THE DOPAMINERGIC NIGRO-STRIATAL PATHWAY AND MECHANISMS OF DRUG ACTION

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This is to certify that the

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R. E. Moore

Major professor

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ABSTRACT

THE DOPAMINERGIC NIGRO-STRIATAL PATHWAY
AND MECHANISMS OF DRUG ACTION

By

Philip Friedrich Von Voigtlander

Anatomical, biochemical and electrophysiological evidence indicates that the substantia nigra and the corpus striatum are connected with reciprocal neuronal pathways. One of these, the nigro-striatal projection, has been shown to contain high concentrations of dopamine. This compound apparently serves a neurotransmitter role at the terminals of these neurons in the corpus striatum. A number of drugs are thought to alter the activity of the nigro-striatal pathway. It was the purpose of this study to examine the effects of these compounds upon this pathway by directly monitoring dopamine release from the caudate nucleus and with a behavioral test using mice with unilateral, chemically induced destruction of this projection.

The release of dopamine from the caudate nucleus was monitored in the following manner. Cats were prepared for ventricular perfusion with cannulas inserted in the lateral ventricles and a catheter in the cerebroaqueduct. H^3-dopamine was injected into the lateral ventricle over the caudate nucleus. After 15 minutes, perfusion of the ventricular system was commenced. Two hours later 1 ml perfusates were collected at 2 or 10 minute intervals and analyzed for H^3-dopamine. During the collection of one or more perfusates, the nigro-striatal neurons were activated by electrical stimulation

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and/or drugs were administered by ventricular perfusion or intravenous injection. In some experiments, the axons of these neurons were electrolytically lesioned either 2-8 weeks before or during the ventricular perfusion.

Electrical activation of the nigro-striatal neurons resulted in an increased rate of release of H^3-dopamine into ventricular perfusates as did ventricular perfusion of amantadine, d-amphetamine, l-amphetamine or tyramine. Chronic lesions of the nigro-striatal neurons markedly reduced the efflux of H^3-dopamine induced by these drugs. Acute lesions similarly disrupted the efflux induced by amantadine and d-amphetamine but not that induced by tyramine. Amantadine and d-amphetamine potentiated the release of H^3-dopamine induced by electrical stimulation of the nigro-striatal neurons; tyramine did not. Thus, amantadine and d-amphetamine alter the disposition of dopamine by a mechanism that is dependent upon the activity of the nigro-striatal neurons. Tyramine releases dopamine from these terminals independently of nerve activity.

Haloperidol, a drug proposed to indirectly activate the nigro-striatal neurons, failed to alter H^3-dopamine efflux when perfused through the cerebroventricular system or given intravenously. Similar results were obtained with intravenous administration of bulbocapnine and apomorphine. These results fail to support the concept that these agents indirectly alter dopamine release.

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striatal projection, 6-hydroxydopamine was injected unilaterally into the striatum of mice. This treatment resulted in a marked reduction in ipsilateral forebrain dopamine concentrations without altering 5-hydroxytryptamine concentrations or causing extensive tissue damage. Mice with these lesions displayed preferential turning toward the lesioned side; sham lesioned mice and those lesioned in the striatum by the injection of ethanol did not. The rate of ipsilateral turning in mice with 6-hydroxydopamine lesions was increased by treatment with d-amphetamine and certain other psychomotor stimulants. However, low doses of apomorphine or L-dopa, drugs which are thought to stimulate dopaminergic receptors directly and indirectly through the formation of dopamine respectively, caused the lesioned mice to turn to the non-lesioned side. This contralateral turning could not be blocked by α-methyltyrosine as could the ipsilateral turning induced by d-amphetamine. This response to apomorphine developed over the course of several days after the injection of 6-hydroxydopamine. Chronic treatment with L-dopa suppressed the contralateral turning response to apomorphine. These observations are consistent with the hypothesis that the loss of dopamine from the striatum results in a supersensitivity to dopamine agonists due to an increased number of dopaminergic receptors. Mice with unilateral 6-hydroxydopamine-induced lesions in the striatum may serve as a useful model to detect drugs with dopamine-agonist properties.

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THE DOPAMINERGIC NIGRO-STRIATAL PATHWAY AND MECHANISMS OF DRUG ACTION

By

Philip Friedrich Von Voigtlander

A THESIS

Submitted to

Michigan State University

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Department of Pharmacology

1972

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INTRODUCTION

The great complexity of the central nervous system results in considerable difficulty for those studying the normal as well as the altered function of this system. This complexity is present not only on the anatomical and histological level but also on the functional level. Feedback circuits involving numerous neurons as well as those acting within the individual neuron may act to control the output of the system. Also, the dynamic nature of the nervous system in the form of the plasticity of neuronal relationships may alter or normalize altered function.

To contend with the problems inherent in the study of the mechanisms whereby drugs affect the central nervous system, it is useful to combine several different approaches. That is, by utilizing biochemical, behavioral, electrophysiological and anatomical techniques, it is possible, despite the complexity of the system, to formulate a clearer understanding of mechanisms of drug action. Furthermore, the application of these diverse techniques to delineate specific pathways within the central nervous system allows the pharmacologist the opportunity to study the effects of drugs upon relatively simple systems. Obviously such a broad approach requires the joint efforts of many individuals;

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This thesis is an attempt to combine several different approaches to the study of the action of drugs upon the dopaminergic nigro-striatal pathway. This pathway and the reciprocal striato-nigral tracts have previously been demonstrated and traced by anatomical, biochemical and electrophysiological techniques. Furthermore, pharmacological data implicate the nigro-striatal pathway as a site of action of several classes of compounds.

The dopamine-containing fibers of the nigro-striatal pathway were originally traced by Andén and coworkers (1964, 1965) using the histofluorescence microscopic technique of Falck and Hillarp. Their results were surprising in light of the failure of classical lesion degeneration and electron microscopic techniques to reveal a nigro-striatal projection.

Afifi and Kaelber (1965), using the Nauta staining technique for degenerating nerve fibers, were unable to trace a pathway to the striatum from the lesioned substantia nigra. Likewise, electron microscopic examination of the caudate nucleus after substantia nigra lesions failed to reveal any degenerating nerve terminals (Adinolfi, 1967). However, the results of both of these studies are in direct opposition to the conclusions of more recent investigations. Using the improved Fink and Heimer staining procedure, Moore et al. (1971) traced a degenerating nerve bundle from the substantia

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nigra to the caudate nucleus of the cat after making electrolytic lesions of the former structure. Furthermore, biochemical analysis of the ipsilateral caudate nuclei after electrolytic lesions of the fibers of this pathway revealed a marked decrease in dopamine concentrations and in the activities of tyrosine hydroxylase and dopa decarboxylase. Lesions in this proposed dopaminergic projection to the striatum caused a similar decrease in caudate dopamine concentrations to those induced by ventral tegmental lesions in the region of the substantia nigra (Poirier and Sourkes, 1965) as well as mimicking the effect of the ventral tegmental lesions upon tyrosine hydroxylase and dopa decarboxylase activity (Goldstein et al., 1969[a]). The electron microscopic studies of Adinolfi (1967)are also in contrast to more recent investigations; Hökfelt and Ungerstedt (1969) reported that following nigro-striatal pathway lesions there was not only a loss of dopamine fluorescence in the ipsilateral striatum, but also the degeneration of small, granule-containing nerve terminals as observed by the electron microscope. The nigral origin of striatal innervation has been confirmed by retrograde degeneration studies. Bédard and coworkers (1969) and Moore et al. (1971) reported that large striatal and discrete nigro-striatal pathway lesions both result in the ipsilateral loss of the cell bodies in the substantia nigra. Thus, the majority of the anatomical and biochemical evidence indicate the presence of a dopamine-

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Electrophysiological studies also support the existence of a dopaminergic nigro-striatal pathway. Connor (1968) observed that electrical stimulation of the substantia nigra could inhibit units in the caudate nucleus that were previously stimulated with homocysteic acid. His data suggest that the nigral input to the caudate nucleus is inhibitory in nature. By direct application of minute amounts of chemicals to small populations of neurons combined with simultaneous microelectrode recording, the technique of microiontophoresis (Bloom et al., 1965) is useful in determining if a substance is inhibitory or excitatory to neurons. By utilizing this technique, McLennan and York (1967) determined that the effect of dopamine upon resting caudate units was primarily inhibitory. Furthermore, microiontophoretic application of dopamine was capable of blocking stimulation-induced activity of caudate units. Other studies, however, make it difficult to determine if the nigral inputs to the striatum are strictly inhibitory. Frigyesi and Purpura (1967) have recorded both a fast (3-4 msec) antidromic and a slow (15-20 msec) orthodromic depolarizing response in the caudate in response to substantia nigra stimulation. Other workers (Hull et al., 1970) reported that the most common response in the caudate to single nigral shocks was a complex of excitatory and inhibitory postsynaptic

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potentials; they claimed that neither response was antidromic. The most unifying conclusion one may draw from all of these studies is that dopamine may indeed be an inhibitory neurotransmitter at the terminals of a nigrostriatal pathway, but that there may also be an excitatory projection from the substantia nigra to the corpus striatum. The concept of a second non-dopaminergic excitatory input from the substantia nigra to the striatum is further supported by the work of Feltz (1971[a,b]). He reports that low intensity stimulation of substantia nigra evokes inhibition of caudate units, whereas higher intensity stimulation evokes increased firing in the caudate.

In addition to nigro-striatal fibers, there also appear to be projections from the striatum to the substantia nigra. Here, again, the electrophysiological data appears contradictory. Frigyesi and Purpura (1967) presented evidence for an excitatory striatal input to the substantia nigra. Goswell and Sedgwick (1971), however, have reported evidence for the striato-nigral input being inhibitory; they argue that it is only by stimulus spread to the internal capsule that caudate stimulation leads to excitatory postsynaptic potentials in the substantia nigra. Other workers also report a direct inhibitory pathway from the caudate nucleus to the substantia nigra (Yoshida and Precht, 1971); low intensity stimulation of the caudate produced long latency (15-20 msec) inhibitory postsynaptic potentials and positive field potentials with cessation of

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firing in the ipsilateral substantia nigra. Furthermore, this response was blocked by low doses of picrotoxin (Precht and Yoshida, 1971). Classical lesion degeneration studies (Nimi et al., 1970; Johnson and Rosvold, 1971) demonstrate that large numbers of fibers from the caudate nucleus do, indeed, terminate in the substantia nigra. Electron microscopic studies (Kemp, 1969; Grofová and Rinvik, 1970) also show degenerating terminals in the substantia nigra following caudate lesions. This loss of boutons containing elongated vesicles was accompanied by a marked depletion of gamma-aminobutyric acid from the substantia nigra (Kim et al., 1971), suggesting that the inhibitory input from the caudate might be mediated by this neurotransmitter. In this context, however, it is important to note that Olivier et al. (1970) have traced a cholinesterase-containing pathway from the striatum to the globus pallidus and substantia nigra and that electron microscopic studies (Gulley and Wood, 1971) have shown small clear vesicle-containing boutons characteristic of cholinergic terminals in the substantia nigra. Thus, as was the case with the caudate inputs from the substantia nigra, the caudate-fugal fibers to the substantia nigra may not be strictly inhibitory or excitatory. Nevertheless, the demonstration of both nigro-striatal and striato-nigral fiber systems makes possible a reciprocal, neuronally-mediated feedback between these two structures.

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requires the severing of both the nigro-striatal pathway and the rubro-olivary-cerebellar-rubral loop. Thus, it appears that the dopaminergic nigro-striatal pathway is an important system for the maintenance of normal postural and locomotor control in primates; the loss of this projection is related to marked deficits in these behaviors. Studies in rats (Andén et al., 1966) and mice (Lotti, 1971) have shown that animals with large unilateral striatal lesions display motor asymmetries when treated with certain drugs. However, these lesions are not, of course, specific for the dopaminergic pathway. More recent studies (Ungerstedt, 1971[a]) have shown that when selective unilateral lesions of the dopaminergic nigro-striatal projection are made, rats manifest locomotor asymmetries with certain drug treatments. Furthermore, bilateral lesions of the substantia nigra block the increased locomotor activity of rats treated with amphetamine (Iversen, 1971). The mechanism by which the dopaminergic system affects locomotor activity is not altogether clear; however, Ohye et al. (1970) have shown that after sectioning this projection the spontaneous activity of units in the striatum increases. Likewise, Andén and coworkers (1971[a]) suggest that the balance between alpha and gamma motor neuron excitability may be altered following destruction of this projection. Thus, as in primates, the dopaminergic projection from the substantia nigra to the striatum plays an important role in modulating motor activity of rodents. This similarity suggests the usefulness of these latter species

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In the course of this discussion of the nigro-striatal pathway, the presence of high concentrations of dopamine in these neurons has been tacitly assumed to indicate a neurotransmitter role of this compound at the terminals of these fibers. Basic to this idea is the release of dopamine upon stimulation of the nigro-striatal neurons; however, early attempts to monitor this release met with only limited success (McLennan, 1964; Vogt, 1969). In later studies electrical stimulation of nigro-striatal neurons was shown to increase the rate of depletion of striatal dopamine after synthesis inhibition by alpha-methyltyrosine (Arbuthnott et al., 1970) and to release dopamine in vitro (Ng et al., 1971). With the use of H^3-dopamine to label the periventricular catecholamine stores, it has been possible to demonstrate the neuronally mediated release of this amine during stimulation of either the cell bodies in the substantia nigra or the terminals in the caudate nucleus (Von Voigtlander and Moore, 1971). These studies provide the groundwork for further investigations into the mechanisms of dopamine release and the mechanisms whereby drugs may interact with the dopaminergic nigro-striatal pathway. It may also be possible with the recent tracing of the compact nigro-striatal fiber bundle in the cat (Moore et al., 1971) to find more effective sites for evoking dopamine release.

Several lines of evidence indicate that the dopaminergic nigro-striatal pathway may be an important site of drug action.

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The observation of Weissman and coworkers (1966) that inhibition of catecholamine synthesis with α-methyltyrosine resulted in the blockade of amphetamine-induced stimulation, is particularly important. This result suggests that ongoing norepinephrine and/or dopamine synthesis is necessary for amphetamine to exert stimulant effects. More recent studies (Thornburg, 1972) using dopamine-β-hydroxylase inhibitors to selectively block norepinephrine synthesis suggest that this amine may not be as important as dopamine for the amphetamine hypermotility response. Furthermore, Simpson and Iversen (1971) have shown that lesions of the substantia nigra greatly reduce the response to amphetamine, implicating a nigral pathway as a site of the effect of amphetamine.

The mechanism whereby the dopaminergic nigro-striatal pathway functions in the amphetamine response is not entirely clear. Glowinski and Axelrod (1965) demonstrated that this compound could decrease brain tissue concentrations of H^3-catecholamines whether it was given before or after the tracer. Thus, amphetamine might release catecholamines or block their transport into the tissue stores. Carr and Moore (1970[a]) have demonstrated that after labeling the periventricular amine stores with H^3-dopamine, the ventricular perfusion of amphetamine results in marked increase in the rate of H^3-dopamine outflow. Certain other psychomotor stimulants had a similar effect (Carr and Moore, 1970[b]). From these studies the mechanism by which amphetamine increases dopamine

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The antiparkinsonian drug, amantadine (Walker et al., 1972) has also been suggested to act through dopaminergic mechanisms (Grelak et al., 1970; Scatton et al., 1970). However, as is the case with amphetamine, it is difficult to determine if amantadine actively releases dopamine or acts by some other mechanism. Several other antiparkinsonian compounds have been shown to interfere with dopamine uptake in vitro (Coyle and Snyder, 1969[a]; Farnebo et al., 1970). The mechanisms by which anti-parkinsonian drugs as well as more potent psychomotor stimulants might alter dopamine release and/or reuptake is thus still open to question. By altering the rate of neurogenic release of dopamine one might be able to test which of these mechanisms is more important. That is, if a drug actively releases dopamine, it would be expected to act independently of nerve activity; however, if a drug either facilitates dopamine release or blocks the reuptake of released dopamine, it would be strictly dependent on the ongoing neurogenic release of dopamine to exert these effects.

It has been suggested that other compounds indirectly alter the rate of dopamine release from the terminals of the nigro-striatal pathway. The ability of neuroleptic agents of the phenothiazine, butyrophenone and diphenylbutylpiperidine

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classes to induce parkinsonian-like symptoms is well known (Simpson, 1970: Huber et al., 1971). Because Parkinson's disease involves a loss of dopaminergic striatal innervation, it is possible that neuroleptic-induced parkinsonism may result from a dopaminergic blockade (Hornykiewicz, 1966). These drugs have been shown to increase dopamine turnover in experimental animals (O'Keefe et al., 1970) and man (Chase et al., 1970). Andén and coworkers (1970) have proposed that the blockade of postsynaptic dopamine receptors is causally related to the ability of the neuroleptic agents to increase dopamine turnover. They reason that the blockade of striatal dopamine receptors results in an activation of the nigro-striatal neurons via a negative feedback neuronal loop leading to an increased dopamine release and thus an increased turnover. This interesting hypothesis, however, remains to be confirmed. No one has been able to demonstrate that neuroleptics increase dopamine release from the intact brain. If such an increase does occur, it should be blocked by severing the nigro-striatal fibers and potentiated by drugs that enhance the neurogenic release of dopamine.

Apomorphine, a drug that reverses parkinsonian symptoms (Cotzias et al., 1970) has been hypothesized to directly stimulate dopamine receptors in the striatum (Ernst, 1965). Andén et al. (1967) have shown that this putative dopamine agonist decreases dopamine turnover in

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the striatum. Thus, apomorphine causes some behavioral and neurochemical changes that are opposite to those caused by neuroleptic agents. It has been suggested (Andén et al., 1967) that the stimulation of dopamine receptors by apomorphine results in an inhibition of firing of the nigro-striatal neurons thereby decreasing dopamine release and turnover. This concept of a neuronal feedback altering dopamine release has not been directly tested. If apomorphine does, indeed, decrease firing over the nigro-striatal pathway, it would be expected to act in an antagonistic manner to drugs that increase extracellular dopamine concentrations by mechanisms dependent on ongoing dopamine release.

It is the purpose of this thesis to describe experiments performed to further elucidate the mechanisms by which drugs act directly and indirectly upon the dopaminergic nigro-striatal pathway. Specifically, the mechanisms whereby psychomotor and antiparkinsonian stimulants increase extracellular dopamine concentrations have been investigated. To accomplish this, techniques of stimulating and lesioning the nigro-striatal pathway have been developed. Likewise, an attempt has been made to utilize these techniques to determine if neuroleptics and apomorphine indirectly alter dopamine release. A simple behavioral model for studying the effects of drugs upon the dopaminergic nigro-striatal neurons has also been developed.

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METHODS

Ventricular perfusion studies

Domestic cats (2-3 kg) of either sex were anesthetized with nitrous oxide (80%), oxygen (20%) and methoxyflurane (0.2-0.4%) using a closed circuit gas anesthetic machine (Lundy Rochester model, Heidbrink Co.) attached to a sealed anesthesia box. After induction, the animal was removed from the box and placed in dorsal recumbency with its muzzle covered by a cone connected to the anesthetic machine. A mid-ventral incision was made through the skin and muscles over the trachea, the trachea incised and a cannula inserted. The anesthesia was then continued with the anesthetic machine attached directly to the tracheal cannula. The cat was placed in a small animal stereotaxic unit (model 1404, David Kopf Instruments). A mid-dorsal incision was made from the level of the supraorbital processes to the atlas and the cervical muscles reflected and cut away to expose the supraoccipital region of the skull. The fascia and dural covering of the cord were reflected and the spinal cord sectioned just above the first vertebra. The tracheal cannula was immediately attached to a small animal respirator (model 672, Harvard Apparatus Co.) which was adjusted to 20 cycles/ minute and

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an appropriate tidal volume. Anesthesia was maintained with 80% nitrous oxide and 20% oxygen by fitting the anesthesia machine to the respirator input. One-eighth inch diameter holes were drilled through the skull at A 16.5, L 3.5 left and L 3.5 right (Snider and Niemer, 1961) and 22 gauge stainless steel screw type cannulas (model 201, David Kopf Instruments) inserted in the lateral ventricles to a depth of H +8. The supraoccipital region of the skull was removed and bone wax packed into the cut edge. The cerebellum was carefully lifted until the cerebroaqueduct could be visualized and a cannula (5 cm x 2 mm outside diameter polyethylene, with a 5 mm silastic cuff) was then carefully inserted into the aqueduct (see Figure 1a). Wounds and pressure points were infiltrated with 2% lidocaine and the gas anesthesia terminated. Five µc H^3-dopamine (New England Nuclear, 9.5-12.4 c/mM) or 2.5 µc H^3-dopamine (Amersham/ Searle, 2 c/mM) in a volume of 5 microliters was then injected into one of the lateral ventricular cannulas and flushed in with 10 microliters of artificial cerebrospinal fluid (Pappenheimer et al., 1962). In one series of experiments 2.5 µc C^{14}-urea (New England Nuclear, 0.27 mc/mM) was injected in a volume of 20 microliters prior to the H^3-dopamine. After 15 minutes, perfusion of the ventricular system with artificial cerebrospinal fluid at a rate of 0.1 ml/min was commenced using a Harvard Compact infusion pump. Two hours later, 1 ml perfusates were collected into tubes containing 0.1 ml 5 N acetic acid and 0.1 mg sodium ascorbate at 2 or 10 minute

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Figure 1a. Schematic view of a cat brain prepared for cerebroventricular perfusion.

The ventricular system is shown in gray and black. The striped areas represent the substantia nigra and caudate nucleus. The inflow cannula is shown projecting into the lateral ventricle and the outflow catheter is placed into the cerebroaqueduct. Also illustrated are electrodes placed in the caudate nucleus and substantia nigra.

Figure 1b. Saggital view of apparatus used for intra-striatal injections.

The head mold which surrounds and immobilizes the mouse's head is represented by the cross-hatched area. The anterior-posterior and lateral coordinates of the injection site are specified by the location of the guide cannula, the depth of the injection by the cuff on the microliter syringe needle. The gray area at the tip of the needle represents the corpus striatum.


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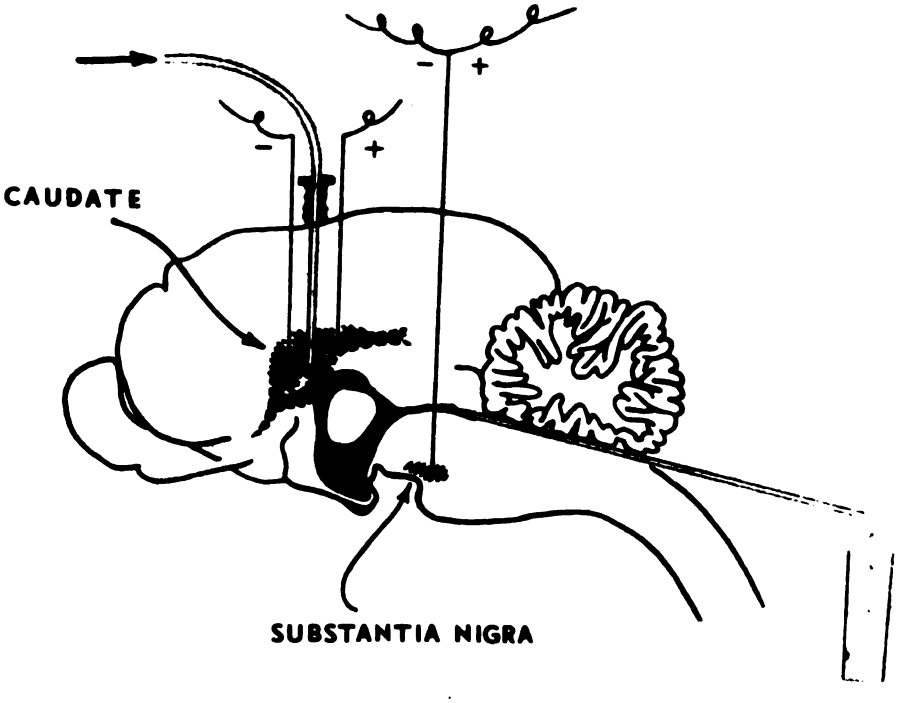

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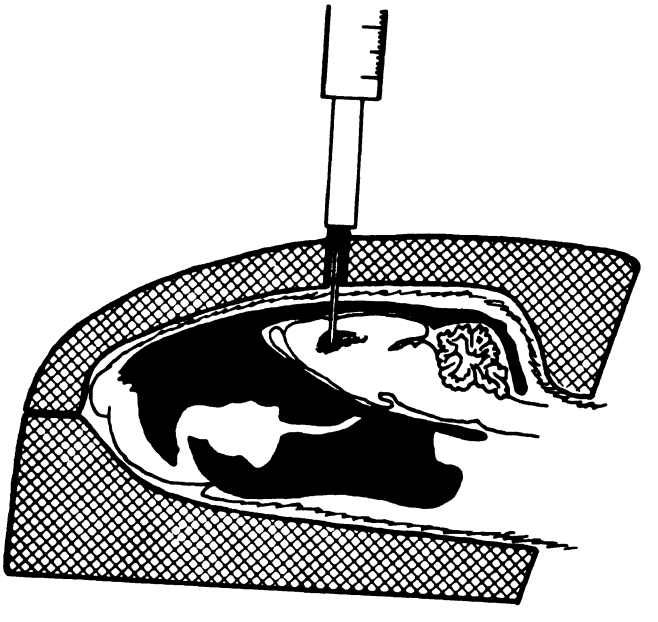

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intervals using perfusion inflow rates of 0.5 ml/min and 0.1 ml/min respectively. During the collection of some perfusates, drugs were added to the perfusion inflow or injected intravenously into the femoral vein. In some experiments monophasic square wave (1 msec duration) electrical stimulation (Grass S-4 Stimulator and Grass Constant Current Unit) was applied to the caudate nucleus with 2 electrodes (Model NE-200, David Kopf Instruments) placed 5 mm apart; the cathode and anode were placed at L 4.0 and H +5 and A 18.0 and A 13.0 respectively. The electrodes were inserted at a 24° angle to avoid puncturing the ventricle. In other experiments electrical stimulation was applied to sites in the diencephalon with a single bipolar electrode (Model NE-200, David Kopf Instruments). Electrolytic lesions were also produced with 3 mA anodal direct current passed through electrodes aimed at A 10, L 3.0 and H -2.5 and H -3.5 for 1 minute at each point with the stereotaxic serving as the cathode. These lesions were made either 2-8 weeks before or during the collection of perfusates.

The procedure for the push-pull cannula perfusions was the same as for the previously described ventricular perfusions except the ventricular inflow cannula was replaced with a push-pull cannula (Gaddum, 1961) and no cerebroaqueduct cannula was used. This push-pull cannula was constructed with an 18 gauge outer outflow needle and a concentrically fitted 23 gauge inner inflow cannula. A

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perfusion inflow rate of 0.5 ml/min was used and perfusion outflow was adjusted to this rate by raising or lowering a syphon attached to the outflow cannula.

When appropriate the entire experiment was repeated using the opposite lateral ventricle. In experiments which involved chronic or acute unilateral diencephalic lesions, the opposite non-lesioned side served as the control. In cats with chronic lesions, the entire process of H^3-dopamine injection and perfusion were repeated twice on each side.

The brain was removed and fixed in 10% formalin for later gross and histological examination to verify the positions of the inflow cannulas, electrodes and lesions. In some experiments, the caudate nuclei and septal nuclei were removed and weighed for biochemical analysis after first perfusing the circulatory system with 2 liters of saline. The remainder of the brain was then fixed in formalin.

Rectal temperature was monitored and maintained at 37.5 $\pm$ 0.5°C with an electric heating pad. In some experiments arterial blood pressure was monitored from the femoral artery with a Statham physiological pressure transducer and Grass polygraph.

Mouse turning behavior studies

Male mice weighing 20-25 gm (Spartan Farms) were used throughout these experiments. A head mold for accurate and

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reproducible placement of intra-striatal injections was made in the following manner. A mouse was sacrificed and modeling plastic (Polyform Products Co.) was encased around its head and neck. The plastic was baked to hardness, the block cut horizontally, and the mouse removed. The interior dimensions of the hardened plastic block were thus molded to conform to the head of a 20-25 gm male mouse (See Figure 1b). Frontal sections of a mouse head were used to determine the skull coordinates overlying the corpus striatum and the distance from the center of the striatum to the surface of the skull. In this manner, it was determined that the center of the corpus striatum was approximately 3 mm below the skull surface at 1.5 mm lateral to the midline and 5 mm anterior to the occipital suture. This region has the approximate stereotaxic coordinates in the mouse brain of A 4, L 2, H + 2.5 (Montemurro and Dukelow, 1972). Two 20 gauge stainless steel cannulas were then mounted in the left and right side of the head mold so that they would make contact with the mouse head at these anterior and lateral coordinates. A 10 microliter syringe (Model 701-N, Hamilton) was fitted with a nylon cuff so that when the needle was inserted through the guide cannula on the head mold, only 3 mm of needle protruded. A 26 gauge needle which protruded less than 0.5 mm beyond the guide was used to pierce the skin and the skull.

The intra-striatal injection procedure was performed in the following manner. Mice were anesthetized in a beaker containing cotton soaked with methoxyflurane. The mouse head

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was swabbed with 70% ethanol and placed in the head mold. The 26 gauge needle was inserted through the left or right guide to pierce the underlying skin and skull. The needle was withdrawn and the microliter syringe inserted. Four microliters of distilled water containing 0.8 µg sodium ascorbate and 8 or 16 µg 6-hydroxydopamine hydrobromide or 30 µg 5,6-dihydroxytryptamine creatinine sulfate was then injected over a 30-60 sec period; the microliter syringe was then slowly withdrawn. In other experiments, 8 microliters of 95% ethanol was injected over a 60 sec period. This technique allows one operator to inject 16-20 mice per hour.

Two types of sham injections were made. For cortical sham lesions, injections were made following the same procedure except that an extra 2 mm cuff was added to the microliter syringe. Thus, 6-hydroxydopamine lesions were made in the cerebral cortex overlying the corpus striatum. Sham lesions in the striatum were made by injecting 4 microliters of distilled water containing 0.8 µg sodium ascorbate.

At various intervals (generally 10 days or more) after the intra-cranial injection, the mice were observed individually and their behavior quantified in the following manner. The animal was placed in a 3 liter beaker which was painted white and illuminated from below. The beaker was contained in a sound attenuating box with a one-way window

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in the top. The observer could then view the mouse in an environment relatively free of extraneous distraction. The number of times which the mouse made full 360 turns to the right and to the left were recorded during a 2 minute period immediately after the mouse was placed in the beaker. Turns to the right and left were recorded as positive and negative turns respectively. The results reported are the sums (net turns) of the right (+) and left (-) turns. Thus, mice which consistently turned to the left more than to the right have negative net turn scores.

Some groups of mice were decapitated 10 days or more after the intra-cranial injection and their brains analyzed for norepinephrine, dopamine and 5-hydroxytryptamine. The forebrain was separated by a cut through the corpora quadrigemina and then sectioned midsaggitally into the left and right forebrain. Four left or 4 right forebrains were pooled and weighed for biochemical analysis.

Some mice were sacrificed for gross and histological confirmation of the striatal injection site. First the skull was exposed and a dissecting microscope with an eye-piece micrometer was used to measure the distance from the needle lesion on the skull to the midsaggital and occipital sutures. The brain was then removed and placed in 10% formalin.

Histological techniques

After at least 48 hours of fixation the brains were

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removed from the formalin and dissected. Histologic sections fifty microns thick of the mouse forebrains were cut horizontally on a frozen section microtome (American Optical 880). Sections that showed visible needle tract lesions were saved for staining and microscopic examination. The positions of the ventricular cannulas and caudate electrodes in the cat brains were determined by visual inspection. Cat hemi-diencephalons were sectioned in the horizontal plane. Sections cut through electrode tracts and electrolytic lesions were stained by the following method. The sections were placed on clean microscope slides. The slide was flooded with buffered cresyl violet stain (Humason, 1967) for twenty minutes and washed quickly with 70% and then 95% ethanol. Dehydration was completed by flooding the slide with isopropanol for three minutes. After the alcohol had evaporated, the stained tissue was mounted to the slide with diatex (Scientific Products). The sections were then examined under a 10x dissecting microscope with an eyepiece micrometer to determine the size and location of the lesions and electrode tracts.

Biochemical analyses of perfusates and tissues

In some of the experiments, the total radioactivity of the perfusates was estimated. One hundred µl of each perfusate were transferred into glass scintillation vials containing a toluene-ethanol-2,4-diphenyloxasole (7:3, 0.5%

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2,5-diphenyloxasole) scintillator. The radioactivity was then determined in a Beckman LS-100 liquid scintillation counter with direct readout module. The counts were corrected for counting efficiency for these total perfusates as well as all other samples counted; hence, the data presented are in units of absolute radioactivity (dpm or nc). The background was subtracted and for the H^3-dopamine and H^3-3-methoxytyramine fractions, a factor for recovery of a standard was applied to correct for losses during the separation procedures.

The initial separation performed on the perfusates was the alumina extraction of the catechol compounds. For this purpose 0.1 ml of 0.2 M disodium ethylenediamine tetra-acetate plus 6 drops of an alumina suspension (approximately 100 mg of aluminum oxide) were added to 5 ml centrifuge tubes containing the collected perfusates. The pH of the contents of each tube was then adjusted to 8.5-8.6 with 5 N, 1 N and 0.1 N potassium hydroxide. The tubes were then shaken for five minutes in an Eberbach horizontal tube shaker followed by a five minute centrifugation at 1800 x g. The resulting supernatant fluid containing the non-catechol compounds was then aspirated and in some experiments saved for further analysis. The alumina containing the adsorbed catechols was then washed twice, once with 2 ml water and once with 1 ml water. These washes involved the same shaking and centrifuging steps as previously outlined. After the second wash,

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the catechols were eluted from the alumina with 1 ml 0.2 N acetic acid. The acid and alumina were shaken for ten minutes, centrifuged for five minutes and the eluate aspirated off and saved. One-hundred µl of the eluate were counted as previously described.

In a number of experiments the alumina supernatant (non-catechol fraction) was further separated into an amine fraction and a non-amine fraction by cationic exchange on Dowex 50 resin, H^+ form, 100-200 mesh. The Dowex was formed into 6 mm x 40 mm free flowing columns and the alumina supernatants, after having been adjusted to pH 6 with 0.2 N acetic acid, were poured on the columns. After the sample had run through, 5 ml water was added; these two fractions contained the non-amine portion of the non-catechol fraction, the deaminated-O-methylated metabolites. In some experiments 1 ml of this fraction was saved and counted in 10 ml of modified Bray's solution (6 gm of 2,5-diphenyloxasole and 100 gm of naphthalene per liter of dioxane). The amines were then eluted from the ion exchange resin with 5 ml of a 1:1 solution of 95% ethanol and 6 N hydrochloric acid. One ml of this fraction, containing the O-methylated amines was added to 10 ml of Aquasol liquid scintillator (New England Nuclear) and the samples counted in the scintillation spectrophotometer.

In initial experiments using H^3-dopamine, the alumina eluate was separated into a H^3-dopamine fraction and a H^3-norepinephrine fraction by selective elution from a 6 mm x

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40 mm column of Dowex 50, 200-400 mesh. After the flow rates of the columns were adjusted to 5-7 drops per minute, the Dowex was changed to Na^+ form by adding 25 ml of 0.1 M sodium phosphate, pH 6.5 buffer, followed by 5 ml water. The samples were prepared by the addition of 100 µg of dopamine, each in a volume of 10 µl. Before being added to the columns, the samples were adjusted to pH 6 with 1.0 N and 0.1 N potassium hydroxide. After the sample had run through the column, 5 ml of water, 8 ml 1.0 N hydrochloric acid, 10.0 ml 1.0 N hydrochloric acid and 4.0 ml 1:1 6.0 N hydrochloric acid-95% ethanol solution were added in succession. The first two fractions presumably contained the deaminated catechols, the 10.0 ml 1.0 N hydrochloric acid eluate contained the H^3-norepinephrine peak and the 4.0 ml hydrochloric acid -ethanol contained the H^3-dopamine; 1.0 ml of each of these fractions was transferred to scintillation vials containing Aquasol and counted in the liquid scintillation spectrophotometer.

In perfusion experiments where chronic or acute diencephalic lesions were made, the caudate nuclei were removed after the experiment and analyzed for dopamine, 5-hydroxytryptamine and H^3-dopamine in the following manner. The tissue was homogenized in 6.0 ml cold n-butanol. The homogenate was poured into a 50 ml glass centrifuge tube containing 0.5 gm sodium chloride and the homogenizer tube rinsed with 6.0 ml cold n-butanol. The 12 ml of butanol

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homogenate were stored at 0°C until analysis. For the assay three standards covering the range of anticipated values (1.0, 2.0 and 4.0 µg dopamine and 0.125, 0.25 and 0.5 µg 5-hydroxytryptamine) and a blank were prepared with 12 ml n-butanol and 0.5 gm sodium chloride. One µc H^3-dopamine (Approximately 0.02 µg dopamine) was added to one of the standards. The standards, blanks and tissue homogenates were then shaken on an Eberbach horizontal tube shaker for 5 minutes followed by a 5 minute centrifugation at 1800 x g. Ten ml of the upper layer (butanol) were carefully aspirated and transferred to another set of 50 ml centrifuge tubes containing 40 ml of heptane and 3.0 ml 0.01 N hydrochloric acid. The remaining butanol, salt and aqueous phase were discarded. The heptane containing tubes were shaken for 5 minutes and spun at 1800 x g for 5 minutes. The upper phase (heptane) was then carefully aspirated and discarded. The lower phase was transferred to a set of 5 ml centrifuge tubes and spun for 5 minutes at 1800 x g. Again, any upper phase (heptane) remaining was aspirated and discarded. For the 5-hydroxytryptamine assay, 1 ml of the lower phase (aqueous) was added to quartz cuvettes containing 0.4 ml concentrated hydrochloric acid. The cuvettes were placed in an Aminco Bowman spectrophotofluorometer with the activating wave length set at 295 nm; the emitted fluorescence at 550 nm wave length was read. The amount of 5-hydroxytryptamine in each tissue sample was calculated directly from the regression line of the 3 standards. For the dopamine assay

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1.0 ml of the aqueous phase from each sample, standard and blank was added to 1.0 ml 0.01 N hydrochloric acid. Likewise, 0.25 ml of the aqueous phases from 4 samples were pooled and added to 1.0 ml 0.01 N hydrochloric acid (tissue blank) as was 0.25 ml of each of the three standards and blank (standard blank). One ml of 0.5 M potassium phosphate buffer (pH 8.0) was added to each tissue sample, standard and blank. Then 0.2 ml of an aqueous 0.5% sodium periodate solution was added to all tubes except the tissue and standard blanks to which were added 0.2 ml distilled water. Exactly two minutes later 1.0 ml of an alkaline sulfite solution (10 ml 0.265% sodium sulfite plus 90 ml 5 N sodium hydroxide) followed by 3.5 ml glacial acetic acid was added to each tube. The tubes were then capped and placed in a boiling water bath for 30 minutes after which they were cooled in ice. An aliquot from each tube was added to a quartz cuvette and the fluorescent emission at 385 nm in response to activation at 325 nm read. As with the 5-hydroxytryptamine assay, the amount of dopamine in each tissue sample was calculated directly from the regression line of the standards. H^3-dopamine was analyzed from 0.25 ml of the remaining aqueous phase of the solvent extraction by adding 0.75 ml distilled water to the sample and separating the catechols by alumina extraction as described for the analysis of perfusates.

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5-hydroxytryptamine and in some cases dopamine was carried out in exactly the same manner except the range of standards used was 0.2, 0.4 and 0.6 µg for both the amines and no H^3-dopamine standard was included.

The extraction of dopamine and norepinephrine from some mouse hemi-forebrains and of norepinephrine from cat septal nuclei was effected in the following manner. The tissues were homogenized in 2.0 ml cold 0.4 N perchloric acid and kept in ice for 30 minutes. The tubes were spun for 5 minutes at 14,000 x g and the supernatant was collected. The pellet was rehomogenized in 2.0 ml 0.4 N perchloric acid, kept on ice for 15 minutes and recentrifuged at 14,000 x g for 5 minutes. The supernatants were combined and stored at 0° C until analyzed. After thawing, 0.5 ml 0.2 M disodium ethylenediaminetetraacetic acid was added to each sample, standard and blank. The blank consisted of only 4.0 ml 0.4 N perchloric acid whereas the 3 standards had appropriate amounts of catecholamines added (0.2, 0.4, 0.6 µg dopamine or 0.1, 0.2, 0.3 µg norepinephrine for mouse hemi-forebrains and 0.02, 0.04, 0.08 µg norepinephrine for cat septal nuclei). The pH of each sample was raised to 4.0 with 10 N, 1.0 N and 0.1 N potassium hydroxide and the sample cooled in ice for 10 minutes. The resulting precipitate of potassium perchlorate was compacted by centrifugation at 14,000 x g and the supernatant decanted to a 20 ml beaker containing 10 drops of an alumina suspension (approximately

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170 mg aluminum oxide) while stirring with a glass impeller. The pH of the sample was then carefully increased to 8.6 with 1.0 M and 0.2 M potassium carbonate. After stirring the sample an additional 5 minutes, the supernatant was discarded and the alumina transferred to a 15 ml stoppered centrifuge tube containing 5 ml distilled water. The tubes were shaken for 5 minutes, centrifuged at 1800 x g for 2 minutes and the water aspirated and discarded. This water wash was then repeated. After aspiration of the second wash, 4.0 ml of 0.2 N acetic acid were added to the alumina and the samples shaken for 10 minutes and centrifuged at 1800 x g for 2 minutes. The supernatant was aspirated and saved. Two ml of this fraction were assayed for dopamine in exactly the same manner as that previously described. The norepinephrine assay proceeded in the following manner. A two ml aliquot of each sample was used as a blank, the remaining 2 ml was run as the sample. The pH of each sample and blank was raised to 6.5 with 1.0 and 0.2 M potassium carbonate; then 0.4 ml of 0.1 M potassium phosphate buffer (pH 6.5) was added to each tube. Next, 0.05 ml of distilled water was added to each of the blanks and 0.05 ml of a 0.24% potassium ferricyanide solution was added to the samples. Exactly 2 minutes later 0.25 ml of an alkaline ascorbate solution (1 ml 2% sodium ascorbate + 9 ml 5 N sodium hydroxide) was added to every tube. An aliquot from each tube was transferred to a quartz cuvette and the fluorescence at 510 nm in response to 390 nm

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excitation recorded. After subtracting the blank values the amounts of norepinephrine were calculated directly from the regression line of the standards.

Drugs used

In the course of these investigations the following drugs were perfused through the ventricular system and/or injected intravenously during ventricular perfusion: amantadine hydrochloride, d-amphetamine sulfate, l-amphetamine sulfate, apomorphine hydrochloride, bulbocapnine hydrochloride, fluphenazine dihydrochloride, haloperidol, tyramine hydrochloride and vasopressin U.S.P. The perfused concentrations are indicated in terms of molarity; the injected doses refer to the salts where appropriate. Apomorphine, bulbocapnine and haloperidol were dissolved in 5 ml propylene glycol for intravenous injection.

The following drugs were dissolved in normal saline immediately before use in the mouse turning behavior studies: amantadine hydrochloride, d-amphetamine sulfate, l-amphetamine sulfate, apomorphine hydrochloride, caffeine, clonidine, morphine sulfate, magnesium pemoline, pipradrol hydrochloride, L-5-hydroxytryptophan methyl ester and methylphenidate. Other compounds were suspended in 1% methylcellulose: amfonelic acid, L-dopa, L-3-methoxytyrosine and 1-[3,4-(methylenedioxy) benzyl]-4-(2-pyrimidyl) piperazine (ET-495).

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Statistical methods

Means and standard errors of all grouped data were calculated on an Olivetti Underwood-Programma 101 desk computer. Statistical comparisons were by the Student's t test utilizing paired comparisons where appropriate. Linear regression analysis was performed by the method of least squares. "t" Values and regression coefficients for which $P < .05$ were considered statistically significant.

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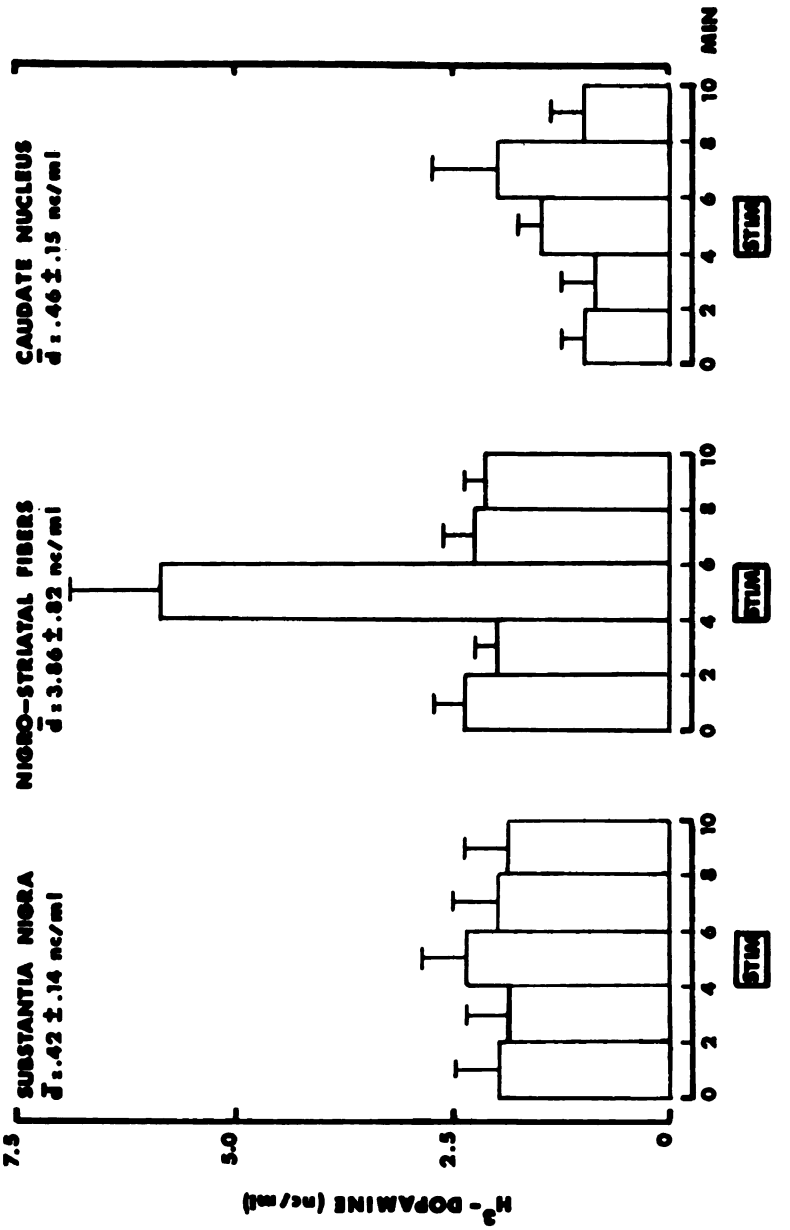
RESULTS

I. Ventricular perfusion: stimulation of nigro-striatal neurons.

Previous studies (Von Voigtlander and Moore, 1971) have demonstrated that after labeling the caudate nucleus of the cat with H^3-dopamine, electrical stimulation of either the substantia nigra or caudate nucleus results in an increase in H^3-dopamine release into ventricular perfusates. Figure 2 compares the release of H^3-dopamine evoked by stimulation of these regions with that evoked by stimulation of a specific site in the diencephalon (A 10, L 3, H -3.5; Snider and Niemer, 1961). In each case stimulation resulted in a statistically ($P < .05$) significant increase in the concentration of H^3-dopamine in the perfusion effluent during the period of stimulation as compared to the period immediately before stimulation using the paired t test. Electrical stimulation in the area of the diencephalic nigro-striatal fibers, however, resulted in a many fold greater release of H^3-dopamine than did stimulation in the other two regions. It is also noteworthy that while stimulation of substantia nigra or the nigro-striatal fibers resulted in the release of H^3-dopamine only during the period of stimulation, that direct stimulation of the caudate nucleus evoked a release

Figure 2. Effects of 2 minutes of electrical stimulation of substantia nigra, nigro-striatal fibers and caudate nucleus on ventricular effluent concentrations of H^3-dopamine.

The height of each bar represents the mean concentration (vertical lines denote 1 standard error) of H^3-dopamine in the ventricular perfusates collected over 2 minute periods from at least 4 cats. When each region was stimulated (1 msec pulses of 350-400 μA intensity at a frequency of 30-50 Hz) the perfusate concentration of H^3-dopamine was significantly ($P < .05$) greater than that just before stimulation. $\bar{d}$ Values represent the mean ($\pm$ 1 standard error) difference between H^3-dopamine concentration in the perfusate before and during stimulation.


SUBSTANTIA NIGRA
d̄ = .42 ± .14 nc/ml
NIGRO-STRIATAL FIBERS
d̄ = 3.86 ± .82 nc/ml
CAUDATE NUCLEUS
d̄ = .46 ± .15 nc/ml
STIM
MIN
H³- DOPAMINE (nc/ml)
7.5
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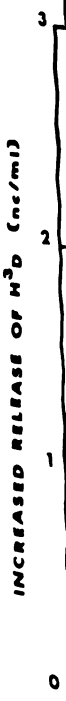
If the release of H^3-dopamine induced by electrical stimulation is a neuronally mediated event one might expect the effect to be dependent upon the frequency of stimulation. Figure 3 compares the frequency-release curves for substantia nigra, nigro-striatal and caudate nucleus stimulation-induced release of H^3-dopamine. Stimulation at all three sites results in a maximal release of H^3-dopamine with pulses of 30-50 Hz; 100 Hz is in each case less effective. It appears, therefore, that the mechanism which is responsible for this stimulation-induced release is capable of following frequencies of 30-50 Hz but not 100 Hz.

Since the diencephalic stimulation was far more effective than either stimulation of substantia nigra or caudate nucleus, the specificity of this effect was investigated. If the nigro-striatal fibers were involved in this evoked H^3-dopamine release, then only stimulation in the region of these fibers should result in this marked release. Figure 4 illustrates the results of a series of experiments in which a stimulating electrode was placed at A 10, L 3 and H -1.5, stimulation applied for 2 minutes and the electrode lowered by 1 mm steps with stimulation repeated at each level. Thus, in each experiment summarized electrical stimulation was applied at A 10, L 3, and H -1.5, -2.5, -3.5, -4.5 and -5.5. In each of four experiments, the greatest increase in H^3-dopamine release occured during stimulation at the H -3.5 level with

Figure 3. The increases in H^3-dopamine released into ventricular perfusates upon electrical stimulation of nigro-striatal fibers, caudate nucleus and substantia nigra at various frequencies.

Nigro-striatal (O) and substantia nigra (Δ) stimulation were 1 msec pulses of 200 μA intensity. Caudate nucleus (□) stimulation was 1 msec pulses of 400 μA intensity. Increased release of H^3-dopamine (H^3D) is the mean difference between the ventricular effluent concentration during the 2 minute period of stimulation and that during the 2 minute period just before stimulation in a total of 8 experiments. Solid symbols denote increases that are statistically ($P < .05$) significant. Vertical lines denote 1 standard error. Standard errors smaller than the associated point are not noted.



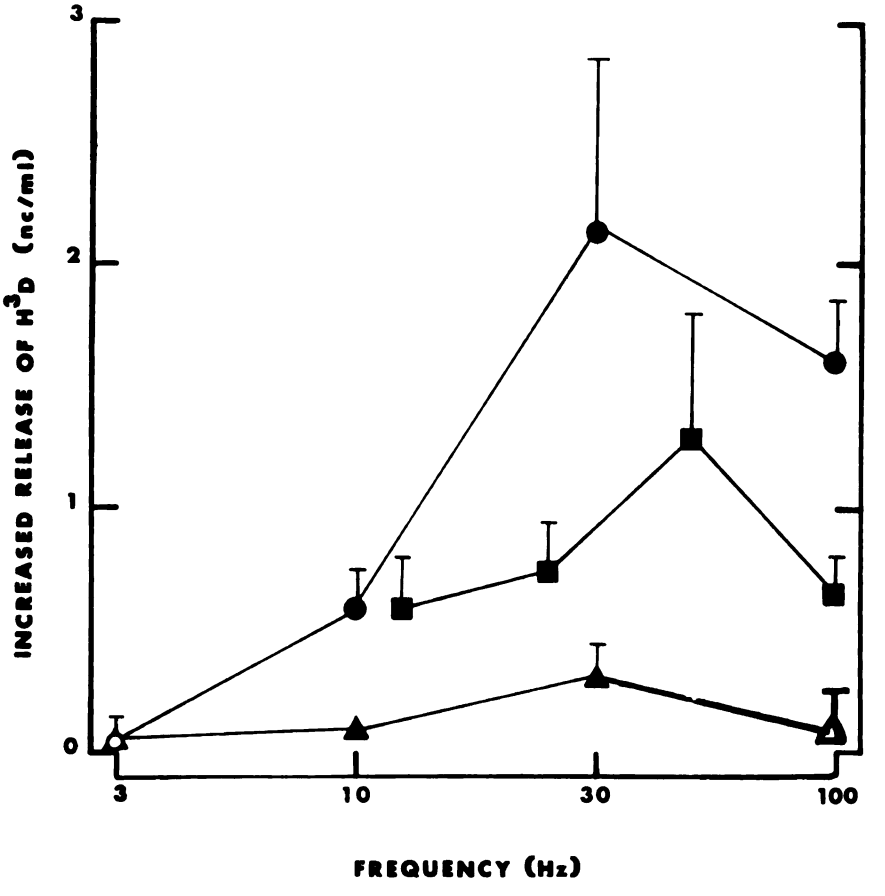

INCREASED RELEASE OF H^3D (nc/ml)
3
2
1
0
3
10
30
100
FREQUENCY (Hz)

Figure 4. The effects of electrical stimulation at various points near the nigro-striatal fibers upon the release of H^3-dopamine into ventricular perfusates.

The histological section demonstrates the points that were stimulated (2 min of 400 μA, 1 msec pulses at 30 Hz). To the left are shown the increases in H^3-dopamine perfusate concentrations elicited by stimulation in each of the four experiments. The right-hand column gives the mean ± 1 standard error of the increase induced by stimulation at each level.

Electrode depth	Increased release of [^{3}H] dopamine (nCi/ml)				
	Exp. 1	Exp. 2	Exp. 3	Exp. 4	$\bar{x} \pm 1$S.E.
−1.5	0.51	0.83	0.49	0.12	0.49 ± 0.15
2.5	1.20	6.64	5.37	1.18	3.60 ± 1.41
−3.5	2.71	7.01	5.65	1.94	4.33 ± 1.20
−4.5	1.21	1.22	1.38	0.07	0.97 ± 0.30
−5.5	0.27	0.30	−0.11	0.05	0.13 ± 0.10

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the second greatest release evoked by stimulation at H -2.5. Similar experiments were performed in which the stimulating electrode was placed at A 10, L 2 and the previously mentioned depths. The mean (± 1 standard error) increases in H^3-dopamine release evoked in two experiments were 0.32 ± 0.53, 0.11 ± 0.06, 0.05 ± 0.01 and 0.02 ± 0.31 nc/ml at -1.5, -2.5, -3.5 and -4.5 respectively. Similarly, in 2 experiments in which the electrode was initially placed at A 10, L 4, H -1.5 and lowered, the following results were obtained: increased release of H^3-dopamine- 0.10 ± 0.33, -0.20 ± 0.11, 0.09 ± 0.04 and -0.05 ± 0.38 at -1.5, -2.5, -3.5 and -4.5 respectively. Therefore, a marked increase in H^3-dopamine release could be evoked only by stimulation at A 10, L 3, and H -2.5 and -3.5. These coordinates include the medial fibers of the nigro-striatal pathway (Moore et al., 1971). All further references to nigro-striatal stimulation refer to stimulation at A 10, L 3 and H -3.5.

In the data thus far presented, the values reported as H^3-dopamine have been those of the alumina eluate fraction. Nevertheless, H^3-norepinephrine or other catechol metabolites of H^3-dopamine might also appear in this fraction. Accordingly, in one series of experiments this fraction was further separated by ion-exchange chromatography into H^3-dopamine, H^3-norepinephrine and H^3-deaminated catechol fractions. This separation revealed that during the non-stimulation periods the radioactivity of the alumina eluate

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fraction consisted of 80% H^3-dopamine, 18% H^3-deaminated catechols and 2% H^3-norepinephrine. The effect of nigro-striatal stimulation upon these individual fractions is shown in Figure 5. It is evident that the marked increase in radioactivity in the alumina eluate fraction induced by stimulation is almost exclusively associated with an increase in H^3-dopamine concentration. All further references to H^3-dopamine concentration refer to the alumina eluate fraction.

The alumina supernatant fraction was also separated by ion-exchange chromatography into H^3-deaminated-O-methylated and H^3-O-methyl amine fractions. Figure 6 illustrates the effect of nigro-striatal stimulation upon the concentrations of H^3-labelled compounds in these fractions. There is a small but nevertheless statistically significant increase in the H^3-O-methylated amine fraction during the period of stimulation. This change is quite small as compared to the simultaneous marked increase in H^3-dopamine release (Figure 5). The concentrations of H^3-deaminated-O-methylated products were not changed significantly by stimulation.

II. Ventricular perfusion: mechanism by which drugs increase H^3-dopamine efflux.

Previous studies (Carr and Moore, 1970[a,b]) have demonstrated that perfusion of amphetamine and certain other psychomotor stimulants through the cerebroventricular system

Figure 5. The effect of nigro-striatal pathway stimulation on the concentrations of H^3-dopamine, H^3-deaminated catechols (H^3-DC) and H^3-norepinephrine (H^3-NE) in the alumina eluate of ventricular perfusates.

Each bar represents the mean concentration of the H^3-compound in perfusates collected during successive 2 minute periods in a total of 4 experiments (vertical lines denote 1 standard error). During the indicated period electrical stimulation of 1 msec 400 μA pulses at 30 Hz was applied to the nigro-striatal fibers. H^3-dopamine concentration in effluent during period of stimulation is statistically different ($P < .05$) than that during the period immediately before stimulation ($\bar{d}=4.30\pm1.30$ nc/ml).

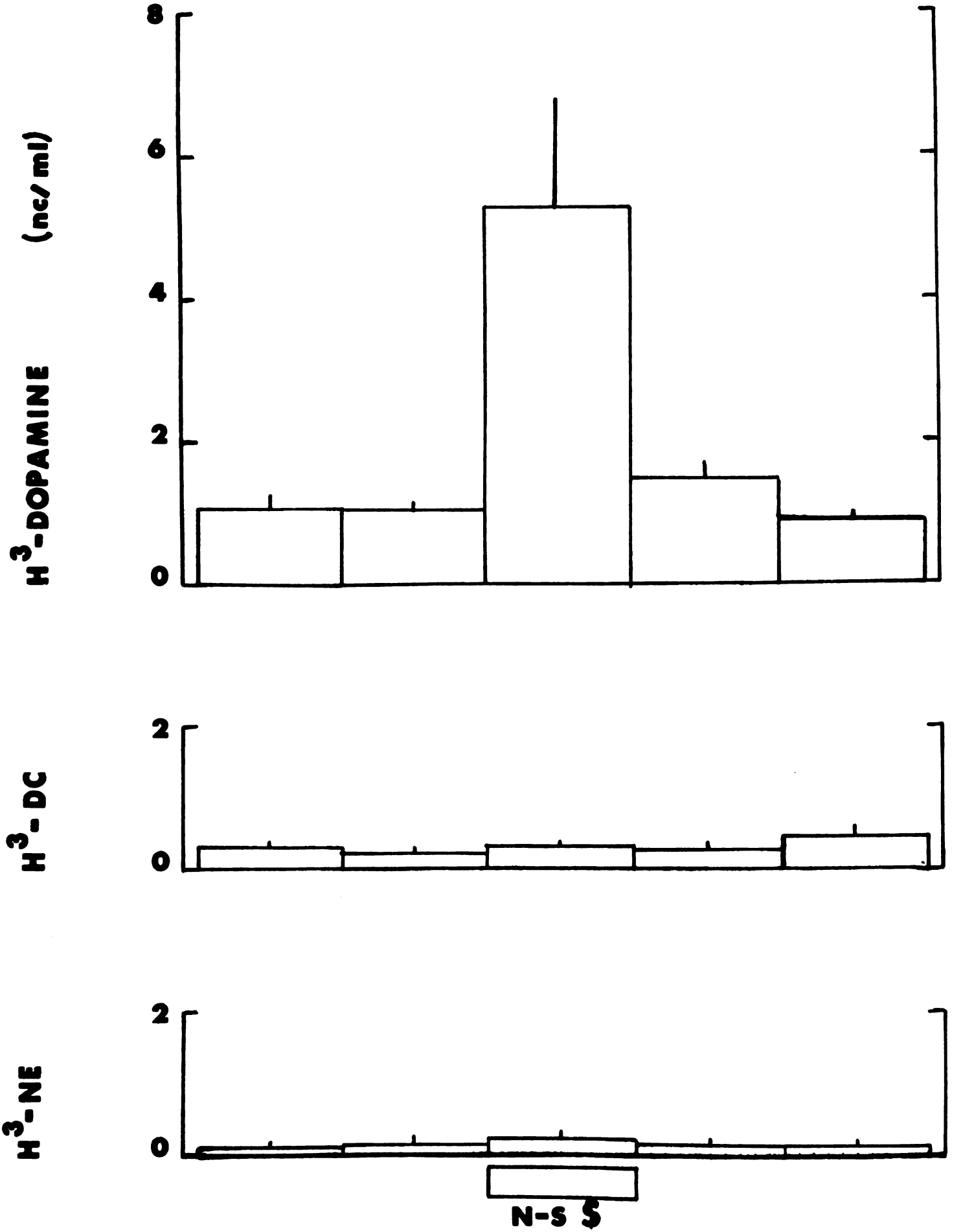
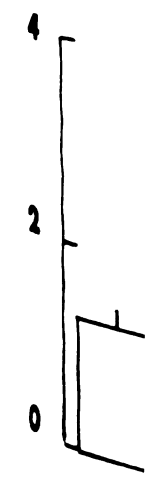
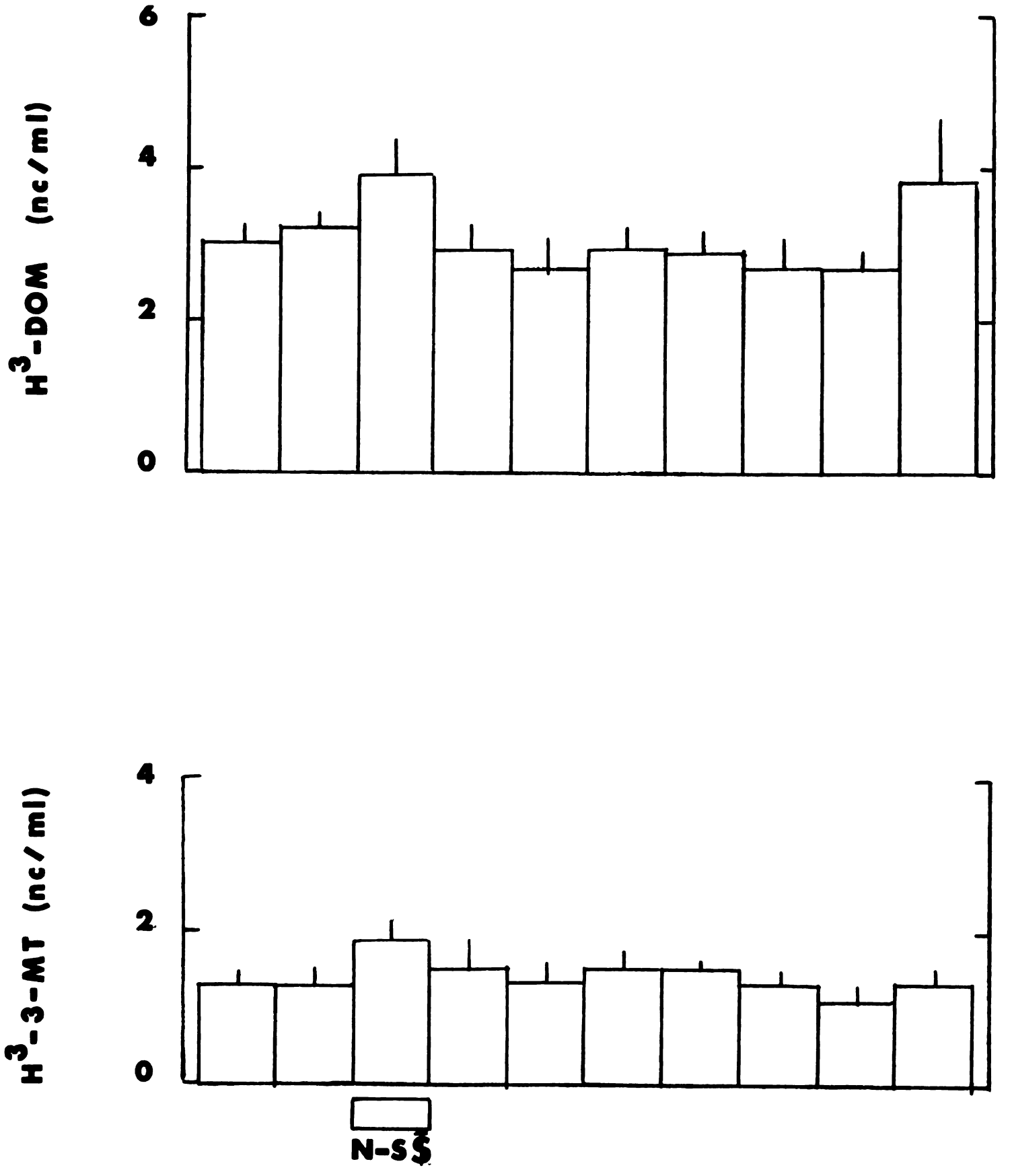

8
6
4
2
0
H3-DOPAMINE (nc/ml)
2
0
H3-DC
2
0
H3-NE
N-S $

Figure 6. The effect of nigro-striatal pathway stimulation on the concentrations of H^3-deaminated O-methylated (H^3-DOM) and H^3-O-methylated amine (H^3-3-MT) metabolites of H^3-dopamine in ventricular perfusates.

Each bar represents the mean concentration of H^3-compound in perfusates collected during successive 2 minute periods in a total of 4 experiments (vertical lines denote 1 standard error). During the indicated period electrical stimulation of 1 msec 400 μA pulses at 30 Hz was applied to the nigro-striatal fibers. H^3-3-MT concentration in effluent during the period of stimulation is statistically different ($P < .05$) than that during the period immediately before stimulation ($\bar{d}=0.50\pm0.15$ nc/ml).




6
4
2
0
H^3-DOM (nc/ml)
4
2
0
H^3-3-MT (nc/ml)
N-S$

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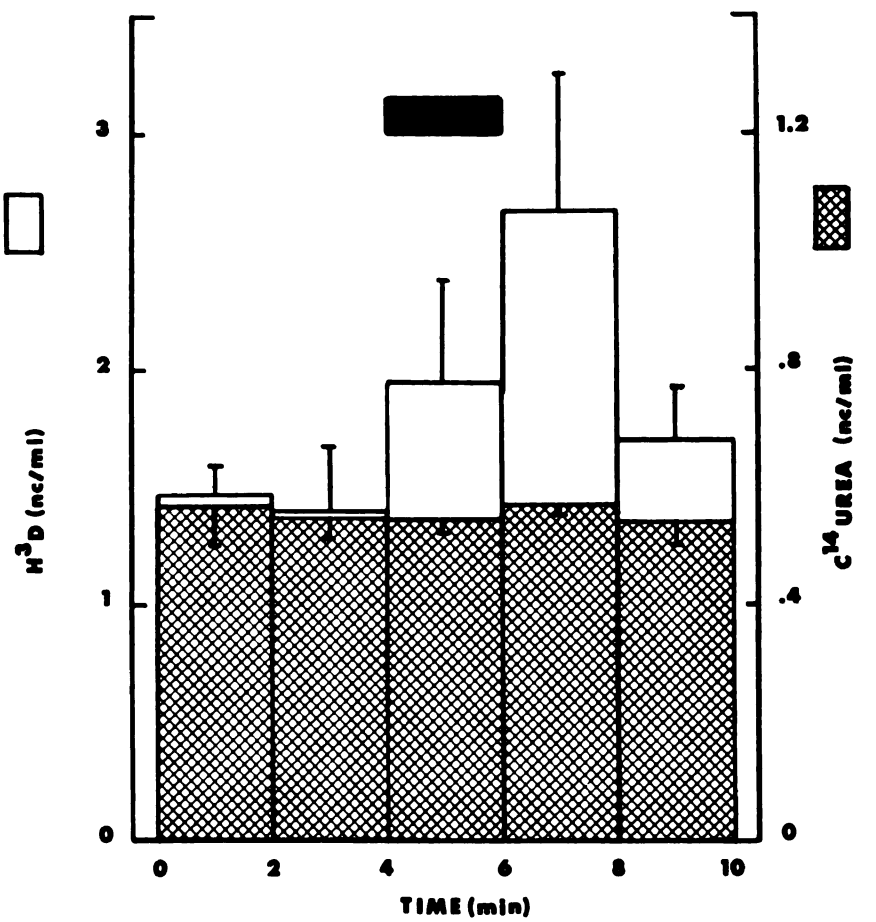
results in an increased efflux of H^3-catecholamines in cats pre-labeled with H^3-dopamine or H^3-norepinephrine. However, the mechanisms and cellular source of this release was not determined. It was demonstrated that this release of catecholamines into the ventricular system was fairly specific; amphetamine perfusion failed to alter the efflux of C^{14}-urea or C^{14}-inulin. The results summarized in Figure 7 demonstrate that amantadine perfusion may also result in a selective increase in H^3-dopamine efflux. Addition of this compound to the perfusion inflow for a 2 minute period caused a significant increase in H^3-dopamine outflow concentrations without altering the concentration of C^{14}-urea in the ventricular effluent.

Figure 8 compares the abilities of amantadine, d-amphetamine, l-amphetamine and tyramine to increase H^3-dopamine efflux into ventricular perfusates. The amphetamine isomers and tyramine appear considerably more potent than amantadine; however, since the slope of the amantadine curve differed from the others, true potency ratios between amantadine and the other compounds could not be calculated. Comparison of the regression lines for the amphetamine isomers revealed d-amphetamine to be 3-4 times as potent as l-amphetamine over the range of concentrations tested. Thus, all four drugs cause dose-related increases in H^3-dopamine efflux.

Since amantadine, amphetamine and tyramine all increase H^3-dopamine efflux into ventricular perfusates, it was of interest to determine if the outflow concentrations of the

Figure 7. Effects of amantadine upon the concentrations of H^3-dopamine and C^{14}-urea in cerebroventricular perfusates.

The heights of the open bars and the cross-hatched bars represent the mean concentrations of H^3-dopamine (H^3D) and C^{14}-urea respectively in a total of 4 experiments (vertical lines denote one standard error). During the two minute period indicated by the solid horizontal bar, amantadine (5.4×10^{-4}M) was added to the artificial cerebrospinal fluid. The sum of the H^3-dopamine concentrations in the 2 samples collected during and immediately following amantadine perfusion is statistically different ($P < .05$) than the sum of the concentrations in the 2 samples collected before amantadine perfusion ($\bar{d}=1.79\pm0.47$ nc/ml).


3
2
1
0
1.2
.8
.4
0
0
2
4
6
8
10
TIME (min)
H3D (nc/ml)
C14 UREA (nc/ml)

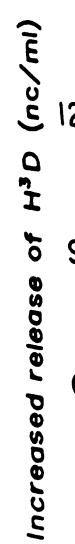
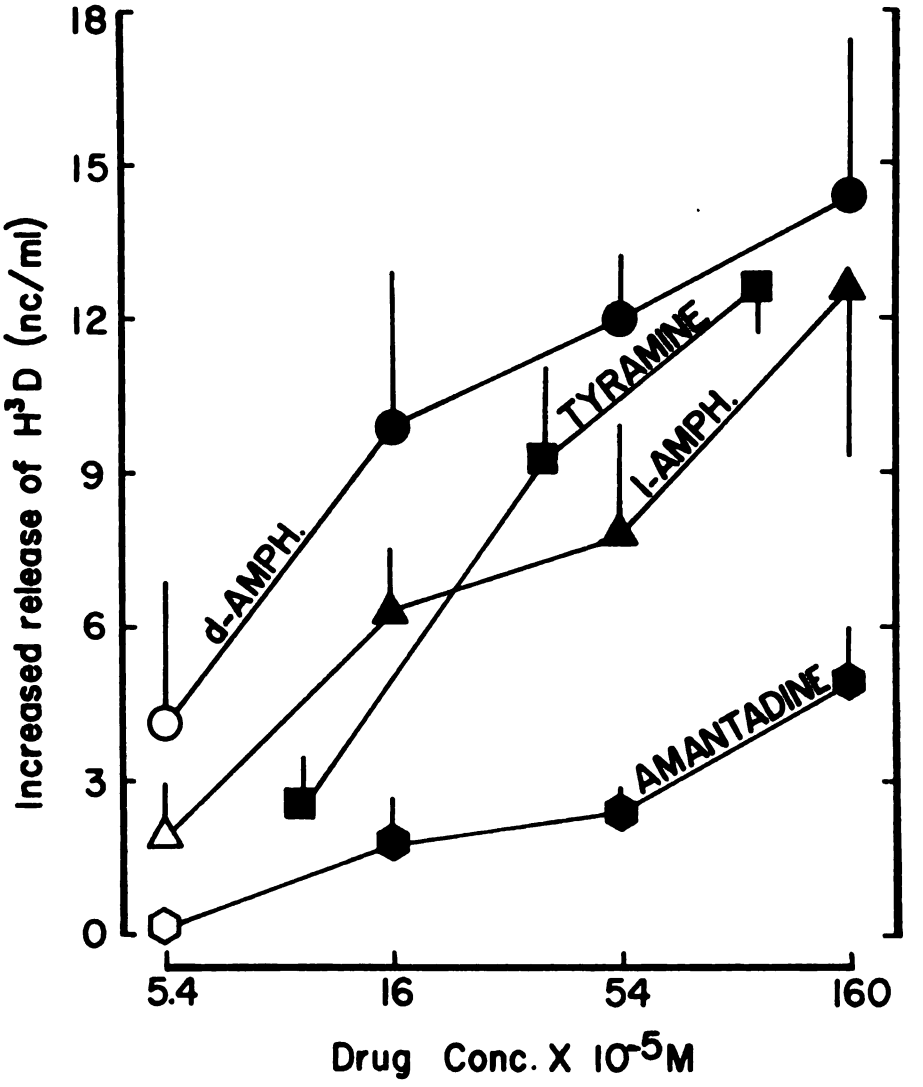


Figure 8. Increased efflux of H^3-dopamine (H^3D) induced by perfusion of various concentrations of d or l-amphetamine, amantadine or tyramine.

The drugs were included in the ventricular inflow for a period of 2 minutes. The increased release of H^3D was calculated by summing the perfusate outflow concentrations of H^3D for the 2 minute period of drug perfusion plus the 2 minute period immediately after and subtracting from this sum the concentration of H^3D effluxing during the 4 minutes before drug perfusion. Each point is the mean of at least 4 experiments, the vertical lines represent one standard error. Solid symbols designate statistically significant increases ($P < .05$).


18
15
12
9
6
3
0
Increased release of H^3D (nc/ml)
d-AMPH.
TYRAMINE
l-AMPH.
AMANTADINE
5.4
16
54
160
Drug Conc. X 10^{-5}M

H^3-dopamine

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H^3-dopamine metabolites were altered by these drugs. Figure 9 shows that amantadine perfusion had no effect upon the release of either H^3-deaminated-O-methylated or H^3-O-methylated amine metabolites of H^3-dopamine. However, Figures 10 and 11 demonstrate that amphetamine and tyramine were both capable of increasing the perfusate concentrations of the O-methylated amine but not the deaminated-O-methylated metabolites of H^3-dopamine. These increases are, however, quite small as compared to the large increases in H^3-dopamine evoked by the same concentrations of the drugs.

One approach to determining the cellular origin of the H^3-dopamine released by these drugs is to cause the selective destruction of the possible site and determine what effect the lesion has upon the drug effect. Since earlier studies (Figure 4; Moore et al., 1971) localized the dopaminergic nigro-striatal fibers in the diencephalon, it was possible to make reasonably selective lesions of those fibers. Table 1 summarizes the results of chronic unilateral nigro-striatal pathway lesions. The regional specificity of these lesions is indicated by their failure to alter septal weight or norepinephrine concentrations. Likewise, the cellular selectivity of the lesion is illustrated by the fact that neither caudate nucleus weight nor 5-hydroxytryptamine concentration was affected despite the marked decrease in dopamine concentration in the caudate

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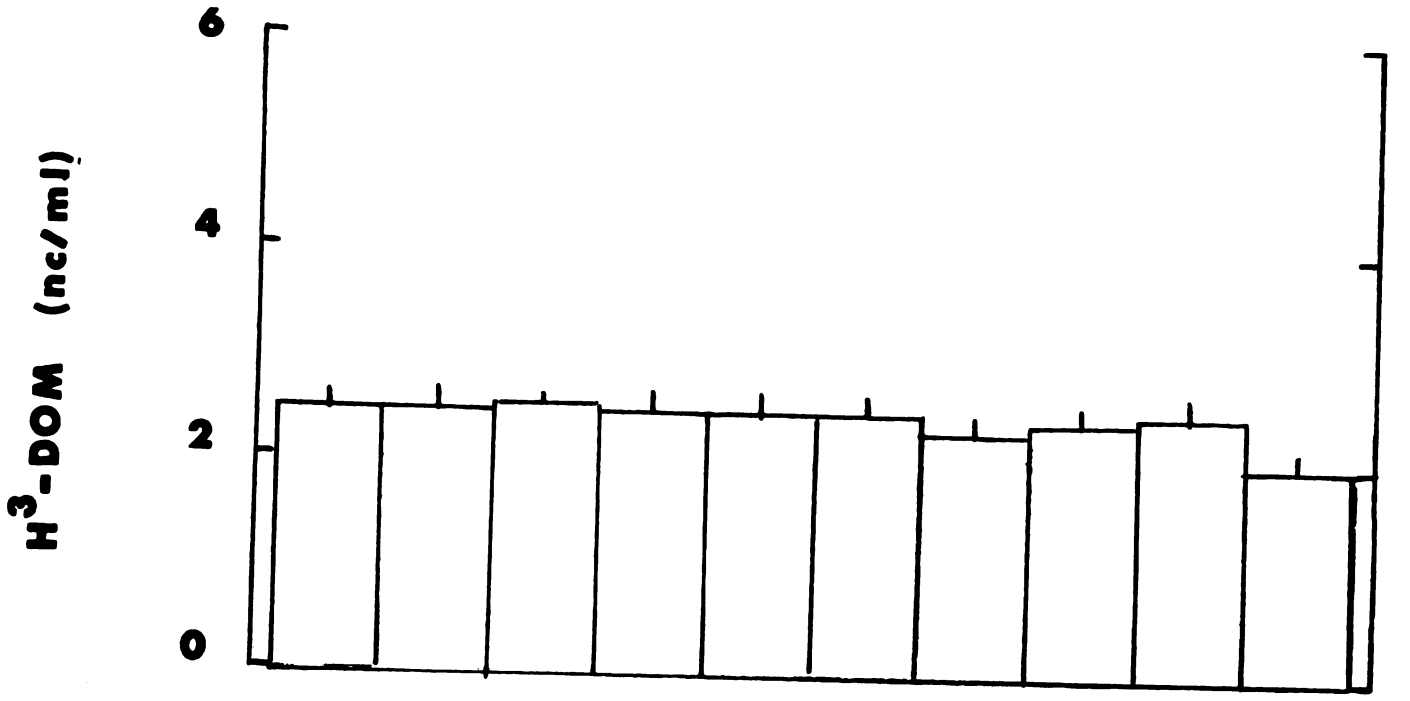
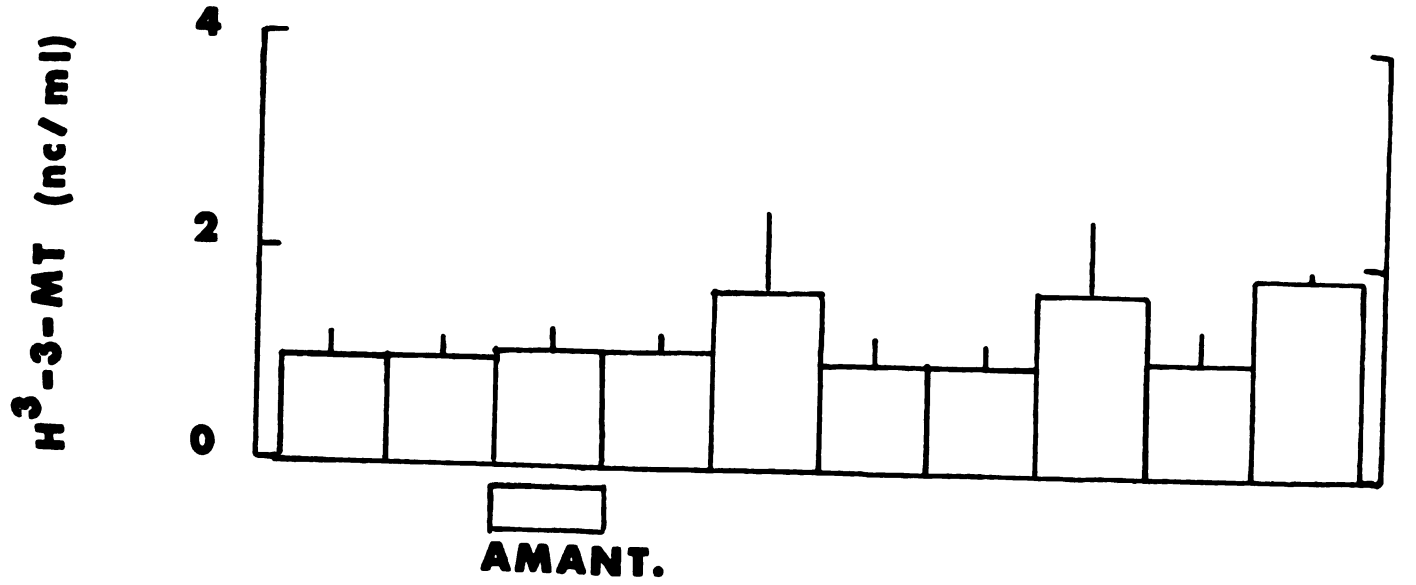
Figure 9. Effects of amantadine (AMANT) upon the concentrations of H^3-deaminated O-methylated (H^3-DOM) and H^3-O-methylated amine (H^3-3-MT) metabolites of H^3-dopamine in ventricular perfusates.

The height of each bar represents the mean concentration of the H^3-compounds in successive 2 minute perfusate samples from 4 experiments (vertical lines denote 1 standard error). During the indicated period amantadine (5.4×10^{-4} M) was perfused through the ventricular system.


6
4
2
0
H^3-DOM (nc/ml)


4
2
0
H^3-3-MT (nc/ml)
AMANT.

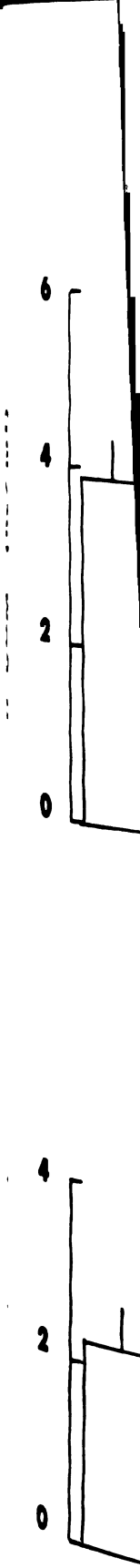
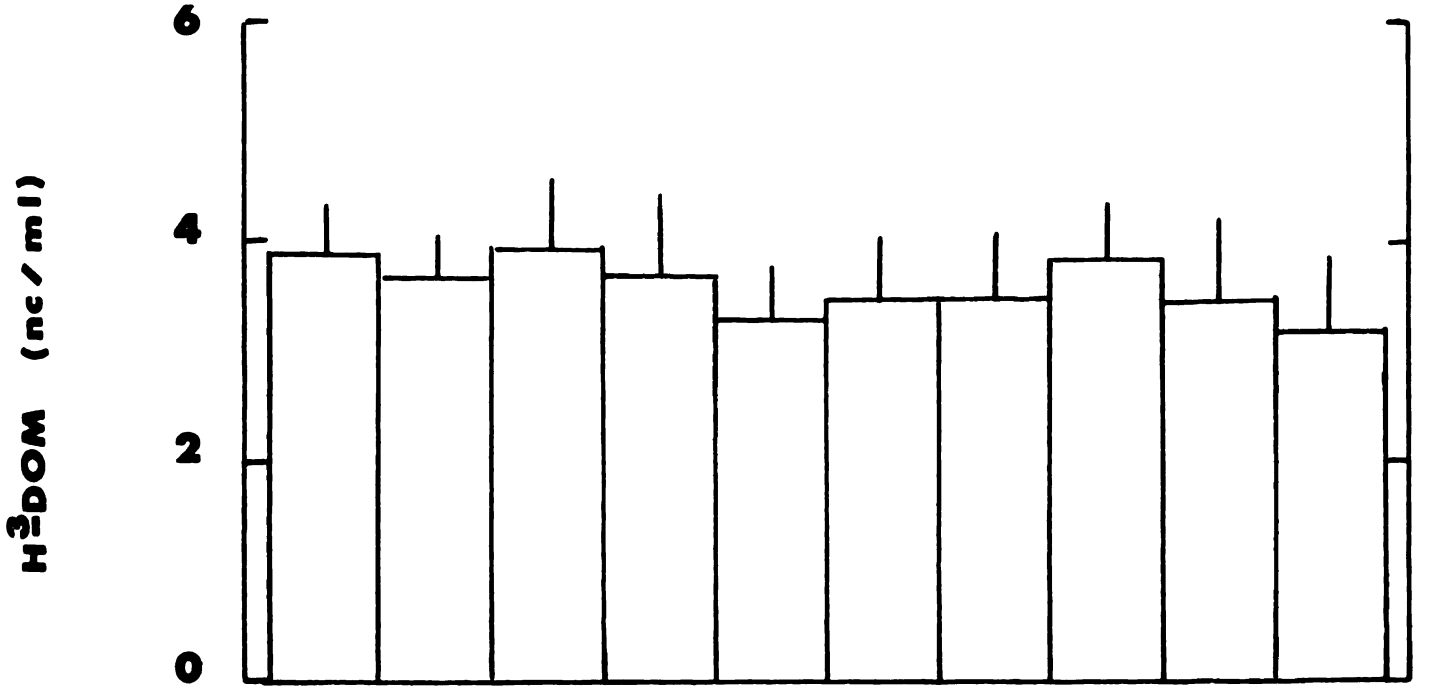


Figure 10. Effects of d-amphetamine (AMPH) upon the concentrations of H^3-deaminated O-methylated (H^3-DOM) and H^3-O-methylated amine (H^3-3-MT) metabolites of H^3-dopamine in ventricular perfusates.

The height of each bar represents the mean concentration of H^3-compounds in successive 2 minute perfusate samples from 4 experiments (vertical lines denote 1 standard error). During the indicated period d-amphetamine (1.6×10^{-4} M) was perfused through the ventricular system. Combined concentration of H^3-3-MT in perfusates collected during the 2 minute periods of and immediately after drug perfusion is statistically different ($P < .05$) from the combined concentration in the two samples collected immediately before drug perfusion ($\bar{d}=1.09\pm0.34$ nc/ml).


6
4
2
0
H³DOM (nc/ml)

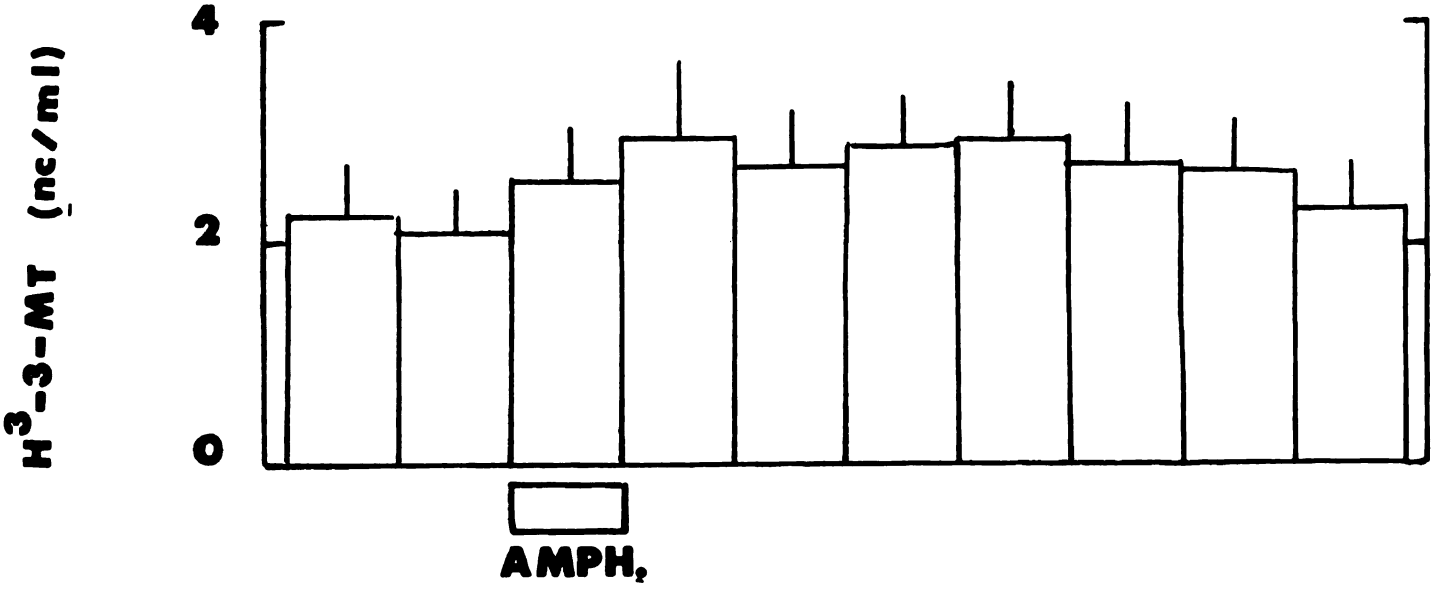

4
2
0
H³-3-MT (nc/ml)
AMPH,

Figure 11. Effects of tyramine upon the concentrations of H^3-deaminated O-methylated (H^3-DOM) and H^3-O-methylated amine (H^3-3-MT) metabolites of H^3-dopamine in ventricular perfusates.

The height of each bar represents the mean concentration of H^3-compounds in successive 2 minute perfusate samples from 4 experiments (vertical lines denote 1 standard error). During the indicated period tyramine (3.2×10^{-4} M) was perfused through the ventricular system. Combined concentration of H^3-3-MT in perfusates collected during the 2 minute periods of and immediately after drug perfusion is statistically different ($P < .05$) from the combined concentration in the two samples collected immediately before drug perfusion ($\bar{d}=0.63\pm0.19$ nc/ml).

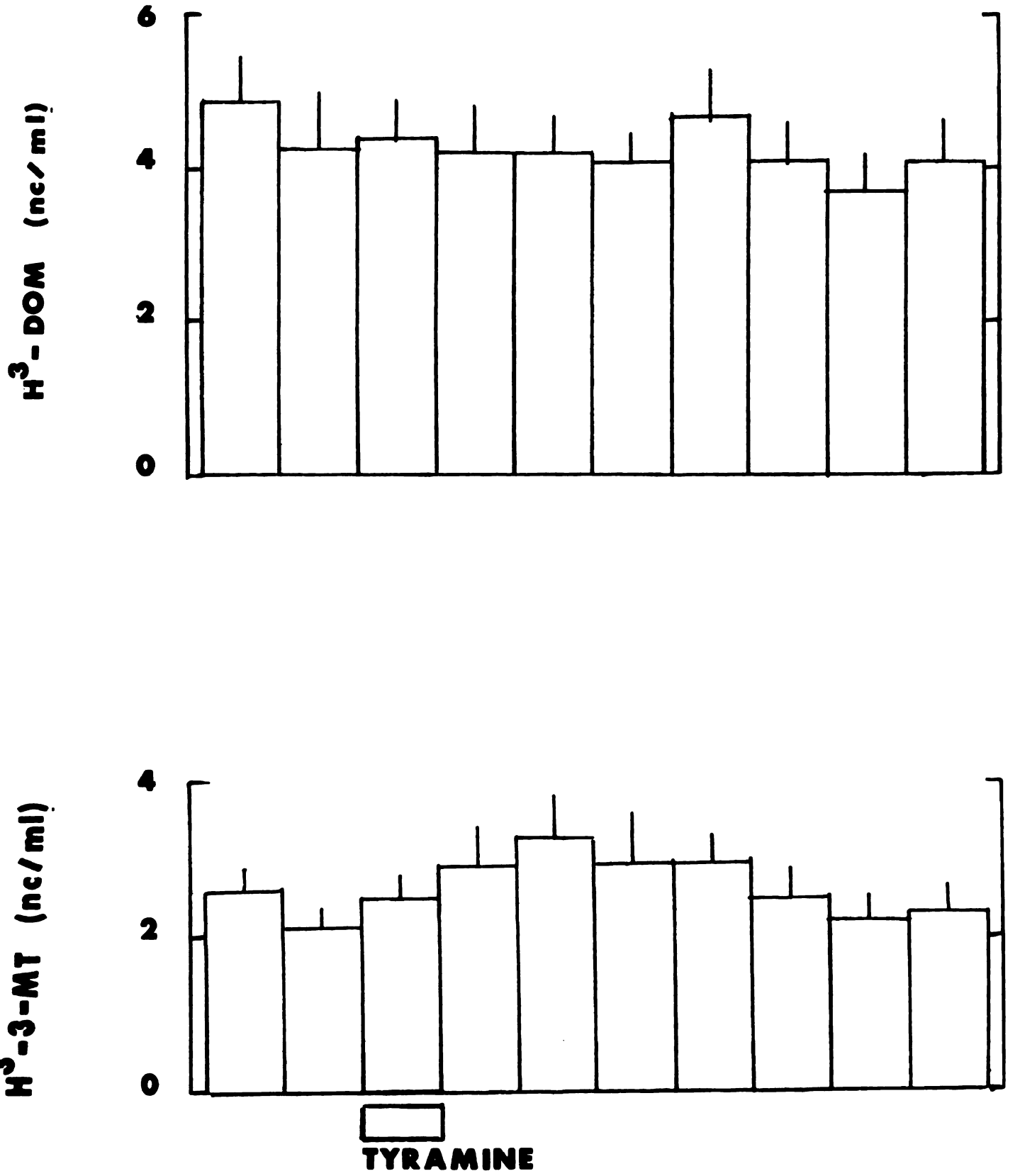

6
4
2
0
H^3-DOM (nc/ml)
4
2
0
H^3-3-MT (nc/ml)
TYRAMINE

Table 1. Effects of chronic nigro-striatal lesions on forebrain endogenous amines and H^3-dopamine uptake and retention.

Amines and Tissue Weights	Control	Lesioned

Table 1. Effects of chronic nigro-striatal lesions on forebrain endogenous amines and H^3-dopamine uptake and retention.

Amines and Tissue Weights	Control	Lesioned
Weight of septum (mg)	37.70 ± 3.30	38.30 ± 5.20
Norepinephrine in septum (µg/gm)	0.56 ± 0.09	0.35 ± 0.05
Weight of caudate nucleus (mg)	199.30 ± 7.30	190.20 ± 7.80
5-Hydroxytryptamine in caudate (µg/gm)	1.05 ± 0.14	0.85 ± 0.09
Dopamine in caudate (µg/gm)	12.43 ± 0.89	1.05 ± 0.25*
H^3-dopamine in caudate (µc/gm)	5.28 ± 0.64	0.40 ± 0.10*
Dopamine specific activity (µc/ug)	0.41 ± 0.06	0.56 ± 0.15

Cats were lesioned in either the left or right nigro-striatal pathway 2-8 weeks before being perfused and sacrificed.

*Statistically different from control ($P < .05$); n= 6-12.

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nucleus on the side of the lesion. Concomitant with the loss of endogenous dopamine was a parallel decrease in the ability of the tissue to take up and retain H^3-dopamine, suggesting that this labeled amine is distributed almost exclusively to the dopaminergic neurons.

Figure 12 shows a typical frontal section through the diencephalon of a cat with a chronic nigro-striatal lesion. This lesion encompasses the regions previously shown to be most sensitive to electrical stimulation.

Figure 13 compares the efflux of H^3-dopamine elicited by perfusion of amphetamine, amantadine and tyramine ipsilateral to and contralateral to chronic unilateral nigro-striatal lesions. The resting efflux of H^3-dopamine during the 2 periods prior to drug perfusion was significantly ($P < .05$) lower on the side of the lesion (1.48 ± 0.20 nc/2 min) than on the side opposite the lesion (2.42 ± 0.29 nc/2 min). It is apparent from the figures that H^3-dopamine efflux evoked by these drugs is markedly reduced by these selective nigro-striatal lesions. This is confirmed by the statistical comparisons of the H^3-dopamine efflux evoked from the lesioned and non-lesioned sides presented in Table 2; for each of the drugs H^3-dopamine efflux was significantly reduced on the lesioned side as compared to the opposite non-lesioned control side.

The tissue weights and amine concentrations for tissues taken from cats with chronic unilateral diencephalic lesions

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Figure 12. Frontal section of diencephalon of cat with chronic nigro-striatal lesion.

Four weeks after the lesion was made the cat was perfused and sacrificed. Frontal sections of the diencephalon containing the lesion were stained with cresyl violet and mounted.


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Figure 13. Comparison of H^3-dopamine efflux evoked by ventricular perfusion of d-amphetamine, amantadine and tyramine contralateral to and ipsilateral to unilateral chronic nigro-striatal lesions.

Chronic lesions were made in the nigro-striatal pathway 2-8 weeks before the cats were perfused. The lateral ventricles contralateral to (upper panels) and ipsilateral to (lower panels) the lesion were perfused with either d-amphetamine (1.6×10^{-4} M), amantadine (5.4×10^{-4} M) or tyramine (3.2×10^{-4} M) during the indicated 2 minute periods. Each bar represents the mean H^3-dopamine concentration in consecutive samples from at least 4 experiments. The vertical lines are 1 standard error. See Table 2 for statistical analysis of results.

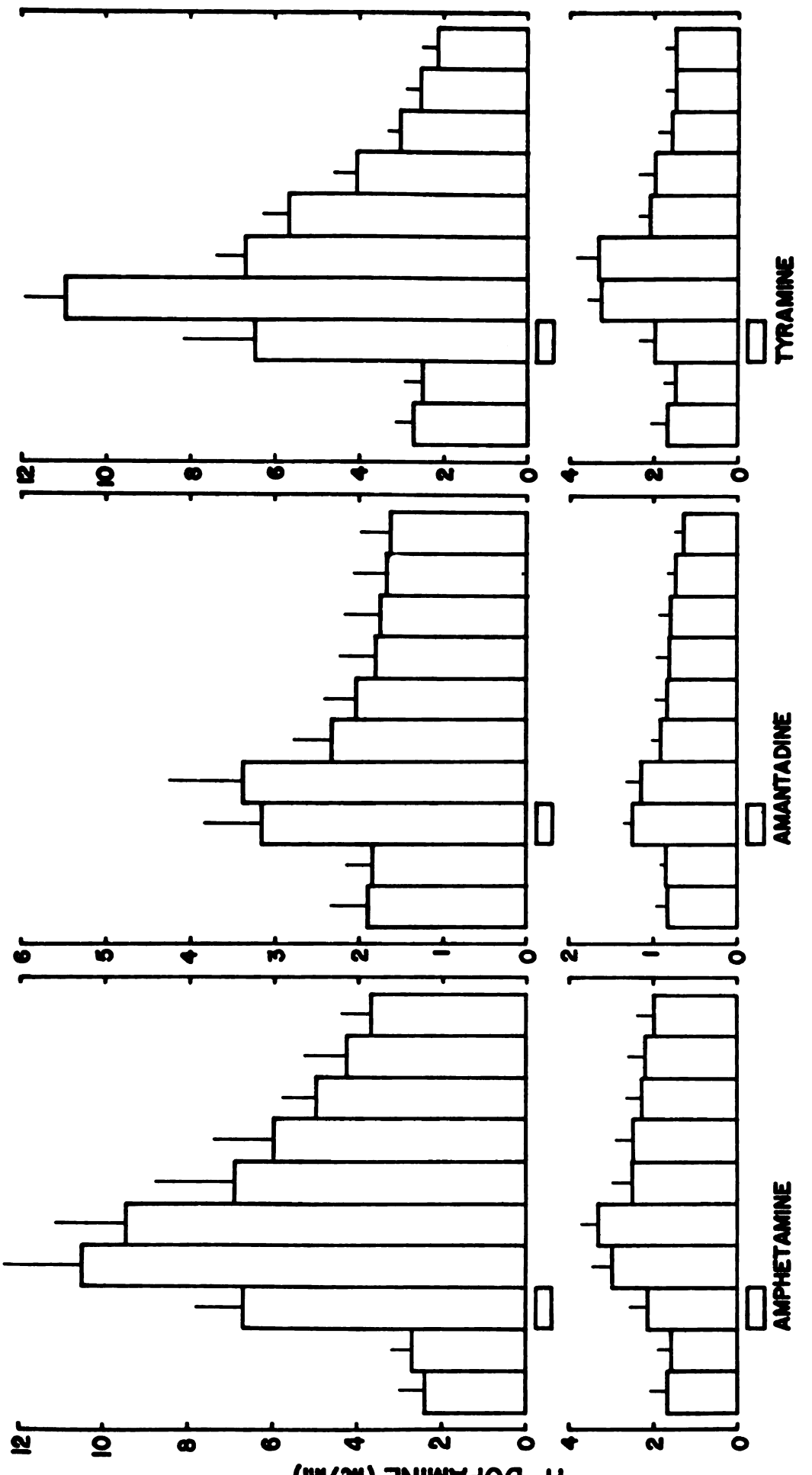
H³ DOPAMINE (nc/ml)
AMPHETAMINE
AMANTADINE
TYRAMINE

Table 2. A summary of increases in H^3-dopamine efflux induced by perfusion of d-amphetamine, amantadine and tyramine contralateral to and ipsilateral to chronic nigro-striatal lesions.

Table 2. A summary of increases in H^3-dopamine efflux induced by perfusion of d-amphetamine, amantadine and tyramine contralateral to and ipsilateral to chronic nigro-striatal lesions.

Drug	Increased efflux of H^3-dopamine (nc/ml)		
	Control side	Lesioned side	Control-lesioned
d-Amphetamine (1.6×10^{-4} M)	12.18 ± 2.46^a	1.60 ± 0.41^a	10.58 ± 2.19^b
Amantadine (5.4×10^{-4} M)	2.01 ± 0.48^a	0.66 ± 0.08^a	1.35 ± 0.56^b
Tyramine (3.2×10^{-4} M)	12.27 ± 2.94^a	2.10 ± 0.30^a	10.17 ± 2.80^b

Drugs were perfused through a lateral ventricle contralateral to (control side) or ipsilateral to (lesioned side) a chronic nigro-striatal pathway lesion. Increased efflux was calculated by subtracting the concentration of H^3-dopamine in the 2 samples collected before drug perfusion from the concentrations in the 2 samples collected during and immediately following drug perfusion in a total of at least 4 experiments.

[a]Increased efflux of H^3-dopamine is statistically significant ($P < .05$).

[b]Increased efflux of H^3-dopamine from lesioned side is significantly reduced ($P < .05$).

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that failed to completely destroy the region of the nigro-striatal fibers are summarized in Table 3. These lesions failed to significantly alter any of the measured parameters on the lesioned side as compared to the control side with the exception of a modest decrease in 5-hydroxytryptamine concentration in the caudate nucleus. Figures 14 and 15 illustrate the effect upon H^3-dopamine efflux of perfusing d-amphetamine and tyramine contralateral to and ipsilateral to these chronic lesions that failed to alter endogenous dopamine concentration. Although efflux from the lesioned side appears somewhat lower, examination of Table 4 reveals that these differences are not statistically significant. Thus, lesions that lowered 5-hydroxytryptamine concentrations but failed to significantly alter dopamine concentrations did not affect the release of H^3-dopamine by d-amphetamine or tyramine.

Since the site of H^3-dopamine release induced by these drugs had been localized to the nigro-striatal neurons, it was possible to study the mechanism of this effect by altering the activity of this pathway. One means of decreasing the impulse flow reaching the dopaminergic terminals in the striatum would be to acutely section the axons of the nigro-striatal neurons. In this manner, it might be possible to determine if a given drug relied upon ongoing nerve activity to exert an effect on dopamine disposition or if it released dopamine directly and independently of nerve activity.

[illegible] the nigro-striatal fibers on forebrain endogenous amines and H^3-dopamine uptake and retention.

Amines and Tissue Weights	Control	Lesioned
Weight of septum (mg)		

Table 3. Effects of chronic diencephalic lesions that failed to completely destroy the nigro-striatal fibers on forebrain endogenous amines and H^3-dopamine uptake and retention.

Amines and Tissue Weights	Control	Lesioned
Weight of septum (mg)	40.0 ± 7.80	42.5 ± 9.4
Norepinephrine in septum (µg/gm)	.72± 0.12	0.87± 0.18
Weight of caudate nucleus (mg)	179.5 ± 21.5	187.8 ± 26.1
5-Hydroxytryptamine in caudate (µg/gm)	1.57± 0.11	1.04± 0.23*
Dopamine in caudate (µg/gm)	13.41± 1.09	8.71± 2.42
H^3-dopamine in caudate (µc/gm)	4.31± 1.04	3.12± 0.51

Cats were lesioned in either the left or right diencephalon 2-8 weeks before being perfused and sacrificed.

*Statistically different from control ($P < .05$); $n=4$.

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Figure 14. Comparison of H^3-dopamine efflux evoked by ventricular perfusion of d-amphetamine (AMPH) contralateral to and ipsilateral to unilateral chronic lesions that failed to destroy completely the nigro-striatal fibers.

These diencephalic lesions which spared the dopaminergic nigro-striatal fibers were made 2-8 weeks before the cats were perfused. The lateral ventricles contralateral to (upper panel) and ipsilateral to (lower panel) the lesion were perfused with d-amphetamine (1.6×10^{-4} M) during the indicated 2 minute period. Each bar represents the mean H^3-dopamine concentration in consecutive samples from 4 experiments. The vertical lines are 1 standard error. See Table 4 for statistical analysis.

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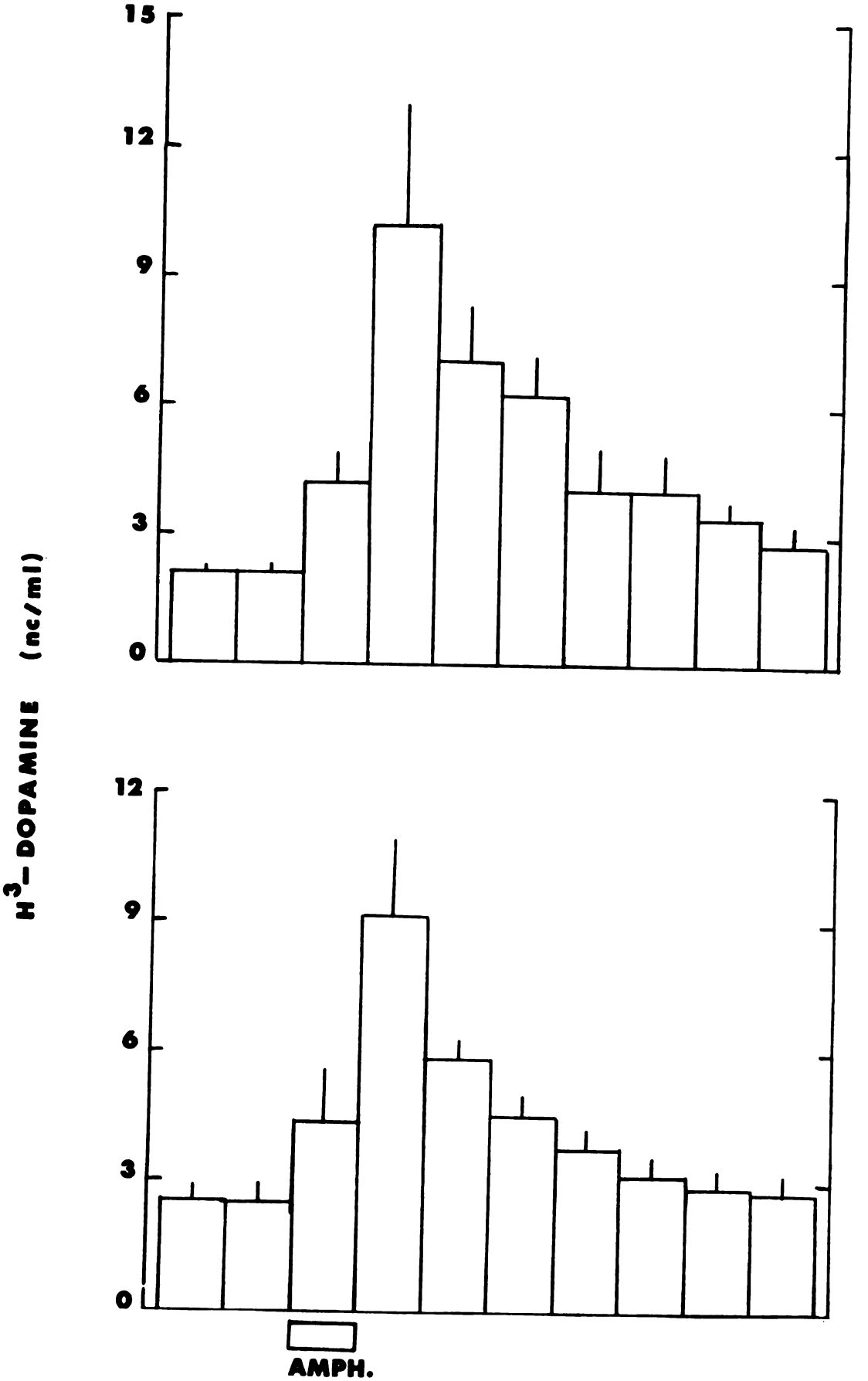
H^3-DOPAMINE (nc/ml)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H3- DOPAMINE (nc/ml)
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AMPH.

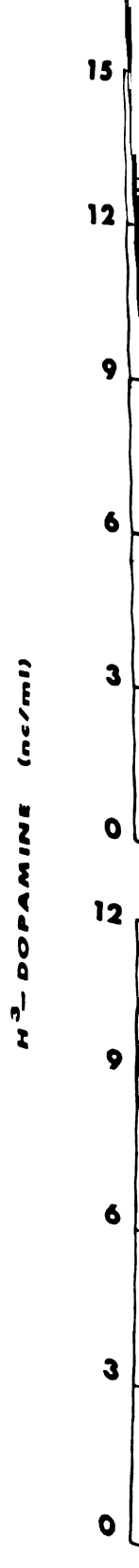
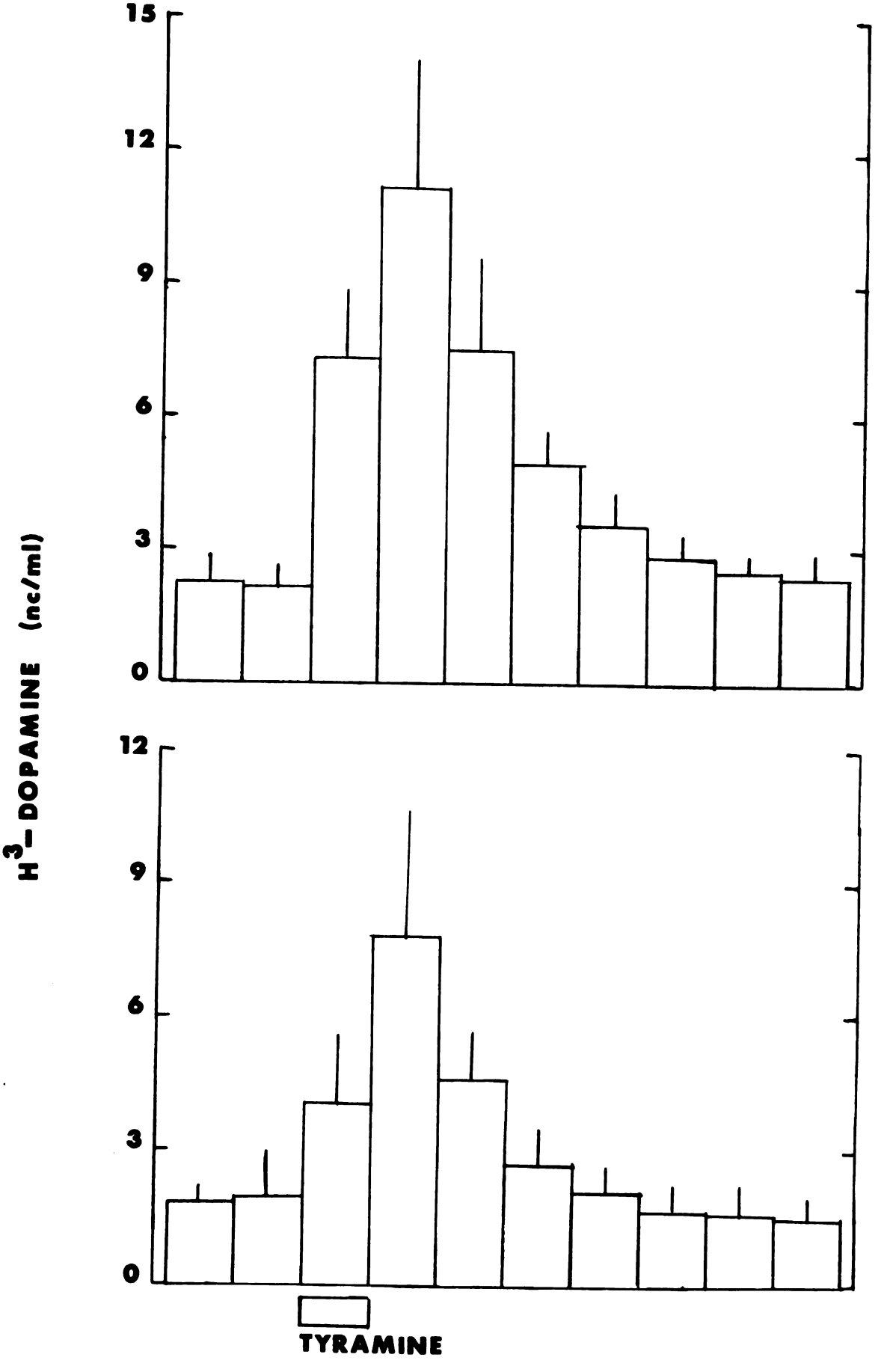


Figure 15. Comparison of H^3-dopamine efflux evoked by ventricular perfusion of tyramine contralateral to and ipsilateral to unilateral chronic lesions that failed to completely destroy the nigro-striatal fibers.

These diencephalic lesions which spared the dopaminergic nigro-striatal fibers were made 2-8 weeks before the cats were perfused. The lateral ventricles contralateral to (upper panel) and ipsilateral to (lower panel) the lesion were perfused with tyramine (3.2×10^{-4}M) during the indicated 2 minute period. Each bar represents the mean H^3-dopamine concentration in consecutive samples from 4 experiments. The vertical lines are 1 standard error. See Table 4 for statistical analysis.


15
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H^3- DOPAMINE (nc/ml)
TYRAMINE

Table 4. A summary of increases in H^3-dopamine efflux induced by perfusion of d-amphetamine and tyramine contralateral to and ipsilateral to chronic diencephalic lesions that failed to completely destroy the nigro-striatal fibers.

Drug

Table 4. A summary of increases in H^3-dopamine efflux induced by perfusion of d-amphetamine and tyramine contralateral to and ipsilateral to chronic diencephalic lesions, that failed to completely destroy the nigro-striatal fibers.

Drug	Increased efflux of H^3-dopamine (nc/ml)		
	Control side	Lesioned side	Control-Lesioned
d-Amphetamine (1.6×10^{-4} M)	10.82 ± 3.63*	8.49 ± 2.20*	2.33 ± 2.49
Tyramine (3.2×10^{-4} M)	13.74 ± 3.46*	8.52 ± 3.44*	5.18 ± 3.43

Drugs were perfused through a lateral ventricle contralateral to (control side) or ipsilateral to (lesioned side) a chronic diencephalic lesion. Increased efflux was calculated by subtracting the concentration of H^3-dopamine in the 2 samples collected before drug perfusion from the concentration in the 2 samples collected during and immediately following drug perfusion in a total of 4 experiments.

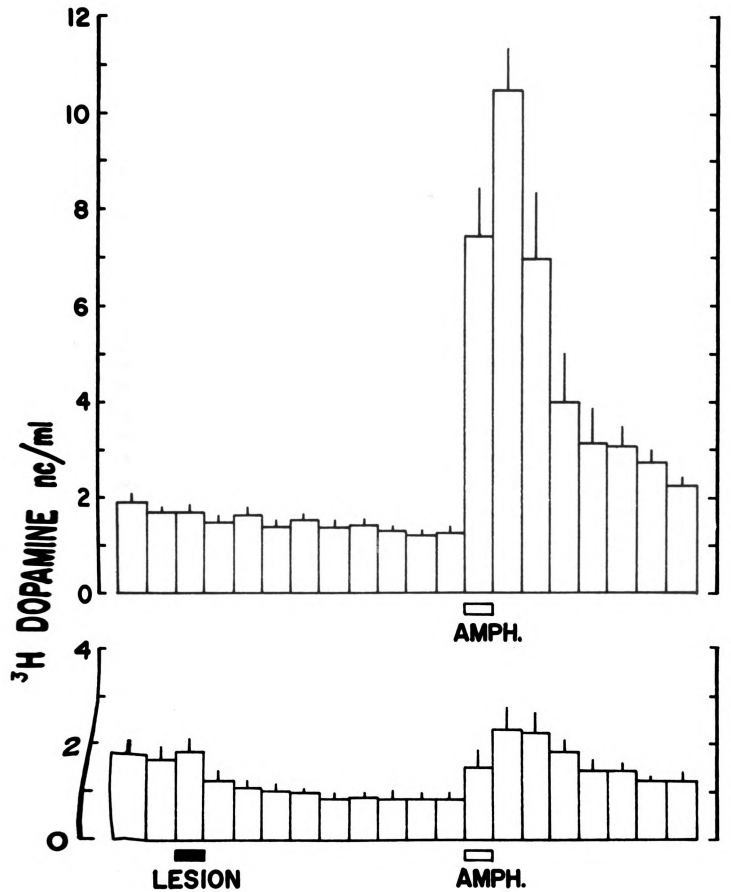
*Increased efflux of H^3-dopamine is statistically significant ($P < .05$).

Figure 16 illustrates the results of a series of experiments in which d-amphetamine was perfused either in the absence of an acute nigro-striatal pathway lesion or 20 minutes after such a lesion was made. The acute lesion resulted in a marked decrease in the efflux of H^3-dopamine elicited by d-amphetamine. When these experiments were repeated using amantadine the lesion resulted in a similar effect (Figure 17). However, when tyramine was perfused after acute nigro-striatal lesions, it elicited a release of H^3-dopamine of the same magnitude as in the absence of an acute lesion (Figure 18). The statistical analysis in Table 5 verifies that acute nigro-striatal lesions did, indeed, significantly lower the efflux of H^3-dopamine evoked by d-amphetamine and amantadine, whereas the response to tyramine perfusion was unaltered.

Since chronic lesions of the nigro-striatal fibers decrease caudate dopamine concentrations (Table 1), it was therefore of interest to determine what effect acute lesions in this region had upon this parameter. Table 6 summarizes the results of such experiments. Acute nigro-striatal lesions failed to alter caudate weight or 5-hydroxytryptamine concentrations; while the dopamine concentrations in the caudate nucleus on the acutely lesioned side increased significantly. This increase in endogenous dopamine concentration was reflected by a significant decrease in dopamine specific activity. Thus, in contrast to the chronic

Figure 16. Comparison of H^3-dopamine efflux evoked by ventricular perfusion of d-amphetamine (AMPH) contralateral to and ipsilateral to a unilateral acute nigro-striatal lesion.

The upper panel shows the effect of d-amphetamine (1.6×10^{-4} M) perfusion on H^3-dopamine efflux from the control side (no acute lesion). The lower panel illustrates the effect of the same concentration of d-amphetamine perfused 20 minutes after an acute lesion (solid horizontal bar) of the nigro-striatal fibers. Each vertical bar represents the mean H^3-dopamine concentration in successive samples from 4 experiments; the vertical lines are 1 standard error. See Table 5 for statistical analysis of data.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^{3}H DOPAMINE nc/ml
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LESION
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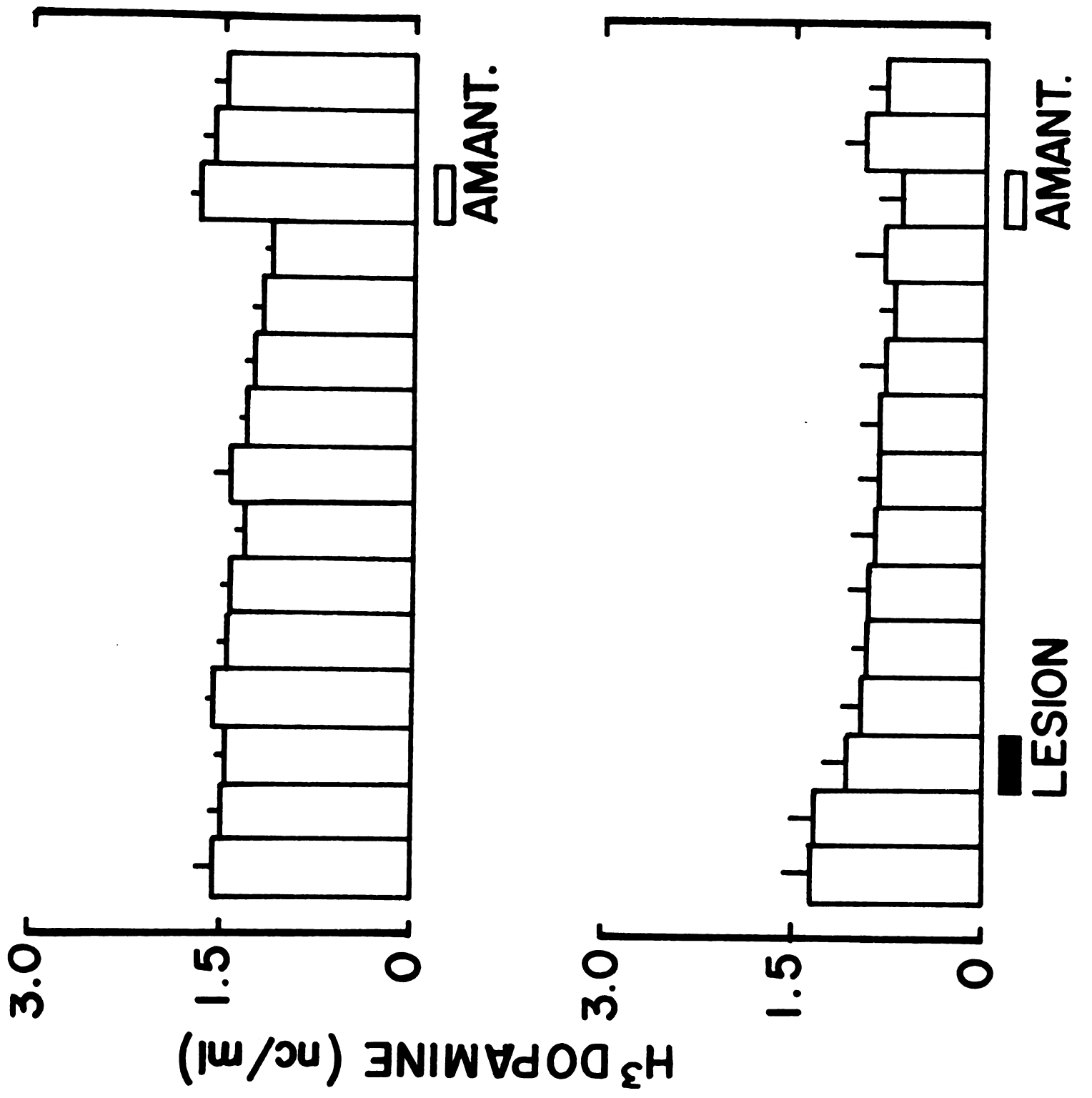
Figure 17. Comparison of H^3-dopamine efflux evoked by ventricular perfusion of amantadine (AMANT) contralateral to and ipsilateral to a unilateral acute nigro-striatal lesion.

The upper panel shows the effect of amantadine (1.6×10^{-4} M) perfusion on H^3-dopamine efflux from the control side (no acute lesion). The lower panel illustrates the effect of the same concentration of amantadine perfused 20 minutes after an acute lesion (solid horizontal bar) of the nigro-striatal fibers. Each vertical bar represents the mean H^3-dopamine concentration in successive 2 minute samples from 4 experiments; the vertical lines are 1 standard error. See Table 5 for statistical analysis of data.


H³ DOPAMINE (nc/ml)
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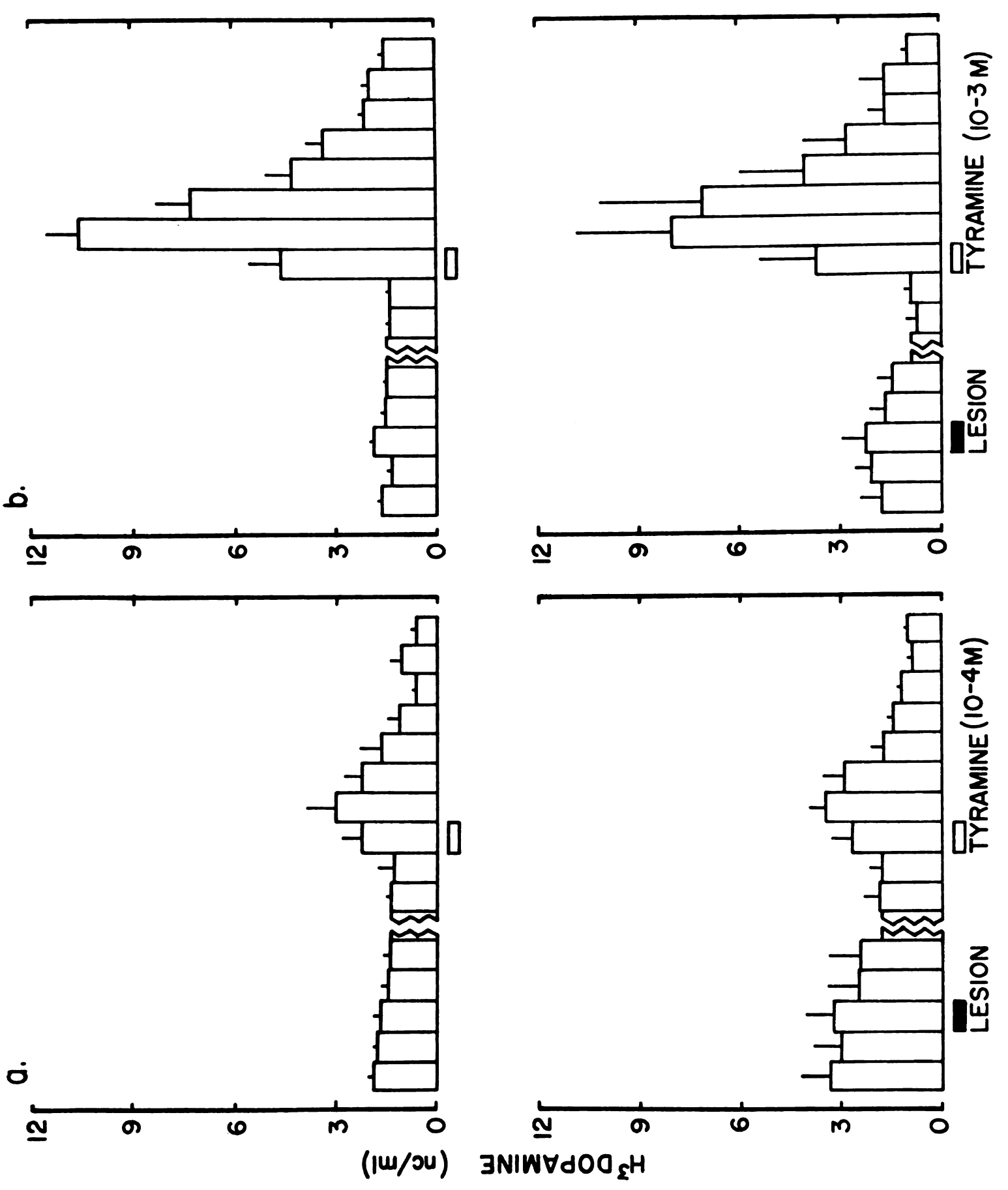
AMANT.
LESION

Figure 18. Comparison of H^3-dopamine efflux evoked by ventricular perfusion of tyramine contralateral to and ipsilateral to a unilateral acute nigro-striatal lesion.

The upper panels illustrate the effects of tyramine (10^{-4} M, left; 10^{-3} M, right) perfusion upon dopamine efflux from the control side (no acute lesion). The lower panels show the effects of the same concentrations of tyramine perfused 20 minutes after an acute lesion (solid horizontal bar) of the nigro-striatal fibers. Each vertical bar represents the mean H^3-dopamine concentration in successive 2 minute samples from 4 experiments; the vertical lines are 1 standard error. See Table 5 for statistical analysis of data.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H^3 DOPAMINE (nc/ml)
LESION
TYRAMINE (10-4M)
TYRAMINE (10-3M)

Table 5. A summary of increases in H^3-dopamine efflux induced by perfusion of d-amphetamine, amantadine and tyramine contralateral to and ipsilateral to acute nigro-striatal lesions.

Drug	Increased efflux of H^3-dopamine (nc/ml)		
	Control side	Lesioned side	Control-Lesioned
d-Amphetamine (1.6×10^{-4} M)	15.57 ± 1.25^a	2.16 ± 0.71^a	13.41 ± 0.75^b
Amantadine (1.6×10^{-4} M)	1.00 ± 0.06^a	0.02 ± 0.06	0.98 ± 0.11^b
Tyramine (1×10^{-4} M)	2.56 ± 0.79^a	2.54 ± 0.29^a	0.02 ± 0.54
Tyramine (1×10^{-3} M)	12.45 ± 0.76^a	10.30 ± 3.83^a	2.15 ± 4.14

Drugs were perfused through a lateral ventricle contralateral to (control side) or ipsilateral to (lesioned side) an acute nigro-striatal pathway lesion. Increased efflux was calculated by subtracting the concentration of H^3-dopamine in the 2 samples collected before drug perfusion from the concentrations in the 2 samples collected during and immediately following drug perfusion in a total of at least 4 experiments.

[a]Increased efflux of H^3-dopamine is statistically significant ($P < .05$).

[b]Increased efflux of H^3-dopamine from lesioned side is significantly reduced ($P < .05$).

Table 6. Effects of acute nigro-striatal lesions on endogenous amines and H^3-dopamine retention in the caudate nucleus.

Amines and Tissue Weight	Control	Lesioned
Caudate weight (mg)	202.6 ± 10.7	207.1 ± 9.4
5-Hydroxytryptamine (µg/gm)	1.81± 0.11	1.72± 0.22
Dopamine (µg/gm)	12.04± 0.61	18.93± 2.11*
H^3-dopamine (µc/gm)	3.21± 0.58	2.90± 0.53
Dopamine specific activity (µc/µg)	0.28± 0.03	0.15± 0.03*

Lesions were made during the collection of perfusates 1-4 hours before sacrifice.

*Statistically different from control ($P < .05$); N=8.

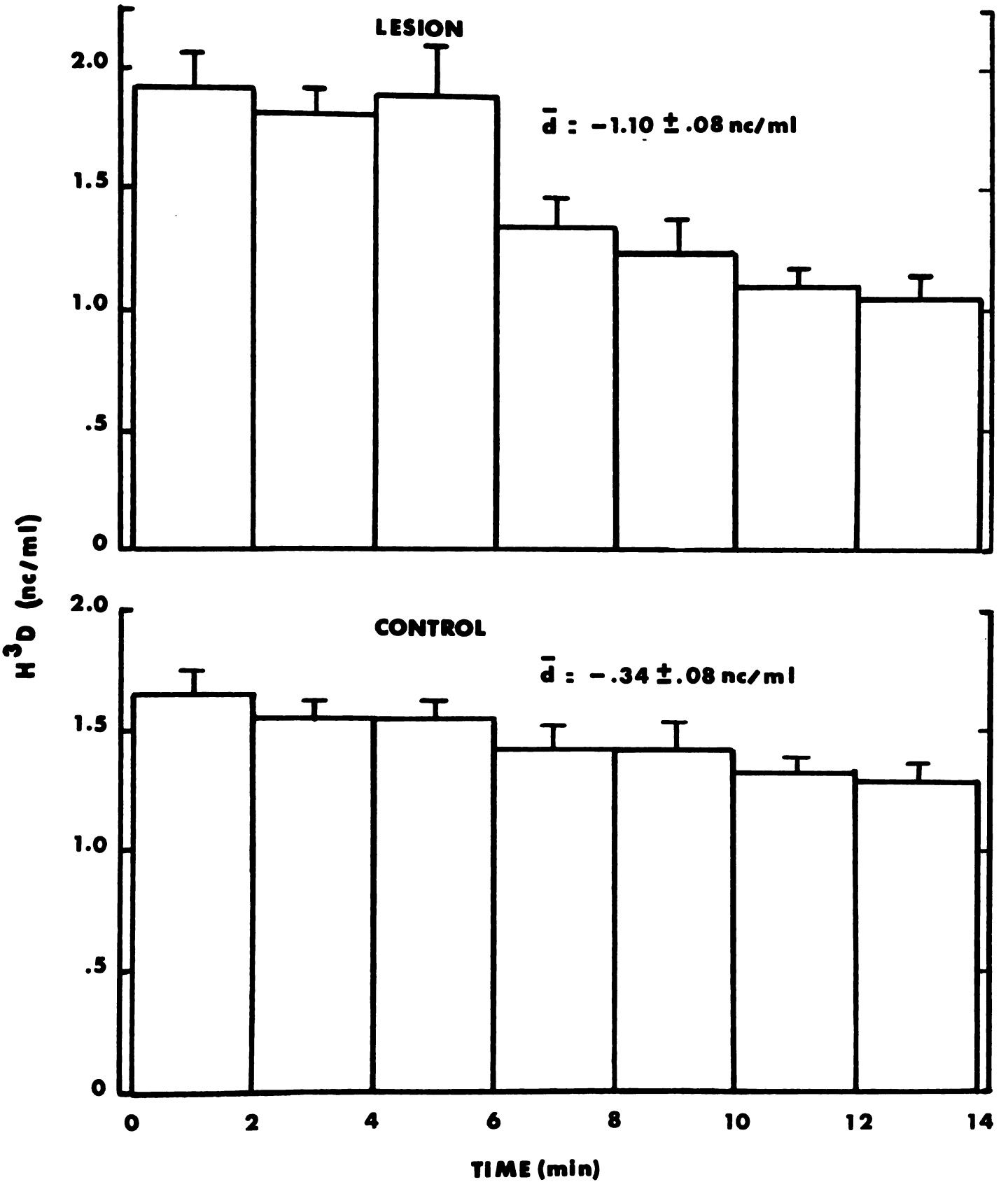
nigro-striatal lesions which lowered endogenous dopamine concentrations without affecting dopamine specific activity, acute lesions increased dopamine concentrations and thereby lowered dopamine specific activity.

Careful examination of Figures 16, 17 and 18 offers a clue as to why dopamine concentrations might increase after acute lesions. In each figure there appears to be a slight decrease in H^3-dopamine release immediately following the lesion. In Figure 19 the data from the last 3 figures are pooled. This figure clearly illustrates that following an acute nigro-striatal lesion, H^3-dopamine release is significantly reduced. The mean efflux of H^3-dopamine before the lesion was 3.73 ± 0.13 nc/ 4 min so that the acute lesion resulted in a $21 \pm 3\%$ greater decrease in H^3-dopamine efflux over that observed during the normal washout. The increase in endogenous dopamine concentrations may thus be related to a lesion-induced decrease in dopamine release. That is, the dopamine that is normally released by ongoing neuronal activity accumulates in the nerve terminals following the acute lesion.

The data summarized in Table 5 suggest that decreasing ongoing impulse activity in the nigro-striatal neurons blocks the ability of d-amphetamine and amantadine to increase extracellular concentrations of H^3-dopamine. If this is correct, then the converse should also be true; increasing impulse activity in the nigro-striatal neurons should potentiate the

Figure 19. Effect of acute nigro-striatal lesions on the release of H^3-dopamine (H^3D) into ventricular perfusates.

Each bar represents the mean H^3-dopamine concentration in perfusates collected during successive 2 minute periods in a total of 16 experiments. During the third collection period of the experiments illustrated in the upper panel, acute electrolytic lesions were made in the nigro-striatal fibers. This resulted in the indicated mean decrease ($\bar{d}$) in H^3-dopamine effluent concentration in the 2 perfusates collected after the lesion as compared to the 2 collected immediately before the lesion. This decrease was statistically different ($P < .05$) than that observed in the control washout as illustrated in the lower panel.


LESION
$\bar{d}$: −1.10 ± .08 nc/ml
CONTROL
$\bar{d}$: −.34 ± .08 nc/ml
H^3D (nc/ml)
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1.0
.5
0
0
2
4
6
8
10
12
14
TIME (min)

ability of d-amphetamine and amantadine to increase H^3-dopamine efflux. One means of increasing this activity is to stimulate the dopaminergic nerve terminals in the caudate nucleus directly. The first two bars in Figure 20 demonstrate the effects of perfusing amantadine at a subthreshold concentration and stimulating the caudate nucleus at a subthreshold intensity separately; the last bars represents the release of H^3-dopamine induced by combined stimulation and amantadine perfusion. The H^3-dopamine efflux elicited by the combination is significantly larger than the sum of their individual effects. Thus, direct stimulation of the caudate nucleus appears to potentiate the effects of amantadine. If this potentiation is related to a stimulation-induced increase in neurogenic release of H^3-dopamine, then it should also be possible to demonstrate it during stimulation of the nigro-striatal fibers in the diencephalon. Figure 21 illustrates the results of 3 series of experiments which combined the perfusion of subthreshold concentrations of amantadine, d-amphetamine or tyramine with subthreshold frequency stimulation of the nigro-striatal fibers. With both amantadine and amphetamine, stimulation combined with drug perfusion caused a greater increase in H^3-dopamine efflux than did the sum of drug perfusion and stimulation alone. This was not the case for tyramine; perfusion of tyramine failed to potentiate the stimulation-induced H^3-dopamine release. Therefore, the increases in H^3-dopamine release evoked by d-amphetamine and

Figure 20. Effects of subthreshold doses of amantadine and subthreshold electrical stimulation of the caudate nucleus upon the ventricular perfusate concentration of H^3-dopamine.

The height of each bar represents the mean increase in H^3-dopamine (H^3D) in the four-minute period immediately following the start of the amantadine (5.4×10^{-5} M) perfusion and/or caudate nucleus stimulation (200 μA, 1 msec and 50 Hz) as compared to the H^3-dopamine concentration during the four-minute period just before drug perfusion and/or electrical stimulation (vertical lines denote 1 standard error as determined from four experiments).

*Indicates that the release is significantly greater ($P < .05$) than the sum of the amantadine and stimulation-induced release.

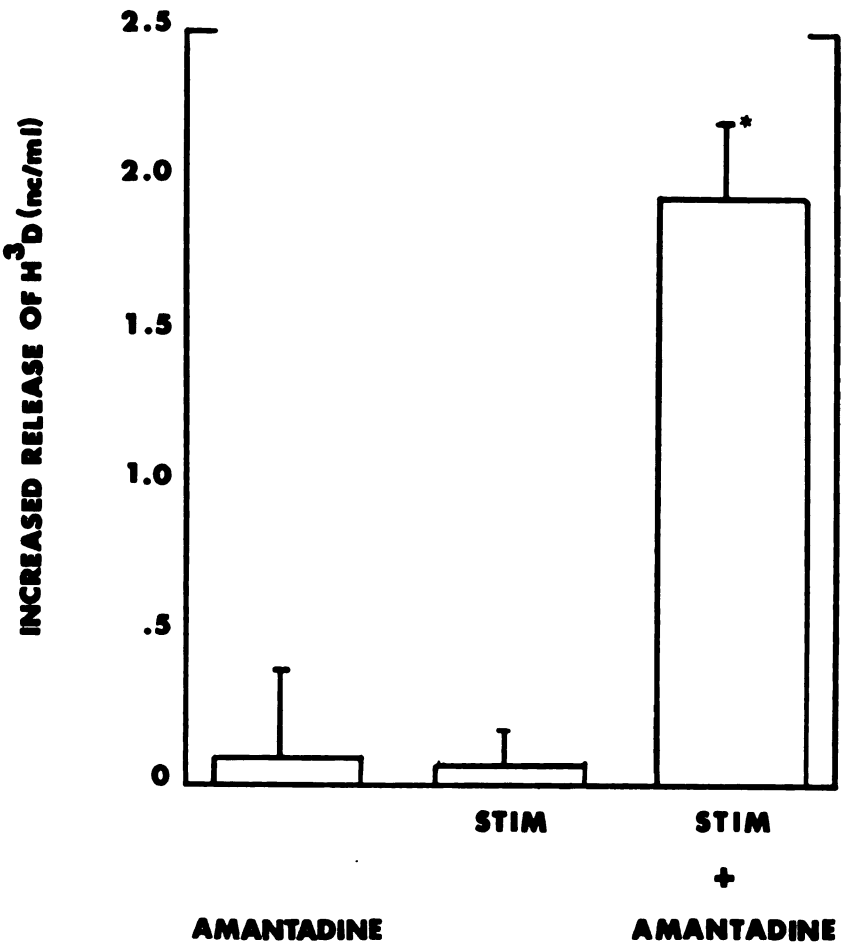
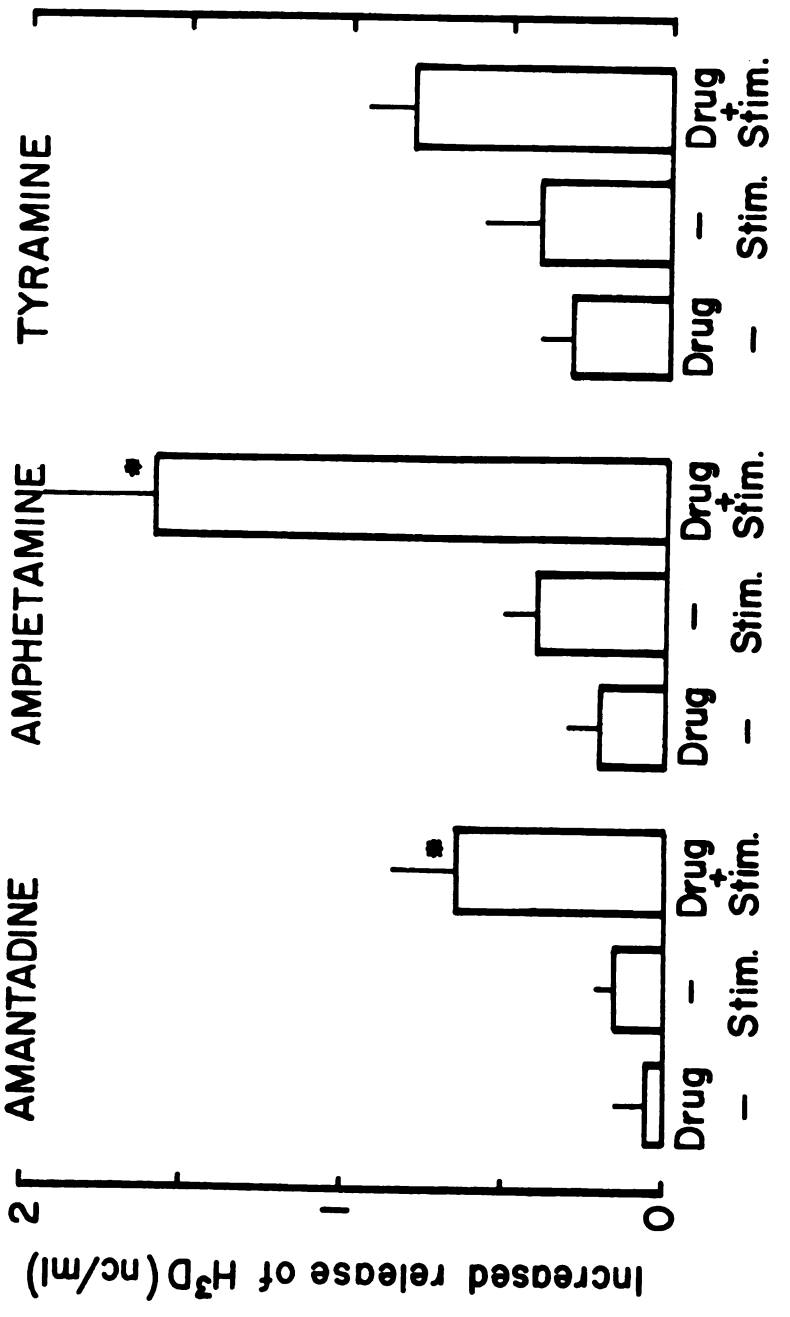

INCREASED RELEASE OF H3D (nc/ml)
2.5
2.0
1.5
1.0
.5
0
*
STIM
STIM
+
AMANTADINE
AMANTADINE

Figure 21. Effects of low concentrations of amantadine, d-amphetamine and tyramine upon the efflux of H^3-dopamine evoked by low frequency nigro-striatal stimulation.

Drug perfusion (amantadine, 5.4×10^{-5} M; d-amphetamine, 5.4×10^{-6} M; tyramine, 1×10^{-5} M) or nigro-striatal stimulation (400 uA, 6 Hz, 1 msec duration pulses) were effected separately or in combination in random order. Increased release of H^3D refers to the mean difference between the H^3-dopamine concentration in the samples collected during the 2 minute period of drug perfusion and/or stimulation minus the concentration in the samples collected during the 2 minute period immediately before drug perfusion and/or stimulation. Vertical lines represent standard errors; n= at least 4.

*Mean increase in H^3-dopamine efflux evoked by combined drug plus stimulation is statistically greater ($P < .05$) than the sum of their effects when presented separately.

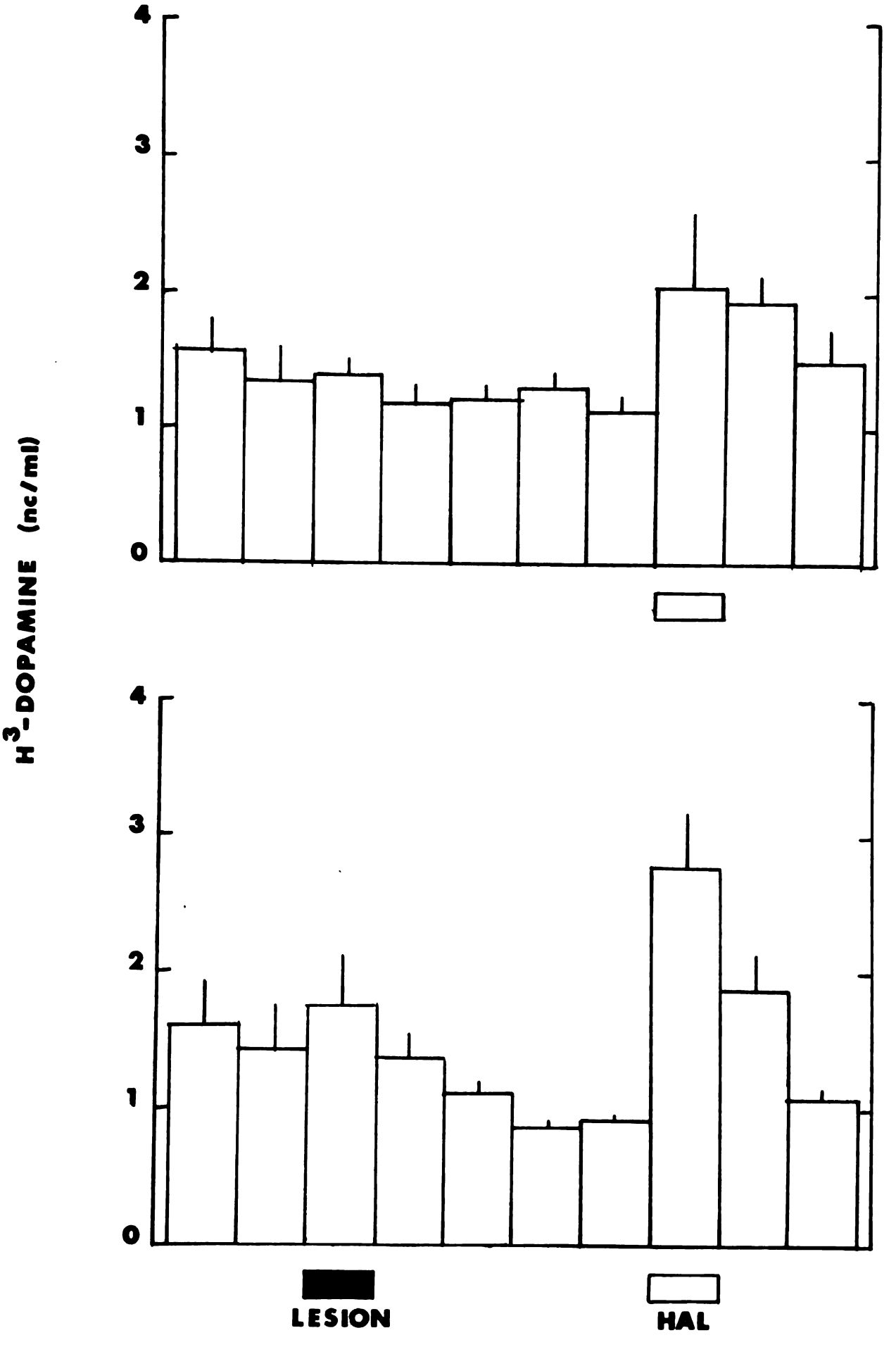

Increased release of H³D (nc/ml)
2
1
0
AMANTADINE
AMPHETAMINE
TYRAMINE
Drug – Stim.
– – Stim.
Drug + Stim.
*

amantadine both of which are blocked by acute lesions of the nigro-striatal fibers are potentiated by stimulation of these neurons, whereas the effect of tyramine, which is not blocked by acute lesions, is not potentiated by stimulation.

On the basis of indirect evidence it has been proposed that neuroleptic drugs (phenothiazines and butyrophenones) increase dopamine release from striatal dopaminergic terminals by an indirect feedback activation of nigro-striatal neurons resulting from blockade of dopaminergic receptors (Andén et al., 1970[a]). Since this effect depends on the activation of the nigro-striatal pathway, acutely lesioning these fibers should block it. To test this hypothesis, haloperidol was perfused. This compound is only soluble in water to a concentration of 4×10^{-5} M; therefore, the drug was dissolved in citric acid (final concentration 10^{-3} M). Figure 22 illustrates 2 series of experiments; one in which haloperidol (10^{-4} M) dissolved in citrate was perfused and a second in which drug perfusion was preceded by an acute nigro-striatal lesion. In both series of experiments perfusion of the haloperidol-citrate solution significantly ($P < .05$) increased H^3-dopamine efflux. Thus, this observed effect was apparently not dependent upon either the ongoing activity or an increased activity of the nigro-striatal fibers. In an effort to determine whether the vehicle or

Figure 22. Effect of acute nigro-striatal lesions upon the efflux of H^3-dopamine into ventricular perfusates evoked by haloperidol (HAL) dissolved in citrate.

The bars represent H^3-dopamine mean concentrations in successive perfusates collected at 2 minute intervals from 4 experiments (vertical lines denote 1 standard error). The upper panel illustrates the effect of haloperidol (10^{-4} M dissolved in 10^{-3} M citrate) upon H^3-dopamine efflux. In the experiments shown in the lower panel, an acute lesion of the nigro-striatal fibers was made during the indicated period and the haloperidol solution perfused 8 minutes later. In both the upper and lower panel, the sum of the H^3-dopamine concentrations in the 2 samples collected during and immediately following drug perfusion is statistically different ($P < .05$) than the sum of the concentrations in the 2 samples collected before drug perfusion (upper panel, $\bar{d}=1.73\pm0.41$ nc/ml; lower panel, $\bar{d}=2.80\pm0.81$ nc/ml).


4
3
2
1
0
H3-DOPAMINE (nc/ml)
4
3
2
1
0
LESION
HAL

the haloperidol itself was responsible for the increased efflux of H^3-dopamine, citrate was perfused alone. The upper panel of Figure 23 shows the results of these experiments. The increase in H^3-dopamine efflux induced by citrate (10^{-3} M) is in contrast to the effect of haloperidol (10^{-5} M) alone which is illustrated in the lower panel of Figure 23; this agent perfused by itself failed to alter the release of H^3-dopamine into ventricular perfusates.

The failure of haloperidol to alter H^3-dopamine release could be due to the route of administration; for this reason the drug was injected intravenously in subsequent experiments. Furthermore, the drug-induced release might be of such low magnitude as to be entirely reclaimed by the amine reuptake system and not appear in the perfusate. For this reason the ventricular system was perfused with d-amphetamine after the injection of haloperidol. If haloperidol causes an indirect activation of the nigro-striatal pathway, then it should potentiate the d-amphetamine-induced efflux of H^3-dopamine. For the purpose of comparison, cats were injected intravenously with either haloperidol, vehicle or apomorphine. The latter agent has been claimed to indirectly inhibit the rate of firing of the nigro-striatal pathway by directly stimulating dopaminergic receptors (Andén et al., 1967). Inspection of Figure 24, however, reveals that neither haloperidol or apomorphine significantly

Figure 23. The effects of citrate and haloperidol (HAL) separately upon the efflux of H^3-dopamine into ventricular perfusates.

The bars represent mean H^3-dopamine concentrations in successive perfusates collected at 2 minute intervals from 4 experiments (vertical lines denote 1 standard error). In the experiments in the upper panel, citrate (10^{-3} M) was perfused during the indicated period; in the lower panel, haloperidol (10^{-5} M) was perfused. The sum of the H^3-dopamine concentrations in the 2 samples collected during and immediately following citrate perfusion is statistically different ($P < .05$) than the sum of the concentrations in the 2 samples collected before citrate perfusion ($\bar{d}=2.12\pm0.59$ nc/ml).

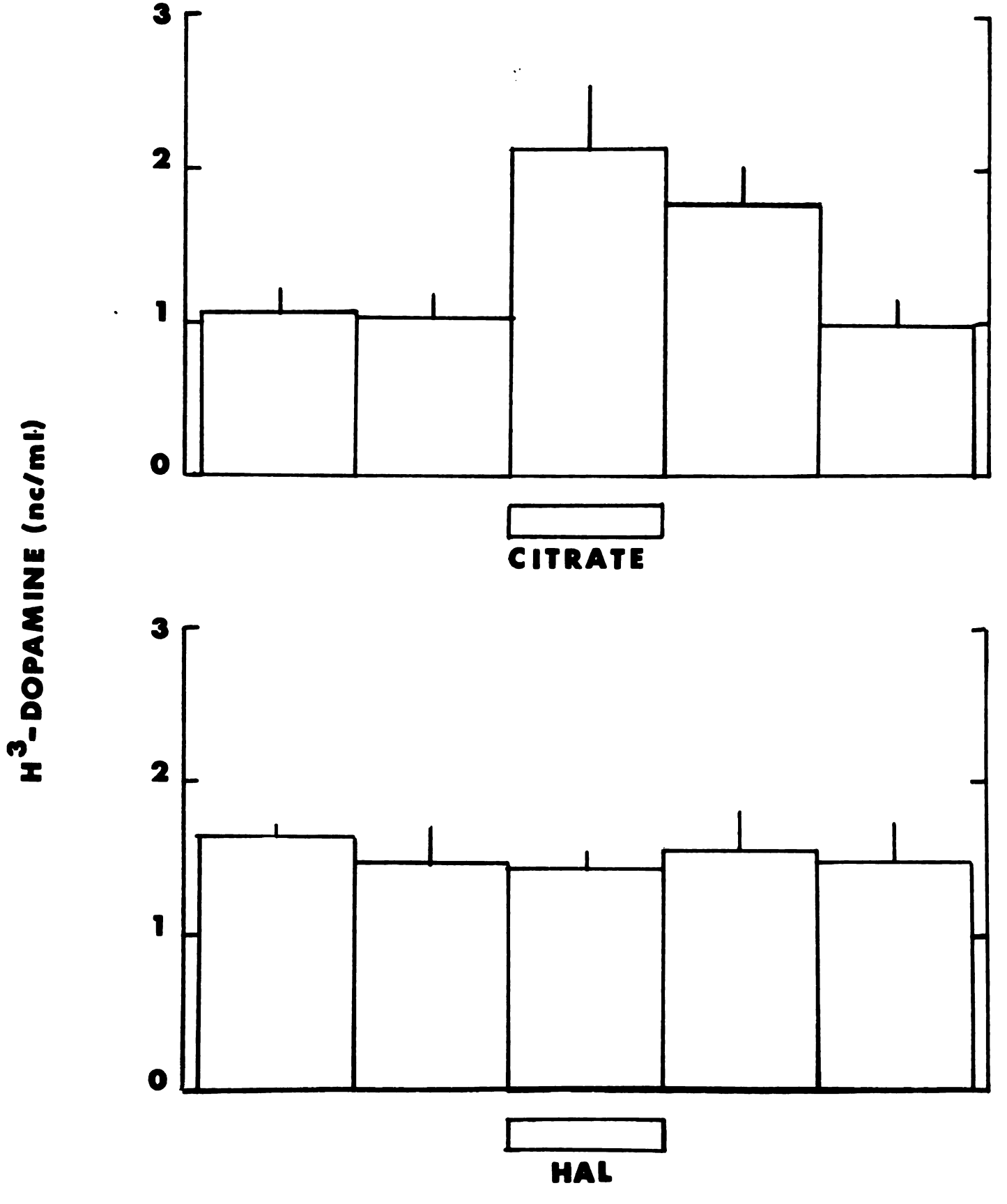
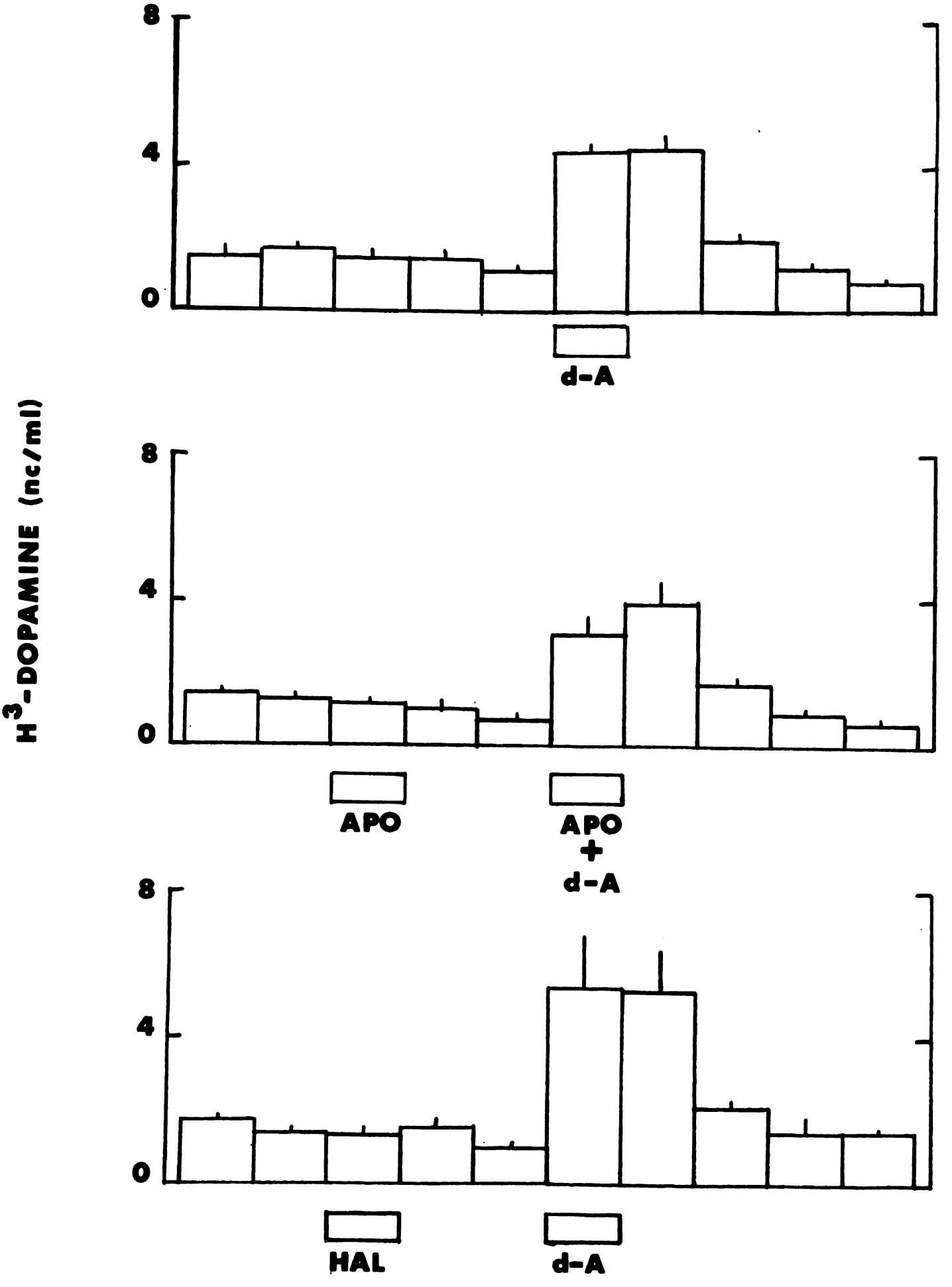

3
2
1
0
CITRATE
3
2
1
0
HAL
H3-DOPAMINE (nc/ml)

Figure 24. The effect of intravenously injected apomorphine (APO) or haloperidol (HAL) upon the efflux of H^3-dopamine into ventricular perfusates evoked by d-amphetamine (d-A) perfusion.

Each vertical bar represents the mean concentration of H^3-dopamine in successive perfusates collected at 10 minute intervals in 4 experiments (the vertical lines denote 1 standard error). The upper panel illustrates the effect of d-amphetamine (1.6×10^{-4} M) perfusion on H^3-dopamine efflux. The middle panel illustrates experiments in which apomorphine (10 mg/kg) was injected during the third collection period and again (5 mg/kg) during the perfusion of d-amphetamine. In experiments summarized in the lower panel, haloperidol (10 mg/kg) was injected during the third collection period, then d-amphetamine perfused. In all three series of experiments, the sum of the H^3-dopamine concentrations in the 2 samples collected during and immediately after d-amphetamine perfusion is statistically different ($P < .05$) than the sum of the concentrations in the 2 samples collected before d-amphetamine perfusion (upper panel, $\bar{d}$= 5.78±0.55 nc/ml; middle panel, $\bar{d}$=4.15±0.92 nc/ml; lower panel, $\bar{d}$=7.45±2.19 nc/ml).


8
4
0
d-A
H³-DOPAMINE (nc/ml)
8
4
0
APO
APO
+
d-A
8
4
0
HAL
d-A

altered H^3-dopamine release nor did either compound significantly ($P < .05$) alter the efflux of H^3-dopamine induced by d-amphetamine perfusion.

In an effort to determine if apomorphine or haloperidol might be altering the release of dopamine metabolites, the alumina supernatant fraction was analyzed for H^3-O-methylated amines and H^3-deaminated-O-methylated compounds. Figure 25 indicates that neither drug altered the perfusate concentration of the H^3-O-methylated amines nor did they alter the increase in this fraction induced by d-amphetamine perfusion. Likewise, the lack of effect of these compounds upon the perfusate concentration of H^3-deaminated-O-methylated metabolites of H^3-dopamine is shown in Figure 26. Thus, intravenous injections of haloperidol or apomorphine did not alter the resting or d-amphetamine-evoked release of H^3-dopamine and its major metabolites. Similarly, bulbocapnine, a potent cataleptic agent (Ernst, 1965), failed to affect H^3-dopamine release (Figure 27). This figure also illustrates that the reduction in H^3-dopamine release induced by acutely lesioning the nigro-striatal fibers was not affected by prior intravenous injection of bulbocapnine. If this agent had been indirectly activating the nigro-striatal pathway, one might expect it to cause an increase in H^3-dopamine release which might be manifested by a more pronounced reduction in H^3-dopamine release coincident with the acute lesion.

Figure 25. The effect of intravenously injected apomorphine (APO) or haloperidol (HAL) upon the efflux of H^3-O-methylated amine metabolites (H^3-3-MT) of H^3-dopamine into ventricular perfusates evoked by d-amphetamine (d-A) perfusion.

Each vertical bar represents the mean concentration of H^3-O-methylated amine metabolites (H^3-3-MT) of H^3-dopamine in successive perfusates collected at 10 minute intervals in 4 experiments (the vertical lines denote 1 standard error). The upper panel illustrates the effect of d-amphetamine (1.6×10^{-4} M) perfusion on H^3-O-methylated amine metabolites (H^3-3-MT) of H^3-dopamine efflux. The middle panel illustrates experiments in which apomorphine (10 mg/kg) was injected during the third collection period and again (5 mg/kg) during the perfusion of d-amphetamine. In experiments summarized in the lower panel, haloperidol (10 mg/kg) was injected during the third collection period, then d-amphetamine perfused. In all three series of experiments the sum of the H^3-3-MT concentrations in the 2 samples collected during and immediately after d-amphetamine perfusion is statistically different ($P < .05$) than the sum of the concentrations in the 2 samples collected before d-amphetamine perfusion (upper panel, $\bar{d}=1.52\pm0.27$ nc/ml; middle panel, $\bar{d}=0.99\pm0.29$ nc/ml; lower panel, $\bar{d}=1.51\pm0.46$ nc/ml).

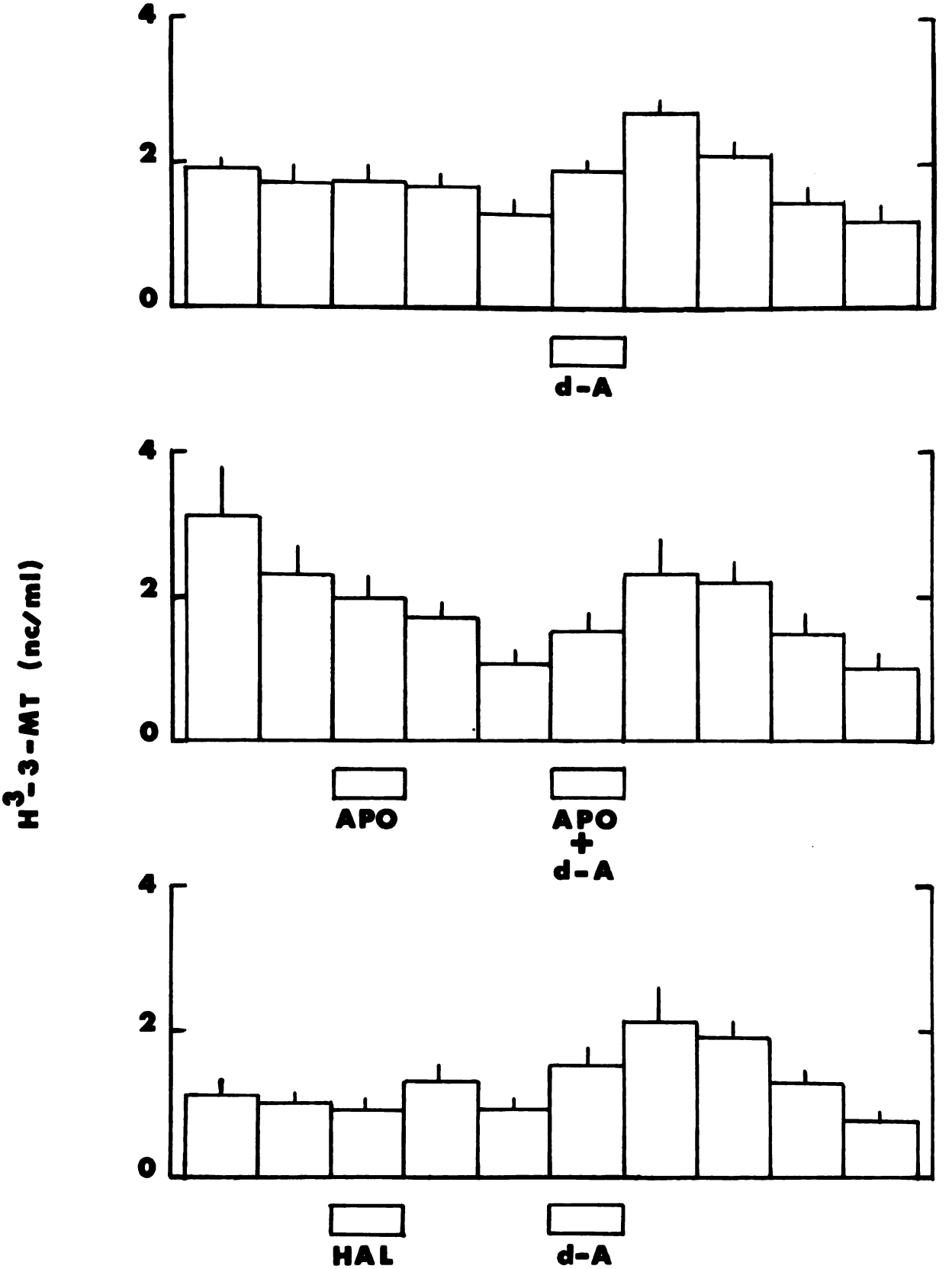

4
2
0
d-A
4
2
0
APO
APO
+
d-A
4
2
0
HAL
d-A
H^3-3-MT (nc/ml)

Figure 26. The effect of intravenously injected apomorphine (APO) or haloperidol (HAL) upon the efflux of H^3-deaminated O-methylated metabolites of H^3-dopamine into ventricular perfusates evoked by d-amphetamine (d-A) perfusion.

Each vertical bar represents the mean concentration of H^3-deaminated O-methylated metabolites of H^3-dopamine in successive perfusates collected at 10 minute intervals in 4 experiments (the vertical lines denote 1 standard error). The upper panel illustrates the effect of d-amphetamine (1.6×10^{-4} M) perfusion on H^3-deaminated O-methylated metabolites of H^3-dopamine efflux. The middle panel illustrates experiments in which apomorphine (10 mg/kg) was injected during the third collection period and again (5 mg/kg) during the perfusion of d-amphetamine. In experiments summarized in the lower panel, haloperidol (10 mg/kg) was injected during the third collection period, then d-amphetamine perfused.

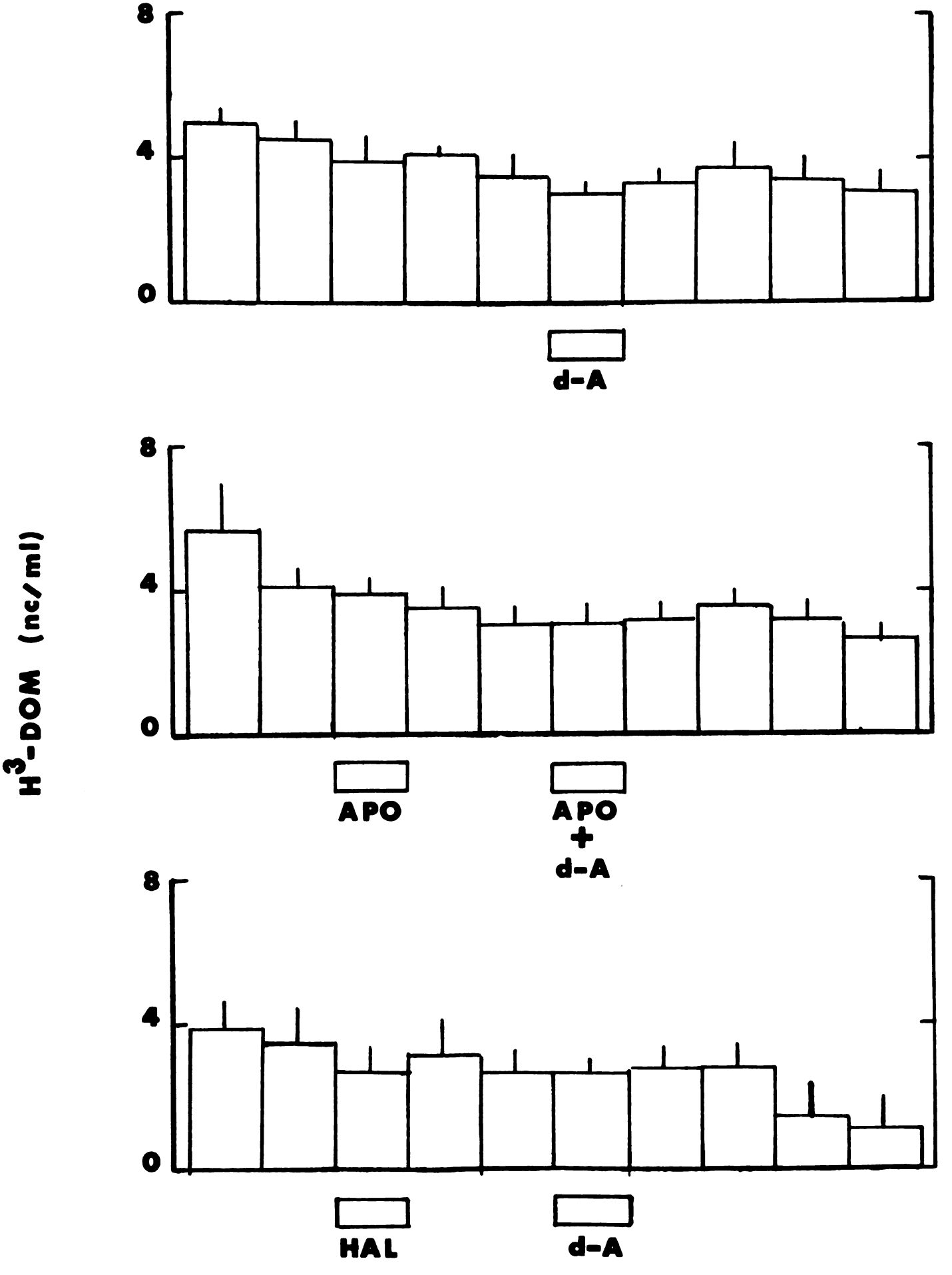
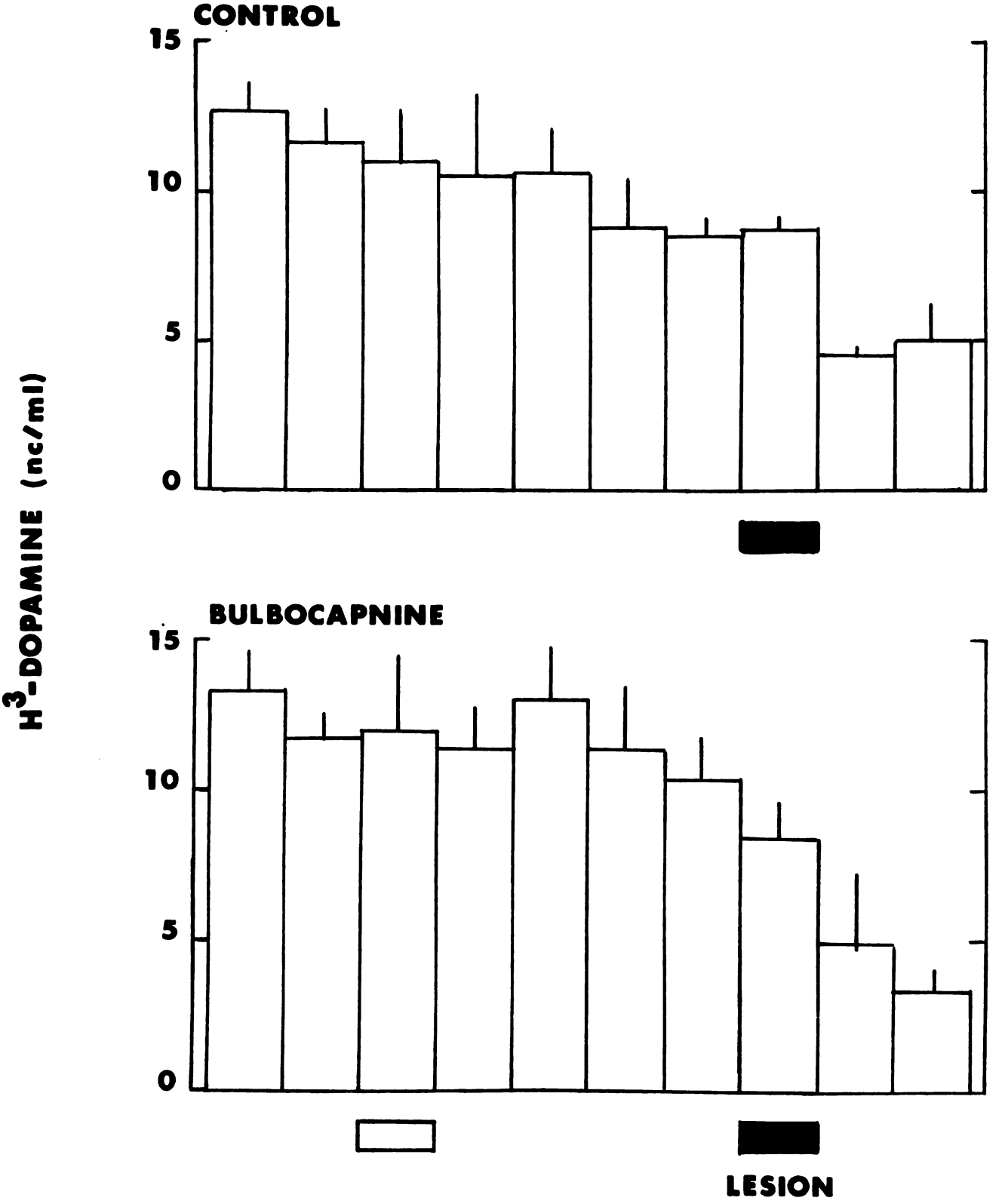

8
4
0
d-A
8
4
0
APO
APO
+
d-A
H^3-DOM (nc/ml)
8
4
0
HAL
d-A

Figure 27. Effect of intravenously administered bulbocapnine and acute nigro-striatal lesions on ventricular effluent concentrations of H^3-dopamine.

The vertical bars represent H^3-dopamine mean concentrations in successive perfusates collected at 10 minute intervals in 4 experiments (vertical lines denote 1 standard error). Solid horizontal bars mark the collection periods during which acute electrolytic lesions were made. In the experiments summarized in the lower panel bulbocapnine (15 mg/kg) was injected during the third collection period.


CONTROL
15
10
5
0
BULBOCAPNINE
15
10
5
0
H^3-DOPAMINE (nc/ml)
LESION

Although neither the brain stimulation nor drug perfusions that were effective at increasing the outflow of H^3-dopamine altered systemic blood pressure consistently, it was, nevertheless, of interest to determine if acute changes in blood pressure might alter the efflux of H^3-dopamine into ventricular perfusates. For this purpose vasopressin was injected intravenously during the collection of perfusates. In these experiments (n=4) the average mean blood pressure during the 2 minutes after vasopressin injection increased 24.0 $\pm$ 4.5 mm Hg; however, as illustrated in Figure 28, the efflux of H^3-dopamine was unaltered. Thus, mean blood pressure changes of up to 24 mm Hg do not appear to alter H^3-dopamine efflux.

III. Push-pull cannula perfusions.

In all the previous perfusion experiments, the technique of ventricular perfusion from the anterior horn of the lateral ventricle to the cerebroaqueduct was used. This method entails the perfusion of not only the terminals of the nigro-striatal fibers but also the structures lining the third ventricle and cerebroaqueduct. For this reason an attempt was made to adapt the push-pull cannula for the selective perfusion of the surface of the caudate nucleus. Figure 29 is a plot of the concentrations of H^3-dopamine and metabolites appearing in push-pull cannula perfusates

Figure 28. The effects of intravenously administered vasopressin (ADH) on ventricular effluent concentrations of H^3-dopamine.

The vertical bars represent mean H^3-dopamine concentrations in successive perfusate samples collected at 2 minute intervals in 4 experiments (vertical lines denote 1 standard error). During the indicated period vasopressin (0.5 units) was injected.

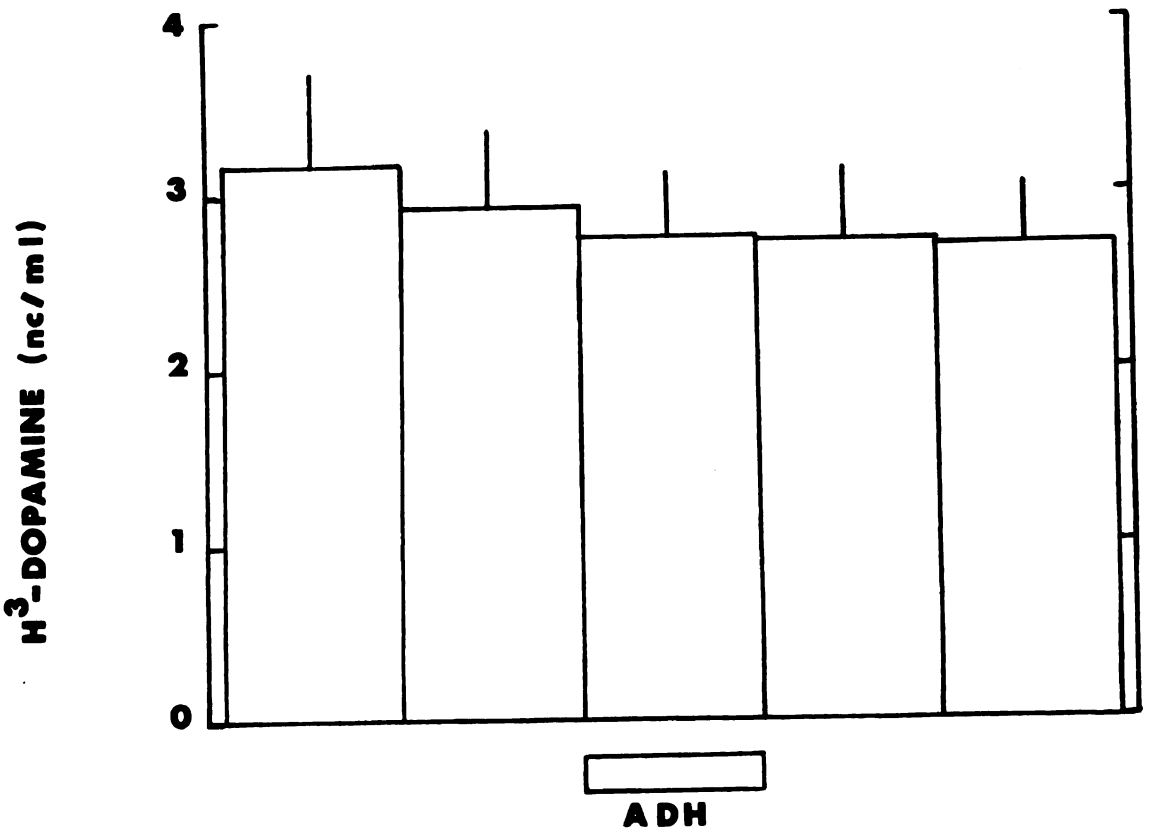
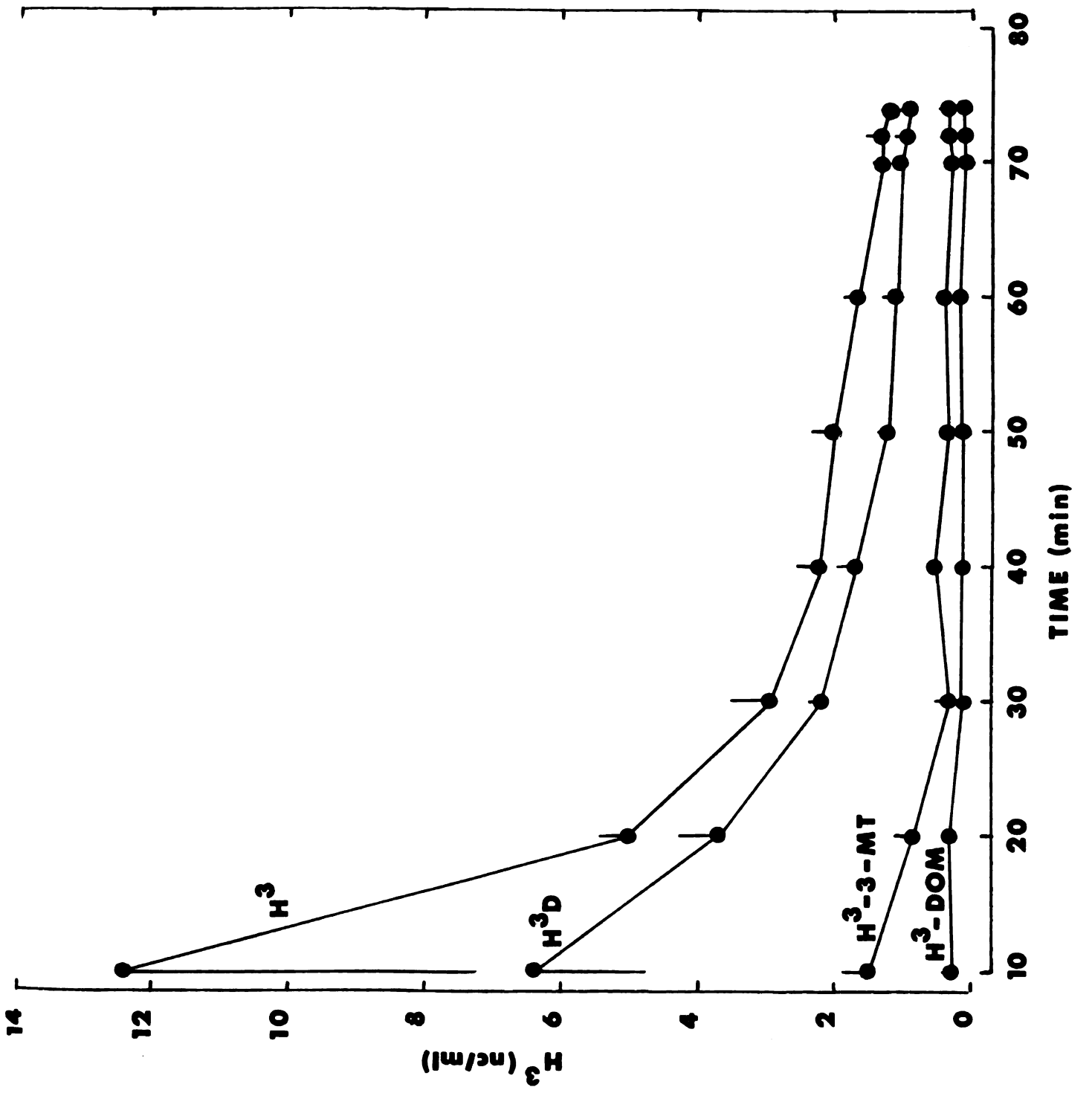

4
3
2
1
0
H3-DOPAMINE (nc/ml)
ADH

Figure 29. Washout of H^3-compounds following labeling of the caudate nucleus with H^3-dopamine and subsequent perfusion of the lateral ventricle with a push-pull cannula.

Each point represents the mean total radioactivity (H^3), H^3-dopamine (H^3-D), H^3-O-methylated amine (H^3-3-MT) or H^3-deaminated O-methylated metabolites (H^3-DOM) in perfusates collected from 4 experiments. Vertical lines denote 1 standard error.


H^3
H^3D
H^3-3-MT
H^3-DOM
H^3 (nc/ml)
0
2
4
6
8
10
12
14
10
20
30
40
50
60
70
80
TIME (min)

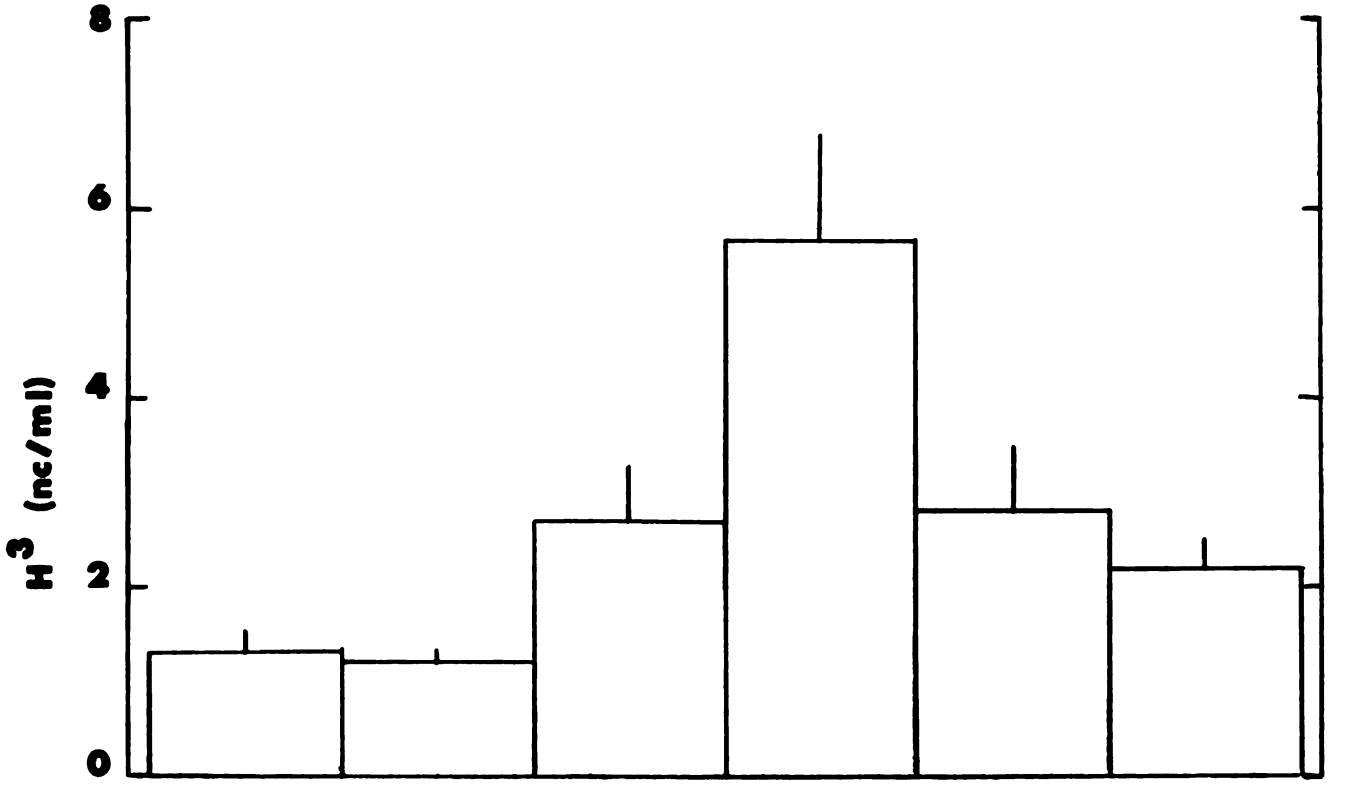
at various times after ventricular injection of H^3-dopamine. H^3-dopamine accounted for most of the radioactivity in the perfusates; after 50 minutes of perfusion the rate of efflux of this amine was relatively stable. H^3-O-methylated amines and H^3-deaminated-O-methylated metabolites of H^3-dopamine were present in much smaller amounts than the parent amine.

If d-amphetamine was perfused through the push-pull cannula after labeling the caudate nucleus with H^3-dopamine and perfusing for 76 minutes, a marked increase in H^3-efflux occurred (Figure 30, upper panel). This increase was almost completely accounted for by an increased concentration of H^3-dopamine (Figure 30, lower panel). The analysis of the H^3-noncatechol fraction, illustrated in Figure 31, demonstrated a slight but significant increase only in the H^3-O-methylated amine fraction; the perfusate concentration of H^3-deaminated-O-methylated metabolite of H^3-dopamine did not increase during or after d-amphetamine perfusion. Thus, the selective perfusion of the caudate nucleus yielded results similar to those obtained when the whole ventricular system was perfused.

Unfortunately, problems developed with the push-pull cannula perfusion technique. Frequently the outflow cannula became plugged with either tissue of the caudate nucleus or with choroid plexus. In attempting to avoid these problems the cannula was occasionally not lowered far enough, so that the corpus callosum rather than the lateral

Figure 30. Effect of d-amphetamine (AMPH) perfusion on H^3- and H^3-dopamine concentrations in push-pull cannula perfusate from the lateral ventricle.

Vertical bars represent mean concentrations in successive perfusates collected at 2 minute intervals from 4 experiments. During the indicated period, d-amphetamine (1.6×10^{-3} M) was added to the perfusion inflow. For both H^3- and H^3-dopamine the sum of the concentrations in the 2 samples collected during and immediately after drug perfusion is significantly different ($P < .05$) than the sum of the concentrations in the 2 samples collected before drug perfusion (H^3-, $\bar{d}=5.55\pm1.50$ nc/ml; H^3-dopamine, $\bar{d}=5.12\pm1.57$ nc/ml).


8
6
4
2
0
H3 (nc/ml)

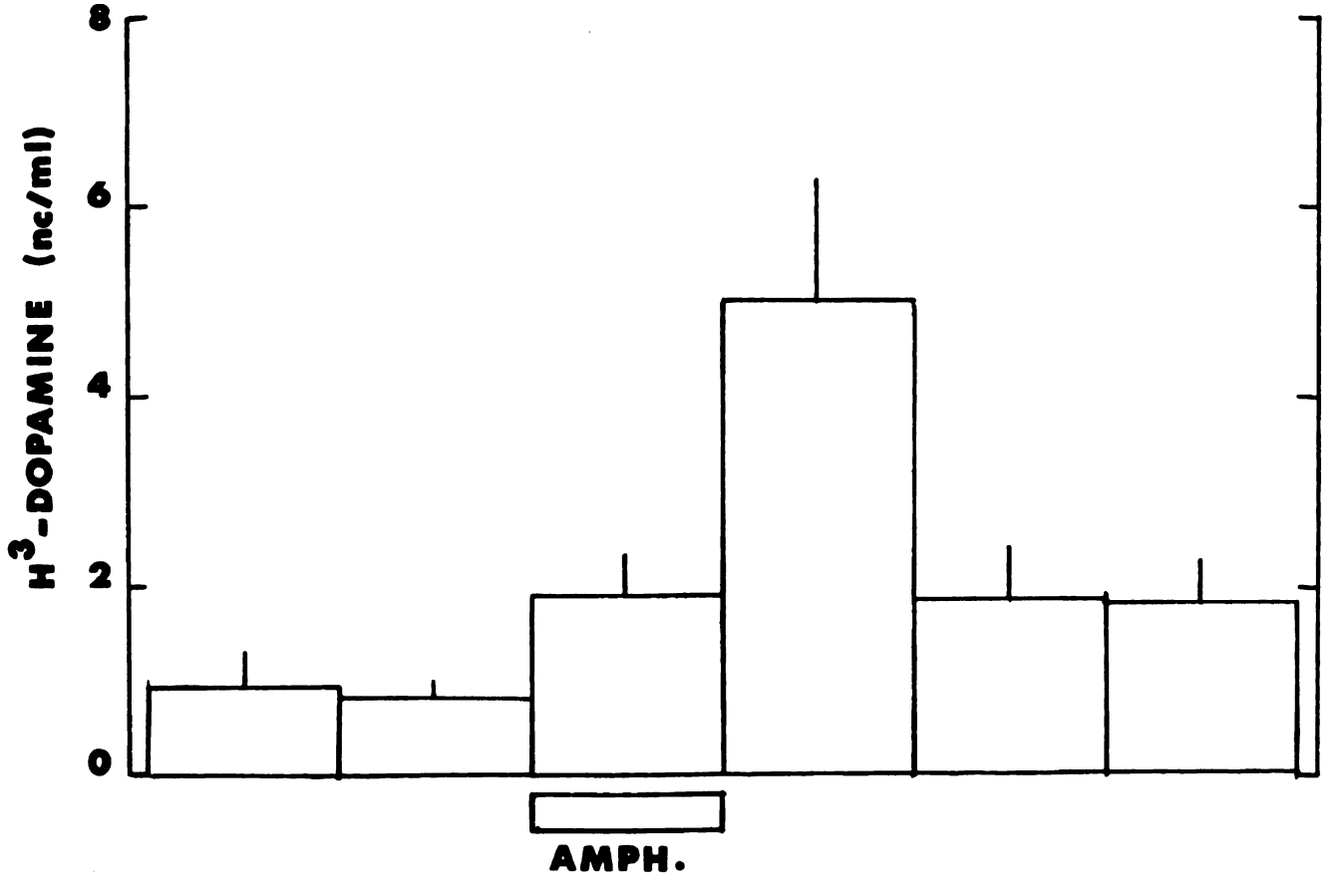
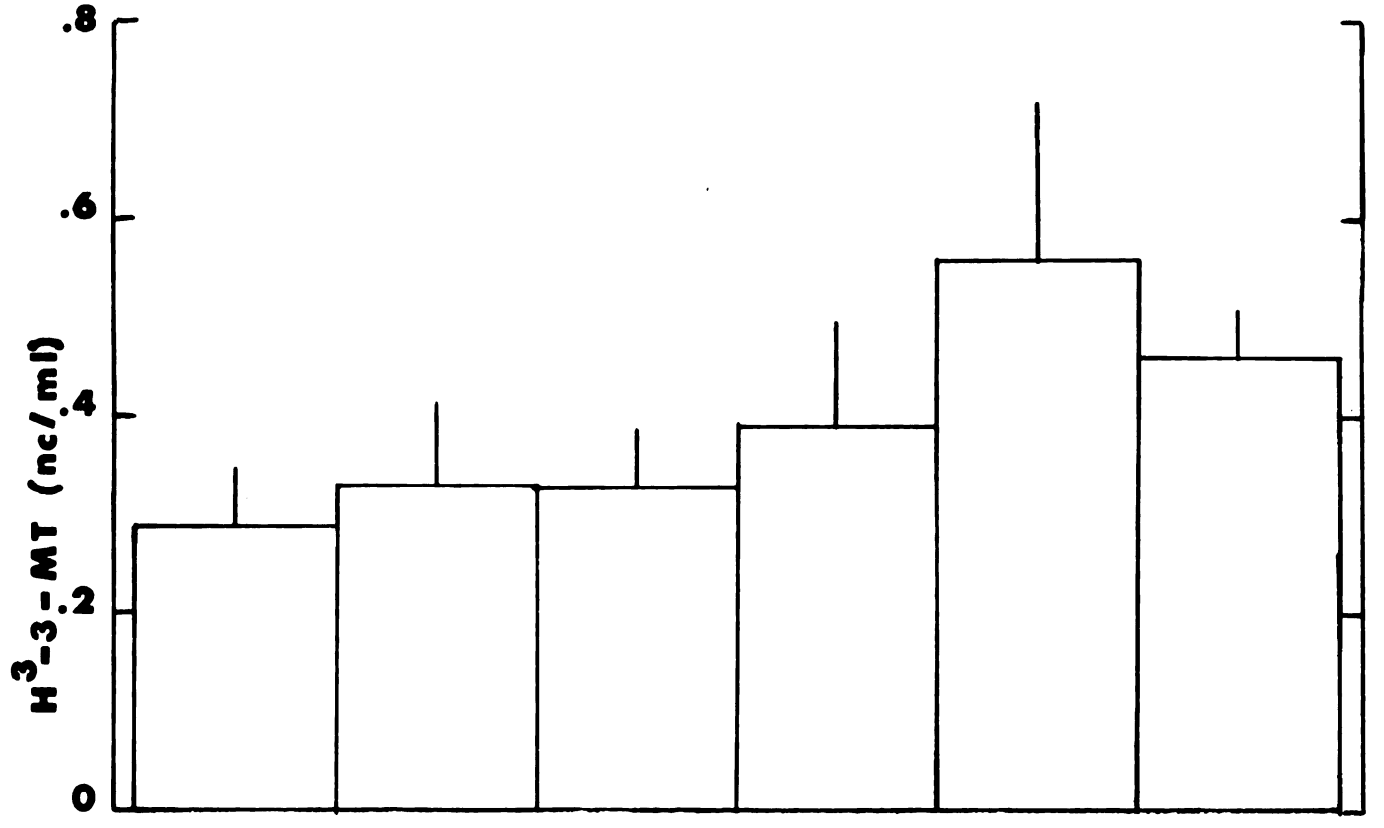
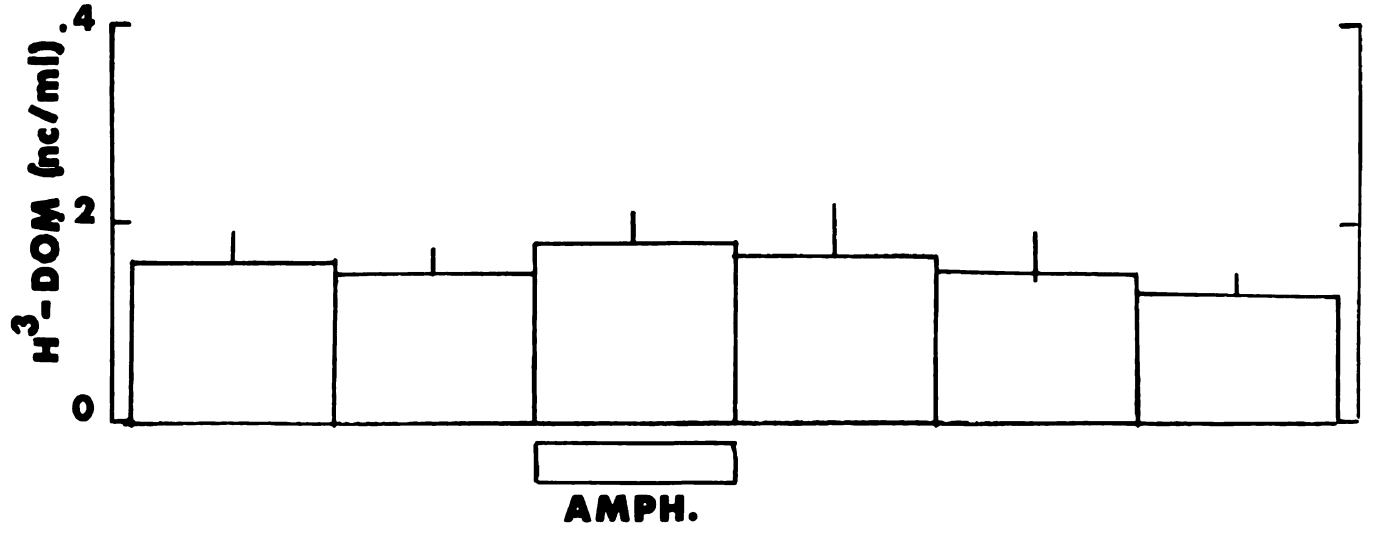

8
6
4
2
0
H3-DOPAMINE (nc/ml)
AMPH.

Figure 31. Effect of d-amphetamine (AMPH) perfusion on H^3-O-methylated amine (H^3-3-MT) and H^3-deaminated-O-methylated (H^3-DOM) metabolites concentrations in push-pull cannula perfusate from the lateral ventricle.

Vertical bars represent mean concentrations in successive perfusates collected at 2 minute intervals from 4 experiments. During the indicated period, d-amphetamine (1.6×10^{-3} M) was added to the perfusion inflow. The sum of the concentrations of H^3-3-MT in the 2 samples collected during the 2 periods after drug perfusion is significantly different ($P < .05$) than the sum of the concentrations in the 2 samples collected before drug perfusion ($\bar{d}=0.39\pm0.11$ nc/ml).


.8
.6
.4
.2
0
H^3-3-MT (nc/ml)


.4
2
0
H^3-DOM (nc/ml)
AMPH.

ventricle was perfused. All of the experiments in which the caudate nucleus was damaged or the cannula plugged were discarded. When the corpus callosum rather than the caudate nucleus was labeled with H^3-dopamine, d-amphetamine perfusion was not capable of altering the release of the amine (Figure 32). These results are reminiscent of those in Figure 13 in which d-amphetamine was perfused over the dopaminergically denervated caudate nucleus; despite a significant resting release the drug-induced release was markedly decreased. Thus, in both cases the resting release appeared to arise from an amphetamine insensitive source.

Another series of push-pull cannula perfusions were, however, successfully completed. Figure 33 illustrates that perfusion of fluphenazine increased the efflux of total radioactivity, consisting primarily of H^3-dopamine. This effect was accompanied by a slight increase in H^3-O-methylated amine concentration and variable changes in the H^3-deaminated-O-methylated fraction (Figure 34). Thus, in contrast to haloperidol, this potent neuroleptic appeared to be capable of altering dopamine disposition in the cat.

IV. Behavioral studies

The intra-striatal injection technique described in the method section proved to be reasonably reliable and

Figure 32. Effect of d-amphetamine (AMPH) perfusion on H^3-dopamine concentration in push-pull cannula perfusates from the corpus callosum.

Vertical bars represent the mean H^3-dopamine concentrations in successive perfusates collected at 2 minute intervals in 4 experiments; vertical lines denote 1 standard error. During the indicated period, d-amphetamine (1.6×10^{-3} M) was included in the ventricular inflow.

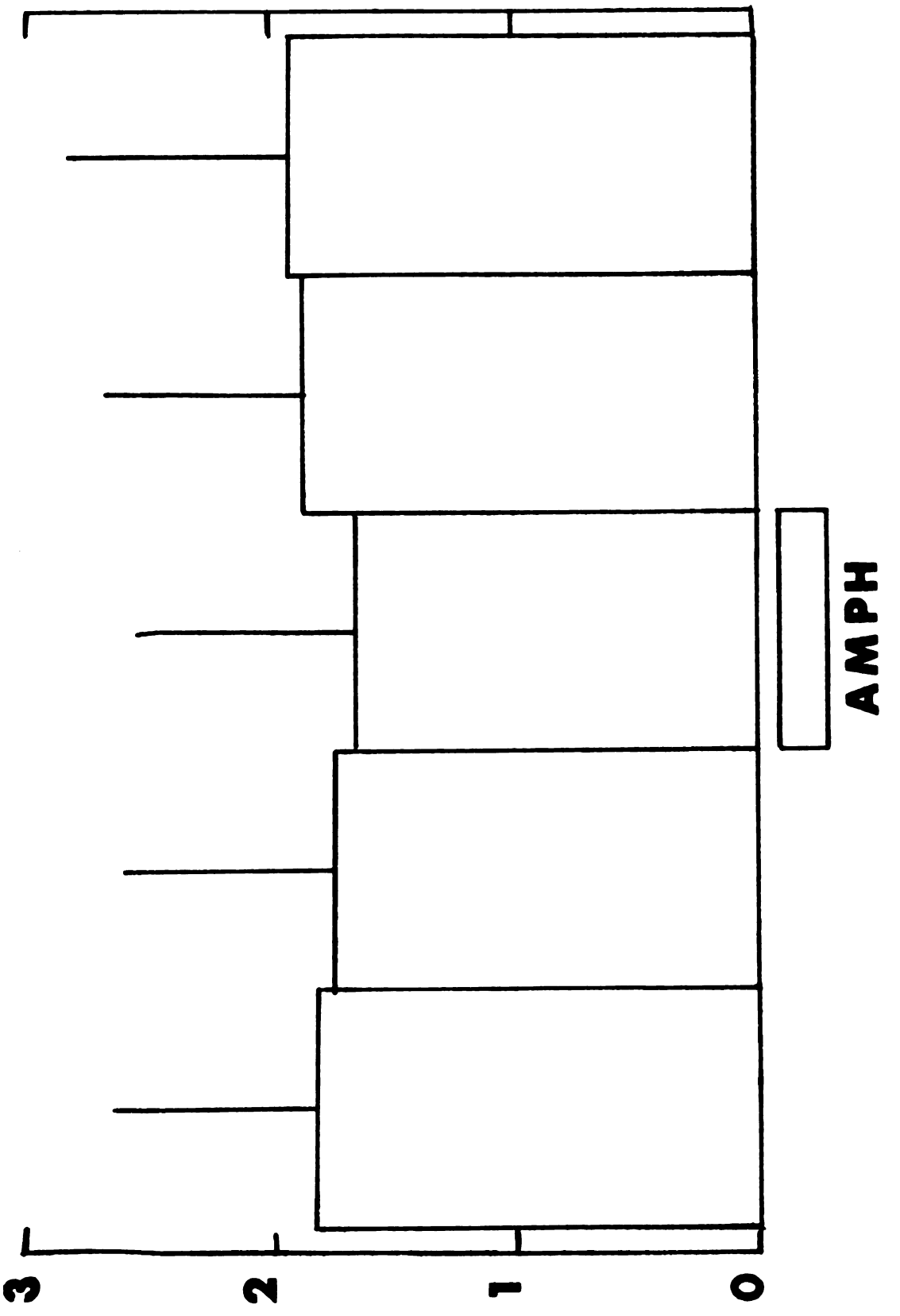
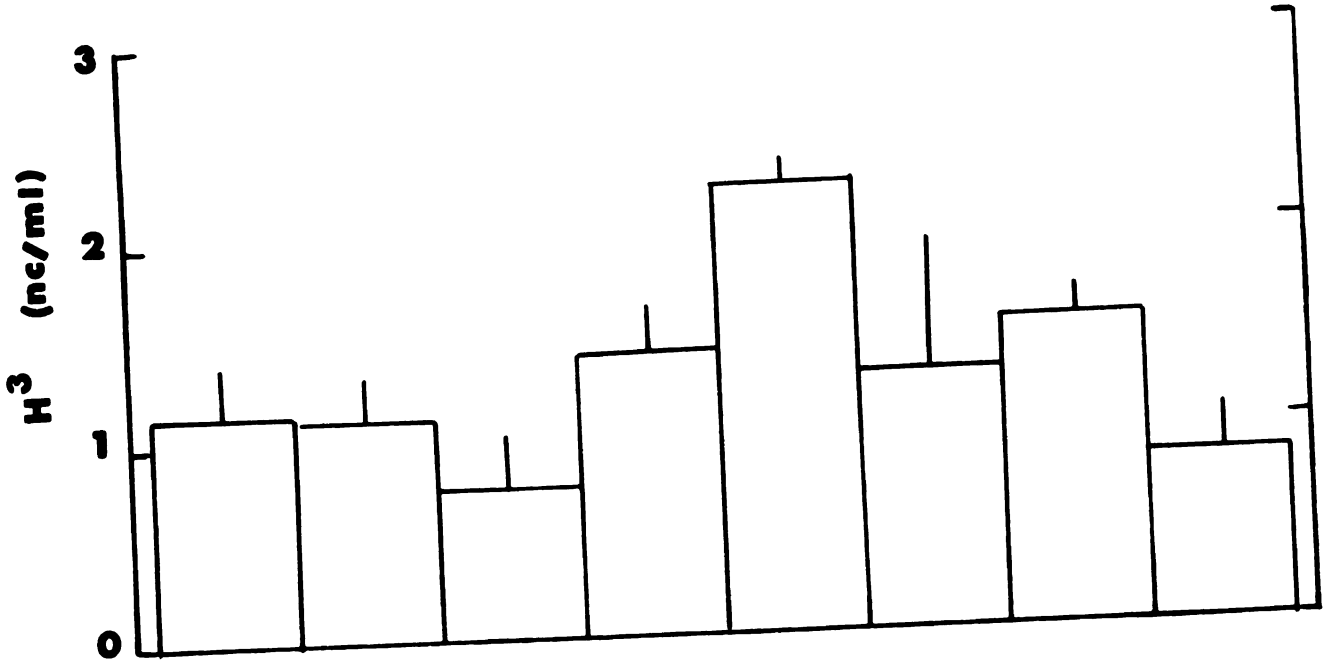

AMPH
0
1
2
3
H³-DOPAMINE (nc/mm/)

Figure 33. Effect of fluphenazine (FLUPHEN) perfusion on total radioactivity (H^3) and H^3-dopamine concentrations in push-pull cannula perfusates from the lateral ventricle.

Vertical bars represent mean concentrations in successive perfusates collected at 2 minute intervals from 4 experiments. During the indicated periods, fluphenazine (10^{-3} M) was added to the perfusion inflow. For both H^3- and H^3-dopamine, the sum of the concentrations in the 2 samples collected during drug perfusion is significantly different ($P < .05$) than the sum of the concentrations in the 2 samples collected before drug perfusion (H^3-, $\bar{d}$= 1.52 ± 0.15 nc/ml; H^3-dopamine, $\bar{d}=0.72 \pm 0.22$ nc/ml).


3
2
1
0
H3 (nc/ml)

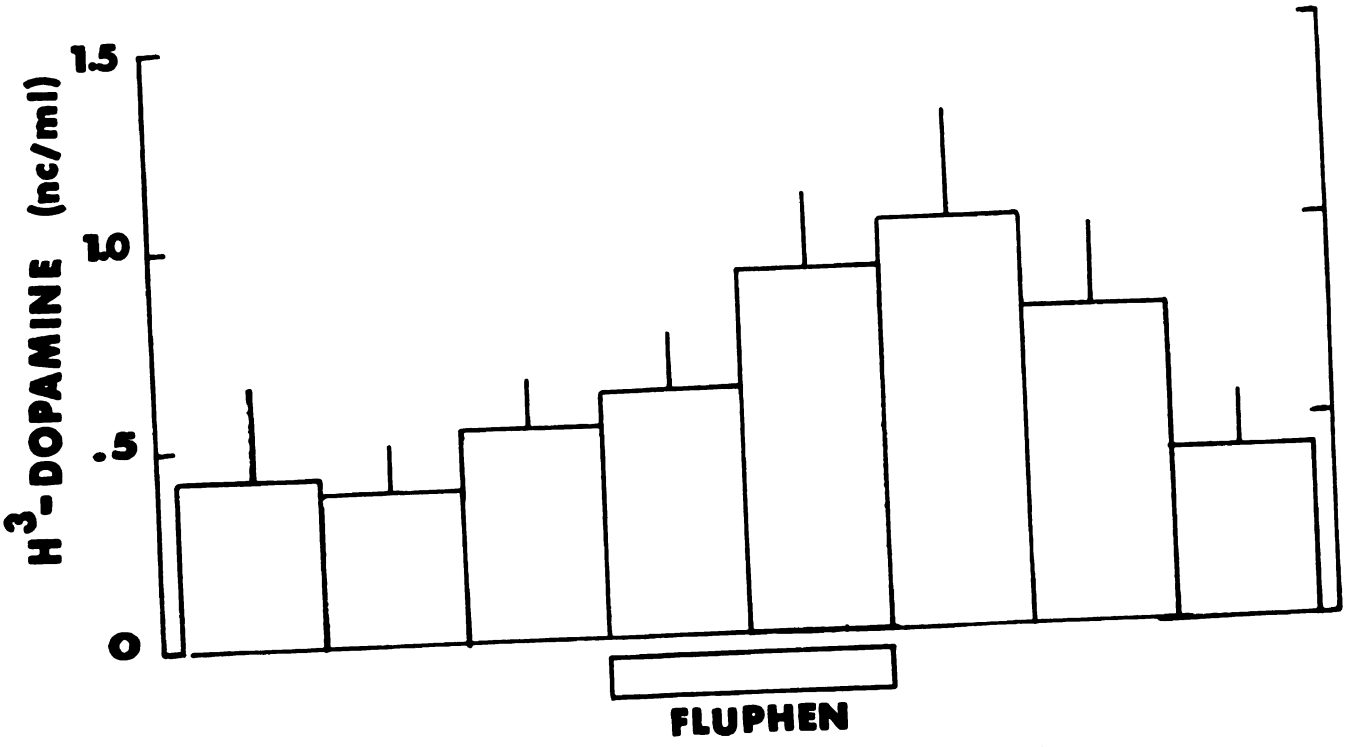
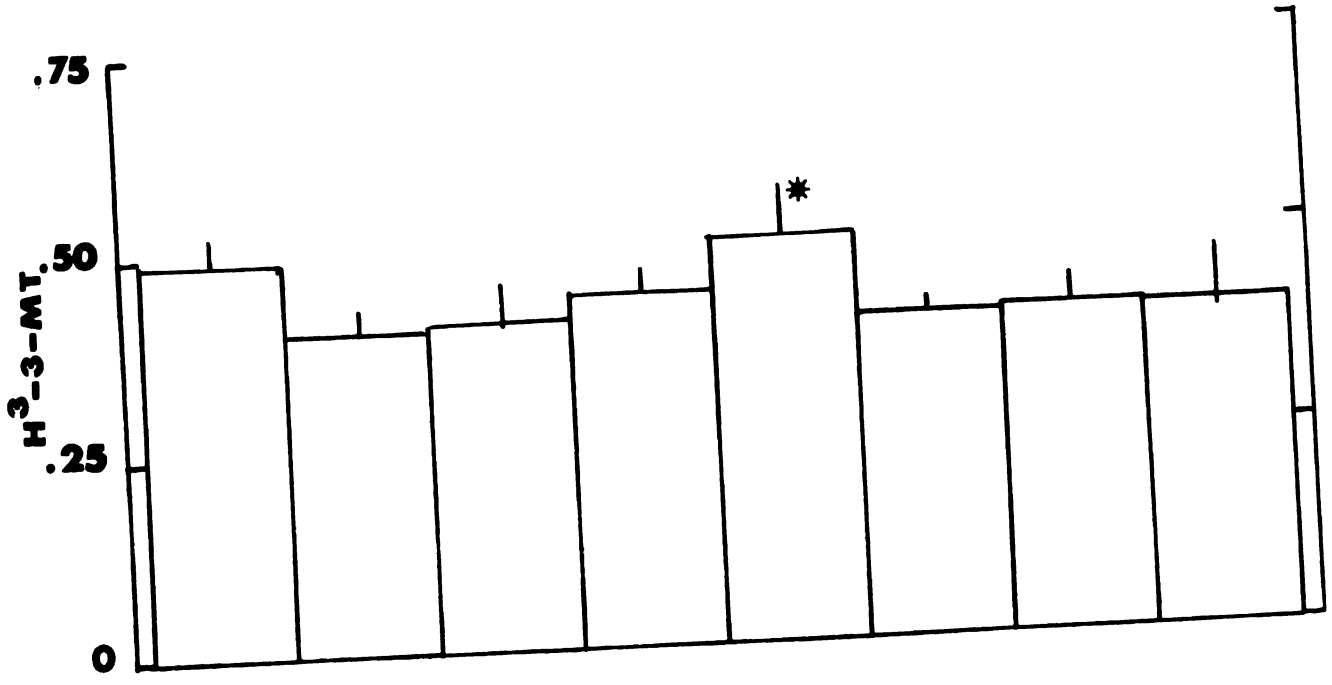
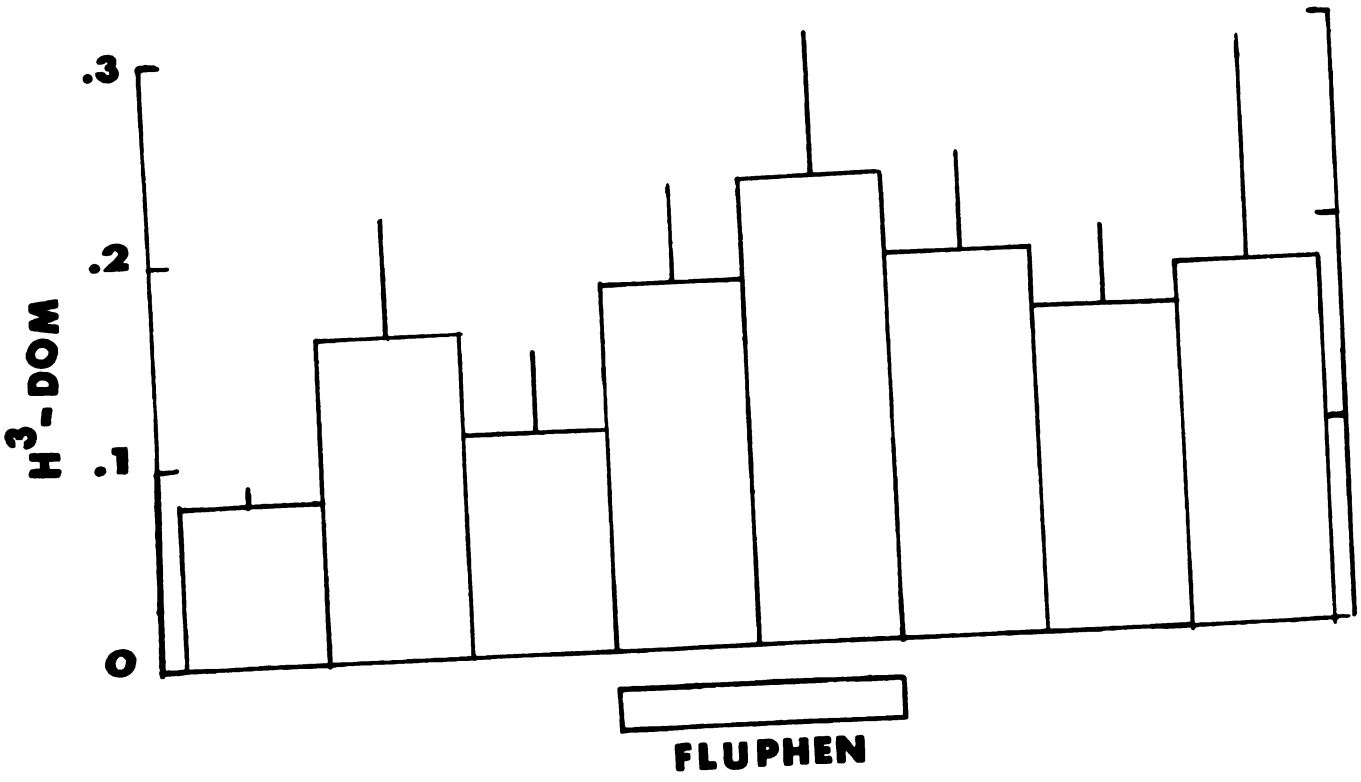

1.5
1.0
.5
0
H3-DOPAMINE (nc/ml)
FLUPHEN

Figure 34. Effect of fluphenazine (FLUPHEN) perfusion on H^3-O-methylated amine (H^3-3-MT) and H^3-deaminated-O-methylated metabolite (H^3-DOM) concentrations in push-pull cannula perfusates from the lateral ventricle.

Vertical bars represent mean concentration in successive perfusates collected at 2 minute intervals from 4 experiments. During the indicated periods, fluphenazine (10^{-3} M) was added to the perfusion inflow.


.75
.50
.25
0
H^3-3-MT.
*


.3
.2
.1
0
H^3-DOM
FLUPHEN

atraumatic. Anterior and lateral skull coordinates of the injection site were determined in 140 mice. The mean coordinates (± 1 standard error) were 1.6 ± 0.1 mm lateral of the midsagittal suture and 5.7 ± 0.1 mm anterior of the occipital suture. Two weeks after the injection of 16 µg of 6-hydroxydopamine in 4 µl, non-specific damage to the tissue appeared to be minimal; in most tissues sectioned, no needle tract was grossly visible. Figure 35 is a photograph of a frontal section in which the tract was apparent on gross examination. Microscopic examination of the tract revealed some old hemmorhage and inflammatory cell infiltration; however, this was localized to the tract. It should be noted that the normal trabecular appearance of the corpus striatum is not generally altered.

Ten days or more after the injection of 8 µg of 6-hydroxydopamine into the left striatum, forebrain dopamine concentrations and to a lesser extent norepinephrine concentrations were lowered on the side of the injection; 5-hydroxytryptamine concentrations were, however, unaltered (Table 7). Observation of mice (n=80) with left striatal lesions induced by 8 µg 6-hydroxydopamine revealed that 66% of these mice turned to the left more than to the right (net ipsilateral turning). Figure 36 illustrates the effects of several drugs upon the net turning of these selected mice. Amantadine and the d and l isomers of amphetamine significantly increased ipsilateral turning, whereas, apomorphine caused

Figure 35. Frontal section of mouse brain through corpus striatum.

Two weeks after the injection of 16 µg 6-hydroxydopamine into the left striatum, mice were sacrificed and the brains fixed in formalin. They were then sectioned and the needle tracts located. This is a typical cresyl violet stained section showing the vertical needle tract through the cerebral cortex, corpus callosum and into the left corpus striatum.

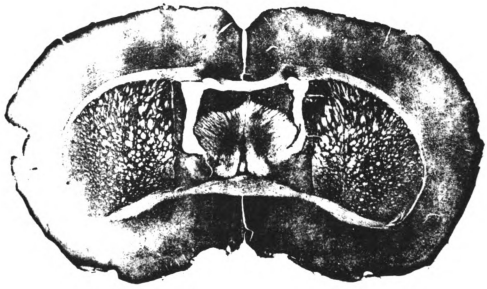


Table 7. Effects of left intra-striatal injection of 8 µg 6-hydroxydopamine on forebrain amine concentrations.

Amine (µg/gm)	Left	Right
Dopamine	0.43 ± 0.05*	0.77 ± 0.05
Norepinephrine	0.16 ± 0.01*	0.21 ± 0.02
5-Hydroxytryptamine	0.56 ± 0.01	0.60 ± 0.01

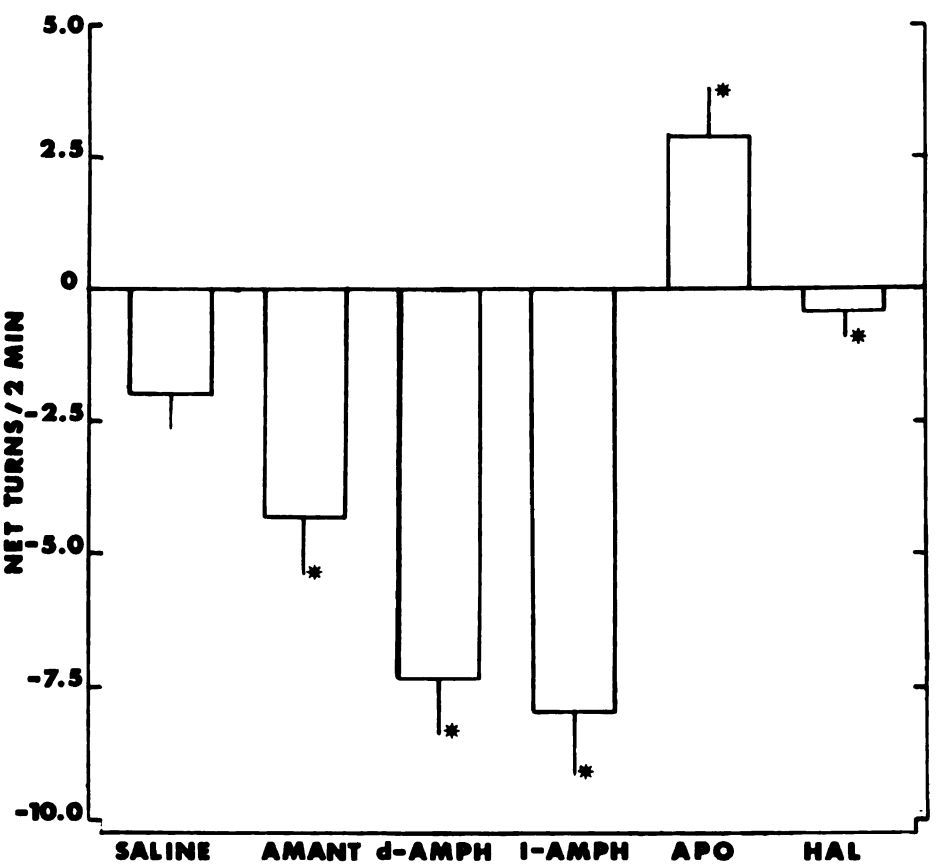
The mice were sacrificed 10 days or more after the injection. Left and right forebrains were grouped by fours for amine analysis.

*Left forebrain concentration significantly less than right forebrain concentration ($P < .05$). n=4-8.

Figure 36. Effects of various drugs upon the turning of mice with left striatal 6-hydroxydopamine (8 µg) lesions.

Ten days or more after the lesions were made, mice were injected with saline, amantadine (AMANT, 40 mg/kg), d-amphetamine (d-AMPH, 1 mg/kg), l-amphetamine (l-AMPH, 2.5 mg/kg), apomorphine (APO, 6 mg/kg) or haloperidol (HAL, 0.25 mg/kg) and observed 30 minutes later. Each bar represents the mean of the scores of 16 mice; the vertical lines denote 1 standard error. In this and subsequent figures describing mouse turning behavior, positive and negative numbers indicate net turning to the right and left respectively. All statistical comparisons were made by the Student's t test for grouped data.

*Significantly different than saline controls ($P < .05$).


5.0
2.5
0
-2.5
-5.0
-7.5
-10.0
NET TURNS/2 MIN
SALINE
AMANT
d-AMPH
l-AMPH
APO
HAL

the mice to reverse to contralateral turning. Haloperidol markedly reduced ipsilateral turning.

Since not all of the mice injected with 8 µg 6-hydroxydopamine displayed ipsilateral turning and the induced reductions in dopamine concentrations were less than 50% it was reasoned that an increase in the dose of 6-hydroxydopamine was indicated. Table 8 presents the effects of the intra-striatal injection of 16 µg 6-hydroxydopamine upon forebrain amines. This dose resulted in a marked reduction in dopamine concentration accompanied by a modest decrease in norepinephrine; 5-hydroxytryptamine concentrations were unaltered. Observation of mice with lesions induced by 16 µg 6-hydroxydopamine (n=88) revealed that 88% of these mice displayed net ipsilateral turning. Thus, there appeared to be a correlation between reduced dopamine concentrations and ipsilateral turning; when the dose of 6-hydroxydopamine was increased both the magnitude of dopamine reduction and the percentage of mice turning ipsilaterally increased.

To examine the possible correlation between unilateral reduction of forebrain dopamine and ipsilateral turning, mice were injected in the left or right striatum with 6-hydroxydopamine. Ten days later, these mice plus a group of untreated animals were observed and grouped by fours according to their turning scores. They were then sacrificed by groups and the hemi-forebrains analyzed for dopamine. There was a significant correlation between the unilateral

Table 8. Effects of left intra-striatal injection of 16 µg 6-hydroxydopamine on forebrain amine concentrations.

Amine (µg/gm)	Left	Right
Dopamine	0.14 ± 0.02*	0.87 ± 0.08
Norepinephrine	0.15 ± 0.01*	0.21 ± 0.02
5-Hydroxytryptamine	0.55 ± 0.01	0.53 ± 0.03

The mice were sacrificed 10 days or more after the injection. Left and right forebrains were grouped by fours for amine analysis.

*Left forebrain concentration significantly less than right forebrain concentration ($P < .05$). n=4-12.

loss of dopamine and ipsilateral turning (Figure 37). Mice with reduced dopamine concentrations in the right striatum turned to the right whereas mice with reduced dopamine concentrations in the left striatum turned to the left. Untreated mice displayed neither significant net turns (-0.4 ± 0.8/2 min) nor significant differences in hemi-forebrain dopamine concentrations (left-right, -0.01 ± 0.05 µg/gm).

Since a high percentage of the mice with lesions induced by 16 µg 6-hydroxydopamine manifested ipsilateral control turning, it was not necessary to select animals for further drug studies. Dose-response curves for the effect of several agents upon the turning of mice with left striatal lesions are illustrated in Figure 38. Amantadine, d-amphetamine and l-amphetamine all increased the rate of ipsilateral turning in a dose-dependent fashion; apomorphine and L-dopa caused significant contralateral turning at doses of 0.06 mg/kg and 10 mg/kg respectively.

A third drug, ET-495 also elicited contralateral turning. The time courses of contralateral turning induced by this compound is compared to those induced by L-dopa and apomorphine in Figure 39. Two minutes after ET-495 the mice exhibited a dose-dependent contralateral turning response. Thirty minutes after injection animals receiving the highest dose appeared quite depressed and failed to display net contralateral turns. Nevertheless, this dose

Figure 37. Correlation between unilateral dopamine loss and ipsilateral turning.

Control mice (O) and mice with 6-hydroxydopamine lesions in the left (□) or right (Δ) corpus striatum (10 days before) were observed for 2 minutes and their net turns recorded. The mice were grouped by fours according to their net turning scores. Those with the most positive scores were grouped together and those with the most negative scores were grouped together. The left and right forebrain dopamine concentrations were determined for the grouped mice. Each point on the graph represents the mean net turn score of 4 mice and the difference in their pooled right and left forebrain dopamine concentrations.

*Correlation is statistically significant ($P < .05$).

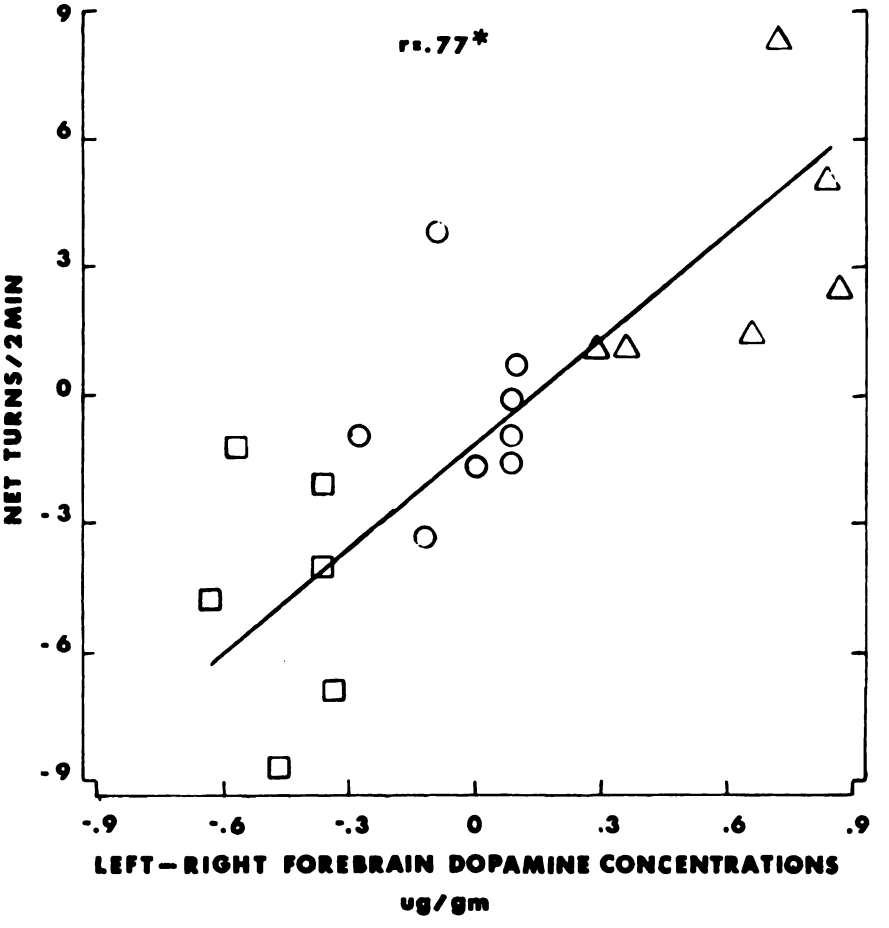
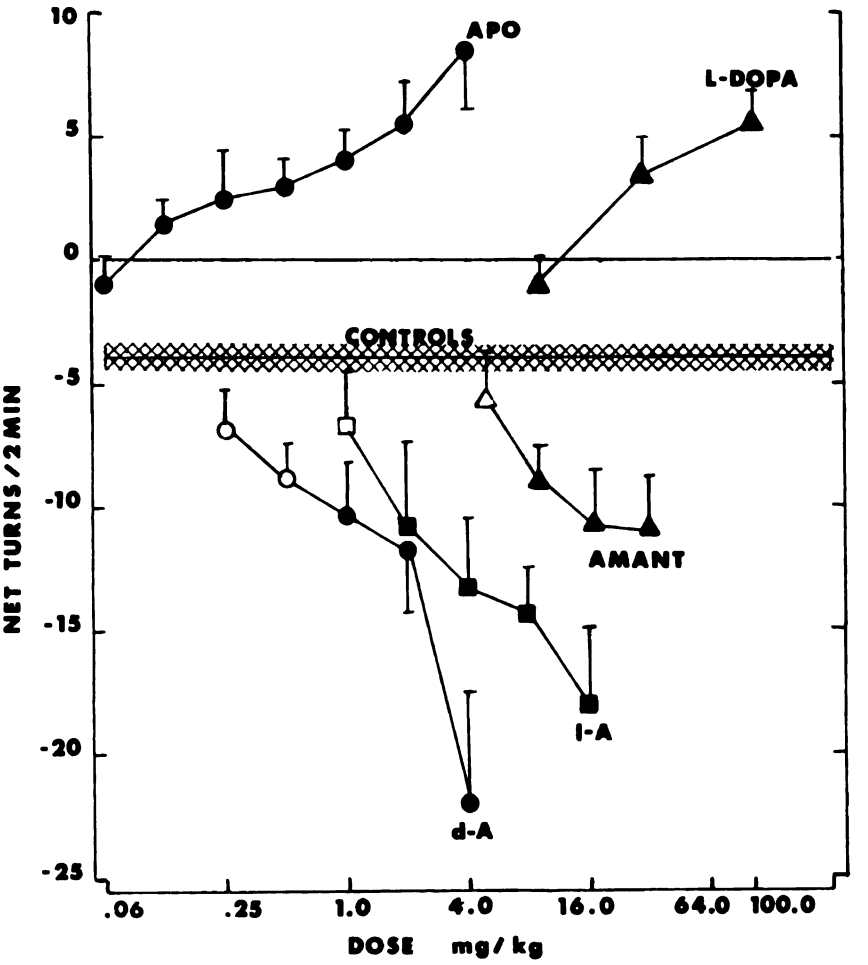
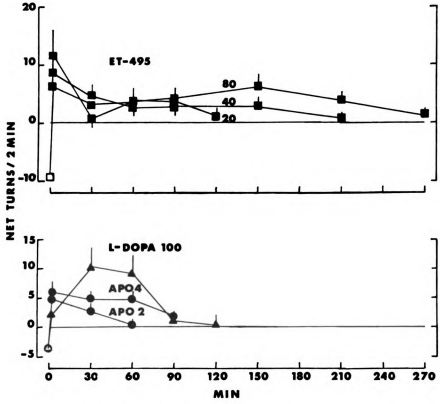

r=.77*
9
6
3
0
-3
-6
-9
NET TURNS/2MIN
-.9
-.6
-.3
0
.3
.6
.9
LEFT-RIGHT FOREBRAIN DOPAMINE CONCENTRATIONS
ug/gm

Figure 38. Effects of various drugs upon the turning of mice with left striatal 6-hydroxydopamine (16 µg) lesions.

Ten days or more after the lesions were made, mice were injected with the indicated doses of d-amphetamine (d-A), l-amphetamine (l-A), amantadine (AMANT), apomorphine (APO) or L-dopa and observed 30 minutes later. The horizontal line and crosshatching represent the mean and standard error of the pooled controls (n=88). Each symbol represents the mean of the scores of at least 10 mice and the vertical lines denote 1 standard error. Solid symbols are significantly different than the controls ($P < .05$).


10
5
0
-5
-10
-15
-20
-25
NET TURNS / 2 MIN
APO
L-DOPA
CONTROLS
AMANT
l-A
d-A
.06
.25
1.0
4.0
16.0
64.0
100.0
DOSE mg / kg


20
10
0
-10
ET-495
80
40
20
NET TURNS/2 MIN
15
10
5
0
-5
L-DOPA 100
APO 4
APO 2
0
30
60
90
120
150
180
210
240
270
MIN

produced the longest duration of action, which was approximately 3 times that of an approximately equieffective dose of L-dopa. There was a definite latent period before L-dopa produced a maximal effect. On the other hand, apomorphine, like ET-495, had a rapid onset but a short duration of action.

Amphetamine and amantadine, both of which increased ipsilateral turning, are psychomotor stimulants. The selectivity of this turning response was investigated by examining the effects of several other psychomotor stimulants. Pipradrol, amfonelic acid, methylphenidate and caffeine all significantly increased ipsilateral turning in mice with left unilateral 6-hydroxydopamine lesions (Figure 40). The following drugs, however, failed to significantly increase ipsilateral or contralateral turning 30 minutes after injection: morphine sulfate (1, 10 or 100 mg/kg, n=10), clonidine (0.1, 1 or 10 mg/kg, n=10), 3-methoxytyrosine (100 mg/kg, n=10, turning measured at 2, 30, 60, 120 min after injection), dopamine (100 mg/kg, n=10, turning measured at 5 and 30 min after injection) and L-5-hydroxytryptophan-methyl ester (200 mg/kg, turning measured at 2, 30 and 60 min after injection).

Blockade of a drug-induced response by α-methyltyrosine suggests the involvement of ongoing catecholamine synthesis for the response. The data in Figure 41 indicate that α-methyltyrosine blocked

Figure 39. Time courses of the contralateral turning response of mice with left striatal 6-hydroxydopamine lesions to ET-495, L-dopa and apomorphine.

Ten days or more after the injection of 6-hydroxydopamine, mice were observed, injected with ET-495 (20, 40 or 80 mg/kg), L-dopa (100 mg/kg) or apomorphine (APO, 2 or 4 mg/kg) and observed at 2 minutes and at 30 or 60 minute intervals thereafter. Each point is the mean of the score of 10 mice; the vertical lines denote 1 standard error. Solid points differ significantly ($P < .05$) from control.

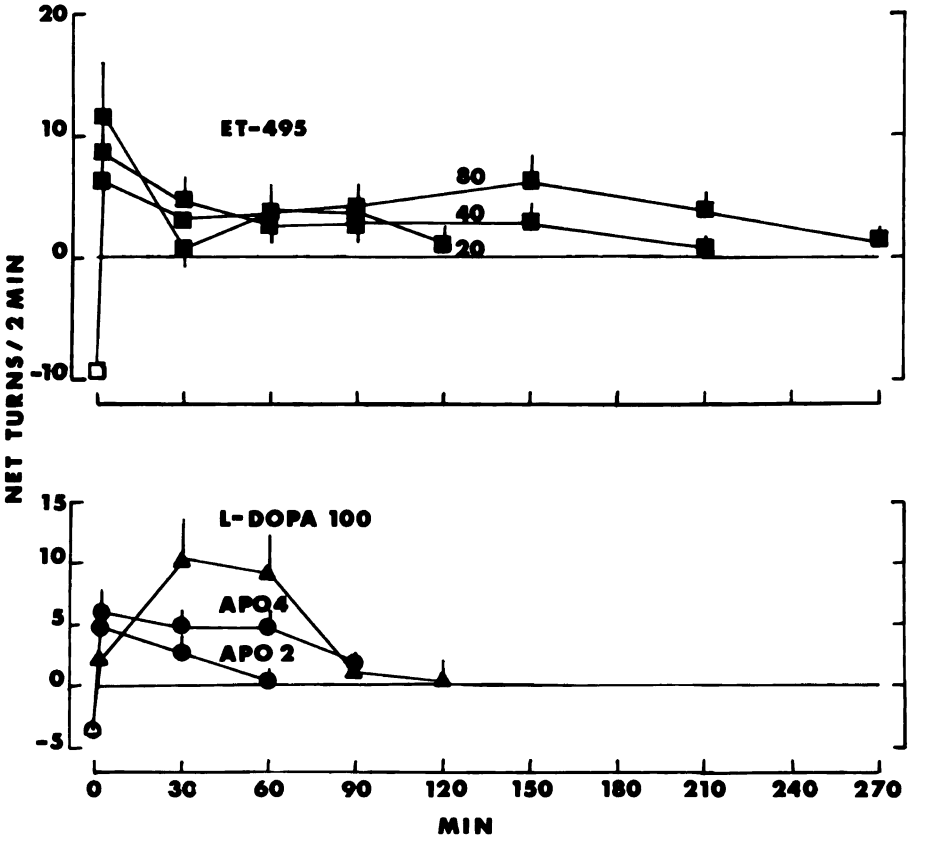

20
10
0
-10
ET-495
80
40
20
NET TURNS / 2 MIN
15
10
5
0
-5
L-DOPA 100
APO4
APO 2
0
30
60
90
120
150
180
210
240
270
MIN

Figure 40. Effect of various psychomotor stimulants on turning of mice with left striatal 6-hydroxydopamine lesions.

Ten days or more after the lesions were made, the mice were injected with saline, pipradrol (PIP), amfonelic acid (NCA), methylphenidate (METHYLPHEN), caffeine (CAFF) or d-amphetamine (d-AMPH) and observed 30 minutes later. Each bar represents the mean score of at least 10 mice and the vertical lines denote 1 standard error.

*Significantly different from control ($P < .05$).

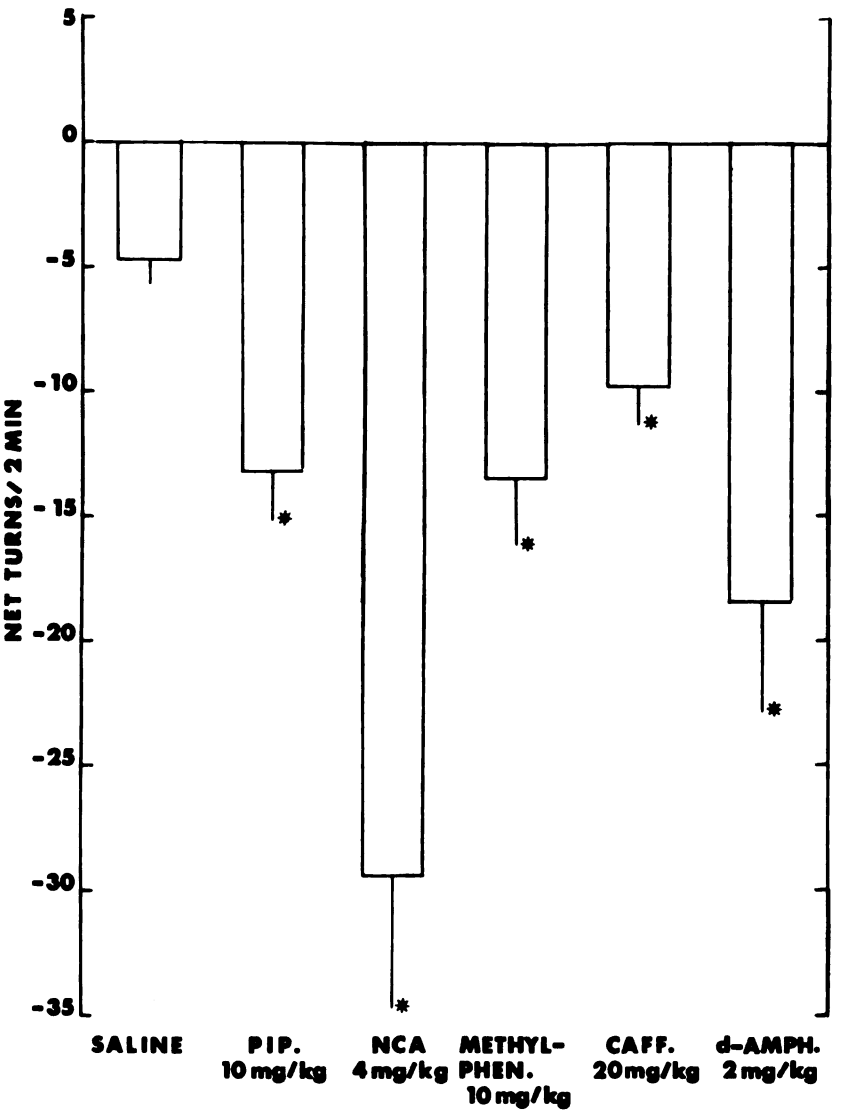
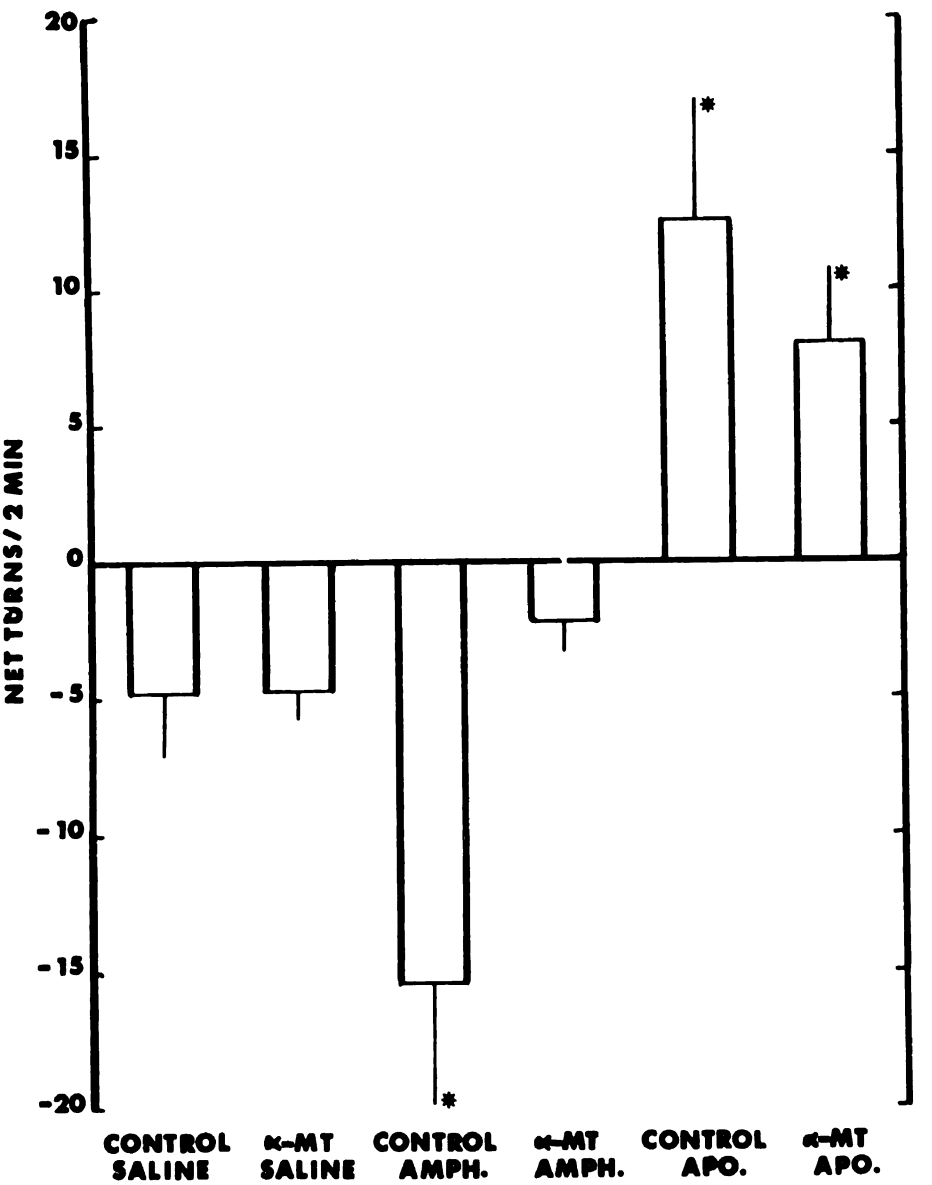

5
0
-5
-10
-15
-20
-25
-30
-35
NET TURNS/2 MIN
SALINE
PIP. 10 mg/kg
NCA 4 mg/kg
METHYL-PHEN. 10 mg/kg
CAFF. 20 mg/kg
d-AMPH. 2 mg/kg

Figure 41. Effect of α-methyltyrosine upon turning evoked by d-amphetamine and apomorphine in mice with left striatal 6-hydroxydopamine lesions.

Ten days after the lesions were made, the mice were fed either a control diet or a diet containing 0.4% α-methyltyrosine for 4 hours. Thirty minutes later they were injected with saline, d-amphetamine (AMPH, 2 mg/kg) or apomorphine (APO, 2 mg/kg) and observed 30 minutes later. Each bar represents the mean turning score of 10 mice and the vertical lines denote 1 standard error.

*Significantly different from control saline group ($P < .05$).


20
15
10
5
0
-5
-10
-15
-20
NET TURNS/2 MIN
*
*
*
CONTROL SALINE
α-MT SALINE
CONTROL AMPH.
α-MT AMPH.
CONTROL APO.
α-MT APO.

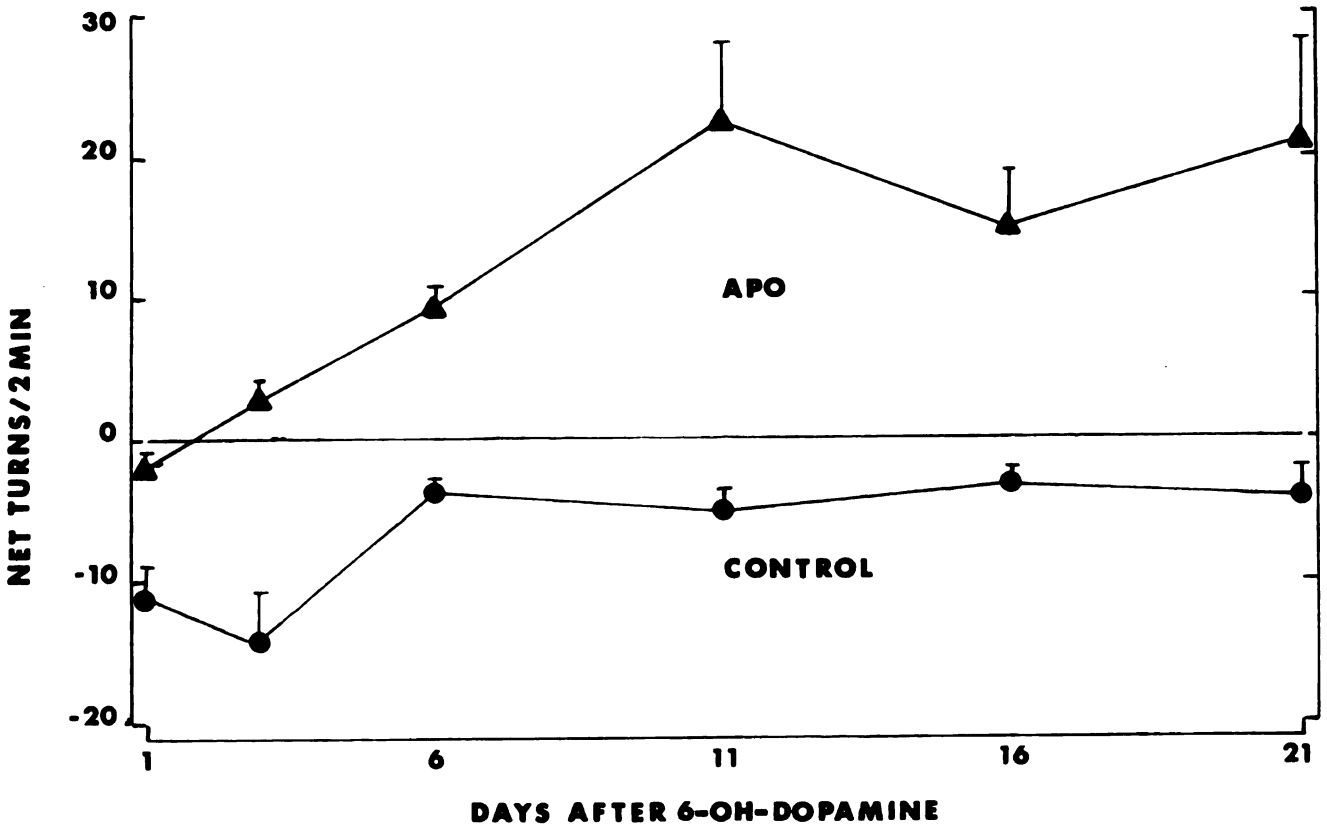
the ipsilateral turning in response to d-amphetamine whereas the contralateral turning induced by apomorphine was not significantly ($P < .05$) altered. Thus, d-amphetamine depends upon synthesis of catecholamines to elicit a turning response while apomorphine does not.

Because the contralateral response was independent of catecholamine synthesis the mechanism of this effect was studied further. Figure 42 shows the time course of the development of the apomorphine-induced contralateral turning and compares this time course to that of the ipsilateral control turning after left striatal 6-hydroxydopamine lesions. The response to apomorphine developed over the course of several days, reaching a maximum at day 11 and remaining fairly stable through day 21. During the first 6 days after lesioning the control turning decreased markedly and remained stable thereafter. Thus, during the same time period that the mice appeared to be partially compensating for the lesion they were developing a contralateral turning response to apomorphine.

The contralateral turning response induced by apomorphine may result from a postsynaptic supersensitivity to dopamine agonists in the dopaminergically denervated striatum (Ungerstedt, 1971[a]). If this supersensitivity is induced by the loss of dopamine from the striatum, it should be possible to suppress it by maintaining dopamine concentrations in this region. Specifically, chronic treatment of

Figure 42. Time course of the effect of left striatal 6-hydroxydopamine lesions upon control turning and turning evoked by apomorphine.

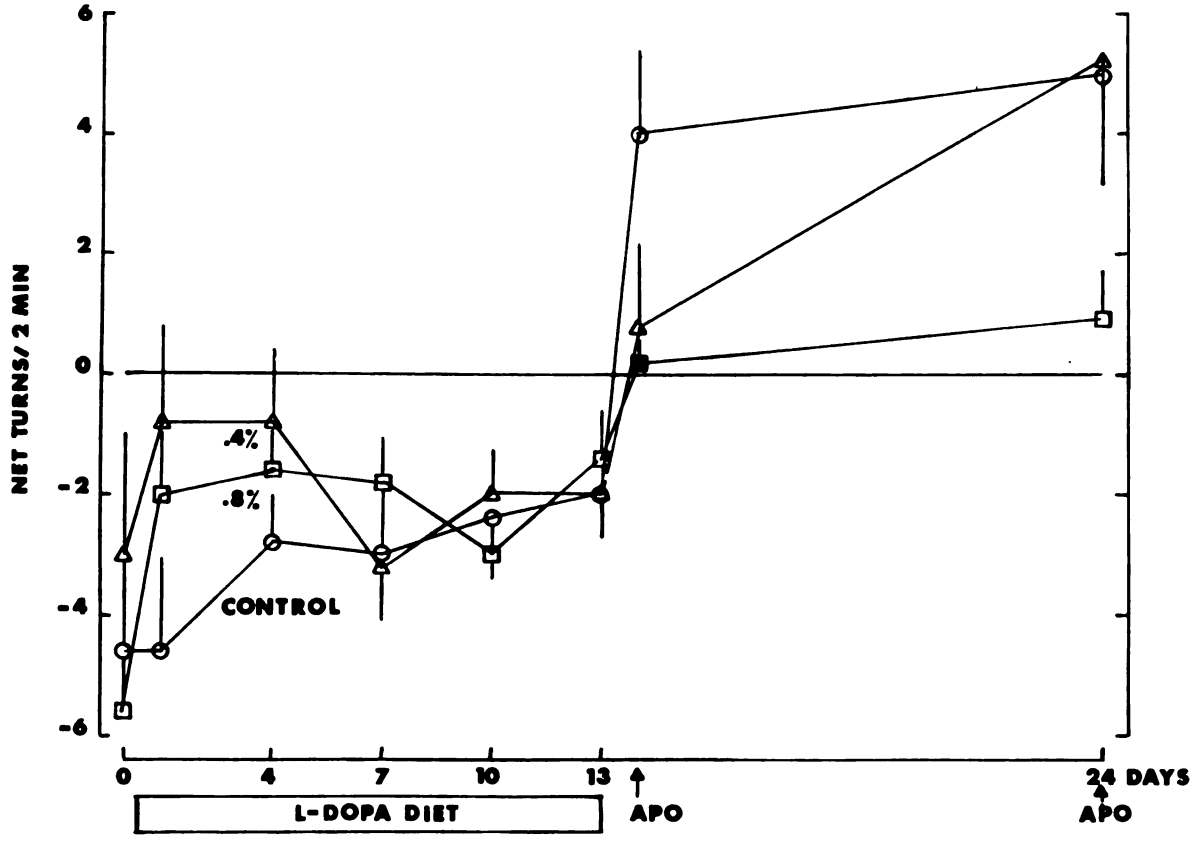
Different groups of mice were lesioned and observed at various time intervals thereafter, first as controls, then 30 minutes after the injection of apomorphine (APO, 6 mg/kg). Each point represents the scores of at least 10 mice and the vertical lines denote 1 standard error.


30
20
10
0
-10
-20
APO
CONTROL
1
6
11
16
21
NET TURNS/2MIN
DAYS AFTER 6-OH-DOPAMINE

mice with L-dopa might be expected to decrease the response to apomorphine if the loss of dopamine is the factor which stimulates and maintains supersensitivity. Figure 43 illustrates experiments designed to test this hypothesis. Commencing ten days after left intra-striatal injection of 16 µg 6-hydroxydopamine mice were either maintained on a control diet or fed a diet containing 0.4% or 0.8% L-dopa for 13 days and then switched back to control diet. Immediately before they were placed on the L-dopa or control diets (day 0) and at 3 day intervals while on the diets, the mice were observed for turning behavior. Then 24 hours after the termination of the diet (day 14) the mice were injected with apomorphine and observed 30 minutes later. Apomorphine injection and observation were repeated on day 24. Surprisingly, while on the L-dopa diets, the mice failed to display contralateral turning. This was despite daily intake of approximately 600 and 1200 mg/kg of drug for those on the 0.4% and 0.8% diets respectively. However, when challenged with apomorphine after receiving the 0.8% diet for 14 days, the mice displayed significantly less contralateral turning then did the control animals. This trend was also apparent, although not statistically significant, in the mice that received the 0.4% L-dopa diet. For neither of the L-dopa diets did the response to apomorphine differ from that of the control animals on the tenth day after terminating the drug diet. The depression of the

Figure 43. Effect of chronic L-dopa diets upon contralateral turning evoked by apomorphine in mice with left striatal 6-hydroxydopamine lesions.

Ten days after 6-hydroxydopamine injection the mice were placed on L-dopa (0.4% or 0.8%) or control diets for 13 days. On the 14th and 24th days the mice were injected with apomorphine (APO, 4 mg/kg). Turning was observed just before and at 3 day intervals during the chronic diet period as well as 30 minutes after apomorphine injection on days 14 and 24. Each symbol represents the mean of 10 animals, vertical lines denote 1 standard error. Solid symbol indicates statistical difference ($P < .05$) from control.


NET TURNS/ 2 MIN
6
4
2
0
-2
-4
-6
.4%
.8%
CONTROL
0
4
7
10
13
24 DAYS
L-DOPA DIET
APO
APO

response to apomorphine thus appears to be a transient effect associated with the L-dopa diet; after removal from the diet the mice appear to regain their responsiveness.

Several types of controls were used to determine the relationship of drug-induced turning in mice with 6-hydroxydopamine lesions to the loss of dopamine brought about by this agent. First of all, it was determined that mice receiving no intra-cerebral injections failed to display net control turning (see discussion of Figure 37). The next important question to answer was whether the turning response might be due to damage to the cerebral cortex induced by passage of the needle through this structure or by the back flux of 6-hydroxydopamine into it. To test this possibility injections of 6-hydroxydopamine were made directly into the cerebral cortex, 2 mm above the usual injection site in the corpus striatum. These cortical 6-hydroxydopamine lesions resulted in a modest decrease in ipsilateral forebrain dopamine concentrations (injected side, 0.54 $\pm$ 0.10 µg/gm; side opposite injection, 0.84 $\pm$ 0.09 µg/gm; n=5). The mice so lesioned, failed to display significant ($P < .05$) net turns when injected with saline, amantadine, d-amphetamine or apomorphine (Table 9). Thus, neither needle nor 6-hydroxydopamine induced damage to the cortex could account for the locomotor asymmetries of mice with unilateral striatal 6-hydroxydopamine lesions.

Table 9. Effects of various drugs on turning of mice with cerebral cortical 6-hydroxydopamine lesions.

Treatment	Net turns/2 min
Saline	0.1 ± 1.1
Amantadine 20 mg/kg	-0.6 ± 0.8
d-Amphetamine 4 mg/kg	-1.9 ± 1.5
Apomorphine 4 mg/kg	1.4 ± 0.5

Sixteen µg 6-hydroxydopamine was injected into the cerebral cortex above the corpus striatum. Ten days or more later and 30 minutes after drug injection the mice were observed for turning behavior. n=10.

A third group of controls consisted of mice which received left intra-striatal injections of 4 µl of the distilled water-ascorbate vehicle. Ten days or more after injection the mice were injected with various drugs and observed 30 minutes later (Table 10). These mice failed to display significant control turning. Furthermore, neither d-amphetamine, apomorphine, L-dopa nor L-5-hydroxytryptophan had any effect. Not only did these sham lesions fail to produce locomotor asymmetry, but they also failed to significantly alter forebrain amine concentrations (Table 11). To further investigate if any locomotor asymmetry might be induced by non-specific damage to the corpus striatum, left intra-striatal injections of 8 µl of 95% ethanol were used (Table 12). Ten days after striatal ethanol lesions the mice exhibited marked contralateral turning. Apomorphine, L-dopa and L-5-hydroxytryptophan failed to induce either further increases in contralateral turning or ipsilateral turning; rather the animals' contralateral turning was depressed. Treatment with d-amphetamine however, resulted in a switch from contralateral to ipsilateral turning. Examination of forebrain amine concentrations in these mice revealed marked ipsilateral reductions of dopamine and 5-hydroxytryptophan with little effect upon norepinephrine (Table 13).

Since only mice with 6-hydroxydopamine lesions displayed contralateral turning in response to putative dopamine agonists

Table 10. Effects of various drugs on turning of mice with striatal sham lesions.

Treatment		Net turns/2 min
Control		-0.7 ± 1.3
d-Amphetamine	4 mg/kg	0.3 ± 2.7
Apomorphine	4 mg/kg	0.3 ± 0.3
L-dopa	100 mg/kg	1.0 ± 0.5
L-5-Hydroxy-tryptophan	200 mg/kg	0.7 ± 0.5

Four µl of distilled water containing 0.8 µg sodium ascorbate was injected into the left striatum of mice. Ten days or more later and 30 minutes after drug injection the mice were observed for turning behavior. n=10.

Table 11. Effects of left striatal sham injection on forebrain amine concentrations.

Amine (µg/gm)	Left	Right
Dopamine	0.77 ± 0.07	0.79 ± 0.06
5-Hydroxy-tryptamine	0.58 ± 0.02	0.56 ± 0.02

The mice were sacrificed 10 days or more after the injection of 4 µl of distilled water containing 0.8 µg sodium ascorbate. Left and right forebrains were grouped by fours for amine analysis. n=4.

Table 12. Effects of various drugs on turning of mice with striatal ethanol lesions.

Treatment		Net turns/2 min
Control		7.2 ± 2.1
d-Amphetamine	4 mg/kg	-8.0 ± 2.0*
Apomorphine	4 mg/kg	0.6 ± 0.5*
L-Dopa	100 mg/kg	0.6 ± 0.6*
L-5-Hydroxy-tryptophan	200 mg/kg	1.3 ± 0.9*

Eight µl of 95% ethanol was injected into the left striatum. Ten days or more later and 30 minutes after drug injection the mice were observed for turning behavior.

*Differ significantly from controls ($P < .05$), n=10.

Table 13. Effects of left striatal injection of 8 µl ethanol on forebrain amine concentrations.

Amine (µg/gm)	Left	Right
Dopamine	0.26 ± 0.05*	0.81 ± 0.01
5-Hydroxytryptamine	0.47 ± 0.03*	0.55 ± 0.01
Norepinephrine	0.25 ± 0.03	0.27 ± 0.02

The mice were sacrificed 10 days or more after the injection. Left and right forebrains were grouped by fours for analysis.

*Left forebrain concentration significantly less than right forebrain concentration ($P < .05$), n=4.

it was of interest to determine if specific destruction of other types of neurons in the striatum would result in locomotor asymmetries. Baumgarten and coworkers (1972) have reported that 5,6-dihydroxytryptamine causes a long-lasting depletion of 5-hydroxytryptamine from various brain regions. The data in Table 14 illustrates that mice with left striatal lesions induced by injection of 30 µg 5,6-dihydroxytryptamine, like mice with non-specific striatal lesions, display contralateral control turning which is significantly reversed by d-amphetamine. L-Dopa, apomorphine and ET-495 depressed contralateral turning and decreased overall activity. 5-Hydroxytryptophan was without effect. 5,6-Dihydroxytryptamine reduced both dopamine and 5-hydroxytryptamine on the side of the injection (Table 15). Gross examination of the brains 14 days after injection of 5,6-dihydroxytryptamine revealed considerable tissue disruption along the needle tract and a brown staining through the ipsilateral striatum. These observations suggest that 5,6-dihydroxytryptamine may not be as specific an amine depletor as 6-hydroxydopamine.

Table 14. Effects of various drugs on turning of mice with striatal 5,6-dihydroxytryptamine lesions.

Treatment		Net turns/2 min
Control		5.1 ± 1.5
d-Amphetamine	4 mg/kg	-5.0 ± 2.3*
Apomorphine	4 mg/kg	0.2 ± 0.7*
L-Dopa	100 mg/kg	1.1 ± 0.5*
ET-495	40 mg/kg	0.3 ± 0.4*
L-5-Hydroxy-tryptophan	200 mg/kg	3.6 ± 1.5

Thirty µg of 5,6-dihydroxytryptamine were injected into the left striatum. Ten days or more later and 30 minutes after drug injection the mice were observed for turning behavior.

*Differ significantly from controls ($P < .05$), n=10.

Table 15. Effects of left intra-striatal injection of 30 µg 5,6-dihydroxytryptamine on forebrain amine concentrations.

Amine (µg/gm)	Left	Right
Dopamine	0.26 ± 0.07*	0.76 ± 0.02
5-Hydroxytryptamine	0.37 ± 0.04*	0.52 ± 0.04

The mice were sacrificed 10 days or more after the injection. Left and right forebrains were grouped by fours for amine analysis.

*Left forebrain concentration significantly less than right forebrain concentration ($P < .05$), n=4.

DISCUSSION

The interpretation of the results of these cerebroventricular perfusion experiments is dependent upon a knowledge of the anatomical and cellular distribution of the injected H^3-dopamine. Earlier studies (Carr and Moore, 1969) demonstrated that H^3-catecholamines, when injected into the anterior lateral ventricle of the cat in a volume of 10 µl, distribute primarily to the ipsilateral caudate nucleus. Lesser amounts of radioactivity were localized in the hypothalamus while essentially no radioactivity was contained in the contralateral caudate nucleus. Subcellular distribution studies (Green et al., 1969) have indicated that the ventricularly injected H^3-catecholamines assume a similar cellular and subcellular distribution as the endogenous amines. Thus, it would appear likely that the tracer amounts of H^3-dopamine used in these experiments localize primarily to the dopaminergic nerve terminals in the caudate nucleus. This possibility is supported by data showing that in cats with chronic nigro-striatal lesions the caudate nucleus ipsilateral to the lesion suffered a parallel decline in endogenous dopamine concentration and H^3-dopamine uptake. These effects were observed without signs of generalized damage as might be indicated by decreases

in tissue weight or 5-hydroxytryptamine concentrations. This correlation between dopamine concentration and H^3-dopamine uptake suggests a similar site of localization in the tissue for the endogenous amine and the tracer. However, the data from three different types of perfusion experiments indicate that the major portion of the resting efflux of H^3-dopamine may not arise from this site. When chronic lesions of the nigro-striatal fibers were made, H^3-dopamine injected and the ventricles subsequently perfused, the resting efflux of H^3-dopamine during the control collection periods was only moderately (39%) decreased as compared to the efflux from the control non-lesioned side. Similarly, when acute lesions of the nigro-striatal fibers were made, the concurrent decrease in H^3-dopamine efflux amounted to 21% as compared to that effluxing during the control periods. Lastly, when the push-pull cannula was used to label and perfuse the corpus callosum, an area devoid of dopaminergic nerve terminals, a steady resting efflux of H^3-dopamine into the perfusates was observed. However, in each of these three series of experiments the release of H^3-dopamine into the perfusates by d-amphetamine was either abolished or greatly reduced. Thus, it appears that while H^3-dopamine is localized primarily in the dopaminergic nerves, small amounts are also present in the non-dopaminergic areas lining the ventricles; the drug-induced H^3-dopamine release arises from the former pool whereas the majority of the resting efflux appears to originate from the

latter.

The release of H^3-dopamine induced by electrical stimulation of the diencephalon appears to originate specifically from dopaminergic nerve terminals. The most effective electrode sites for stimulation-induced release of H^3-dopamine coincided with the established location of the nigro-striatal pathway (Moore et al., 1971). Furthermore, electrolytic lesions at these sites resulted in a marked depletion of dopamine from the caudate nucleus without significantly altering 5-hydroxytryptamine or norepinephrine concentrations. It appears unlikely that the release of H^3-dopamine induced by electrical stimulation of the nigro-striatal pathway originates from the wall of the third ventricle since placement of the stimulating electrode 1 mm closer to the ventricle resulted in a loss of this response.

The magnitude of the release of H^3-dopamine in response to nigro-striatal fiber stimulation is considerably greater than that induced by stimulation of either the substantia nigra or the caudate nucleus. The reason for this is not entirely clear although several possibilities exist. The fibers of the nigro-striatal pathway constitute a more compact structure than either the caudate nucleus or the substantia nigra so that stimulation of the former structure depolarized a greater number of neurons and caused a greater efflux of H^3-dopamine. A second

possibility is that the placement of the electrode in the substantia nigra may not have been optimal for stimulating cells, the terminals of which are present on the ventricular surface of the caudate nucleus. Riddell and Szerb (1971) have suggested that stimulation of the more rostral substantia nigra (A 5.5) is more effective in releasing dopamine than stimulation of the more caudal areas as was performed in these experiments. A further possibility that might explain not only the relatively small release of H^3-dopamine induced by stimulation of the substantia nigra but also that induced by stimulation of the caudate nucleus is that stimulation of these areas may have activated non-dopaminergic nigro-striatal systems which may inhibit dopamine release by a presynaptic mechanism. In light of this hypothesis, the results of Bartholini and Pletscher (1971) are particularly relevant. These workers demonstrated that intra-ventricular administration of anticholinergic agents led to an increase in homovanillic acid concentrations in the striatum. If a cholinergic input to the dopaminergic terminals exerted a tonic suppression of dopamine release, then anticholinergic drugs might block this suppression producing an increased dopamine release and subsequent metabolism of the amine. Behavioral studies (Fuxe et al., 1970) demonstrating that anticholinergic drugs may potentiate the effects of dopamine agonists also support this hypothesis, as does the observation that d-tubocurarine potentiates

C^{14}-dopamine release in response to stimulation of the caudate nucleus (McKenzie and Gordon, 1972). Further research, perhaps utilizing some of the techniques described here, may prove useful in the investigation of the mechanism by which the dopaminergic nigro-striatal system may be modulated by cholinergic inhibition. For example, if anticholinergic drugs block the tonic inhibition of dopamine release, then the perfusion of the caudate nucleus with solutions containing these agents or cholinergic agonists would be expected to increase and to decrease dopamine release respectively. Such effects should also be altered by acute lesions of the nigro-striatal fibers.

Early studies with amphetamine (McLean and McCartney, 1961; Stein, 1964) indicated that this drug might interact with brain catecholamines. This contention has been further supported by studies demonstrating that some of the behavioral effects of amphetamine can be blocked by the inhibition of dopamine and norepinephrine synthesis in animals (Weissman et al., 1966) and man (Jönsson, 1969). By process of elimination, recent reports have focused attention upon dopamine in particular; selective inhibition of norepinephrine synthesis does not block some of the behavioral effects of amphetamine and related compounds (Thornburg, 1972). It has also been suggested that amantadine may act through dopaminergic mechanisms (Grelak et al., 1970). Indeed, this compound has been shown to block dopamine uptake into striatal

tissue *in vitro* (Thornburg and Moore, 1971; Baldessarini *et al.*, 1972). However, as was the case with amphetamine (Glowinski and Axelrod, 1965), it was not clear whether the *in vivo* effects of this compound on dopamine disposition were due to a direct release of dopamine (Grelak *et al.*, 1970) or an indirect mechanism such as the blockade of reuptake of released dopamine. The data reported here do not support the former hypothesis; if amantadine and amphetamine act to release dopamine directly, this effect should be independent of the rate of firing of the dopaminergic neurons; it is not. In fact, acute sectioning of the nigro-striatal fibers blocked the action of both compounds and electrical stimulation of the fibers potentiated amantadine-and *d*-amphetamine-induced dopamine efflux. The latter results appear in agreement with *in vitro* studies (Farnebo *et al.*, 1971; Farnebo, 1971) in which these compounds increased the release of H^3-catecholamines from electrically stimulated brain slices. Since amantadine and amphetamine rely upon the activity of the nigro-striatal fibers for their action, it is possible that they may act to facilitate dopamine release by one of at least two mechanisms. α-Adrenergic blocking agents have been shown to increase the stimulation-induced release of both norepinephrine and dopamine-β-hydroxylase from spleen (DePotter *et al.*, 1971). Farnebo and Hamberger (1971) also claim that phentolamine and phenoxybenzamine at doses too low

to block reuptake may increase the release of norepinephrine from isolated irises in response to electrical field stimulation. d-Amphetamine and amantadine, however, are not generally thought of as having α-adrenergic blocking properties. An alternative explanation is that these drugs might facilitate dopamine release by making the amine more available for release. Besson et al. (1971) have suggested that amphetamine might act to increase cytoplasmic dopamine concentrations, whether this would lead to a greater release of dopamine per nerve impulse is not clear. Before such questions may be answered, the mechanism whereby neurogenic release of dopamine occurs must be elucidated. The data presented in this study offer no grounds on which to reject the hypothesis that amantadine and amphetamine facilitate neurogenic dopamine release. But, based upon in vitro studies demonstrating that these compounds do block dopamine transport into striatal tissue (Thornburg and Moore, 1971; Coyle and Snyder, 1969), the simplest interpretation of these data is that amantadine and d-amphetamine act primarily to block the reuptake of neurogenically released dopamine.

The results with tyramine perfusion indicate that if amantadine and amphetamine did act primarily by directly releasing dopamine, such a release would be observable in this system. Tyramine increased H^3-dopamine release regardless of the firing of the dopaminergic pathway, suggesting a direct

release mechanism. The terminals of this projection were, however, the source of the released H^3-dopamine as indicated by the chronic lesion studies. Thus, tyramine appears to directly release dopamine from the terminals of the nigro-striatal pathway whereas amphetamine does not. This apparent difference between the mechanisms of tyramine and amphetamine on striatal dopamine release may be related to the differential uptake of the compounds. Baldessarini and Vogt (1971) reported that while tyramine is actively taken up into striatal tissue, d-amphetamine is not. Therefore, tyramine may be actively taken into the terminals and displace dopamine independently of the neurogenic release mechanism. The latter postulate is supported by *in vitro* studies demonstrating that tyramine may release H^3-norepinephrine independently of the calcium dependent release mechanism (Thoenen *et al.*, 1969).

The studies involving acute lesions of the nigro-striatal fibers also provide us with some other interesting observations. Coincident with the lesion there is a 21% decrease in H^3-dopamine release. This might be taken to indicate that 21% of the H^3-dopamine in control perfusates was the result of neurogenic release from dopaminergic terminals. Although it is possible that the induced increases in endogenous striatal dopamine and consequent reduction in H^3-dopamine specific activity was the cause of the reduction in H^3-dopamine efflux, the data from the drug-induced release

studies do not support this hypothesis. For example, the releasing effect of tyramine was not reduced by the acute lesion, indicating that the decrease in H^3-dopamine specific activity is probably not sufficient to decrease the amount of H^3-dopamine appearing in the perfusate. Furthermore, the effects of amantadine and amphetamine were blocked by acute lesions, which correlates well with the idea of a decreased neurogenic release induced by the lesion.

An increase in endogenous dopamine concentrations after acutely lesioning the nigro-striatal fibers has also been reported by other workers (Faull and Laverty, 1969; Andén et al., 1971[h]; Nyback, 1972). These observations appear to be in opposition to the concept that striatal dopamine concentrations are strictly controlled by dopamine synthesis. Rather, it appears that the steady state dopamine concentrations are shifted to a lower than maximal level by the rapid rate of release of the amine. When acute lesions of the nigro-striatal axons are made, dopamine release is markedly reduced and the amine concentration increases until feedback inhibition of tyrosine hydroxylase halts it at a new, higher steady state concentration.

Although d-amphetamine was not compared to the l-isomer in terms of mechanism, dose-response relationships for both drugs were studied. The data from this comparison appears to be at variance with the in vitro study of Snyder and Coyle (1969[h]) who reported that the 2 isomers were approximately

equipotent in their ability to block dopamine uptake into striatal homogenate preparations. The results of the present study indicate that d-amphetamine is 3 to 4 times as potent as the l-isomer in increasing H^3-dopamine efflux. This result is in good agreement with a recent report that d-amphetamine was 3 to 4 times as potent as l-amphetamine in blocking the accumulation of H^3-dopamine into striatal crude synaptosomes (Ferris and Maxwell, 1972). Similar results (d-isomer 5 times as potent as l) have been obtained by Thornburg (personal communication). Further evidence for a stereoselective uptake system for amines in the striatum is provided by a recent study (Horn and Snyder, 1972) showing a 4 fold difference in the ability of d-tranylcypromine and l-tranylcypromine to block dopamine uptake into striatal preparations.

Neuroleptic drugs of the phenothiazine and butyrophenone classes have been shown to alter brain dopamine metabolism. An increased homovanillic acid concentration has been noted in the brains of cats, monkeys and rodents after treatment with these compounds (O'Keefe et al., 1970; Fuentes and Del Rio, 1972). After inhibition of monoamine oxidase this increase in dopamine metabolism is manifested by increased brain concentrations of 3-methoxytyramine (Scheel-Krüger, 1972). Since increased dopamine metabolism induced by neuroleptic agents is not accompanied by corresponding decreases in brain dopamine concentrations (O'Keefe et al., 1970),

increased synthesis of dopamine must also occur. This effect has been thoroughly documented (Persson, 1970). Thus, neuroleptic agents may be said to increase dopamine turnover. Andén and coworkers (1971[b]) have suggested that the blockade of dopamine receptors is causally related to the neuroleptic-induced increase in dopamine turnover. Indeed, York (1972) has demonstrated a reasonably selective blockade of the neuronal responses to microiontophoretically applied dopamine by prior application of chlorpromazine. Thus, neuroleptic agents may block dopamine receptors and thereby cause an increased firing of the nigro-striatal pathway, leading to an increased release and metabolism of dopamine. This hypothesis is supported by experiments demonstrating that acute lesions of the nigro-striatal pathway block the increased depletion of dopamine after synthesis inhibition (Andén et al., 1971[b]) as well as the increased synthesis of C^{14}-dopamine (Nybäck and Sedvall, 1971) induced by haloperidol and chlorpromazine. The results reported here indicate that ventricular perfusion of 10^{-3} M fluphenazine or 10^{-4} M haloperidol dissolved in 10^{-3} M citrate moderately increase H^{3}-dopamine efflux. However, neither perfusion with 10^{-5} M haloperidol nor intravenous administration of high doses of this compound were capable of altering this parameter. The results obtained with high concentrations of fluphenazine in the perfusion medium may represent a blockade of dopamine uptake by this compound

rather than an activation of the nigro-striatal neurons. Horn and coworkers (1971) have demonstrated that phenothiazine neuroleptics block dopamine uptake. However, haloperidol at concentrations of up to 10^{-4} M does not appear to block dopamine transport *in vitro* (Katz and Chase, 1971). In these experiments, haloperidol was dissolved in citric acid and then diluted with artificial cerebrospinal fluid to a concentration of 10^{-4} M haloperidol and 10^{-3} M citrate. Although perfusion of the ventricles with this solution increased dopamine efflux, 10^{-3} M citrate also elicited a similar effect, while 10^{-5} M haloperidol (with no citrate) was ineffective. Thus, haloperidol at concentrations of 10^{-5} M and 10^{-4} M appears to have no effect on H^3-dopamine release. The modest increase in H^3-dopamine efflux induced by citrate may be related to the sequestration of calcium. Thoenen *et al.* (1969) have reported that decreased calcium concentrations in the bathing media led to increased H^3-norepinephrine efflux from the iris *in vitro*. This may be due to an increased membrane permeability in the absence of calcium (Shanes, 1958).

The apparent failure of haloperidol to alter H^3-dopamine release from the cat brain is puzzling. On one hand, it is possible that this represents a true species difference since most experiments demonstrating neuroleptic-induced alterations in dopamine turnover have utilized the rat. However, O'Keefe *et al.* (1970) have shown that neuroleptics

do alter dopamine metabolism in the cat. Another alternative is that the present cerebroventricular perfusion technique, because of the high non-specific background efflux of H^3-dopamine, is not sensitive enough to measure the small physiological changes in dopamine release that may be elicited by receptor blockade. It is also possible that changes in dopamine turnover may not always be indicative of alterations in amine release. For example, neuroleptics may increase dopamine metabolism by making the amine more available to monoamine oxidase. This may lead to an increase in dopamine synthesis by disinhibition of tyrosine hydroxylase. Further experiments are necessary to answer these questions. One approach would be to utilize a different species for ventricular perfusion and to analyze the perfusates for endogenous dopamine by sensitive gas chromatograph-mass spectrometric techniques (Koslow et al., 1972). If in this experimental situation neuroleptics still failed to alter dopamine release, the relationship between increased dopamine turnover and increased release would have to be seriously questioned.

Central administration of 6-hydroxydopamine has been shown to result in the degeneration of noradrenergic and dopaminergic neurons (Breese and Traylor, 1970). This effect appears to be relatively specific as 5-hydroxytryptamine (Bloom et al., 1969; Ungerstedt, 1971[b]) and acetylcholine (Consolo et al., 1972) containing neurons are not affected.

The results of this study also indicate the specificity of the action of 6-hydroxydopamine since the direct injection of 6-hydroxydopamine into the striatum of mice markedly lowered forebrain dopamine concentrations without altering 5-hydroxytryptamine concentrations. Although no attempt was made in this study to determine the distribution of the injected 6-hydroxydopamine, Ungerstedt (1968) has demonstrated that when 12 µg of the salt are injected in a volume of 4 µl into the corpus striatum of the rat, loss of dopamine fluorescence is limited to an area approximately 2 mm in diameter. This corresponds roughly to the size of the mouse striatum. Considering the marked depletion of dopamine induced by the 16 µg 6-hydroxydopamine injections reported here, it seems likely that the entire striatum was affected. The microscopically visible damage, however, was limited to the needle tract itself.

The ipsilateral turning seen 10 days after unilateral 6-hydroxydopamine injection into the corpus striatum appears to be specifically related to the 6-hydroxydopamine-induced lesion in the striatum. Neither cortical 6-hydroxydopamine injections nor striatal ethanol or 5,6-dihydroxytryptamine injections elicited this effect. In fact, the 2 latter treatments resulted in contralateral turning. The increased ipsilateral turning seen after treatment with amphetamine may not be as specific, since this effect could be elicited in mice with non-specific lesions of the striatum induced by

ethanol or 5,6-dihydroxytryptamine injections. Furthermore, ipsilateral turning could be elicited by other psychomotor stimulants (methylphenidate and pipradrol) that alter catecholamine release as well as one (caffeine) that reportedly does not (Carr and Moore, 1970[b]). It is interesting to note that morphine, when given at doses that markedly stimulate locomotor activity in mice (Rethy et al., 1971) and alter dopamine metabolism (Fukui and Takagi, 1972), had no effect upon net turns of mice with unilateral 6-hydroxydopamine lesions. Generally, however, it appears that psychomotor stimulants will induce ipsilateral locomotion even in mice with non-specific unilateral damage of the striatum.

In contrast to the drug-induced ipsilateral turning of mice with unilateral 6-hydroxydopamine lesions, the drug-induced contralateral turning of these mice is quite specific. Only apomorphine, ET-495 and L-dopa elicited this effect. Furthermore, these drugs elicited contralateral turning only in mice with striatal 6-hydroxydopamine lesions. These 3 agents are thought to act as dopamine receptor stimulants (Andén et al., 1967; Corrodi et al., 1971). It is of interest to note some of the drugs which failed to evoke this effect. Clonidine, a hypothesized noradrenergic receptor stimulant (Andén et al., 1970[b]), did not elicit contralateral or ipsilateral turning even following high doses; this suggests that noradrenergic mechanisms may not be directly involved in these responses. Likewise, 5-hydroxytryptophan, a precursor

of 5-hydroxytryptamine was without effect on these animals. Hence, serotonergic mechanisms may not be directly involved. 3-Methoxytyramine has been suggested as a possible precursor of dopamine (Bartholini et al., 1971); however, in this study 3-methoxytyramine failed to mimic the dopamine agonists. Thus, it seems unlikely that significant amounts of dopamine are formed from this drug in the striatum. Despite reports to the contrary (Goldstein et al., 1968; Michaels and McCann, 1972) systemically administered dopamine does not appear to enter the brain in significant amounts; high doses failed to elicit contralateral turning. Unilateral injection of dopamine into the striatum does, however, result in contralateral turning (Cools, 1971). Thus, it appears that the primary utility of this technique is the detection of compounds with direct dopaminergic agonist properties.

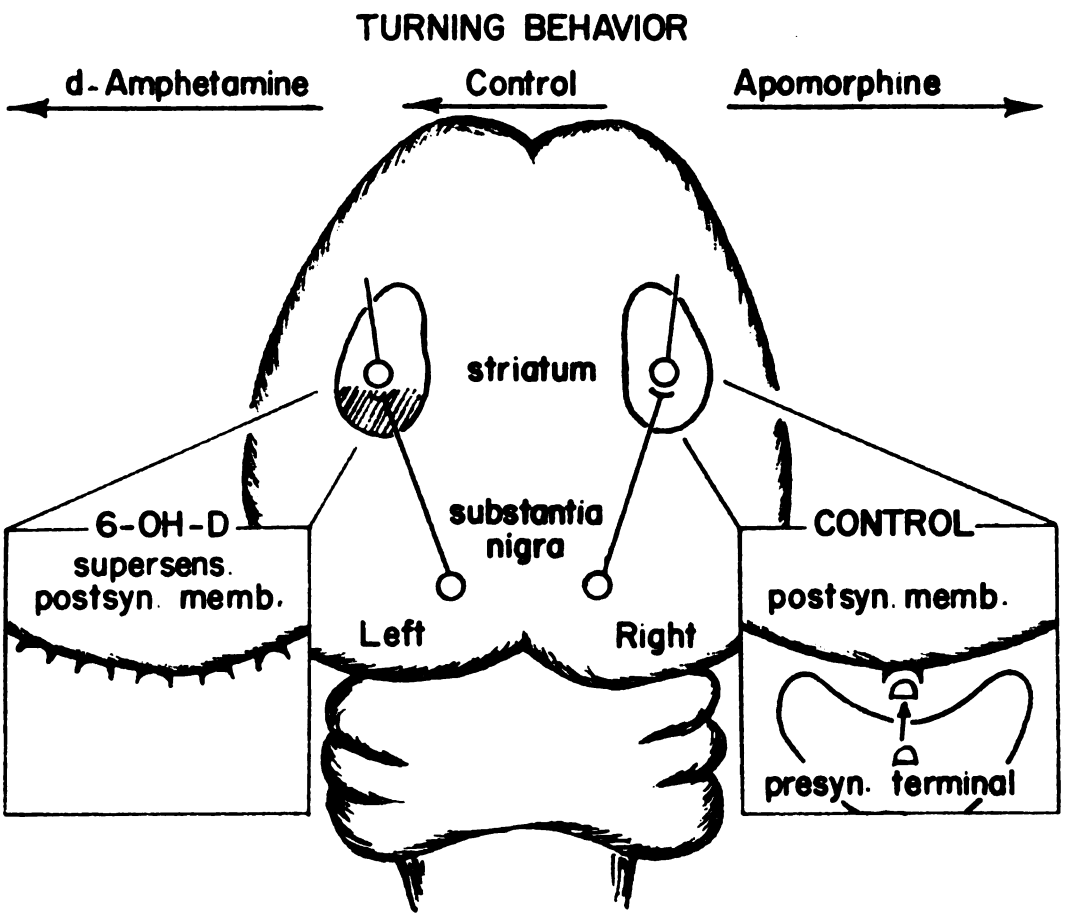
The doses of apomorphine and L-dopa required to elicit contralateral turning of mice with striatal lesions induced by 6-hydroxydopamine were considerably below those necessary to stimulate motor activity in normal rodents (Maj et al., 1972; Smith, 1963). This suggests that the mice with unilateral 6-hydroxydopamine lesions developed a supersensitivity to these agonists. Indeed, Ungerstedt (1971[a]) has concluded from experiments using rats with nigro-striatal lesions that contralateral turning is related to a postsynaptic supersensitivity. Denervation supersensitivity may be either

presynaptically or postsynaptically mediated. The former is apparently related to the loss of the reuptake of amines after loss of the nerve terminal (Brimijoin et al., 1970) and has been observed in the central nervous system (Boakes et al., 1971). Postsynaptic supersensitivity in the sympathetic nervous system requires several days to develop (Trendelenburg et al., 1970) whereas the presynaptic type induced by 6-hydroxydopamine (Nadeu et al., 1971) is complete within a few hours. The presumed supersensitivity observed in this study is most likely of the postsynaptic type as indicated by the several day period necessary for its development. Figure 44 illustrates how this supersensitivity to dopamine agonists may result in contralateral turning when mice are treated with these agents. A similar mechanism has been postulated for increased sensitivity to amphetamine in mice treated chronically with α-methyltyrosine (Dominic and Moore, 1969) and rats treated chronically with reserpine (Stolk and Rech, 1968).

The investigation of the mechanisms involved in post-synaptic supersensitivity has been rather limited. It is generally believed that certain trophic factors arising from the presynaptic terminal control the postsynaptic sensitivity. Recently, Drachman and Witzke (1972) have shown that chronic electrical stimulation of the denervated rat diaphragm inhibits increased sensitivity to acetyl-choline. They suggest that the contractions induced by the neurotransmitter may be one of the trophic factors controlling

Figure 44. Relationship of proposed postsynaptic supersensitivity to the contralateral turning induced by apomorphine in mice with unilateral striatal lesions.

The illustration depicts the bilateral nigro-striatal projection. The terminals in the left striatum have been destroyed by the injection of 6-hydroxydopamine. The enlarged view of the synaptic region from the left striatum lacks a presynaptic terminal in contrast to the right striatum, where a dopamine (D) containing terminal is represented. Note, however, that in the absence of a dopamine containing nerve terminal that the number of postsynaptic receptors (crescents) has increased on the lesioned side. A predominence of dopamine-action in one striatum over the other apparently causes the mouse to turn toward the deficient side (ipsilateral turning). Drugs such as amphetamine, which potentiates the effects of dopamine, increase the rate of ipsilateral turning. Drugs that interact directly with dopamine receptors however, cause a predominence of dopamine-action on the lesioned side because of the increased number of postsynaptic receptors. Apomorphine, for example, causes the mouse to turn away from the lesioned side (contralateral turning). This proposed mechanism of postsynaptic supersensitivity may account for the differential effects of amphetamine and apomorphine on mouse turning behavior.


TURNING BEHAVIOR
d-Amphetamine
Control
Apomorphine
striatum
substantia nigra
6-OH-D
supersens. postsyn. memb.
CONTROL
postsyn. memb.
Left
Right
presyn. terminal

postjunctional supersensitivity. In an analogous fashion, the experiments with chronic L-dopa diets have shown that dopaminergic postsynaptic supersensitivity in the striatum may be inhibited by replacing the lost neurotransmitter. The mechanism of this altered sensitivity and its suppression by chronic treatment with L-dopa merits a great deal more study. With an understanding of such mechanisms it might be possible to suppress or evoke supersensitivity in the central nervous system by the use of rational drug combinations or by alternating drugs of different mechanisms. These manipulations might be useful therapeutically. For example, it is possible that parkinsonian patients develop a similar type of supersensitivity, but that during treatment with L-dopa, it is suppressed. If this suppression of supersensitivity could be blocked, more dramatic therapeutic effects might be expected with L-dopa treatment.

The delineation of the dopaminergic nigro-striatal pathway and the development of experimental tools such as the cerebroventricular perfusion technique and 6-hydroxydopamine selective denervation have greatly facilitated the study of the mechanisms whereby drugs affect this pathway. Many of these mechanisms remain to be fully understood; considerable work remains to be done. At the same time, as other central pathways are neurochemically delineated, similar techniques may prove useful for elucidating their interaction with centrally active drugs.

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