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# Differential Visualization of Dopamine and Norepinephrine Uptake Sites in Rat Brain Using [$^3$H]Mazindol Autoradiography[1]

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## Abstract

**Mazindol is a potent inhibitor of neuronal dopamine (DA) and norepinephrine (NE) uptake. DA and NE uptake sites in rat brain have been differentially visualized using [$^3$H]mazindol autoradiography. At appropriate concentrations, desipramine (DMI) selectively inhibits [$^3$H]mazindol binding to NE uptake sites without significantly affecting binding to DA uptake sites. The localization of DMI-insensitive specific [$^3$H]mazindol binding, reflecting DA uptake sites, is densest in the caudate-putamen, the nucleus accumbens, the olfactory tubercle, the subthalamic nucleus, the ventral tegmental area, the substantia nigra (SN) pars compacta, and the anterior olfactory nuclei. In contrast, the localization of DMI-sensitive specific [$^3$H]mazindol binding, representing NE uptake sites, is densest in the locus coeruleus, the nucleus of the solitary tract, the bed nucleus of the stria terminalis, the paraventricular and periventricular nuclei of the hypothalamus, and the anteroventral thalamus. The distribution of DMI-insensitive specific [$^3$H]mazindol binding closely parallels that of dopaminergic terminal and somatodendritic regions, while the distribution of DMI-sensitive specific [$^3$H]mazindol binding correlates well with the regional localization of noradrenergic terminals and cell bodies. Injection of 6-hydroxydopamine, ibotenic acid, or colchicine into the SN decreases [$^3$H]mazindol binding to DA uptake sites in the ipsilateral caudate-putamen by 85%. In contrast, ibotenic acid lesions of the caudate-putamen do not reduce [$^3$H]mazindol binding to either the ipsilateral or contralateral caudate-putamen. Thus, the DA uptake sites in the caudate-putamen are located on the presynaptic terminals of dopaminergic axons originating from the SN.**

Synaptic inactivation of the catecholamines, dopamine (DA) and norepinephrine (NE), is achieved primarily by high-affinity uptake into nerve terminals. While the DA and NE uptake processes have similar substrate specificities, a variety of evidence indicates substantial differences in the molecular structure of these complexes (Horn, 1976; Ross, 1976). Drugs such as benztropine have somewhat greater affinity for the DA uptake site (Horn et al., 1971), whereas tricyclic antidepressants, such as desipramine (DMI), are much more potent in inhibiting NE uptake (Horn, 1973; Eckhardt et al., 1982). [$^3$H]DMI labels binding sites associated with the neuronal NE uptake system in membrane homogenates prepared from brain and peripheral tissues (Hrdina et al., 1981; Langer et al., 1981; Lee and Snyder, 1981; Lee et al., 1982, 1983; Rehavi et al., 1982). Furthermore, *in vitro* autoradiography using [$^3$H]DMI visualizes NE uptake sites (Biegon and Rainbow, 1983). Sodium-sensitive cocaine binding to striatal membranes may in part label DA uptake sites (Kennedy and Hanbauer, 1983), although the relatively low affinity of cocaine for the binding sites makes a detailed evaluation difficult.

Mazindol, a clinically employed appetite suppressant, potently inhibits both NE and DA uptake (Heikkila et al., 1977; Hyttel, 1982). [$^3$H]Mazindol labels both DA and NE uptake sites in rat brain homogenates (Javitch et al., 1983; 1984). DMI at appropriate concentrations selectively abolishes [$^3$H]mazindol binding to NE uptake sites without significantly affecting binding to DA uptake sites (Javitch et al., 1984). In the present study, we have differentially visualized sites associated with NE and DA uptake utilizing [$^3$H]mazindol autoradiography in the presence or absence of DMI.

## Materials and Methods

[$^3$H]Mazindol (11 Ci/mmol) was supplied by New England Nuclear, Boston, MA. Sources of other drugs were as described previously (Javitch et al., 1984). Male Sprague-Dawley rats (150 to 200 gm) were anesthetized with pentobarbital and perfused via the left ventricle of the heart with 0.9% NaCl, 50 mM sodium phosphate, pH 7.5, followed by 50 mM sodium phosphate and 0.3 M sucrose. Brains were removed, embedded in brain paste, and rapidly frozen at −70°C on microtome chucks. Sections (8 μM) were cut at −15°C and thaw-mounted on gelatin-coated slides. The slides were dessicated and then stored at −20°C. For autoradiographic studies, sections were preincubated at 4°C for 5 min in 50 mM Tris buffer (pH 7.9 at 4°C, containing 120 mM NaCl, 5 mM KCl) and then incubated for 40 min at 4°C in the assay buffer (50 mM Tris, pH 7.9, at 4°C, 300 or 120 mM NaCl, 5 mM KCl) with 4 nM [$^3$H]mazindol in the presence or absence of inhibitors. Nonspecific binding was determined in the presence of 1 μM mazindol. DMI-sensitive specific [$^3$H]mazindol is defined as the difference between total binding and binding in the presence of 0.3 μM DMI. DMI-insensitive binding is the difference between [$^3$H]mazindol bound in the presence of 0.3 μM DMI and the amount bound in the presence of 1 μM unlabeled mazindol (see "Results"). After two consecutive 1-min washes in the same buffer, the slides were dipped in water and immediately dried under a stream of cold dry air. Autoradiograms were generated by apposing the slides to LKB Ultrofilm for 5 to 6 weeks at 4°C (Unnerstall et al., 1982). Following autoradiography, tissue was stained with 0.1% toluidine blue. Density of silver grains on Ultrofilm was quantified by computer-assisted microdensitometry and converted to femtomoles of [$^3$H]mazindol bound per milligram of protein (Kuhar et al., 1984).

Coronal brain sections at the level of the caudate-putamen were used for saturation analysis, drug inhibition, and ion concentration experiments. Total and nonspecific binding for each concentration or condition were averaged from two sections from each of two brains. Tissue sections were wiped off the slide with a Whatman GF/B glass fiber filter and counted using liquid

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scintillation spectrometry. Saturation analysis of binding used 1, 2, 6, 10, 25, and 50 nM [$^3$H]mazindol. $K_D$ and $B_{max}$ values were calculated from saturation binding data using an iterative curve fitting program (McPherson, 1983). Binding of [$^3$H]mazindol to tissue homogenates was assayed as described previously (Javitch et al., 1984).

For lesion studies, 4 μg of colchicine (Sigma), 15 μg of ibotenic acid (Regis), or 8 μg of 6-hydroxydopamine hydrobromide (6-OHDA) (Sigma) in 2 μl of 0.9% NaCl were injected into the center of the left caudate-putamen or the left substantia nigra (SN) using stereotaxic coordinates measured from the interaural line. To confirm the location of the needle tip, a dye was injected into an age-matched rat, and the brain was dissected and inspected visually. Coronal sections at the level of the caudate-putamen were obtained as described above 7 to 14 d after the injections.

## Results

*Characteristics of [$^3$H]mazindol binding.* To ensure that [$^3$H]mazindol labels DA uptake sites in rat brain slices, the pharmacological properties of [$^3$H]mazindol binding to brain slices at the level of the caudate-putamen were evaluated. The striatum contains predominantly DA uptake sites with relatively few NE uptake sites (Table II; Figs. 2 and 3). [$^3$H]Mazindol binds saturably and with high affinity to striatal slices in accordance with results obtained previously using striatal homogenates (Javitch et al., 1983; 1984). Specific binding of 4 nM [$^3$H]mazindol at 300 mM NaCl is about 70% of total binding in the caudate-putamen. Scatchard analysis reveals a single component of binding with a dissociation constant ($K_D$) of 15 nM and a maximal number of binding sites ($B_{max}$) of 7.8 pmol/mg of protein. These values agree closely with those obtained in striatal homogenates (Javitch et al., 1984) as well as with the potency of mazindol as an inhibitor of DA uptake (Hyttel, 1982). Specific [$^3$H]mazindol binding to brain slices is dependent upon sodium with half-maximal binding at 250 mM NaCl (Fig. 1). By contrast, potassium chloride fails to augment binding.

The conclusion that [$^3$H]mazindol binding to striatal slices is associated with DA uptake sites is supported by the potency of various uptake inhibitors in inhibiting binding (Table I). Nomifensine and benztropine, two inhibitors of DA uptake (Hyttel, 1982), potently reduce [$^3$H]mazindol binding to striatal slices, as does mazindol itself. By contrast, 0.3 μM DMI, which potently inhibits NE but not DA uptake (Hyttel, 1982), has negligible effect on specific [$^3$H]mazindol binding to striatal slices. These results confirm that binding of [$^3$H]mazindol to striatal slices has the same properties as binding in striatal homogenates, which is associated with DA uptake sites (Javitch et al., 1983, 1984).

Comparable experiments assessing [$^3$H]mazindol binding associated with NE uptake sites were not performed in tissue slices, since in nonstriatal parts of the brain, levels of specific [$^3$H]mazindol binding are too low to permit accurate quantification by liquid scintillation spectrometry. However, in homogenates of the cerebral cortex with 1 μM mazindol employed to define nonspecific binding, inhibition of specific [$^3$H]mazindol binding by DMI is biphasic with a high-affinity component displaying a $K_i$ of about 10 nM (Fig. 2). This high-affinity component reflects binding to NE uptake sites (Javitch et al., 1984). A concentration of 0.3 μM DMI maximally inhibits the high-affinity component of [$^3$H]mazindol to cortical membranes without affecting the lower affinity component. In cerebral cortical homogenates, specific [$^3$H]mazindol binding to NE uptake sites, assessed using 0.3 μM DMI to define nonspecific binding, is saturable and displays a single component with a $K_D$ of about 4 nM and a

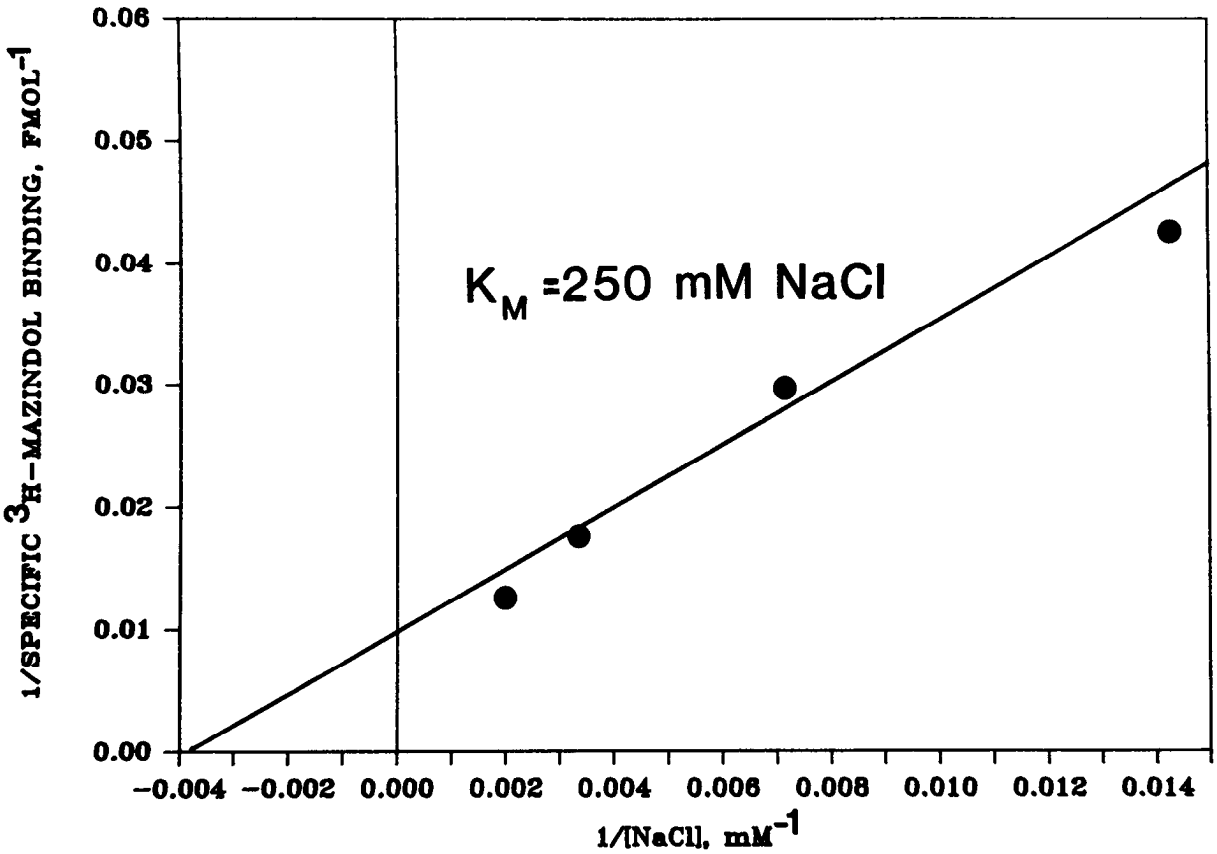


*Figure 1.* Effect of NaCl on specific [$^3$H]mazindol binding to slices of rat caudate-putamen. Binding experiments were performed with 4 nM [$^3$H]mazindol and 1 μM unlabeled mazindol to define nonspecific binding. Sodium chloride concentration was varied from 0 to 500 mM. No specific binding was seen in the absence of NaCl or in the presence of increasing concentrations of KCl up to 500 mM. Results are the mean of two separate determinations performed in duplicate. The line of best fit was determined by linear regression ($r = 0.98$).

$B_{max}$ of 150 fmol/mg of protein. Furthermore, 0.3 μM DMI has a negligible effect on specific [$^3$H]mazindol binding to DA uptake sites in striatal homogenates (Fig. 2) or striatal slices (Table I).

*Autoradiographic localization of [$^3$H]mazindol binding in rat brain.* The distribution of specific [$^3$H]mazindol binding sites in rat brain is summarized in Table II. DMI at 0.3 μM is used to abolish selectively the noradrenergic component and 1 μM unlabeled mazindol to block both NE and DA uptake sites (Fig. 2; see "Materials and Methods").

TABLE I

*Effect of drugs on specific [$^3$H]mazindol binding to rat corpus striatum*

[$^3$H]Mazindol binding to tissue sections was assayed as described in "Materials and Methods" in the presence of 300 mM NaCl and the indicated concentration of inhibitors. Values are expressed as percentage of binding in the absence of inhibitor and are the means of two determinations performed in duplicate, which varied less than 15%. $K_i$ values for the inhibition of [$^3$H]DA uptake into striatal synaptosomes and [$^3$H]mazindol binding to striatal homogenates are from Javitch et al. (1984).

| Drug | Concentration of Inhibitor (μM) | Percentage of Total Specific [$^3$H] Mazindol Binding to Striatal Slices | $K_i$ | |
|---|---|---|---|---|
| | | | [$^3$H]DA Uptake Striatal Synaptosomes (μM) | [$^3$H]Mazindol Binding Striatal Homogenates (μM) |
| Mazindol | 0.02 | 53 | 0.016 | 0.023 |
| Nomifensine | 0.10 | 44 | 0.043 | 0.084 |
| Benztropine | 0.20 | 47 | 0.098 | 0.22 |
| DMI | 0.30 | 94 | 8.1 | 12.2 |

The densest localization of DMI-insensitive specific [$^3$H]mazindol binding occurs in the caudate-putamen, the nucleus accumbens, and the olfactory tubercle (Figs. 3 and 4). In the caudate-putamen, specific binding is seen only in gray matter. Also densely labeled are the subthalamic nucleus, the ventral tegmental area (VTA), the SN, pars compacta (Fig. 5), and anterior olfactory nuclei. Substantial autoradiographic silver grains occur in the SN pars reticulata, the locus coeruleus, the nucleus of the solitary tract, the lateral habenula, the supraoptic and paraventricular nuclei of the hypothalamus, the amygdala, the dorsal raphe nucleus, the bed nucleus of the stria terminalis, and the median eminence. Low levels of DMI-insensitive specific [$^3$H]mazindol binding are seen in the arcuate and periventricular nuclei of the hypothalamus, the thalamus, the mamillary complex, superficial layers of the cerebral cortex, the lateral septum, globus pallidus, and dentate gyrus.

The distribution of DMI-sensitive [$^3$H]mazindol sites differs markedly from that observed for DMI-insensitive [$^3$H]mazindol binding (Fig. 3). Thus, the highest density of DMI-sensitive [$^3$H]mazindol binding occurs in the locus coeruleus and the nucleus of the solitary tract. High levels of binding are also detected in the paraventricular and periventricular nuclei of the hypothalamus, the bed nucleus of the stria terminalis, and the anteroventral nucleus of the thalamus. Substantial grain densities occur in the ventral medullary region, superficial layers of the cerebral cortex, the median eminence, the habenula, the lateral septum, the supraoptic nucleus, the amygdala, the dorsal raphe nucleus, the mamillary nucleus, the interpeduncular nucleus, the hippocampus and dentate gyrus, the zona incerta, and

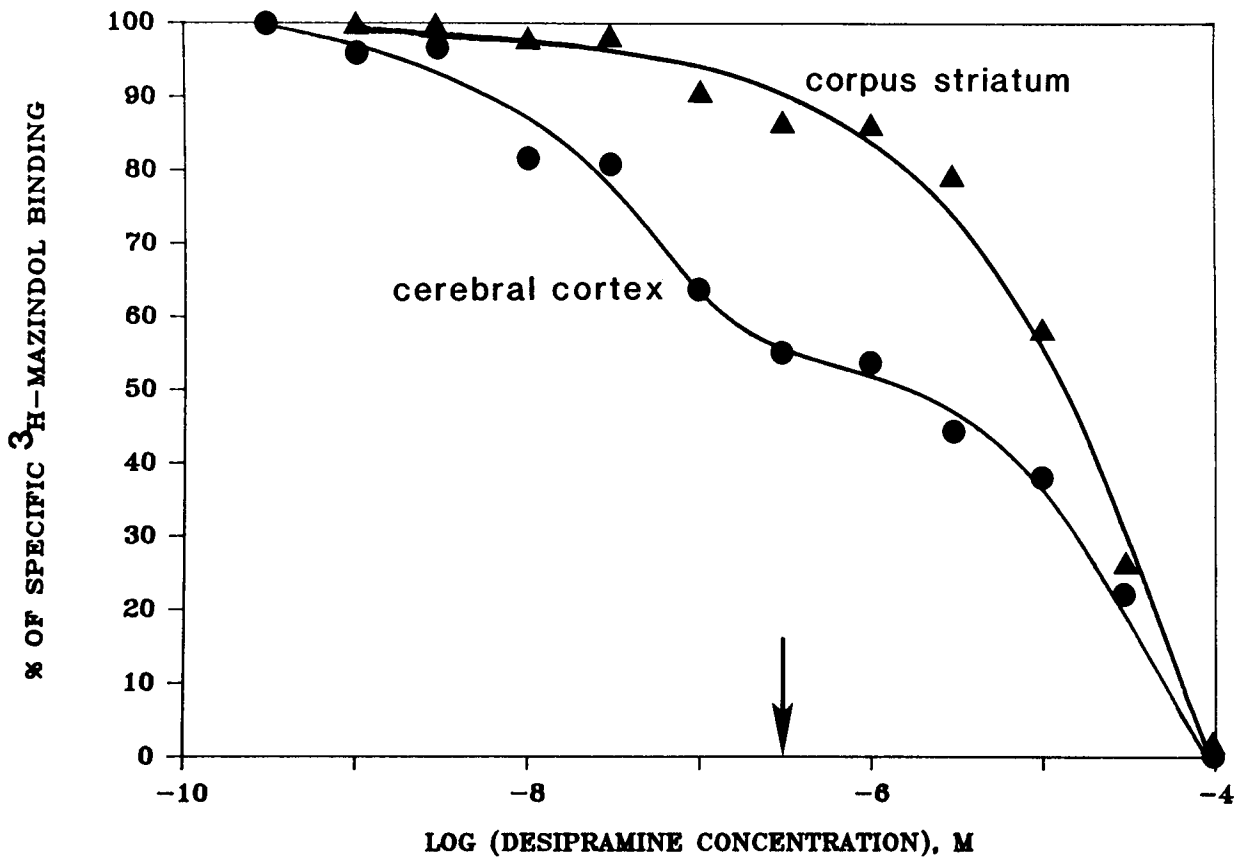


*Figure 2.* Inhibition of specific [$^3$H]mazindol (4 nM) binding by unlabeled DMI in rat cerebral cortical and striatal membranes. Specific binding was calculated by subtracting nonspecific binding in the presence of 100 μM DMI from the total binding and is expressed as a percentage of binding in the absence of unlabeled DMI. The *arrow* indicates 0.3 μM DMI, the concentration used to inhibit the high-affinity component of binding to cortical membranes without substantially inhibiting the low affinity component of binding to cortical membranes or binding to striatal membranes. Results are the mean of two separate determinations performed in triplicate.

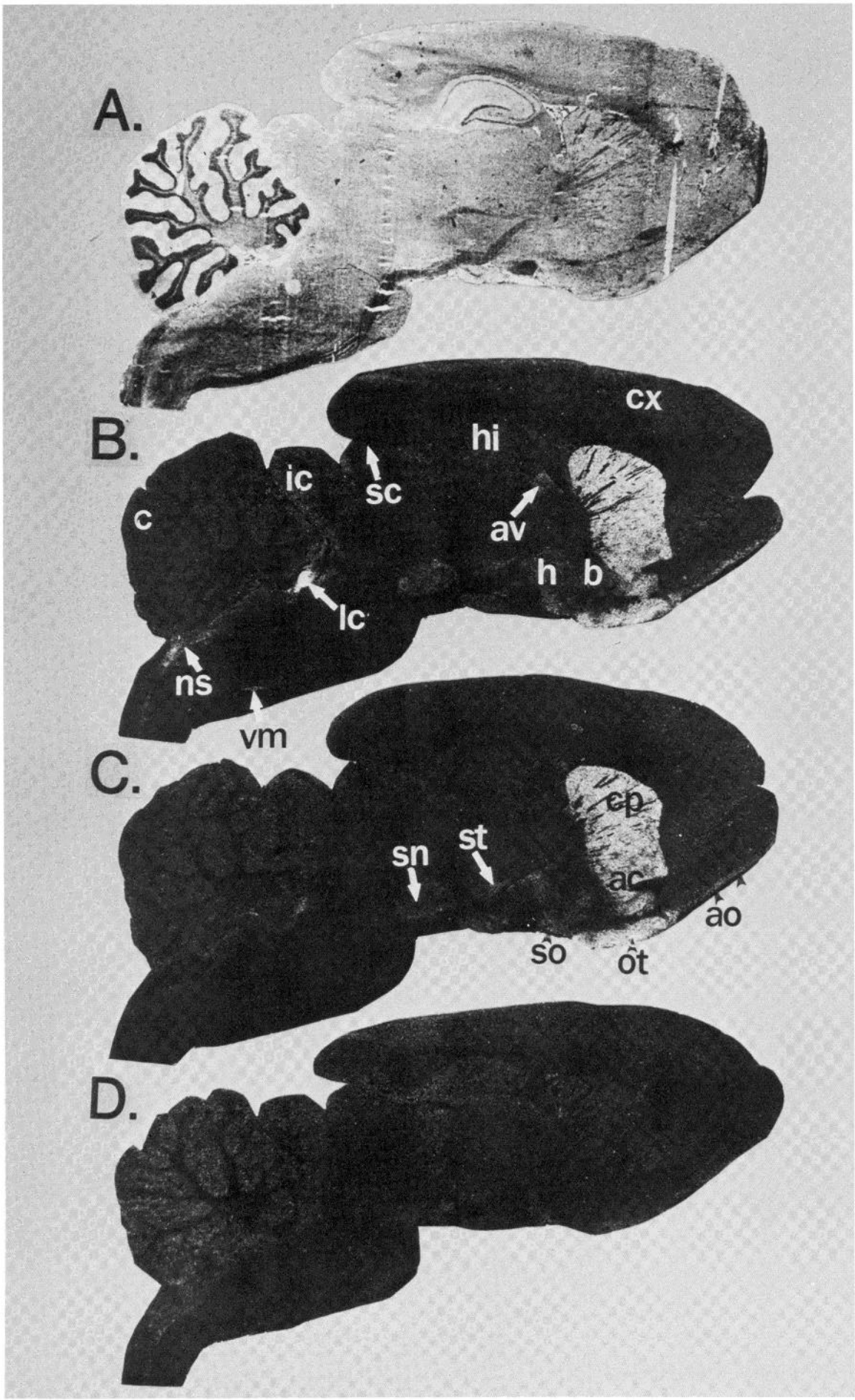


*Figure 3.* Autoradiograms of [$^3$H]mazindol binding sites in sagittal sections of rat brain. *A*, Serial section stained with 0.1% toluidine blue. *B*, High levels of [$^3$H]mazindol binding to NE uptake sites are seen in the locus coeruleus, the nucleus of the solitary tract, the ventral medullary region, and the anteroventral

TABLE II

*Regional localization of [$^3$H]mazindol binding to DA and NE uptake sites*

[$^3$H]Mazindol (4 nM) binding to tissue sections was assayed as described in "Materials and Methods" in the presence of 300 mM NaCl. Since the $K_D$ for [$^3$H]mazindol binding to NE uptake sites is about 25% of its $K_D$ for DA uptake sites, the values presented represent roughly half the maximal number of NE uptake sites but only one-eighth the number of DA uptake sites. Binding levels in each region were determined using computer-assisted microdensitometry (Kuhar et al., 1984). Values represent the means of determinations in 2 to 5 areas in representative sections from each of two animals, which varied less than 20%. Density is described on a scale from 0 to 5 where each level is defined as follows: 0, 0 to 0.04 pmol/mg protein; 1, 0.04 to 0.2 pmol/mg protein; 2, 0.2 to 0.5 pmol/mg protein; 3, 0.5 to 0.9 pmol/mg protein; 4, 0.9 to 1.5 pmol/mg protein; 5, 1.5 to 2 pmol/mg protein. Values for density of [$^3$H]DMI binding are from Biegon and Rainbow (1983).

| Brain Region | DMI-insensitive [$^3$H]Mazindol Binding to DA Uptake Sites | DMI-sensitive [$^3$H]Mazindol Binding to NE Uptake Sites | [$^3$H]DMI Binding to NE Uptake Sites |
|---|---|---|---|
| Caudate-putamen | 5 | 1 | 1 |
| Nucleus accumbens | 5 | 1 | 1 |
| Olfactory tubercle | 5 | 1 | ND[a] |
| Subthalamic nucleus | 4 | 0 | ND |
| VTA | 4 | 1 | ND |
| Anterior olfactory nuclei | 3 | 2 | ND |
| SN | | | |
| Pars compacta | 3 | 0 | 0 |
| Pars reticulata | 2 | 1 | ND |
| Median eminence | 2 | 2 | ND |
| Locus coeruleus | 2 | 5 | 3 |
| Nucleus tractus solitarus | 2 | 4 | ND |
| Lateral habenula | 2 | 2 | ND |
| Supraoptic nucleus | 2 | 2 | 2 |
| Paraventricular nucleus | 2 | 3 | 2 |
| Amygdala | 2 | 2 | ND |
| Dorsal raphe | 2 | 2 | 2 |
| Bed nucleus stria terminalis | 2 | 3 | 3 |
| Anteroventral thalamus | 1 | 3 | 3 |
| Ventral thalamus | 1 | 1 | 1 |
| Mamillary nucleus | 1 | 2 | 2 |
| Frontal cortex | 1 | 2 | 1 |
| Arcuate nucleus | 1 | 2 | 3 |
| Periventricular nucleus | 1 | 3 | 2 |
| Lateral septum | 1 | 2 | 1 |
| Globus pallidus | 1 | 0 | 0 |
| Dentate gyrus | 1 | 2 | 2 |
| Ventral medullary region | 0 | 2 | ND |
| Hippocampus | 0 | 2 | 1 |
| Interpeduncular nucleus | 0 | 2 | 2 |
| Zona incerta | 0 | 2 | 2 |
| Central gray | 0 | 2 | 2 |
| Superior colliculus | 0 | 2 | 2 |
| Inferior colliculus | 0 | 1 | 1 |
| Cerebellar cortex | 0 | 1 | 1 |
| Medial habenula | 0 | 2 | ND |
| Medial septum | 0 | 1 | 1 |

[a] ND, not determined.

the central gray matter. We observe lower levels of binding in the inferior colliculus, the medial septum, the cerebellar cortex, the ventral thalamus, the VTA, the caudate-putamen, the nucleus accumbens, and the olfactory tubercle.

*Effects of lesions on [$^3$H]mazindol binding to caudate-putamen.* The most prominent density of [$^3$H]mazindol binding throughout the brain occurs in the caudate-putamen. To ascertain whether these binding sites occur on terminals of the nigrostriatal dopaminergic pathway, a series of lesions was performed. Ibotenic acid, colchicine, or 6-OH DA was stereotaxically injected unilaterally into the SN. Each of these injections elicits a profound reduction of [$^3$H]mazindol binding in the ipsilateral caudate-putamen (Table III; Fig. 6). Injections of saline alone into the SN have no effect on [$^3$H]mazindol binding to the ipsilateral caudate-putamen.

Unilateral injections of ibotenic acid into the caudate-putamen do not affect [$^3$H]mazindol binding to the ipsilateral or contralateral caudate-putamen in adjacent sections from the same brains. These same lesions lower [$^3$H]captopril binding to angiotensin-converting enzyme by 80 to 90% in the ipsilateral caudate-putamen, reflecting a localization of the enzyme to a striatonigral pathway (Strittmatter et al., 1984).

## Discussion

[$^3$H]Mazindol labels both DA and NE uptake sites (Javitch et al., 1983; 1984). The $K_D$ and $B_{max}$ values as well as drug potencies in blocking [$^3$H]mazindol binding to brain slices agree with values obtained in brain homogenates (Javitch et al., 1983, 1984). Moreover, ionic requirements for binding to slices and homogenates are similar and resemble those for [$^3$H]DA accumulation into striatal synaptosomes (Holz and Coyle, 1979).

It is possible to separate the dopaminergic and noradrenergic components of [$^3$H]mazindol binding using 0.3 $\mu$M DMI, a concentration sufficient to abolish binding to NE uptake sites without significantly affecting binding to DA uptake sites (Javitch et al., 1984). The regional distribution of DMI-sensitive specific [$^3$H]mazindol binding closely resembles the localization of noradrenergic cell bodies and terminal projections as demonstrated by dopamine $\beta$-hydroxylase immunohistochemistry and catecholamine histofluorescence (Fuxe, 1965; Ungerstedt, 1971; Hartman et al., 1972; Hartman, 1973; Swanson and Hartman, 1975; Moore and Bloom, 1979; Biegon and Rainbow, 1983; Lindvall and Bjorklund, 1983). In contrast, the DMI-insensitive component of specific [$^3$H]mazindol binding parallels the distribution of dopaminergic neurons and their projections (Fuxe, 1965; Moore and Bloom, 1978; Lindvall and Bjorklund, 1983).

The high levels of DMI-insensitive specific [$^3$H]mazindol binding to caudate-putamen, nucleus accumbens, and olfactory tubercle are to uptake sites on the terminals of DA neurons originating in the SN and VTA. This conclusion is supported by the success of colchicine, ibotenic acid (Schwarcz et al., 1979), or 6-OHDA (Breese and Traylor, 1979) lesions of the SN/VTA region to profoundly decrease DMI-insensitive specific [$^3$H]mazindol binding to the ipsilateral caudate-putamen.

High levels of DMI-insensitive specific [$^3$H]mazindol binding are seen in the subthalamic nucleus, which contains moderate levels of DA (Versteeg et al., 1976) and catecholamine fibers (Brown et al., 1979; Meibach and Katzman, 1979). [$^3$H]Mazindol binding to the SN pars reticulata reflects the substantial levels of DA uptake into dendrites of this region (Geffen et al., 1976). High levels of [$^3$H]mazindol binding to DA uptake sites occur in the SN pars compacta and VTA, the origins of the mesotelencephalic dopaminergic system.

thalamus. *C*, [$^3$H]Mazindol binding in the presence of 0.3 $\mu$M DMI to inhibit binding to NE uptake sites. High levels of binding to DA uptake sites are seen in the caudate-putamen, the nucleus accumbens, the olfactory tubercle, the subthalamic nucleus, and the SN, pars compacta. *D*, [$^3$H]Mazindol binding in the presence of 1 $\mu$M unlabeled mazindol to inhibit binding to NE and DA uptake sites. [$^3$H]Mazindol (4 nM) autoradiography was performed in the presence of 300 mM NaCl. *ac*, nucleus accumbens; *ao*, anterior olfactory nuclei; *av*, anteroventral thalamus; *b*, bed nucleus of the stria terminalis; *c*, cerebellum; *cp*, caudate-putamen; *cx*, cerebral cortex; *h*, hypothalamic regions; *hi*, hippocampus; *ic*, inferior colliculus; *lc*, locus coeruleus; *ns*, nucleus of the solitary tract; *ot*, olfactory tubercle; *sc*, superior colliculus; *sn*, substantia nigra; *so*, supraoptic nucleus; *st*, subthalamic nucleus; *vm*, ventral medullary region.

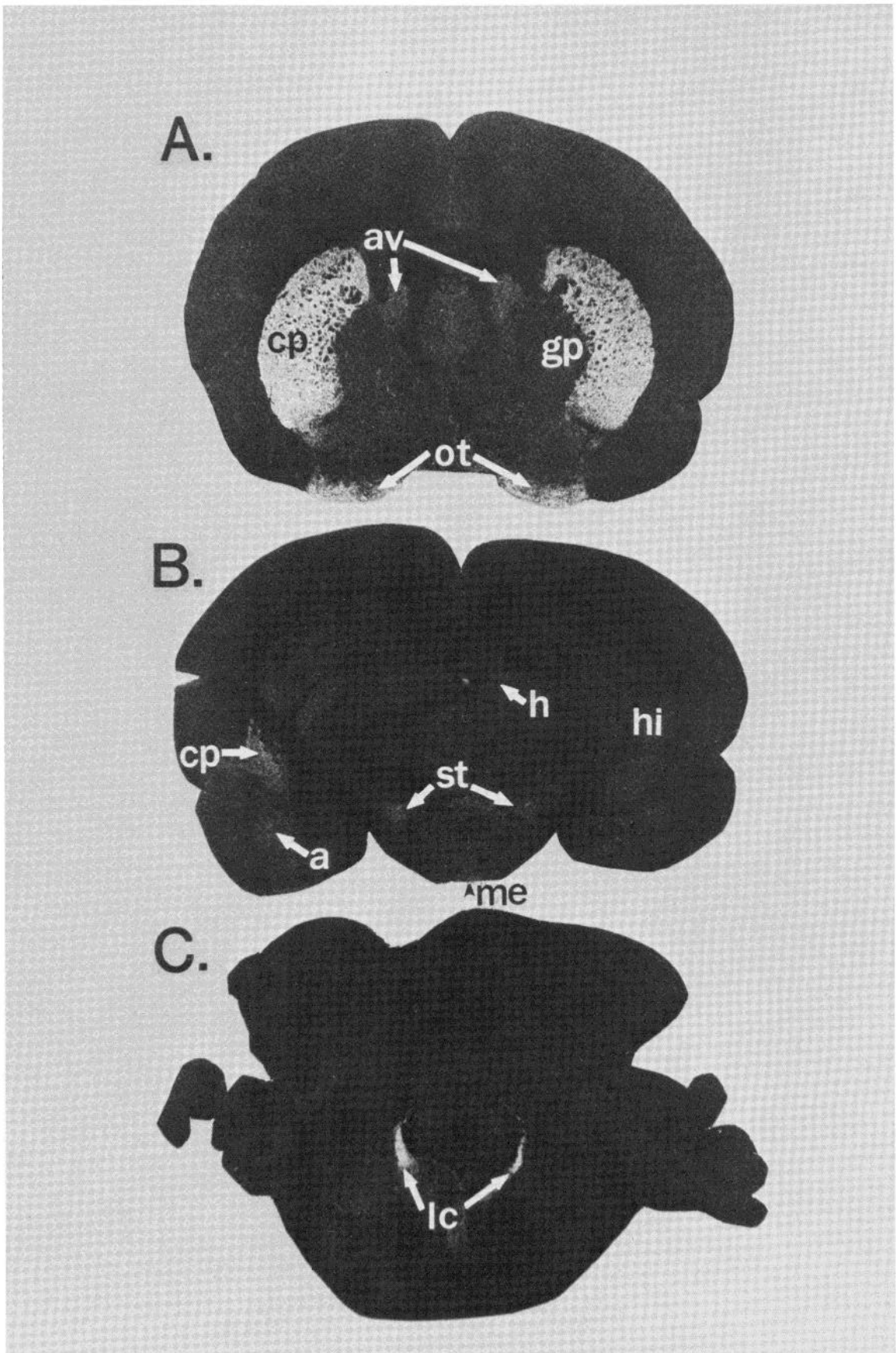


*Figure 4.* Autoradiogram of [$^3$H]mazindol binding sites in coronal sections of rat brain. *A,* High levels of binding are seen in the caudate-putamen, the olfactory tubercle, and the anteroventral thalamus. *B,* High levels of binding are seen in the subthalamic nuclei and the caudate-putamen. *C,* Intense binding is seen in the locus coeruleus. [$^3$H]Mazindol (4nM) autoradiography was performed in the presence of 300 mM NaCl. *a*, amygdala; *av*, anteroventral thalamus; *cp*, caudate-putamen; *gp*, globus pallidus; *h*, habenula; *hi*, hippocampus; *lc*, locus coeruleus; *me*, median eminence; *ot*, olfactory tubercle; *st*, subthalamic nucleus.

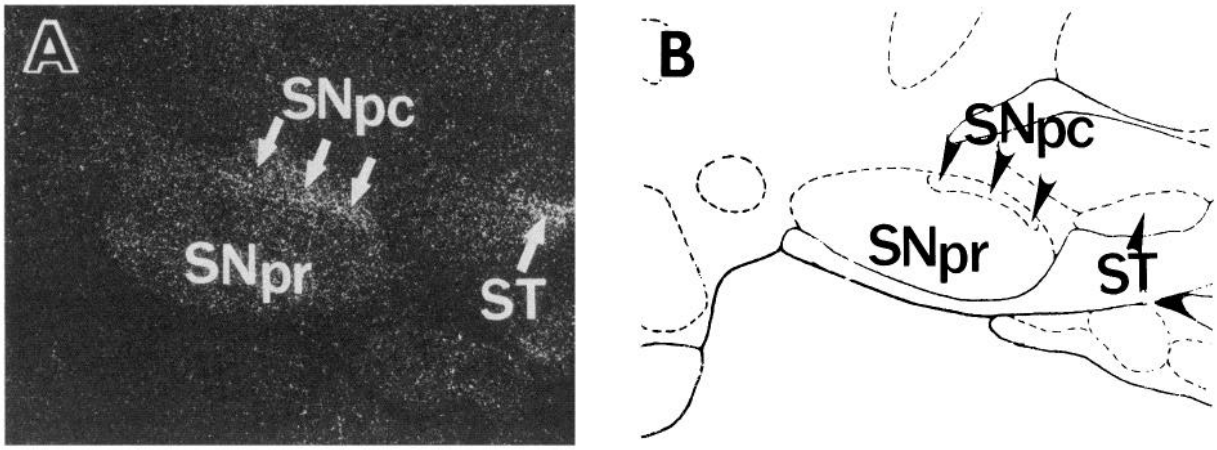


*Figure 5.* Autoradiogram of [$^3$H]mazindol binding sites in a sagittal section at the level of the substantia nigra and subthalamic nucleus of the rat brain. *A*, High levels of binding are seen in the substantia nigra, pars compacta, and the subthalamic nucleus. Moderate binding is seen in the substantia nigra, pars reticulata. *B*, Schematic representation of tissue section from *A*, adapted from the data of Paxinos and Watson (1982). [$^3$H]Mazindol autoradiography (4 nM) was performed in the presence of 300 mM NaCl. *SNpc*, substantia nigra, pars compacta; *SNpr*, substantia nigra, pars reticulata; *ST*, subthalamic nucleus.

TABLE III

*Effect of lesions on [$^3$H]mazindol binding*

[$^3$H]Mazindol (4 nM) binding to tissue sections was performed as described in "Materials and Methods" in the presence of 120 mM NaCl. Lesions were performed as described in "Materials and Methods." Binding levels were determined using computer-assisted microdensitometry (Kuhar et al., 1984).

| Treatment | [$^3$H]Mazindol binding (fmol/mg of protein) in Caudate-Putamen | |
|---|---|---|
| | Control side | Lesion side |
| SN lesions | | |
| Ibotenic acid ($n$ = 3) | 440 ± 80[a] | 60 ± 30[b] (14%)[c] |
| Colchicine ($n$ = 3) | 550 ± 150 | 90 ± 80[b] (16%) |
| 6-OHDA ($n$ = 1) | 560 | 100 (18%) |
| Saline-control ($n$ = 1) | 450 | 430 (96%) |
| Caudate-putamen lesions | | |
| Ibotenic acid ($n$ = 3) | 490 ± 50 | 480 ± 100 (98%) |

[a] Mean ± SE.

[b] $p < 0.005$ compared to control side.

[c] Numbers in parentheses, percentage of binding on the lesion side relative to the control side.

While DA concentrations in these regions are only 10 to 20% of those in the caudate-putamen, specific [$^3$H]mazindol binding to DA uptake sites in these cell body containing areas is 30 to 50% of that in the caudate-putamen. This finding fits with studies showing a higher ratio of DA uptake to endogenous DA concentration in cell body areas than in terminal areas (Geffen et al., 1976; Beart et al., 1979; Beart and McDonald, 1980). Proportionally higher amine uptake in cell bodies than in terminals may reflect ongoing synthesis of uptake sites at the rough endoplasmic reticulum of the cell body prior to their transport to terminal areas. Transmitter presumably accumulates primarily in mature secretory granules of terminals.

While comparable concentrations of DA are found in the caudate-putamen and median eminence, the ratio of [$^3$H]mazindol binding to DA uptake is 10 times higher in caudate-putamen, agreeing with the proportionally lower [$^3$H]DA uptake into synaptosomes prepared from median eminence (Cuello et al., 1973; Cuello and Iversen, 1973; Demarest and Moore, 1979). As DA is released from tuberohypophyseal dopaminergic neurons into the portal blood vessels, there may be less need to inactivate synaptic levels of transmitter via reuptake. Fewer uptake sites may explain the difficulty in destroying DA cells in the tuberohypophyseal system with 6-OHDA (Cuello et al., 1974). The low level of [$^3$H]mazindol binding to DA uptake sites in the median eminence is paralleled by similar low levels in the arcuate nucleus, the site of origin of the tuberohypophyseal system.

Other regions with substantial DMI-insensitive specific [$^3$H]mazindol binding correlate with the projection of DA neurons. The lateral habenula contains a dense collection of catecholaminergic axons, as well as substantial DA concentrations (Lindvall and Bjorklund, 1983; Versteeg et al., 1976). Tyrosine hydroxylase immunohistochemistry has also demonstrated a catecholaminergic fiber system in this location (Lindvall and Bjorklund, 1983). Furthermore, DMI-insensitive DA uptake has been measured in the lateral habenula (Lindvall and Bjorklund, 1983).

The lateral septal nucleus is innervated by a dopaminergic projection from the VTA as is the bed nucleus of the stria terminalis (Moore and Bloom, 1978; Lindvall and Bjorklund, 1983). There is also a dopaminergic innervation of the amygdala originating in the VTA and medial SN (Lindvall and Bjorklund, 1983). Binding to DA uptake sites in hypothalamic regions, the thalamus, and septum is probably associated with the periventricular and incertohypothalamic DA systems (Lindvall and Bjorklund, 1983). DMI-insensitive [$^3$H]mazindol binding to locus coeruleus presumably reflects uptake sites on the terminals of dopaminergic neurons in the mesopontine system (Lindvall and Bjorklund, 1983). Cerebral cortical areas are innervated by the mesocortical dopaminergic system (Moore and Bloom, 1978; Lindvall and Bjorklund, 1983), explaining DMI-insensitive [$^3$H]mazindol binding to cerebral cortex. The anterior olfactory nuclei are also innervated by a projection from the medial SN and VTA (Lindvall and Bjorklund, 1983).

DMI-sensitive specific [$^3$H]mazindol binding is densest in the locus coeruleus, the site of origin of many noradrenergic neurons (Moore and Bloom, 1979; Lindvall and Bjorklund, 1983). The very high levels of binding to NE uptake sites in this cell body area may reflect the same sort of factors that operate in DA cell body areas. The high densities of binding in the nucleus of the solitary tract also may be associated with catecholamine cell bodies.

Although catecholaminergic systems have been visualized by tyrosine hydroxylase and dopamine $\beta$-hydroxylase immunohistochemistry and catecholamine histofluorescence, [$^3$H]mazindol autoradiography provides several advantages over these techniques. Autoradiography independently and quantifiably labels both norepinephrine and dopamine uptake systems. Furthermore, the concerted

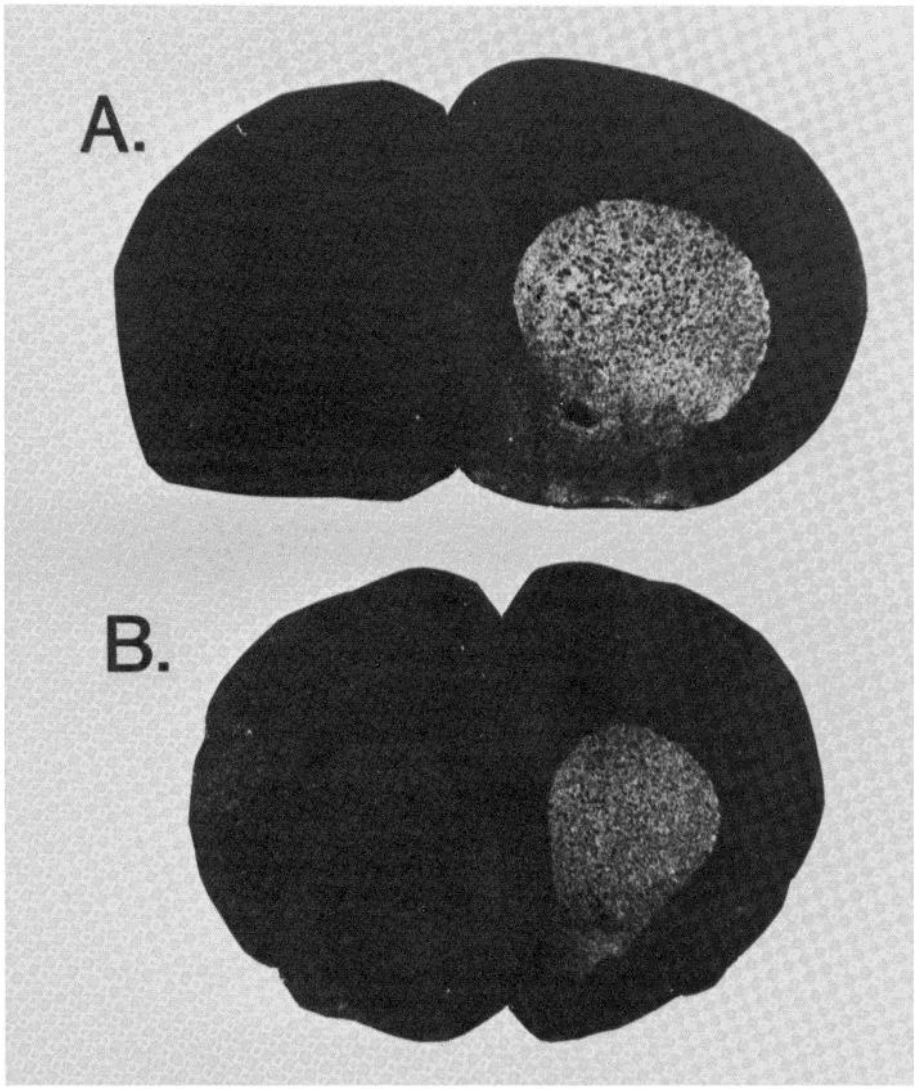


*Figure 6.* Autoradiograms of [$^3$H]mazindol binding sites in coronal sections of rat brain at the level of the caudate-putamen following unilateral lesions of the right SN. *A*, Unilateral 6-OHDA lesion of the SN. *B*, Unilateral ibotenic acid lesion of the SN. See the legend of Table III for experimental details.

use of these three methods in adjacent sections would visualize the synthetic capability, transmitter levels, and transmitter inactivation potential of catecholaminergic neurons and projections.

Specific [$^3$H]mazindol binding to both DA and NE uptake sites is stable in frozen tissue. Thus, [$^3$H]mazindol autoradiography may be useful in studying changes in the catecholaminergic systems in clinical syndromes. Sodium-sensitive [$^3$H]cocaine binding to striatal homogenates declines in patients with Parkinson's Disease (Pimoule et al., 1983). Using [$^3$H]mazindol autoradiography, it would be possible to study the pattern of degeneration of dopaminergic terminals in Parkinson's Disease and other disease states.

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