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Parkinsonism-inducing neurotoxin, *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine: Uptake of the metabolite *N*-methyl-4-phenylpyridine by dopamine neurons explains selective toxicity

(substantia nigra/caudate–putamen/nucleus accumbens/locus ceruleus/[^{3}H]mazindol autoradiography)

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ABSTRACT ***N*-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) produces neuropathological and clinical abnormalities in humans, monkeys, and mice that closely resemble idiopathic parkinsonism. *N*-Methyl-4-phenylpyridine (MPP^+), a metabolite of MPTP formed by monoamine oxidase B, is accumulated into striatal and cerebral cortical synaptosomes by the dopamine and norepinephrine uptake systems, respectively, whereas MPTP itself is not accumulated. The potencies of drugs in inhibiting [^{3}H]MPP^+ or [^{3}H]dopamine uptake into striatal synaptosomes are very similar, as are potencies in inhibiting [^{3}H]MPP^+ or [^{3}H]norepinephrine uptake into cortical synaptosomes. The K_m values for [^{3}H]MPP^+ uptake are 170 and 65 nM and the V_{max} values are 2 and 0.1 nmol/g of tissue per min in rat striatum and cortex, respectively, similar to values for [^{3}H]dopamine uptake. Autoradiography of accumulated [^{3}H]MPP^+ in slices of rat brain shows high densities in the caudate–putamen and nucleus accumbens. Furthermore, blockade of dopamine uptake by mazindol prevents MPTP-induced damage to nigrostriatal dopamine neurons, indicating that MPP^+ concentration into dopamine neurons explains their selective destruction by MPTP.**

N-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is a by-product of the chemical synthesis of an analog of the opiate meperidine. In humans MPTP produces apparently irreversible symptoms clinically similar to those found in parkinsonism (1–4). After MPTP administration to humans, monkeys, and mice, pathological lesions and neurochemical changes are prominent in nigrostriatal dopaminergic neurons (1–11), although other catecholaminergic neurons are also affected (5, 7, 10).

MPTP neurotoxicity is dependent on its conversion by monoamine oxidase B (MAO B) to *N*-methyl-4-phenylpyridine (MPP^+) (12, 13). Deprenyl or pargyline, selective MAO B inhibitors, block the conversion of MPTP to MPP^+ in brain mitochondrial preparations (14). Pretreatment of mice (15) and monkeys (16) with MAO inhibitors prevents the neurotoxic action of MPTP.

[^{3}H]MPTP binds with high affinity to receptor-like sites that reflect the initial recognition sites for the neurotoxic process (17). Thus, the chemical specificity of the binding sites parallels the ability to elicit neurotoxicity (17). Also, [^{3}H]MPTP binding sites are concentrated in human substantia nigra and caudate, whereas these regions have substantially fewer binding sites in rats (17), in accordance with the lesser neurotoxicity of MPTP in this species (18–22). The autoradiographic localization of binding sites for [^{3}H]MPTP resembles that of [^{3}H]pargyline (an MAO B inhibitor) (17, 23, 24) as well as the localization of MAO B determined immunohistochemically (25, 26). Moreover, the potencies of selective MAO B inhibitors in competing for [^{3}H]MPTP binding are similar to their potencies as MAO inhibitors (17, 24). Thus, MPTP binding sites appear to reflect MAO B.

While levels of MAO B are high in human substantia nigra and caudate, MAO B also occurs in numerous other parts of the brain (17, 23–26), and thus MPP^+ is formed from MPTP throughout the brain (12, 13). Accordingly, though the selective binding of MPTP and production of MPP^+ by MAO B is a necessary first step in eliciting toxicity, it cannot account for the selective effects on catecholamine neurons. In a preliminary study, we showed that [^{3}H]MPP^+, but not [^{3}H]MPTP, is accumulated via the dopamine neuronal uptake system (27). This report describes how both the dopamine and norepinephrine uptake systems transport [^{3}H]MPP^+ and provides evidence establishing that MPP^+ accumulation by dopaminergic neurons is required for MPTP neurotoxicity.

MATERIALS AND METHODS

[^{3}H]MPP^+ (85 Ci/mmol; 1 Ci = 37 GBq) and [^{3}H]MPTP (85 Ci/mmol) were supplied by S. Hurt of New England Nuclear; MPTP and MPP^+, by S. Markey of the National Institutes of Health (Bethesda, MD); and [^{3}H]mazindol, by T. C. Kung of New England Nuclear. Other reagents were obtained from commercial sources.

For uptake experiments, corpus striatum or cerebral cortex from male Sprague–Dawley rats (150–250 g, Charles River Breeding Laboratories) was homogenized in ice-cold 0.3 M sucrose in a glass homogenizer with a Teflon pestle, using 20 or 5 vol for corpus striatum or cerebral cortex, respectively. The homogenate was centrifuged at 1000 × *g* for 10 min. The supernatant was centrifuged at 12,000 × *g* for 20 min. The second pellet was resuspended with a pestle in 20 or 5 vol of 0.3 M sucrose for corpus striatum or cerebral cortex, respectively. This suspension was used in [^{3}H]MPP^+ or [^{3}H]dopamine uptake experiments. Twenty-five microliters of the suspension was added to 475 μl of uptake buffer containing 120 mM NaCl, 5 mM KCl, 11 mM glucose, 50 mM Tris·HCl (pH 7.4 at 37°C), 1 nM [^{3}H]MPP^+ or [^{3}H]dopamine, and various tested drugs. After incubation at 37°C for 6 min the uptake was terminated by the addition of 4 ml of ice-cold buffer to each tube and filtration of the mixture through glass-fiber filters (Schleicher & Schuell no. 32). Filters were washed with two consecutive 2-ml aliquots of buffer. Radioactivity remaining on the filters was measured by liquid

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Abbreviations: MPTP, *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MPP^+, *N*-methyl-4-phenylpyridine; MAO, monoamine oxidase.

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scintillation spectrometry. To correct for passive diffusion and adsorption to membranes and filters, control samples were incubated in the presence of 10 μM mazindol. Active uptake was calculated by subtracting from the total cpm the number of cpm accumulated in the presence of 10 μM mazindol and was expressed as nmol per g of tissue per min. K_m, V_{max}, and K_i values were calculated from saturation and drug competition data by using an iterative curve-fitting program (28).

Autoradiographic studies of accumulated [^{3}H]MPP$^+$ utilized 300-μm coronal sections of rat brain cut with a Vibratome. The sections were incubated for 30 min at 37°C in uptake buffer with 5 nM [^{3}H]MPP$^+$. The sections were washed twice for 5 min in uptake buffer, then placed on glass slides and frozen on dry ice. The slides were dried under reduced pressure and then apposed to tritium-sensitive film (^{3}H-Ultrofilm, LKB) for 2 days at 4°C (29). Autoradiographic studies with [^{3}H]mazindol used slide-mounted mouse brain sections as described previously (30). The binding of [^{3}H]mazindol to striatal homogenates was assayed as described (31). Protein was measured by the method of Lowry *et al.* (32).

RESULTS

Characteristics of [^{3}H]MPP$^+$ Uptake by Synaptosomal Preparations. Rats were used for most uptake experiments because of the greater availability of tissue, although similar saturable and pharmacologically similar [^{3}H]MPP$^+$ uptake is observed with mice. [^{3}H]MPP$^+$ accumulation is temperature dependent, with negligible specific uptake at 4°C, and is linear with time up to 8 min, after which it begins to plateau. Sodium is required for [^{3}H]MPP$^+$ uptake, with negligible accumulation when sodium is replaced by potassium. [^{3}H]MPP$^+$ accumulation is linearly dependent on tissue concentration between 0.16 and 2.5 (striatum) and 0.6 and 10 (cortex) mg (original wet weight) of tissue per assay tube.

[^{3}H]MPP$^+$ accumulation is saturable (Fig. 1), with half-maximal uptake at 170 and 65 nM and V_{max} values of 2 and 0.1 nmol/g of tissue per min in striatal and cortical synaptosomes, respectively. Eadie–Hofstee analysis indicates a single component of [^{3}H]MPP$^+$ accumulation in both brain regions. In parallel experiments measuring the accumulation of [^{3}H]dopamine, the K_m for [^{3}H]dopamine uptake in striatum and cortex is 150 and 74 nM, respectively, while the respective V_{max} values for the two brain regions are 2 and 0.1 nmol/g of tissue per min. The 20-fold higher V_{max} for [^{3}H]MPP$^+$ and [^{3}H]dopamine in the corpus striatum than the cerebral cortex reflects the greater density of striatal catecholamine uptake sites. Thus, [^{3}H]MPP$^+$ has an affinity similar to that of dopamine for the dopamine and norepinephrine uptake systems and is accumulated by the two systems to the same extent. In contrast, uptake of [^{3}H]MPTP into striatal or cerebral cortical synaptosomal preparations is less than 5% that of [^{3}H]MPP$^+$ (Fig. 1).

Pharmacological Specificity of [^{3}H]MPP$^+$ Uptake. An extensive series of drugs inhibits [^{3}H]MPP$^+$ and [^{3}H]dopamine uptake into striatal homogenates with identical potencies (Table 1). These drugs also show the same potencies in inhibiting [^{3}H]MPP$^+$ or [^{3}H]norepinephrine uptake into cerebral cortical synaptosomes. Mazindol, the most potent uptake inhibitor examined, is 10 times more potent in cerebral cortex than in corpus striatum, whether [^{3}H]MPP$^+$ or [^{3}H]catecholamine is the uptake substrate. In contrast, desipramine is almost as potent as mazindol in the cerebral cortex, but only 1/1000th as potent in the corpus striatum, consistent with its selectivity for the norepinephrine rather than the dopamine uptake process. Citalopram, a very potent inhibitor of serotonin but not catecholamine uptake, is quite weak in reducing [^{3}H]MPP$^+$ accumulation in both striatum and cortex. MPP$^+$ has about the same potency as dopamine in both tissues. MPTP is only 1/10th as potent as MPP$^+$ in blocking [^{3}H]MPP$^+$ or [^{3}H]dopamine accumulation into the corpus striatum and the cerebral cortex.

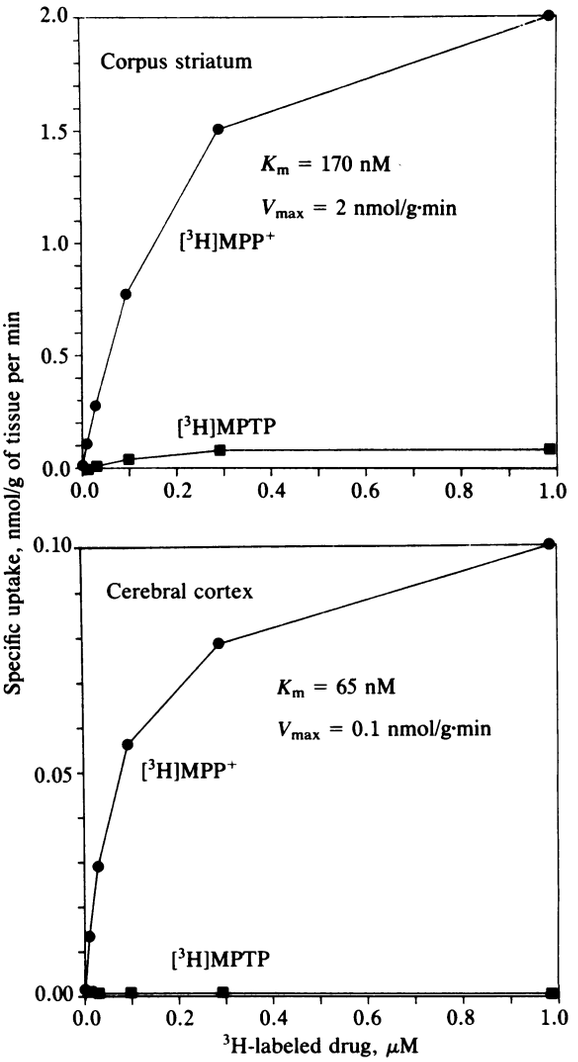

FIG. 1. Saturation of specific uptake of [^{3}H]MMP$^+$ and [^{3}H]MPTP into striatal and cortical synaptosomes. Each point represents the mean of two experiments each performed in triplicate. Nonspecific uptake was measured in the presence of 10 μM mazindol.

Autoradiographic Localization of [^{3}H]MPP$^+$ Accumulated by Brain Slices. Autoradiographic grains associated with accumulated [^{3}H]MPP$^+$ in brain slices are highly concentrated in the corpus striatum and nucleus accumbens, with much lower levels seen in the cortex (Fig. 2). Uptake in slices is sodium dependent and potently inhibited in the corpus striatum by mazindol but not by desipramine or citalopram (data not shown).

Prevention of MPTP Destruction of Dopamine Neurons in the Mouse Corpus Striatum by Inhibition of Dopamine Uptake. To monitor the destruction of dopamine neurons by MPTP, we have measured the binding of [^{3}H]mazindol, which labels uptake sites on dopamine neurons (refs. 30 and 31; Table 2). Treatment of male C57BL mice (20–25 g) with intraperitoneal injections of MPTP (30 mg/kg) once daily for 7 days lowers striatal [^{3}H]mazindol binding by 75%, similar to the extent of dopamine neuronal destruction monitored by levels of endogenous dopamine and by [^{3}H]dopamine uptake (8). Autoradiographic analysis confirms that MPTP mark-

Table 1. Potencies of drugs inhibiting [^{3}H]MPP$^+$ and [^{3}H]catecholamine uptake into rat striatal and cortical synaptosomes

	K_i, μM			
	Corpus striatum		Cerebral cortex	
Drug	[^{3}H]MPP$^+$	[^{3}H]Dopamine*	[^{3}H]MPP$^+$	[^{3}H]Norepinephrine†
Mazindol	0.020	0.016	0.003	0.002
Nomifensine	0.070	0.043	0.004	0.005*
Benztropine	0.095	0.098	0.31	0.22
DITA	0.103	0.076	0.015	0.012
EXP-561	0.14	0.24†	0.035	0.015‡
Dopamine	0.16	0.15§	0.12	0.074§
MPP$^+$	0.17	0.14§	0.064	0.12§
Norepinephrine	0.33	0.18	0.15	0.10
Serotonin	1.6	1.5	3.3	3.2
MPTP	2.3	5.1§	0.47	0.60§
Desipramine	5.2	8.1	0.007	0.007
Citalopram	33	37	31	5.8*

Drug effects were examined on [^{3}H]MPP$^+$ and [^{3}H]dopamine uptake into striatal and cortical synaptosomes prepared from rat brain. The values are the means of two to four experiments, each performed in triplicate, and varied less than 10%. DITA, [3′,4′-dichloro-2(2-imidazolin-2-yl-thio]-acetophenone hydrobromide; EXP 561, 1-amino-4-phenylbicyclo[2,2,2]octane.
*Values are from Hyttel (33) except where noted.
†Values are from Lee *et al.* (34) except where noted.
‡Values are from Wong *et al.* (35).
§Values were obtained by using [^{3}H]dopamine as described in *Materials and Methods*.

edly reduces [^{3}H]mazindol binding in the mouse caudate, nucleus accumbens, and olfactory tubercle (Fig. 3).

In accordance with the findings of Heikkila *et al.* (15), pretreatment with the MAO inhibitor pargyline (10 mg/kg) prevents MPTP-induced dopaminergic terminal loss as reflected by the preservation of [^{3}H]mazindol binding (Table 2). In addition, mazindol (10 mg/kg), a potent inhibitor of dopamine and norepinephrine uptake, given 30 min prior to each daily injection of MPTP, also prevents MPTP neurotoxicity. By contrast, similar treatment with desipramine, which blocks norepinephrine but not dopamine uptake, fails to prevent MPTP toxicity in the corpus striatum.

DISCUSSION

The present findings provide a model to explain the parkinsonism-like MPTP neurotoxicity (Fig. 4). MPTP first binds with receptor-like high affinity to MAO B, which transforms it to MPP$^+$. Active accumulation of MPP$^+$ but not MPTP by the dopamine neuronal uptake system then concentrates MPP$^+$ within dopamine neurons, accounting for their selective destruction. The prevention of MPTP-induced damage to the nigrostriatal dopaminergic neurons of mice by the dopamine uptake blocker mazindol demonstrates the requirement of MPP$^+$ transport for neurotoxicity.

Immunohistochemical studies have localized MAO B to rat brain astrocytes and serotonin-containing but not catecholaminergic neurons (25, 26). Autoradiographic studies show no change in the number of [^{3}H]MPTP binding sites in the substantia nigra of parkinsonian brains, although histology and other biochemical and autoradiographic markers show a dramatic loss of dopamine neurons (unpublished observations). Accordingly, MAO B presumably converts MPTP to MPP$^+$ in astrocytes, inconsistent with suggestions that MPP$^+$ is formed and trapped within neurons (12).

If MPTP is converted to MPP$^+$ within astrocytes, the metabolite must escape from glia by diffusion or perhaps by damaging the cells. Dopamine and norepinephrine neurons would then accumulate the toxic metabolite. Since MPP$^+$ is accumulated by the dopamine uptake system with the same K_m and V_{max} as dopamine, it should be concentrated within terminals as much as dopamine itself, to levels several

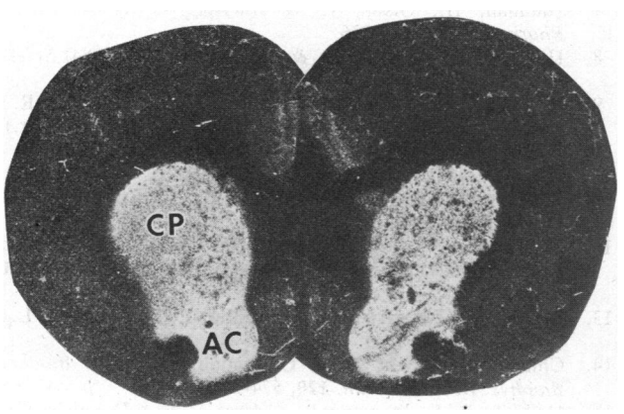

FIG. 2. Autoradiogram of accumulated [^{3}H]MPP$^+$ (5 nM) in a coronal section of rat brain. Note high density of [^{3}H]MPP$^+$-associated grains in the caudate–putamen (CP) and nucleus accumbens (AC). Uptake in the presence of 100 μM mazindol was negligible.

Table 2. Prevention of MPTP-induced destruction of dopamine neurons

	[^{3}H]Mazindol binding to striatal membranes		
	pmol/mg of protein		
Pretreatment	Saline	MPTP	% of control
Saline	2.3 ± 0.2 (3)	0.7 ± 0.1* (5)	28
Mazindol	2.1 ± 0.3 (5)	1.9 ± 0.3 (4)	93
Pargyline	2.4 ± 0.4 (4)	2.1 ± 0.1 (4)	87
Desipramine	2.3 ± 0.4 (4)	0.7 ± 0.2* (5)	32

Male C57BL mice were injected once daily for 7 days with MPTP (30 mg/kg) or saline. Thirty minutes prior to this injection mice received mazindol (10 mg/kg), pargyline (10 mg/kg), desipramine (10 mg/kg), or saline. Seven days after the last drug treatment, the mice were killed by cervical dislocation and the corpora striata were assayed for [^{3}H]mazindol binding as described previously (31). [^{3}H]Mazindol (5 nM) was incubated with striatal membranes in the presence of 300 mM NaCl. Values represent mean ± SD of (n) determinations.
*$P < 0.001$ compared with saline control.

thousandfold above the extracellular concentration (36). Exactly how MPP^+ then damages dopamine neurons is unclear. MPP^+ is cytotoxic (37) and can interfere with mitochondrial respiration (38).

The caudate–putamen, nucleus accumbens, and locus ceruleus contain the highest number and density of catecholamine uptake sites in the brain (30). Thus, the enhancement by MPTP of 2-deoxyglucose accumulation into the substantia nigra, the ventral tegmental area containing the cell bodies of those dopamine neurons terminating in the nucleus accumbens, and the locus ceruleus (39) corresponds with the regions expected to accumulate MPP^+ most avidly. The accumulation of MPP^+ by noradrenergic neurons fits with the marked effects of MPTP on norepinephrine metabolism both in the brain (5, 7, 10) and in the peripheral sympathetic nervous system (10). The accumulation of MPP^+ by mesolimbic dopaminergic neurons is supported by the depletion of [^{3}H]mazindol binding to dopamine uptake sites in the nucleus accumbens. Further support for MPTP toxicity to cells with catecholamine uptake systems comes from a recent report of MPTP-induced damage to dopamine neurons in the rabbit retina (40) and MPTP treatment selecting for mutant PC-12 cells lacking catecholamine uptake (41).

Although metabolic effects of MPTP are seen in many catecholaminergic systems (5, 7, 10), cell degeneration in monkeys is most prominent in the nigrostriatal dopaminergic system even though mesolimbic neurons concentrate MPP^+ as well as nigrostriatal neurons (5, 8). Perhaps species differences in the regional density of catecholamine uptake coupled with regional differences in MAO B density can account for the specific pattern of neuronal degeneration seen in primates. Alternatively, the mesolimbic dopamine neurons are less sensitive to the toxic action of intraneuronal MPP^+.

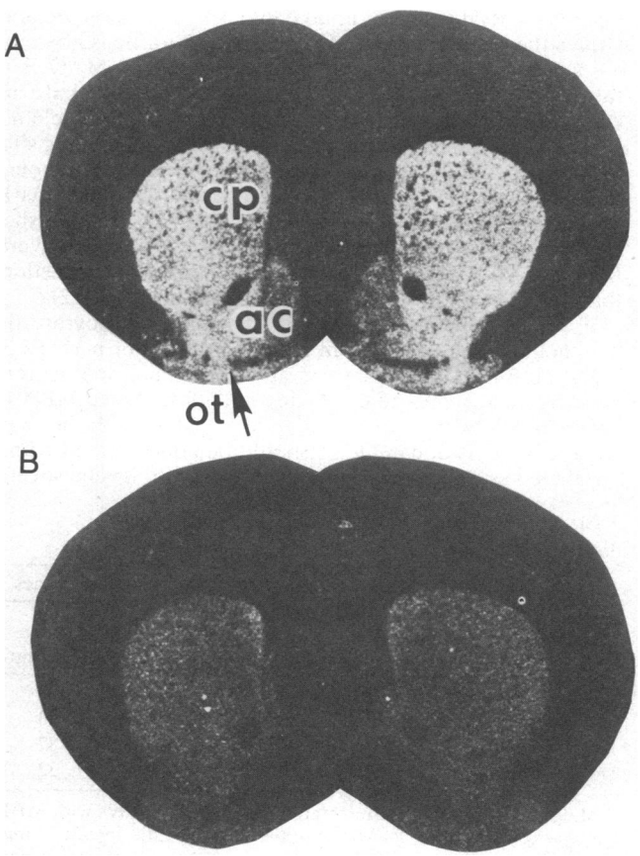

FIG. 3. Autoradiography of [^{3}H]mazindol (10 nM) binding to coronal sections of brain from C57BL mice. (*A*) Control animal, (*B*) animal treated daily for 7 days with MPTP (30 mg/kg). See legend to Table 2 for details. Mice were killed 7 days after the last injection. Note high binding to caudate–putamen (cp), nucleus accumbens (ac), and olfactory tubercle (ot) in normal mouse (*A*) and the depletion of binding to all three areas in MPTP-treated mouse (*B*).

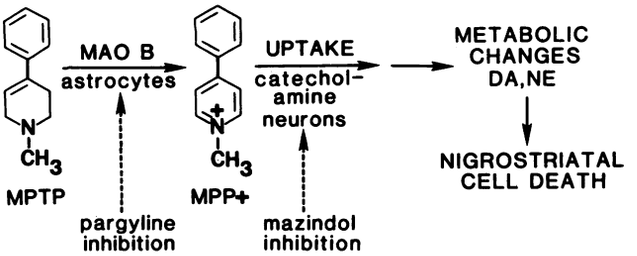

FIG. 4. Proposed mechanism of MPTP neurotoxicity. DA, dopamine; NE, norepinephrine.

It has been suggested that environmental toxins similar to MPTP cause idiopathic parkinsonism (42). The accumulation of MPP^+ into norepinephrine neurons and into mesolimbic dopaminergic neurons has bearing on these speculations, since in idiopathic parkinsonism, norepinephrine neurons in the locus ceruleus and dopamine neurons in the ventral tegmental areas as well as the substantia nigra are affected (43–45).

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1. Davis, G. C., Williams, A. C., Markey, S. P., Ebert, M. H., Caine, E. D., Reichert, C. M. & Kopin, I. J. (1979) *Psychiatry Res.* **1**, 249–254.
2. Langston, J. W., Ballard, P., Tetrud, J. W. & Irwin, I. (1983) *Science* **219**, 979–980.
3. Langston, J. W. & Ballard, P. (1984) *Can. J. Neurol. Sci.* **11**, 160–165.
4. Burns, R. S., Markey, S. P., Phillips, J. M. & Chiueh, C. C. (1984) *Can. J. Neurol. Sci.* **11**, 166–168.
5. Burns, R. S., Chiueh, C. C., Markey, S. P., Ebert, M. H., Jacobowitz, D. M. & Kopin, I. J. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 4546–4550.
6. Langston, J. W., Forno, L. S., Rebert, C. S. & Irwin, I. (1983) *Brain Res.* **292**, 390–394.
7. Hallman, H., Olson, L. & Jonsson, G. (1984) *Eur. J. Pharmacol.* **97**, 133–136.
8. Heikkila, R. E., Hess, A. & Duvoisin, R. C. (1984) *Science* **224**, 1451–1453.
9. Heikkila, R. E., Cabbat, F. S., Manzino, L. & Duvoisin, R. C. (1984) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **43**, 743 (abstr.).
10. Wallace, R. A., Boldry, R., Schmittgen, T., Miller, D. & Uretsky, N. (1984) *Life Sci.* **35**, 285–291.
11. Sershan, H., Reith, M. E. A., Hashin, A. & Lajtha, A. (1984) *Eur. J. Pharmacol.* **102**, 175–178.
12. Markey, S. P., Johannessen, J. N., Chiueh, C. C., Burns, R. S. & Herkenham, M. A. (1984) *Nature (London)* **311**, 464–467.
13. Langston, J. W., Irwin, I., Langston, E. B. & Forno, L. S. (1984) *Neurosci. Lett.* **48**, 87–92.
14. Chiba, K., Trevor, A. & Castagnoli, N. (1984) *Biochem. Biophys. Res. Commun.* **120**, 574–578.
15. Heikkila, R. E., Manzino, L., Cabbat, F. S. & Duvoisin, R. C. (1984) *Nature (London)* **311**, 467–469.
16. Langston, J. W., Irwin, I. & Langston, E. B. (1984) *Science* **225**, 1480–1482.
17. Javitch, J. A., Uhl, G. R. & Snyder, S. H. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 4591–4595.
18. Chiueh, C. C., Markey, S. P., Burns, R. S., Johannessen,

J. N., Pert, A. & Kopin, I. J. (1984) *Eur. J. Pharmacol.* **100,** 189–194.
19. Boyce, S., Kelly, E., Reavill, C., Jenner, P. & Marsden, C. D. (1984) *Biochem. Pharmacol.* **33,** 1747–1752.
20. Chiueh, C. C., Markey, S. P., Burns, R. S., Johannessen, J., Jacobowitz, D. M. & Kopin, I. J. (1983) *Pharmacologist* **25,** 131.
21. Steranka, L. S., Polite, L. N., Perry, K. W. & Fuller, R. W. (1984) *Res. Commun. Subst. Abuse* **4,** 315–323.
22. Sahgal, A., Andrews, J. S., Biggins, J. A., Cordy, J. M., Edwardson, J. A., Keith, A. B., Turner, J. D. & Wright, C. (1984) *Neurosci. Lett.* **48,** 179–184.
23. Wieczorek, C. M., Parsons, B. & Rainbow, T. C. (1984) *Eur. J. Pharmacol.* **98,** 453–454.
24. Parsons, B. & Rainbow, T. C. (1984) *Eur. J. Pharmacol.* **102,** 375–377.
25. Levitt, P., Pintar, J. E. & Breakefield, X. O. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 6385–6389.
26. Pintar, J. E., Levitt, P., Salach, J. I., Weyler, W., Rosenberg, M. B. & Breakefield, X. O. (1983) *Brain Res.* **276,** 127–139.
27. Javitch, J. A. & Snyder, S. H. (1984) *Eur. J. Pharmacol.* **106,** 441–447.
28. McPherson, G. A. (1983) *Comput. Programs Biomed.* **17,** 107–114.
29. Unnerstall, J. R., Niehoff, D. L., Kuhar, M. J. & Palacios, J. M. (1982) *J. Neurosci. Methods* **6,** 59–73.
30. Javitch, J. A., Strittmatter, S. M. & Snyder, S. H., *J. Neurosci.*, in press.
31. Javitch, J. A., Blaustein, R. O. & Snyder, S. H. (1984) *Mol. Pharmacol.* **26,** 35–44.
32. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265–275.
33. Hyttel, J. (1982) *Neuropsychopharmacol. Biol. Psychol.* **6,** 277–295.
34. Lee, C.-M., Javitch, J. A. & Snyder, S. H. (1982) *J. Neurosci.* **2,** 1515–1525.
35. Wong, D. T., Mollory, B. B. & Bymaster, F. B. (1977) *Neuropharmacology* **16,** 11–15.
36. Hamberger, B. & Masuoka, D. (1965) *Acta Pharmacol. Toxicol.* **22,** 363–368.
37. Swartzbek, R. A. (1974) *Proc. Br. Weed Control Conf.* **12,** 851–856.
38. Lewin, R. (1984) *Science* **225,** 1460–1462.
39. Palacios, J. M. & Wiederhold, K. M. (1984) *Brain Res.* **301,** 187–191.
40. Wong, C. G., Tucker, G. S. & Hamasaki, D. I. (1984) *Neurosci. Abstr.* **10,** 890.
41. Bitler, C. M., Zhang, M.-B. & Howard, B. D. (1984) *Neurosci. Abstr.* **10,** 364.
42. Calne, D. B. & Langston, J. W. (1983) *Lancet* **ii,** 1457–1459.
43. Alvord, E. C., Jr., Forno, L. S., Kusske, J. A., Kauffman, R. J., Rhodes, J. S. & Goetowski, C. R. (1974) in *Second Canadian-American Conference on Parkinson's Disease, Advances in Neurology*, eds. McDowell, F. H. & Barbeau, A. (Raven, New York), Vol. 5, pp. 175–193.
44. Greenfield, J. G. & Bosanquet, F. D. (1953) *J. Neurol. Neurosurg. Psychiatry* **16,** 213–226.
45. Uhl, G. R., Hedreen, J. C. & Price, D. L. (1983) *Neurology* **33,** 131.