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The Effect of Prenatal Treatment With MPTP or MPP^+ on the Development of Dopamine-Mediated Behaviors in Rats

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WEISSMAN, E. M., A. B. NORMAN, S. F. CALDERON, E. M. ZUBRYCKI, M. M. EL-ETRI, M. T. SHIPLEY AND P. R. SANBERG. *The effect of prenatal treatment with MPTP or MPP^+ on the development of dopamine-mediated behaviors in rats.* PHARMACOL BIOCHEM BEHAV **34**(3) 545–551, 1989.—Systemic exposure to the neurotoxin MPTP produces a Parkinsonian syndrome in man and primates, but not in adult rats. However, embryonic rat dopamine neurons in cell cultures are selectively destroyed by MPTP. This study examined whether similar effects on dopamine neurons occur in vivo, by studying dopamine-mediated behaviors in rats prenatally treated with MPTP or its active metabolite MPP^+. Pregnant rats were injected daily with MPTP, MPP^+, or vehicle from gestational day (E)13 until birth. There were time-dependent increases in spontaneous locomotor and rearing activity. Offspring of both the MPTP and MPP^+ groups were hyporesponsive to d-amphetamine (1 mg/kg IP) at postnatal day 21. This hyporesponsiveness persisted at postnatal day 50 in the pups from MPTP-treated mothers. However, the striatal concentration of dopamine and its metabolites DOPAC and HVA were not significantly affected by the prenatal MPTP or MPP^+ treatments. Both MPTP and MPP^+ groups had significantly increased stereotypic responses to apomorphine (0.2 mg/kg SC) on both postnatal days 21 and 50. These results demonstrated persistent postsynaptic supersensitivity to dopaminergic agonists following prenatal MPTP/MPP^+ treatment. That fetal rats develop long-term sequelae after prenatal exposure to MPTP/MPP^+ suggests a different sensitivity of the immature rat dopamine neurons than in adult rats. Understanding this difference may provide useful information in the development of animal models of Parkinson's Disease.

MPTP MPP^+ Dopamine development Apomorphine Amphetamine Supersensitivity
Locomotor activity Stereotypy Parkinson's Disease HPLC

SYSTEMIC exposure to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) produces a Parkinsonian syndrome in man and in primates. The mechanism by which selective damage occurs to the dopaminergic neurons with cell bodies in the substantia nigra has been the subject of much study. MPTP neurotoxicity is dependent on its conversion to 1-methyl-4-phenylpyridine (MPP^+) by monoamine oxidase B (MAO-B) in primates (11) and mice (6,13). MPP^+ is accumulated by striatal synaptosomes via the dopamine (DA) neuronal uptake system (7, 8, 21, 22). The mechanism of subsequent cellular toxicity remains unclear, but may involve inhibition of the oxidation of selective substrates in mitochondrial respiration (4,24).

Rats are much less sensitive to the neurotoxic effects of MPTP than are primates (1,5). This may be a result of decreased availability of the toxic metabolite, MPP^+, to nigrostriatal tissues in rats. One possible explanation for this difference is that rodents clear MPTP and its toxic metabolites much more rapidly from brain tissues than primates (9). It has also been suggested MAO-B may function as an enzymatic blood-brain barrier (10). The cerebral microvasculature in rat contains more MAO-B than in the primate. MAO-B oxidizes MPTP to MPP^+, which may cause local damage to the endothelial cells, but prevent further distribution of MPP^+ to neural tissue and preclude the more detrimental neurotoxic damage. Alternatively neuromelanin has been shown to bind MPTP and MPP^+, and may serve as a reservoir which slowly releases toxic MPP^+. Albino rats have a lower concentration of neuromelanin in the substantia nigra than primates, possibly resulting in decreased accumulation of MPTP or MPP^+ in the

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substantia nigra, thereby decreasing subsequent neurotoxicity (3).

Although DA neurons in mature rat brain are resistant to MPTP in vivo, the drug does produce selective toxic effects on explants of rat embryonic dopaminergic mesencephalic cells in culture (14,21). It is possible that the in vitro sensitivity of embryonic rat dopaminergic neurons to MPTP also occurs in vivo. The fetal environment features an immature blood-brain barrier and altered metabolism which could permit the more rapid production and distribution of MPP^+ to striatal neurons, with subsequent increased neurotoxicity. We, therefore, assessed the effects of in vivo prenatal exposure on the nigrostriatal DA system by studying DA-mediated behaviors in rats following chronic prenatal exposure to MPTP or MPP^+.

METHOD

Timed pregnant Sprague-Dawley rats (Zivic-Miller) were injected daily from embryonic day 13 until birth with either MPTP or MPP^+ (10 mg/kg IP, Research Biochemicals Inc., Natick, MA) or an equal volume of 0.9% normal saline vehicle. Two pregnant females comprised each treatment group. Both male and female offspring from the six litters were weighed on days 5, 8, 10, 12 and 14, and the number of pups whose eyes were opened were recorded daily.

Both spontaneous nocturnal activity and pharmacologic responses were measured in offspring in postnatal day 21. Spontaneous nocturnal activity and pharmacologic responses were also measured in a separate group of offspring from the same litters on postnatal day 50. Spontaneous nocturnal activity was recorded overnight in computerized Digiscan-16 Animal Activity Monitors Version 2.5 [Omnitech Electronics, Columbus, OH; (18)] for 12 hours at hourly intervals from 6 p.m.–6 a.m. Only data from the peak activity period 8 p.m.–10 p.m. were used in data analysis. The monitors measured three different types of activity: 1) ambulation, 2) rearing, and 3) stereotypy. The parameters of ambulation (18) and their units were horizontal activity (counts), total distance (cm), movement time (sec), rest time (sec), average speed (cm/sec), number of movements (counts), and average distance per move (cm). The parameters of rearing (20) were vertical activity (counts), number of vertical movements (counts), and vertical time (sec). Parameters of nonambulatory movements (19) were stereotypy time (sec), and number of stereotypic movement (counts).

Pharmacologic responses were assessed the day following the spontaneous activity recording using the same subjects. Subjects were divided into two test groups comprising approximately one-half the offspring of each treatment group (prenatal MPTP, MPP^+, or control). After being habituated to the boxes for 30 minutes, the first test group received d-amphetamine (1 mg/kg IP, Sigma Chemical Co., St. Louis, MO) and locomotor activity was measured in Digiscan activity monitors for ninety minutes using five-minute recording intervals. The second test group received apomorphine (0.2 mg/kg SC, Sigma Chemical Co., St. Louis, MO) and visual stereotypy ratings according to the scale of Creese and Iversen (2) were recorded for the first minute of each five-minute observation period for a total of 45 minutes. Three days later, the test groups were reversed. Group 1 received apomorphine and group 2 was given d-amphetamine in the identical doses and manner described above.

Statistics

Mean weights were compared using a Student's *t*-test at each day. Eye opening data were analyzed using one-way analysis of variance comparing the mean day of eye opening for the three treatment groups. Data from individual pups were considered as independent variables in these analyses. For spontaneous nocturnal activity, Digiscan locomotor variables from each of the three groups, i.e., MPTP, MPP^+, and control, were compared using analysis of variance of the mean values during the peak activity period. Post hoc comparison tests were used to determine the significance between groups. In pharmacologic testing, the order in which drugs were administered did not alter behavioral responses (data not shown), therefore, data from the two groups receiving the same drug were combined for further data analysis. d-Amphetamine data was analyzed using a two-way analysis of variance (ANOVA) of the mean values for each activity variable for eight 5-minute intervals following drug administration. Apomorphine-induced stereotypy ratings were analyzed using nonparametric statistics. A Kruskall-Wallis ANOVA compared mean stereotypy scores for 40 minutes following drug administration.

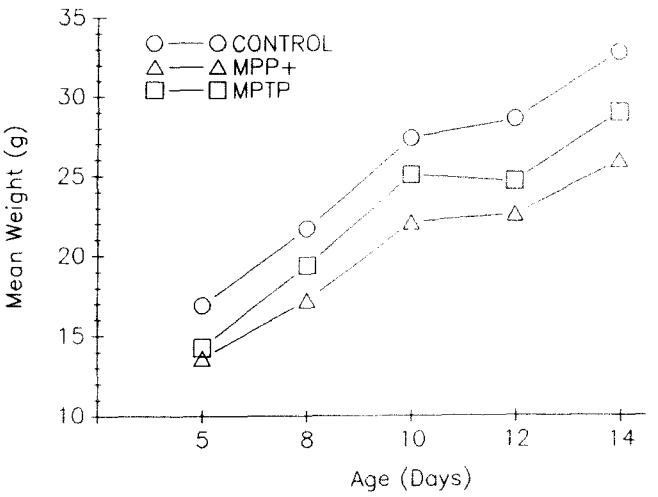

FIG. 1. Mean body weight of rat pups treated with MPTP, MPP^+, or vehicle. Values represent mean weights (g) of all rats in each treatment group. At each day, both MPTP and MPP^+ treatment groups weighed significantly less than controls ($p<0.001$).

Chromatographic Conditions and Procedure

HPLC apparatus. HPLC determinations were achieved using a

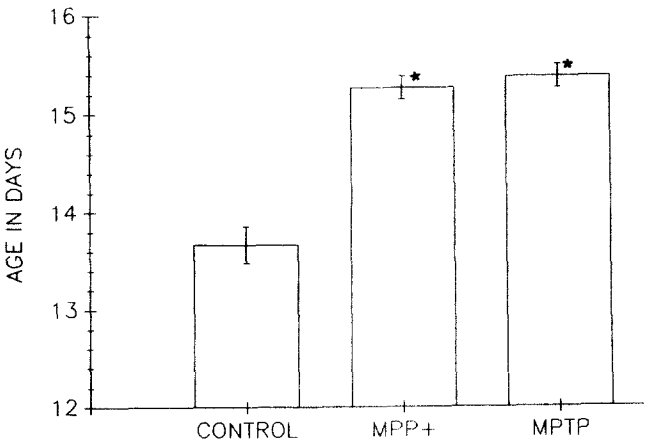

FIG. 2. Age of eye opening. Bars represent mean age of eye opening in days ±SEM in rats treated with prenatal MPTP, MPP^+, or vehicle (*$p<0.001$).

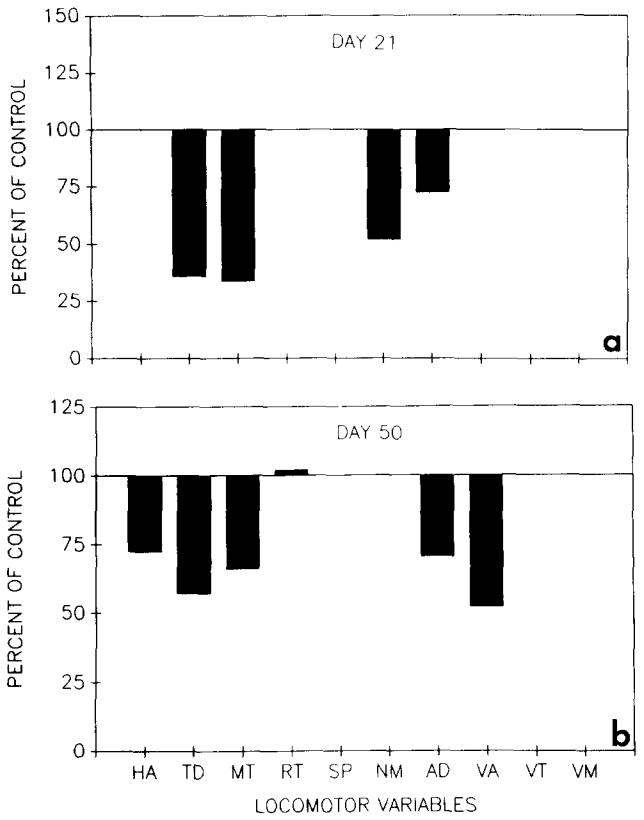

FIG. 3. Activity Print: Spontaneous nocturnal activity during the peak activity period, 8 p.m.–10 p.m., in rats treated prenatally with MPTP. Bars represent percent of control values for those variables that differed significantly ($p<0.05$) from controls as measured in Digiscan Activity Monitors. All bars represent variables which were significantly different from control values. At day 21, mean control values ±SEM were: HA 13,868±2448; TD 873±219; MT 204±50; RT 7010±56; SP 4.3±0.2; NM 575±106; AD 1.4±0.1; VA 1404±420; VT 672±196; VM 317±89. At day 50, mean control values ±SEM were: HA 9896±793; TD 3301±481; MT 315±36; RT 6860±51; SP 10.2±0.4; NM 300±31; AD 10.5±0.7; VA 373±67; VT 176±37; VM 51±9 (HA: horizontal activity; TD: total distance; MT: movement time; RT: rest time; SP: average speed; NM: number of movements; AD: average distance per move; VA: vertical activity; VT: vertical time; VM: number of vertical movements).

computerized Bioanalytical Systems (West Lafayette, IN) Model 200 Liquid Chromatograph. The reversed-phase (C_{18}) ion-pair separation was performed on a 100×3.2 mm Biophase ODS 3-μm column (Bioanalytical Systems). Electrochemical detections were obtained using a Bioanalytical Systems Model LC-4B electrochemical detector and a glassy carbon working electrode which is kept at a constant potential of 0.75 or 0.70 V vs. Ag/AgCl reference electrode. The sensitivity of the detector was kept at 10.0 nA full scale. The column and the detector were kept in a constant temperature environment of 40°C and 41°C, respectively.

Materials. Monochloroacetic acid, octyl sodium sulfate, norepinephrine bitartarate (NE), 3-hydroxytyramine hydrochloride (DA), homovanillic acid (HVA), serotonin (5-HT), 5-hydroxyindole-3-acetic acid (5-HIAA), 3,4-dihydroxyphenylacetic acid (DOPAC), 5-hydroxytryptophan (5-HTP), L-*B*-3,4,dihydroxyphenylanaline (L-DOPA) and the internal standard 3,4-dihydroxybenzylamine hydrobromide (DHBA) were obtained from Sigma Chemical Co. L-cysteine, Na_2EDTA, perchloric acid and acetonitrile (HPLC grade) were obtained from Fisher Scientific.

The striatum. A low ionic-strength buffer of pH 2.75 was used for the separations of the eight biogenic amines plus the internal standard (DHBA) in about 20-min run. The mobile phase consists of 0.06 M monochloroacetic acid, 1.3 mM octyl sodium sulfate and 0.1 mM Na_2EDTA in the aqueous phase and 4.2% acetonitrile in the organic phase. The mobile phase was filtered using a 0.22 micron filter, degassed with helium and kept at 35°C. The flow rate was kept at 1 ml/min. Working standard solutions containing DOPAC, HVA, L-DOPA, 5-HT and NE (5 ng/50 μl), DA (25 ng/50 μl) and 5-HIAA, and 5-HTP (2.5 ng/50 μl) were made up in 0.1 M perchloric acid containing 0.1% cysteine as an antioxidant. The internal standard solution containing 5 ng/50 μl of DHBA was also made up in 0.1 M perchloric acid containing 0.1% cysteine.

Extractions of the biogenic amines were carried out as follows. Rats were decapitated and the striata as well as the olfactory bulbs were rapidly dissected and immediately frozen on dry ice, then stored at −80°C until assay. The striata of each rat were individually homogenized in a 750 ml of 0.1 M perchloric acid containing 0.10% cysteine and 15 ng of the internal standard (DHBA). Homogenization was performed in Eppendorf polypropylene tubes at 0°C for 1 min. Homogenates were centrifuged at 1360×g for 5 min at 4°C. For HPLC injection, 100 μl of the supernatant were diluted with a 100 μl of the homogenization solvent. Typically, 10 μl of this solution were injected into the HPLC. For peak height measurements, the same volume of 10 μl of standards containing a constant amount (0.2) of the internal standard DHBA were injected. Standards were injected between the runs to account for any loss in sensitivity of the working electrode which was kept at 0.75 V. Quantitation was done by comparing peak heights of unknowns to standards.

The retention times of the biogenic amines and the internal standard under the described chromatographic conditions are: DOPAC (1.8 min), L-DOPA (2.1 min), NE (2.5 min), 5-HIAA (2.9 min), DHBA (4.7 min), HVA (5.1 min), 5-HTP (6.2 min), DA (7.7 min) and 5-HT (21 min).

The olfactory bulb. For the determination of the biogenic amines in the olfactory bulb, the above described procedure was modified as follows. The olfactory bulbs of each rat were homogenized in a 200 ml of 0.1 M perchloric acid containing 0.1% cysteine and 4 ng of DHBA as the internal standard. Extractions of the amines were done in a similar manner to that used in the case of the striata. Under the same chromatographic conditions used for the analyses of the biogenic amines in the striata, it was not possible to detect the earlier eluting peaks including DOPAC. Therefore, full measurements of the amines in the olfactory bulb were done in two separate runs. In one run, the same mobile phase described above was used in the determination of the indoles including serotonin but at a constant potential of 0.70 V.

For the determinations of catecholes including DOPAC, a major metabolite of dopamine, the above mobile phase was increased in pH to 2.9 but the acetonitrile composition was lowered to 1.5% and that of octyl sodium sulfate was also decreased to 1.25 mM to improve separation. The potential of the working electrode was kept constant (0.70 V).

The retention times of the amines using the modified mobile phase at a flow rate of 0.9 ml/min are: DOPAC (3.1 min), L-DOPA (3.7 min), NE (4.1 min), 5-HIAA (5.6 min), DHBA (9.2 min), HVA (10.6 min), 5-HTP (12.9 min) and DA (18.5 min). Under these conditions, serotonin is retained very strongly on the column. When eluted, serotonin appears broad and of a retention time of about 50 min.

A working standard solution containing 2.5 ng/50 μl of DOPAC, L-DOPA, NE, 5-HIAA, HVA, DA, 5-HTP and 5 ng/50 μl of 5-HT were made up as described earlier. Typically, 15 μl of either individual sample or standard containing a constant

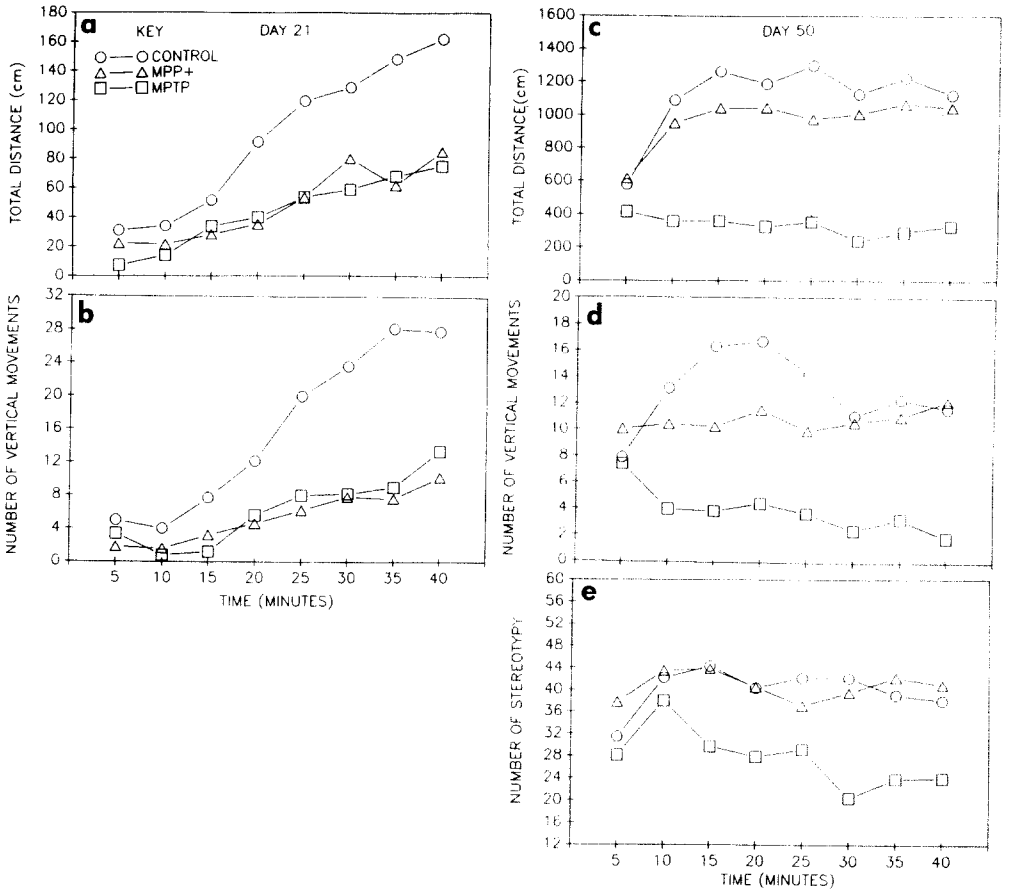

FIG. 4. Amphetamine-stimulated activity in pups treated with prenatal MPTP, MPP^+, or vehicle, at postnatal days 21 and 50. Graphs illustrate representative trends in ambulation and rearing behavior at postnatal day 21, and ambulation, rearing, and stereotypic behavior at postnatal day 50, as measured in Digiscan Activity Monitors (see text). On day 21, both MPTP and MPP^+ groups are significantly hyporesponsive compared to controls. On day 50, the MPP^+ group is similar to controls and only the MPTP treatment group is hyporesponsive to d-amphetamine.

amount (0.3 ng) of the internal standard DHBA were injected into the HPLC. Injections of standards and quantitations were done as described earlier.

RESULTS

Development

Prenatal exposure to either MPTP or MPP^+ produced a significant decrease in body weight compared to controls on all measured days (p's<0.001, Fig. 1). Age of eye opening was delayed by approximately 1.5 days compared to controls (analysis of variance, $p<0.001$; Fig. 2). Prenatal treatment with MPTP/MPP^+ did not affect the number of pups per litter (data not shown).

Spontaneous Activity

As shown in Fig. 3, offspring treated prenatally with MPTP displayed significant hypoactivity during the peak nocturnal activity period (8–10 p.m.). This effect was more pronounced at postnatal day 50 than at postnatal day 21. At both postnatal days 21 and 50, most parameters of ambulation were decreased compared to controls, including total distance, movement time, number of movements, and average distance per movement ($p\leq0.05$; Fig. 3a). There were no significant differences in rearing and stereotypy between MPTP and control groups. However, at postnatal day 50, rearing behavior was also affected, showing decreased vertical activity. An additional ambulation parameter, horizontal activity, was also decreased ($p<0.05$; Fig. 3b).

Less pronounced hypoactivity compared to controls in rats receiving treatment prenatally with MPP^+ did not reach significance at either test day (data not shown).

Drug-Induced Behaviors

As shown in Table 1, at postnatal day 21, both MPTP and MPP^+ treatment groups were hyporesponsive to amphetamine compared to controls, as evidenced by a decrease in ambulatory and rearing behaviors. Stereotypy measures were not significantly different from controls.

However, at postnatal day 50, only the prenatal MPTP group was significantly hyporesponsive when treated with d-amphetamine. Ambulation, rearing, and stereotypy were all decreased compared to controls. In contrast, rats treated prenatally with MPP^+ showed no significant difference in their response to amphetamine compared to controls. These data are further illustrated in Fig. 4 which shows the time course of the effect of a dose of amphetamine on activity. Representative parameters of ambu-

TABLE 1

AMPHETAMINE-STIMULATED BEHAVIOR IN MPP^+- AND MPTP-TREATED ANIMALS[1]

	Control	MPP^+	MPTP
Day 21			
TD	766.8 ± 156.5	386.6 ± 141.2*	360.4 ± 100.3*
MT	239.2 ± 57.8	198.0 ± 101.9	143.0 ± 60.5*
VA	366.6 ± 115.3	139.0 ± 73.8*	105.6 ± 37.6*
VT	179.0 ± 66.1	51.8 ± 27.8*	46.6 ± 21.6*
VM	128.5 ± 42.8	43.0 ± 22.8*	49.6 ± 18.2*
Day 50			
HA	16771.4 ± 2772.4	15399.4 ± 2843.4	6631.7 ± 2741.1*
TD	8897.0 ± 1868.5	7752.5 ± 1750.3	2671.8 ± 1310.4*
MT	694.1 ± 137.1	700.4 ± 157.1	288.3 ± 137.3*
AD	167.4 ± 31.9	142.4 ± 33.3	50.1 ± 18.7*
VA	656.2 ± 182.0	407.4 ± 123.8	153.1 ± 83.2*
VT	164.0 ± 37.3	129.2 ± 35.4	56.4 ± 31.52*
VM	103.3 ± 25.8	85.9 ± 25.7	31.0 ± 16.7*
ST	536.0 ± 74.5	519.6 ± 77.3	297.4 ± 97.3*
NS	319.8 ± 31.7	325.4 ± 31.9	221.1 ± 60.7*

[1]Data represent mean responses ±S.E.M., *$p<0.05$. (TD: total distance; MT: movement time; VA: vertical activity; VT: vertical time; VM: number of vertical movements; HA: horizontal activity; AD: average distance travelled per move; ST: stereotypy time; NS: number of stereotypies.)

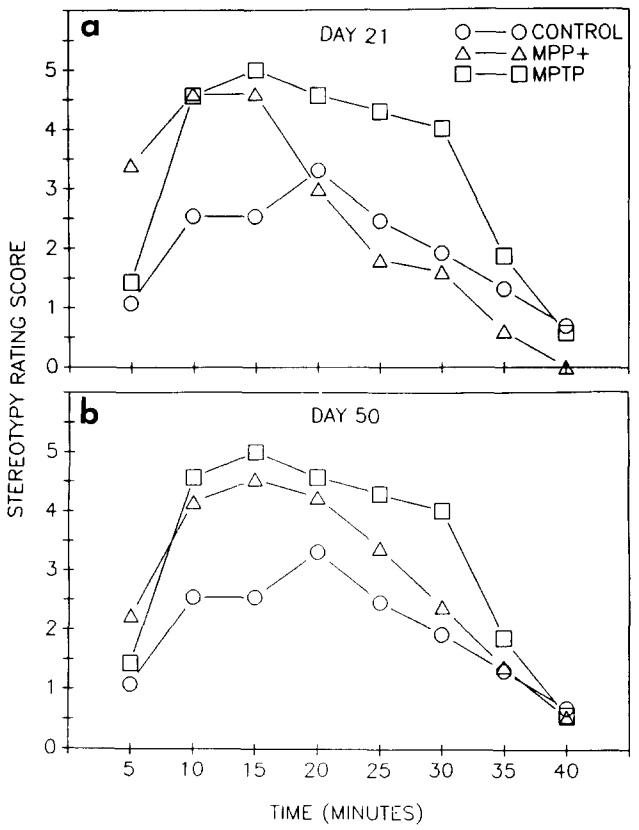

FIG. 5. Apomorphine-induced stereotypy in rats treated prenatally with MPTP, MPP^+, or vehicle, at postnatal days 21 and 50. Graphs represent visual stereotypy ratings for the first minute of each five-minute interval. The mean of the total stereotypy ratings over forty minutes were compared using nonparametric statistics and increases in stereotypy were significant for both treatment groups on both test days ($p<0.02$).

lation and rearing are shown at postnatal days 21 (Fig. 4a and b) and 50 (Fig. 4c and d). The decrease in stereotypy only reached significance at day 50 and is shown in Fig. 4e. Patterns for variables shown (total distance, number of vertical movements, and number of stereotypy) are typical for the other variables which were also significantly different from controls.

Rats exposed in utero to MPTP, MPP^+, or vehicle were also treated with apomorphine 0.2 mg/kg SC at postnatal days 21 (Fig. 5a) or 50 (Fig. 5b). Stereotypy peaked at 10–15 minutes postinjection and subsided in approximately forty minutes. Both treatment groups demonstrated significantly higher stereotypy scores over 40 minutes compared to controls on both test days [day 21, $H(2,16)=7.48$, $p<0.025$; day 50, $H(2,33)=10.1$, $p<0.01$].

As shown in Table 2, the striatal concentrations of DA in control rats were approximately 4.4 μg/g original wet weight of tissue. This is comparable to DA levels previously observed in the striatum of rat pups at three weeks postnatal (16). The striatal DA concentrations were not significantly different in rat pups treated prenatally with MPTP or MPP^+. Furthermore, the striatal levels of the DA metabolite DOPAC were not significantly different in the MPTP or MPP^+-treated rats compared to controls. The striatal concentration of another metabolite of DA, HVA, was also not significantly different from control values in MPP^+-treated rats. There was a statistically significant decrease from control levels in the concentration of HVA in the striatum of MPTP-treated rats, although the decrease was only 12% lower than control values. We also investigated the effects of prenatal MPTP and MPP^+ treatment on a different population of dopaminergic neurons located in the rat olfactory bulb (12). There were also no significant effects of MPP^+ or MPTP treatment on the level of DA or its metabolites in the olfactory bulb (Table 2).

DISCUSSION

Exposure in utero to either MPTP or its metabolite MPP^+ resulted in short-term developmental delays, and produced long-term alterations in behavioral reactions to apomorphine and

TABLE 2

EFFECT OF PRENATAL TREATMENT WITH MPTP AND MPP^+ ON DOPAMINE DOPAC AND HVA LEVELS IN NEONATAL RAT STRIATUM AND OLFACTORY BULB

	Dopamine (ng/g tissue)	DOPAC (ng/g tissue)	HVA (ng/g tissue)
Striatum			
Control (n = 10)	4361.5 ± 156.5	1039.1 ± 44.0	880.4 ± 35.1
MPTP (n = 8)	4084.0 ± 103.7	1027.9 ± 69.0	774.9 ± 16.5*
MPP^+ (n = 10)	4194.9 ± 97.9	1136.7 ± 66.2	868.2 ± 90.5
Olfactory Bulb			
Control (n = 10)	47.8 ± 7.6	22.7 ± 2.8	55.0 ± 2.6
MPTP (n = 8)	46.8 ± 3.4	21.7 ± 2.2	52.4 ± 2.6
MPP^+ (n = 10)	45.5 ± 7.2	23.6 ± 3.1	52.7 ± 5.2

Pregnant rats were injected daily with MPTP or MPP^+ (10 mg/kg IP) or saline as control from day 12 of gestation until parturition. At 23 days postnatal, the pups were decapitated, the striata and olfactory bulbs removed and quickly frozen and stored at −80° until assay. Individual striata and olfactory bulbs were prepared and amines assayed by HPLC as described in the Method section.

*Significantly different from control levels, $p<0.01$, two-tailed t-test.

amphetamine. Behavioral studies in mice suggest that there is a correspondence between behavioral and biochemical responses to MPTP/MPP^+ treatment (23). We, therefore, studied spontaneous and drug-induced behaviors in rats exposed to MPTP and MPP^+ in utero to examine functional changes.

Prenatal exposure to either MPTP or MPP^+ produced generalized delays in early development. Both drugs caused decreased birth weight and delayed eye opening. Body hair growth, though not quantified, was also delayed. That both MPTP and MPP^+ produced these generalized effects suggests both drugs crossed the placenta and that they operated via similar mechanisms. These developmental delays were not associated with visibly observable long-term changes in adult development.

MPP^+ is the active metabolite of MPTP, and in the same doses as MPTP might be expected to produce an equal or greater effect. However, MPTP produced a more pronounced behavioral change than MPP^+. MPP^+ caused less severe damage, evidenced by the lack of a significant decrease in spontaneous nocturnal behavior relative to controls observed following prenatal MPP^+ treatment (in contrast to a significant decrease in spontaneous behavior observed in the MPTP group). This result might be explained by considering drug distribution. Since MPP^+ is a charged molecule, it is possible that more of the drug was bound to protein, reducing its free concentration. Being a charged molecule, it is also possible that less drug was able to cross the placental barrier, decreasing the concentration reaching the fetuses.

Changes in DA-mediated behavior were, however, evident in the MPP^+ group where alterations in response to d-amphetamine treatment were observed. It is possible that a higher threshold level of adaptations had to occur in the DA system before decreases in spontaneous activity were seen and compensatory mechanisms may be sufficient to regulate spontaneous activity in these cases. However, even these milder effects could be uncovered by the pharmacologic challenge. Similarly, MPTP administration in adult mice produced no overt effects on spontaneous behaviors, but markedly enhanced the behavioral effects of stress or dopamine receptor antagonists (25).

The mechanism of action of d-amphetamine is to enhance the release of DA from dopaminergic terminals. Lack of response to d-amphetamine could indicate destruction of dopaminergic neurons. All MPTP and MPP^+ animals were responsive to d-amphetamine, but the magnitude of the response was less than in controls. These results indicated an intact presynaptic dopaminergic system following prenatal treatment with MPTP or MPP^+. The decreased response to d-amphetamine might suggest either a reduction in the DA available presynaptically for release or a decreased sensitivity of the postsynaptic DA receptors.

We, therefore, tested integrity of the postsynaptic DA receptors by measuring responses to apomorphine, a directly acting DA-receptor agonist and measured directly the concentration of DA and its metabolites in the striatum of rat pups treated prenatally with MPTP and MPP^+. Apomorphine induced stereotypy increased relative to controls in offspring treated prenatally with MPTP or MPP^+ on both postnatal days 21 and 50. Persistent postsynaptic supersensitivity was thereby demonstrated. Thus, neither MPTP nor MPP^+ treatments interfered with the cellular mechanisms determining DA receptor supersensitivity (15).

However, the striatal concentration of DA and its metabolites DOPAC and HVA were not greatly affected by prenatal treatment with MPTP or MPP^+. The change in HVA levels observed in the MPTP-treated rats was very small, but may have indicated changes at earlier time points which were normalizing at this time postnatal. Therefore, the behavioral hyposensitivity of the rats in response to amphetamine at the particular ages tested did not appear to be due to a reduction either in the content of DA or turnover of DA in the striatum. The lack of effect of MPTP or MPP^+ on the dopaminergic neurons in the olfactory bulb demonstrate the general resistance of dopaminergic systems in the rat having differential morphology and developmental time course (12).

It is unclear why there was an apparent supersensitivity to apomorphine when the spontaneous turnover of DA in the striatum was apparently normal. However, it has been reported that prenatal and early postnatal treatment with DA receptor antagonists can produce persistent changes in the sensitivity of dopaminergic receptors (17). MPTP produces a reversible decrease in DA content of the striatum in rats (5). If MPTP and MPP^+ were to elicit a similar reversible effect on the developing DA neurons in the embryonic rats, then it might be postulated that a lack of dopamine may have produced a persistant supersensitivity of postsynaptic neurons to DA receptor agonists.

Although the observed effects on behavior are consistent with changes in the sensitivity of striatal dopaminergic systems, the possibility cannot be ignored that deficits in peripheral catecholamine systems might mediate some of the observed behavioral responses.

Our data also suggest that behavioral recovery occurred between postnatal days 21 and 50 following the apparently less severe functional deficits resulting from prenatal treatment with MPP^+. At postnatal day 21, rats treated with prenatal MPP^+ were hyporesponsive to d-amphetamine, but at day 50, their responses were similar to controls. Evidently, neurons became able to balance presynaptic stimulation with their postsynaptic response. The mechanism may involve increased availability of releasable dopamine, further sensitization of postsynaptic receptors, or both. If more DA were being released, one would expect the postsynaptic supersensitivity to apomorphine to eventually be abolished.

In contrast, rats treated prenatally with MPTP had a more pronounced functional change presumably in dopaminergic neurotransmission, evidenced by decreases in both spontaneous noc-

turnal behavior and amphetamine-induced behavior. In this group, there was no evidence of recovery to control levels by postnatal day 50. In fact, more locomotor activity parameters were affected at day 50 than at day 21.

Early studies in cell culture indicated that MPTP was toxic to explants of rat embryonic mesencephalic (DA precursor) cells (14). We observed important differences in the response to MPTP treatment in fetal rats as compared with previous studies in adult rats (5). In mature rats MPTP produces a brief, reversible decrease in DA turnover in the striatum (5). In contrast, the present data suggest that although there are no marked effects on the development of striatal dopaminergic neurons, immature rats display persistent alterations in behaviors mediated by dopaminergic systems following MPTP/MPP^+ treatment.

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