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REPEATED ADMINISTRATION OF *N*-METHYL-4-PHENYL 1,2,5,6-TETRAHYDROPYRIDINE TO RATS IS NOT TOXIC TO STRIATAL DOPAMINE NEURONES

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Abstract—*N*-Methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP) (10 mg/kg/day i.p.) was administered to rats for 16 days, which were then observed for a further 9–11 days. MPTP administration did not alter spontaneous locomotor activity or amphetamine (2.5 mg/kg ip)-induced locomotion. Apomorphine (0.25 mg/kg sc) did not alter locomotion in control rats but increased activity in MPTP treated animals. The stereotyped response to apomorphine (0.25 mg/kg sc) and amphetamine (2.5 and 5.0 mg/kg ip) was unaltered by MPTP administration. The striatal content of dopamine, HVA and DOPAC was unaltered by MPTP intake. The uptake of [^{3}H]dopamine and [^{3}H] 5HT in striatal synpatosomes was not changed by MPTP. The results suggest that MPTP, in the dose used, is not toxic to nigro-striatal dopamine neurones in the rat. This contrasts with its neurotoxic actions in monkeys and man.

Four drug addicts who self-administered *N*-methyl-4-phenyl-4-propionoxy piperidine (MPPP)† contaminated with 2.5–3.2% of *N*-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP) intravenously, developed a persistent Parkinsonian syndrome within 1 week of administration [1]. The patients exhibited akinesia, tremor, rigidity and a stooped posture as observed in idiopathic Parkinson's disease. The symptoms responded to treatment with L-DOPA (plus a peripheral decarboxylase inhibitor), although two patients required additional therapy with the dopamine agonists bromocriptine or lisuride. A previous case reported by Davis *et al.* [2] describes how a student developed marked Parkinsonism after injecting MPPP contaminated with MPTP. The student died as a result of drug overdose. At necropsy, there was a selective degeneration of neuronal cell bodies in the zona compacta of substantia nigra as observed in idiopathic Parkinson's disease. There was, however, no cell loss in the locus coeruleus in contrast to idiopathic Parkinson's disease. A single eosinophilic body, perhaps representing a Lewy body, was found.

In subsequent experiments the administration of MPTP to rhesus monkeys for 5–9 days caused persistent akinesia, rigidity, tremor and flexed posture [3]. The primate Parkinsonian syndrome which is associated with a persistent fall in CSF HVA concentrations was reversed by administration of L-DOPA. At necropsy a 90% decrease in the dopamine content of the caudate/putamen was found, together with a marked loss of pigmented neurones in the pars compacta of substantia nigra.

Clearly, MPTP is selectively neurotoxic for those dopamine cells located in the zone compacta of substantia nigra of man and primates. However, there is a limit to the extent to which biochemical or pharmacological determinations can be carried out in these species. In the present study we have attempted to demonstrate neurotoxic effects of MPTP in the rat. We find that in contrast to man and monkeys MPTP does not appear neurotoxic in the rat.

MATERIALS AND METHODS

Drug administration. Male Wistar rats (350 ± 6 g; Bantin & Kingman Ltd.) were housed in groups of 5 under standard conditions of laboratory lighting and temperature (12 hr light–dark cycle; 22 ± 2°) with free access to food and water.

Animals received daily interperitoneal (i.p.) injections of either *N*-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP; Aldrich Chemical Co.) (10 mg/kg) or vehicle solution for 16 days. The dose of MPTP used was based on a calculation of the highest daily intake of MPTP by drug addicts (*ca.* 1 mg/kg; Langston *et al.* [1]) multiplied by a factor of 10 to compensate for the generally higher drug metabolism of rodents and to ensure adequate concentrations to potentially achieve a lesion. MPTP was mixed with tartaric acid (10:1 by weight) and dissolved in distilled water. Control animals received a tartaric acid solution alone (= 1 mg/kg ip daily).

Behavioural tests were performed on the animals both during drug administration and following drug withdrawal. Biochemical examinations were performed 9–11 days after drug withdrawal.

Spontaneous locomotor activity. Spontaneous

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† Abbreviations: MPTP, *N*-methyl-4-phenyl-1,2,5,6-tetrahydropyridine. MPPP, *N*-methyl-4-phenyl-propionoxy piperidine. HVA, homovanillic acid. DOPAC, 3,4-dihydroxyphenylacetic acid. L-DOPA, L-3,4-dihydroxyphenylalanine.

locomotor activity was assessed in individual animals in the holeboard apparatus as previously described by Clow *et al.* [4]. Activity was measured by counting the number of crosses, dips and rears occurring in a 3 min observation period. Five animals from each group were assessed at each test point and at least 7 days had elapsed before retesting any animal. Statistical analysis was performed using the Kruskal–Wallis H test for non-parametric data; when significant H values were obtained a Mann-Whitney U test was performed between groups.

Spontaneous locomotor activity also was measured using automated activity cages fitted with two photoelectric cells and connected to a PET 4002 computer (designed and built within the Department by Mr. H. C. Bertoya). The spontaneous locomotor activity of 20 individual animals from each group was measured over a 60 min period following introduction into activity cages.

Amphetamine- and apomorphine-induced locomotion. Following withdrawal from MPTP treatment the locomotor response of animals to both intraperitoneal administration of (+) -amphetamine sulphate (2.5 mg/kg ip; SKF Ltd.) and subcutaneous (sc) administration of apomorphine hydrochloride (0.25 mg/kg sc; MacFarlan Smith Ltd.) was assessed.

Animals were placed in individual photocell activity cages and allowed a 60 min acclimatisation period after which the dopamine agonists were administered. Activity was recorded for the subsequent 60 min following apomorphine administration and 240 min following amphetamine administration.

Statistical analysis of locomotor activity in control and MPTP treated animals was carried out using a one-way analysis of variance; Student's *t*-test was used for single point comparisons.

Stereotyped behaviour. The stereotyped response to (+)-amphetamine sulphate (2.5 and 5.0 mg/kg ip) and apomorphine hydrochloride (0.25 mg/kg sc) was assessed according to the four point scoring system described by Costall and Naylor (1975) [5]. Animals were housed in individual perspex cages (30 × 25 × 20 cm) and stereotypy was assessed over a 30 sec period at a time of peak drug effect, which was 45 min following amphetamine administration and 20 min following apomorphine administration.

Striatal dopamine, HVA and DOPAC content. Rats were killed by cervical dislocation and decapitation. The brains were removed and placed on ice and the striata were immediately dissected out and frozen at −20°. The paired striata were weighed and homogenised in 0.4 M $HClO_4$ using a Teflon glass homogeniser. Standards were prepared using cerebellar tissue in place of striatal homogenates. Tissue preparations were centrifuged at 2000 rpm for 20 min at 4°. The supernatant was decanted onto Sephadex G10 columns to separate dopamine from HVA and DOPAC. Dopamine was assayed by the fluorimetric technique of Earley and Leonard [6]. HVA and DOPAC were assayed using semi-automated fluorimetric technique of Westerink and Korf [7]. Statistical analysis was performed using Student's *t*-test between groups.

Uptake of ^{3}H-DA and ^{3}H-5HT into striatal synaptosomes. Rats were killed by cervical dislocation and decapitation and brains removed and dissected on ice. Pooled striatal tissue (4 rats per group) was weighed and homogenised in 0.32 M sucrose containing 1 mM EDTA and 50 mM Tris–HCl using a Teflon glass homogeniser. Tissue homogenates were centrifuged at 1500 rpm for 5 min at 4°. The supernatant then was spun at 10,000 rpm for 10 min at 4°, the pellet resuspended in 0.32 M sucrose and again centrifuged. The resulting pellet was suspended in 20 vol of Krebs bicarbonate (bubbled with 95% O_2/5% CO_2) containing 0.5 μM pargyline and 0.5 μM desimipramine.

Synaptosomal suspension (100 μl) was incubated at 37° with 5×10^{-7} M ^{3}H-5HT or 5×10^{-7} M ^{3}H-DA (sp. act.; 17.0 Ci/mmole and 13.5 Ci/mmole respectively; Amersham International) for 5 min, immediately filtered over Whatman GF/B filters using a millipore vacuum filtration and washed with 2 × 4 ml ice-cold Krebs bicarbonate. Blanks were carried out using the same procedure but on ice at 0°.

Radioactivity on the filters was counted using a Packard 2425 liquid scintillation spectrometer at an efficiency of approx. 40%. The protein content of tissue preparations was assessed using the method of Lowry *et al.* [8].

RESULTS

Animal weights. Animals receiving MPTP (10 mg/kg/day ip) for 16 days did not gain weight during the treatment period. (Table 1). On drug withdrawal MPTP treated animals started to gain weight such that after 1 week their body weight was not different from that of control animals.

Spontaneous locomotor activity. Administration of MPTP (10 mg/kg ip) for 16 days did not alter the

Table 1. The effect of intraperitoneal (ip) administration of MPTP (10 mg/kg/day ip) to rats for 16 days followed by drug withdrawal on body weight compared to vehicle treated animals

Treatment group	Body weight (g) 0	1 week	2 weeks	1 week following withdrawal
Control	361 ± 9	378 ± 9	387 ± 8*	396 ± 7*
MPTP	334 ± 7	340 ± 7†	344 ± 7†	368 ± 7*

Values are expressed as the mean (± 1 S.E.M.) body weight for 20 animals per group. Statistical analysis was performed using Student's *t*-test between groups.

* $P < 0.05$. Comparison of animal weights at various time intervals with pretreatment weights.

† $P < 0.05$. Comparison between control and MPTP rats.

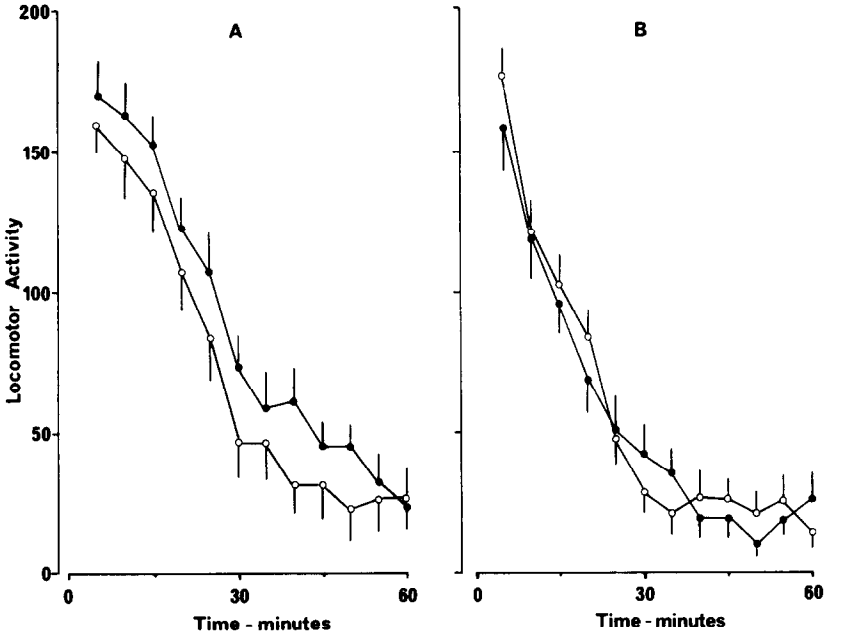

Fig. 1. The effect on spontaneous locomotor activity assessed using photocell cages of (A) the intraperitoneal (ip) administration to rats of MPTP (10 mg/kg ip) for 16 days and (B) 9 days following withdrawal of MPTP treatment in comparison to vehicle injected control animals. (●), Control; (○), MPTP (10 mg/kg/day ip). No significant difference between groups using one way analysis of variance and Student's *t*-test.

spontaneous locomotor activity of animals compared to control rats when assessed using photocell cages (Fig. 1A). Nine days following cessation of MPTP treatment spontaneous activity again was unchanged when compared to control animals (Fig. 1B).

Using the hole board apparatus, activity was assessed at intervals during the study (Fig. 2). Measurement of crosses, dips and rears showed no difference with respect to time between MPTP-treated animals and control rats. However, the number of crosses performed by animals receiving MPTP were consistently lower than observed for control

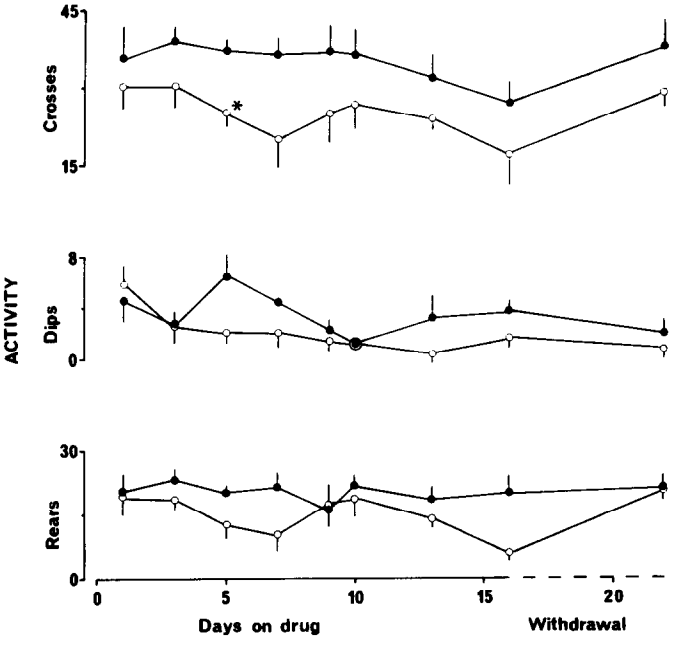

Fig. 2. The effect of intraperitoneal (ip) administration of MPTP (10 mg/kg ip) for 16 days followed by 6 days drug withdrawal on crosses, dips and rears assessed in a holeboard apparatus. (●), Control; (○), MPTP (10 mg/kg/day ip). Values are expressed as the mean (± 1 S.E.M.) activity for 5 animals in each group. Statistical analysis was performed using Kruskal–Wallis H test; for significant H-values a Mann Whitney 'U' test was performed. * $P<0.05$. Comparison between control and MPTP rats.

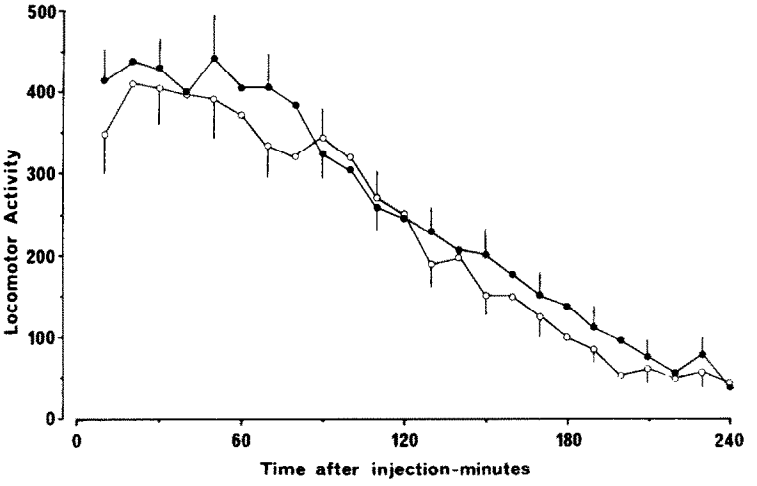

Fig. 3. The effect of intraperitoneal administration to rats of MPTP (10 mg/kg ip) for 16 days followed by 9 days drug withdrawal on amphetamine (2.5 mg/kg ip)-induced locomotor activity assessed using photocell cages in comparison to vehicle treated control animals. (●), Control; (○), MPTP. Values are expressed as mean (± 1 S.E.M.) activity per 10 min period for 20 animals in each group. Pre-injection activity for control and MPTP was 54 ± 18 and 28 ± 10, respectively. No significant difference was observed between groups.

animals although this was only statistically significant on day 5 of treatment.

Amphetamine- and apomorphine-induced locomotor activity. Amphetamine (2.5 mg/kg ip) administration caused a marked increase in locomotor activity in both rats treated with MPTP (10 mg/kg ip) for 16 days and then withdrawn for nine days, and in control animals. Activity was the same for both groups of animals over the 4 hr period following amphetamine administration (Fig. 3).

Administration of a threshold dose of apomorphine (0.25 mg/kg sc) produced a short-lived increase in locomotor activity between 20–35 minutes after injection in control rats. In animals treated with MPTP (10 mg/kg ip) for 16 days then withdrawn for 11 days apomorphine (0.25 mg/kg sc) caused a greater initial increase in locomotor activity (Fig. 4).

Apomorphine- and amphetamine-induced stereotyped behaviour

Administration of apomorphine (0.25 mg/kg sc; 20 min previously) produced stereotyped behaviour in control animals consisting of continuous sniffing, discontinuous locomotor activity and occasional biting or licking (Table 2). Administration of MPTP to rats for 16 days produced no change in stereotyped response to apomorphine when compared to vehicle treated control animals (Table 2).

Administration of amphetamine (2.5 and 5.0 mg/kg ip; 45 min previously) produced stereotyped behaviour in both control and MPTP treated animals which did not differ between the groups (Table 2).

Striatal dopamine, HVA and DOPAC concentrations. Measurement of striatal dopamine, HVA and DOPAC concentrations 13 days following withdrawal from a 16 day period of administration of MPTP revealed no difference between control and MPTP treated animals (Table 2).

Uptake of ^{3}H-dopamine and ^{3}H-5HT by striatal synaptosomes. The uptake of ^{3}H-dopamine and ^{3}H-5-HT into striatal synaptosomes from rats treated with MPTP for 16 days and then withdrawn for 19 days was not different from values found in tissue from control animals (values obtained from a single experiment, ^{3}H-dopamine: control 36.1 pmoles/hr

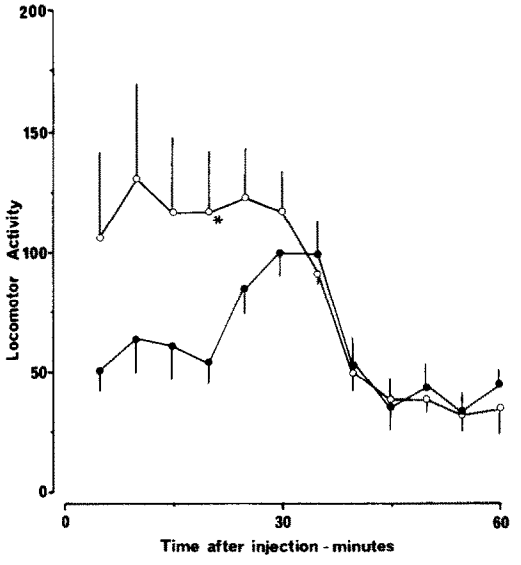

Fig. 4. The effect of intraperitoneal administration to rats of MPTP (10 mg/kg ip) for 16 days followed by 11 days drug withdrawal on locomotor activity induced by the subcutaneous (sc) administration of apomorphine (0.25 m/kg sc) assessed using photocell cages in comparison to vehicle treated control animals. (●), Control; (○), MPTP. Values are expressed as mean (± 1 S.E.M.) activity per 5 min period for 20 animals in each group. Pre-injection activity for control and MPTP rats are 64 ± 11 and 46 ± 11, respectively. Statistical analysis was performed using one-way analysis of variance and Student's *t*-test between groups.
* $P<0.05$ comparison between control and MPTP rats.

Table 2. The effect of intraperitoneal (ip) administration of MPTP (10 mg/kg/day ip) for 16 days followed by 13–16 days drug withdrawal on stereotyped behaviour induced by subcutaneous (sc) administration of apomorphine (0.25 mg/kg sc) and intraperitoneal (ip) administration of amphetamine (2.5 and 5 mg/kg ip) and on striatal DA, HVA and DOPAC concentrations compared to control animals.

	Stereotypy			Striatal concentration			
Treatment Group	Amphetamine (mg/kg)		Apomorphine (mg/kg)	DA (μg/g)	HVA (ng/g)	DOPAC (ng/g)	HVA/DOPAC ratio
	2.5	5.0	0.25				
Control	1.8 ± 0.2	2.7 ± 0.4	2.7 ± 0.2	8.34 ± 0.48	569 ± 70	746 ± 37	0.76 ± 0.07
MPTP	1.7 ± 0.2	2.0 ± 0	2.5 ± 0.3	8.47 ± 0.61	458 ± 23	778 ± 72	0.61 ± 0.03

Stereotyped behaviour was assessed according to Costall and Naylor [5]. The results are expressed as the mean ± 1 S.E.M. for 6 animals in each group. Striatal DA, HVA DOPAC levels were measured by the technique of Westerink and Korf [7]. Values are expressed as mean ± 1 S.E.M. for 8 animals in each group. No significant difference was found between control and MPTP groups. Student's *t* test for parametric data; Mann Whitney U test for non-parametric data.

mg protein; MPTP treated 36.5 pmoles/hr/mg protein ^{3}H-5-HT: control 40.0 pmoles/hr/mg protein; MPTP treated 37.7 pmoles/hr/mg protein).

DISCUSSION

The ability of MPTP to produce a persistent Parkinsonian syndrome in man, associated with a loss of dopamine containing cells in zona compacta of substantia nigra, suggests a selective neurotoxic action. This is confirmed by the ability of MPTP to induce identical changes in the rhesus monkey. However, in contrast, in the rat a neurotoxic action on brain dopamine neurones is not apparent.

We administered MPTP to rats in doses far higher than those employed in monkey experiments or received by the affected drug addicts and for longer periods of time. However, apart from a failure to gain weight there was no obvious difference between animals receiving MPTP and the control group. The absence of a neurotoxic effect of MPTP was suggested by the lack of effect on spontaneous locomotion as judged using both photocell cages and a holeboard apparatus. This was confirmed by the ability of amphetamine to induce identical increases in locomotor activity in both groups of animals presumably by releasing dopamine from intact presynaptic stores. Interestingly, a threshold dose of apomorphine was able to induce a greater change in locomotor activity in MPTP treated animals than in control animals. This might reflect a change in the sensitivity of post-synaptic dopamine receptors as a result of MPTP administration, but this was not reflected in the results of the stereotypy experiments. Apomorphine and amphetamine induced equivalent stereotyped behavioural responses in MPTP treated and control animals, suggesting that no functional change had occurred in either presynaptic or post-synaptic dopamine neuronal events. The conclusion from these experiments is that in the rat the administration of MPTP does not alter motor behaviour.

The difference between the effects of administration of MPTP to rats compared to monkeys and man was apparent also in the results of the biochemical experiments. Compared to the profound depletion of dopamine and HVA observed in primates, MPTP administration did not alter the striatal dopamine, HVA or DOPAC concentrations or the ratio of metabolites. The functional integrity of presynaptic dopamine terminals in striatum was suggested also by the normal level of [^{3}H]DA uptake occurring after MPTP treatment. So biochemically there was no evidence for a loss of nigro-striatal dopamine neurones.

Why does MPTP not exhibit the same selective neurotoxic effect in the rat as is seen in monkeys and man? The dose used in the present study may not have been sufficiently high to produce toxic levels of MPTP owing to the rapid clearance of drug in the rat. This would seem unlikely, however, since drug intake was sufficient to prevent growth in the animals. MPTP may not penetrate into brain in the rat as in man or monkeys. Again, this would seem unlikely since species variations of this kind are rare and since drug penetration is more dependent on the physiochemical properties of the drug molecule. Species differences in blood flow to different brain areas causing variation in the concentration of MPTP achieved in substantia, might occur. However, there are two other possible reasons which would seem to provide a more reasonable explanation for the failure of MPTP to exert toxicity in the rat. The dopamine-containing neurones of substantia nigra in the rat may not be sensitive to the toxic actions of MPTP in contrast to monkey and man. Many reasons could be advanced for this, including differences in metabolic rate and variations in the extent of protective mechanisms (superoxide dismutase, catalase, glutathione peroxidase) although there is no evidence to support this suggestion. Another explanation is that MPTP itself is not the active moiety, but that some metabolite is responsible for nigral cell death. Species variation in the extent and routes of metabolism of xenobiotics are common. However, if this is the case, why is this toxic species selective for dopamine containing neurones? Indeed, why are the dopamine containing cells of substantia nigra destroyed but not those in the adjacent ventral tegmental area? Such a selective effect might suggest the formation of the toxic species occurs within substantia nigra.

In conclusion, we could not demonstrate a toxic action of MPTP (in the dose employed over a period of up to 27 days) on dopamine containing neurones

in the rat brain. This contrasts with the toxic effects of this substance in monkeys and man but it is in agreement with the recent report by Chieuh *et al.* [9] which also failed to demonstrate toxicity in the rat and guinea-pig.

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