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## Behavioural effects and supersensitivity in the rat following intranigral MPTP and MPP<sup>+</sup> administration

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Unilateral intranigral injections of MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) and MPP<sup>+</sup> (1-methyl-4-phenylpyridine) were given to young rats and unilateral intranigral injections of MPTP were given to old rats. MPTP in old rats and MPP<sup>+</sup> in young rats induced ipsiversive circling for at least one week after injection and contraversive circling after the systemic administration of apomorphine; the number of D-2 receptors ( $B_{\max}$ ) in the striatum of the injected hemisphere increased compared with that of control rats. MPTP in young rats induced only short-lasting ipsiversive circling and no contraversive circling after apomorphine; the number of striatal D-2 receptors did not increase. These results suggest that the neurotoxicity of MPTP is age-dependent in the rat, and that MPTP has neurotoxic effects on the nigrostriatal dopaminergic system in old rats and induces dopamine receptor supersensitivity in the denervated striatum.

MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine); MPP<sup>+</sup> (1-methyl-4-phenylpyridine); Substantia nigra; Circling behaviour; Ageing; (Supersensitivity)

### 1. Introduction

Exposure of human subjects and non-human primates to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) causes a Parkinsonian syndrome accompanied by destruction of dopamine-containing neurones in the substantia nigra pars compacta (SNc; see Langston, 1987). The brains of rodents, in particular those of rats, are much less sensitive to the neurotoxic effects of MPTP than the brains of primates are. The systemic administration of MPTP to rats failed to cause neurotoxicity (Chiueh et al., 1984), and the intranigral administration of MPTP to rats did not induce lasting damage to the dopaminergic nigral neurones (Chiueh et al., 1984; Bradbury et al., 1986) or lasting behavioural effects (Welzl and Lange, 1986). Studies on the mechanisms of MPTP toxic-

ity have shown an important role of the enzyme monoamine oxidase B (MAO B), which oxidizes MPTP to 1-methyl-4-phenylpyridine (MPP<sup>+</sup>); MPP<sup>+</sup> accumulates in dopaminergic neurones and appears to kill the cells by interfering with their energy metabolism (see Langston, 1987). The neurotoxicity of MPTP seems to be age-dependent. For example, MPTP produces severe damage in the substantia nigra and causes marked motor impairments in old mice whereas it has only minor effects in young mature mice (Gupta et al., 1986). The present study examined the behavioural effects of unilateral injections of MPTP and MPP<sup>+</sup> into the SNc and the age dependence of MPTP neurotoxicity in the rat.

### 2. Materials and methods

Young adult (aged 4–5 months) and old (aged 22–24 months) male BD IX rats were used. Both

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young ( $n = 8$ ) and old ( $n = 8$ ) rats received a unilateral injection of  $50 \mu\text{g}/1 \mu\text{l}$  MPTP (Research Biochemicals Inc., Wayland, MA) into the SNC through cannulas. Young adult ( $n = 8$ ) and old ( $n = 8$ ) control rats received a saline solution which was equimolar to the MPTP solution. Another group of young rats ( $n = 8$ ) was given unilateral intranigral injections of  $4 \mu\text{g}/1 \mu\text{l}$   $\text{MPP}^+$  iodide (Research Biochemicals Inc., Wayland, MA) and control animals ( $n = 8$ ) received injections of an equimolar NaI solution. The stereotaxic coordinates for the intranigral injection corresponded to König and Klippel (1963) coordinates: A 2420, V - 2.4, L 1.6.

The circling behaviour of all groups was recorded automatically with the animals in a square box ( $0.5 \times 0.5 \text{ m}$ ). Recordings were made for 30 min on the 1st and 7th day after the intranigral injection and on the 7th day for 30 min after the s.c. injection of apomorphine ( $0.5 \text{ mg/kg}$ ; Woelm Pharma, Eschwege, F.R.G.).

Ten days after the intranigral injection the rats were decapitated and their brains were rapidly removed onto ice. The striatum was dissected out and placed into ice-cold  $50 \text{ mM}$  Tris-HCl buffer ( $\text{pH } 7.6$ ). The specific binding of  $[^3\text{H}]$ spiperone ( $18\text{--}19 \text{ Ci/mmol}$ ; concentrations between  $0.1$  and  $1.0 \text{ nM}$ ; Amersham International, U.K.) in tissue homogenates of the striatum was determined by the method of Leysen et al. (1978); a final tissue suspension of  $1$  in  $800 \text{ w/v}$  in an incubation buffer containing  $50 \text{ mM}$  Tris-HCl and  $120 \text{ mM}$  NaCl ( $\text{pH } 7.6$ ) was used. The striatum of the injected hemisphere and of the intact hemisphere of each rat was assayed separately. Non-specific binding of  $[^3\text{H}]$ spiperone was defined by the incorporation of  $10^{-5} \text{ M}$  ( $\pm$ )-sulpiride (Delagrangé, Paris, France) in the incubation buffer. All determinations were carried out in triplicate. The number of binding sites ( $B_{\text{max}}$ ) and the apparent equilibrium constant ( $K_d$ ) were determined by Eadie-Hofstee analysis.

### 3. Results

The unilateral intranigral injection of MPTP induced strong ipsiversive circling (i.e. towards the

side of injection) in both young adult and old rats on the 1st day after injection (see fig. 1). Seven days after the MPTP injection, spontaneous ipsiversive circling behaviour was still present in old rats whereas young adult rats did not circle preferentially in either direction. Young adult and old control rats injected with saline showed no asymmetry in their circling behaviour. Old rats that had received unilateral MPTP injections circled contraversively when injected with apomorphine 7 days after the injection; young rats did not show contraversive circling after the administration of apomorphine (see fig. 1). The unilateral intranigral injection of  $\text{MPP}^+$  I produced ipsiversive circling on the 1st and 7th day after injection. The sys-

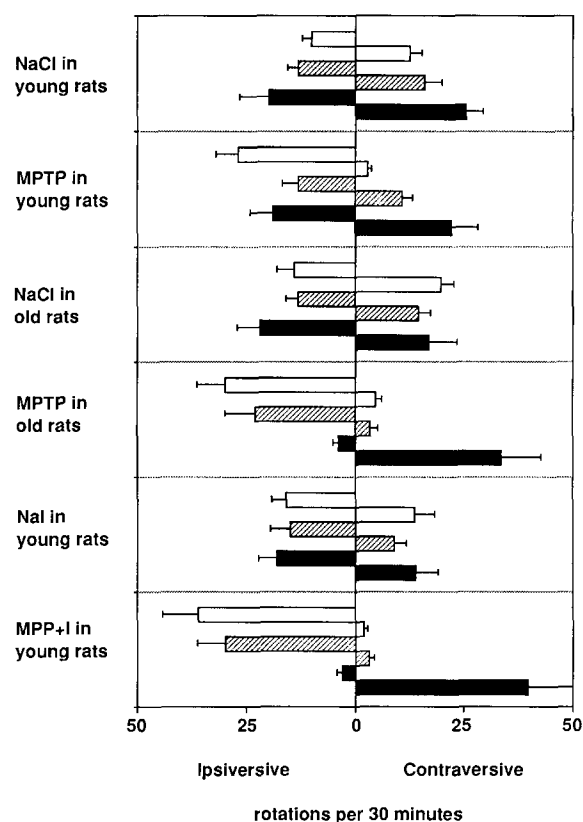


Fig. 1. Ipsiversive and contraversive circling on day 1 (white bars) and day 7 (hatched bars) after unilateral injection of MPTP,  $\text{MPP}^+$  I and control solutions into the substantia nigra pars compacta of young adult and old rats, and apomorphine-induced circling on day 7 after injection (black bars). Mean values  $\pm$  S.E. are shown.

TABLE 1

Number ( $B_{\max}$ ) and affinity ( $K_d$ ) of D-2 receptors, identified by specific [ $^3$ H]spiperone binding, in the striatum of the injected and intact hemispheres on day 10 after unilateral intranigral injection of MPTP, MPP<sup>+</sup> and control solutions in young adult and old rats.

Treatment	No. of animals	Injected hemisphere		Intact hemisphere	
		$B_{\max}$ (pmol/g wet weight of tissue)	$K_d$ (nM)	$B_{\max}$ (pmol/g wet weight of tissue)	$K_d$ (nM)
NaCl in young rats	8	23.1 ± 0.6	0.18 ± 0.01	23.6 ± 0.6	0.16 ± 0.01
MPTP in young rats	8	22.8 ± 0.7	0.18 ± 0.01	24.1 ± 0.9	0.18 ± 0.01
NaCl in old rats	8	21.6 ± 0.6	0.14 ± 0.01	21.1 ± 0.8	0.16 ± 0.01
MPTP in old rats	8	28.8 ± 0.7 <sup>a</sup>	0.15 ± 0.01	22.8 ± 0.4	0.14 ± 0.01
NaI in young rats	8	22.1 ± 0.6	0.19 ± 0.01	23.8 ± 0.7	0.17 ± 0.01
MPP <sup>+</sup> I in young rats	8	28.5 ± 0.8 <sup>a</sup>	0.18 ± 0.01	22.9 ± 0.6	0.18 ± 0.01

<sup>a</sup>  $P < 0.05$  in comparison with the control group, Mann-Whitney U-test. Values are means ± S.E.

temic administration of apomorphine induced contraversive circling in these rats (see fig 1).

In old rats the number ( $B_{\max}$ ) of D-2 receptors, identified by specific [ $^3$ H]spiperone binding, in the striatum of the injected hemisphere was higher after the MPTP injection than after the saline injection (see table 1). In young rats there were no differences between the number of D-2 receptors in the experimental and control animals. The number of D-2 receptors in the striatum of the injected hemisphere increased after the MPP<sup>+</sup>I injections in comparison with the number after the NaI injections. Increased D-2 receptor numbers were confined to the injected hemisphere; there were no differences between the number of D-2 receptors in the contralateral striata of the experimental and control groups (see table 1). Changes in receptor affinity ( $K_d$ ) were not observed (see table 1).

#### 4. Discussion

Unilateral lesions within the nigrostriatal dopaminergic system of the rat induce circling behaviour which is thought to reflect an imbalance of dopaminergic activity in the striata. The rat rotates towards the side of the lesion, i.e. away from the hemisphere with the higher striatal dopaminergic activity. Behavioural supersensitivity is manifested by the animal rotating in a

direction contralateral to the side of the lesion after the systemic administration of dopamine agonists, and appears to be due to supersensitivity of the denervated striatal dopamine receptors (Ungerstedt, 1971; Creese et al., 1977).

MPTP appears to have only a short-lasting depressive effect on nigral dopaminergic neurones in young rats since the unilateral injection of MPTP into the SNC induced ipsiversive circling for only a short period. In contrast, MPP<sup>+</sup> caused ipsiversive circling for at least 7 days as well as behavioural supersensitivity and striatal D-2 receptor supersensitivity as measured by increased [ $^3$ H]spiperone binding. These behavioural results reflect biochemical alterations following the intranigral administration of MPTP and MPP<sup>+</sup> to the rat. There is no loss of striatal dopamine or its metabolites after an infusion of MPTP whereas MPP<sup>+</sup> markedly reduces striatal dopamine levels (Bradbury et al., 1986; Sun et al., 1988).

In this study apomorphine-induced contraversive circling in young rats treated with MPP<sup>+</sup> and old rats treated with MPTP was limited compared to the high number of rotations observed following the administration of apomorphine to rats with unilateral 6-hydroxydopamine lesions (e.g. Ungerstedt, 1971; Creese et al., 1977). A possible explanation for this difference is the ratio between the number of striatal dopamine receptors in the injected and intact hemispheres. Creese et al. (1977) showed that behavioural supersensitivity increased concomitantly with receptor su-

persensitivity. These authors described a mean increase in [ $^3\text{H}$ ]haloperidol binding of 50% in the striatum on the side of the 6-hydroxydopamine lesion compared with the contralateral, non-lesioned side. In contrast, the results of the present study showed that [ $^3\text{H}$ ]spiperone binding in the striatum on the side of the MPTP or  $\text{MPP}^+$  lesion increased by only about 25% (see table 1).

Other authors have observed apomorphine-induced ipsilateral circling after unilateral nigral  $\text{MPP}^+$  lesions (Sun et al., 1988). This is possibly the result of destruction of not only the dopaminergic neurones in the pars compacta but also non-dopaminergic neurones in the pars reticulata of the substantia nigra, since the  $\text{MPP}^+$  doses used by Sun et al. (1988) were higher than those used in the present study. It has been shown that apomorphine causes ipsilateral rather than contralateral circling in rats after a non-specific unilateral lesion of the substantia nigra (Costall et al., 1976). Altar et al. (1986) reported that there was no contralateral circling in response to dopamine agonists when nigrostriatal projections had been destroyed by  $\text{MPP}^+$  injections into the medial forebrain bundle in the rat although the dopamine concentrations in the ipsilateral striatum were decreased by 95%. These results can be explained by the finding that high doses of  $\text{MPP}^+$  destroy GABAergic striato-nigral fibres (Altar et al., 1986). In rats with supersensitive striatal dopamine receptors following a unilateral dopaminergic lesion, circling behaviour to apomorphine is blocked by a lesion of the descending GABAergic pathway (Marshall and Ungerstedt, 1977).

Ipsiversive circling was observed in old rats for at least one week after the unilateral injection of MPTP and the systemic administration of apomorphine induced contraversive circling; the number of D-2 receptors was increased in the striatum of the lesioned hemisphere. These results suggest that the neurotoxicity of MPTP is age-dependent in the rat. MPTP has toxic effects in the nigrostriatal dopaminergic system of old rats and induces receptor supersensitivity in the denervated striatum. Previous attempts to induce lasting changes with MPTP in the rat may have been unsuccessful because young animals were used.

Other studies have also provided evidence that the effects of MPTP increase with age. Jarvis and Wagner (1985) showed that neonatal rats were resistant to the dopamine-depleting effects of MPTP whereas the toxin produced a 65% depletion of striatal dopamine in young adult rats. The enhanced toxicity of MPTP in older animals can be a toxicodynamic or toxicokinetic effect. In the former case nigrostriatal neurones of older animals may be more sensitive to the effects of MPTP, in the latter case greater quantities of MPTP or its metabolite  $\text{MPP}^+$  may reach the target area. Differences between the striatal dopamine depletion induced by intracerebroventricularly administered  $\text{MPP}^+$  have not been observed in old and young mice (Irwin et al., 1988), suggesting that old nigrostriatal neurones are not more sensitive to  $\text{MPP}^+$  and that higher concentrations of the toxin account for the age-related effects. A possible explanation for the age dependence of MPTP toxicity is the increase of MAO B activity in the brain with age. The neurotoxic effects of MPTP have been shown to be dependent on its conversion to  $\text{MPP}^+$  by MAO B (see Langston, 1987). In the rat, central MAO B activity increases over at least the first 2 years of life (Benedetti and Keane, 1980). In the mouse, striatal  $\text{MPP}^+$  concentrations increase with the age of the animals injected with MPTP (Langston et al., 1987).

The present study suggests that the intranigral injection of  $\text{MPP}^+$  in the rat damages the nigrostriatal dopamine system and produces D-2 receptor supersensitivity in the denervated striatum. The neurotoxic effects of MPTP appear to be age-dependent in the rat. The results suggest that, in old rats, MPTP destroys dopaminergic neurones in the substantia nigra and induces receptor supersensitivity in the striatum whereas it has only a short-lasting depressive effect on nigral dopaminergic neurones in young rats.

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