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Effects of MPTP on lever-pressing for light extinction in rats

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Abstract

Rats were daily treated for seven days with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) at a dose of 20 mg/kg/day, i.p. Seven days after treatment withdrawal, the rats were individually tested in a brightly lit apparatus containing two levers: an active lever allowing periods of darkness, and an inactive one. The test was performed over two consecutive days, in 20-min sessions. While control rats had a higher number of total active lever pressings than inactive lever pressings, this was not the case for MPTP-treated rats. Control rats decreased their useless active lever pressings and inactive lever pressings across the two sessions, but MPTP-treated rats did not do either. The absence of the differential effect in rats injected with MPTP may be due to a reduction in reinforcement mechanisms caused by the mild depletion of dopamine in the striatum.

Keywords: Light extinction test; MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine); Dopamine; Striatum; Substantia nigra

1. Introduction

It is widely known that administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which was first supposed to simulate the actions of heroin, leads to permanent parkinsonism in humans and primates (Burns et al., 1984; Clarke et al., 1989; Colosimo et al., 1992) by causing selective destruction of the dopaminergic neurons of the substantia nigra pars compacta (Burns et al., 1983; Tetrud and Langston, 1989).

Administration of this substance to laboratory rodents also induces biochemical and cytomorphological modifications in the striatum and in the substantia nigra (Gerlach et al., 1991; Klemfuss et al., 1986; Reinhard and Nichol, 1986; Russ et al., 1991; Sayre, 1989; Singer et al., 1987). Nevertheless, the central nervous system of rodents, particularly the albino rat, is considered to be the most insensitive to the neurotoxin (Arai et al., 1990; Brooks et al., 1989; Melnick et al., 1986). MPTP easily crosses the blood-brain barrier, but this protoxin must then be activated before it exerts its neurotoxic actions. Its neurotoxic major active metabolite, MPP^+ (1-methyl-4-phenylpyridinium ion), is unable to cross the blood-brain barrier. The biotransformation of MPTP to MPP^+ requires monoamine oxidase type B (Chiba et al., 1984). The resistance of rodents may lie in the fact that their blood brain barrier involves endothelial cells with a high monoamine oxidase type B content (Riachi and Harik, 1988). In this case MPTP is transformed to MPP + inside the endothelial cell and cannot reach the dopaminergic cells. Moreover, the storage of MPP^+ in the brain is partly due to the presence of neuromelanine (D'Amato et al., 1986), whose level in the albino rat brain is low.

Although in the mouse the initial dopamine depletion in the striatum is about 70–90%, the deficits appearing after MPTP administration are reversible. Dopamine levels remain low for one to two months after the end of MPTP administration and then progressively revert to normal after five to six months (Chiueh et al., 1986; Ricaurte et al., 1986).

While the neurohistological and biochemical effects of MPTP have been widely studied, most behavioural studies have concentrated on the sensorimotor (Lange, 1989; Leroux-Nicollet and Costentin, 1986; Weihmuller et al., 1989) and olfactory aspects (Dluzen and Kreutzberg, 1993; Doty et al., 1992). The present study assessed the effects of

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MPTP on performance of a novel task, in which rats exposed to a high level of illumination had access to a lever permitting light extinction. Low doses of MPTP were used which did not severely disable the rats in terms of motor function. The purpose of the present study was to determine whether MPTP has an effect on reinforcement mechanisms. While dopamine is known to be involved in reinforcement mechanisms as revealed by intracranial self-stimulation and other tests (Wise, 1978), it was not been done in a light extinction test, which should be highly motivating for this nocturnal animal.

2. Materials and methods

2.1. Subjects

34 male Wistar rats IOPS/Han (Iffa Credo, France), weighing 200–220 g at the start of the experiment, were used for the study. They were housed in groups of four in a climate-controlled room with a 12-h light:dark cycle (lights on at 07:00). The animals were allowed access to food and water ad libitum.

2.2. Apparatus

The apparatus consisted of a brightly lit wire-mesh cage with a transparent front door (50 × 40 × 37 cm). The light level in the test cage was 1200 lux. Two levers were included in the device: an active lever which allowed access to a 30-s period of darkness when pressed and an inactive lever, which did not provide darkness. During periods of darkness, pressing the active lever did not prolong the period of darkness. A TV camera permitted observation of the rats from a neighbouring room and recording of the experiment on video-tape.

2.3. Drug treatments

MPTP (RBI, Bioblock, Strasbourg, France) was dissolved in 0.1 N hydrochloric acid neutralized to pH 5 and diluted in a saline solution which served as the vehicle control. The animals were randomly divided into two groups: the MPTP-treated group ($n = 18$), daily injected with 20 mg/kg, for seven consecutive days; the control group ($n = 16$), injected over the same period. MPTP and saline were administered i.p. in a volume of 1 ml/kg body weight.

In both groups, 12 animals were assessed behaviorally while the rest were used for the neurochemical analyses.

2.4. Behavioural testing

Seven days after the series of injections, these 12 rats were tested in the light-extinction test, on two consecutive days. During these two sessions, the rats were placed individually in the cage for 20 min and could learn to control the light. The number of active lever pressings during periods of brightness (AL_{ON}) and darkness (AL_{OFF}) and for both periods (AL_T) and the number of inactive lever (IL) pressings were recorded.

The paired t-test (2-tailed) was used to evaluate the repeated measurements whereas the unpaired t-test (2-tailed) was used to compare the two groups in each session.

2.5. Neurochemical analyses

The other rats were killed by decapitation. The brains were rapidly removed and dissected on an ice-cooled glass plate. Frozen samples were homogenized in 0.1 N $HClO_4$, containing 4 mM Na-metabisulphite and 1 mM EDTA. Levels of dopamine, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in the homogenates were measured using high pressure liquid chromatography (HPLC, Water instruments, 715 ultra Wisp, 510 solvent delivery System and 460 electro-chemical detector). A μ Bondapack phenyl column (Water Ass.) was used for the separation. The mobile phase consisted of 3% methanol in 0.1 M Na-phosphate buffer pH 2.5 and 1 mM 1-octane sulphonic acid (PIC B-8 Waters).

The non-parametric Mann-Whitney U-test was used to compare MPTP-treated to control rats. One sample was lost in each group for the substantia nigra measurements.

3. Results

3.1. Neurochemical analyses

As shown in Table 1, MPTP-treated rats had lower concentrations of dopamine in the striatum ($U = 3$, 4.6 df, $P < 0.05$) but not in the substantia nigra ($U = 5$, 3.5 df, $P > 0.05$). On the contrary, DOPAC levels were increased in the substantia nigra ($U = 0$, 3.5 df, $P < 0.05$), but not in the striatum ($U = 5$, 4.6 df, $P > 0.05$). HVA was not changed in the striatum ($U = 6$, 4.6 df, $P > 0.05$).

Table 1
Mean (±S.E.M.) concentrations in ng/mg protein of dopamine and metabolites in the striatum and the substantia nigra in MPTP and control rats (Mann-Whitney U-test, [a] $P < 0.05$)

Treatment	Dopamine	DOPAC	HVA
Striatum			
Control ($n = 4$)	112.27 ± 17.18	15.14 ± 2.59	6.52 ± 0.71
MPTP ($n = 6$)	79.08 ± 5.26 [a]	11.07 ± 0.67	5.55 ± 0.31
Substantia nigra			
Control ($n = 3$)	10.32 ± 0.84	1.07 ± 0.05	
MPTP ($n = 5$)	11.86 ± 0.78	1.41 ± 0.03 [a]	

Table 2
Mean (±S.E.M.) total number of lever pressings on the active (AL_T) and inactive lever (IL) during two sessions of testing for MPTP-treated ($n = 12$) and control ($n = 12$) rats (paired t-test, [b] $P < 0.005$)

Treatment	Session	AL_T	IL
Control	1	31.33 ± 5.13	15.17 ± 2.70 [b]
	2	13.75 ± 2.91	8.25 ± 1.78 [b]
MPTP	1	20.75 ± 4.47	16.58 ± 3.33
	2	11.75 ± 3.73	9.58 ± 2.76

3.2. Behavioral analyses

As shown in Table 2, at the end of the first session, control rats had a higher number of pressings on the AL_T than on the IL ($t = 4.70$, 11 df, $P < 0.001$). The same pattern persisted in session 2 of testing ($t = 4.35$, 11 df, $P < 0.01$). The AL_{ON}/AL_T ratio was at chance level in session 1 for control rats but increased in session 2 ($t = 4.47$, 11 df, $P < 0.01$) (Table 3). Both AL_{ON} ($t = 5.07$, 11 df, $P < 0.001$) pressings and AL_{OFF} ($t = 4.96$, 11 df, $P < 0.001$) pressings decreased across days of testing (Table 3).

In contrast to the results found with control rats, there was no difference in lever-pressing rate on the AL_T as opposed to the IL during session 1 ($t = 1.62$, 11 df, $P > 0.05$) and session 2 ($t = 0.91$, 11 df, $P > 0.05$) for MPTP-treated rats (Table 2).

However, MPTP did not decrease AL_T (session 1: $t = 1.56$, 22 df, $P > 0.05$; session 2: $t = 0.42$, 22 df, $P > 0.05$) or IL (session 1: $t = 0.33$, 22 df, $P > 0.05$; session 2: $t = 0.41$, 22 df, $P > 0.05$) rates in comparison to the control group (Table 2). There was a decrease in the MPTP group for AL_{ON} pressings across the two days of testing ($t = 2.59$, 11 df, $P < 0.05$) but not for AL_{OFF} pressings ($t = 1.38$, 11 df, $P > 0.05$) (Table 3). The AL_{ON}/AL_T ratio did not increase across days of testing for MPTP-treated rats ($t = 1.30$, 11 df, $P > 0.05$), although the value in session 2 was similar to that for control rats (Table 3). There was no difference ($P > 0.05$, t-test) between the MPTP and the control group for AL_{ON} and AL_{OFF} pressings.

Table 3
Mean (±S.E.M.) number of lever pressings on the active lever while the light was on (AL_{ON}) or off (AL_{OFF}) and AL_{ON}/AL_T ratio during two sessions of testing for MPTP-treated ($n = 12$) and control ($n = 12$) rats (paired t-test, [a] $P < 0.05$, [b] $P < 0.005$)

Treatment	Session 1	Session 2
AL_{ON}		
Control	14.17 ± 1.88	8.00 ± 1.33 [b]
MPTP	10.25 ± 1.68	5.75 ± 1.43 [a]
AL_{OFF}		
Control	17.17 ± 3.71	5.75 ± 1.69 [b]
MPTP	10.50 ± 2.95	6.00 ± 2.47
AL_{ON}/AL_T		
Control	0.51 ± 0.04	0.63 ± 0.04 [b]
MPTP	0.56 ± 0.04	0.68 ± 0.08

4. Discussion

In control rats, as early as session 1, the total number of pressings on the active lever (AL_T), which is the sum of the number of pressings when that lever procured reinforcement (AL_{ON}) and when it did not (AL_{OFF}), was higher than the number of lever pressings on the inactive lever (IL). The distribution between AL_{ON} and AL_{OFF} pressings at that time was approximately even. In session 2, the AL_{ON}/AL_T ratio increased, an indication that the rats had learned to lever-press more often on the active lever when it was on than when it was off. This was achieved in spite of the fact that the number of AL_{ON} pressings did not increase but decreased across test days. This result probably means that the rats had habituated to the light and were less motivated to extinguish it. However, in session 2, AL_T pressings were still higher than IL lever pressings, meaning that the active lever still had reinforcing properties.

Rats injected with MPTP had lower concentrations of dopamine in the striatum but not in the substantia nigra (Table 1). This result probably indicates that MPP + had entered the synaptic terminal at the level of the striatum through the dopamine uptake system but was not carried back to the cell body. There was an increase in the levels of DOPAC, the main metabolite for presynaptic dopamine metabolism. No change occurred in levels of the main metabolite for postsynaptic dopamine metabolism, HVA. Thus, interruption of dopaminergic neurotransmission had probably occurred in the absence of neurodegeneration of cell bodies. The albino rat, in contrast to human and non-human primates (Burns et al., 1984; Clarke et al., 1989; Colosimo et al., 1992), is known to be resistant to the degenerative effect of MPTP.

The mild depletion of striatal dopamine concentrations appeared to have functional effects. The dose regimen of MPTP was not sufficient to cause important motor deficits, as indicated by the absence of a significant decrease in AL_T or IL pressings. However, there was a slight decrease in AL_T pressings for MPTP-treated rats so that, contrary to control rats, in this case a significant difference between AL_T and IL rates was not found. The absence of an AL_T-IL differential effect is an indication that the active lever for rats injected with MPTP had not acquired reinforcing properties.

It is well known that dopamine is involved in brain reinforcement mechanisms as determined in intracranial self-stimulation and appetitive tests (Beninger, 1983; Wise, 1978). It appears that dopamine may also be involved in the light extinction test. Intracranial self-stimulation in dopamine-rich brain areas is conducive to high lever pressing rates. These rates are diminished by neuroleptic drugs that block dopaminergic neurotransmission. It has been proposed that dopamine antagonists reduce brain reward mechanisms (Wise, 1978). In the present test, light extinction was rewarding for normal rats, as indicated by the

higher number of AL_T pressings in comparison to IL pressings. MPTP administration eliminated this tendency.

While control rats had lower AL_{ON} and AL_{OFF} pressings across days of testing, MPTP-treated rats had lower AL_{ON} but not lower AL_{OFF} lever pressings. The lack of an effect on AL_{OFF} lever pressings was due to slightly lower pressings in session 1 rather than to higher lever pressings in session 2. This may also be due to an MPTP-related effect on reinforcement mechanisms, because some AL_{OFF} pressings may be a secondary consequence of AL_{ON} pressings directly leading to reinforcement. While control rats had a higher AL_{ON}/AL_T ratio on the second day of testing, rats injected with MPTP did not. This was not due to a lower ratio in session 2, and so no conclusion can be drawn concerning an MPTP-related effect on learning. It remains to be determined to what extent the test design can be adapted so that AL_{ON} pressings remain stable or increase.

A light-extinction test might be an interesting tool for studying the cognitive disorders which can appear in parkinsonism induced by various neurotoxic agents, and for screening new drugs for recovery of behavioral functions.

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