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A novel effect of MPTP: the selective suppression of paradoxical sleep in cats

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We studied the effect of MPTP on sleep–wakefulness cycle in cats. Five mg/kg *n*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) was administered i.p. for 5 consecutive days. Electrocorticographic, electrooculographic and electromyographic recordings were performed before (5 days), during (5 days) and after (14 days) the treatment. Total selective paradoxical sleep deprivation was observed from the first injection. This effect lasted 6–9 days after the last dose, while the relative amount of slow wave sleep increased. The Berg–Fourier analysis showed no significant change in the EEG power spectra of slow-wave sleep during the paradoxical sleep deprivation compared to control period. Recurrence of paradoxical sleep was parallel to the disappearance of the motor symptoms. Histopathological investigation showed neuronal loss mainly in the substantia nigra. Our present study suggests a complex behavioral effect of MPTP.

n-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) has been known to induce irreversible Parkinson syndrome in humans and monkeys[1,3] and reversible Parkinson-like effects in laboratory animals such as cats[16]. It affects the dopaminergic system mainly[2], but alters other catecholaminergic structures as well, such as the serotoninergic nucleus raphe and the noradrenergic locus coeruleus[5,10,12]. MPTP also produces acute hallucination[16] suggesting a more complicated spectrum of its behavioral effects. Since MPTP has been investigated from the point of view of the Parkinsonian symptoms, our knowledge of the non-Parkinsonian effects of this neurotoxin is rather limited.

The control of the sleep–waking cycle is known to be influenced by tonic changes in monoaminergic transmission[8]. The theory is widely accepted that switching off the monoaminergic transmission by the reduction in neuronal activity of serotoninergic raphe and noradrenergic locus coeruleus is an important component of the paradoxical sleep (PS) mechanism[6,7]. In turn, the cellular deficits in the raphe and locus coeruleus induced by MPTP suggested that it could induce changes in the sleeping behavior. The reversibility of the behavioral and biochemical effects of MPTP in cats could provide a good chance for studying the MPTP-induced modifications in sleep in correlation with the development and recovery of the Parkinsonian-like symptoms in the motor activity. This assumption is supported by the fact that Parkinsonian patients also have marked changes in the sleep–waking cycle[4]. In our present study we report on MPTP-induced selective and complete PS deprivation and its relations to the behavioral and histological effects of MPTP treatment.

The studies were carried out in 4 adult cats of both sexes (two males, two females, weight: 3.4–4.8 kg). The implantation was done under Nembutal anesthesia (50 mg/kg, i.p.). Gold-plated screw electrodes were inserted symmetrically into the skull above the left and right somatosensory, associative, temporal and occipital cortices to record electroencephalogram (EEG). For electromyography (EMG), two stainless-steel electrodes were placed into the neck muscles. Two screw electrodes were also implanted into the left frontal cavity for electrooculographic (EOG) recording. A recovery period of 3–5 days was allowed following the implantation.

The cats were kept individually in 150 × 70 × 80 cm large boxes with a Plexiglas window (150 × 70 cm) on the front side. The box was continuously lighted by a 1.5 W light bulb. The room temperature was between 20 and 25 °C. Cats were recorded in their cage. To habituate the animals to their environment they were placed there one week before the implantation. Full-day recordings were performed with 0.5-h-long intermissions, one at 08.00 h and the other at 13.00 h, when the animals were fed milk and meat ad libitum.

The cats were injected intraperitoneally with 5 mg/kg

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MPTP HCl (RBI) dissolved in 2 ml sterile distilled water for 5 consecutive days at 08.00 h. Respecting to the PS deprivatory effect of MPTP, the experimental session was divided into 4 parts: (1) control period before MPTP treatment (C), (2) PS deprivation during MPTP treatment (D I), (3) PS deprivation following MPTP treatment (D II) and (4) PS recovery period (R). The durations of these periods were the following: C, 5 days; D I, 5 days; D II, 6–9 days; R, 6–9 days.

The sleep stages: wakefulness (W), drowsiness (D), slow-wave sleep (SWS) and PS were defined using the usual polygraphic criteria[11]. The duration of sleep stages was obtained with 1 min accuracy, shorter periods were added to the previous one.

For statistical analysis we calculated the relative amount of the different sleep stages per hour each day. The length of the individual phases and their number per hour each day were also obtained. The data were analyzed by an unpaired two-tailed Student's *t*-test (IBM t-ease).

The EEG power spectra of the sleep stages in the four different periods (C, D I–II and R) was compared by an O.T.E. Biomedica two channel 1263–1264 Berg–Fourier analyzer between 0 and 32 Hz frequency range.

The behavioral changes were tested each day at the time of intermissions in recording. Changes of spontaneous movements, pupillary changes, ventilation pattern and weight loss were recorded.

Following the experiments, the animals were anesthetized by Nembutal (50 mg/kg) and perfused with 500 ml fixative solution (4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4)) intracarotideally, then the brains were removed and kept in the same fixative solution for one week. Coronal sections of the brains including cerebral cortex, striatum, diencephalon, mesencephalon, oral pons and medulla oblongata were dehydrated and embedded in paraffin. Five 15-μm-thick sections were stained by Luxol fast blue, Cresyl violet and hematoxylin-eosine for light microscopic examination.

The MPTP treatment caused a total PS deprivation that occurred immediately after the first injection. This PS deprivation lasted for 11–14 days. After the reappearance, the amount of PS generally reached the control (C) level within two days generally. The PS latency was longer after the reappearance of PS: it was 39 min in C and 107.6 min in R. The relative amount of PS showed no significant change during R compared to C, while phase length had a tendency to increase and the number of phases decreased significantly ($P < 0.05$) (Fig. 1, PS).

Along with the PS deprivation, the relative amount of SWS increased significantly ($P < 0.05$) during the treatment period (D I). The relative amount of SWS reached its baseline (C) already in D II and remained at

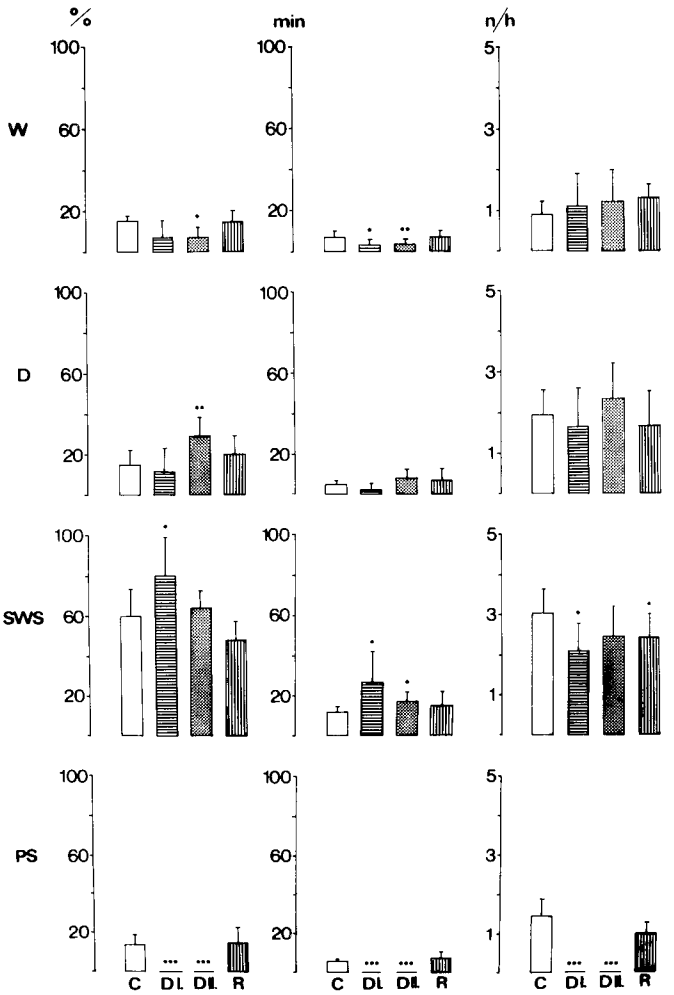

Fig. 1. Effect of MPTP on sleep–wakefulness cycle of cats. %, relative amount of phases; min, length of the single phases in minutes; n/h, number of phases per hour; W, wakefulness; D, drowsiness; SWS, slow-wave sleep; PS, paradoxical sleep; C, control; D I, PS deprivation during MPTP treatment; D II, PS deprivation following MPTP treatment; R, paradoxical sleep recovery period. $^{o}P < 0.05$; $^{oo}P < 0.005$; $^{ooo}P < 0.001$.

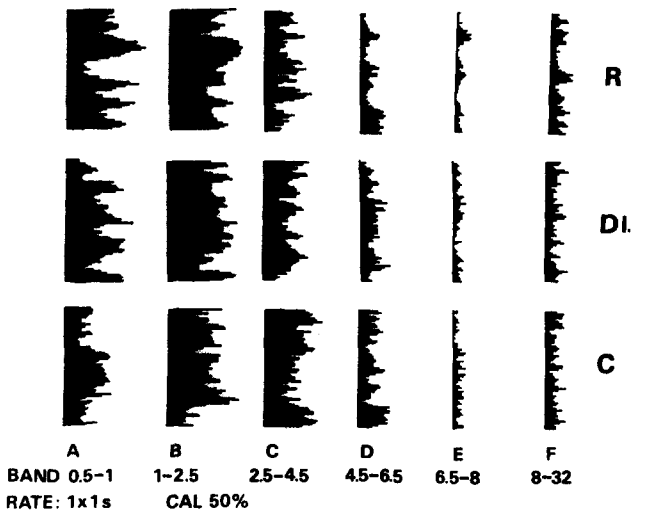

Fig. 2. Comparison of the EEG power spectra of slow-wave sleep (SWS) by Berg-Fourier analysis in the control (C), paradoxical sleep deprivation (D I), and paradoxical recovery periods (R) showed no significant change.

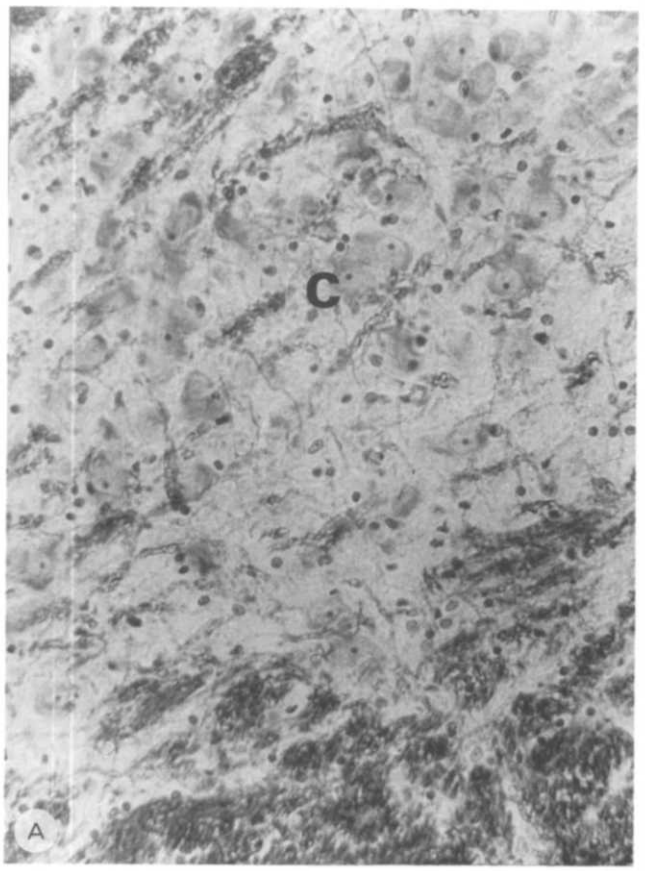

Fig. 3A. *For legend see Fig. 3B.*

that level without significant change. The phase length increased during DI ($P < 0.05$), and showed a decrease in D II, though it remaind slightly elevated even in R. The number of phases decreased significantly in D I ($P < 0.05$) and elevated in D II, though they still remained under the control value in R (Fig. 1, SWS).

The relative amount of D decreased during the MPTP treatment (D I), while it increased above the control value during the post-treatment period (D II) and showed no significant change in R compared to C. The phase length and the number of phases had the same tendency (Fig. 1D).

The relative amount and the phase length of W had a tendency to decrease during D I, and reached the level of C gradually in R. The number of phases showed the opposite tendency. These changes, however, were not significant (Fig. 1W).

The Berg–Fourier analysis of the different stages showed no difference comparing the EEG power spectra of C, D I–II, R, neither did the power of the delta waves decrease in SWS following MPTP treatment (Fig. 2).

The MPTP treatment caused pupillary dilatation within 3–5 min after the injection lasting for 2–3 h on the first day. From the 3rd to the 4th day they remained constant and disappeared 1–2 days after finishing the treatment. In one animal, the injections caused transient hyperventilation for 1–2 h.

The other symptoms developed from the 1st to the 2nd day of the treatment. Lack of spontaneous vocalization, reduction in the amount of movements, difficulty in initiation of motor actions, weight loss and hypersalivation were observed. The motor activity of the cats was

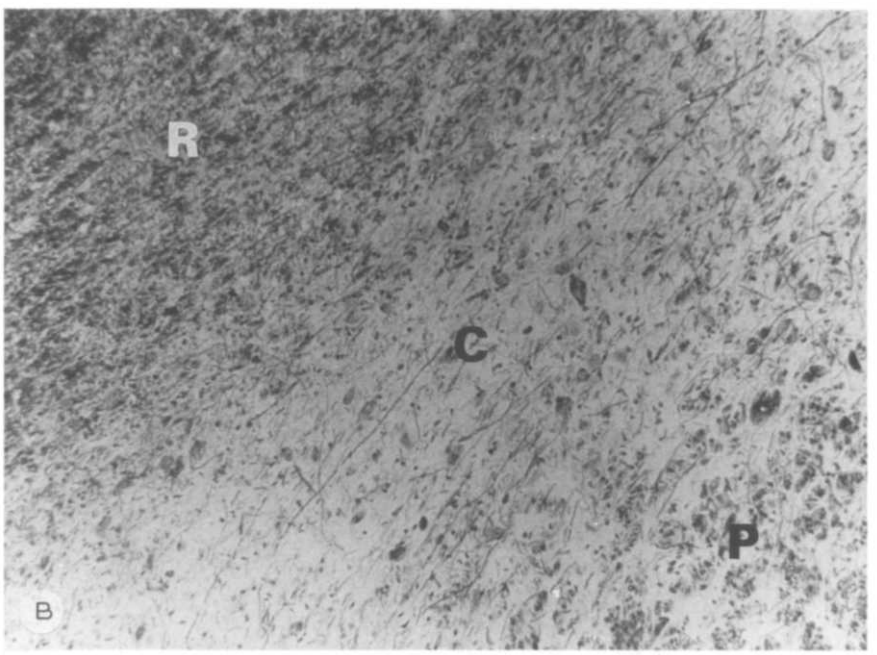

Fig. 3B. Middle third of the substantia nigra in control cat (A) and in an MPTP treated cat (B). B shows nearly complete cell loss in the compact zone. P, cerebral peduncle; C, compact zone of the substantia nigra; R, reticular formation of the mesencephalon.

specifically changed in each animal. Performing some movements, the cats tended to remain in a fixed position for several minutes. They preferred resting with extended hind limbs, which were hard to flex. In two animals we observed transient trembling of head, tail and limbs. All these symptoms worsened up to the last dose of MPTP. Following the treatment, the symptoms remained stable for 6–8 days, and improved till the end of the experiments, when still some reduction in the amount of movements could be observed.

The histopathological examination showed degeneration of the lateral two thirds of the substantia nigra. The most severely affected portion was the middle third of the compact zone with apparent sparing of the medial third including the ventral tegmental area. Contrary to the control cats, where 30–35 nerve cells were found in 1 mm^2 of a 15-μm-thick section in the lateral two thirds of the substantia nigra, the number of nerve cells was reduced to 0–6 cells/mm^2 in MPTP treated animals. Less marked cell degeneration was found in locus coeruleus, nucl. basalis Meynert and in the dorsal nucleus of the vagal nerve.

In our present study we report on MPTP-induced PS deprivation without typical PS deprivation symptoms (hallucination, aggressive behavior, sensory disabilities, etc.). The lack of rebound phenomena after the reappearance of PS suggests a reduced physiological need for PS after MPTP administration. An efficient trigger mechanism for PS is originated from the acetylcholinergic group of the dorsal part of the gigantocellular tegmental field[13–16]. The destruction of these cells could explain the depriving effect of PS caused by MPTP. However, specific histochemical studies have not been made on the cholinergic neurons in this study. Thus the possibility of their role cannot be excluded.

The SWS period preceding PS is characterized by an increase in delta waves[11], which could be a result of the destruction of dopaminergic pathways, since dopamine is known to inhibit SWS sleep[9]. The MPTP-induced PS deprivation was not a result of delta wave reduction in SWS, as it was shown by the EEG power spectra analysis. It is supported by the fact that the amount of SWS increased during the PS deprivation period indicating a compensatory mechanism. In the PS recovery period, the SWS/PS balance was also restored, so the regulatory mechanisms of the sleep–waking cycle were reversibly affected by MPTP.

The development of Parkinsonian symptoms required 1–3 days from the beginning of the treatment, while the PS deprivation started immediately after the first injection. This also suggests that the destruction of monoaminergic neurons is not substantially important for the PS deprivation or that even a low amount of *n*-methyl-4-phenyldihydropyridinium ion (MPP^+) generated from MPTP is able to modify the function of the monoaminergic neurons involved. On the other hand, the reappearance of PS and the recovery started at the same time, suggesting a synchrony in the restoration of Parkinsonian-like effects and PS deprivation.

Accordingly, the MPTP-induced PS deprivation indicates a complex effect of this neurotoxin. The final behavioral changes of MPTP treatment are probably the result of interfering effects of motor deficits and PS deprivation. Therefore MPTP induced Parkinsonism in cats is different to some extent from the human disease, where total PS deprivation does not occur. Otherwise, MPTP treatment provides a novel method for PS deprivation in cats without the stressful awakening of the animal.

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