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Changes in Motor Activity and the Sleep–Waking Cycle in an MPTP Model of Parkinson's Disease in Mice

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Mice with previously implanted electrodes for recording the cortical electroencephalogram and electromyograms underwent all-day baseline videopolysomnography with a 12/12 light cycle, after which animals received doses of 24 or 48 mg/kg of the neurotoxin precursor MPTP (1-methyl-4-phenyl-1.2.3.7-tetrahydropyridine) or physiological saline (controls) and recording was continued for 14 days. At the end of the experiments, morphological monitoring of the extent of lesions to the dopaminergic system was performed. Increases in motor activity and the total duration of waking during the dark part of the night were seen after administration of MPTP, as compared with controls. These changes were accompanied by decreases in the durations of rapid and (at the level of a tendency) slow-wave sleep. These changes were apparent by recording day 7 and were significant by day 14; they were more marked after treatment at a dose of 48 mg/kg than after the smaller dose. No changes were seen during the light period of the day. Morphological monitoring demonstrated a 70% reduction in the number of dopamine-containing neurons in the compact zone of the substantia nigra after administration of MPTP (48 mg/kg) and a 35% drop after MPTP (24 mg/kg).

Keywords: Parkinson's disease, experimental models, motor activity, sleep–waking cycle.

The search for early markers for Parkinson's disease is an important task in the diagnosis of neurodegenerative diseases. Parkinson's disease is known to be accompanied by a wide spectrum of impairments to the sleep–waking cycle affecting, according to some reports, up to 98% of patients [4]. These impairments include symptoms of sleeplessness (insomnia), excessive daytime drowsiness (hypersomnia), parasomnia, impairments to respiratory and motor activity during sleep, etc. Insomnias in Parkinson's disease include decreases in the total duration and effectiveness of nocturnal sleep, along with frequent nocturnal waking. Changes in the REM sleep phase* are often noted: suppression of REM sleep, nightmares and behavioral impairments (REM sleep behavioral disorders, RBD). Hypersomnic symptoms and RBD are the earliest precursors of Parkinson's disease, arising several years and sometimes decades (the published re-

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* The rapid eye movement (REM, paradoxical) phase of sleep is a special state of the body in warm-blooded animals, which occurs periodically during sleep (every 90 min in adults) and characterized by very high levels of brain activity, complete suppression of tonic muscle tone (interrupted by episodic phasic twitches) and irregularity in the cardiac and respiratory rhythms). This is the state in which dreams are dreamed. Its evolutionary origin, functional significance, and molecular mechanisms remain mysterious despite more than half a century of very intense study [1, 3].

cord is 28 years!) before the onset of motor disorders [9, 15]. However, the mechanisms underlying the development of sleep–waking cycle disorders in Parkinson's disease remain essentially unknown [4, 5, 15].

Progress in studies of the pathogenesis of neurodegenerative disorders depends primarily on the development of suitable experimental models. One widely used model is based on the use of 1-methyl-4-phenyl-1.2.3.7-tetrahydropyridine (MPTP), a model of parkinsonism in mice. Black C57BL/7 mice are given systemic doses of the neurotoxin MPTP, which selectively lesions the dopaminergic system. The effects of this toxin depend on the dose and administration regime. Thus, recent studies have shown that two doses of MPTP (12 mg/kg) with a 2-h interval imitates the preclinical, while treatment with four doses simulates the early clinical stage of parkinsonism [17]. However, changes in the sleep–waking cycle in these models in comparison with controls have not been studied. This was the aim of the present work.

Methods

Experimental protocols were approved by the Ethics Committees of the institutes taking part in the study. C57BL/7 mice aged 2.5–3 months weighing 25–30 g were anesthetized with Avertin and subdural electrodes were implanted for chronic recording of the electroencephalogram (EEG) from the frontotemporal neocortex and electromyograms (EMG) of the posterior cervical muscles. Animals were placed in small individual sound-proofed boxes fitted with highly sensitive modular video cameras connected to a video recorder, whose output as in turn connected to the USB port of a computer. Animals were kept in a 12/12 light regime (bright white light from 09:00 to 21:00, faint red light from 21:00 to 09:00) at a temperature of 24–27°C with unrestricted access to water and food. Continuous EEG and EMG recordings were made in conditions of free mobility by permanent connection of each animal via a flexible cable and rotating socket to the input of a digital electroencephalograph whose output was in turn connected to a computer USB port. Recording, examination, and analysis of EEG and EMG traces, along with video monitoring and video tracking to provide quantitative assessments of the level of motor activity in arbitrary units per hour of recording time, were performed using a set of special programs.

After one week, 24-h baseline recordings were made of the EEG and EMG, motor activity, and behavior, after which two- and four-dose treatments with MPTP were given (MPTP is a precursor of a neurotoxin which lesions the dopaminergic system) at a dose of 12 mg/kg in 0.3 ml s.c. Control animals received analogous injections of 0.3 ml of physiological saline. The interval between doses was 2 h. Over the next two weeks, continuous all-day recordings of the EEG, EMG, behavior (video monitoring), and motor activity (video tracking) were made.

The resulting polysomnograms (EEG, EMG) were analyzed visually using 20-sec epochs. Standard criteria were used to identify the states of waking, slow-wave sleep, and REM sleep [14]. Statistical analysis was run using nonparametric methods in GraphPad/Prism 4.02 (Friedman and Kruskal–Wallis analysis of variance, post hoc Dunn test, and the Wilcoxon and Mann–Whitney tests).

At the end of the experiments, animals were anesthetized with urethane (>1 g/kg, i.p.) and subjected to transcardiac perfusion with 0.2 M phosphate buffer pH 7.4 in physiological saline (0.9% NaCl solution) (phosphate-buffered saline) followed by 4% paraformaldehyde in 0.2 M phosphate buffer. Fixed brains were harvested and post-fixed with 4% paraformaldehyde in 0.2 M phosphate buffer and incubated in 30% sucrose solution in phosphate-buffered saline. Brains were frozen in isopentane at –40°C. Frontal sections of thickness 40 μm were then cut. Each fourth section of thickness 40 μm containing the substantia nigra (SNpc) was sequentially incubated in phosphate-buffered saline containing: 1) mouse monoclonal antibodies to tyrosine hydroxylase (1:200) (TH, Sigma, USA) with 2% normal horse serum and 0.3% Triton X100 (Sigma, USA) at 4–8°C for 12 h; 2) biotinylated antibodies to mouse immunoglobulins (1:200) (Vector Laboratories, USA) at 20°C for 2 h; 3) avidin-biotin complex conjugated with horseradish peroxidase (Vector Laboratories) at 20°C for 1 h. Peroxidase was then detected using 0.03% diaminobenzidine tetrahydrochloride solution with 0.05% H_2O_2 (Sigma, USA). Stained sections were mounted on slides and embedded in 50% glycerol. TH-immunopositive neuron bodies were counted using an Olympus IX81 microscope (Japan) fitted with a Märzhäuser motorized slide stage (FRG) controlled by computer and a digital Olympus DP72 camera. Cells were counted on the computer monitor using the "Cell*" program (Olympus Soft Imaging Solution GmbH). Cells were counted in the compact zone of the substantia nigra (substantia nigra/pars compacta, SNpc) and the ventral tegmental area (VTA).

Results

Video tracking results (Fig. 1) revealed a consistent increase in total motor activity in mice after administration of toxin in the dark period of the day. Animals given toxin (4 × 12 mg/kg; $n = 7$) showed progressive increases in motor activity in the dark (active) period of the day, such that by the end of the experiment the level was significantly greater (+40%; $p < 0.05$) than baseline. Mice given the smaller neurotoxin dose (2 × 12 mg/kg; $n = 7$) showed no change in motor activity (results not shown). In control animals ($n = 8$), there was an insignificant variation in this level around the baseline value. There were no changes in activity levels from baseline during the light period of the day either in animals given injections of neurotoxin or in control animals (results not shown).

Analysis of polysomnography results showed that the total duration of waking in the dark part of the day after administration of MPTP at both doses (2 × 12 and 4 × 12 mg/kg, s.c.) underwent a gradual increase, reaching signifi-

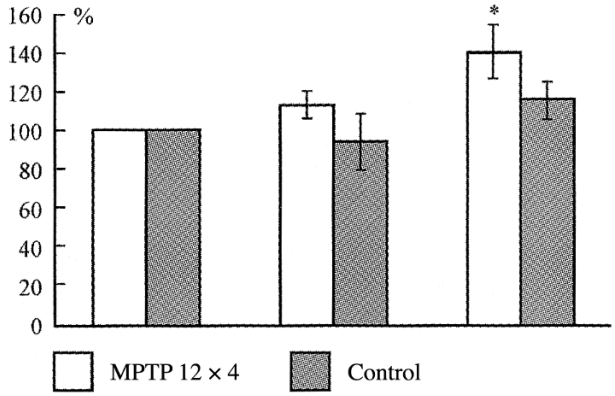

Fig. 1. Motor activity in mice during the dark (active) period of the day, video tracking data. The ordinate shows the total level of motor activity (taking the baseline level as 100%); the abscissa shows activity in baseline conditions and on days 7 and 14 after administration of toxin. Light columns show animals given toxin (4 × 12 mg/kg); dark columns show controls; *$p < 0.05$ compared with baseline, $n = 6$ (Dunn's test).

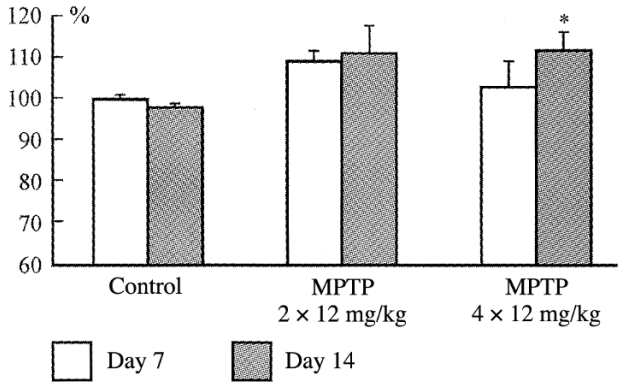

Fig. 2. Content of waking during the dark period of the day, EEG and EMG measures. The plots show percentage changes in the total duration of waking after the 12-h dark period relative to the baseline level, which was taken as 100% in controls and on days 7 and 14 after administration of the toxin MPTP (2 × 12 and 4 × 12 mg/kg). Light columns show day 7 and dark columns show day 14. *$p < 0.05$ compared with baseline, $n = 6$.

cance by day 14 ($p < 0.05$), while controls showed insignificant oscillations of this parameter around the baseline level (Fig. 2). The proportion of slow-wave sleep during the dark part of the day after administration of MPTP at both doses underwent a gradual decrease, though this did not reach significance because of the wide spread in the data ($p = 0.57$; $n = 7$); at the same time, this parameter in control animals oscillated close to the baseline level (Fig. 3). The content of REM sleep in the dark part of the day after the high dose of MPTP also decreased; this decrease reached significance by day 14 ($p < 0.05$), though in controls it showed only minor variations close to the baseline level (Fig. 4). During the light period of the day (results not shown), there were no changes in the sleep–waking cycle as compared with control mice.

Morphological monitoring showed a 35% decrease in the number of TH-immunopositive neurons in the SNpc after two injections of neurotoxin and a 70% decrease after four injections, without any marked change in the number of stained cells in the ventral tegmental area of the midbrain (VTA) (Fig. 5).

Discussion

The experiments reported here support data [17] showing a decrease in the number of TH-immunopositive neurons in the SNpc two weeks after administration of 2 × 12 and 4 × 12 mg/kg of the neurotoxin MPTP. The smaller dose decreased the number of cell bodies by 35% (compared with 27% in [17]), while the larger dose produced a 70% reduction (compared with 43% in [17]). The difference in the effects seen in our studies from those reported in [17] can probably be explained in terms of the larger number of animals used for histological analysis in the study cited ($n = 20$, compared with $n = 4$ in our experiments), such that individual variation played a significant role in our experiments. It also remains possible that surgery and anesthesia had long-term sequelae, increasing the sensitivity of the animals to the toxin.

The changes in motor activity and the sleep–waking cycle found here in the MPTP model of the preclinical and early clinical stages of Parkinson's disease in mice allow us to approach the conclusion that two and four s.c. doses of 12 mg/kg of toxin, despite moderate (in the former case) and significant (in the latter) decreases in the numbers of TH-immunopositive neurons in the SNpc, did not induce any marked impairments to the sleep–waking cycle. In the light, mainly inactive period of the day, toxin-treated animals slept just like control individuals. During the active period of the day, toxin-treated animals appeared to experience motor discomfort, such that they became more "hurried" (which is also characteristic of patients with Parkinson's disease[#]); this was confirmed by examination of video recordings. This was also reflected as an increase in the total duration of waking, due to a decrease in the proportions of both phases of sleep during the dark period of the day.

Patients in the clinical stage of Parkinson's disease, when more than half the SNpc neurons have degenerated, also show decreases in the total durations of sleep (by 18%) and REM sleep (two-fold) as compared with healthy subjects [16]. However, these effects arise during the night, which is the inactive period in humans); in the daytime, these patients show excessive drowsiness. Thus, despite the external similarity, impairments to nocturnal sleep in patients with Parkinson's disease cannot be compared directly with our results in models of the early stages of "mouse" parkinsonism.

Our results are quite unexpected given the important role of the ascending dopaminergic system in controlling the sleep–waking cycle [1, 4, 13]. We might expect from

[#] "The first qualities of Parkinsonism which were ever described were those of *festination* (hurry) and *pulsion* (push). Festination consists of an acceleration (and with this, an abbreviation) of steps, movements, words, or even thoughts..." [6].

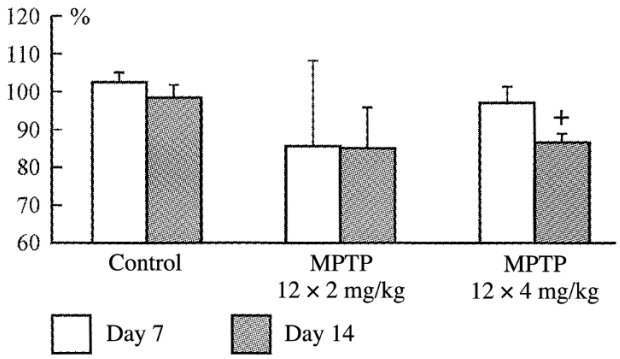

Fig. 3. Content of slow-wave sleep in control animals and mice given MPTP. For further details see caption to Fig. 2; $^{+}p = 0.056$ compared with baseline, $n = 6$.

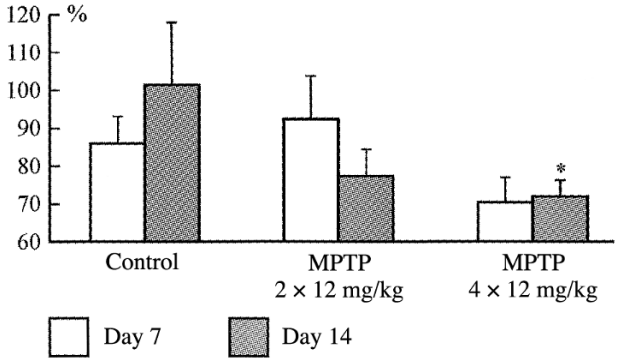

Fig. 4. Content of REM sleep during the dark period of the day. For further details see caption to Fig. 2. $^{*}p < 0.05$ compared with baseline, $n = 6$.

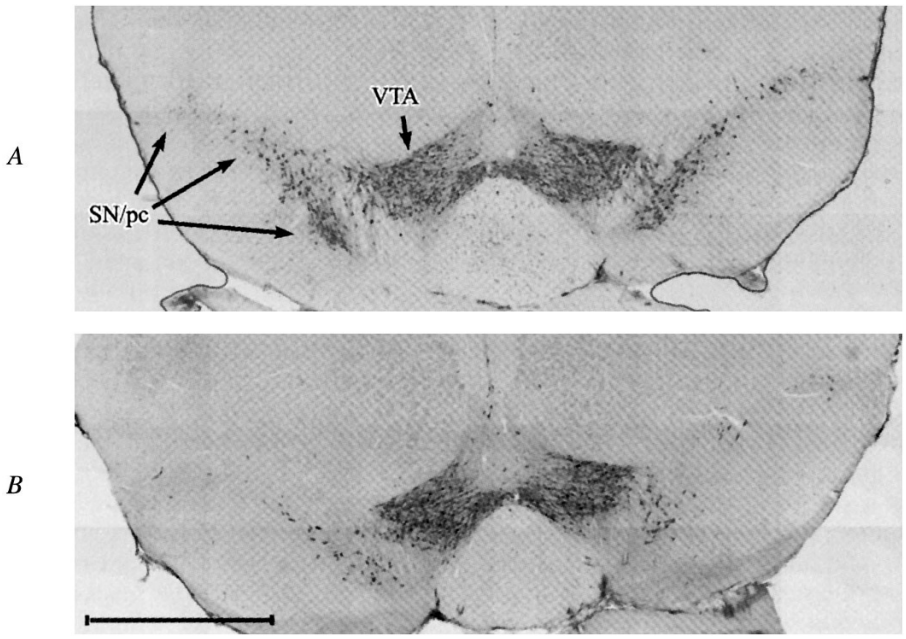

Fig. 5. Frontal sections of the brains of mice, immunohistochemical staining for the dopaminergic neuron marker protein TH in controls (*A*) and two weeks after systemic administration of toxin MPTP at a dose of 4 × 12 mg/kg (*B*). A decrease in the quantity of TH-immunopositive neurons can be seen in the compact zone of the substantia nigra (SNpc) after administration of toxin. This effect was weaker in the central part of the ventral tegmental area (VTA) of the midbrain. Scale bar at left: 1 mm.

this role that partial lesioning of SNpc neurons leads to a decrease rather than an increase in the proportion of waking in the active period of the day (the direct effect of lesioning) and a decrease in the proportion of REM sleep – not in the dark, but in the light, inactive, period (the indirect effect) [4]. MPTP at a dose of 4 × 12 mg/kg has been found to evoke significant specific motor impairments (decreased walking distance and number of rearings in the open field, shortening of steps) [17].

Thus, the marked morphological and specific motor impairments in this model of Parkinson's disease *are not accompanied by* especially marked changes in the sleep–waking cycle. It can be suggested that the descending dopaminergic system is functionally much more susceptible to the damaging action of the neurotoxin MPTP than the ascending. High levels of functional resistance of the ascending activatory systems of the lisencephalic brains of rodents to neurotoxins have been noted in other studies [1, 2]. Thus, the experiments reported in [7] showed that rats given local intracerebral injections of saporin-containing neurotoxins, which allow "targeted" degradation of chemically specific neuron bodies, *does not lead to* significant impairments to

the sleep–waking cycle. The lesions produced by these authors affected up to 75% of histaminergic neurons in the tuberomammillary nuclei of the posterior hypothalamus, along with 90% of noradrenergic cells in the locus ceruleus and cholinergic basal nuclei of the forebrain, with almost no effect on the surrounding cells. Simultaneous lesioning of one, two, and even three activating systems in the same animals was found to lead at 20 days to only minimal changes in the sleep–waking cycle. Of these changes, the main were twofold decreases in the content of waking on transition from the light period of the day to the dark and in REM sleep in the light part of the day [7]. This suggests that the other ascending activatory systems (of which there are tens [3]) take on the lost functions and compensate for the loss of toxin-lesioned cells. However, there are also other possibilities; these effects remain essentially unstudied [1, 2].

Overall, the development of experimental models of parkinsonism by lesioning dopaminergic neurons in the SNpc and VTA has produced very contradictory results in relation to similarities with symptoms of sleep–waking cycle impairment typical of patients with Parkinson's disease. Thus, partial lesioning of all the dopaminergic systems induced in C57BL/7 mice by five days of once-daily systemic injections of 25 mg/kg MPTP led to no more than a moderate increase in the content of REM sleep during some of the hours recorded [14]. These effects appeared 20 days after administration of toxin and disappeared at 40 days, despite irreversible 30% lesioning of dopaminergic neurons in the SNpc [11]. It is interesting that the selective pharmacological increase in synaptic dopamine content (via systemic administration of its reuptake inhibitor) in this model led to suppression of REM sleep, which was significantly more marked than in control mice. An analogous effect was also produced by administration of the noradrenaline reuptake inhibitor desipramine, an antidepressant. At the same time, another antidepressant, the serotonin reuptake inhibitor citalopram suppress REM sleep in both experimental and control mice to identical extents. The muscarinic receptor agonist arecoline induced an increase in the content of REM sleep in experimental but not control mice. Overall, mice with lesions to the dopaminergic system were much more sensitive to administration of pharmacological agents modulating the aminergic and cholinergic systems of the brain than control animals [10].

In summary, we can note that the mouse MPTP model of parkinsonism appears to be entirely suitable for studies of the specific motor symptoms of Parkinson's disease and can, with caution, be used for investigations of the sleep–waking cycle at the early stages of disease. Other authors have recently come to this same conclusion [5, 8, 12, 13], as no neurotoxic model of the presymptomatic stage of Parkinson's disease with sleep disturbance has yet been found. New models of Parkinson's disease need to be developed and studied. Thus, considering the important role of RBD as a factor preceding the development of synucleinopathy in 80% of patients, models of this impairment have been developed by lesioning the sublaterodorsal nucleus of the pons or the gigantocellular reticular nucleus of the medulla oblongata in adult cats, rats, and mice, as well as in juvenile rats. In marmosets, systemic doses of MPTP, inducing minor motor lesions during waking, led to the appearance of muscle tone during REM sleep – a precursor of RBD [15].

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