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Early Stages of Parkinson's Disease: Comparative Characteristics of Sleep–Wakefulness Cycle in Patients and Model Animals

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Abstract—The results of study on the sleep–wakefulness cycle in experimental models of the preclinical and early clinical stages of Parkinson's disease are presented and compared with clinical examples. The conclusion is made that the enhancement of behavioral activity and decrease in the total duration of the slow-wave and paradoxical sleep in model animals occur at the same circadian period of the secretion of pineal melatonin as sleep disorders in patients.

Keywords: sleep–wakefulness, Parkinson's disease, experimental models
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Parkinson's disease (PD) is among the most socially important diseases, and research in the biological mechanisms of its pathogenesis (along with those Alzheimer's disease, depression, and stroke pathogeneses) is an urgent task facing all the modern sciences dealing with the brain, as emphasized President of the Russian Academy of Sciences at the *Brain* Scientific Session in December 2009 [1]. For reasons still unknown, this disorder entails very slow (decades-long) but steadily progressive degeneration of dopaminergic neurons in the pars compacta of the substantia nigra (SNpc) of the midbrain projecting their axons to the striatum. For many years it progresses asymptomatically because of compensatory mechanisms, and it is not until the late stage of the disease, when less than half of the initial number of dopamine-containing neurons is left and the level of dopamine in the striatum supplied by these neurons drops fourfold, that motor and, later, cognitive disorders occur[1]. However, it is too late to begin treatment at this stage, and the world history of medicine knows no PD patient that was cured. Modern medicine can only mitigate the symptoms of PD and, in some cases, slightly slow down the disease progress. Therefore, development of adequate experimental models and search for early markers are topical tasks today [3, 4].

Like no other disease, PD is accompanied by an outstandingly diverse spectrum of sleep disorders, which are found in 45–98% of patients (according to different sources) and include insomnias, parasomnias, hypersomnias, and other disorders of sleep and wakefulness [5, 6]. The most important of them are manifestations of hypersomnias (excessive daytime sleepiness (EDS) and the so-called "sleep attacks") and disorders of behavior in rapid-eye-movement (REM) phase of sleep[2] (REM behavior disorders,

[1] "The first, originally described characteristics of parkinsonism are *fidgeting* (festination) and *propulsions* (jerkings). The fidgeting is expressed in speeding-up (and simultaneous shortening) of steps, movements, pronunciation of words, and even thoughts" [2].

[2] Rapid-eye-movement (REM) or paradoxical sleep phase is a specific state of the body of homoeothermic animals that periodically occurs during sleep (in humans, every 1.5 h) and is characterized by an extremely high brain activity, complete suppression of the tonic muscle tone (occasionally interrupted by phasic jerks) and irregular cardiac and respiratory rhythms. This is the state in which dreams occur. The evolutionary origin, functional role, and molecular mechanisms of REM sleep remain mysterious notwithstanding more than 50 years of intense studies [7–9]. The terms slow sleep and REM sleep have about a dozen synonyms each (slow-wave/fast-wave sleep, orthodoxical/paradoxical sleep, non-REM/REM sleep, telencephalic/rhombencephalic sleep, quiet/activated sleep, etc.). There is no commonly accepted English terminology yet. In the original Russian paper, the pairs of terms recommended by A.M. Vein (1928–2003), member of the Russian Academy of Medical Sciences, the founder of the Russian school of "sleep medicine" and human sleep physiology, are used.

RBDs). EDS and RBD, along with anosmia (drastic deterioration of the olfactory function, see [10]), are early predictors of PD and sometimes manifest themselves years or even decades before the onset of motor disorders. As many as half PD patients suffer from EDS, and disorders of REM-sleep behavior are found in about one-third of PD patients [11].

Progress in the study of the pathogeneses of neurodegenerative disorders primarily depends on the development of appropriate experimental models. The 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) model of parkinsonism is one of commonly accepted models. This model appeared owing to seven drug addicts from California with manifest symptoms of parkinsonism described in 1983. Those young persons were found to practice intravenous injections of unpurified meperidine, a synthetic analogue of heroine containing a large amount of MPTP as a by-product. Subsequent careful studies have shown that the MPTP molecule, which is highly lipophilic, easily crosses the blood–brain barrier, penetrates into astrocytes, and is converted into the 1-methyl-4-phenylpyridine ion (MPP^+) by monoamine oxidase B (MAOB). This ion binds with dopamine active transporter (DAT), a highly affine integral membrane protein, thereby being transported into dopaminergic neurons and eventually enters mitochondria. There, it inhibits complex 1 (related to the enzyme NADH-ubiquinone oxydoreductase) of the mitochondrial electron transport chain and, as a result, uncouples oxidative phosphorylation. This, in turn, interferes with ATP production and leads to an increased extracellular calcium level and formation of free radicals/reactive oxygen species, which interact with cell proteins, nucleic acids, lipids, and other molecules to cause lesions and eventual death of the neurons; i.e., dopamine neurotoxicity occurs [12].

The toxicity of MPTP considerably varies in different mammalian species, mainly depending on the brain content of MAOB. This content is high in carnivores and primates and low in rodents, and this enzyme is almost absent in rats (which accounts for their high resistance to rodent control procedures). Therefore, the Parkinsonian phenotype is formed in cats and monkeys after treatment with about 15-fold lower doses of the proneurotoxin than in mice. In rats, it is practically impossible to cause signs of parkinsonism in chronic experiments with systemic administration of MPTP [13].

However, a model of parkinsonism in Ñ57BL/6 mice has been developed; at present, it is generally accepted and has been validated by the International Society of Neurobiology and Psychopharmacology. In this model, Ñ57BL/6 mice are systemically injected with the MPTP proneurotoxin, which, as mentioned above, selectively destroys the dopaminergic system. The effect of MPTP depends on the dosage and injection protocol. Recently, it has been shown that two injections of 12 mg/kg MPTP at a 2-h interval mimic the preclinical stage of parkinsonism, and four injections, its early clinical state [14, 15].

We have used these models to study the sleep–wakefulness cycle [16]. Electrodes were preliminarily implanted (under avertin anesthesia) to mice for recording a cortical electroencephalogram, and round-the-clock background video polysomnography was carried out at a 12L/12D illumination cycle. After that, the mice were subcutaneously injected with 12 × 2 or 12 × 4 mg/kg of MPTP or physiological saline (control), and the recording was continued on days 7 and 14 after the injection. After the experiments were completed, we performed the morphological assessment of the degree of destruction of the nigrostriatal dopaminergic system. We found increased motor activity and total duration of wakefulness in the dark period of the nychthemeron one to two weeks after the MPTP injection as compared to the control group. Accordingly, the durations of the REM sleep and slow-wave (SW) sleep were decreased. These changes appeared as soon as day 7 of recording and became significant on day 14; they were greater after injection of a total MPTP dose of 48 mg/kg (12 × 4 mg/kg) than at the lower total dose. During the light period of the day, no changes were observed. The morphological examination showed a 70% decrease in the number of dopamine-containing neurons in the substantia nigra pars compacta after injection of 48 mg/kg of MPTP and a 35% decrease, after injection of 24 mg/kg (12 × 2 mg/kg) of MPTP.

Figure 1 shows, as an example, hypnograms of the same mouse before and 14 days after the MPTP injection. As seen from Fig. 1 (left), wakefulness accounted for 59% of the duration of the night background recording; SW sleep, for 35.5%; and REM sleep, for 5.5% of it. Two weeks after the MPTP injection (Fig. 1, right), the duration of wakefulness increased to 69% due to prolongation of the periods of activity, and the durations of SW and REM sleep decreased to 27 and 4%, respectively.

Figure 2 (left) shows a hypnogram (three stages of SW sleep, according to AASM–2007[3] of a 64-year-old female patient without PD symptoms. Despite an increased number of awakenings during sleep (nine, including four awakenings longer than 3 min each), the total duration of wakefulness in the period from

[3] According to the 2007 classification of the American Academy of Sleep Medicine (AASM), the SW sleep phase is subdivided into three stages [17], where stage 3 comprises stages 3 and 4 according to the generally accepted Rechtschaffen and Kales's (1967) classification.

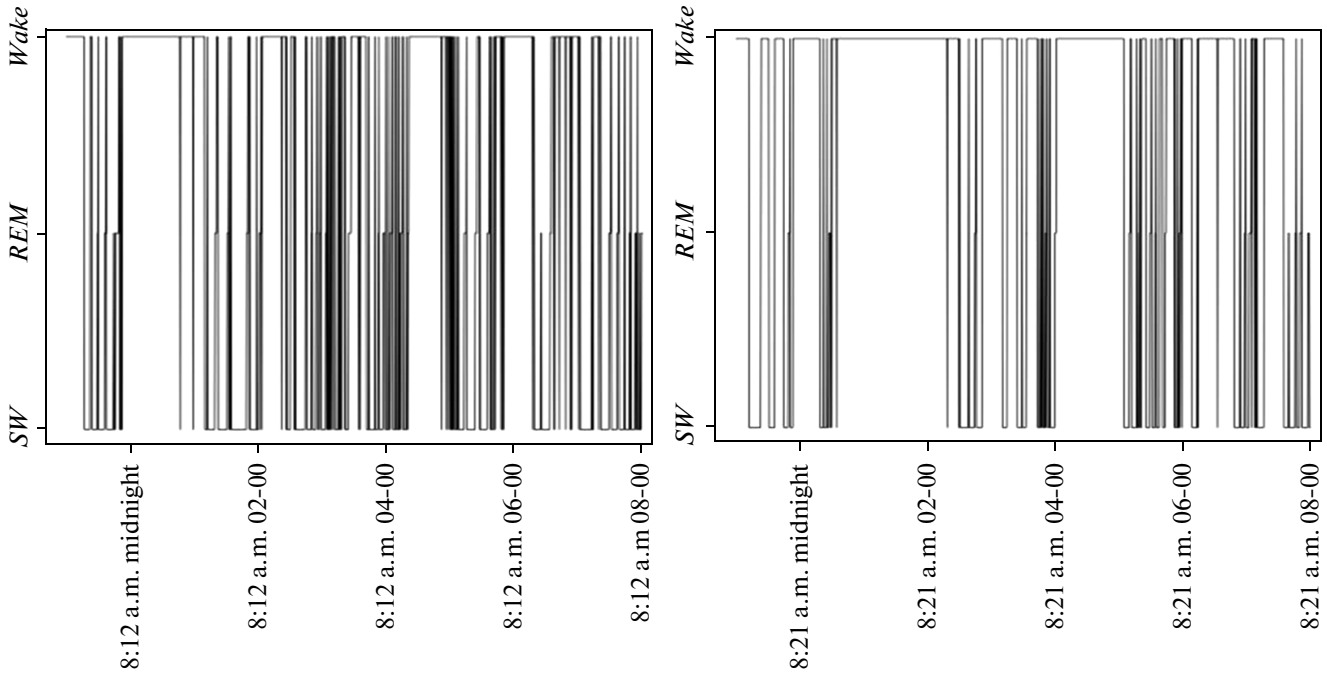

Fig. 1. Representative hypnograms of a mouse recorded during a 12-h "dark" (dim red light) period in a chamber: left, baseline; right, two weeks after a subcutaneous injection of 48 mg/kg (4 × 12 mg/kg) of the dopaminergic proneurotoxin MPTP. The abscissa shows the time of day. The ordinate, from the top down: Wake, wakefulness; REM, REM sleep phase; SW, SW sleep phase. Note wider "light" areas in the right panel compared to the left one, which reflects longer periods of wakefulness.

going to sleep to awakening in the morning was slightly longer than 20 min, and the proportions of the deep SW sleep (stage 3, 18% of the total duration of sleep) and REM sleep (22%) remained within the normal limits. A hypnogram of a 65-year-old male patient with stage 1 of PD according to Hoehn and Yahr (1967) [10] is shown for comparison in Fig. 2 on the right. As seen in the hypnogram, the number of awakenings (24, including six awakenings longer than 3 min each) and the total duration of wakefulness during the period of night sleep were increased; accordingly, the total duration of sleep was decreased. We found decreased durations of both stage 3 of SW sleep (11%, almost 1.5 times shorter than the normal value of 15–20%) and REM sleep (also 11%, two times shorter than the normal value of 20–25%). This patient had no manifest respiratory disturbances during sleep (apnea/hypopnea), but he had a distinct early symptom, periodic movements of extremities during SW and REM sleep.

Another patient, 58 years of age, who was diagnosed with REM behavior disorder associated with α-synucleionopathy and obstructive sleep apnea/hypopnea of severe degree (Fig. 3) also displayed considerably increased durations of periods of wakefulness during night sleep (in total, longer than 1 h); in the given case, this was associated with respiratory disturbance and periodic movements of extremities. In this patient, the decrease in the proportions of stage 3 of SW sleep and REM sleep phase was smaller (to 14 and 18%, respectively).

The literature data show an even greater increase in the total duration of wakefulness during the time spent in bed in patients with clinical stages of PD treated with levodopa and other drugs. Accordingly, this results in decreased total duration of night sleep (by 20%) and, especially, duration of REM sleep (by half) compared to control values [18].

Thus, polysomnographic data show that the changes in the sleep pattern in PD patients are generally similar to those observed in experiments on mouse MPTP models of the preclinical and early clinical stages of parkinsonism in nighttime. Mice are nocturnal animals; therefore, if one correlates the changes in sleep parameters with circadian rhythmicity, the resultant relationships for PD patients and experimental mice are not similar to each other. However, if one correlates these changes with the rhythm of pineal melatonin release, which is secreted in the nighttime in both humans and animals, similarity is obvious. Disturbance of the pineal melatonin secretion plays a role in both the pathogenesis of PD and its treatment with levodopa preparations [19, 20]. This provides grounds for further use of these models in studying early predictors of PD and development of methods for its treatment.

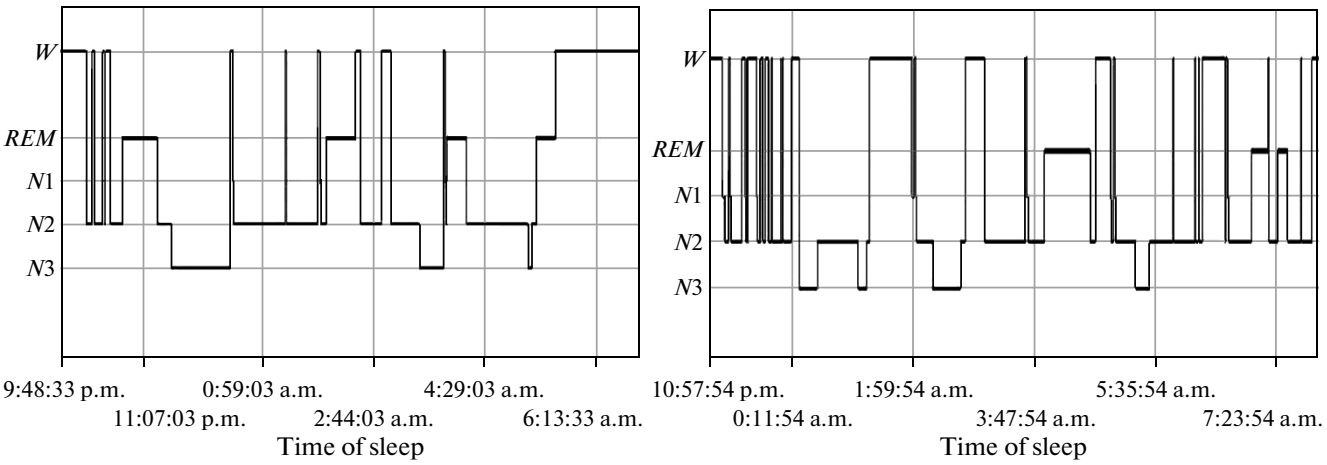

Fig. 2. Representative hypnograms of two patients (data provided by E.A. Lyashenko and M.G. Poluektov). Left, a 64-year-old female patient without symptoms of Parkinson's disease; right, a 65-year-old male patient with stage 1 of Parkinson's disease. See Fig. 1 for denotations. It can be seen that long awakenings are more numerous and much longer in the right hypnogram than in the left one.

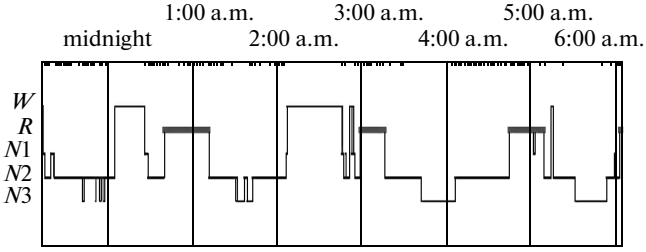

Fig. 3. A hypnogram of a 58-year-old male patient diagnosed with α-synucleionopathy complicated with REM behavior disorder and obstructive sleep apnea/hypopnea of severe degree (data provided by A.L. Kalinkin). See Fig. 1 for denotations. Two long awakenings during the period of sleep can be seen.

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SPELL: 1. apnea, 2. hypopnea, 3. synucleionopathy, 4. levodopa, 5. polysomnographic, 6. preclinical