LITERATURE CITED

1. E. G. Bogdanov, "Neurophysiological mechanisms of regulation of nociceptive responses of arterial pressure and their pharmacologic correction," Author's abstract of dissertation for the degree of Candidate of Medical Sciences, Leningrad (1987).
2. A. V. Dmitriev, The Psychopharmacology of Emotional Stress and of Zoosocial Interaction [in Russian], Leningrad (1975), pp. 29-35.
3. A. A. Zaitsev, Neuropharmacologic Aspects of Pain [in Russian], Leningrad (1982), pp. 108-127.
4. A. A. Zaitsev and Yu. D. Ignatov, The Neuropharmacology of Analgesics [in Russian], Leningrad (1986), pp. 30-43.
5. Yu. D. Ignatov and A. A. Zaitsev, Byull. Éksp. Biol. Med., No. 8, 201 (1985).
6. O. Calvillo and M. Ghighone, Neurosci. Lett., 64, 335 (1986).
7. S. Fielding and H. Lal, Med. Res. Rev., 1, 97 (1981).
8. J. Heym, M. E. Trulson, and B. L. Jacobs, Eur. J. Pharmacol., 74, 117 (1981).
9. Y.-Y. Lai and S. H. H. Chan, Exp. Neurol., 78, 38 (1982).
10. J. C. Liebeskind, G. Guilbaud, J. M. Besson, and J. L. Oliveras, Brain Res., 50, 441 (1973).
11. D. J. Mayer, Prog. Neuro-Psychopharmacol. Biol. Psychiat., 8, 557 (1984).
12. C. Posz, M.-L. Persson, T. Archer, et al., Eur. J. Pharmacol., 137, 107 (1987).
13. A. F. Sullivan, M. R. Dashwood, and A. H. Dickenson, Eur. J. Pharmacol., 138, 169 (1987).
14. T. L. Yaksh, Pharmacol. Biochem. Behav., 22, 845 (1985).

ANALYSIS OF THE STRUCTURE OF SLEEP AND WAKING IN RATS WITH A PARKINSONIAN SYNDROME INDUCED BY 1-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE AND BY OXOTREMORINE

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Disturbances of the structure of sleep and of its qualitative characteristics are often observed in patients with parkinsonism. In particular, in individual patients a decrease is observed in the relative contribution of fast sleep [7], delta-sleep [1], and reduction of "sleep spindles" and their restoration after treatment with levodopa [2, 12, 14], have been observed in individual patients. At the same time there is evidence that some clinical manifestations of the parkinsonian syndrome disappear during sleep in the relaxed state and are intensified during sustained wakefulness. However, the mechanism of the connection between the functional state and the symptom-forming process in parkinsonism still remains unclear.

The discovery of the neurotoxin MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), which can damage nigrostriatal dopaminergic neurons and reproduce the most essential features of the parkinsonian syndrome [1, 9], has enabled the particular features of development of parkinsonism to be assessed from new standpoints.

The aim of this investigation was to study the structure of the sleep—waking cycle in an experimental parkinsonian syndrome induced by systemic administration of MPTP or of oxotremorine.

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TABLE 1. Extrapyramidal Disturbances in Rats after Three Daily Injections of 30 mg/kg of MPTP and a Single Injection of 1 mg/kg of Oxotremorine (M ± m)

Extrapyramidal disturbance	Experimental conditions	MPTP	Oxotremorine
Oligokinesia (motor activity in 2 min)	Before injection of substance	22,7±3,2	35,3±2,4
	After injection of substance 1.5 h	9,8±2,8*	5,5±2,3*
	6 h	10,2±1,3*	14,1±1,8
Rigidity (presence of "kyphosis" sign	After injection of substance 1.5 h	++	+—
	6 h	++	—
Time during which periodic tremor was observed, min		34,0±7,2	52,0±5,4

Legend. Here and Table 2: asterisk indicates that differences are significant compared with control at $p < 0.05$ level; ++) marked effect, —) no effect, +—) variable effect.

TABLE 2. Effect of MPTP and Oxotremorine on Sleep—Waking Cycle in Rats, Recorded for 6 h (M ± m)

Parameter	Control	MPTP	Oxotremorine
Latent period of falling asleep, min.	22,1±5,3	96,6±2,1*	84,6±5*
PPS, %	9,4±1,6	0,4±0,01*	1,8±0,1*
SWS, %	48,6±4,5	17,1±2,4*	32,2±8*
Waking, %	42,1±4,6	82,6±2,4*	66,0±9,1*
"Sleep spindles" mean number in transition period of cycle	7,4±1,7	0	6,4±2,8

EXPERIMENTAL METHOD

Experiments were carried out on noninbred albino rats weighing 370-450 g, aged 4-8 months, with chronically implanted electrodes. The electrodes were implanted under pentobarbital anesthesia into the sensomotor cortex, dorsal hippocampus, caudate nucleus, and nuclei raphe, taking coordinates from the stereotaxic atlas [5], and also into the neck muscles. Experimental parkinsonism was induced by injection of MPTP (30 mg/kg intraperitoneally, once a day for 3 days) or by a single intraperitoneal injection of oxotremorine (1 mg/kg). The experiments began 5-7 days after the operation and were conducted according to the following scheme: 1st day) recording of the basic electroencephalogram (EEG) without injection of any substances, 2nd and 3rd days) injection of MPTP without recording of the EEG, 4th day) recording of the EEG 1.5 h after injection of MPTP. The EEG was recorded against the background of oxotremorine 1.5 h after a single injection of the compound. The EEG and electromyogram were recorded in unrestrained animals, on a "Neirograf-18" electroencephalograph, for subsequent processing by BAS-161 neurocomputer ("O.T.E. Biomedica," Italy). The duration of recording was 6 h. During analysis of the sleep—waking cycles, the latent period of falling asleep, the relative percentage of waking episodes, of slow-wave sleep (SWS), and of the paradoxical phase of sleep (PPS) were determined from changes in the hippocampal and cortical EEG and EMG of the cervical muscles. During the periods of waking and PPS, spectral analysis of the EEG was carried out. The mean number of "sleep spindles" per unit time was determined in the transition period from SWS to waking. Manifestations of symptoms of parkinsonism were assessed according to the presence of tremor, muscle rigidity (the "kyphosis" sign), motor activity (the open field test), and autonomic disturbances.

The results were subjected to statistical analysis by Student's (in Welch's modification) and the Wilcoxon—Mann—Whitney tests [3].

EXPERIMENTAL RESULTS

The results showed that 5-10 min after a single injection of MPTP the rats developed transient tremor and autonomic disturbances: hypersalivation, lacrimation, piloerection, exophthalmos, and hypothermia. After 10-15 min clonicotonic convulsions lasting 1-1.5 h were observed, against the background of which periodic tremor persisted for

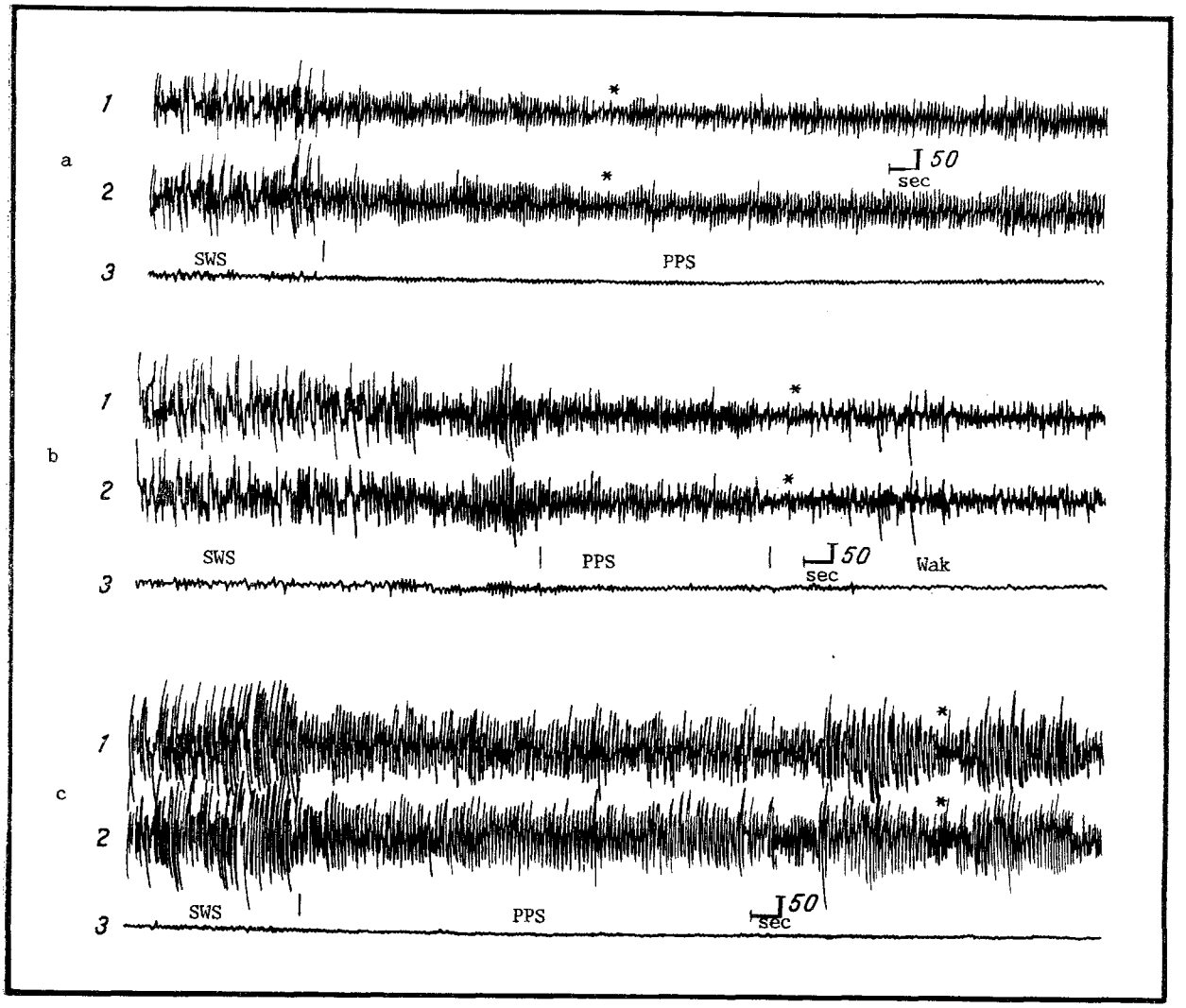

Fig. 1. Electrical activity recorded in sensomotor cortex (1) and dorsal hippocampus (2) and electromyograms of cervical muscles (3) in intact rats (a), after repeated injections of MPTP (30 mg/kg, intraperitoneally, daily for 3 days) (b), and after a single injection of oxotremorine (1 μg/kg, intraperitoneally) (c), recorded for 5 h. SWS) Slow-wave sleep (tonic component of theta-rhythm noted). Wak) Waking. Calibration: 50 μV, 1 sec.

30-40 min. After the end of the seizures, extrapyramidal disturbances developed, with oligokinesia and rigidity. In response to repeated injections of MPTP, the extrapyramidal disturbances became more lasting, and continued for more than 6 h, with mainly akineticorigid symtoms, and the seizure period with episodes of tremor was shortened.

Systemic administration of the muscarinic acetylcholine receptor agonist oxotremorine caused marked tremor of the forelimbs and trunk 5-10 min after injection. The tremor lasted 50-55 min and was accompanied by the development of autonomic disturbances: hypersalivation, diarrhea, exophthalmos, and lacrimation. Oligokinesia and rigidly developed 30-40 min after injection of oxotremorine. After injection of oxotremorine, a predominantly tremoro-akinetic form of extrapyramidal disturbances was observed, the symptomatology was less stable than after injection of MPTP, and it weakened 2-2.5 h after injection of the substance (Table 1).

Continuous recording of the EEG in intact animals for 6 h revealed distinct alternation of sleep—waking cycles, with the presence of two phases of sleep: SWS and PPS. Administration of MPTP for 3 days led to a marked disturbance of the structure of the sleep—waking cycle with reduction of PPS or even its complete disappearance in individual animals, a decrease in the contribution of SWS, and an increase in the relative contribution of waking and a sharp decrease in the number of "sleep spindles" (Table 1). Under the influence of oxotremorine the relative contribution of SWS and waking underwent a smaller change than after injection of MPTP, whereas the number of "sleep spindles" remained unchanged compared with the control (Table 2).

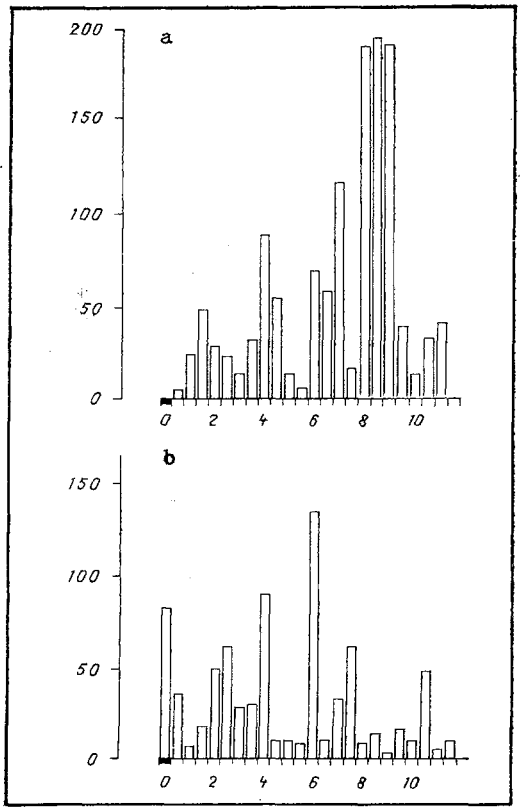

Fig. 2. Frequency power spectra of electrical activity recorded in dorsal hippocampus during paradoxical phase of sleep in intact animals (a) and after injection of MPTP (b). Abscissa, frequency of waves; ordinate, power of individual spectra (in μV^2).

Under the influence of both substances the greatest changes thus took place in the characteristics and structure of PPS. Under the influence of MPTP, for instance, the number of episodes of PPS during 6 h of observation fell from 8-12 in the normal state to 1-2 during the experiment, the duration of one episode was reduced to 20-30 sec (normal 3-4 min), and the first episode of PPS did not appear until after 4.5-5 h of recording.

Analysis of slow rhythmic activity (SRA) of the sensomotor cortex and dorsal hippocampus of intact rats during the period of PPS led to the distinction between two types of SRA: a tonic component (t-SRA), consisting of brief episodes, with low frequency (4-7 Hz) and amplitude, and a phasic component (p-SRA) with a frequency of 8-12 Hz, characterized by definite regularity and high amplitude, as a rule over 100 μV (Fig. 1a), and accompanied by twitches and a rapid eye movement.

MPTP differed in its effect on these two components of SRA during PPS, for it caused the almost complete disappearance of the p-SRA component, with a decrease in its amplitude and regularity, but with potentiation of t-SRA (Fig. 1b). By contrast, under the influence of oxotremorine potentiation of p-SRA and reduction of t-SRA were observed (Fig. 1c).

These results confirm the view that the structure of the hippocampal and cortical SRA of rats in the PPS period consists of two components: t-SRA and p-SRA [10, 11, 13]. It is claimed [6] that p-SRA during PPS is generated and controlled by cholinergic mechanisms. Potentiation of p-SRA under the influence of oxotremorine confirms the cholinergic nature of this component and is in agreement with the observed potentiation of the phasic manifestations during PPS by eserine [8] and their abolition by atropine [8] and imipramine [6].

The study of the power spectrum of the hippocampal EEG of intact rats during PPS revealed a dominant peak in most animals in the 7-9 Hz range (Fig. 2a). Under the influence of MPTP this dominant peak disappeared and the power spectrum increased in the 4-6 Hz band; this correlates with the observed change in the relative contribution of t-SRA and p-SRA during PPS.

The experiments thus showed that extrapyramidal disturbances of different types develop as a result of the use of MPTP and oxotremorine: with predominance of the akineticorigid form after MPTP and of the

tremoroakinetic form after oxotremorine. Meanwhile, against the background of the extrapyramidal symptomatology, under the influence of both substances the organization of the sleep—waking cycles was disturbed, changes in PPS being most stable and consistent in direction. Meanwhile analysis of SRA in the PPS period revealed opposite actions of MPTP and oxotremorine on t-SRA and p-SRA. Potentiation of p-SRA by oxotremorine confirms results showing that the tremoro-akinetic syndrome induced by this substance is generated and controlled by cholinergic mechanisms. Reduction of p-SRA and potentiation of t-SRA after administration of MPTP are evidence that the mechanism of formation of the akinetico-rigid manifestations of the parkinsonian syndrome under the influence of the neurotoxin is unconnected with any direct strengthening of the functions of the cholinergic system, but has a more complex polyneurotransmitter nature.

LITERATURE CITED

1. A. M. Vein, V. L. Golubev, and Yu. E. Berzin'sh, Parkinsonism [in Russian], Moscow (1981), pp. 136-140.
2. N. A. Vlasov, Parkinsonism [in Russian], Moscow (1974), pp. 97-103.
3. F. C. Mills, Statistical Methods, Pitman, London (1955).
4. Y. Arai, Y. Toyoshima, H. Kinemuchi, et al., Neurosci. Lett., 70, No. 2, 266 (1986).
5. J. Bureš, M. Petran, and J. Zachar, Eelctrophysiological Methods in Biological Research, Prague (1960).
6. H. Deportere, Neuropsychobiology, 18, 160 (1987).
7. A. Friedman, Neurol. Neurochir. Pol., 11, No. 3, 289 (1977).
8. M. Jouvet, Cholinergic Mechanisms, ed. by P. G. Waser, New York (1975), pp. 455-475.
9. I. J. Kopin, S. P. Markey, R. S. Burns, et al., Recent Developments in Parkinson's Disease, New York (1986), pp. 165-173.
10. M. M'Harzi and P. Monmaur, Exp. Neurol., 89, No. 2, 361 (1985).
11. P. Monmaur, Experientia (Basel), 37, 261 (1981).
12. F. M. Puca, A. Bricolo, and G. Turella, Electroenceph. Clin. Neurophysiol., 35, No. 3, 327 (1973).
13. T. E. Robinson, R. C. Kramis, and C. H. Vanderwolf, Brain Res., 124, 544 (1977).
14. J. Scott, F. Snyder, R. J. Wyatt, and T. N. Chase, Nature, 228, 999 (1970).