10. D. Edgar and Y. Barde, "Neuronal growth factors," Trends Neurosci., 6, No. 2, 260-262 (1983).
11. L. Green and E. Shooter, "The nerve growth factor," Annu. Rev. Neurosci., 3, 385-387 (1980).
12. D. Kligman, "Isolation of a protein from bovine brain which promotes neurite extension from chick embryo cerebral cortex neurons in defined medium," Brain Res., 250, No. 2, 93-100 (1982).
13. R. Levi-Montalchini, "Developmental neurobiology and the natural history of NGF," Annu. Rev. Neurosci., 5, 341-356 (1982).
14. F. Pearce, B. E. Banks, and D. V. Baunthorpe, "The isolation and characterisation of nerve growth factor from the venom of Vipera russeli," in: NGF and Its Antiserum, E. Zaimis (ed.), Athlore Press, London (1978), pp. 3-18.
15. M. E. Schwab and H. Thoenen, "Early effects of nerve growth factor on adrenergic neurons," Cell Tissue Res., 158, No. 4, 543-553 (1975).
16. S. Varon, "Trophic and specifying factors," Adv. Cell Neurobiol., 2, No. 1, 115-163 (1985).
17. J. Weiss, "The occurrence of nerve growth factor in teleost fishes," Experientia, 24, No. 3, 763-767 (1968).

NEUROPHYSIOLOGICAL ANALYSIS OF THE EFFECTS OF PARTIAL PARADOXICAL SLEEP DEPRIVATION

T. N. Oniani, N. D. Lortkipanidze, L. M. Maisuradze, and L. T. Oniani

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A neurophysiological study was made of the effects of partial and complete paradoxical sleep deprivation by substituting episodes of active wakefulness for spells of paradoxical sleep (PS) of the same duration in the sleep–wake cycle. Neither accumulated need for paradoxical sleep (culminating in increased onset of PS during deprivation), PS rebound during the post-deprivation period, nor dissociation of the stages of paradoxical sleep resulting in their intervening individually at unaccustomed points in the sleep–wake cycle were observed during our experimental procedure. The phenomenon of self-deprivation, increased heart rate, eye movements, and pontogeniculooccipital (PGO) action potentials also failed to occur during the post-deprivation period. It is postulated that PS requirement and the need for periods of wakefulness stem from the same neurochemical alterations.

INTRODUCTION

Awareness of the varying neurophysiological picture of sleep patterns [2, 4] let research workers concentrate on identifying the functional significance of spells of paradoxical sleep (PS) [6, 10]. Paradoxical sleep deprivation (PSD) techniques were mainly used for this purpose, together with analysis of the changes taking place in the integrative activity of the central nervous system (CNS), during both the experimental procedure and the post-deprivation period. A fairly complex set of effects indicative of impairments in normal brain activity and behavior resulting from PSD have now been described in animals [3, 5, 7-9, 11, 17, 18], including the following disorders in particular: nonspecific raised excitability in brain structures, facilitation of evoked epileptic activity, onset of aggression, hyperphagia, hypersexuality, impaired memory trace consolidation, etc. These findings have mostly been obtained from PSD experiments on rats, however. The swimming test was used during these experiments, moreover; this has always been viewed with doubt [12, 19] and has recently been confirmed as stressful to the organism [13, 15]. It remains, nonetheless, the most widespread method for investigating neurophysiological and neuropsychological aspects of the sleep–wake cycle as a whole and the functional significance of PS in particular. This could largely account for the fact that many questions still remain unanswered [20], such as whether omission of PS from the sleep–wake cycle raises brain structure excitability, whether PSD brings about intensification of general and specific motivation, and whether a PS deficit

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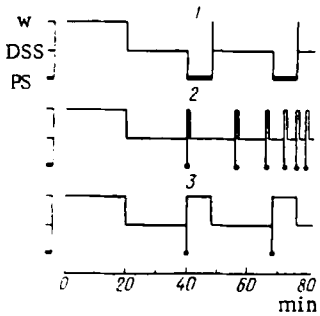

Fig. 1. Scheme of paradoxical sleep deprivation (PSD) experiments using different techniques; frame 1) pattern of prevailing sleep–wake cycle; 2) PSD with brief arousal; 3) PSD, substituting episode of waking for spell of PS of the same duration; W) waking; DSS) deep slow-wave sleep; PS) paradoxical sleep.

affects learning and memory. Nor has it been established whether conditions of PS deprivation in general or total deprivation can be achieved in reality [7].

An answer to these questions may be found by analyzing findings obtained by selective PSD while limiting the stress-promoting factors as far as possible. Of the various non-pharmacological techniques suitable in this respect, the traditional method of PSD (brief arousal of the animal with minimal emotional overlay as each spell of PS sets in) appeared the most promising [7, 9]. Much more frequent onsets of PS spells in the course of deprivation and rebound phenomenon during the post-deprivation period were observed when using this method, however, resulting from accumulated need for the physiological state of PS – a factor leading to dissociation of the different stages of PS during partial deprivation as produced using the traditional method. These then re-emerge in isolation at other stages of the sleep–wake cycle [7], thereby disrupting the normal course of the cycle.

More suitable PSD techniques are thus being currently sought for both neurophysiological investigation of the sleep–wake cycle and for clinical purposes. On the basis of findings on competitive interaction between PS and waking in the cycle [7], the similarities between these two physiological brain states in electrocorticogram pattern [4, 10], and characteristics of neuronal activity within the wider-ranging brain structures [16, 14], we set ourselves the task of producing partial PSD by replacing the PS stage by a period of wakefulness of equal length within the sleep–wake cycle and studying whatever changes in brain activity this produced.

METHODS

Experiments were conducted on 10 cats of either sex weighing 3-3.5 kg. Metal electrodes were chronically implanted in different structures of the cerebral cortex, the cervical and oculomotor muscles in order to record electrographic patterns of the sleep–wake cycle, as well as in different nuclei of the hypothalamus and the mesencephalic reticular formation (for stimulation of active structures). The operation for implanting electrodes was performed under Nembutal-induced anesthesia (30-35 mg/kg).

Electroneocorticograms and electrohippocampograms were recorded using unipolar techniques: the reference electrode was fastened either to the top of the occipital bone, or to the frontal sinus area. Bipolar electrodes are used to record electromyograms and electrooculograms. Experiments investigating the sleep–wake cycle and involving PSD were conducted in a special experimental chamber once the animals had recovered from post-operation trauma, requiring about 5-8 days. Deprivation consisted of arousing the animal after its transition from slow-wave sleep to PS. The onset of PS was determined by the changes in electrical activity taking place in the visual region of the neocortex, the hippocampus, and the cervical muscles, as well as eye movements. Above-threshold sensory (acoustic or electrocutaneous) stimulation was used to arouse the animal from PS, or alternatively electrical stimulation applied directly to the active midbrain and diencephalic structures (0.8-1.5 mA, 0.1 msec, 100-200 Hz).

The scheme for producing PSD is shown in Fig. 1. Changes in the pattern of the sleep–wake cycle under the effects of PSD consisting of brief arousal (Fig. 1, frame 2) and subsequent preservation of the waking spell for the main duration of the stage of PS (frame 3). Changes occurring during deprivation and the post-deprivation period were compared with data appertaining to the sleep–wake cycle prior to the start of PSD (see Fig. 1, frame 1). By so doing it was considered that the first set of experiments would yield data on need for PS accumulating in relation to deprivation, in the form of increased frequency of PS spells and a higher total amount of PS during the post-deprivation period. The second series was to help establish whether spells of PS can be entirely replaced by episodes of waking of the

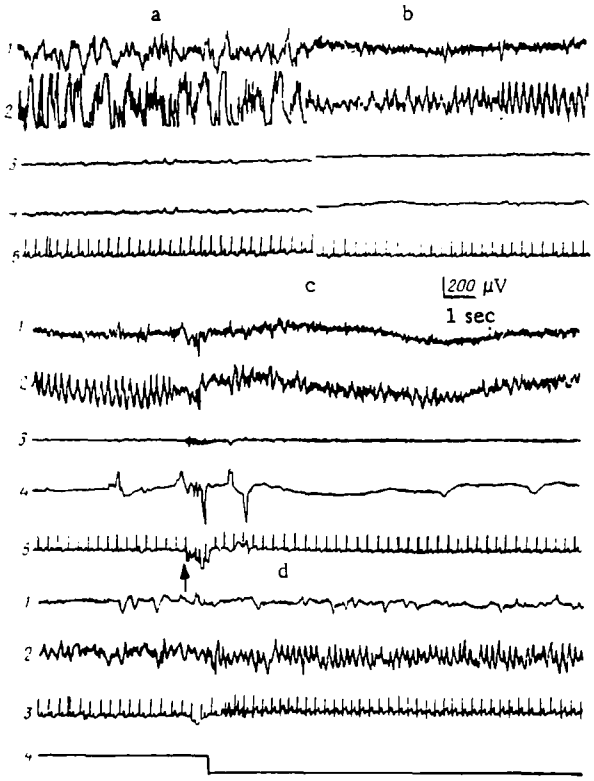

Fig. 2. Changes in the electrical activity of neo- and archipaleocortex, cervical muscle tension, eye movements, and heart rate during paradoxical sleep deprivation (PSD): a) deep, slow-wave sleep; b) transition between the latter and PS; c) PSD during sensory stimulation (point of signal presentation arrowed); d) PSD by arousal produced by electrical stimulation of the hypothalamus (setting up electrical stimulation corresponds to downturn of tracing); a-c, tracing 1) recording from neocortical sensorimotor region; 2) from dorsal hippocampus; 3 and 4) from cervical and oculomotor muscle, respectively; and 5) electrocardiogram (ECG); d, tracing 1) recording from visual region of the cortex; 2) from dorsal hippocampus; 3) ECG; and 4) marks electrical stimulation.

same length during the sleep–wake cycle.

The cats were given a fatal dose of Nembutal once experiments had ended. The brain was fixed by perfusing it with neutral formalin via the carotid arteries. Electrode sites were verified on 20-50 μm brain slices.

Results were subjected to statistical processing, involving calculation of mean values and standard deviations from the mean. Significance of differences of mean values were found using Student's t test.

RESULTS

Changes in the Electrographic Profile of the Sleep–Wake Cycle during Deprivation. Behavioral and electroencephalographic manifestations of all stages of the sleep–wake cycle were duly found to develop in coordination in the usual type of experimental chamber to which the animal had properly adapted. Slow, high-amplitude electrical potentials were recorded during deep slow-wave sleep (the usual precursor of PS) in both the neocortex and the hippocampus; these arose when atonia affected the cervical muscles (with the animal lying curled in a ball, resting its head against a firm support) and no rapid eye movements occurred (see Fig. 2a). The transition from slow-wave sleep to PS generally took place over a fairly short period of time; high-amplitude slow-wave potentials then disappeared in the neocortex and hippocampus (see Fig. 2b). Irregular slow waves in the theta rhythm range remained in the

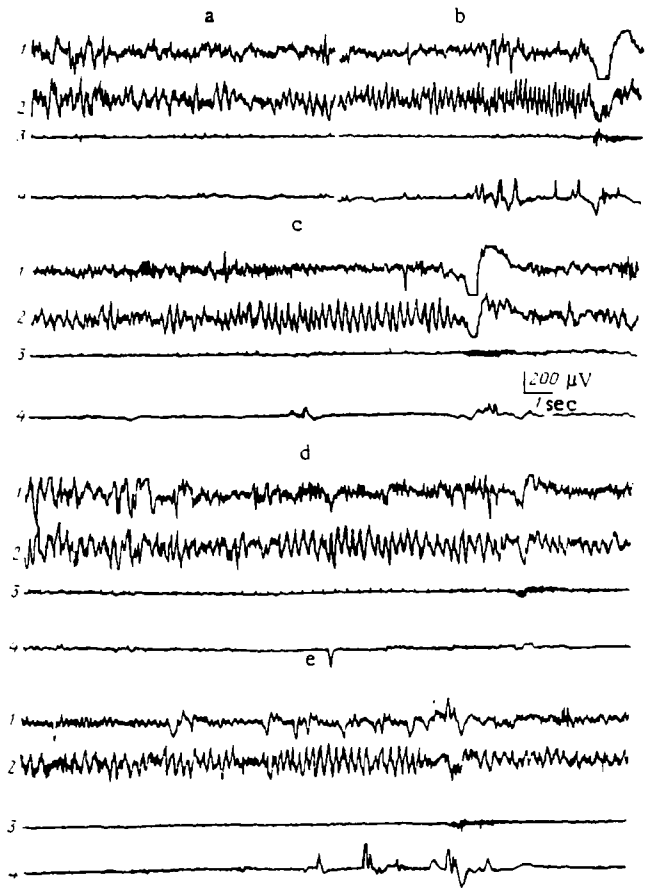

Fig. 3. Changes in the electrical activity of neo- and archi-paleocortex, cervical muscle tension, and eye movements during deprivation and self-deprivation of paradoxical sleep (PS); a) superficial slow-wave sleep; b) transition from the latter to PS and PSD; c) onset and deprivation of PS following waking period; e) onset of PS following waking spell and self-deprivation in respect to this; tracing 1) recording from visual cortex; 2) from dorsal hippocampus; and 3 and 4) from cervical and oculomotor muscles, respectively.

hippocampus at this point, but not in the neocortex. Theta rhythm then rose gradually in the hippocampus to peak once rapid eye movements had set in (see Fig. 2c, start of tracing). While the animal could be aroused free of stress by sensory stimulation or by applying electrical stimulation directly to the brain at this point, the most marked electrographic effects consisted of pronounced hippocampal theta rhythm inhibition, partial recovery of skeletal muscle tonus, and suppression of rapid eye movements (see Fig. 2c). Meanwhile, heart rate appeared to undergo no special change.

Hippocampal electrical activity changed after arousing the animal in relation to the nature of the waking period once PS phases had been replaced by spells of waking. Theta rhythm was regular and irregular, respectively, when in a state of ordinary and active wakefulness. The pitch of hippocampal theta rhythm could reach the level characteristic of PS when affective arousal was produced in response to electrical stimulation of the activating columnar structures, in combination with manifestations of motivational behavior (either attentiveness or fear – see Fig. 2d).

One characteristic effect produced by brief arousal during PSD was a gradual reduction in the latency of the ensuing spell of PS; while preliminary reinstatement of deep slow-wave sleep was first required in order for the ensuing PS phase to occur, spells of PS could occur following only recently reinstated superficial slow-wave sleep (Fig. 3a) or even wakefulness (Fig. 3b) during the course of protracted deprivation. Onset of PS at a fixed point during the sleep–wake cycle accordingly grew much more frequent and the number of associated arousal stimuli increased (see Fig. 4b).

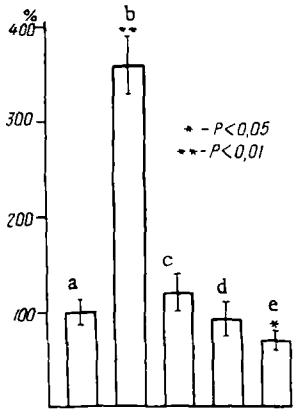

Fig. 4

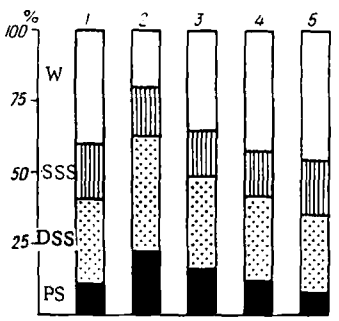

Fig. 5

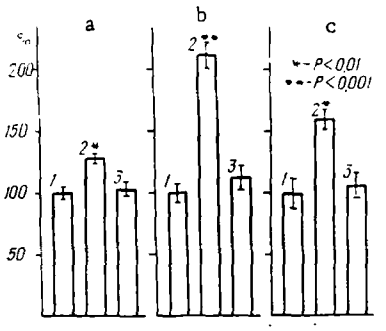

Fig. 6

Fig. 4. Effects of paradoxical sleep deprivation (PSD) using different techniques on the frequency of paradoxical phases occurring during 24-h sleep–wake cycle. Diagram shows PS phases as a % of control tracings (a), during PSD with brief arousal in response to sensory stimulation (b), during deprivation by substituting episodes of non-affective waking for spells of PS (c), by substituting episodes of active waking for PS spells (d), and finally by replacing PS spells with episodes of affective waking in response to electrical stimulation of the lateral hypothalamus (e). Shows mean values and standard deviations from these. Single asterisk: significance of differences (compared with a): $P < 0.05$; double asterisk: $P < 0.01$.

Fig. 5. Effects of paradoxical sleep deprivation (PSD) using different techniques on the relationship between different stages in the 24 h sleep–wake cycle; column 1) control; 2) after PSD produced by traditional method; 3) by the method of replacing intervals of PS by spells of non-affective, 4) active, and 5) affective wakefulness in response to electrical stimulation of the lateral hypothalamus; SSS: superficial slow-wave sleep. Remaining notations as for Fig. 1.

Fig. 6. Effects of paradoxical sleep deprivation (PSD) produced by different techniques on phasic components of paradoxical sleep; a) ECG rate; b) pontogeniculooccipital spikes; c) EEG of oculomotor muscle potentials; column 1) in control tracings (= 100%); 2) following PSD produced by traditional techniques; and 3) by the technique of replacing spells of PS by episodes of wakefulness. Epoch of analysis: 40 sec. Remaining notations as for Fig. 4.

The phenomenon of self-deprivation set in characteristically as a consequence arising from more or less protracted PSD produced by traditional techniques. It consisted basically of the animal waking spontaneously without sensory or brain stimulation with the onset of the initial brief spell of PS. Self-deprivation could develop either when PS began following deep slow-wave sleep, or when the latter was either preceded by superficial slow-wave sleep (Fig. 3c) or occurred against a background EEG characteristic of wakefulness (Fig. 3d).

A different picture emerged when, during an average period of PS, the waking period was sustained artificially after waking the animal from incipient PS, thus substituting the waking state for spells of PS. Under these conditions, the increased frequency of PS compared with the normal cycle was much less pronounced than with PSD produced by brief arousal and sometimes absent altogether. Level of this effect depended on that of the degree of arousal produced. Frequency of the onset of PS in comparison with the controls could increase imperceptibly when simple non-affective arousal replaced PS (see Fig. 4c), but did not approach the level produced by PSD associated with brief arousal (Fig. 4b), while the frequency of PS spells during the sleep–wake cycle produced by PSD associated with active and affective arousal conformed (Fig. 4d) with that observed in the control cycle or else fell below this level (see Fig. 4a and e, respectively).

Changes in the Structure of the Sleep–Wake Cycle during the Post-Deprivation Period. Pronounced quantitative changes occurred in both frequency and duration of PS spells during

the post-deprivation sleep–wake cycle when PSD took the form of brief arousal and the procedure lasted between several hours and a few days. In all, this led to an increase in total PS without radically changing overall duration of slow-wave sleep. The phenomenon of selective PS rebound once the deprivation procedure had ended stood out with particular clarity during the first 3-5 h (see Fig. 5, column 2). During the post-deprivation period and after the onset of rebound, incipient spells of PS often terminated, depending on the type of self-deprivation at work. Initially, episodes of self-deprivation ended with behavioral arousal, but later spells of PS could terminate without any such arousal occurring.

When episodes of wakefulness were substituted for spells of PS, PS rebound was much less pronounced during the post-deprivation period than following PSD consisting of brief arousal, or else failed to occur at all; an actual reduction in PS was observed occasionally (see Fig. 5). The process described also depended on the level of wakefulness replacing spells of PS. Post-deprivation rebound was reduced in the case of peaceful, non-affective waking. No increase in the frequency and duration of PS occurred during the post-deprivation period when spells of PS were replaced by episodes of active wakefulness ("arousing" stimuli produced responses of attentiveness and watchfulness).

Other changes occurring during the post-deprivation sleep–wake cycle could also be discerned by analyzing the electrographic picture obtained. Accordingly, heart rate (Fig. 6a) and frequency of pontogeniculooccipital spikes and of eye movements (Fig. 6b and c, respectively) measured during PS after the onset of selective rebound increased substantially compared with equivalent parameters on control tracings. Parameters then declined to control levels once the PS rebound phase had ended. No such changes in patterns of heart rate, eye movements, and pontogeniculooccipital potentials was observed during the post-deprivation phase of the sleep–wake cycle when episodes of active wakefulness had replaced those of PS.

DISCUSSION

Examination of our findings showed that PSD produced by traditional techniques enables the onset of complete PS to be put off for fairly long periods. This inevitably leads to an accumulation of unsatisfied PS requirement, however, which in turn produces a big increase in the incidence of PS phases during deprivation and shapes selective rebound in the post-deprivation period. Dissociation processes as described in the literature occur under the effects of accumulated need for PS, moreover; these would suggest that individual components of PS could emerge at other stages in the sleep–wake cycle. We believe, however, that involvement of the phenomenon of self-deprivation should be taken into account when considering the "disassociation" aspects of selective PSD. Auto-deprivation of PS can, in fact, set in without any noticeable behavioral reactions occurring. Occasionally auto-deprivation is unaccompanied even by the slightest recurrence of neck muscle tension. It may thus be seen that spells of PS begin and end with both coordinated and uncoordinated (synchronized and unsynchronized) electrical activity occurring in the neocortex under these circumstances. The impression of the PS components recorded developing in juxtaposition with other stages of the sleep–wake cycle may therefore be misleading. Hence, briefly arousing the animal as each spell of PS sets in does not ensure partial or complete PSD.

Increased incidence of PS phases during PSD and the phenomenon of partial PS rebound during the post-deprivation period do not occur when substituting episodes of active wakefulness for spells of PS of equal duration during the sleep–wake cycle. Nor were increased pontogeniculooccipital spikes, eye movements, or heart rate observed during PS in the post-deprivation period under this experimental setup. When creating PSD by this replacement technique, neither incidence of auto-deprivation of PS nor dissociation of individual components of the physiological brain state leading to manifest impairment of the normal sleep–wake cycle were observed. The foregoing would suggest that partial and complete PSD can only be produced by substituting episodes of active wakefulness for spells of PS of equal duration.

These findings would lead us to conclude that episodes of (especially active) wakefulness replace spells of PS and supply satisfactory biological need for these periods of PS. Biological requirement for PS and wakefulness manifesting as neurochemical alterations occurring in the brain juxtaposed against slow-wave sleep could be identical. At present it can be confidently stated that neurochemical changes producing biological need for different stages of sleep do exist, but no data are available on changes creating a need for the waking state. Reliable evidence for the existence of a biological requirement for waking states has now been assembled in our laboratory [1] and was referred to in presenting this hypothesis.

LITERATURE CITED

1. T. N. Oniani and G. R. Akhvlediani, "Effects of some monoamine oxidase inhibitors on form of the sleep–wake cycle," Fiziol. Zh. SSSR, 73, No. 3, 332-337 (1987).
2. E. Aserinsky and N. Kleitman, "Regularly occurring periods of eye motility and concomitant phenomena during sleep," Science, 118, No. 3062, 273-274 (1953).
3. H. B. Cohen and W. C. Dement, "Sleep: changes in threshold to electroconvulsive shock in rats after deprivation of paradoxical phase," Science, 150, No. 3701, 1318-1319 (1965).
4. W. Dement, "The occurrence of low voltage, fast electroencephalogram patterns during behavioral sleep in the cat," Electroencephalogr. Clin. Neurophysiol., 10, No. 2, 291-296 (1958).
5. W. Dement, "The effect of dream deprivation," Science, 131, No. 3601, 1705-1707 (1960).
6. W. Dement, "Recent studies on the biological role of rapid eye movement sleep," Am. J. Psychiat., 112, No. 2, 404-408 (1965).
7. W. Dement, "Sleep deprivation and the organization of the behavioral states," in: Sleep and the Maturing Nervous System, C. D. Clemente et al. (eds.), Academic Press, New York–London (1972), pp. 219-261.
8. W. C. Dement, P. Henry, J. Cohen, and J. Fergusson, "Studies on the effect of REM deprivation in humans and animals," in: Sleep and Altered States of Consciousness, Proc. Assoc. for Research in Nervous and Mental Disorders, S. S. Kety et al., (eds.), Williams & Wilkins, Baltimore–New York (1967), pp. 456-468.
9. M. Jouvet, "Behavioral and EEG effects of paradoxical sleep deprivation in the cat," Proc. Int. Congr. Physiol. Sci., 87, No. 12, 344-353 (1967).
10. M. Jouvet, "Neurophysiology of the states of sleep," Physiol. Rev., 47, No. 2, 117-177 (1967).
11. B. Morden, G. Mitchell, and W. Dement, "Selective REM sleep deprivation and compensation phenomena in the rat," Brain Res., 5, No. 3, 339-349 (1967).
12. R. D. Ogilvie and R. J. Broughton, "Sleep deprivation and measures of emotionality in rats," Psychophysiology, 13, No. 3, 249-260 (1976).
13. T. N. Oniani, "Does paradoxical sleep deprivation disturb memory trace consolidation?," Physiol. Behav., 33, No. 5, 687-692 (1984).
14. T. N. Oniani, G. G. Beradze, L. B. Gvetadze, and S. D. Manjavidze, "The dynamics of unit activity in hippocampus and mesencephalic reticular formation in the sleep-wakefulness cycle," Neurosci. Lett., Supp. 1, 335 (1978).
15. T. N. Oniani and N. D. Lortkipanidze, "Effect of paradoxical sleep deprivation on the learning and memory," in: Neurophysiology of Motivation, Memory and Wakefulness-Sleep Cycle, T. N. Oniani (ed), Metsniereba, Tbilisi, Vol. 4 (1985), pp. 9-52.
16. J. M. Siegel, "Unit activity in the reticular formation and REM sleep," in: The Function of Sleep, R. Drucker-Colin et al. (eds.), Academic Press, New York (1979), pp. 31-71.
17. M. A. Sloan, "The effect of deprivation of rapid eye movement (REM) sleep on maze learning and aggression in the albino rat," J. Psychiat., Res., 9, No. 5, 101-111 (1972).
18. P. Vimont-Vicary, D. Jouvet-Monnier, and F. Delorme, "Effets EEG et comportement des privations de someil paradoxical chez le chat," Electroencephalogr. Clin. Neurophysiol., 20, No. 3, 439-449 (1966).
19. G. W. Vogel, "A review of REM sleep deprivation," Arch. Gen Psychiat., 32, No. 6, 749-761 (1975).
20. W. B. Webb, "Selective and partial deprivation of sleep," in: Sleep: Physiol., Biochem., Psychol., Pharmacol., Clin. Implicat. 1st Eur. Congr. Sleep Res., W. P. Koella and P. Levin (eds.), Karger, Basel (1972), pp. 176-185.