A Qualitative and Quantitative Assessment of the Sleep-Wakefulness Cycle Architecture in the Post-Paradoxical Sleep Deprivation Period

Lia M. Maisuradze, Nani D. Lortkipanidze,
Marine D. Eliozishvili, Niko T. Oniani, Tengiz N. Oniani

Department of Neurobiology of Sleep-Wakefulness Cycle, I. Beritashvili Institute of Physiology,
Georgian Academy of Sciences, Tbilisi, Georgia

The aim of this study was to assess post-deprivation sleep-wakefulness cycle (SWC) architecture following two versions of paradoxical sleep deprivation (PSD): rapid eye movements (REM) and ponto-geniculo-occipital (PGO) spikes. Mature cats were chronically implanted with electrodes for SWC registration during baseline, deprivation and recovery days. Differences between the two versions are expressed in the quantity and quality of sleep phases. Thus, the paradoxical sleep (PS) rebound and enhancement of its intensity clearly testifies to the pressure from the unsatisfied PS need accumulated in the course of the REM-deprivation period. Although following PGO-deprivation an increase in the amount of PS is available, quantitatively it is markedly less than PS rebound after REM-deprivation, and PS quality does not differ from baseline; in contrast to REM-deprivation, there is a significant slow-wave sleep (SWS) rebound in quality and quantity that leads to the increase in the amount of PS. However, the absence of changes in the post-deprivation ratio of deep slow-wave sleep (DSWS) and PS in relation to total sleep time indicates that an increase of the total amount of PS is not only a consequence of enhanced PS need, but also an increased SWS that suppresses wakefulness. The enhanced intensity of brain mechanisms during SWS takes up the responsibility for PS triggering. It is suggested that this occurs because of the pressure from the system of SWS on that of wakefulness, which becomes weak and is unable to control and delay the triggering of PS; having been released from the control of wakefulness, PS follows DSWS in full-developed episodes.

CURRENT CLAIM: Quantitative and qualitative variations in the postdeprivation SWC architecture are different following two versions of PS elimination, REM- and PGO-deprivation.

One of the most important events in the history of sleep research has become the discovery of a sleep period with rapid eye movements (REM) in man (Aserinsky and Kleitman, 1953), the so-called third state of the brain (Jouvet, 1962). A similar stage of sleep with a desynchronization of electroencephalogram (EEG), a pronounced hippocampal theta rhythm, ponto-geniculo-occipital (PGO) spikes, skeletal muscles atonia, sporadic jerking of parts of the body and face, periodic movement of the paws, toes, tail, tongue, whiskers and vibrissae (Jouvet, 1999) was found in cats as well (Dement, 1958) and was named by Jouvet et al. (1959) as paradoxical sleep (PS). It is a periodical phenomenon and the ultradian periodicity (PS appears several times in 24 h) of various species is different (Orem and Barnes, 1980); in the majority of species, it takes about one-fourth of total sleep time. For example, in cats, PS is repeated every 25-30 min and the mean duration of each episode is 5 min (Jouvet, 1999).

In order to define a function and/or the functions of PS, quite different techniques are applied to achieve its elimination from the sleep-wakefulness cycle (SWC). The first method, an instantaneous awakening of a sleeping subject immediately at the onset of PS, is considered to be a classic one (Dement, 1960), which has also been successfully employed in different modifications at the present time (Endo et al., 1998; Nykamp et al., 1998; Oniani et al., 2001a).

Cessation of PS deprivation (PSD) procedures are accomplished by a variety of methods and, with the exception of some (Kitahama and Valatx, 1980; Oniani et al., 1988a, 2001b; Nykamp et al., 1998; Maisuradze et al., 1999), is followed by the so-called PS rebound (Dement, 1960); i.e., a compensatory increase of its total amount in the post-deprivation period (Jouvet et al., 1964; Vimont-Vicary et al., 1966; Morden et al., 1967; Cohen, 1972; Dement, 1972; Van Hulzen and Coenen 1980; Kushida et al., 1984; Kovalzon and Tsibulsky, 1984; Oniani et al., 1988b; Vogel et al., 1990; Endo et al., 1998; Adrien, 2001). At the same time, depending on the employed methods for PSD, both an increase of slow-wave sleep (SWS) amount (Vimont-Vicary et al., 1966; Oniani et al., 2001a) and suppression of its intensity (Beersma et al., 1990), or lack of any considerable changes in SWS (Kovalzon and Tsibulsky, 1984; Oniani et al., 1988a, 1988b) are observed. However, the evidence available in the literature chiefly deals with a quantitative assessment of PS rebound and amount and intensity of SWS (Borbély, 1982; Dijk et al., 1987; Beersma et al., 1990; see Ferrara et al., 1999), although it is suggested that observed changes in the EEG power spectra during increased PS need may be a sign of an altered quality of PS (Endo et al., 1998); furthermore, while considering the post-deprivation period, it has not been taken into account at what moment (before PS onset or upon the appearance of precisely which signs of PS) the awakening stimuli had been delivered.

Correspondence: Lia M. Maisuradze, Ph.D., Department of Neurobiology of Sleep-Wakefulness Cycle, 14, Gotua str., 380060 Tbilisi, Georgia, Tel: 995-32-371154, Fax: 995-32-252280, E-mail: liamais@postmaster.co.uk.
Maisuradze et al. (2001) made a comparison of two versions of PSD, REM- and PGO-deprivation, taking into account the following circumstances: 1) REMs (basic parameter of PS) start to develop in the cat (Dement, 1958) after the onset of desynchronized electrical activity in the neocortex and well-expressed hippocampal theta rhythm (Jouvet, 1967b), i.e., significantly later after PGO spikes; 2) PGO spikes (earliest messengers of PS onset approaching) start arising during deep SWS (DSWS) (Dement, 1972). Particular attention has been devoted to the study of SWC structure in the deprivation period. The main finding was the PS-onset elimination, as well as partial loss of DSWS during PGO-deprivation in contrast to REM-deprivation. The present work is, as it were, a logical continuation of the above-said investigation, for on the basis of a comprehensive analysis of the post-deprivation structure of SWC, we made our purpose to assess not only quantitative but also qualitative changes observable in the recovery period, i.e., after the cessation of each deprivation manipulation as a consequence of REM- and PGO-deprivation.

METHODS

The experiments were carried out in ten mature cats (three females and seven males) with body mass 3-3.5 kg. For a polygraphic recording of SWC, metallic electrodes with an uninsulated tip, 150-200 μ in diameter, were inserted in various areas (sensorimotor and visual) of the cerebral cortex, dorsal hippocampus, lateral geniculate body, posterior hypothalamus, cervical and oculomotor muscles; heart rate was also recorded. An indifferent electrode of silver was fixed to the cranial occipital crest. Surgery for the electrode implantation was done under Nembutal narcosis (30-35 mg/kg); the coordinates were selected according to the atlas of Jasper and Ajmone-Marsan (1954).

Recordings of electrical activity from the cerebral cortex (ECoG) and dorsal hippocampus (EHG) (led monopolarly), lateral geniculate body (LGB), oculomotor muscles (EMG) (led bipolarly) and of electrocardiogram (ECG) were made on the “Medicor” electroencephalograph.

When the animals recovered completely from surgery (6-9 days) experiments for recording SWC in the baseline, deprivation and post-deprivation periods were conducted in an ordinary, comfortable-for-the-animal, experimental chamber adjusted for both visual observation of the animal’s general behavior and recording of the SWC structure. After the animal had been completely adapted to the experimental set (as well as to a cable recording system), baseline SWC records were continuously made for several (3-4) days. After the establishment of a baseline structure of SWC, PSD experiments were started.

PSD was performed by a non-pharmacological “classic” method (Dement, 1960) by means of instantaneous awakening of a sleeping animal. For this procedure, in separate series of experiments, two versions were employed: REM- and PGO-deprivation. In the case of the first version, the animal was awakened at the appearance of first REMs on the face of PS; in the second version, awakening was performed after the appearance of PGO spikes exclusively on the face of DSWS (at this moment, the animal should experience atonia of the cervical muscles, as well as synchrony of behavioral and EEG parameters, necessary for full-fledged development of normal SWS). Thus, in the case of PGO-deprivation, the deep stage of SWS also suffered partial deprivation.

Each cat received both experimental procedures, REM-deprivation as well as PGO-deprivation. In one group of animals (n=5), REM-deprivation was done first, then PGO-deprivation, and vice-versa in the other group (n=5). Before each deprivation version, we recorded the baseline SWC structure. In the process of deprivation, using either versions, awakening of animal was performed by direct electrical stimulation of the posterior hypothalamus (100 imp/sec; 2-5 V; 0.1-3 msec) to elicit a short-lasting episode of wakefulness; thereafter, the animal was allowed to go to sleep before the appearance of the above-indicated phasic parameters corresponding to the deprivation versions. As soon as the deprivation manipulations were over (duration of each version equaled 12 or 24 h) continuous recording of post-deprivation SWC was made for 24 h (the intervals between different deprivation conditions were no less than five days). The animals were fed at the same time, keeping a standard diet before beginning recording procedures (with the purpose to exclude any side effect of excess or deficit of food intake).

To assess the effects of the two versions of deprivation, the post-deprivation SWC structure was compared with the baseline. The results obtained after PGO-deprivation were also compared with the respective data determined by REM-deprivation. To this end, total duration of various SWC phases (wakefulness, light SWS (LSWS), DSWS and PS) was determined at the baseline as well as in the post-deprivation period, and a percent ratio was established; frequency of occurrence of SWS and PS phases was calculated and their latency and mean duration were determined.

SWS quality was estimated by the changes of electrical activity in the sensorimotor area of the neocortex (the so-called “sleep-spindles” and delta-rhythm amplitude were calculated). As to PS quality, it was defined by prominence of the hippocampal theta rhythm, numbers of REMs and PGO spikes, as well as heart rate. Epoch of analysis for PGO spikes, REMs, and “sleep spindles” equaled 40 sec, while for delta-rhythm amplitude, heart rate and frequency of the hippocampal theta rhythm equaled 5 sec.

Quantitative data were statistically treated and validity of differences were determined by Student’s t-test.

RESULTS

Effect of REM-deprivation on the SWC in the Post-deprivation Period

Study of the SWC post-deprivation structure after the cessation of REM-deprivation has shown a synchronous development of behavioral and EEG correlates of each SWC phase. Following active and passive wakefulness, drowsiness passes into a full-value behavioral sleep (animal curling up). Respectively, it also alters EEG; during transition of a drowsy state to SWS there appear single, so-called “sleep spindles” in EcoG, while in the hippocampus, the theta rhythm gets...
disturbed and irregular slow waves appear instead. On the face of full-value behavioral sleep, there regularly arise slow and high-amplitude potentials in the neocortex as well as in the hippocampus. During the transition from SWS to PS, compared to the baseline, a short episode of time is observable when the high-amplitude slow potentials disappear from both the neocortex and hippocampus. Then, the latter gradually enhances the theta rhythm, which is most prominent at the appearance of REMs. The PS latency (Figure 1A) is shorter than in the baseline records, whereas DSWS latency (Figure 1B) did not change significantly. At the very beginning of recording the post-deprivation SWC, there occurred the animal’s self-awakening from PS once or twice—the so-called phenomenon of PS self-deprivation (Oniani et al., 1988b, 1988c; Maisuradze et al., 2000). Similar fragments ended with a partial short-lasting recovery of tonic activity of cervical muscles. After two cases of this type, PS developed in full measure.

In the post-deprivation period, pronounced qualitative and quantitative changes were noted in the SWC structure. In particular, total length of wakefulness decreased (Figure 2A), while that of PS increased (Figure 2D), i.e., a PS rebound occurred; in regard to the amount of LSWS and DSWS, they did not tangibly differ from the baseline (B and C on Figure 2). The increase in the numbers of PS-onset was not statistically significant (Figure 3A, Column 2), but the mean duration of PS episodes was considerably increased in comparison with the baseline (Figure 3B, Column 1).

The data we obtained concerning DSWS have shown that it does not vary from the baseline by relevant parameters; in particular, the numbers of DSWS onset (Figure 4A, Column 2), their mean duration (Figure 4B, Column 2), and the DSWS quality estimated by number of “sleep-spindles” in the sensorimotor area of the neocortex (Figure 5A, Column 2) as well as by the delta-rhythm amplitude (Figure 5B, Column 2), did not undergo any reliable changes.

PS quality was determined by the frequency of PGO spikes, REMs, hippocampal theta rhythm, and heart beats during each episode of PS. All the enumerated parameters appeared to be significantly increased statistically (especially PGO spikes and REMs) (Figure 6A, B, C, Column 2) as compared to the baseline respective values (Figure 6A, B, C, Column 1). At this time, one could observe frequent occurrence of the episodes of PS during which there was an especially violent development of the somatic parameters exclusively characteristic of PS (such as twitching of ears, paws, muscles of the body and face, movement of whiskers and vibrissae). The indicated signs regained the baseline level somewhat later after the termination of PS rebound, i.e., their excess was noted even after PS had regained the baseline amount (i.e., the increase in PS intensity dragged on, in spite of its total duration regaining the baseline level).

**Effect of PGO-deprivation on the SWC in the Post-deprivation Period**

A polygraphic recording of the SWC structure following the cessation of PGO-deprivation has demonstrated that the post-deprivation period is realized in quite a normal way and takes...
all the steps of its organization; the sequence of development of various SWC phases is not disturbed and the EEG and behavioral correlates develop synchronously in accordance with triggering and developing each phase of the cycle. The moments of PS self-deprivation, in contrast to REM-deprivation, were lacking altogether. The PS latency was the same as in baseline records (Figure 1A, Column 3), while that of DSWS during the first two hours of the recovery period was decreasing (Figure 1B, Column 3), though in the subsequent 2-h intervals, regained the baseline value. At the same time, an enhancement of spindle-like activity (Figure 5A, Column 3) and delta-rhythm amplitude (Figure 5B, Column 3) was noted in the sensorimotor area of the neocortex and expressive synchronization of high-amplitude slow waves was observed in the hippocampus. The frequency of DSWS-onset reliably exceeded, statistically (Figure 4A, Column 3), its baseline meaning (Figure 4A, Column 1); mean duration of DSWS episodes, however, remained stable (Figure 4B, Column 3).

Percent ratios of various SWC phases in the post-deprivation period show that the total amount of PS has increased (Figure 2C, Column 3) and total duration of wakefulness has decreased (Figure 2A, Column 3), while the decrease of LSWS was not remarkable (Figure 2B, Column 3). In regard to PS, its total duration did increase (Figure 2D, Column 3) compared to baseline (Figure 2D, Column 1), but it was less than PS rebound observed following the offset of REM-deprivation (Figure 2D, Column 2). The number of PS phases increased (Figure 3A, Column 3) compared to baseline (Figure 3A, Column 1), but it did not vary from the number of PS-onsets after termination of the first version of deprivation (Figure 2A, Column 2). As to the mean duration of PS episodes (Figure 3B, Column 3), it did not differ from the basal values (Figure 3B, Column 1), but was (5 min±0.7) shorter (p<0.05) than the PS length after REM-deprivation (7 min±0.4).

As far as the frequency of PGO spikes, REMs, and hippocampal theta-rhythms are concerned, they did not undergo any statistically significant changes (Figure 6A, B, C) compared with the respective baseline indices at PS (Figure 6A, B, C, Column 1), although there was a tendency toward an increase of the average of PGO spikes during the increased amount of PS after PGO-deprivation. Comparison with the data obtained during PS rebound following termination of REM-deprivation has shown that the frequency of PGO spikes (20±2.2 vs. 26±0.7, p<0.02) and REMs (9±0.7 vs. 13±1.2, p<0.02) is lower after PGO-deprivation, while there is a tendency toward a decrease of frequency of the hippocampal theta-rhythm (0.05<p<0.1). Here, it should also be mentioned that during PS development there were no episodes with remarkable somato-vegetative correlates (the number of fragments with sporadic jerking of the paws, toes, movements of the ears, whiskers, vibrissae and tail, as well as the heart rate did not exceed the similar fragments in baseline records), and, respectively, the changes were more feeble than during PS rebound and even after its offset, in the case of REM-deprivation.

The results we obtained in relation to wakefulness show that both the numbers of PS episodes and their mean duration
increase, compared to a baseline level, after termination of the two versions of deprivation. However, calculations of cases of transition of DSWS to PS and spontaneous awakenings from DSWS in relation to the occurrence of DSWS episodes in the post-deprivation SWC have demonstrated that after PGO-deprivation, the percentage of spontaneous awakenings from DSWS is less (Figure 7, Column 3) than in the baseline (P<0.02) and following REM-deprivation cessation (p<0.05). Correspondingly, the DSWS episodes that develop after the cessation of PGO-deprivation more frequently pass into PS than in the baseline and after REM-deprivation. No difference was found in the cited cases between the baseline and post-deprivation period after REM-deprivation (Figure 7, Column 2); i.e., after the cessation of PGO-deprivation, the number of complete cycles increases while that of incomplete cycles decreases, and abnormal cycles are lacking in the post-deprivation SWC.

**DISCUSSION**

Analysis of our results reveals that even though the regular alternation of various SWC phases is not disturbed in the post-deprivation periods, their percent ratio either after REM- or PGO-deprivation remarkably differs from the baseline data. This, above all, manifests itself in the quantity and quality of sleep phases (SWS and PS). Thus, the PS rebound found following REM-deprivation and increased PS intensity (PS is rich in emotional coloring—an increase in the frequency of PGO spikes, REMs, hippocampal theta-rhythm and heart beats) clearly testifies to the pressure from the unsatisfied need for PS, accumulated in the course of REM-deprivation procedures; neither the amount nor the quality of SWS undergoes any changes, since this phase is not affected during REM-deprivation (Maisuradze et al., 2001).

As to the general increase of SWS after the termination of PGO-deprivation, in particular, an increase in the number of DSWS phases, its total amount and intensity (the latter is expressed in the abundance of spindle-like activity and enhancement of delta-rhythm amplitude) is beyond doubt, since in the course of PGO-deprivation there occurs, though partial, elimination of that fragment of DSWS at which the triggering of PS for its subsequent development should be formed (Jouvet, 1967a; Ursin, 1968; Oniani et al., 1999, 2000; Maisuradze et al., 2001).

Rebound of SWS, because of its selective deprivation, was also noted in the work by Ferrara et al. (1999), accentuating the fact that in their experiments, they made an effort not to elicit more or less prolonged fragments of wakefulness during deprivation and employed, for awakening, the technique of acoustic stimulation. A significant increase of "delta-sleep" following its elimination through this technique is described in the earlier published work of Danilin (1974). With regard to the increase in PS, the total amount in the post-deprivation SWC after cessation of PGO-deprivation, in comparison with the baseline (though it is less than following REM-deprivation), might be considered a result of a more frequent occurrence of PS episodes and not the increase of their mean duration (which remains within the baseline ranges). It could be thought of as a consequence of accumulation of the need for PS, since its phases are altogether absent during the PGO-deprivation period (Maisuradze et al., 2001). It is, however, necessary to take into account the circumstance that 1) after the termination of PGO-deprivation, the increased frequency and enhanced intensity in DSWS phases are chiefly followed by PS phases rather than by wakefulness episodes, and the total amount of wakefulness decreases because of the pressure from DSWS, and 2) post-deprivation ratios of SWS and PS (Figure 8A, Column 3), and even of DSWS and PS (Figure 8B, Column 3), in the total sleep time revealed no shifts after cessation of PGO-deprivation compared to baseline (Figure 8, Column 1).

Bearing this circumstance in mind, one can easily conclude that the increase in the total duration of post-deprivation of PS is not only a consequence of enhanced PS pressure, but also of increased, in amount and intensity, DSWS through the suppression of wakefulness. The reciprocal interrelationship between wakefulness and PS found, in the intact animal’s (cat) undisturbed SWC (Dement, 1972; Oniani et al., 1988b, 2001b), that it is namely in DSWS that the ground is prepared for the formation and triggering of PS (Svorad and Karmanova, 1966; Jouvet, 1967b; Ursin, 1968; Oniani et al., 1988b, 1999,

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**Figure 7.** Percentage of spontaneous awakenings from DSWS in baseline and following REM-deprivation. ****: p<0.02, compared to baseline; • p<0.05, compared to REM-deprivation.

**Figure 8.** Percent ratio of A: SWS/PS, and B: DSWS/PS amounts in relation to total sleep time in baseline (Column 1) and a 4-h post-deprivation period following REM-deprivation (Column 2) and PGO-deprivation (Column 3).
And, though in the duration of each phase of post-deprivation DSWS is not increased after PGO-deprivation, the enhanced intensity of brain mechanisms takes up the responsibility for the PS triggering. This occurs because of the pressure from the SWS system on wakefulness. The latter becomes weak and is unable to control and delay the triggering of PS (Oniani et al., unpublished observations), which, having been released from the control of wakefulness, follows DSWS phases in full-developed episodes.

The analysis of our results deserves special interest in the aspect of evaluating the quality of PS. The possibility of measuring the PS intensity by REMs frequency is rejected according to Beersma et al. (1990), suggesting that the experimental manipulations in relation to the need for PS reveal no consecutive changes in the density of REMs. In some studies, there is an indication of the coincidence of REM density and duration of PS episodes in sleep (Zimmerman et al., 1980); in other studies, the dependence of REM density on other parameters is considered (Antonioli et al., 1981; Gennaro and Ferrara, 2000), or analysis of the data is made only from the quantitative point of view (Dijk et al., 1987). Did not Aserinsky (1969) consider the REM density as an index of sleep saturation? Besides, it is namely the number of REMs that is considered as one of the indices for the sleep need and its saturation (Aserinsky, 1973; Feinberg et al., 1980; Lucidi et al., 1996). Therefore, it is curious why one cannot judge the intensity of PS by REM frequency. On the other hand, in the cited works, the experiments were performed on human subjects in whom it is naturally impossible to record PGO spikes and dorsal hippocampal theta rhythms. In our present experiments as well as in the earlier (Oniani et al., 1988a, 1988b) and recently (Maisuradze et al., 1999; Oniani et al., 1999, 2001b) published works conducted on cats, the intensity of PS episodes was judged namely by its emotional coloring.

PS saturated with PGO spikes and REMs, as well as an increase in the frequency of hippocampal theta-rhythm occurrence and heart rate found in the present work following REM-deprivation, clearly testify to the enhancement of emotional tension and intensity of brain mechanisms that are responsible for PS development, which is proof of an increase of PS degree. Evaluating the quality of this sleep phase by the mentioned parameters after cessation of PGO-deprivation, it can be convincingly stated that this version entails no qualitative shifts in PS in the post-deprivation period. In this aspect, the results of human experiments are of interest, indicating that SWS deprivation results in a reduction of REM frequency at PS (Gennaro et al., 2000).

Thus, quantitative and qualitative PS rebound is greater in the post-deprivation SWC following REM-deprivation than after cessation of PGO-deprivation caused by the accumulation of the specific need for PS; partial DSWS loss evoked by PGO-deprivation must retard the formation of the specific PS need, which may affect the extent of PS rebound in the post-deprivation SWC. It is suggested that enhanced DSWS, in amount and intensity, through suppression of activity of the brain mechanisms responsible for maintaining wakefulness entails only PS quantity but not quality rebound. However this quantitative rebound is not as selective because 1) in the post-deprivation SWC, there occurred an increase in DSWS also because of partial deprivation of this SWS stage during PGO-deprivation, and 2) the percentage ratio of DSWS and PS remains stable. For a reasonable analysis of PSD post-deprivation effects in relation to the SWC in general and the architecture of sleep in particular, it is necessary to specify the exact moments (either before PS formation or following PS onset) at which the awakening of a sleeping animal is performed. The results presented and analyzed in this paper acquire special significance, testifying to the existence of differences between the two versions of PS elimination, REM- and PGO-deprivation, which is sure to be reflected in the post-deprivation SWC architecture.

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