Behavioural Brain Research 205 (2009) 482–487

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Small platform sleep deprivation selectively increases the average duration of rapid eye movement sleep episodes during sleep rebound

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ARTICLE INFO

Article history:
Received 29 May 2009
Received in revised form 24 July 2009
Accepted 3 August 2009
Available online 7 August 2009

Keywords:
Sleep
REM sleep deprivation
Platform method
EEG
Stress
Circadian rhythm

ABSTRACT

The single platform-on-water (flower pot) method is extensively used for depriving rapid eye movement sleep (REMS). Detailed comparison of sleep–wake architecture, recorded during the rebound period after spending three days on either a small or large platform, could separate the effects of REMS deficit from other stress factors caused by the procedure. A further aim of the study was to find the most characteristic REMS parameter of the rebound originating from REMS deficit. Rats were kept on a small or large platform for 72 h. Their fronto-parietal electroencephalogram, electromyogram and motility were recorded during the 24 h rebound at the beginning of the passive phase. A similar period of a home cage group was also recorded. The most typical differences between the two rebound groups were the increased cumulative time and longer average duration of REMS episodes without significant change in the number of these episodes of the small platform animals during the passive phase. Results obtained by cosinor analysis were in accordance with the findings above. Since we did not find any difference in the average duration of REMS episodes comparing the large platform rebound group and the home cage group, we concluded that the increased mean duration of REMS episodes is a selective marker for the rebound caused by small platform sleep deprivation, while other changes in sleep architecture may be the consequence of stress and also some sleep deficit.

1. Introduction

The flower pot (also called platform-on-water) method was first described by Jouvet et al. [1] in 1964 as a simple and efficient way for depriving rapid eye movement sleep (REMS) of cats. Cohen and Dement adapted this method for rats [2] a year later. In this protocol, animals are placed onto a round platform surrounded by water, and whereas muscle atony is typical for REMS, they fall into the water and awaken immediately as they switch to REMS. Two platforms are usually used: a smaller one, on which the animal can not perform REMS, and a bigger one for control, on which the animal can curl, consequently can reach this sleep stage. Several problems and proposals to solve them have been published on this method As an example, Hicks et al. [3] have pointed out the fact that size of the animal basically influences the outcome of REMS deprivation performed with a platform of a given diameter. However, the fundamental difference between sleep patterns on the small and large platform is the cessation of REMS by the small platform [4], Grahnstedt and Ursin [5] have shown that slow wave sleep (SWS) is also strongly affected by platform sleep deprivation. Moreover, this protocol is very stressful for the animals [6–8]. Several suggestions were made in the literature to reduce some stressors: for instance, the multiple platform technique [9,10] attempted to abolish immobilization stress, or the modified multiple platform technique [11] which was designed to reduce stress originating from social isolation and the establishment of hierarchy, but none of them could reassuringly avoid or separate the effects of nonspecific stressors from the effects of sleep loss. Although using a large platform can also cause mild sleep deprivation, it does not abolish REMS [4], moreover, it is nearly as stressful for the animal as using a small platform as proved by measuring either Selye's classical indices, plasma ACTH and corticosterone levels or the oxidative stress in the frontal cortex [6–8,12]. Hence, using a large platform as a control for small platform can be suitable to filter out the effects of nonspecific stressors, such as. immobilization, social isolation, new environment and wetness, and to separate it from the effects of sleep loss.

The changes of sleep architecture during REMS deprivation performed by the platform technique [4,5,10,13,14] as well as during and after repeated REMS deprivation-rebound periods [15,16] are extensively studied phenomena. Although several types of platform methods have been studied so far, the rebound was never selective,

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doi:10.1016/j.bbr.2009.08.004

i.e. beyond REMS, other sleep stages were also changed compared to baseline. Differences in the sleep architecture following either a small or large platform deprivation throughout 24 h at the first rebound day was never studied, although it could clarify the specialties of the rebound of each sleep stage after such a selective treatment. Comparison of sleep architecture after REMS deprivation by the two types of platform could also provide a time interval, in which the rebound is selective for REMS. Thus, our results could give a tool to separate the changes in sleep architecture of the rebound day caused by sleep loss from the effects of several stress factors originating from the platform sleep deprivation procedure.

2. Materials and methods

All animal experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and the National Institutes of Health "Principles of Laboratory Animal Care" (NIH Publications No. 85-23, revised 1985), as well as specific national laws (the Hungarian Governmental Regulations on animal studies, December 31, 1998). Permission was obtained from the local ethical committees. Animals were male Wistar rats, weighing 347.7 ± 4.8 g (mean ± SE) at the start of the REMS deprivation.

2.1. Surgery

Rats were equipped with electroencephalographic (EEG) and electromyographic (EMG) electrodes as described earlier [17]. Briefly, stainless steel screw electrodes were implanted epidurally over the left frontal cortex (L: 2.0 mm and A: 2 mm to bregma) and left parietal cortex (L: 2.0 mm and A: 2.0 mm to lambda) for fronto-parietal EEG recordings. The ground electrode was placed over the cerebellum. In addition, EMG electrodes (stainless steel spring electrodes embedded in silicon rubber, Plastics One Inc., Roanoke, VA, USA; length = 50 mm, d = 1.2 mm with the silicon rubber) were placed into muscles of the neck. Surgery was performed under 2% halothane anesthesia (Fluotec 3) using a Kopf stereotaxic instrument.

After surgery, rats were kept in single cages in the recording chamber, maintained at a 12/12 h light/dark cycle (lights on from 10:00 to 22:00 h, daylight type fluorescent tubes, 18 W, approximately 150 lx) at an ambient temperature of 21 ± 1 °C and relative humidity of 40–50%. After a 7-day recovery period, in order to habituate the animals to the recording conditions, rats were attached to the polygraph by a flexible recording cable and an electric swivel, fixed above the cages, permitting free movement of the animals. To assess motor activity, electromagnetic transducers were used in which potentials were generated by movements of the recording cable as described earlier [17]. Habituation period was 7 days long.

2.2. Groups

Animals were randomly divided into three groups as follows:

Home cage (HC) group (n = 8): 23 h polysomnographic recordings were made under undisturbed conditions in their own home cages.
Small platform rebound (SPR) group (n = 7): 23 h polysomnographic recordings were made after spending 72 h on a small platform.
Large platform rebound (LPR) group (n = 6): 23 h polysomnographic recordings were made after spending 72 h on a large platform.
Food and water was available ad libitum for all animals throughout the whole experiment.

2.3. REM sleep deprivation

Animals in SPR and LPR group were detached from the cable and were placed on a round platform situated in the middle of a round water tank at lights on (diameter: 41 cm, each rat in individual water tank and platform). The diameter of the small and the large platform was 6.5 and 13 cm, respectively, their height was 18.5 cm. The surface of both platforms was 0.5 cm above the water level. Rings were placed 4.5 cm under the surface of the platform to help the animals get out of the water, as we found in preliminary experiments that some animals had difficulties in climbing back to the large platform. It was verified that the animals did not spend any time on the ring, except a few seconds when climbing back onto the platform. In order to record effects of all stress factors caused by the procedure, data from the platform rebound groups and from the HC group were also compared. The HC animals were kept under undisturbed conditions during the whole experiment. After spending 72 h on platforms, animals in SPR and LPR groups were reattached to their home cage recording cable just after the lights on.

2.4. Recording

Since animals in the SPR and LPR groups were removed from platforms at lights on and then reattached to the cable, the first hour of the recording would be incomplete, hence the recordings were started 1 h after light onset. All EEG, EMG and motor activity were recorded for 23 h. Rats were not disturbed throughout the recordings. Data were stored on computer for further analysis.

2.5. Vigilance analysis and scoring

The vigilance states were classified by SleepSign for Animal sleep analysis software (Kissei Comtec America, Inc., USA) for 4 s periods over 23 h as follows: active wakefulness (AW), the EEG is characterized by low amplitude activity at beta (14–30 Hz) and alpha (8–13 Hz) frequencies accompanied by high EMG and motor activity; passive wakefulness (PW), the EEG is characterized by low amplitude activity at beta (14–30 Hz) and alpha (8–13 Hz) frequencies accompanied by high EMG activity; light slow wave sleep (SWS1), high voltage slow cortical waves (0.5–4 Hz) interrupted by low voltage fast EEG activity (spindles 6–15 Hz) accompanied by reduced EMG and motor activity; deep slow wave sleep (SWS2), continuous high-amplitude slow cortical waves (0.5–4 Hz) with reduced EMG and motor activity; intermediate stage of sleep (IS), a brief stage just prior to REMS and sometimes just after it, characterized by unusual association of high-amplitude spindles (mean 12.5 Hz) and low-frequency (mean 5.4 Hz) theta rhythm; rapid eye movement sleep (REMS), low amplitude and high frequency EEG activity with regular theta waves (5–9 Hz) accompanied by silent EMG and motor activity with occasional twitching [17]. After the automatic scoring, recordings were visually revised. The following data were calculated: time spent in each sleep stage per hour; total time spent in slow wave sleep (TSWS) per hour: time spent in SWS1 plus SWS2; time spent in SWS2 in the percent of time spent in TSWS per hour (SWS2%); time spent awake per hour (total wake, TW): time spent in AW plus PW; average duration of REMS episodes per hour; number of REMS episodes per hour (REMS number). In order to exclude short REMS attempts (sRa), a REMS episode was defined as a period of REMS lasting for ≥16 s and not interrupted by ≥16 s of other vigilance state [13,18]. The average duration and number of REMS episodes including sRa-s per hour were also calculated.

2.6. Statistical analysis

Statistical analysis was carried out by STATISTICA 7.0 (Statsoft Inc., Tulsa, OK, USA). Values of vigilance states were evaluated by repeated measures ANOVA for measures with two main factors: treatment (non-repeated; HC, SPR and LPR) and time (repeated, hourly values in 2–24 h, 2–6 h, 7–12 h, 2–12 h and 13–24 h intervals) for sleep analysis. One-way ANOVA was also calculated for values of every sleep parameters studied in every hour (factor: treatment, non-repeated, HC, SPR and LPR). Zeitgeber time = 0 was at lights on. Tukey's honest significant difference test was used for post-hoc comparisons after ANOVA significance for factor treatment. Table 1 and figures show the results of Tukey's post hoc test after ANOVA significance for factor treatment. Amplitude, mesor (mean of the fitted curve) and acrophase (time of peak of the fitted curve) values (±confidence limits, p = 0.05) were calculated by single cosinor analysis [19] using the Time Series Analysis Seriel Cosinor 6.0 Lab View program (Expert Soft Technologie, 1996–2004). The period length was set to 24 h and the 0 h was at lights on. Data in all figures are expressed as mean ± SEM.

3. Results

3.1. Sleep parameters

Evaluation of the rebound day in its total duration revealed several differences in sleep parameters of the three groups. For the summary of this chapter (significant differences obtained by Tukey's post hoc test) see Table 1. ANOVA results ($F_{2,18}$ values) are shown in Table 2. REMS architecture is indicated in Fig. 1A–C. Several differences were found between the sleep parameters of the SPR group and the HC group throughout the whole rebound period studied. Generally, sleep architecture of the LPR group showed significant differences compared to the SPR and the HC group in the passive and active phase, respectively. Figures of time spent in AW, PW, SWS1, SWS2, IS, TSWS and TW per hour, SWS2%, the average duration and number of REMS episodes including short REMS attempts as well as table of ANOVA results ($F_{2,18}$ values for treatment-time interactions) of sleep stages and other sleep parameters are attached as a supplementary material. The only alteration between the analysis of REMS episodes including or excluding short REMS attempts was the following: the lengthening of REMS episodes in the SPR group compared to the HC group in the active phase was significant only when short REMS attempts were included.