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Research Report

Theta activity in the waking EEG is a marker of sleep propensity in the rat

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Abstract

In humans, EEG power in the theta frequency band (5–8 Hz) during quiet waking increases during sleep deprivation (SD), and predicts the subsequent homeostatic increase of sleep slow-wave activity (SWA; EEG power between 0.5 and 4.0 Hz). These findings indicate that theta power in waking is an EEG variable, which reflects the rise in sleep propensity. In rodents, a number of short sleep attempts, as well as SWA in the waking EEG increase in the course of SD, but neither variable predicts the subsequent homeostatic increase of EEG SWA during recovery sleep. To investigate whether there is an EEG marker for sleep propensity also in rodents, the EEG of the rat was recorded during 6 h SD in the first half of the light period (SDL, $n = 7$). During SDL, power of the waking EEG showed an increase in the delta (1.5–4 Hz) and low theta (5–6.5 Hz) band. Based on the neck muscle EMG, wakefulness was subdivided into active (high EMG activity) and quiet (low EMG activity) waking. During quiet waking, the theta peak occurred at 5.5 Hz, the frequency at which the increase of EEG power during SD was most pronounced. This increase was due to higher amplitude of theta waves, while wave incidence (frequency) was unchanged. Correlation analysis showed that the rise in EEG power in the 5–7 Hz band during SD predicted the subsequent enhancement of SWA in non-rapid eye movement sleep. The analysis of data of a further batch of rats which were sleep deprived for 6 h after dark onset (SDD, $n = 7$) revealed a significant increase in theta-wave amplitude during the SD and a tendency for a similar, positive correlation between the increase of theta power (5–7 Hz) and subsequent SWA. The results indicate that in rats, as in humans, a specific waking EEG frequency, i.e., theta power in quiet waking is a marker of sleep propensity.

Theme: Neural basis of behavior
Topic: Biological rhythms and sleep

Keywords: Theta EEG activity; Waking EEG; Sleep homeostasis; Sleep regulation; EMG; Behavior

1. Introduction

Sleep propensity rises progressively during waking and declines monotonically during sleep. The level of EEG slow-wave activity (SWA; EEG power between 0.5 and 4.0 Hz) increases proportionally to the duration of prior wakefulness, and thus reflects the homeostatic aspect of sleep regulation [5,11,26]. This fundamental property of sleep has been demonstrated for a wide variety of different species [6,25].

In the two-process model of sleep regulation, the time course of the rise of the homeostatic Process S was derived from the level of SWA in non-rapid eye movement (NREM) sleep preceding and following a waking episode [1,5]. In humans, a marker of Process S was identified also in the waking EEG. Thus, an increase of delta or theta power occurred in the course of wakefulness [2,3,9,12,22,28]. Further evidence for a link between theta activity in the waking EEG and sleep homeostasis was provided by a between-subject correlative study [12]. The rate of increase of theta power during 40 h of waking correlated positively with the increase of SWA in NREM sleep. Moreover, theta

Abbreviations: ANOVA, analysis of variance; EEG, electroencephalogram; EMG, electromyogram; NREM, non-rapid eye movement; SEM, standard error of the mean; SD, sleep deprivation; SDL, sleep deprivation in the light period; SDD, sleep deprivation in the dark period; SWA, slow-wave activity

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activity in the waking EEG paralleled subjective sleepiness [2,12,15,20,21,24].

In animals, an EEG correlate for sleep pressure during waking has not been reported. Rodents exhibit a variety of waking behaviors including active exploration and quiescence with low locomotion. These differences in behavior are reflected in the EEG [29]. During active waking, associated with voluntary movements, the EEG is desynchronized, dominated by fast frequencies and regular theta activity, while quiet waking is characterized by a mixed pattern with slower waves [14,16,29].

The changes in arousal level and EEG activity are interrelated because both depend on the tonic depolarizing input from the brainstem [18,23]. Several neurotransmitters play a major role in the "arousal brain system" [18]. The neurons comprising the ascending reticular activating system, as well as thalamocortical neurons, use glutamate as a transmitter; pontomesencephalic neurons use acetylcholine, while locus coeruleus neurons, projecting in a diffuse manner from the brainstem to the entire forebrain, are adrenergic [18]. Activation of these nuclei results in the blockage of synchronized low-frequency oscillations in the thalamocortical system that are typical for NREM sleep, and in the generation of fast activity, typical for wakefulness [23].

We hypothesized that an increase of sleep propensity could be manifested in the EEG during quiet wakefulness also in rodents in the course of prolonged wakefulness. To test this hypothesis, we subdivided the waking epochs during a 6-h sleep deprivation (SD) into quiet waking and active waking on the basis of EMG activity, analyzed the changes in the power spectrum, and investigated whether the changes were related to the increase of SWA during recovery sleep.

2. Materials and methods

2.1. Animals

The local governmental commission for animal research approved the experiments. Adult male albino rats of the Sprague–Dawley strain (n = 14) with a mean body weight 277.5 ± 5.1 (SEM) g were used. The animals were kept individually in Macrolon cages (53 × 34 × 37 cm) with food and water available ad libitum, and maintained on a 12-h light–12-h dark cycle (light from 8.00 to 20.00 h; 7 W OSRAM Dulux EL energy saving lamp, approximately 30 lx). Ambient temperature was maintained at 21–22 °C. Under deep pentobarbital anesthesia (Nembutal sodium, 80 mg/kg i.p., volume approximately 0.5 ml), the rats were implanted with gold-plated miniature screws (0.9 mm diameter) that served as EEG electrodes. The right parietal electrode was implanted 5.5 mm lateral to the midline, 2.5 mm posterior to the bregma, and referenced to the electrode above the cerebellum (2 mm posterior to the lambda, on midline).

Two gold wires (diameter 0.2 mm) inserted into the neck muscles served to record the electromyogram (EMG). The electrodes were connected to stainless steel wires that were fixed to the skull with dental cement. At least 10 days were allowed for recovery. The rats were connected to the cable and amplifier via a swivel one day before the baseline was recorded.

2.2. Experimental protocol and data acquisition

The EEG and the EMG were recorded continuously. A 24-h baseline day was followed by 6 h sleep deprivation (SD) starting at light onset (8:00 a.m., SDL, n = 7) and 18 h recovery. To investigate whether the timing of the intervention would play a role, a second batch of rats was sleep deprived for 6 h beginning at dark onset (8:00 p.m., SDD, n = 7). SD was performed by introducing a variety of objects (e.g., nesting material, pieces of wood, paper, and tissue) into the cage, and by tapping on the cage whenever the animal appeared drowsy or the EEG exhibited slow waves. Halfway through the SD, the cages were exchanged to provide additional stimulation [17,30]. Due to the increased occurrence of EEG artifacts during this manipulation, the second 2-h interval of SD (hours 3–4) was excluded from the analysis. The rats were never disturbed when they were spontaneously awake, or during feeding and drinking.

The EEG and the EMG signals were amplified (amplification factor approximately 2000), conditioned by analog filters (high-pass filter: −3 dB at 0.016 Hz; low-pass filter: −3 dB at 40 Hz, less than −35 dB at 128 Hz) sampled with 512 Hz, digitally filtered (EEG: low-pass FIR filter 25 Hz; EMG: band-pass FIR filter 20–50 Hz) and stored with a resolution of 128 Hz. EEG power spectra were computed for 2-s epochs by a Fast Fourier Transform (FFT) routine (linear detrending, Hanning window, 0.5 Hz resolution). Frequencies between 0.5 and 25 Hz were analyzed. Period–amplitude analysis was performed on the 5–7 frequency band. The raw EEG signal was band-pass filtered (5–7 Hz, 4th order Chebyshev type II filter, forward and reverse filtering), and the amplitude of positive peaks of individual theta waves, and their number per second (frequency) were computed.

2.3. Vigilance states

Two vigilance states, NREM sleep and waking were scored for 4-s epochs. They were determined off-line by visual inspection of the parietal EEG and the EMG records and EEG power in the slow-wave range (0.75–4.0 Hz). Special care was taken to exclude from the waking EEG those epochs during SD where clear signs of NREM sleep (i.e., delta waves concomitant with low EMG activity) were evident. Epochs containing EEG artifacts were excluded from spectral analysis (Table 1). The sleep deprivation procedure was effective, since only 0.3 ± 0.1 (n = 7) and 1.8 ± 0.9 min (n = 7) of NREM sleep occurred during SDL and SDD, respectively.