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Research report

Evidence for 2-stage models of sleep and memory: Learning-dependent changes in spindles and theta in rats

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ABSTRACT

What processes are involved in the formation of enduring memory traces? Sleep has been proposed to play a role in memory consolidation and the present study provides evidence to support 2-stage models of sleep and memory including both non-rapid eye movement (NREM) and rapid eye movement (REM) sleep. Previous research has shown REM sleep increases following avoidance learning and memory is impaired if REM deprivation occurs during these post-training periods indicating that REM sleep may have a role in memory consolidation processes. These discrete post-training periods have been termed *REM sleep windows* (RSWs). It is not known whether the electroencephalogram has unique characteristics during the RSW. Further investigation of the RSW was one of the primary goals of this study. We investigated the epidural-recorded electrophysiological learning-related changes following avoidance training in rats. Theta power increased in the learning group during the RSW, suggesting that theta is involved in memory consolidation during this period. Sleep spindles subsequently increased in slow wave sleep (SWS). The results suggest that both NREM and REM sleep are involved in sleep-dependent memory consolidation, and provide support for existing 2-stage models. Perhaps first theta increases to organize and consolidate material via hippocampal–neocortical dialogue, followed by subsequent refinement in the cortex by spindles during SWS.

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1. Introduction

The role of sleep in memory consolidation has been a contentious topic in the neurosciences [39]. Particular stages of sleep and their unique electrophysiological characteristics have been implicated in neural plasticity. However, neither the time-course of the learning-dependent changes in sleep has been well characterized, nor the relative contributions of the various sleep stages to memory consolidation processes are well understood. Paradoxical or rapid eye movement (REM) sleep plays a significant role in the formation of new memories [28]. During REM sleep following learning, memories may be consolidated by long-term potentiation (LTP)-like processes [1]. Without REM sleep, performance improvements are smaller in humans [27,35] and animals [25,14]. Changes in the number of REMs and in theta activity, which characterize REM sleep, have been observed in humans following learning [9,30,33]. Thus, REM sleep contributes to memory processing.

Following learning, increases in REM sleep have been observed [25]. In rats, these increases do not occur homogeneously across an entire 24-h period (or more) following training. Rather, REM increases occur in discrete periods, which have been termed REM sleep windows (RSWs). RSWs are periods of sleep characterized by increased number of REM sleep episodes, thereby increasing the total duration of REM sleep following learning [28,25]. The timing of the RSW systematically varied as a function of animal strain [31], type of memory task [34,36] and the training regime [36]. The RSW has typically been observed to be limited in duration to about 4 h. REM sleep deprivation for a period of 4 h during but not outside of the RSW impaired memory [28]. The electrophysiological learning-related changes during the RSW have yet to be investigated, and were the major focus of this investigation.

In humans, REM sleep deprivation and reduced rapid eye movements resulted in memory impairment [26,27]. Memory consolidation may be enhanced during learning-dependent increases in REM sleep. During REM sleep, ensembles of hippocampal neurons were reactivated in the same pattern as during initial learning [41]. P-waves (the pontine component of the ponto-geniculo-occipital circuit that triggers REMs) and REMs in the rat were correlated with theta power during REM sleep [15]. Thus, learning-dependent increases in REMs and theta may reflect brain activation

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involved in memory consolidation. It is not known whether learning-dependent changes in theta power accompany increases in REM sleep during the RSW following learning.

More recently, the role of Stage 2 sleep in memory consolidation has been investigated. In humans, Stage 2 sleep makes up about 60% of a night's sleep and is characterized by sleep spindles, k-complexes and less than 30% delta wave activity. The electroencephalogram (EEG) appears more synchronized and generally slower than waking. Muscle activity is lower than waking, but higher than the muscle atonia observed in REM sleep. In humans, Stage 2 sleep deprivation impairs simple procedural memory performance on tasks such as the pursuit rotor [32], and increases in Stage 2 sleep have been observed following periods of simple procedural learning [8,9]. Sleep spindles characterize Stage 2 sleep, and until recently, their function has remained elusive. Following periods of simple procedural learning [8,9] or verbal learning [4,11,25] increases in sleep spindles have been observed in humans. In animals, increases in sleep spindles in a 60-min period immediately following associative learning (odor-reward pairing task) have also been observed [7].

The goal of the present study was to investigate the learning-dependent changes in EEG spectral power to better characterize the changes in brain activity which may reflect memory consolidation processes across the entire 24 h post-training day. We examined the hypothesis that increases in theta power and sleep spindles would occur in the RSW following learning. This investigation may provide additional information about the processes involved in sleep-dependent memory consolidation and provide experimental evidence to support a 2-stage model for sleep-dependent memory consolidation proposed in humans [29] and animals [3,24]. Thus, according to this model both REM and non-rapid eye movement (NREM) sleep may be involved in memory consolidation.

2. Methods

2.1. Methods summary

Twenty male Sprague–Dawley rats weighing 250–300 g were implanted with four EEG and two electromyogram (EMG) electrodes. Animals were housed with *ad libitum* food and water and kept on a 12 h light–dark cycle. After 14 days of recovery, 3 days of acclimatization, and 24 h of baseline (BL) recording, animals were trained on the two-way avoidance task for 100 trials (50 trials/day) and re-tested for 25 trials on day 3. EEG was recorded for 22 h after training on both training days 1 and 2. EEG was stage scored as REM, SWS or wake, analyzed using a fast Fourier transform (FFT) algorithm, and sleep spindles were automatically counted. Rats in the learning group (LG) ($n = 8$) avoided foot shock on 60% of the test trails. The remaining rats ($n = 12$) were assigned to the non-learning group (NLG).

2.2. Animals and housing

Twenty male Sprague–Dawley rats (Charles River, Saint-Constant, Quebec, Canada) weighing 250–300 g at the time of surgery were housed individually in opaque plastic cages (20 cm × 30 cm × 18 cm high) with food and water available *ad libitum*. Lights were on from 11:00 AM to 11:00 PM, and off from 11:00 PM to 11:00 AM. The experimental protocol was approved by the Trent University ethics review board.

2.3. Surgery

Anaesthesia was maintained with the use of isofluorane gas (0.5 L/min breathable oxygen with 1.5–3.0% isoflurane/O^2 maintained) delivered via a stereotaxic-mounted nosepiece. A nosepiece and ear bars restrained the head once Bregma and Lambda were levelled. Four EEG and four EMG electrodes were implanted. Each EEG electrode consisted of a stainless steel screw implanted into the skull attached to a 32 gauge (0.20 mm diameter), 2 cm long Teflon-coated stranded stainless steel wire. The two anterior screws were placed at stereotaxic coordinates 2 mm anterior and ±2.5 mm laterally from Bregma. The two posterior screws were placed at stereotaxic coordinates 3.5 mm posterior and ±3.0 mm laterally from Bregma. Posterior placement was overlying the hippocampus to better visualize theta. Two 32-gauge, 3 cm long bare stranded stainless steel wires were implanted into the neck muscles bilaterally. Each wire was crimped into a gold connector socket and routed to a six-channel connector affixed to the skull with dental acrylic. Each rat was administered an intramuscular injection of antibiotic and given liquid acetaminophen orally for post-operative pain relief. All rats were allowed 15 days to recover from the surgical procedure and to become acclimatized to the cables attached to the skull connector.

2.4. Sleep recording and analysis

Sleep was recorded after the recovery period and after three consecutive days in the recording chamber with the skull connector attached to the recording cable. The first sleep recording session lasted 24 h and served as a baseline recording. During the recording sessions, the electrodes were connected from the skull connector on the rat to a commutator (Plastics One Inc.) which was then connected to a digital Sandman (Putritan Bennett Inc.) Suzanne™ polygraphic amplifier. Bipolar EEG recordings were made by referencing one of the two posterior sites to the anterior sites. The remaining posterior site served as an isolated ground electrode. EEG and EMG recordings were taken at 120 samples/s with a low frequency cut off at 0.18 Hz and a high frequency cut off at 42 Hz. A 60 Hz notch filter was also used. Sleep recording took place for 23–24 h starting between 10:00 and 11:00 AM. The recording apparatus consisted of a clear Plexiglas recording chamber (25 cm × 25 cm × 50 cm high). Food, water and bedding were made available throughout the recording sessions.

For sleep scoring and analysis EEG was subsequently filtered using a 0.5 Hz high-pass filter and a 30 Hz low-pass filter. EMG was filtered using a 1 Hz high-pass filter and a 50 Hz low-pass filter. Sleep records were scored in 30 s epochs as wake, SWS or REM sleep when at least 50% of the epoch met the following criteria: wake – EEG appeared desynchronized, high frequency, low amplitude accompanied by elevated EMG activity; SWS – the appearance of sleep spindle activity, low frequency, high amplitude EEG, accompanied by lower EMG activity; REM sleep – the EEG was characterized by synchronized theta activity (6–10 Hz), accompanied by lower EMG activity and the disappearance of sleep spindle and slow wave activity. Sleep spindles are high amplitude (>0.25 μV) phasic waxing and waning ("fusiform") events in the EEG from 12–16 Hz, typically lasting from 0.5 s up to about 3 s. The transition between SWS and REM sleep, which is characterized by usually brief (<15 s) high amplitude bursts of sigma and theta activity were included with REM sleep scores. Transition sleep was not scored separately due to its short duration and was included with REM as this is a period marked by increased P-wave activity (a physiological characteristic of REM sleep) related to avoidance learning observed by Datta [6]. Sleep stage scoring was compared to stage scoring conducted in 10-s epochs to evaluate the reliability of scoring in 30-s epochs. One hundred and sixty-two 30-s epochs were selected from baseline recordings from six different rats. Of the 162 epochs, there were 54 of each wake, SWS and REM (54 × 3 = 162). The 30-s epoch was rescored instead as three 10-s epochs. Rescoring was done by an experimenter who was blind to the original scoring of the 30-s epochs. Based on the 162 cases, the 30-s and 10-s scoring methods were in almost perfect (92.0%) agreement (Kappa = .880, $p < 0.0000001$).

The frequency content of the EEG was analyzed using FFT power spectral analysis. Only movement artifact-free EEG was included in the analysis. Movement artifact was identified using an automatic computer algorithm which identified periods of EEG in two separate passes for slow activity (1–6 Hz) and fast activity (18–60 Hz) that was high amplitude and at least 2 s in duration. To accomplish this, the average amplitude of 3600 s of "background" EEG was calculated. When a window of at least 2 s of EEG within each 3600 s of EEG exceeded four standard deviations beyond the average amplitude, the 2 s window was labelled as artifact and subsequently merged with adjacent artifact events if closer than 0.10 s apart. The FFT analysis was done in 2 s overlapping windows (75% overlap), averaged into 30 s epochs so that each power value was represented in the time scale used for sleep stage scoring. Four frequency bins were used including delta (1–5 Hz), theta (6–10 Hz), sigma (11–16 Hz) and beta (17–20 Hz) identified by Corsi-Cabrera et al. [5]. The spectral power values were then log transformed and multiplied by 10 to obtain units in decibels (dB), then averaged across each sleep–wake state within each 4 h recording period. EEG power for each sleep stage, in each 4 h period in each frequency bin was expressed as the change from baseline. When the assumption of sphericity was violated for ANOVA analyses, the degrees of freedom were adjusted using a Huynh-Feldt correction factor.

Sleep spindles were counted during SWS for the entire recording period on the baseline, training day 1 and training day 2 using a computerized algorithm. Sleep spindles were identified if they occurred in the 12–16 Hz range, were greater than 100 μV in amplitude, had a minimum duration of 0.15 s, maximum duration of 3 s and a minimum inter-spindle interval of 0.25 s. Spindles identified with an inter-spindle interval of less than 0.25 s were merged and counted as a single spindle. The difference from baseline spindle density (number of spindles/min) was calculated for each 4 h period on training days 1 and 2. Only sleep spindles detected during movement artefact-free EEG using the same algorithm described above were included in the spindle count.

2.5. Behavioural procedures

Behavioural training and testing began at 9:00 AM and ended by 11:00 AM using a two-way shuttle shock avoidance task in a dimly lit room. The shuttle apparatus had two identical compartments (50 cm × 25 cm × 20 cm high). Each compartment

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