Validation of a Closed-Loop Sensory Stimulation Technique for Selective Sleep Restriction in Mice

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Abstract— **Experimental manipulation of sleep in rodents is an important tool for analyzing the mechanisms of sleep and related disorders in humans. Sleep restriction systems have relied in the past on manual sensory stimulation and recently on more sophisticated automated means of delivering the same. The ability to monitor and track behavior through the electroencephalogram (EEG) and other modalities provides the opportunity to implement more selective sleep restriction that is targeted at particular stages of sleep with flexible control over their amount, duration, and timing. In this paper we characterize the performance of a novel tactile stimulation system operating in closed-loop to interrupt rapid eye movement (REM) sleep in mice when it is detected in real time from the EEG. Acute experiments in four wild-type mice over six hours showed that a reduction of over 50% of REM sleep was feasible without affecting non-REM (NREM) sleep. The animals remained responsive to the stimulus over the six hour duration of the experiment.**

I. Introduction

Since circadian and homeostatic modulation of sleep is similar across different mammalian species, animal models may be useful in unraveling the mechanisms of sleep in humans. The use of animal models, particularly rodents, in sleep research provides scientists with the opportunity to investigate the genetic and neurobiological changes underlying sleep abnormalities.

Total sleep deprivation (TSD) has long been used for investigating sleep regulation mechanisms and the effects of sleep loss. The main effects of TSD on rodent sleep are a prominent increase in electroencephalogram (EEG) slow wave activity (SWA; 0.75-4Hz oscillations) during non-rapid eye movement (NREM) sleep as well as theta activity (6-9 Hz oscillations) in rapid eye movement (REM) sleep [1]. However, TSD is not an adequate model for interrupted sleep in humans, which does not always feature total sleep loss. Hence, selective sleep deprivation has been investigated in animal models and humans to evaluate its effects on physiology.

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Selective sleep deprivation protocols permit comparison of the roles of each state of sleep on behavior and cognition. Deprivation of NREM (REM) sleep is followed by NREM (REM) rebound during the recovery period [2]. However, deprivation of either state may affect the other one as well. REM sleep deprivation (REM SD) is not 100% selective and results in REM rebound and extensive suppression of SWA in NREM [2]. Manual experimental methods have been employed to target particular stages of sleep that may involve gentle handling [3] or cage movement [4]. To avoid the need for human supervision and intervention, other methods have been devised, especially for REM SD: for instance, the "flower pot" or "multiple platform" method, in which the animal is placed on a platform and falls into a basin when it becomes atonic during REM sleep. This method essentially deprives the animal of all REM sleep and is highly stressful [5].

Programmable computer-controlled devices have been employed to detect sleep state automatically from the EEG and rouse the animal using some form of stimulation, such as cage shaking [6,7], a rotating disk over water [8] or a slowly rotating stir bar on the cage floor [9]. Automated sleep restriction may provide greater flexibility and selectivity compared to previous techniques, and perhaps limit the confounding effects of hormonal stress. However, each manipulation technique has advantages and limitations related to convenience, intrusiveness, cost, and efficacy. Here, we test the feasibility of a novel sleep restriction technique in mice. The basis of this technique is to detect the onset of a targeted sleep state (REM or NREM) from EEG signals using a computer algorithm and apply tactile stimulation in the form of vibrations transmitted through the cage floor to rouse the animal. Using this system, we can implement selective sleep restriction relatively easily and with greater flexibility over the proportion of sleep loss. The application of our technique for selective REM SD in mice is described in this paper.

II. Methods

A. Animals, care and protocols

All experimental procedures in this study were conducted with the approval of the Institutional Animal Care and Use Committee (IACUC) at the University of Kentucky. The experiments were performed on adult male wild type mice (C57BL/6J, Jackson Labs), the most widely used inbred strain (4-6 weeks old, n = 4). Each animal was housed independently with 14h/10h light/dark (ambient temperature: 20 ± 2°C, humidity: 50 ± 10%) and free access to food and

water at all times. A baseline EEG recording of 6 h duration (1-7 P.M.) during subjective night was acquired from mice and used to tune an automated REM sleep detector. An experimental session was performed at a later date, at the same time of day and for the same duration, in which tactile stimulation was applied whenever REM sleep onset was detected from the EEG.

B. Surgical implantation and signal acquisition

Electrodes for monitoring brain and muscle activity were implanted under 2.5% isoflurane anesthesia. A head-mounted preamplifier (8201; Pinnacle Technology, Inc, Lawrence, KS) was affixed directly over bregma using four miniature silver screws that serve as two differential cortical EEG derivations with a common reference and ground. Teflon-coated leads were inserted bilaterally into the dorsal neck muscle posterior to the skull to provide an electromyogram (EMG). Then, the animals were allowed to recover and adapt for two weeks before collecting data.

Our chronic acquisition system includes tethered EEG/EMG (8206; Pinnacle Tech., Lawrence, KS), with a USB camera (Microsoft LifeCam VX-6000) and infrared (IR) illumination source to enable continuous video recording across light and dark periods. Input signals were digitized at 14 bits and a sampling rate of 400 Hz under software control (Sirenia^{TM}, Pinnacle Tech.). A custom LabVIEW^{TM} interface (National Instruments) captured video in synchrony with EEG/EMG acquisition. EEG/EMG signals were analyzed in real time using LabVIEW to detect REM sleep onset and trigger stimulation for REM SD. A baseline recording was first processed offline to tune the REM classifier. This was then used to detect REM sleep onset in real time and trigger the stimulation system in closed-loop. The trigger signal was recorded synchronously with the EEG and EMG as an additional data stream.

C. Sleep scoring and REM detector training

Training data were selected from a baseline recording in each mouse (after allowing it to adapt to the recording cage for two days) to determine transition to REM sleep based on EEG/EMG features. The vigilance state was manually scored based on well-established criteria using a video-EEG viewer in 4 s epochs as Wake, REM and NREM. Wake is identified by low amplitude, desynchronized EEG and relatively high amplitude EMG. NREM stages have increasingly prominent delta ($\boldsymbol{\delta}$: 0.5-4 Hz) while REM has a prominent theta oscillation ($\boldsymbol{\theta}$: 6-9 Hz) similar to activity during Wake; EMG amplitude is low in both REM and NREM. Hence, spectral band power estimates from EEG and EMG were used as features to construct an automated REM sleep detector for each animal. The mean power from band-pass filtered EMG (80-100 Hz) was used to detect low muscle tone in sleep. Within sleep, the $\boldsymbol{\delta/\theta}$ band power ratio was estimated to detect the onset of REM sleep. Thus, REM detection thresholds were established for both features using the baseline recording and manual scores. These feature thresholds were used to detect REM sleep onset during the REM SD experiment.

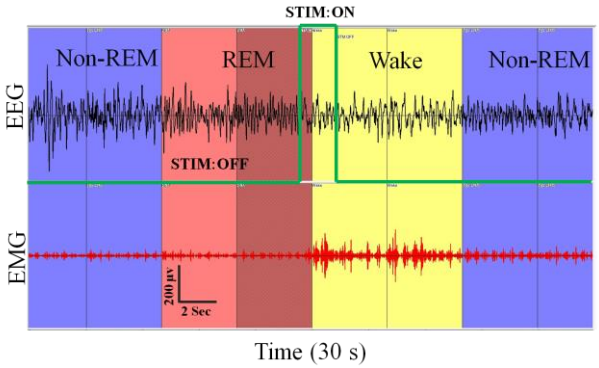

Figure 1. Snapshot of EEG and EMG during a NREM-REM state transition during sleep interrupted by a brief tactile stimulus. The animal is aroused briefly as seen from the elevated EMG amplitude, and then drifts back into NREM sleep.

D. Real time REM sleep detection and stimulation

REM SD experiments were performed using a closed-loop system that applies a vibratory tactile stimulus to the animal when REM sleep is detected. Eight equally spaced button-type shaftless vibration motors (No. 1638, Pololu Corporation, Las Vegas, NV, USA) are attached to the underside of a rubber pad on the floor of the animal's cage. Each motor (10 mm diameter, 2 mm thickness) vibrates with an amplitude of 0.75 g at 12,000 r.p.m. when driven by a 3 V DC supply. The vibration is transmitted to the animal's body via the pad and produces tactile stimulation. A LabVIEW program calculates frequency band power features from EEG and EMG in 1 s epochs (4 s moving average) and activates the stimulation when preset thresholds on the EEG/EMG features are crossed indicating that REM sleep onset has been detected. The stimulation is automatically stopped when the state has changed and the animal is awake (see Fig. 1). Using this technique we were able to selectively reduce the proportion of REM sleep dramatically without affecting NREM sleep. The performance of the system in detecting REM sleep in real time and reducing its proportion was verified against manual scoring of the data from the experiment.

III. Results

First, we evaluate the performance of the real-time REM sleep detector for each animal. Then we assess the effects of closed-loop sensory stimulation on REM and NREM sleep.

A. Assessment of real-time REM sleep detection

A human scorer inspected EEG, EMG and video data for each mouse during the experimental stimulation phase and determined vigilance state in sequential 4 s epochs. The state of the stimulation trigger was not visible to the scorer. Stimulation onset and offset times were also extracted from the recordings. To assess the performance of real-time REM detection in each animal, true REM incidents (bouts of continuous REM) as determined by visual scoring were compared against stimulation times. Numbers of the following events were determined for each recording: 1. True Positive (TP) detections, i.e., REM bouts that overlapped with stimulation onset; 2. True Negative (TN) events, i.e., NREM or Wake bouts (i.e., other than REM) in which stimulation was already off or switched off; and 3. False Positive (FP) detections, i.e., NREM or Wake epochs in which stimulation was activated or already on. These counts were combined into two commonly used performance measures:

$$\text{Sensitivity} = TP / (TP + FN) \quad (1)$$

$$\text{Positive predictive value } (PPV) = TP / (TP + FP) \quad (2)$$

In addition, depending on the choice of threshold or time constants related to filtering and the 4 s resolution of manual scoring, there can be a finite delay from the true onset of REM to when the detector is triggered. The REM detection latency was estimated for each TP detection as the time between REM onset and stimulation onset. A summary of detector metrics is presented in Table I.

B. Performance of REM sleep restriction system

To study the effectiveness of closed-loop REM SD system, sleep parameters were estimated and compared for the baseline and experimental REM SD recordings. Visual scores from each animal in baseline and REM SD phases were used to estimate percent time spent in REM, NREM, and Wake as well as the distribution of bout duration for each state. In Fig. 2 (Upper) we plot the cumulative distribution of bout duration for each state of vigilance (data from all animals are pooled together). It shows that stimulation drastically reduced REM bout duration while its effect on NREM and Wake is relatively small. In Fig. 2 (Lower) the mean % time spent in each state ($n = 4$ mice) is shown for the baseline and stimulation phases. The % time in NREM is almost intact while % REM is clearly reduced and % Wake increased. The amount of REM loss appears to be compensated by a gain in Wake.

Table I. Evaluation of REM detection performance.

Animal	Sensitivity (%) [Total REM bouts]	PPV (%) [Total detections]	Latency (s) (mean ± s.d.)
1	72.4 [254]	88.0 [209]	8.2 ± 4
2	93.5 [77]	38.3 [188]	7.9 ± 8
3	98.3 [114]	65.1 [172]	6.7 ± 4
4	95.7 [117]	72.7 [154]	6.7 ± 3
Mean ± s.e.m.	**95.8 ± 6**	**66.0 ± 10**	**7.4 ± 0.4**

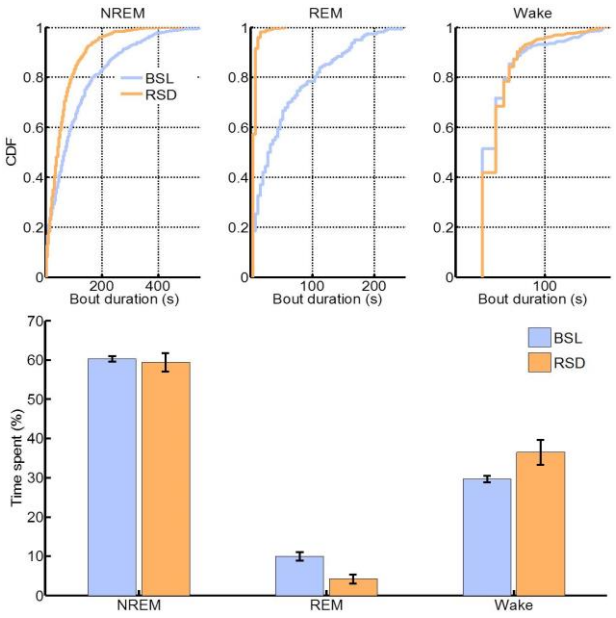

Figure 2. Effects of closed-loop sensory stimulation on sleep. **Upper:** Cumulative distribution function (CDF) of bout durations in each state (data pooled from all four mice). **Lower:** Comparison of mean percent time spent in each state for baseline and stimulation periods. Error bars represent standard error of the mean ($n = 4$).

IV. Discussion

Sleep is a delicate and complex dynamical process. Disruption of sleep due to stress, injury, medication, disease, lifestyle, and environmental factors can have serious health consequences. Experimental manipulation of sleep can help us understand how sleep and health are inter-related and discover new treatments for sleep-related disorders. Many methods have been proposed for sleep restriction in rodents, and each one comes with unique trade-offs—ease of implementation, flexibility, stressfulness, intrusiveness, efficacy, arousal threshold, adaptation over time—that must be considered in selecting one that is appropriate for the research question under investigation. New additions to the arsenal of techniques for sleep manipulation are therefore always welcome.

In this paper a promising new technique for selective sleep restriction in mice was evaluated that employs vibratory tactile stimulation triggered by automated detection of a particular phase of sleep (in this case, REM). As with any closed-loop sleep restriction method, the effectiveness of this technique relies on how sensitive and selective the detector is to REM sleep, the responsiveness of the animal to the stimulus, and whether the animal is likely to get desensitized to the stimulus with repeated exposure to it over the course of the experiment. We consider these factors below in light of our experimental results.

The ability of our algorithm to detect REM sleep in real time is summarized in Table I. The sensitivity of the detector to REM sleep onset was high, over 90 %, in 3 of 4 animals. But this is balanced by a much more moderate specificity (PPV), which ranged from 38 to 73 % in the same animals.

(The situation is reversed in Animal 1, which had relatively poor detection sensitivity but high specificity.) This means that roughly one-third to one-half of all stimulations occurred during a state other than REM (NREM or Wake). Since tactile stimulation does not change the animal's state when awake, the slight reduction in mean NREM bout duration relative to baseline (Fig. 2 Upper) could have been due to these false REM detections. Stimulation during NREM appeared to induce only brief arousal, which may explain why the fragmentation of NREM sleep is not accompanied by a significant reduction in % NREM sleep in Fig. 2 (Lower). Another possible reason for the shorter NREM bouts could be the brain's homeostatic tendency to try to recover lost REM sleep as the protocol is continued. Metrics for Wake show a lower bout duration, which may be due to increased brief arousals during NREM triggered by FP stimulation, but a greater % Wake, which matches the reduction in % REM closely. The other detection metric in Table I is latency. On average it takes about 7 s (two 4 s epochs) for the detector to determine that the animal is in REM sleep and then deliver a stimulation pulse. A possible reason for this delay is the way data was scored. Brief or transitional episodes of REM are manually scored as REM while the detector may wait for a more distinctive signature of REM sleep based on EEG/EMG features and the preset thresholds on them. As a consequence, the protocol only affects prolonged REM bouts and ignores brief episodes. The reduction in median REM bout duration (Fig. 2 Upper) compared to baseline (from 28 s to 4 s) supports this observation.

Taken together, Table I and Fig. 2 indicate that the stimulation protocol produced a reduction in REM sleep of over 50 % on average over a 6 h period. That REM sleep is not eliminated altogether may be attributed in part to the latency of detection. In this preliminary study, we have implemented a simple linear thresholding approach for REM onset detection that is very sensitive to EEG/EMG signal quality, which was poor in Animal 2 and adversely affected detection specificity (PPV). Taking advantage of better supervised machine learning techniques, such as support vector machines or hidden Markov models, could improve the performance of online REM detection and thereby the efficacy of REM sleep restriction.

The effectiveness of this system was examined for selective disruption of REM sleep in mice in acute experiments 6 h in duration. Besides the limitations on performance of the detector, the animal could become desensitized and eventually oblivious to the stimulus depending on the frequency and duration over which it is applied. Fig. 3 presents the mean % time spent in REM for each consecutive hour of monitoring during the baseline and RSD protocols. The % time in REM is lowest (1 %) in the first hour and higher but relatively constant (4-6 %) over the next five hours. By comparison the baseline value starts at 10 %, peaks in the mid-afternoon (14 %), and starts to fall as the evening approaches; this is consistent with diurnal variation. It is logical that the achievable reduction in REM later in this period is low simply because the probability of its occurrence is already low under baseline conditions. In conclusion, the efficacy of the stimulation protocol appears to be relatively stable except in the first hour since the animal is naive to the stimulus at that time. This suggests that, in the acute experiments performed, the animal remains responsive to the stimulus without a change in threshold. However, it remains to be seen whether the effect will persist with more frequent application over a longer monitoring period. More experimentation is under way to better characterize the performance and limitations of this new system for chronic selective sleep restriction in mice.

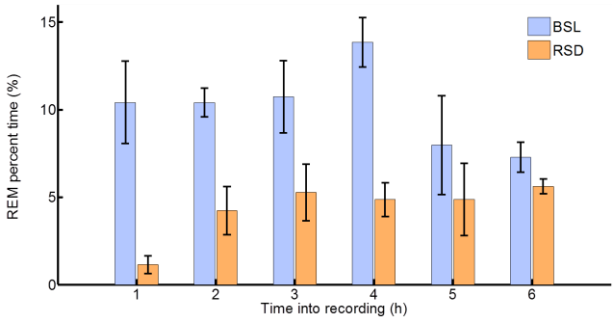

Figure 3. Trends in mean hourly percent time spent in REM during the baseline (BSL) and REM SD (RSD) stimulation protocol.

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