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Long-term effects of sleep deprivation on neuronal activity in four hypothalamic areas

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ABSTRACT

Lack of adequate sleep has become increasingly common in our 24/7 society. Unfortunately diminished sleep has significant health consequences including metabolic and cardiovascular disease and mental disorders including depression. The pathways by which reduced sleep adversely affects physiology and behavior are unknown. We found that 6 h of sleep deprivation in adult male rats induces changes in neuronal activity in the lateral hypothalamus, the paraventricular nucleus, the arcuate nucleus and the mammillary bodies. Surprisingly, these alterations last for up to 48 h. The data show that sleep loss has prolonged effects on the activity of multiple hypothalamic areas. Our data indicate also that measuring electroencephalographic slow wave activity underestimates the amount of time that the hypothalamus requires to recover from episodes of sleep deprivation. We propose that these hypothalamic changes underlie the well-established relationship between sleep loss and several diseases such as metabolic disorders, stress and depression and that sufficient sleep is vital for autonomic functions controlled by the hypothalamus.

1. Introduction

In humans, sleep loss can have widespread detrimental effects on health and is shown to be a risk factor for several diseases (Lim and Dinges, 2010; Grandner et al., 2010; Ma et al., 2015; Schmid et al., 2015; Aguirre, 2016). Chronic sleep loss is associated with deficits in attention, cognition, immune function, metabolism, mood, and cardiovascular function (Grandner et al., 2010; Ma et al., 2015; Schmid et al., 2015). For example, insufficient sleep is associated with decreases in the satiety hormone leptin, increases in the hunger-stimulating hormone ghrelin and increases in appetite (Spiegel et al., 2009; Hanlon and Van Cauter, 2011; Schmid et al., 2015). Combined with our modern obesogenic environment, this physiological state leads to overeating and ultimately overweight and obesity (Schmid et al., 2015). Even one night of sleep loss can acutely impair both executive functions and cognitive learning (Lim and Dinges, 2010) and alter metabolic hormonal balance (Leproult et al., 1997; Spiegel et al., 2009; Hanlon and Van Cauter, 2011). On the other hand, sleep loss can have a profound positive effect on mood in clinically depressed patients (Dallaspezia and Benedetti, 2015).

fMRI studies in humans investigating the effects of sleep deprivation on health have focused mainly on cortical structures leaving the potential contribution of subcortical brain areas largely unexplored (Ma et al., 2015). The hypothalamus plays a major role in regulating physiological and behavioral responses by sensing the body's internal state and altering the electrical activity of hypothalamic neurons, thereby regulating the activity of other brain areas in order to maintain homeostasis (Sternson, 2013). Within the hypothalamus, the lateral hypothalamus (LH) as well as the arcuate nucleus (ARC) integrate diverse systemic and neuronal inputs in order to regulate metabolism and feeding behavior (Berthoud and Münzberg, 2011) while the paraventricular nucleus (PVN) and the mammillary bodies (MB) have been involved respectively in the control of the endocrine component of the stress response (Füzesi et al., 2016) and learning and memory (Vann and Aggleton, 2004). Because all the physiological functions controlled by these hypothalamic nuclei are known to be adversely affected by sleep loss (Lim and Dinges, 2010; Grandner et al., 2010; Ma et al., 2015; Schmid et al., 2015; Aguirre, 2016), we investigated in adult Wistar rats the acute and long-term effects of a 6-h of sleep deprivation (SD) on electrical multi-unit activity (MUA), as a measure of neuronal activity in the LH (n = 20 animals), the PVN (n = 7), the ARC (n = 8) and the MB (n = 7; Fig. S1). In addition, electroencephalogram (EEG) and electromyogram (EMG) recordings were performed simultaneously in order to investigate changes in sleep and waking before, during, and after SD.

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2. Materials and methods

2.1. Animals

A total of 63 male Wistar rats (approximately 300 g at the time of surgery) were used for this study. The animals were purchased from Charles river. All animal experiments were approved by of the Ethics Committee of the Leiden University Medical Center and were carried out in accordance with the EU Directive 2010/63/EU on the protection of animals used for scientific purposes.

2.2. In vivo multi-unit activity, EEG, and EMG recordings

In vivo multiunit activity (MUA) and EEG and EMG were recorded as described previously (Meijer et al., 1998; Deboer et al., 2003, 2007). In brief, for the MUA recordings, stainless steel tripolar electrodes (0.125 mm diameter; Plastics One, Inc., Roanoke, VA) were implanted in each animal under deep anesthesia. For differential recordings, two electrodes were directed toward the targeted hypothalamic structure with 0.4-mm space between the electrodes. The third electrode was placed in the cortex as a reference electrode. The electrodes were placed to record from the LH (relative to Bregma: 1.8 mm posterior and 2 mm lateral; depth: 8.15 mm), PVN (relative to Bregma: 2 mm posterior and 0.84 mm lateral; depth: 7.67 mm; 5 degrees relative to vertical), ARC (relative to Bregma: 3.8 mm posterior and 0.26 mm lateral; depth: 9.8 mm; 5° relative to vertical), and MB (relative to Bregma: 3.8 or 4.8 mm posterior and 1 mm lateral; depth: 9.1 mm). The coordinates were adapted from (Paxinos and Watson, 1982).

For EEG, electrodes were screwed into the skull above the dura over the right cortex (2.0 mm lateral to the midline and 3.5 mm posterior to Bregma) and cerebellum (at the midline and 1.5 mm posterior to lambda). For EMG recordings, two wires with suture patches were inserted in the tissue between the skin and the neck muscle.

The animals were connected to the recording system via a flexible cable and counterbalanced swivel system, and the animals were acclimated to the setup in continuous darkness for at least one week prior to the start of the recording. The animals' behavioral activity (drinking and locomotion) was recorded continuously in order to obtain an estimate of the circadian rhythm.

Neuronal activity in the hypothalamic structures was amplified approximately 40,000 ×, band-pass filtered (500–5000 Hz, − 40 dB/decade), and processed further offline. Online, a window discriminator converted the action potentials into electronic pulses. A second window discriminator was set at a higher level to detect artifacts caused by the animal's movements. Action potentials and movement-related artifacts were counted in 10-s epochs. The analog EEG and EMG signals, which were recorded continuously, were amplified approximately 2000 ×, band-pass filtered (0.5–30 Hz, − 40 dB/decade), and digitized at 100 Hz. All data were recorded simultaneously and stored on a computer hard disk. The stability of the multi-unit signal and EEG recording was evaluated daily by visually inspecting the signal using an oscilloscope; the circadian rhythm in the signal and the amplitude of the EEG were monitored for 7 days before the baseline data were collected. After the experiments, the animals were sacrificed in order to verify the recording sites. To mark the location of the electrode tip, current was passed through the electrode, and the brain was perfused with a buffered solution containing 4% paraformaldehyde and 8% potassium ferrocyanide.

The brains were removed, post-fixed overnight in 4% paraformaldehyde, and cryo-protected in 30% sucrose solution. Free-floating coronal sections (40 μm thickness) were cut on a freezing microtome. The sections were stained with cresyl violet, mounted on gelatinized slides, dried, dehydrated in increasing gradients of ethanol, cleared in toluene, and cover-slipped with Depex. Of the 63 animals used, the recording electrodes were positioned in the correct location as follows: 20 in the LH, 7 in the PVN, 8 in the ARC, and 7 in the MB (2 in the medial mammillary nucleus, 1 in the lateral mammillary nucleus, and 4 in the pre-mammillary nucleus).

Offline, the EEG power density spectra were calculated in 10-s epochs corresponding to the 10-s epochs of the action potentials of the targeted hypothalamic structure using a fast Fourier transform (FFT) routine within the frequency range of 0.25–25.0 Hz in 0.1 − Hz bins. EMG signals were integrated over 10-s epochs. Three vigilance states—wakefulness, NREM sleep, and REM sleep—were determined visually based on standardized EEG/EMG criteria for rodents (Deboer et al., 2003, 2007; Stenvers et al., 2016). Wakefulness was scored when the EMG showed an irregular, high-amplitude pattern and the EEG signal was low in amplitude with relatively high activity in the theta band (6–9 Hz). NREM sleep was scored when EMG amplitude was low and the EEG amplitude was higher than during wakefulness, with high values in the slow wave range (1–4 Hz). REM sleep was scored when the amplitude of the EMG and EEG were low and the EEG showed relatively high values in the theta range. Epochs containing artifacts in the SCN electrical signal or in the EEG signal (observed during the scoring of the vigilance states) were excluded from our analysis of the neuronal activity and EEG spectral analysis.

All MUA data and EEG power density data were calculated relative to the respective mean values recorded during NREM sleep over a 24-hour period.

2.3. Sleep deprivation

A previously validated method using an enriched, novel environment (Gompf et al., 2010; Zhang et al., 2014) was used to stimulate spontaneous exploratory wakefulness without inducing stress. SD was performed during the first 6 h in the subjective day. The experiments were performed in continuous darkness under constant temperature and humidity conditions. Clean bedding, food, water, climbing toys, and novel nesting materials were used as stimuli to stimulate wakefulness. During the 6-hour SD episode, the animals were monitored via their online EEG signal. Whenever the animals appeared to be entering NREM sleep—or if an increase in slow wave amplitude was observed—new toys were introduced to the cage of the animal.

2.4. Statistical analysis

Data were analyzed using SigmaStat version 12.0. All summary data are reported as the mean ± S.E.M. Statistical significance was determined using a repeated-measures ANOVA, with time, neuronal activity, sleep state, and power density considered as repeated measures coupled to Dunnett post hoc analysis in the case of significance (Fig. 1A, D, G, J; Fig. 2; Fig. 3A, D, G, J; Fig. 4A, C, E, G; Fig. 5), paired Student's *t*-test (Fig. 1B, E, H, K; Fig. 3B, E, H, K), one-way ANOVA (Fig. 1C, F, I, L; Table S1; Fig. 3C, F, I, L; Fig. 4B, D, F, H) or simple linear regression (Figs. 6, 7; Fig. S2, Fig. S3). *p*-values are indicated in the text and the figure legends. Differences were considered significant when $p < 0.05$.

3. Results

To avoid any possible confounding effects of light and/or post-operative stress, the recordings were performed in continuous darkness at least one week following surgery. During the baseline 24 h, all four structures showed clear circadian rhythmicity, with high neuronal activity during the subjective night and low activity during the subjective day (Fig. 1A, D, G, J). After this initial 24-hour baseline period, the animals were gently sleep-deprived for 6 h and then recorded for an additional 42 h. During SD, all four structures had a sustained increase in neuronal firing (Fig. 1B, E, H, K). In contrast, after SD, each structure had a unique response. In the LH, a sustained decrease in activity was observed for 18 h (Fig. 1A) leading to an average 6.6% reduction in total MUA relative to baseline (Fig. 1C). After that, the normal circadian pattern recovered (Fig. 1A) with no significant alterations in the 2nd