Sleep EEG Changes After Middle Cerebral Artery Infarcts in Mice: Different Effects of Striatal and Cortical Lesions

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**Study Objectives:** Hemispheric stroke in humans is associated with sleep-wake disturbances and sleep electroencephalogram (EEG) changes. The correlation between these changes and stroke extent remains unclear. In the absence of experimental data, we assessed sleep EEG changes post 7 days of focal cerebral ischemia of different extensions in mice.

**Design:** Following electrode implantation and baseline sleep-wake EEG recordings, mice were submitted to sham surgery (control group), 30 minutes of intraluminal middle cerebral artery (MCA) occlusion (striatal stroke), or distal MCA electrocogulation (cortical stroke). One and 12 days after stroke, sleep-wake EEG recordings were repeated. The EEG recorded from the healthy hemisphere was analyzed visually and automatically (fast Fourier analysis) according to established criteria.

**Measurements and Results:** Striatal stroke induced an increase in non-rapid eye movement (NREM) sleep and a reduction of rapid eye movement sleep. These changes were detectable both during the light and the dark phase at day 1 and persisted until day 12 after stroke. Cortical stroke induced a less-marked increase in NREM sleep, which was present only at day 1 and during the dark phase. In cortical stroke, the increase in NREM sleep was associated in the wake EEG power spectra, with an increase in the theta and a reduction in the beta activity.

**Conclusion:** Cortical and striatal stroke lead to different sleep-wake EEG changes in mice, which probably reflect variable effects on sleep-promoting and wakefulness-maintaining neuronal networks.

**Keywords:** Stroke, sleep, EEG, wakefulness, cortex, striatum

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**INTRODUCTION**

SLEEP-WAKE DISTURBANCES (SWD) ARE FREQUENTLY OBSERVED IN ABOUT 20% TO 40% OF STROKE VICTIMS.\(^1\) 3 SWD CORRESPOND TO A VARIETY OF SYMPTOMS, including insomnia, hypersomnia (increased sleep need), excessive daytime sleepiness (EDS), and fatigue, which may persist over months to years. The presence of poststroke SWD has been linked with poorer functional outcome after stroke.\(^3\)^\(^4\)

Sleep electroencephalographic (EEG) changes are also frequent after stroke. In supratentorial strokes, a reduction of sleep efficiency and rapid eye movement (REM) sleep is most often observed.\(^5\)^\(^7\) Both an increase and a decrease in deep (slow-wave) non-REM (NREM) sleep have also been reported.\(^8\)^\(^10\) Finally, sleep-spindle activity can be reduced ipsilaterally, contralaterally, and bilaterally to thalamic and extrathalamic hemispheric lesions.\(^5\)^\(^7\)^\(^10\)^\(^11\) Some of these sleep EEG changes correlate with stroke extension and stroke outcome.\(^5\)^\(^11\) Similarly, sleep EEG changes have been also shown to be linked with outcome, including neuropsychologic functions, after traumatic brain injury.\(^12\)

The association of SWD with poor functional outcome after stroke is currently unexplained but may—at least in part—be explained by a role of sleep in synaptic plasticity. Studies in humans and in animals suggest that sleep loss may impair learning and memory processes, which in turn are largely dependent on synaptic plasticity.\(^13\)^\(^14\) Sleep also induces an increase in overall brain protein synthesis,\(^15\)^\(^16\) which may influence synaptic remodeling, and is associated with changes in expression of genes implicated in synaptic remodeling and protein synthesis.\(^17\) Finally, sleep may also play a role in recovery by reducing all kinds of neurotoxic activities (glutamate, nitric oxide, detoxification, etc.) and overall metabolism demand.

To test the hypothesis of a direct link between sleep and stroke recovery, one could assess the effects of sleep manipulations on outcome in animal models of stroke. In order to perform such a study, knowledge about the effects of stroke on sleep EEG is necessary. Surprisingly, in a literature search (Medline; key words included sleep, NREM, REM, stroke, ischemia, mice, rats, and rodents), we could not find any study dealing with this question. We therefore decided to assess the effect of cortical and striatal ischemia on sleep-wake EEG in a mouse model of hemispheric stroke.

**METHODS**

**Animals and Experimental Protocol**

All experiments were conducted with governmental approval according to local guidelines for the care and use of laboratory animals. Twelve male C57BL/6J mice, aged 12 weeks (21-27 g), were used. Animals were kept under 12-hour light/12-hour dark cycle (lights on at 8 AM).

**Implantation of EEG and Electromyogram Electrodes and Baseline Sleep Recordings**

Under anesthesia with ketamine (5% (Ketasol-50®): 5 μL/g) / xylazine (2% (Rompun®): 3.3 μL/g), 4 EEG and 2 electromyogram (EMG) electrodes were implanted. Gold-plated miniature screws served as EEG electrodes and were bilaterally inserted in
the skull 2 mm lateral to midline/2 mm posterior to bregma and 2 mm lateral to midline/2 mm anterior to lambda (corresponding to frontal and parietal cortical lobes) and secured with dental cement. Gold wires served as EMG electrodes and were inserted into the neck muscles. After surgery, mice were allowed 11 days of recovery before being connected to pivotable recording leads for adaptation. A 24-hour baseline EEG/EMG (12 hours light, 12 hours dark) was recorded in all mice. EEG and EMG signals were amplified, digitized, sampled at 256 Hz, digitally filtered, and stored at 128 Hz on optical disks for later analysis. Only data from animals with good signal-to-noise ratio in the EEG and EMG were used for further experiments.

Induction of Focal Cerebral Ischemia

The day after the baseline recordings, mice were attributed to the following groups (n = 4 animals/group): (1) sham surgery, (2) intraluminal middle cerebral artery (MCA) occlusion, and (3) transcranial coagulation of a peripheral MCA branch. Animals were anesthetized with ketamine (5% [Ketasol-50®]: 5 μL/g) / xylazine (2% [Rompun®]: 3.3 μL/g). Rectal temperature was maintained between 36.5°C and 37.0°C using a feedback-controlled heating system. Intraluminal MCA occlusion was induced as previously described, using an 8-0, silicon-coated, (Xantopren: Bayer Dental, Osaka, Japan) nylon monofilament (Ethicon, Norderstedt, Germany) that was introduced into the common carotid artery through a small arteriotomy and advanced through the internal carotid artery up to the bifurcation at which the MCA goes off. MCA blood flow was thereby occluded. Thirty minutes later, the monofilament was retracted and reperfusion was reestablished. Transcortical MCA electrocoagulation was performed as previously described by Wiessner et al. A peripheral MCA branch was coagulated under the operation microscope through a small opening of the skull. Control animals not undergoing MCA occlusion were anesthetized identically to ischemic mice. An incision was made at the animal’s neck, and the common carotid artery was isolated but not cut. Animals were placed back into their cages once they have recovered from anesthesia. The 3 groups (intraluminal MCA occlusion, transcranial MCA coagulation, sham surgery) were examined successively.

Neurologic Assessments

On days 1 and 12 after the stroke, the animals’ motor behavior was carefully evaluated using a 5-point scale ranging from 0 = normal function to 4 = absence of spontaneous motor activity.

Poststroke Sleep Recordings

One day (acute phase) and 12 days (subacute phase) after the stroke, bilateral 24-hour EEG/EMG recordings were performed. After the last EEG/EMG recording, animals were reanesthetized and decapitated, and the brains were removed and stored at -80°C.

Histology

Brains were cut on a cryostat into coronal 18-μm sections. For documentation of brain infarcts, sections 2 mm apart were stained with cresyl violet. On these sections, brain lesions were outlined. In animals subjected to 30 minutes of MCA occlusion, densities of viable neurons were determined in the striatum ipsilateral and contralateral to the lesion and expressed as a percentage of surviving cells (for details see Hermann et al). For distal MCA occlusion, infarct volumetric analyses were performed in an additional subgroup of 4 animals, which were subjected to distal MCA occlusion and sacrificed 3 days after the stroke event. Because of volume shifts, precise infarct measurements cannot be performed reliably in the subacute stroke stage, particularly when brain lesions are small.

Sleep EEG

After amplification and filtering the EEG/EMG signals, EEG power spectra were computed for consecutive 4-second epochs in the hemisphere ipsilateral and contralateral to the stroke by a fast Fourier transform routine. EEG spectra between 0.5 and 25 Hz were analyzed. For each epoch, NREM sleep (low-EMG and high-EEG amplitude, high delta = 0.75-4.0 Hz activity) and REM sleep (low-EMG and low-EEG amplitude, high theta = 6.25-9.0 Hz activity) were scored visually. Vigilance states were expressed as a percentage of artifact-free recording time.

Statistics

All values in the text are given as means ± SEM. Differences in sleep stages and spectral EEG activities among baseline, acute, and subacute stroke stages were compared by analysis of variance for repeated measures. Significance levels were set at ≤ 5%.

RESULTS

Sham Controls

Histology and Motor Behavior

Animals subjected to sham surgery did not reveal any brain lesions and did not show any motor deficits.

Sleep EEG

Sleep EEG at baseline and after sham surgery are shown in Figure 2. All mice exhibited a marked diurnal preference for sleep. During the light period at baseline, mice slept for approximately 63% of the recorded 12 hours (54.3% ± 3.3% NREM sleep, 8.8% ± 0.8% REM sleep) and, during the dark period, approximately 35% of the time was spent asleep (31.1% ± 4.7% NREM sleep, 4.1% ± 0.7% REM sleep). Sham surgery did not influence the sleep EEG. Spectral EEG powers did not reveal any differences between baseline recordings and recordings performed after sham surgery during total sleep time, NREM, or REM sleep.

Striatal Stroke (Intraluminal MCA Occlusion)

Histology

The localization of brain lesions after intraluminal MCA occlusion is shown in Figure 1. As in previous studies from our group (e.g., see Wang et al), neuronal injury was reproducibly found in the striatum of animals subjected to 30 minutes of intraluminal MCA occlusion. Only a small percentage of neurons survived in this region, as shown by histologic analysis (13.37 ± 3.03 cells/25000 μm²). In none of the animals, histologic injury was observed in the cerebral cortex.
Motor Behavior

Animals with intraluminal MCA occlusion exhibited normal motor activity. There were no neurologic abnormalities in these mice, particularly no rotation behavior when animals were lifted by their tails (neurological score = 0).

Sleep EEG

One day after intraluminal MCA occlusion, total sleep time in the dark period was significantly increased (47% ± 9% vs 34% ± 6 % at baseline, p = .04). In the light period and 12 days after stroke, differences in total sleep time were not changed compared with baseline. NREM sleep was significantly increased and REM sleep was significantly decreased, both in the light and the dark periods (p < .05; see Figure 2). Amounts of NREM sleep and REM sleep partially recovered 12 days after stroke; during the dark period, sleep EEG had normalized, whereas during the light period, NREM and REM sleep changes were still significant (p < .05).

Sleep EEG Power Spectra

Initially there was a moderate increase in EEG power in the theta (5-7 Hz) range and a decrease in EEG power in the frequency activity above 9 Hz (Figure 3). Changes were seen both in the light and dark phases, but did not reach the statistical significance level. Changes in sleep EEG power spectra were noted mainly during NREM sleep. Sleep EEG changes disappeared 12 days after stroke in the light period. In the dark period, theta power (mainly 3-7 Hz) decreased below baseline, whereas frequency activity above 9 Hz increased above baseline.

Over the hemisphere ipsilateral to the ischemic lesion, there were frequent artifacts, which were excluded from analysis. This may account for larger standard deviations in the power-spectra findings of the lesion hemisphere (Figure 4). Comparisons between healthy and lesion hemispheres did not reveal any significant differences in mean power spectra, regardless of phase of the day (dark, light) or stage after stroke (acute, subacute).

Cortical Stroke (Distal MCA Occlusion)

Histology

The localization of brain infarcts after distal MCA coagulation is shown in Figure 1. MCA occlusion resulted in localized cortical infarcts, which measured 11.67 ± 4.3 mm³. The striatum did not reveal any brain injury.

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Motor Behavior

Animals submitted to distal MCA occlusion also showed normal motor activity. There were no behavioral deficits in these mice (neurologic score = 0).

Sleep EEG Changes

One day after cortical stroke, total sleep time was significantly increased in the dark period (49% ± 7% vs 37% ± 7% at baseline, p = .05). In the light period and 12 days after stroke, total sleep time did not differ compared with baseline. One day after transcortical MCA occlusion, the amounts of NREM sleep were increased. In contrast to striatal strokes, the elevation of NREM sleep was noted only during the dark period (p < .05; Figure 2). These sleep EEG changes completely recovered 12 days after stroke.

Sleep EEG Power Spectra

Initially there was a significant increase in theta (6-9 Hz) and decrease in beta (13.5-18.5 Hz) powers both during the light and dark periods (Figure 3). There were no significant changes in REM sleep. Sleep EEG power spectra almost completely normalized 12 days after stroke.

DISCUSSION

A number of aspects limit studies on sleep EEG in patients with stroke. First, strokes in humans are heterogeneous in terms of size and localization. Second, environmental factors (e.g., noise and light in intensive care units), as well as drug intake and infections, can affect sleep quality. The importance of these factors is proven by the high frequency of sleep disorders among patients in intensive care units who do not have brain damage. Finally, prestroke baseline data are usually unknown. These factors probably account for differences in results of sleep EEG studies in stroke victims, even in those in whom the analysis is restricted to supratentorial hemispheric stroke. In view of these limitations, animal studies are more suitable to assess the effects of stroke on...
The sleep-wake EEG changes after stroke found in our study in mice are at least in part comparable with human data. A reduction of REM sleep and both an increase and a reduction of NREM sleep have, in fact, been reported after hemispheric strokes in humans (see introduction). Changes in sleep-spindle activity, not uncommon in humans with supratentorial strokes (see introduction), cannot be assessed, however, with certainty in mice.

Of particular interest is the (new) observation made in this study of profound sleep-wake EEG changes after striatal brain damage. There is, in fact, evidence that the striatum plays a fundamental role in sleep-wake regulation. The basal ganglia have projections to the ascending reticular activating system. Activity of neurons in the striatum varies throughout the sleep–wake cycle and release of acetylcholine in the basal ganglia is significantly decreased during NREM sleep. Furthermore, complete removal of neocortex and striatum leads to significant changes in sleep behavior in cats. Finally, in a human positron emission tomography study, NREM sleep was associated with a significant reduction in cerebral blood flow throughout the striatum when compared with presleep wakefulness.

The significance of the observed sleep-wake EEG changes in rodent stroke recovery is unclear at this point. The increase in NREM sleep could fit with the theory that sleep may be linked with enhance plasticity effects after brain damage. Recording of spontaneous cortical activity before and after hemispheric lesions in rats has revealed a strong correlation between axonal sprouting and synchronization of neuronal activity. On the other hand, short-term plasticity processes are thought to occur during slow-wave sleep. Studies assessing the effect of sleep-modulating therapies after stroke are needed to test the hypothesis of a causal link between sleep-wake changes and neuronal plasticity occurring after focal brain damage.

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