Published in final edited form as:

Exp Neurol. 2011 December ; 232(2): 168–175. doi:10.1016/j.expneurol.2011.08.020.

Ischemic stroke selectively inhibits REM sleep of rats

Samreen Ahmed^{1,+}, He Meng^{2,+}, Tiecheng Liu¹, Blair Sutton¹, Mark R. Opp^{1,3}, Jimo Borjigin^{1,2,*}, and Michael M. Wang^{1,2,4,*}

¹Department of Molecular and Integrative Physiology, University of Michigan, Ann Arbor, MI 48109-5622

²Department of Neurology, University of Michigan, Ann Arbor, MI 48109-5622

³Department of Anesthesiology, University of Michigan, Ann Arbor, MI 48109-5622

⁴Veterans Administration, Ann Arbor, MI 48105

Abstract

Sleep disorders are important risk factors for stroke; conversely, stroke patients suffer from sleep disturbances including disruptions of non-rapid eye movement (NREM) and rapid eye movement (REM) sleep and a decrease in total sleep. This study was performed to characterize the effect of stroke on sleep architecture of rats using continuous electroencephalography (EEG) and activity monitoring. Rats were implanted with transmitters which enabled continuous real time recording of EEG, electromyography (EMG), and locomotor activity. Baseline recordings were performed prior to induction of either transient middle cerebral artery (MCA) occlusion or sham surgery. Sleep recordings were obtained for 60 hours after surgery to identify periods of wakefulness, NREM, and REM sleep before and after stroke. Spectral analysis was performed to assess the effects of stroke on state-dependent EEG. Finally, we quantified the time in wake, NREM, and REM sleep before and after stroke. Delta power, a measure of NREM sleep depth, was increased the day following stroke. At the same time, there was a significant shift in theta rhythms to a lower frequency during REM and wake periods. The awake EEG slowed after stroke over both hemispheres. The EEG of the ischemic hemisphere demonstrated diminished theta power specific to REM in excess of the slowing seen over the contralateral hemisphere. In contrast to rats exposed to sham surgery which had slightly increased total sleep, rats undergoing stroke experienced decreased total sleep. The decrease in total sleep after stroke was the result of dramatic reduction in the amount of REM sleep after ischemia. The suppression of REM after stroke was due to a decrease in the number of REM bouts; the length of the average REM bout did not change. We conclude that after stroke in this experimental model, REM sleep of rats is specifically and profoundly suppressed. Further experiments using this experimental model should be performed to investigate the mechanisms and consequences of REM suppression after stroke.

Keywords

stroke; sleep; animal models; rat; EEG; REM; NREM; ischemia; physiological monitoring

*Corresponding authors: micwang@umich.edu & borjigin@umich.edu.

⁺These individuals made equal contributions to this work

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Introduction

Stroke patients suffer acute sleep-wake disturbances (Leppavuori, et al., 2002, Vock, et al., 2002), which are often overlooked in the face of significant motor, sensory, and cognitive deficits that appear immediately after brain injury. Accordingly, the sleep electroencephalograph (EEG) of stroke patients is frequently abnormal, and rapid eye movement (REM) sleep is reduced after cortical stroke (Bassetti and Aldrich, 2001, Giubilei, et al., 1992, Hachinski, et al., 1990, Korner, et al., 1986, Muller, et al., 2002). Non-REM (NREM) sleep is variably affected by stroke (Bassetti, et al., 1996, Bassetti and Aldrich, 2001, Korner, et al., 1986, Muller, et al., 2002). These changes in sleep EEG have been seen both acutely (within six hours) and chronically (more than six months) after stroke. Sleep spindle activity, which is a marker of normal sleep architecture, is also suppressed after stroke (Gottselig, et al., 2002). Notably, studies have demonstrated that sleep EEG abnormalities portend poor outcome (Bassetti and Aldrich, 2001, Giubilei, et al., 1992, Muller, et al., 2002, Vock, et al., 2002). An improved understanding of the extent, mechanisms, and reversibility of sleep disturbances after stroke may thus improve patient outcomes.

A full understanding of sleep deficits after stroke requires an integrated approach using both clinical and animal model data; the latter are critical since there are inherent complications to studying the effect of stroke on sleep of human patients. For example, studies demonstrating that sleep disturbances are risk factors for stroke (Munoz, et al., 2006, Yaggi, et al., 2005) and that baseline sleep habits modulate stroke risk (Qureshi, et al., 1997) demonstrate the challenges of proving whether sleep abnormalities discovered after stroke are causative preconditions, or, alternatively, the pathophysiological results of brain injury. In addition, infarcts in patients invariably differ in size and location, which introduces significant variability in study populations. Finally, medications and routine hospital care interfere with sleep and thus complicate human studies; the most ill patients who require intensive care cannot be studied in the acute phase of their illness. On the other hand, studying animal models enables determination of pre-stroke baseline sleep profiles to definitively determine how stroke changes sleep physiology, which is not possible in human studies. Moreover, experimental models allow the induction of controlled infarcts under standardized conditions without interference from medications and variations in the environment.

Two previous animal studies have investigated the relationship between brain ischemia and sleep. In 1975, Sainio and Putkonen (Sainio and Putkonen, 1975) subjected rabbits to global cerebral ischemic and found suppressed NREM and REM sleep 48 hours after brain injury. More recently, Baumann and colleagues (Baumann, et al., 2006) induced focal cerebral ischemia in mice and reported increased NREM after subcortical stroke and a modest suppression in REM sleep after cortical stroke. This study did not examine the effect of combined cortical/subcortical cerebral ischemia which has been associated with sleep disruption in humans. Further, the effect size was modest, limiting the potential for further exploration of mechanism and treatment. Rats are a well-established model for both stroke and sleep research; we therefore investigated the effects of hemispheric focal cerebral ischemia on sleep of rats. We now report that cerebral ischemia induces profound and reproducible changes in sleep, including a dramatic suppression of REM sleep.

Materials and Methods

Transient MCA occlusion model

Protocols were approved by the University of Michigan Animal Care and Use Committee. We studied 18 adult male Sprague-Dawley rats (8 stroke and 10 sham) (400g), purchased

from Harlan. Rats were housed at 21°C under light:dark (L:D) 12:12h conditions, with lights on at 06:00, for at least 2 weeks prior to surgery. Illumination levels were controlled at 400 lux at cage level during the light period. Rats were allowed ad libitum food and water.

At the beginning of the study, animals were implanted with 4ET (DSI) physiological monitors as recommended by the manufacturer. Physiological parameters were recorded for a total of 2–4 weeks prior to induction of stroke or sham surgery. The stroke protocol has been described before (Memezawa, et al., 1992, Meng, et al., 2008). Briefly, animals were subjected to ischemic stroke by filament occlusion of the MCA after a period of baseline physiological monitoring; all surgeries took place between 09:30 and 15:30 during the animals' light cycle. We occluded the right MCA under inhalant anesthesia using a flamed 4–0 intraluminal filament (total anesthesia time was 30 minutes). Animals were allowed to wake up; after 90 minutes, animals were reanesthetized for 10 minutes to remove the filament and allow reperfusion. Animals were returned to monitoring cages after surgery and the EEG and locomotor activity recording was then resumed for a minimum of 60 hours. At the end of the recording period, animals were euthanized and brains removed and cut into 2 mm thick sections which were fixed, paraffin embedded, and finally analyzed by hematoxylin and eosin staining to quantify infarct volumes. Infarct volumes were determined by analysis of imaged brains using NIH Image software. Sham surgery was performed by inserting the filament for 90 minutes into the right carotid artery without advancing the suture to the MCA bifurcation.

Sleep analysis

Continuous recording of ECG, EEG, EMG, body temperature, and locomotor activity data, collected at a sampling rate of 100 Hz, was performed using Dataquest ART software (DSI, Minnesota, USA). Prior to each experimental procedure, baseline data were recorded for at least 3 consecutive days for each rat. Six additional rats were used to determine patterns of sleep during undisturbed baseline periods (not shown) but were not included in stroke studies. For each rat, sleep recordings were manually scored using standard criteria utilizing both the EEG and EMG. Briefly, wake periods were characterized by fast, low voltage unsynchronized EEGs associated with high voltage EMG and cage activity. NREM sleep was defined by high voltage slow activity in the delta range (0.5–4 Hz) associated with low voltage EMG and lack of activity. REM sleep was defined on the basis of low voltage unsynchronized EEGs in the theta range (6–9 Hz) associated with very low voltage EMG and absence of activity. Data recordings could not be obtained while rats were undergoing experimental manipulation; EEG recordings that were deemed indeterminate immediately following experimental manipulation were marked as artifact and removed from data analysis.

Spectral analysis

The EEG was subjected to fast Fourier transformation (FFT) to generate absolute EEG power spectra for each animal and each sleep stage. FFT was applied to 10 second epochs of EEG. Hourly averaged spectra from artifact-free epochs were then computed for NREM, REM, and wake. Power spectra between 0.5 and 20 Hz in 0.5 Hz frequency bins were generated. To account for inter-individual EEG variations, the power spectrum was subjected to a normalization procedure that has been previously described (Baracchi, et al., 2008). Briefly, the power in each frequency bin was expressed as the percentage of total power for each sleep state within the 12 hour light and 12 hour dark period. For some presentations of the data, delta power during NREM sleep was also computed for each hour. Mean power values for the delta (0.5–4.5 Hz) range for every 10 second artifact-free epoch scored as NREM was calculated.

Baseline sleep EEG

Baseline sleep data prior to stroke was obtained for all animals in the stroke study and six additional animals that were not subjected to stroke, which showed that there is minimal intra-individual change in total, NREM, and REM sleep (not shown). On the other hand, these data show that there is significant variation between individuals in sleep; in addition, the relative amounts of NREM and REM sleep were widely dispersed in this group of animals. The contrast between the small intra-individual differences and larger inter-individual differences in sleep confirmed that the most meaningful method to determine the effect of stroke on sleep would be to measure changes in sleep within a single individual before and after stroke.

Statistical analysis

ANOVA was used to analyze group differences in regards to sleep architecture and EEG spectral analysis. Comparisons were made between stroke and sham treated animals, left and right brain EEG, and baseline vs. post-procedure physiological parameters. Differences with a $p < 0.05$ were considered statistically significant.

Results

Post-stroke EEG characteristics

After stroke, the wake EEG over the ischemic (right) hemisphere was attenuated compared to the contralateral hemisphere (Figure 1), as has been described in rats after stroke by Aronowski and colleagues (Kelly, et al., 2006). Sleep EEG during REM and NREM were qualitatively similar to the EEG prior to stroke. The contralateral hemisphere, displayed an EEG pattern during periods of low motor activity (assessed by low EMG activity) that was visually similar to REM and NREM patterns that were measured prior to stroke in both hemispheres.

To more precisely characterize stroke-induced changes, we performed state-specific spectral analysis of the EEG and compared characteristics of the baseline record to the EEG captured over the first 48 hours after stroke (Figure 2). First, we analyzed spectral properties of the NREM, REM and wake EEG over the non-ischemic cortex during three periods: the dark period immediately after stroke, the light period after stroke, and the second 12 hour dark period after stroke. To negate potential differences in lead placement and inter-individual variation, we derived spectra representing differences from the pre-stroke and post-stroke EEG (ie. we compared the day preceding stroke to the days after the stroke).

The non-ischemic NREM EEG showed significant increases in delta power for the first 48 hours after stroke but not sham surgery. The REM EEG over the left side showed slowing throughout the 48 hours post-stroke period, represented by a bimodal difference spectrum (higher at lower theta and lower in the higher theta range).

During waking, left (non-ischemic) EEG spectral changes were significant in both the delta and theta ranges (Figure 2, bottom row). The wake EEG after stroke showed increased delta power, which was significant on the first light period after stroke relative to sham surgery controls. In addition, the theta power was significantly decreased on the first light and second dark period after stroke. In general, these results demonstrate quantitative shifts in the EEG to a slower pattern during all states after stroke; we infer that cerebral ischemia causes an indirect slowing of the wake and sleep EEG in the non-ischemic hemisphere.

Figure 3 shows hour-by-hour analysis of NREM delta power in stroke and sham animals, normalized to pre-surgery values during the same time periods. Increased delta power after

stroke was most sustained (relative to sham controls) in the first dark period after surgery. NREM delta power was not significantly different in stroke animals in the initial few hours of the first light period after stroke, but again increased significantly at the end of the first light period after stroke (Figure 3A). NREM delta power in all animals prior to surgery showed the expected circadian variation with the lowest values in the latter half of the light period and a buildup towards the start of the light period (Figure 3B).

Next, to probe the effect of stroke on the ischemic hemisphere, we compared spectral changes between the right and the left EEGs after stroke. Spectral difference profiles, presented in Figure 4, were derived using a two-step process: we first determined the baseline differences in power spectra for the right hemisphere in response to stroke (not shown), using the states defined by analysis of the left EEG (not shown). Second, the left difference spectra (Figure 2) were subtracted from the right difference spectra to identify the significant changes in the EEG of the ischemic cortex (Figure 4). This procedure was performed to negate minor baseline right-left EEG differences by subtracting both the right and left baselines. As shown in Figure 4, the major stroke-induced EEG difference between the ischemic (right) and non-ischemic (left) cortex was the enhanced suppression of theta power during REM and a delayed increase in delta power during REM during the second dark period. Delta power was increased in excess in the ischemic hemisphere at all times during NREM sleep, but this did not reach statistical significance. However, during the first light period after stroke, there was a marked increase in delta power of the wake EEG on the right, when compared to the left (non-ischemic) side. Our results suggest that the major effect of stroke in the ischemic hemisphere is exaggerated REM EEG slowing, in excess of the slowing described in the hemisphere contralateral to the stroke.

Amounts of post-stroke sleep

To determine whether the length of sleep was perturbed by stroke, we computed total, NREM, and REM sleep time (Figure 5) over the three days prior to stroke and three days after. Duration of time spent in sleep stages during the light period and dark period sleep were analyzed separately. The longitudinal records for animals undergoing stroke (time shown on the x-axis) demonstrated quantitative changes in sleep time that began immediately after stroke.

Figure 5 shows a comparison between sleep times before and after either stroke or sham surgery. We noted a modest but significant decrease (relative to pre-stroke baseline) in total sleep for the first two daytime periods after stroke. In contrast, in sham animals, there was an increase in total sleep the first and second nights, with decreased sleep observed only during the first daytime period. There was no change in NREM sleep after stroke, but sham animals had increased NREM sleep time for the first two nights. The effects of sham surgery on sleep were likely due to anesthesia, which induced a similar pattern of sleep changes (not shown).

Most remarkably, REM sleep time was dramatically reduced during both the day and the night for three continuous days after stroke; in marked contrast, there was no significant change in REM after sham surgery except on the first day after surgery. Sham surgery, but not stroke, lowered the day to night ratio of REM (Figure 5B).

Sleep bouts and sleep power after stroke

The length and number of total sleep and NREM sleep bouts did not differ after animals underwent stroke surgery (not shown). Similarly, the lengths of REM bouts in individual animals did not significantly change (Figure 6A) after stroke or sham surgery. However, the number of REM sleep bouts was significantly reduced after stroke (Figure 6B), indicating

that REM sleep inhibition was driven by reduced number, but not length, of REM sleep bouts.

Discussion

Stroke has been shown to adversely influence sleep in humans, and REM suppression after stroke has been described (Bassetti and Aldrich, 2001, Giubilei, et al., 1992, Hachinski, et al., 1990, Korner, et al., 1986, Muller, et al., 2002). Sleep deficits after stroke have the potential to cause patient distress and may impair recovery. Our ability to rationally devise clinical studies to reverse sleep deficits after stroke could be accelerated using robust animal models. The key finding of our study is that in laboratory rats, the prototype experimental model for the study of stroke and sleep, stroke profoundly and reproducibly suppresses REM sleep.

Due to its larger size, the rat is more amenable to physiological recordings compared to laboratory mice. The ability to precisely record sleep EEGs in longitudinally analyzed individuals provides a powerful approach to define quantitative changes in the EEG after stroke. Our results show stroke-induced slowing of the EEG during all states in both the ischemic and non-ischemic cortex. Spectral analysis further demonstrated that REM slowing after stroke is particularly notable in the ischemic cortex, a finding that suggested that REM sleep might be specifically affected in stroke. The spectral changes in the REM EEG after stroke in the ischemic hemisphere are novel to this animal model. It is likely that similar changes are present in human stroke patients, but that they have not become apparent due to population heterogeneity of the prestroke REM EEG (which are not available in humans).

Our study, compared to the previous study of mice by Baumann and colleagues (Baumann, et al., 2006), demonstrated profound REM suppression. It is difficult to directly compare our results to this previous study, since we utilized a transient ischemia model in rats whereas Baumann et al. used two permanent ischemia models in mice. The infarcts that we induced were more extensive and involve both subcortical and cortical infarction. It is possible that the larger region of infarction and, possibly, reperfusion of the infarct, produced conditions that resulted in more significant and reproducible REM suppression. Our ability to detect strong differences in sleep due to stroke could have also arisen because we compared pre- and post-stroke sleep in the same animals, rather than comparing grouped sleep characteristics which could be attenuated by significant intra-individual variability.

REM sleep bouts are less frequent but of similar length after stroke, suggesting that after stroke, animals fail to transition from NREM to REM sleep; an alternative explanation is that after stroke, NREM sleep could be interrupted before the animal can enter REM. The most commonly discussed model of REM sleep regulation involves two groups of mutually inhibitory pontine neurons which are either REM inducing or REM inhibitory (Jones, 2005, Lu, et al., 2006, McCarley, 2007). Since strokes in these animals strictly affected the forebrain, our results suggest that injury outside of the classical neuronal regulators of sleep could profoundly influence REM onset; in humans, it has been shown that sleep spindles are affected by forebrain stroke that spares the subcortical sleep regulating centers (Gottselig, et al., 2002). Given that a broad literature supports the influence of inflammatory mediators on sleep (Krueger, et al., 2001, Opp, 2005, Opp and Toth, 2003), one potential mechanism for REM inhibition is that humoral factors, such as inflammatory mediators released after stroke, inhibit REM promoting circuits. Indeed, REM sleep is suppressed during pathologies that are characterized by increases in inflammatory mediators such as cytokines (Opp, 2005).

Other alterations in sleep physiology noted in our study could also arise through humoral mechanisms. Analysis of records for 48 hours after the onset of stroke demonstrates that time in NREM sleep is not increased by stroke during this period, but that delta power during NREM sleep, corresponding to sleep depth, is significantly enhanced. Direct injection of cytokines into animals is indeed capable of enhancing NREM delta activity (Yoshida, et al., 2004). It is conceivable that delayed wake EEG slowing (see Figure 2) could be a consequence of a different set of cytokines, since many inflammatory mediators are delayed after the onset of ischemia. Alternatively, delayed cerebral edema, which occurs more than 24 hours after stroke, could participate in later EEG alterations. Clearly, additional experiments will be necessary to determine if stroke-induced alterations in cytokine concentrations in the brain and/or edema are responsible for the physiological disturbances observed in this study.

These studies in rats complement the experimental literature that demonstrates that cortical inputs modulate sleep (Bremer and Terzuolo, 1954, Villablanca, et al., 1976) (Contreras, et al., 1996, Gottselig, et al., 2002). An alternative but not mutually exclusive mechanism of REM inhibition is that stroke causes diffuse injury to cortical neurons that ultimately control REM-regulating neurons. This possibility deserves future experimental attention, but would require modification of the stroke model to selectively target different regions of the rat brain. Analysis of smaller and more selective infarcts in animals could reveal the minimal cortical structures that are required for REM inhibition; the projections of neurons within these structures could then be studied in detail, which is feasible in our experimental model since the neuroanatomy and neurochemistry of sleep has been well-established in rats.

In conclusion, our findings demonstrate that ischemic stroke in rats potentially inhibits REM sleep. The robust and reproducible effect of this model suggests that it may be useful to identify the underlying causes of sleep disturbances in stroke patients and to developing strategies to reverse sleep disturbances which commonly complicate acute stroke care. This model may be applied as a discovery tool to uncover novel pharmacological and environmental manipulations to specifically reverse REM suppression; in patients with acute stroke, reversal of REM deficits could enhance functional recovery and alleviate patient distress from poor quality sleep. It remains to be seen whether prevention of sleep disturbances improves stroke rehabilitation in humans, but given the negative effects of sleep deprivation on human performance, it is a reasonable expectation that modeling long-term sleep disturbances in this rat stroke model may help uncover strategies for improving stroke outcomes by reversing chronic sleep dysfunction.

Acknowledgments

We thank Drs. Helen Baghdoyan, Devin Brown, Ron Chervin, Ralph Lydic, Jack Parent, and Guohua Xi for helpful discussions. Funding was provided by NIH grants NS052681 and NS054724 to MMW and NS057583 to JB. We are also very grateful for the generous support of the Gilmore Fund for Sleep Research and Education.

References

1. Baracchi F, Zamboni G, Cerri M, Del Sindaco E, Dentico D, Jones CA, Luppi M, Perez E, Amici R. Cold exposure impairs dark-pulse capacity to induce REM sleep in the albino rat. *J Sleep Res.* 2008; 17:166–179. [PubMed: 18482105]
2. Bassetti C, Mathis J, Gugger M, Lovblad KO, Hess CW. Hypersomnia following paramedian thalamic stroke: a report of 12 patients. *Ann Neurol.* 1996; 39:471–480. [PubMed: 8619525]
3. Bassetti CL, Aldrich MS. Sleep electroencephalogram changes in acute hemispheric stroke. *Sleep Med.* 2001; 2:185–194. [PubMed: 11311681]

4. Baumann CR, Kilic E, Petit B, Werth E, Hermann DM, Tafti M, Bassetti CL. Sleep EEG changes after middle cerebral artery infarcts in mice: different effects of striatal and cortical lesions. *Sleep*. 2006; 29:1339–1344. [PubMed: 17068988]
5. Bremer F, Terzuolo C. Contribution to the study of the physiologic mechanisms of the maintenance of the waking activity of the brain; interaction of the reticulate formation and of the cerebral cortex in the process of awakening. *Arch Int Physiol*. 1954; 62:157–178. [PubMed: 13171949]
6. Contreras D, Destexhe A, Sejnowski TJ, Steriade M. Control of spatiotemporal coherence of a thalamic oscillation by corticothalamic feedback. *Science*. 1996; 274:771–774. [PubMed: 8864114]
7. Giubilei F, Iannilli M, Vitale A, Pierallini A, Sacchetti ML, Antonini G, Fieschi C. Sleep patterns in acute ischemic stroke. *Acta Neurol Scand*. 1992; 86:567–571. [PubMed: 1481642]
8. Gottselig JM, Bassetti CL, Achermann P. Power and coherence of sleep spindle frequency activity following hemispheric stroke. *Brain*. 2002; 125:373–383. [PubMed: 11844737]
9. Hachinski VC, Mamelak M, Norris JW. Clinical recovery and sleep architecture degradation. *Can J Neurol Sci*. 1990; 17:332–335. [PubMed: 2207892]
10. Jones BE. From waking to sleeping: neuronal and chemical substrates. *Trends Pharmacol Sci*. 2005; 26:578–586. [PubMed: 16183137]
11. Kelly KM, Jukkola PI, Kharlamov EA, Downey KL, McBride JW, Strong R, Aronowski J. Long-term video-EEG recordings following transient unilateral middle cerebral and common carotid artery occlusion in Long-Evans rats. *Exp Neurol*. 2006; 201:495–506. [PubMed: 16814774]
12. Korner E, Flooh E, Reinhart B, Wolf R, Ott E, Krenn W, Lechner H. Sleep alterations in ischemic stroke. *Eur Neurol*. 1986; 25(Suppl 2):104–110. [PubMed: 3758112]
13. Krueger JM, Obal FJ, Fang J, Kubota T, Taishi P. The role of cytokines in physiological sleep regulation. *Ann N Y Acad Sci*. 2001; 933:211–221. [PubMed: 12000022]
14. Leppavuori A, Pohjasvaara T, Vataja R, Kaste M, Erkinjuntti T. Insomnia in ischemic stroke patients. *Cerebrovasc Dis*. 2002; 14:90–97. [PubMed: 12187012]
15. Lu J, Sherman D, Devor M, Saper CB. A putative flip-flop switch for control of REM sleep. *Nature*. 2006; 441:589–594. [PubMed: 16688184]
16. McCarley RW. Neurobiology of REM and NREM sleep. *Sleep Med*. 2007; 8:302–330. [PubMed: 17468046]
17. Memezawa H, Smith ML, Siesjo BK. Penumbra tissues salvaged by reperfusion following middle cerebral artery occlusion in rats. *Stroke*. 1992; 23:552–559. [PubMed: 1561688]
18. Meng H, Liu T, Borjigin J, Wang MM. Ischemic stroke destabilizes circadian rhythms. *J Circadian Rhythms*. 2008; 6:9. [PubMed: 18922153]
19. Muller C, Achermann P, Bischof M, Nirkko AC, Roth C, Bassetti CL. Visual and spectral analysis of sleep EEG in acute hemispheric stroke. *Eur Neurol*. 2002; 48:164–171. [PubMed: 12373034]
20. Munoz R, Duran-Cantolla J, Martinez-Vila E, Gallego J, Rubio R, Aizpuru F, De La Torre G. Severe sleep apnea and risk of ischemic stroke in the elderly. *Stroke*. 2006; 37:2317–2321. [PubMed: 16888274]
21. Opp MR. Cytokines and sleep. *Sleep Med Rev*. 2005; 9:355–364. [PubMed: 16102986]
22. Opp MR, Toth LA. Neural-immune interactions in the regulation of sleep. *Front Biosci*. 2003; 8:d768–779. [PubMed: 12700057]
23. Qureshi AI, Giles WH, Croft JB, Bliwise DL. Habitual sleep patterns and risk for stroke and coronary heart disease: a 10-year follow-up from NHANES I. *Neurology*. 1997; 48:904–911. [PubMed: 9109875]
24. Sainio K, Putkonen PT. Sleep-waking cycle in rabbits after cerebral ischemia. *Electroencephalogr Clin Neurophysiol*. 1975; 39:663–666. [PubMed: 53146]
25. Villablanca JR, Marcus RJ, Olmstead CE. Effects of caudate nuclei or frontal cortex ablations in cats. II. Sleep-wakefulness, EEG, and motor activity. *Exp Neurol*. 1976; 53:31–50. [PubMed: 183969]
26. Vock J, Achermann P, Bischof M, Milanova M, Muller C, Nirkko A, Roth C, Bassetti CL. Evolution of sleep and sleep EEG after hemispheric stroke. *J Sleep Res*. 2002; 11:331–338. [PubMed: 12464101]

27. Yaggi HK, Concato J, Kernan WN, Lichtman JH, Brass LM, Mohsenin V. Obstructive sleep apnea as a risk factor for stroke and death. *N Engl J Med.* 2005; 353:2034–2041. [PubMed: 16282178]
28. Yoshida H, Peterfi Z, Garcia-Garcia F, Kirkpatrick R, Yasuda T, Krueger JM. State-specific asymmetries in EEG slow wave activity induced by local application of TNFalpha. *Brain Res.* 2004; 1009:129–136. [PubMed: 15120590]

Highlights

- Sleep disturbances are common in patients with stroke.
- We used animals to determine the causal relationship between stroke and sleep disturbances
- Stroke in rats caused dramatic reduction in REM.
- Reduction in REM was due to decreased REM bouts.
- Causes and treatment of REM deprivation in stroke should be investigated further.

Figure 1.

Sleep EEG recordings on rats after stroke. Stroke was performed on the right side. A representative awake rat's EEG before (A) and after (B) stroke demonstrates attenuated voltage after stroke over the ischemic cortex. NREM and REM sleep showed no obvious changes over the left (non-ischemic) cortex. EMG demonstrates muscle activity specific to the awake phase.

Figure 2.

Spectral analysis of wake and sleep EEG after stroke. This figure depicts only the left cortex, which is contralateral to the stroke. Periods of wake, NREM and REM sleep before and after stroke were identified and then subjected to Fourier analysis. The power spectra for the day prior to surgery was used as a baseline for each individual animal. The baseline was subtracted from power spectra of EEG records performed during the first night after stroke (left column), the first daytime period (center), and second night after stroke (right). The difference spectra were then averaged for all animals. Spectra for sham animals ($n=10$) are presented in the same panels as animals subjected to stroke ($n=8$). Only animals that experienced more than 24 NREM or REM epochs per hour were used of spectral analysis. Since there was substantial suppression of REM after stroke, the numbers of animals analyzed for REM after stroke were lower than for other states ($n=2$ for Day 0 dark and Day 1 light; $n=3$ for Day 1 dark). One of the sham surgery animals was excluded from REM analysis due to insufficient REM data. Significance is denoted (* $p < 0.05$ and ** $p < 0.01$).

Figure 3.

Temporal evolution of post-stroke NREM delta power. (A) Time course of delta power differences induced by stroke or sham surgery was derived by subtracting baseline delta power (integrated delta power spectrum from specified hourly periods of the circadian cycle on the day prior to surgery) from postsurgical NREM delta power at the same times. Daytime and nighttime periods are shown as open and dark bars, respectively; the first hour 18 represents the onset of the first dark period after stroke or sham surgery. NREM delta power was increased at all time points; differences were significant at times noted (* $p < 0.03$). (B) Circadian variation in NREM delta power in animals prior to stroke or sham operations the day before the operation. These values were used to compute enhancement of NREM delta power after surgery in (A).

Figure 4.

Power spectra changes specific to ischemic cortex. A two-step process was used to determine differences in power induced by stroke and specific for the ischemic cortex. First, a similar analysis as in Figure 2 was performed for the right (ischemic) cortex (not shown), resulting in difference spectra that show changes in the ischemic cortex related to stroke. Next, the difference spectra from Figure 2 were subtracted from the difference spectra from generated from the right hemisphere. The resulting analysis is presented according to state (wake, NREM, and REM) at different times after stroke. Significance is denoted (* $p < 0.05$ and ** $p < 0.01$).

Figure 5. Longitudinal analysis of sleep time after stroke. This analysis shows the effect of stroke or sham surgery on total wake and sleep time compared to presurgery baselines. Grouped analysis of time spent in each state after undergoing right MCA occlusion or sham surgery is displayed. (A) The timeline for the experiment is shown. Dark bars represent nighttime. Sleep was analyzed for at least three days prior to stroke, which was performed on day 0 during the daytime (time window represented by bold bar). After stroke or sham surgery, the animal was returned to monitoring cages; the end of surgery always occurred during the daytime (white bar). Sleep recordings were temporarily halted during surgery; thus, values for sleep during the day on day 0 are not shown. (B) Total, NREM, and REM sleep were recorded over days subsequent to stroke during the day and night. The amount of sleep (see methods) is represented by vertical bars (white bars indicate daytime and dark bars indicate nighttime); days are displayed in the x-axis. Baseline state time was defined as the percentage of time in each state for the day or night prior to stroke. Postsurgery times that differed significantly from baseline state time are denoted by stars and specific p values shown.

Figure 6.

Effect of stroke on the number and length of sleep bouts and sleep power. The average length (A) of REM sleep bouts on the day after stroke or control procedure is displayed. (B) The number of REM bouts as a function of bout length (minutes) is shown per 24 hour period. The control values (white boxes) represent daily numbers of REM bouts prior to surgery. The numbers of bouts of REM after stroke were significantly decreased (red boxes; for 0.2-1.0 minute bouts $p < 0.02$; for 1.2-2.0 minute bouts $p < 0.02$); sham surgery did not change the number of REM bouts.