

# REM sleep as predictor of functional outcome after stroke:

## A translational study

**Cover title:** REM sleep and stroke outcome

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## ABSTRACT

**Study Objectives:** Sleep disturbances are common in acute stroke patients and are linked with a negative stroke outcome. However, it is also unclear which and how such changes may be related to stroke outcome. To explore this link, we performed a sleep-EEG study in animals and humans after ischemic stroke.

**Methods:** (1) Animal-study: 12 male rats were assigned to two groups: ischemia (IS) and sham-surgery (Sham). In both groups, sleep architecture was investigated 24h before surgery and for the following 3 days. (2) Human-study: 153 patients with ischemic stroke participating in the the SAS-CARE prospective, multicentre-cohort study had a polysomnography within 9 days after stroke onset. Functional stroke outcome was assessed by the modified Rankin Scale (mRS) at hospital discharge (short-term-outcome) and at a 3 months follow-up (long-term-outcome).

**Results:** (1) Animal-study: Rapid eye movement (REM) sleep was significantly reduced in the IS group compared to the Sham group. (2) Human-study: Patients with poor short-term functional outcome had a reduction of REM sleep and prolonged REM latency during the acute phase of stroke. REM latency was the only sleep-EEG variable found to be significantly related to short and long-term functional impairment in a multiple linear regression analysis.

**Conclusions:** Acute ischemic stroke is followed by a significant reduction of REM sleep in animals and humans. In humans, this reduction was linked with a bad stroke outcome; in addition, REM latency was found to be an independent predictor of stroke evolution. Potential explanations for this role of REM sleep in stroke are discussed.

**Key words:** sleep, stroke, functional outcome, sleep EEG, REM sleep, sleep disordered breathing, animal model

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## Statement and significance

We performed a study in rodents and humans to understand the impact of ischemic stroke on sleep EEG/architecture and its relationship with outcome. This study shows that 1) REM sleep is significantly reduced after stroke in both animals and humans and 2) decreased REM sleep and increased REM latency are associated in humans with poor outcome. The neurobiological explanation and clinical implications of these observations are discussed but remain speculative at this point.

## INTRODUCTION

Sleep is mediated by several mechanisms which are important for brain and body health.<sup>1</sup> Sleep plays a role not only in the maintenance of wakefulness, but also in energy conservation,<sup>2</sup> thermoregulation,<sup>3</sup> memory consolidation,<sup>4,5</sup> neuronal plasticity,<sup>6, 7</sup> tissue restoration and inflammation.<sup>8,9</sup>

For different reasons sleep could have an impact on stroke evolution and outcome. Sleep is associated with a general decrease in metabolism and a reduction in body temperature<sup>10</sup> which both enhance neuroprotection. Furthermore, sleep plays an important role in modulating inflammation and apoptotic processes,<sup>1, 11, 12</sup> both of which affect stroke evolution. Finally, sleep promotes neuroplasticity, which underlies neurorehabilitation effects.

In humans, several studies have shown detrimental effects of sleep disorders on stroke outcome and conversely favorable effects of treatment of sleep disorders.<sup>13, 14</sup> Siengsukon et al. have shown that stroke

patients, but not healthy controls, improve their performance in an implicit and explicit motor learning task following a period of sleep and that the magnitude of improvement is associated with the amount of time spent in REM sleep.<sup>15, 16</sup> Sarasso et al. have shown that acute logopedic interventions after stroke are accompanied by an overnight improvement of speech which parallels an increase in local slow wave activity.<sup>17</sup> Despite the increasing evidence of a role of sleep for stroke outcome, sleep disorders are neglected and underdiagnosed in stroke patients.<sup>18-20</sup>

In animal models of stroke, sleep disruption/loss has similarly been shown to have a negative impact on stroke outcome and post-stroke neuroplasticity processes.<sup>21</sup> Conversely, sleep promotion was found to improve both post-stroke neuroplasticity and outcome.<sup>22, 23</sup>

Only few studies analyzed sleep architecture changes following stroke in both humans and rodents.<sup>24-28</sup> A reduction of sleep efficiency, total sleep time, sleep spindles, sleep stage 2 and SWS was suggested to be associated with a poor stroke outcome.<sup>29-32</sup> However, it remains unclear whether specific sleep stages may specifically modulate stroke evolution in the acute (ischemic cascade), or chronic phase (neuroplasticity) of stroke.

In this study, we aimed to better characterize the impact of ischemic stroke on sleep architecture and whether specific sleep EEG parameters in the acute phase of stroke might predict its outcome. To do so, we chose a translational approach and assessed sleep EEG changes in rodents and humans.

## **MATERIAL AND METHODS**

### **Animal study**

Male Sprague-Dawley rats (n = 12), 9–11 weeks old and weighing  $300 \pm 50$  g at the time of surgery were used in this study. They were housed under 12-h light/dark cycle (light on 08:00–20:00) with ambient temperature at  $22 \pm 0.5$  °C. Food and water were provided ad libitum. All animal procedures were approved by the Animals Research Committee and the Veterinary Office of the Canton of Bern, Switzerland.

In this study we used rats that had undergone middle cerebral artery occlusion (MCAo), using a permanent occlusion of the artery, which creates a lesion located in the somatosensory cortex (Fig. S1D). Although, this ischemia model does not allow the reperfusion of the artery, which normally happens in stroke patients,<sup>33, 34</sup> it is a reproducible model with low mortality.

### Design

Rats were randomly assigned to two experimental groups: (i) ischemia (IS); and (ii) sham-surgery (Sham). Each experimental group comprised 6 animals, which were sacrificed after 3 days following ischemic stroke (Fig. S1A). Ischemic stroke was performed at the beginning of the dark period, which reflects the morning, i.e., the beginning of the active period for rats. This time point of stroke surgery was chosen since most often in humans ischemic stroke occurs in the morning during the first few hours of wakefulness<sup>35</sup> (Fig.S1A). The EEG/EMG were recorded continuously for 24h for a baseline value (12h:12h dark-light cycle) and for the following 3 days after ischemic stroke in all rats (Fig. S1A, B).

### Sleep-wake and circadian EEG assessments

Sleep EEG/architecture was assessed in both IS and Sham groups 24h before either ischemia or sham surgery, and for the following three days after surgery. The EEG/EMG electrodes were implanted as described in our previous study.<sup>36</sup> The EEG/EMG signals were amplified (Grass Instruments, USA) and digitized at a sampling rate of 100Hz and collected on a PC using VitalRecorder (Kissei Comtec Co. Ltd, Japan). EEG signals were filtered at 0.3 Hz (low pass filter) and 0.1 KHz (high pass filter), respectively, whereas EMG, at 1000 Hz. Vigilance states were scored with the SleepSign software (Kissei Comtec Co. Ltd, Japan), per 10 second epoch window, as wakefulness (W), NREM sleep or REM sleep as previously described.<sup>37</sup> The overage amount of the sleep stages (W, NREM, and REM sleep) were averaged over 3-hour periods for the whole 24-h circadian period of baseline and three consecutive days after ischemia/sham surgeries, separately for IS and Sham groups. The percentage of time spent in wakefulness, NREM and REM was determined for each hour. The total number of sleep bouts and their mean duration were measured across the 24h of baseline and for the following three days after ischemic stroke. The behavioral states were scored using the contralateral hemisphere (healthy hemisphere) as previously described.<sup>36</sup> Polysomnographic recordings started immediately after ischemic or sham surgery, although the analysis of the first 30/40

minutes was excluded because unusual spikes due to isoflurane anesthesia (half-time of isoflurane is <5 minutes<sup>38</sup>) were observed in all animals.

### Ischemic stroke surgery

Stroke was induced by the three-vessel occlusion method (3Vo),<sup>39</sup> which predominantly affects the primary somatosensory cortex, avoiding thalamic, hypothalamic, hippocampal, and midbrain damage (Fig. S1D).<sup>33, 34</sup> 3Vo was performed as previously described.<sup>37</sup> Body temperature was maintained between  $36.5 \pm 0.5^{\circ}\text{C}$  with a heating pad. Sham-operated rats were subjected to the same procedure as well as the same time exposure for anesthesia, approximately 90 minutes, except for the occlusion of the MCA and the common carotid artery (CCA). After surgery rats were returned to their cages and EEG/EMG was resumed until the end of the experiment.

### Evaluation of infarct volume

At the end of the experiment, rats were decapitated while deeply anesthetized (Isoflurane 5%) and brains dissected and frozen immediately in dry ice. Coronal sections of 20 $\mu\text{m}$  were cut on a cryostat at six predefined levels (L) with 1 mm interval (L-1: 2.7mm; L-2: 1.7mm; L-3: 0.7mm; L-4: -0.3mm; L-5: -1.3mm and L-6: -2.3mm from bregma)<sup>40, 41</sup> (Fig. S1C). To determine the volume of the lesion, one section from each level was stained with cresyl violet and digitized. The infarct area was measured as previously described.<sup>37</sup>

## **Human study**

### Design

This study is a part of the prospective multicenter study SAS-CARE-1 (Sleep Disordered Breathing in Transient Ischemic Attack (TIA)/Ischemic Stroke and Continuous Positive Airway Pressure (CPAP) Treatment Efficacy (SAS-CARE); NCT01097967). The aims and design of the study have been described in details in a recent publication.<sup>42</sup> In short, the SAS-CARE is a multicenter trial, which includes 35-75 years old patients with a clinical diagnosis of TIA or ischemic stroke that are admitted to a stroke unit within 48 hours from onset of symptoms. Patients with unstable clinical situation (cardio-respiratory or life-threatening

medical conditions), current or past (within the last 3 months before stroke) CPAP treatment, non-ischemic event (intracerebral/subarachnoid hemorrhage), and coma/stupor were excluded. After 90 days patients with significant obstructive sleep disordered breathing (SDB, AHI  $\geq 20$ ) are treated with CPAP if sleepy or, if not sleepy, randomized to CPAP treatment or no CPAP treatment. The protocol of the study was approved by the local ethics committee and informed consent was obtained from all participants. For the current study we considered only patients with ischemic stroke.

Selected sleep parameters (Table 2) and the following variables were considered: age, sex, cardiovascular risk factors (hypertension, diabetes, dyslipidemia, previous cerebrovascular events, smoking – current or within the past 10 years –, atrial fibrillation and coronary artery disease) body mass index (BMI), sleep-related complaints before stroke (self-reported snoring and Epworth Sleepiness Scale - ESS) assessed within the first week after admission, NIHSS on admission, thrombolysis treatment, topography of current stroke (supratentorial or infratentorial) and etiology according to TOAST-study criteria.<sup>43</sup>

### Sleep and sleep EEG assessments

Sleep was recorded by video-polysomnography with EMBLA Titanium System (Titanium, Embla®Flaga, Reykjavik, Iceland) and included 6 channels of electroencephalogram, electromyogram of submental chin and both tibialis anterior muscles, electrooculogram, nasal airflow, abdominal and thoracic efforts, electrocardiogram, and finger pulse oxymetry. Video-polysomnography (video-PSG) was performed within 9 days after stroke for at least 8 hours (from 8-11:00 PM to 6-8:00 AM) in the stroke unit or in the sleep laboratory. Recordings were scored manually according to standard criteria.<sup>42, 44</sup> Hypopnea was defined as a reduction in airflow by  $\geq 30\%$  for at least 10 seconds associated with oxygen desaturation of  $\geq 3\%$ . Sleep parameters such as total sleep time (TST), sleep onset latency (minutes from lights out to first epoch of any sleep stage), REM latency (minutes from sleep onset to first epoch of REM), sleep efficiency (percentage of TST in total recording time), percentages of sleep stages (N1, N2, N3, and REM) from TST, arousal index (number of arousals per hour of sleep), as well as the apnea-hypopnea index (AHI, number of events per hour of sleep), separate indices for obstructive and central apneas, oxygen desaturation index

(ODI, number of desaturations  $\geq 3\%$  divided by TST), and PLM index (PLMI number of periodic leg movements divided by TST) were included.

### Assessment of neurological outcome

Functional outcome was assessed by the modified Rankin Scale (mRS), which is widely used in clinical practice. The scale ranges from 0 (no symptoms) to 6 (death). A score 2 indicates a slight disability (unable to carry out all previous activities, but able to look after own affairs without assistance). A score 3 indicates a moderate disability, with requirement of some help.<sup>45, 46</sup> Accordingly, and supported by previous studies,<sup>13, 47-52</sup> a good outcome was defined by a mRS  $\leq 2$ , and a poor outcome by a mRS  $> 2$ . Both short- (at discharge) and long-term (3 months after stroke) outcome were assessed. Treatment of SDB was started according to the SAS-CARE study protocol (see above) after the assessment of outcome at 3 months was performed. Clinicians assessing functional outcome were blind to stroke data at admission, as well as sleep study results.

## **Statistical analyses**

### Animal study

Gaussian distribution of values was tested with Kolmogorov–Smirnov test. Data were presented as mean  $\pm$  standard error of the mean (SEM). Differences between groups for sleep changes, REM sleep bouts, and duration of sleep bouts at the baseline value and for the following three days after stroke were evaluated by repeated measures ANOVA. Whenever ANOVA statistical significance was achieved, Fisher's Least Significant Difference (LSD) test was performed. GraphPad Prism6 (GraphPad Prism Software, Inc) was used for statistical analysis. Type I error  $\alpha$  was set at 0.05 ( $p < 0.05$ ).

### Human study

The analysis is based on all patients from the study who had an evaluable baseline video-PSG. Continuous variables were expressed as mean  $\pm$  SD or median (IQR) depending on the distribution. To assess the



relationship between sleep architecture and functional outcome, patients with  $mRS \leq 2$  were compared to those with  $mRS > 2$ , using the unpaired *t*-test or the Mann-Whitney rank sum test for continuous variables, as appropriate. Chi-square test or the Fisher's exact test was used for categorical variables. The relationships between outcomes – mRS (at discharge and at 3 months) – and selected explanatory variables – age, sex, hypertension, diabetes, previous stroke/TIA, stroke topography and etiology, NIHSS on admission, thrombolysis treatment and sleep parameters (sleep efficiency, REM latency, sleep stages, AHI and PLMI) – was assessed with multiple linear regression. In specific cases, models were refined using a backwards variables selection procedure based on Akaike's Information Criterion. In two patients no REM sleep was observed. For statistical purpose, the REM latency in those patients was imputed as the value of the sleep period time (SPT, amount of time available for sleep after sleep onset).

The significance level of all tests was 5% and no correction for multiplicity was employed. Statistical analysis was performed using R 3.1.2 (R Core Team, Vienna, Austria, 2014).

## RESULTS

### Animal study

#### Infarct volume analysis

Infarct size was evaluated in the IS group to confirm the presence of the lesion in the brain resulting from the ischemia inducing surgery. The IS group (infarct size:  $77.79 \pm 5.23 \text{ mm}^3$  (mean  $\pm$  SEM) data not shown) displayed a lesion mainly located in the cortical area (Fig. S1C). As expected, animals subjected to sham surgery did not show any brain lesions.

#### Sleep-wake and circadian EEG variables

At the BL recordings we did not observe any statistical differences concerning the circadian distribution of sleep-wake stages, the distributions and hourly amounts of NREM, REM sleep and waking across the light/dark cycle, between animals that were subsequently randomly divided into the two groups [sham ( $n=6$ ) vs ischemia surgery ( $n=6$ )]. There was no mortality in both groups of animals during the surgery or the recovery time following surgery.

Circadian rhythmicity in rodents is in opposite phase compared to humans. At BL recordings, rats exhibited normal diurnal variation in sleep-wake behaviour, spending less time in wakefulness and more time in both NREM sleep and REM sleep during the light period than during the dark period of the 24h light-dark cycle (% of the recording time for NREM sleep:  $44.7 \pm 1.6$  light period,  $20.3 \pm 0.7$ , dark period; % of the recording time for REM sleep:  $10.3 \pm 0.2$  light period,  $4.9 \pm 0.2$  dark period; % of the recording time for wake:  $47.0 \pm 0.7$  light period,  $71.6 \pm 0.9$  dark period).

The impact of stroke on the circadian distribution of sleep is summarized daily across the 24-h circadian period in the baseline and after three consecutive days after ischemia/sham, for the IS and Sham groups (Fig.1). Visual inspection of data across the postsurgical recording period shows that the circadian distribution of sleep in the IS group was generally flattened over the first 24h following the intervention. Indeed, the IS compared to the Sham group showed a greater proportion of NREM sleep during the dark phase that followed immediately after surgery and which accounts for an active phase for rats. This increase was not observed in the Sham group, although they also received anaesthesia. Moreover, a greater proportion of total wakefulness was also observed in the IS group relative to the Sham group during the light phase, which start 12h after surgery and that accounts for the resting phase for rats. However, sleep-wake behaviour recovered over the subsequent dark-light period of days 2 and 3 after ischemic surgeries. Sham control surgeries had little or no effect on on sleep EEG (Fig.1).

#### Amount of non-REM, REM sleep and waking across the light/dark cycle

Figure 2 displays the amount of NREM sleep, REM sleep and wakefulness assessed separately for the IS and Sham group during three days of postsurgical recordings.

During day 1 post-surgery, the total amount of time spent in sleep (including both NREM and REM sleep) and wakefulness over 24h were unchanged in both IS and Sham groups (Fig. 2B). However, separate analyses of NREM sleep and REM sleep, revealed significant differences over time between the two groups (IS vs. Sham, Fig. 2A).

Notably, NREM sleep was significantly increased in the IS group compared to the Sham group and to the BL recording ( $F(3, 15) = 3.52$   $p = 0.04$  interaction “time x group” Figure 1B). This increase of NREM sleep in the IS group was observed in the early phase of stroke, during the dark period (i.e active phase for rats) (Figure. 1A). However, the total number of NREM sleep bouts and their duration in minutes were unchanged in both groups (Fig. 3).

Conversely, REM sleep was significantly reduced following the 24h BL after surgery in the IS group (rANOVA:  $F(3, 15) = 7.66$   $p = 0.002$  “time ” Fig. 2B). Interestingly when looking at the distributions and hourly amounts of time spent in REM sleep over the first 24h, REM sleep were unchanged between the two groups in the early dark phase after ischemic stroke (Fig. 2A). However, a significant decrease of the percentage of REM sleep was observed during the light period (i.e resting phase for rats), which started 12h after surgery, in the IS group compared to the Sham group (Fig. 2A). In addition, also REM sleep bouts were significantly reduced in the IS group compared to the Sham group, without a change in mean REM bout duration (rANOVA:  $F(3, 15) = 5.003$   $p = 0.01$  interaction “time x group” Fig. 3).

During day 2 post-surgery, no differences were observed on total amount of sleep (Fig. 2) and for the number of total sleep bouts and their duration per minutes (Fig.3) between the IS and Sham groups over the 24h of the day 2 (Fig 2B), i.e when REM and NREM sleep and wakefulness were individually analysed.

During day 3 post-surgery, the total amount of sleep over the 24 of the day 3 was unchanged between groups (Fig.2A). However, when looking at the distributions and hourly amounts of the total sleep time, REM sleep, and NREM sleep, significant differences were observed. Mainly, these differences were found at the beginning of the dark period (48h-54h of the day 3, Fig. 2A) and the start of the light period (60h-69h of the day 3, Fig. 2A). Interestingly, the total number of sleep bouts, particularly NREM sleep bouts were significantly increased in the IS group compared to the BL, without a change in their bout duration at day 3 (Total sleep: rANOVA:  $F(3, 15) = 8.03$   $p = 0.002$  “time”; NREM sleep rANOVA:  $F(3, 15) = 4.05$   $p = 0.002$  “time” Fig. 3).

## Human study

### Patients characteristics and stroke outcome

A total 153 patients with acute ischemic stroke were included. The mean age was  $61 \pm 10$  years and 72% were male. The median NIHSS on admission was 3 (IQR: 1-5). Twenty-three (15%) patients had an infratentorial ischemic lesion involving the cerebellum (in 61% of them) and brainstem (39%). Cardioembolism was the most frequent stroke etiology (36%).

A total of 32 (21.1%) patients received thrombolytic therapy. All patients were hospitalized first in the acute stroke unit. The exact use of drugs was available for 73% of patients. In these 111 patients, 4 (3.6%) used antiepileptic drugs, 6 (5.4%) anxiolytics, and 10 (9%) antidepressants.

The mRS was available for 152 patients (99%) at discharge [median 1 (IQR: 0-1)], and for 118 (77%) patients (88 males,  $60.7 \pm 9.3$  years) at 3 months [median 1 (IQR: 0-1)]. Twenty-four (16%) patients had poor functional outcome at discharge, and only 6 (5%) at 3 months. Characteristics of the patients are shown in Table 1.

### Sleep and sleep EEG assessments

The results of video-PSG recordings during the acute phase are shown in Table 2. The mean total sleep time was  $319 \pm 95$  minutes. Time spent in stage N1 and REM was less than stage N2 and N3. The median AHI and ODI were 15.2 (IQR: 7.3-29.7) and 7.9 (IQR: 2.3-19.6), respectively. Overall, 80% of patients had  $\text{AHI} \geq 5/\text{h}$ ; 40% had an  $\text{AHI} \geq 20/\text{h}$  and 25% an  $\text{AHI} \geq 30/\text{h}$ . Twenty-four (16%) patients had a  $\text{PLMI} \geq 20/\text{h}$  and 6% a  $\text{PLMI} \geq 30/\text{h}$ .

Patients with poor functional outcome ( $\text{mRS} > 2$ ) had a lower sleep efficiency ( $p = 0.006$ ), less amount of REM sleep ( $p = 0.01$ ), longer REM latency ( $p = 0.006$ ) and higher AHI ( $p = 0.04$ ) than patients with good functional outcome ( $\text{mRS} \leq 2$ ).

In a multiple linear regression analysis, age ( $p=0.007$ ), NIHSS on admission ( $p<0.001$ ), and REM latency ( $p=0.006$ ) were found to be predictors of outcome at discharge. The NIHSS on admission ( $p=0.043$ ) and REM latency ( $p=0.036$ ) were also independent predictors of mRS at 3 months (Table 3).

## DISCUSSION

In line with previous published studies,<sup>24-28, 30, 53, 54</sup> we found that ischemic stroke significantly alters sleep quantity and sleep EEG/architecture in both humans and animals.

However, we show for the first time, as main findings of this study that ischemic stroke is associated with 1) a significant reduction of REM sleep observed in both humans and animals during the acute phase of stroke, and that 2) this reduction of REM sleep together with a prolonged REM sleep latency is linked with poor outcome in humans.

In animals we found a significant reduction in the total amount of REM sleep as well as the number of REM sleep bouts in the first 24h following ischemia. This was particularly noticeable during the light period, which started 12h after ischemic surgery representing the resting phase for rats, and recovered over the time of the experiment. REM sleep was unchanged compared to sham surgery after 2 and 3 days from stroke surgery. Similarly, a suppression of REM sleep during the acute phase of stroke was found previously in different animal species.<sup>28, 53, 54</sup> Noteworthy, a reduction of REM sleep was found in rodent models of stroke involving only cortical<sup>28</sup> or subcortical areas but also with a cortical-subcortical extension of the lesion.<sup>53</sup>

Also in humans a reduction in the total amount of REM sleep during the acute phase of stroke was shown by others before.<sup>29, 32,55</sup> The observation of a link between REM sleep and stroke outcome is however new and in line with a recent study published by our team<sup>36</sup> in which REM sleep was found to correlate negatively with infarct volume in rats.<sup>56-58</sup>

As in animal models of stroke, also in humans no clear association was found between REM sleep changes and topography of stroke. In this as well as previous studies REM sleep changes were found following both

brainstem and supratentorial strokes.<sup>59</sup> While this reduction is usually short-lived in most cases, it may persist beyond the first few days following large hemispheric or strategic brainstem lesions.<sup>56-58</sup> It is interesting to stress the fact that data on NREM sleep changes following stroke are less consistent. Some studies found an increase of NREM sleep stage 1 and stage 2,<sup>56-58</sup> while others showed a reduction in NREM sleep stage 2<sup>56-58</sup> and in slow wave sleep.<sup>27</sup> These discrepancies in results could be partially explained by the differences in methodology. Moreover, the abnormalities in sleep/wake cycles may be influenced by many factors: brain lesion/edema can cause dysfunctions in the sleep regulatory centers; sleep-disordered breathing and other sleep disorders (insomnia, restless legs syndrome) that are frequent in stroke patients; comorbidities and others (age, stress, drugs, pain, fever, hospital environmental).<sup>27, 29, 31, 32, 60</sup> It remains also possible that specific locations of stroke (e.g. in thalamus, caudate nucleus, insula, deep or superficial cortex) and/or its extension may have differential effects on sleep macro- and microstructure<sup>56-58,61</sup> and could give new insights into the relationship between this disrupted sleep and stroke outcome. This hypothesis could not be tested in the present study.

The origin of the observed acute reduction of REM sleep could be clarified in our study and remains speculative at this point. Since stroke topography is not relevant, other stroke-related changes must be involved. For example, acute stroke causes the secretion of cytokines from activated microglia at the infarct center<sup>62</sup> during the acute phase of stroke,<sup>63</sup> and cytokines have been shown to suppress REM sleep.<sup>64</sup> These effects could also explain the transitory nature of REM reduction after stroke and the lack of a link with stroke topography.

An additional novel finding is also the observation that REM sleep latency is an independent predictor of stroke short- and long-term outcome. REM latency is influenced by different factors including intra-sleep intrusions of wakefulness, slow wave sleep propensity and circadian factors.<sup>65</sup> An increase in intra-sleep wakefulness, resulting in an increase in REM latency, can be seen in sleep disorders including sleep related breathing disorder (SDB). In fact, SDB was reported to be associated with a poor stroke recovery in some,<sup>51, 66, 67</sup> but not all studies including the present one.<sup>52, 68-70</sup> Conversely, we observed a disruption of circadian

distribution of sleep in during the acute phase of rodent stroke, which may linked with the change in REM sleep latency. Consistent with this interpretation previous studies showed that stroke<sup>71</sup> as well as traumatic brain injury<sup>72</sup> can alter circadian gene expression patterns in both suprachiasmatic nucleus and hippocampus. Tischkau and collaborators observed in particular that stroke shifts the rhythm of Per1 (period circadian protein homolog 1), known to maintain the circadian rhythms in cell transcripts. Peak expression of Per1 occurred 6h earlier following stroke, suggesting a role of the circadian clock in stroke pathophysiology.<sup>71</sup>

Although we performed a comparative study between humans and animals endeavouring to either reduce or compensate limiting features it was not impossible to control all of them. Regarding the animal study, the ischemic lesion induced was located exclusively in the left somatosensory cortex, and it would be interesting to know how ischemic stroke, when localized in different brain areas (i.e only striatal area or cortex and striatum areas), impact sleep and sleep architecture.

Regarding the human study, an unbalanced number of patients with poor and good outcome was available. This is explained by the low stroke severity in our sample and the a priori exclusion of clinically unstable patients. Nevertheless, an association between REM sleep and functional outcome was found even in this sample of mildly affected patients. It remains in addition possible that sleep EEG (including REM) changes may be at least in parts related to the monitoring of patients in the acute stroke unit and by the use of drugs, both of which were not or only partially measured in this study.<sup>73, 74</sup> As psychotropic drugs, in particular the antidepressants, are frequently used by post-stroke patients, it is important to taken into account their effects on sleep for better interpretation the polysomnographic findings.<sup>74-76</sup> Those effects depend upon the type of drug, dosage, time of administration, and duration of the treatment,<sup>77</sup> which were not evaluated in our study. Despite this, the animal model also showed a significant reduction of REM sleep following acute stroke.

## CONCLUSION

Our results showed that acute ischemic stroke is followed by a significant transient reduction of REM sleep in animals, and that the reduction of REM sleep is associated with poor prognosis in humans. Furthermore, an increase in REM latency was found to be an independent predictor of poor outcome. Finally, we also observed in both, humans and animals that sleep architecture and the circadian distribution of sleep are disrupted following an ischemic event and may influence the outcome negatively. These findings suggest the need for further research to understand the role of sleep (and in particular REM sleep) in acute stroke.

#### **List of abbreviations:**

Sham: Sham-surgery

IS: Ischemia

REM: Rapid Eye Movement

NREM sleep: Non-Rapid Eye Movement

N1: NREM sleep stage 1

N2: NREM sleep stage 2

N3: NREM sleep stage 3

EEG/EMG: Electroencephalogram/Electromyogram

3Vo: three-vessel occlusion method of ischemic stroke

MCAo: Middle Cerebral Artery occlusion

CCA: Common Carotid Artery

iCCA: Ipsilateral Common Carotid Artery

TIA: Transient Ischemic Attack

CPAP: Continuous Positive Airway Pressure



SAS-CARE-1: Sleep Disordered Breathing in TIA Ischemic Stroke and Continuous CPAP Treatment Efficacy

BMI: Body Mass Index

Video-PSG: Video-polysomnography

NIHSS: National Institutes of Health Stroke Scale

TST: Total Sleep Time

PLM: Periodic Limb Movements

PLMI: Periodic Limb Movements Index

AHI: Apnea-Hypopnea Index

TOAST: Trial of Org 10172 in Acute Stroke Treatment

ESS: Epworth Sleepiness Scale

mRS: modified Rankin Scale

AHI: Apnea Hypopnea Index

ODI: Oxygen Desaturation Index

SPT: sleep period time

IQR: interquartile range

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**Table 1:** Characteristics of stroke patients

Variables*	Total group (n=153)
<b>Demographic data</b>	
Age, years	61.2 ± 9.6
Male	110 (71.9)
<b>Risk factors</b>	
Hypertension	85 (55.6)
Diabetes	22 (14.4)
Dyslipidemia	98 (64)
Smoking	51 (33.6)
Previous Stroke/TIA	17 (11.2)
Atrial fibrillation	19 (12.4)
Coronary Artery Disease	25 (16.3)
<b>Clinical data</b>	
Body Mass Index, Kg/m <sup>2</sup>	27.7 ± 4.9
Epworth Sleepiness Scale <sup>‡</sup>	6.1 ± 3.7
Self-reported snoring <sup>‡</sup>	113 (74.3)
<b>Stroke data</b>	

NIHSS on admission <sup>†</sup>	3 (1-5)
Thrombolysis	32 (21.1)
Stroke location	
Supratentorial	127 (83)
Infratentorial	23 (15)
Supra- & infratentorial	3 (2)
TOAST	
Large-artery atherosclerosis	18 (11.8)
Cardioembolism	55 (35.9)
Small-vessel occlusion	18 (11.8)
Other determined etiology	6 (3.9)

TIA, transient ischemic attack; NIHSS, National Institutes of Health Stroke Scale

\* Values expressed as means  $\pm$  SD or absolute number (percentage of total)

<sup>†</sup> Values expressed as median (IQR)

<sup>‡</sup> Sleep-related complaints before stroke

Variables*	Total group (n=153)	mRS at discharge <sup>a</sup>		p value
		$\leq 2$ (n = 128)	$> 2$ (n = 24)	
Age, years	61.2 $\pm$ 9.6	60.7 $\pm$ 9.7	63.6 $\pm$ 9	0.14
Male	110 (71.9)	97 (75.8)	13 (54.2)	0.04
NIHSS on admission <sup>†</sup>	3 (1-5)	2 (1-4)	7 (5-12)	< 0.001
TOAST 1/2/3/4 <sup>‡</sup> , n	18/55/18/6	11/48/13/5	7/7/5/1	0.002
Total sleep time, min	319.7 $\pm$ 95	322.7 $\pm$ 94.9	300.2 $\pm$ 95.6	0.42
Sleep efficiency, %	57.6 $\pm$ 18.2	59.4 $\pm$ 18	48.3 $\pm$ 16.7	0.006



Arousals index <sup>†</sup> , no.	19.4 (14.1-27.7)	19.8 (14.8-27.4)	17.1 (11.7-31.6)	0.42
Sleep onset latency, min	53.3 ± 60.3	48.7 ± 55.7	79.2 ± 77.5	0.08
REM latency <sup>†</sup> , min	95 (56.5-151)	84 (54.5-144.1)	125.2 (90.6-296.4)	0.006
N1 sleep, %	10.8 ± 6.6	10.2 ± 5.3	14.3 ± 11	0.05
N2 sleep, %	44.2 ± 9.8	43.9 ± 9.2	46.4 ± 12.3	0.21
N3 sleep, %	27 ± 11.2	27.2 ± 10.8	25.1 ± 13.1	0.20
REM sleep, %	18 ± 7.3	18.7 ± 7	14.1 ± 8	0.01
AHI <sup>†</sup>	15.2 (7.3-29.7)	14.3 (6.8-25.8)	21.9 (13-48.6)	0.04
AHI ≥ 5	123 (80.4)	101 (78.9)	21 (87.5)	0.41
AHI ≥ 30	38 (24.8)	30 (23.4)	8 (33.3)	0.35
Central apnea index <sup>†</sup>	0.1 (0-1)	0.1 (0-0.7)	0.2 (0-2.6)	0.18
Obstructive apnea index <sup>†</sup>	2.2 (0.2-7.5)	2.2 (0.3-6.7)	3.7 (0.2-13)	0.48
ODI <sup>†</sup>	7.9 (2.3-19.6)	7.9 (2.2-19.3)	13 (7.1-23.4)	0.18
PLMI <sup>†</sup>	4.6 (0.2-14.6)	4.4 (0.1-14.6)	4.8 (0.6-8)	0.75

**Table 2:** Sleep parameters at the acute stroke phase according to functional outcome at discharge

PSG, polysomnography; AHI, apnea-hypopnea index; ODI, oxygen desaturation index; PLMI, periodic leg movements index

\*Values expressed as means ± SD or absolute number (percentage of total)

<sup>†</sup>Values expressed as median (IQR)

<sup>‡</sup>Stroke etiology: 1, large-artery atherosclerosis; 2, cardioembolism; 3, small-vessel occlusion; 4, other causes

<sup>a</sup>n =152

**Table 3.** Predictors of functional outcome at discharge and at 3 months after stroke

Variables	Coefficient	Standard error	p-value	
A. mRS at discharge <sup>a</sup>				
Age (years)	0.030	0.011	0.007	
NIHSS on admission	0.135	0.024	< 0.001	
REM latency, min	0.003	0.001	0.006	
B. mRS at 3 months <sup>b</sup>				
NIHSS on admission	0.058	0.028	0.043	
REM latency, min	0.003	0.001	0.036	mRS, Modifi

ed Rankin Scale; NIHSS, National Institutes of Health Stroke Scale

<sup>a</sup>n =152

<sup>b</sup>n = 118

Linear regression analysis, dependent variable: mRS at discharge or mRS at 3 months

Independent variables: Age, sex, diabetes, hypertension, previous stroke/TIA, NIHSS on admission, thrombolysis, infratentorial stroke, large-artery atherosclerosis, cardioembolism and small-vessel occlusion etiology, sleep efficiency, apnea-hypopnea index, REM latency, sleep stages, PLMI, and PSG day from stroke.

## Figure Captions

**Figure 1.** Circadian distribution of sleep/wake cycle at the baseline (BL) and for the following three days after surgery either sham or stroke surgeries. Sleep stages were plotted (each time point is given by averaging 3 consecutive hours) for both groups: IS group and the sham group; wakefulness (W, gray); REM sleep (green); NREM sleep (blue). Black bar on the y-axis indicates the time when surgery was performed at the beginning of the dark period. The black bars on the x-axis indicate the dark portion of the light- dark cycle.

**Figure 2.** Distribution of sleep/wake states across the 24h of baseline (BL) and for the following three days after either sham or stroke surgeries. **(A)** Hourly percentage time spent in wakefulness, total amount of

sleep (including both NREM sleep and REM sleep), and NREM and REM sleep separately. Sham animals (black rectangles) and IS animals (red circles). Data are presented as a mean of three hourly values  $\pm$  SEM. Statistical analyses were performed with rANOVA (factors: group and time) and post hoc analysis, with LSD tests run afterward. The black bar on the x-axis indicates the dark portion of the light-dark cycle. The black bar on the y-axis indicates the time when surgery was performed at the beginning of the dark period. **(B)** Total percentage of the recording time spent in wakefulness, total amount of sleep (including both NREM sleep and REM sleep), and NREM and REM sleep separately across the 24h of baseline (BL) and for the following three days after stroke. Asterisks (\*) indicate a statistically significant difference between IS and Sham group over the time (\* $P \leq .05$ ).

**Figure 3.** Number of wake bouts, total amount of sleep (including both NREM sleep and REM sleep), and NREM and REM sleep bouts and their mean duration in minutes across the BL and for the following three days after stroke. Sham operated animals (black rectangles) and IS animals (red circles). Data are presented as mean values  $\pm$  SEM. Statistical analyses were performed with rANOVA (factors: group and time) and post hoc analysis, with LSD tests run afterward. Asterisks (\*) indicate a statistically significant difference between IS and Sham group (\* $P \leq .05$ ).

## Captions for supplementary materials

**Supplemental Figure I.** Schematic of the experiment design. **(A)** Design for the sleep architecture analysis. Ischemia surgery was performed on day 0, and then the rats were sacrificed after 3 days following ischemic surgery. Baseline state time was recorded 1 day before ischemia for 24h; 12h dark and 12h light. **(B)** Design for sleep architecture. Rats were implanted with EEG/EMG electrodes and then allowed to recover for 4 days, and then connected to a flexible cable and swivel and habituated for 3 days, before EEG/EMG recording. **(C)** Representative sets of brain sections from a rat subjected to ischemia. The infarct areas are delineated by a thin black line. L1 is at 2.7 mm anterior to bregma, and the interval between each level is 1 mm (see methods). **(D)** A magnification of coronal brain section (is at the level of bregma -1.82 mm on the rat brain atlas by Paxinos and Watson, which accounts for L6), showing the distribution of ischemic damage; the white area in the left hemisphere.

Experimental groups: i. IS (n=6); ii. Sham (n=6). Electroencephalogram (EEG); Electromyogram (EMG)

Figure 1

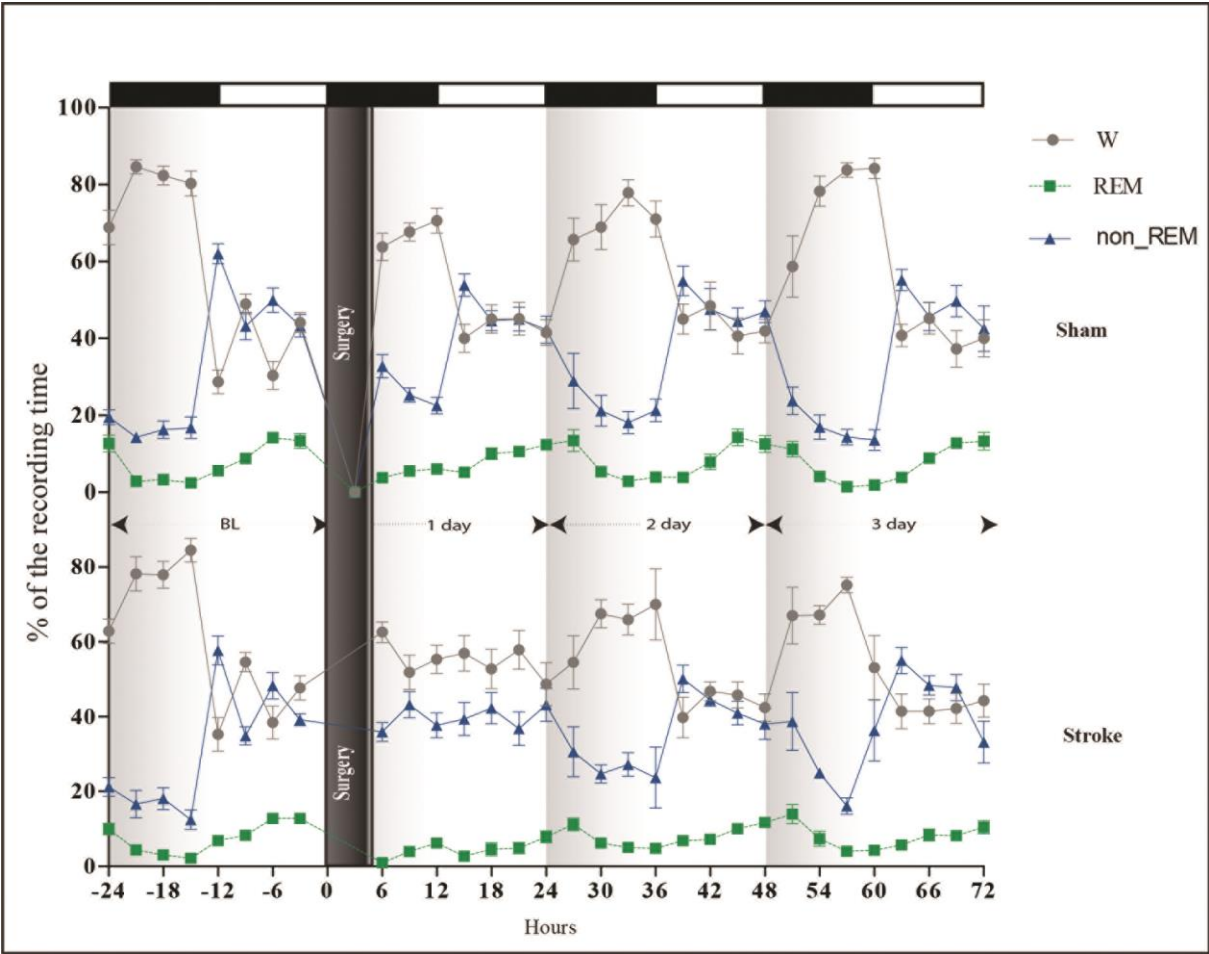


Figure 2

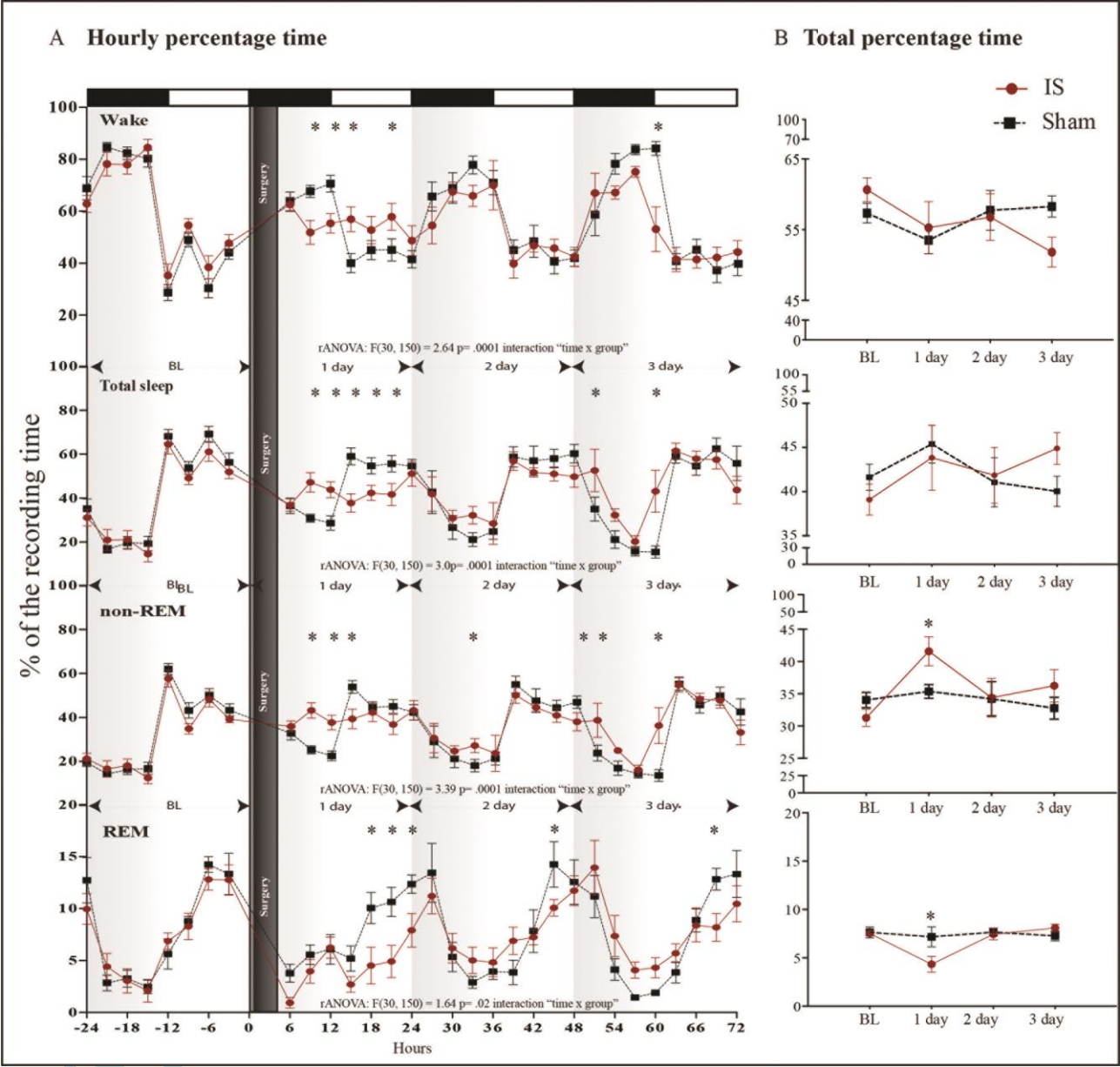


Figure 3

