Thalamic Influence on Slow Wave Slope Renormalization During Sleep

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Objective: Slow waves are thought to mediate an overall reduction in synaptic strength during sleep. The specific contribution of the thalamus to this so-called synaptic renormalization is unknown. Thalamic stroke is associated with daytime sleepiness, along with changes to sleep electroencephalography and cognition, making it a unique "experiment of nature" to assess the relationship between sleep rhythms, synaptic renormalization, and daytime functions.
Methods: Sleep was studied by polysomnography and high-density electroencephalography over 17 nights in patients with thalamic (n = 12) and 15 nights in patients with extrathalamic (n = 11) stroke. Sleep electroencephalographic overnight slow wave slope changes and their relationship with subjective daytime sleepiness, cognition, and other functional tests were assessed.
Results: Thalamic and extrathalamic patients did not differ in terms of age, sleep duration, or apnea–hypopnea index. Conversely, overnight slope changes were reduced in a large cluster of electrodes in thalamic compared to extrathalamic stroke patients. This reduction was related to increased daytime sleepiness. No significant differences were found in other functional tests between the 2 groups.
Interpretation: In patients with thalamic stroke, a reduction in overnight slow wave slope change and increased daytime sleepiness was found. Sleep- and wake-centered mechanisms for this relationship are discussed. Overall, this study suggests a central role of the thalamus in synaptic renormalization.

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Stroke is the leading cause for permanent disability among neurologic disorders and poses major societal challenges worldwide.[1] Stroke aftereffects and recovery have been connected to sleep.[2,3] On the one hand, stroke can lead to excessive daytime sleepiness and cognitive deficits.[4,5] These symptoms are similar to the deteriorating effects of insufficient sleep in healthy individuals,[6] suggesting that stroke may lead to insufficient recovery during sleep. On the other hand, functional recovery after stroke is thought to depend on synaptic plasticity, which in turn has been linked to sleep.[7,8] Sleep is presumed to maintain synaptic homeostasis by promoting an overall reduction in synaptic strength.[9] This so-called synaptic renormalization is necessary to counterbalance the learning-related increases in synaptic strength that occur during wakefulness.[9] Extensive evidence for synaptic renormalization during sleep has been gathered in studies analyzing structural, molecular, and electrophysiological

markers of synaptic strength.[9] For example, animal studies using 2-photon imaging and electron microscopy have demonstrated an increase in dendritic spines, a key marker for synaptic strength, during wakefulness and a decrease during sleep.[10] In humans, synaptic changes seem to be mirrored in slow waves during non–rapid eye movement (NREM) sleep. The amplitude and/or number of slow waves measured as slow wave activity (SWA; electroencephalographic [EEG] power between 1 and 4.5Hz) increases in response to experience-dependent plasticity.[11] Similarly, plastic changes after stroke are reflected in SWA increases in unaffected brain areas.[12,13] The majority of studies on sleep and stroke have focused on mapping these use-dependent changes in SWA but have not examined whether sleep-dependent restoration is altered after stroke. This question can be addressed by analyzing overnight slow wave slope changes, as these have been shown to reflect synaptic renormalization and therefore represent a good marker for the restorative function of sleep.[14–17]

The relationship between sleep and thalamic stroke is intriguing. Given that thalamic stroke is associated with severe excessive daytime sleepiness/hypersomnia, cognitive disturbances, and sleep EEG changes (eg, reduction of sleep efficiency), it poses an ideal model to test the hypothesis of a link between sleep, wakefulness, and cognition.[18,19] Although the thalamus has been shown to be important for the generation of slow waves,[20] it remains unknown to what extent it contributes to synaptic renormalization and restoration during sleep.

As such, the goals of this study were 2-fold: (1) to compare overnight slope changes in thalamic and extrathalamic stroke patients and (2) to examine how overnight slope changes are linked to excessive daytime sleepiness and specific cognitive functions impaired in stroke patients. This study was part of a larger project investigating the role of sleep in poststroke neuroplasticity.

Patients and Methods

Patients and Clinical Characteristics

Patients between 18 and 80 years old, with first-ever stroke, at least one major motor or cognitive deficit (determined by a neurologist), and normal or corrected to normal vision, were considered for the study. Exclusion criteria were concomitant neurodegenerative diseases, known psychiatric diseases, history of documented sleep disorders in the medical record, and inability to follow the procedures of the study. Written informed consent was obtained prior to participation. The study was approved by the local ethics committee and conducted according to the Declaration of Helsinki.

Stroke severity was estimated using the National Institute of Health Stroke Scale (NIHSS)[21]; sleep-disordered breathing was assessed by overnight polysomnography, and its severity was estimated by the apnea–hypopnea index (AHI).[22] These data were assessed as part of the clinical workup and were extracted from patients' reports (Table S1).

Neuroimaging

Lesion topology and extension were identified from the neuroradiology reports and reviewed by a neuroradiologist, who inspected the acute b1000 diffusion-weighted images (DWIs; 4mm slices; Fig 1; Table S1). Patients were stratified as having a thalamic versus extrathalamic stroke. In addition, thalamic patients were classified as having paramedian, inferolateral, tuberothalamic, or posterior choroidal lesions according to Carrera and Bogousslavsky.[23] Lesion volumes were manually delineated on the b1000 DWI maps yielding binary lesion maps using 3D-Slicer.[24] Lesion maps were then simultaneously spatially normalized to Montreal Neurological Institute stereotaxic space and coregistered with a T1 template for older individuals using the unified segmentation algorithm in SPM12.[25] To avoid bias, lesion reconstruction was performed without reference to the results of the behavioral data analysis.

Subjective Sleepiness

Subjective daytime sleepiness was assessed using the Epworth Sleepiness Scale (ESS)[26] at each overnight recording. In addition, momentary sleepiness throughout the experimental procedures was evaluated using the Stanford Sleepiness Scale (SSS)[27] at 3 time points: (1) before the motor and neurocognitive test batteries in the evening, (2) before patients were allowed to sleep, and (3) before the motor and neurocognitive test batteries in the morning (see Table S1).

Neurocognitive Tests

Neurocognitive and motor test batteries were performed in the evening before and the morning after the overnight sleep EEG recording. The following tasks were administered in a random order: Fugl-Meyer[28] for assessing sensorimotor function, timed picture naming (tablet version, adapted from Székely et al[29]) and passage reading[30] for assessing verbal function, Corsi block-tapping forward and reverse (tablet version, adapted from Kessels et al[31]) for assessing visuospatial short-term working memory, and random shape cancellation (tablet version, adapted from Rorden and Karnath[32]) for assessing visuospatial attention. In this article, only the tasks with electronic output, namely the Corsi block-tapping forward and reverse and the random shape cancellation tasks, were analyzed. For both tasks, the center of the tablet (Microsoft [Redmond, WA] Surface Pro, 12.3 inches) was aligned with the participant's midsagittal plane and participants were instructed to tap the screen with a finger. In the Corsi block-tapping task, 9 gray blocks were displayed on a

Extra-Thalamic

Thalamic
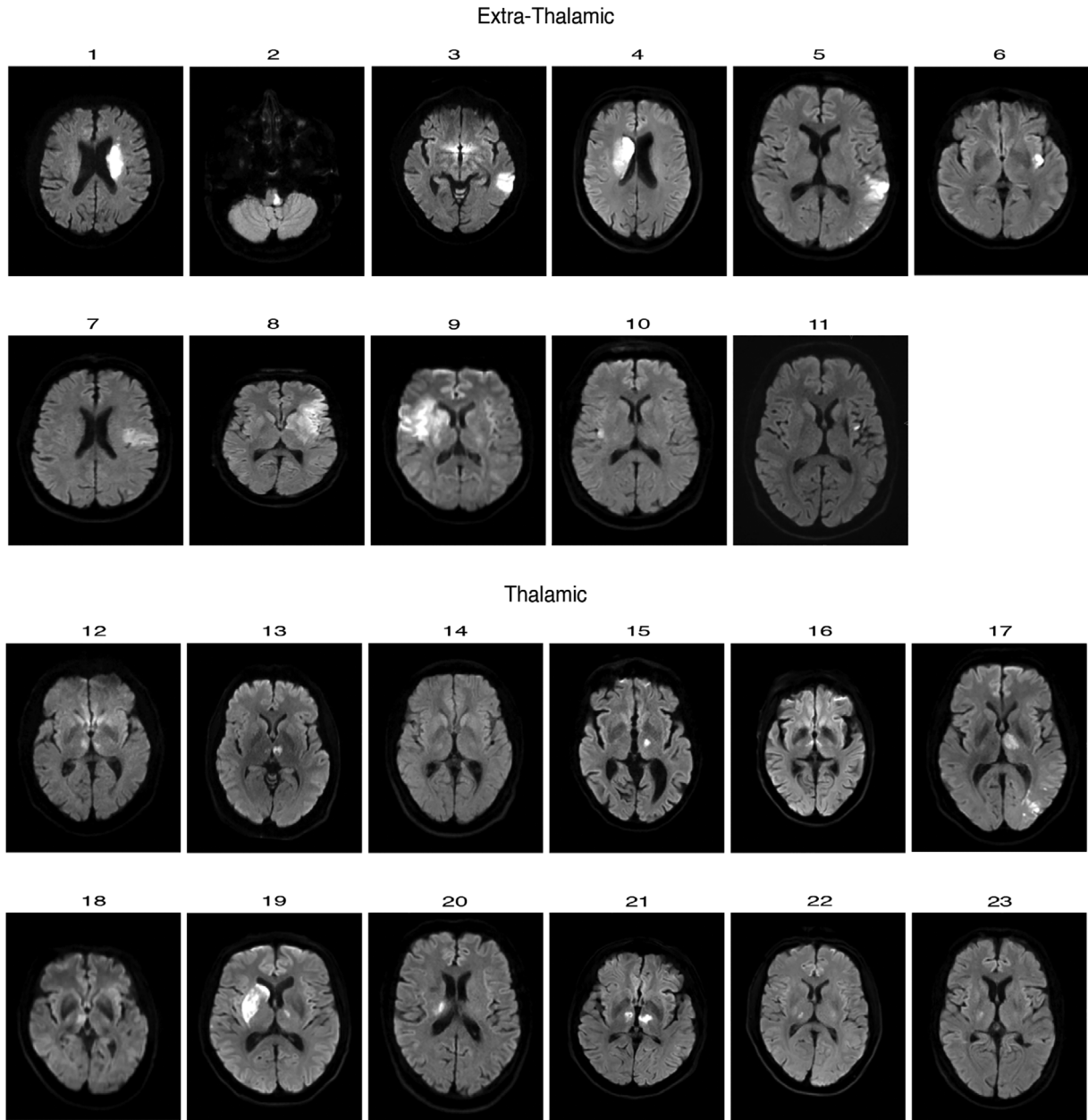
FIGURE 1: Magnetic resonance diffusion-weighted images (b1000 maps) for each included participant. The numbers above the images indicate the patient IDs and correspond to the IDs provided in Table S1.

white background. A sequence of blocks to be tapped was given by sequential coloring of blocks in blue, starting with a sequence of 3 blocks. In the forward task, the participant had to tap the sequence in the same order immediately after the sequence was displayed. In the reverse task, the participant had to tap the sequence in the reverse order. A maximum of 3 trials were given per block sequence of the same length. As soon as the sequence was repeated correctly, a sequence of an increased length was administered. Feedback was given by coloring all blocks in green if the sequence was repeated correctly and in red if the sequence was not repeated correctly before the next

trial was initiated. The block span corresponds to the length of the last correctly repeated sequence. In the random shape cancellation task, a black target object/animal of everyday life to be searched for was displayed on a white background. The participant then had to identify all targets on a field of other objects/animals of everyday life (all displayed in black on a white background). Three difficulty levels were performed. Depending on the difficulty level, 6, 12, or 24 target objects/animals were displayed on a field of 72, 144, or 288 objects/animals in total. For each difficulty level, an omission score was calculated as the percentage of omitted targets relative to the total number of targets.

In addition, a velocity score was computed, corresponding to the time from the start to the last marked object divided by the number of correctly identified targets. Due to many missing test sessions (8–10 evening, 13–15 morning sessions missing depending on the test), evening and morning performance scores were averaged for all tests.

High-Density Sleep EEG

Overnight sleep EEG recordings were performed in the acute (1–15 days poststroke), subacute (1–7 months poststroke), and chronic (>8 months poststroke) stage after the stroke. Due to the low number of patients who returned for a chronic recording, the chronic time point was not analyzed (Fig 2).

A high-density EEG net (Sensor Net for long-term monitoring, 256 channels, Electrical Geodesics Inc, Eugene, OR) was adjusted to the vertex (Cz) of the participant, and electrodes were filled with electrolyte gel (electro-gel; Electro-Cap International, Eaton, OH). During the recording, the signal was referenced to Cz and sampled at 500Hz. For spectral analysis, the sleep EEG was bandpass filtered between 0.5 and 40Hz and downsampled to 128Hz. Sleep stages were scored in 30-second epochs according to modified standard criteria[22] by a sleep expert and reviewed by a second expert. The modifications included the review of additional EEG channels (Fz and parietal electrodes), and the extraction of muscle activity from neck electrodes. Spectral analysis of consecutive 30-second epochs (fast Fourier transformation, Hanning window, average of six 5-second epochs, frequency resolution of 0.2Hz) was performed, and 30-second epochs containing artifacts were rejected by visual inspection and by a semiautomatic approach based on a power threshold in the frequency bands 0.8–4.6Hz and 20–30Hz.[33] Channels with poor quality and below the ears were removed, and the remaining data were rereferenced to an average value across all good quality electrodes. Power for different frequency bands (low SWA, high SWA, theta, alpha, sigma, beta) was calculated by summing up power values in each frequency bin between the lower and upper frequency threshold in hertz and multiplying it by the frequency resolution. Power values were averaged across all artifact-free NREM (stages N2 and N3) sleep epochs. Missing data from excluded electrodes were interpolated using spherical linear interpolation, resulting in 195 electrodes per participant.

### Slow Wave Detection and Analysis of Overnight Slope Changes

To analyze individual slow wave characteristics, slow wave detection was performed using a similar procedure as in Riedner et al.[17] After low-pass filtering below 30Hz, the signal was rereferenced to the average of the 2 mastoids and bandpass filtered (0.5–4.0Hz, stopband 0.1 and 10Hz, Chebyshev type II filter). For each channel separately, negative deflections between 2 zero crossings were identified as slow waves if they were separated by 0.25 to 1.0 seconds. The amplitude was defined as the most negative peak of the signal, and the slope was defined as the amplitude divided by the time from the positive-to-negative zero crossing to the most negative peak. Overnight slope changes were calculated using the same procedure as in Jaramillo et al.[16] In short, waves from the first and last hour of artifact-free NREM sleep (stages N2 and N3) were matched according to their amplitude. Channels that displayed less than 250 matched waves were excluded and interpolated for topographical analyses (spheric linear interpolation). This was the case for 6 nights (n = 4 in the extrathalamic group with 1, 2, 92, and 105 electrodes interpolated and n = 2 in the thalamic group with 1 and 8 electrodes interpolated). This procedure resulted in the inclusion of 1,299 (range = 307–1,961) matched waves on average per night (after averaging across all electrodes). The matched waves were then divided into 5 amplitude quintiles, and overnight slope changes were calculated for each quintile and each electrode. Since the thalamus has been shown to be important for the generation of synchronized, large-amplitude waves,[34,35] we focused our analysis on overnight slope changes of the largest 20% of matched slow waves.

Statistics

Group characteristics were compared using a Wilcoxon rank-sum test. To compare sleep parameters, EEG power across the different frequency bands, and overnight slope changes between the 2 groups, linear mixed-effects models were calculated using restricted maximum likelihood estimation of parameters using the function lmer and the R packages lme4[36] and lmerTest.[37] All models were built based on our hypotheses and in agreement with 2 statistical experts. EEG power and slow wave slope were log-transformed to account for relative differences. Overnight slope changes were calculated as the difference between the log-transformed average slopes of the first and the last hour of NREM sleep. Type III analysis of variance tables of the fitted models were generated using Satterthwaite's degrees of freedom method using the function anova and the R package stats.[38] For the assessment of EEG power and overnight slope change differences across the whole scalp, electrodewise linear mixed-effects models were calculated with statistical nonparametric mapping cluster correction to control for multiple comparisons.[39] In short, the group label (thalamic, extrathalamic) was shuffled randomly, and a linear mixed-effects model was calculated for each electrode. The maximal number of neighboring
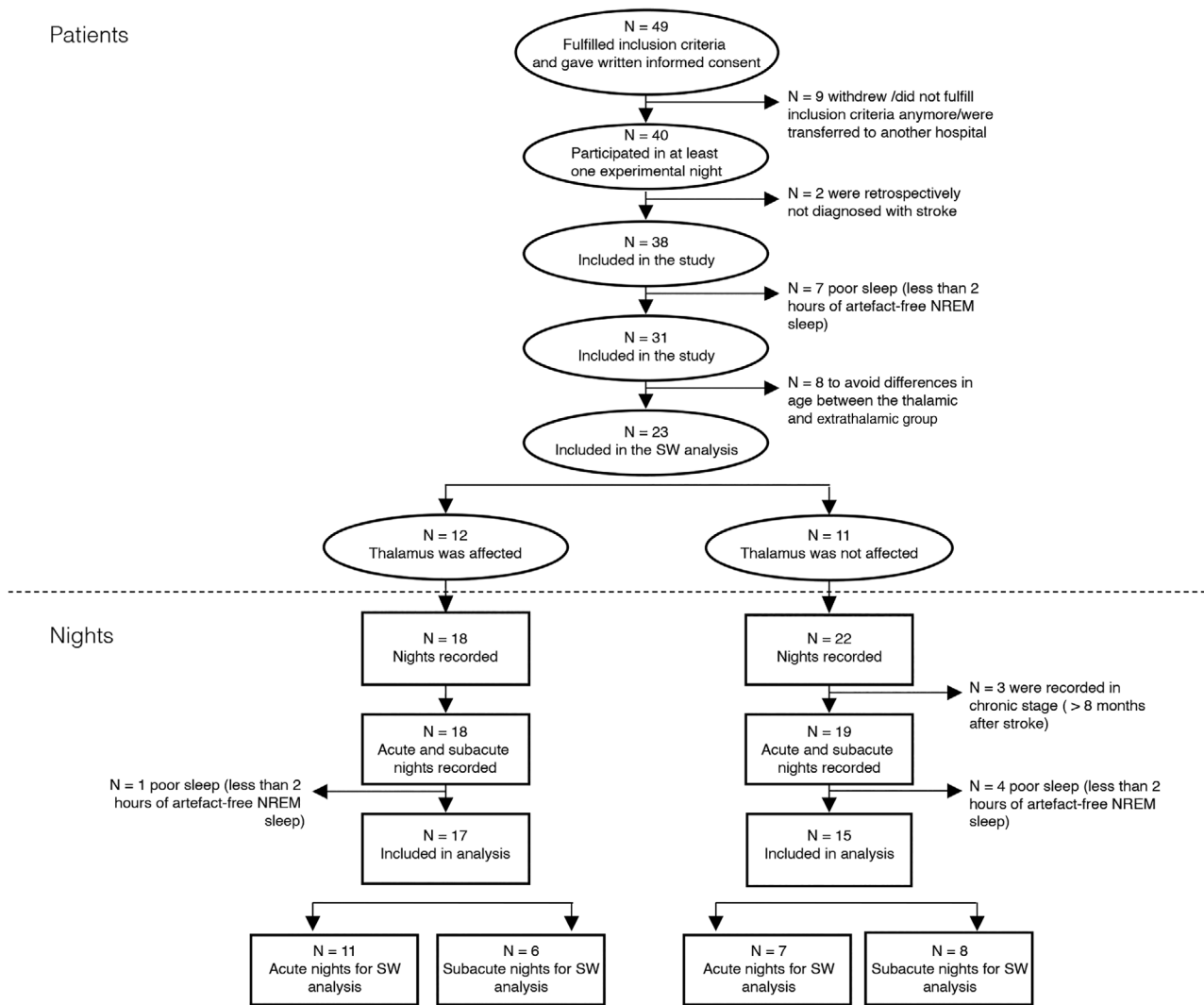
FIGURE 2: Flowchart of excluded patients and nights. The upper part of the figure (ovals) indicates how many participants were excluded and the reasons for exclusion. The lower part indicates how many nights of the included participants were excluded and the reasons for exclusion. NREM = non–rapid eye movement; SW = slow wave.

electrodes with a p value < 0.05 was determined separately for electrodes with positive and negative β coefficients. A total of 5,000 permutations were performed to obtain a distribution of maximal cluster sizes for positive and negative β coefficients, and the threshold was set to the 97.5th percentile. The significance level was set to 0.05. All statistical analyses were performed with R or MATLAB.

Results

Patients and Clinical Characteristics
A total of 49 patients were included in the study. Twenty-six of them were excluded for the following reasons: 9 withdrew, were in an unstable condition, or were transferred to another hospital; 2 were retrospectively not diagnosed with stroke; and 7 exhibited poor sleep in all available nights (<2 hours of artifact-free NREM sleep). Because age was expected to have an effect on the

analyzed sleep and outcome measures, a common age range (40–70 years) was defined for the 2 studied groups (thalamic and extrathalamic). This age-matching lead to further exclusion of 8 patients (4 extrathalamic, <40 years old; 4 thalamic, >70 years old).

A total of 12 patients with thalamic (60.7 $\pm$ 7.2 years, 3 female, NIHSS: 7.7 $\pm$ 6.6, AHI: 22.8 $\pm$ 21.4) and 11 patients with extrathalamic stroke (58.5 $\pm$ 4.6 years, 4 female, NIHSS: 9.0 $\pm$ 7.9, AHI: 16.3 $\pm$ 11.1) were finally considered for this study. The comparison of age, NIHSS, and AHI revealed no significant differences between the 2 groups (Wilcoxon rank-sum test, $p > 0.3$).

Neuroimaging
All 12 patients with thalamic stroke exhibited their lesion core in the thalamus. Specifically, 5 had a paramedian, 5 an inferolateral, and 2 a tuberothalamic lesion core. In

TABLE 1. Comparison of Subjective Sleepiness and Functional Outcome Measures between Thalamic and Extrathalamic Stroke Patients

Measure	Thalamic		Extrathalamic		Stroke Effect		Age Effect		Time Effect		Stroke × Time Effect	
	Mean	SD	Mean	SD	F	p	F	p	F	p	F	p
ESS	8.8	4.0	5.8	2.9	6.3	0.020[a]	0.2	0.686	2.3	0.226	7.3	0.073
SSSE1	2.8	2.0	2.2	1.0	0.3	0.584	0.6	0.435	0.1	0.837	0.2	0.695
SSSE2	4.7	1.8	3.5	1.9	0.9	0.354	0.0	0.904	4.5	0.081	0.3	0.590
SSSM	3.2	2.1	2.1	1.1	0.3	0.571	2.0	0.171	0.3	0.612	0.1	0.729
NIHSS	7.9	6.9	7.7	7.0	0.0	0.985	0.2	0.664	—	—	—	—
CBBS	5.7	0.8	5.3	0.9	3.5	0.076	0.5	0.504	6.4	0.030[a]	1.5	0.249
CBRBS	5.5	1.2	5.1	0.7	0.0	0.997	0.9	0.366	7.3	0.018[a]	4.2	0.061
RSCD1O	2.8	5.4	2.8	4.1	0.0	0.879	0.0	0.986	0.5	0.492	0.0	0.986
RSCD2O	12.2	15.9	3.8	3.8	1.6	0.226	0.1	0.722	0.3	0.611	0.1	0.754
RSCD3O	13.7	19.9	7.5	8.5	0.3	0.621	1.4	0.244	3.3	0.086	0.2	0.701
RSCD1T	5.6	3.7	4.0	0.9	1.7	0.207	2.6	0.121	0.5	0.477	0.6	0.438
RSCD2T	5.3	2.8	4.6	1.6	1.0	0.337	8.1	0.011[a]	2.4	0.139	2.2	0.157
RSCD3T	5.5	1.9	5.2	1.5	0.2	0.163	2.8	0.114	4.8	0.057	0.0	0.870

To account for the repeated measures, a linear mixed-effects model, with the subjective sleepiness or the functional outcome measure tested as dependent variable, age, stroke (thalamic, extrathalamic), time after stroke, and the interaction between stroke and time after stroke as fixed factors, and patient as random factor, was calculated. Because for the NIHSS only the acute time point was available, a simple linear model was calculated (no random factor). Residual analysis of the linear mixed-effects model with RSCD1O as dependent variable indicated that the assumption of normally distributed residuals was violated and these results should be viewed with caution.
[a]Statistically significant.
CBBS = Corsi block-tapping block span; CBRBS = Corsi block-tapping reverse block span; ESS = Epworth Sleepiness Scale; NIHSS = National Institutes of Health Stroke Scale; RSCD1O = random shape cancellation difficulty level 1 omissions; RSCD1T = random shape cancellation difficulty level 1 time; RSCD2O = random shape cancellation difficulty level 2 omissions; RSCD2T = random shape cancellation difficulty level 2 time; RSCD3O = random shape cancellation difficulty level 3 omissions; RSCD3T = random shape cancellation difficulty level 3 time; SSSE1 = Stanford Sleepiness Scale in the evening before the motor and neurocognitive test batteries; SSSE2 = Stanford Sleepiness Scale in the evening before patients were allowed to sleep; SSSM = Stanford Sleepiness Scale in the morning before the motor and neurocognitive test batteries.

addition to the thalamic lesion, 10 of the 12 thalamic patients had associated extrathalamic lesions; 4 patients had lesions in the mesencephalon (1 in the crus cerebri), 4 in the hypothalamus, 2 in the cortex (including temporal and occipital lobes), 2 in the internal capsule, 1 in the hippocampus, and 1 in the basal ganglia. The low number of patients per thalamic/extrathalamic region did not allow a subgroup analysis to be performed.

The 11 extrathalamic patients exhibited lesions in the following structures: 8 in the cortex (including all lobes except the occipital lobe), 5 in the basal ganglia (including 4 in the nucleus lentiformis, and 3 in the caudate nucleus), 2 in the internal caspule, 1 in the external capsule, 1 in the medulla oblongata, and 1 in the cerebral peduncle (some patients had multifocal and extended DWI restrictions in more than 1 location, see Table S1 and Fig 1 for further details).

Lesion volume was significantly lower in thalamic $(3,876.8 \pm 7,371.7 \text{ mm}^3)$ compared to extrathalamic $(20,391.1 \pm 25,608.4 \text{ mm}^3)$ stroke patients (Wilcoxon rank-sum test, $W = 30$, $p = 0.027$).

Subjective Sleepiness
The comparison of subjective sleepiness measures between the 2 groups revealed increased daytime sleepiness as assessed by the ESS in thalamic compared to extrathalamic stroke patients (Table 1).

Neurocognitive Tests
The comparison of neurocognitive tests between the 2 groups revealed no significant differences (see Table 1).

High-Density Sleep EEG
Of the 40 recorded nights in the 23 included patients, 3 nights (all extrathalamic) were excluded because they occurred in the chronic stage and 5 nights (1 thalamic and 4 extrathalamic) due to poor sleep (<2 hours of artifact-free NREM sleep). This led to a final inclusion of 11 acute and 6 subacute nights for

TABLE 2. Comparison of Sleep Parameters between Thalamic and Extrathalamic Stroke Patients

Parameter	Thalamic Mean	SD	Extrathalamic Mean	SD	Stroke Effect F	p	Age Effect F	p
Sleep efficiency, %	68.2	11.5	65.2	11.5	0.616	0.440	1.711	0.206
Sleep latency, min	48.8	31.2	46.9	26.5	0.050	0.824	0.001	0.982
Total recording time, min	561.4	49.9	544.5	40.0	0.006	0.940	7.188	0.012[a]
Total sleep duration, min	381.5	68.2	353.7	61.7	0.178	0.676	0.011	0.919
Wake after sleep onset, min	134.7	66.0	147.5	56.5	1.145	0.295	4.194	0.055
NREM sleep, min	243.8	62.4	244.1	50.0	0.001	0.978	0.010	0.921
REM sleep, min	51.5	33.5	44.4	22.1	0.636	0.432	9.146	0.005[a]

To account for the repeated measures, a linear mixed-effects model, with the sleep parameter tested as dependent variable, age, stroke (thalamic, extrathalamic), time after stroke, and the interaction between stroke and time after stroke as fixed factors, and patient as random factor, was calculated. Only the factor age was significant for certain sleep parameters; all other factors and interactions were not significant ($p > 0.1$) and are therefore not presented.

[a]Statistically significant.

NREM = non-REM; REM = rapid eye movement; SD = standard deviation.

the thalamic group and 7 acute and 8 subacute nights for the extrathalamic group (see Fig 2 for a flowchart of excluded patients and nights).

Sleep parameters extracted from the visual scoring did not differ between the 2 groups (Table 2).

The comparison of low SWA (1–2Hz) between the 2 groups using a linear mixed-effects model is shown in Figure 3. Significant effects for the factors stroke and age were found. These results indicate a reduction in low SWA in thalamic compared to extrathalamic patients, when accounting for age and time after stroke (see Fig 3A). Topographical analysis using the same linear mixed-effects model (see Fig 3B) revealed a large significant cluster of 88 electrodes in which low SWA was reduced in thalamic compared to extrathalamic patients. The electrode cluster spanned across all cortical lobes and was most pronounced in a right frontocentral area (number of electrodes in the left hemisphere: 9 frontal, 1 central, 0 parietal, 7 temporal, 11 occipital; right hemisphere: 26 frontal, 9 central, 10 parietal, 4 temporal, 11 occipital). No significant cluster was found for high SWA (2.2–4.6Hz), theta (5–8Hz), alpha (8.2–10Hz), sigma (12–16Hz), and beta (20–25Hz) frequency bands.

The comparison of overnight slope changes between the 2 groups using a linear mixed-effects model is shown in Figure 4. Overnight slope changes were significantly reduced in thalamic compared to extrathalamic patients, when accounting for age and time after stroke (see Fig 4A). No significant differences were observed when repeating the same analysis for smaller amplitude waves, that is, the 4 smallest amplitude quintiles (data not

shown). Topographical analysis using the same linear mixed-effects model revealed a large significant cluster of 58 electrodes, in which overnight slope changes were reduced in thalamic compared to extrathalamic patients (see Fig 4B). Cluster electrodes were distributed across all lobes of the cortex (number of electrodes in the left hemisphere: 1 frontal, 1 central, 6 parietal, 1 temporal, 8 occipital; right hemisphere: 21 frontal, 5 central, 9 parietal, 4 temporal, 2 occipital). Testing of a slope change reduction in additional clusters in the left (frontal: factor stroke $F[1, 20.4] = 3.21$, $p = 0.088$; central: factor stroke $F[1, 21.9] = 3.19$, $p = 0.088$) and right (occipital: $p > 0.2$) hemispheres indicated no significant differences.

Next, the slope for the first and last hours of artifact-free NREM sleep in the significant cluster was compared between the groups. A linear mixed-effects model with the slope in the first/last hour as dependent variable, age, stroke, time after stroke, and the interaction between stroke and time after stroke as fixed factors, and patient as random factor revealed a significant reduction in the slope in the first hour (factor stroke $F[1,16.5] = 5.45$, $p = 0.032$; factor age $F[1,16.5] = 6.76$, $p = 0.019$; all other factors and interactions $p > 0.3$), but not the last hour ($p > 0.1$) of artifact-free NREM sleep in thalamic patients. To test whether the reduced overnight slope change was related to the reduced slope in the first hour, the same linear mixed-effects model, but with the overnight slope change as dependent variable and the slope in the first hour of artifact-free NREM sleep as an additional fixed factor, was calculated. No significant effect of the slope in the first hour was found ($p > 0.3$),
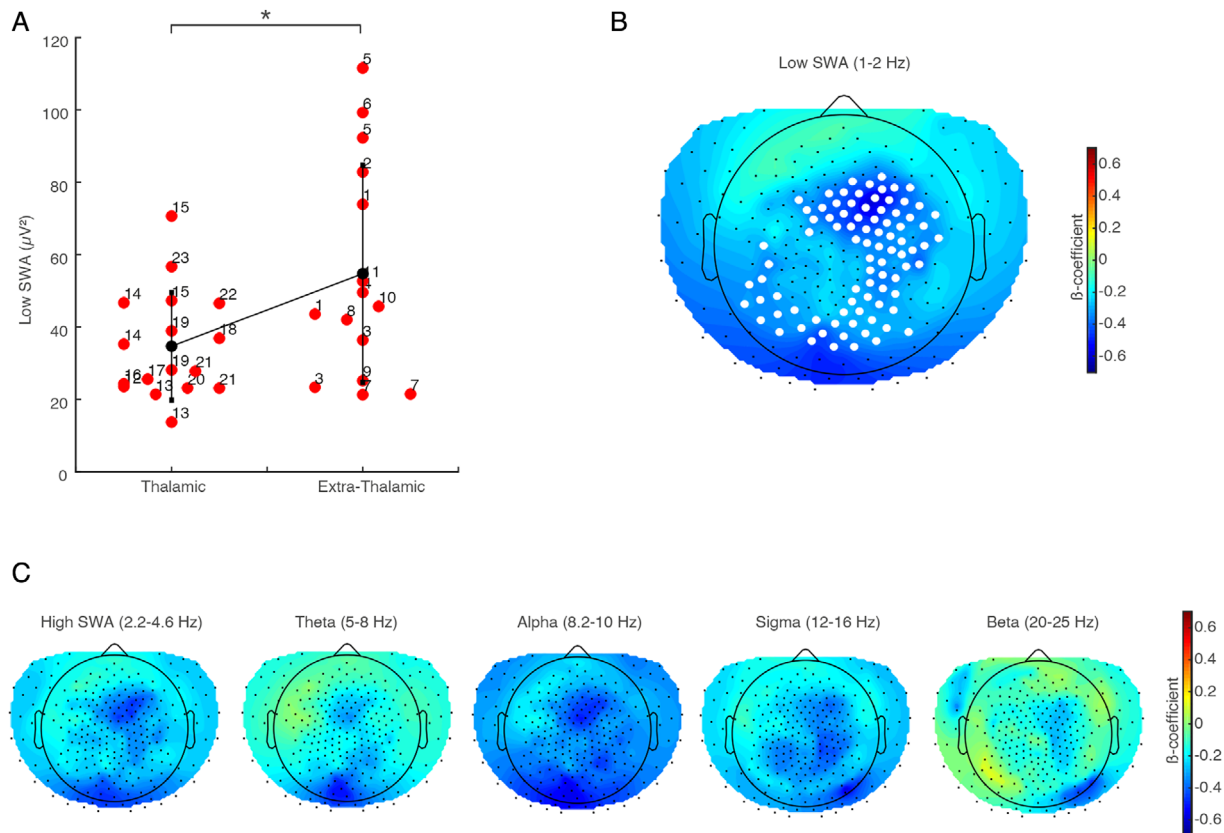
FIGURE 3: Comparison of all night low slow wave activity (SWA) and power in other frequency bands between thalamic and extrathalamic stroke patients. To test significant differences, a linear mixed-effects model, with low SWA (average across all electrodes in [A] and for each electrode in [B]) as dependent variable, age, stroke, time after stroke, and the interaction between stroke and time after stroke as fixed factors, and patient as random factor, was calculated. (A) Red dots show global SWA (average across all electrodes) for each night recording. The numbers beside the dots indicate the patient IDs and correspond to the IDs provided in Table S1. Black dots and error bars represent the mean and standard deviation for each group. The horizontal bar and asterisk denote a significant difference between thalamic and extrathalamic patients (factor stroke $F[1, 19.2] = 6.22$, $p = 0.022$; factor age $F[1,19.1] = 11.8$, $p = 0.003$; all other factors and interactions $p > 0.5$). (B) Topographical representation of standardized β coefficients for the fixed factor stroke. Negative β coefficients (blue) indicate reduced SWA in thalamic compared to extrathalamic patients. Significant electrodes (after statistical nonparametric cluster correction) are indicated with white dots. (C) The same analysis as in B was performed for other frequency bands. Note that no significant electrode cluster was found.

not indicating such an association. The factor stroke was significant, that is, the overnight slope change remained reduced when accounting for the reduced slope in the first hour (factor stroke $F[1, 22.5] = 7.93$, $p = 0.01$). Finally, a relationship between the overnight slope change and low SWA in this cluster was explored using the same linear mixed-effects model, but with low SWA as fixed factor instead of the slope in the first hour. No significant effect for the factor low SWA was found ($p > 0.3$), not indicating such a relationship.

Relationships between Overnight Slope Changes and Subjective Sleepiness/ Neurocognitive Tests

Associations of subjective sleepiness and neurocognitive tests with overnight slope changes were analyzed using

linear mixed-effects models (Fig 5). Subjective sleepiness tended to be higher and performance worse in patients with reduced overnight slope changes. Although statistical significance was only reached for the ESS and SSS in the morning, 10 of 13 outcome measures pointed in the same direction, which is beyond what would be expected by chance (chi-squared test: $\chi [1] = 3.769$, $p = 0.05$).

Discussion

This study provides the first evidence that thalamic stroke is associated with reduced overnight slow wave slope changes compared to extrathalamic stroke, suggesting a role of the thalamus in synaptic renormalization. Synaptic renormalization generally refers to the overall reduction of synaptic strength during sleep, which is key for restoring the brain's learning capacity and proper cognitive
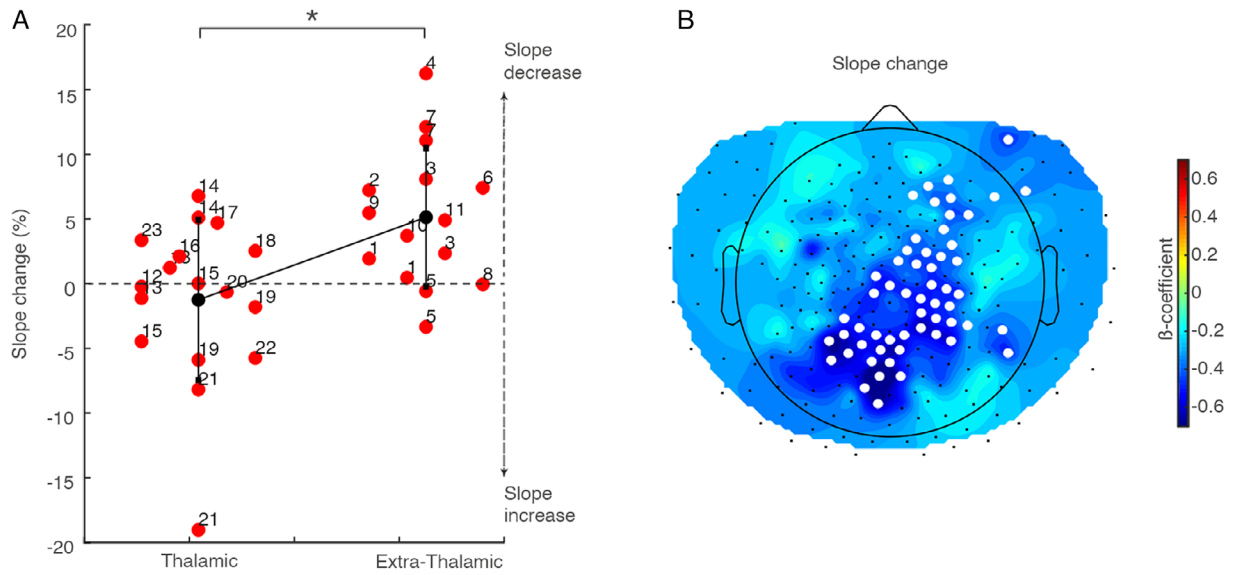
FIGURE 4: Comparison of overnight slow wave slope change between thalamic and extrathalamic stroke patients. To test significant differences, a linear mixed-effects model, with global overnight slope change (average across all electrodes in [A] and for each electrode in [B]) as dependent variable, age, stroke, time after stroke, and the interaction between stroke and time after stroke as fixed factors, and patient as random factor, was calculated. (A) Red dots show global overnight slope change (average across all electrodes) for each night recording. The numbers beside the dots indicate the patient IDs and correspond to the IDs provided in Table S1. Black dots and error bars represent the mean and standard deviation for each group. The horizontal bar and asterisk denote a significant difference between thalamic and extrathalamic patients (factor stroke $F[1, 20.9] = 7.03$, $p = 0.015$; all other factors and interactions $p > 0.4$). (B) Topographical representation of standardized β coefficients for the fixed factor stroke. Negative β coefficients (blue) indicate reduced overnight slope changes in thalamic compared to extrathalamic patients. Significant electrodes (after statistical nonparametric cluster correction) are indicated with white dots.

function after a day of wakefulness.[9] Two mechanisms may underlie this observation: (1) a reduced need for synaptic renormalization after reduced learning-related synaptic potentiation during wakefulness ("wake centered" explanation) or (2) an impairment in the mechanism underlying synaptic renormalization ("sleep centered" explanation). In support of the first, "wake centered" explanation, numerous studies have demonstrated that the thalamus is critical for gating sensory information that is transmitted to the cortex, also referred to as attention.[40] Thus, thalamic lesion may lead to reduced attention, and reduced attention in turn may result in reduced experience-dependent learning and therefore less need for synaptic renormalization. The second, "sleep centered" explanation is supported by the following considerations. Although the mechanism underlying synaptic renormalization is not fully understood, the synaptic homeostasis hypothesis proposes that slow waves may directly contribute to this process.[9] Evidence for an important role of thalamic slow waves for the restorative function of sleep comes from a recent study in which slow waves were induced by optogenetic stimulation of thalamic neurons.[41] Activation of these neurons lead to a faster dissipation of sleep pressure during recovery sleep after sleep deprivation, whereas silencing slowed down this process. Interestingly, in humans auditory closed-loop stimulation has been shown to effectively modulate slow waves and

restoration during sleep.[42] Although the exact mechanism underlying slow wave modulation is not clear, it is likely that it involves the thalamus. Furthermore, in humans both spontaneous slow waves and auditory-evoked slow waves have been shown to originate in frontocentral regions and to involve large cortical areas.[34,42] This fits well with our observation that the reduction in low SWA in thalamic stroke patients was distributed across the whole cortex, but was most pronounced in a frontocentral area. This reduction was specific for low SWA. However, a similar trend of reduced power was observed for the other frequency bands, suggesting that statistical power may have been too low to detect significant differences. Also for spindle power, a reduction has been shown in a previous study comparing thalamic patients to healthy controls.[43] An overall reduction in power might result from reduced neuronal synchronization as has previously been shown in the course of development.[44] Also the overnight slope change was reduced across large areas of the cortex, suggesting a rather global phenomenon. This reduction was specific for large-amplitude waves that have previously been shown to be generated by a mechanism involving the thalamus,[34,35] further suggesting a role of the thalamus in the overnight dynamics of large slow waves. Importantly, the overnight slope change reduction was not associated with the diminished slope in the first hour of artifact-free NREM sleep, nor with low SWA
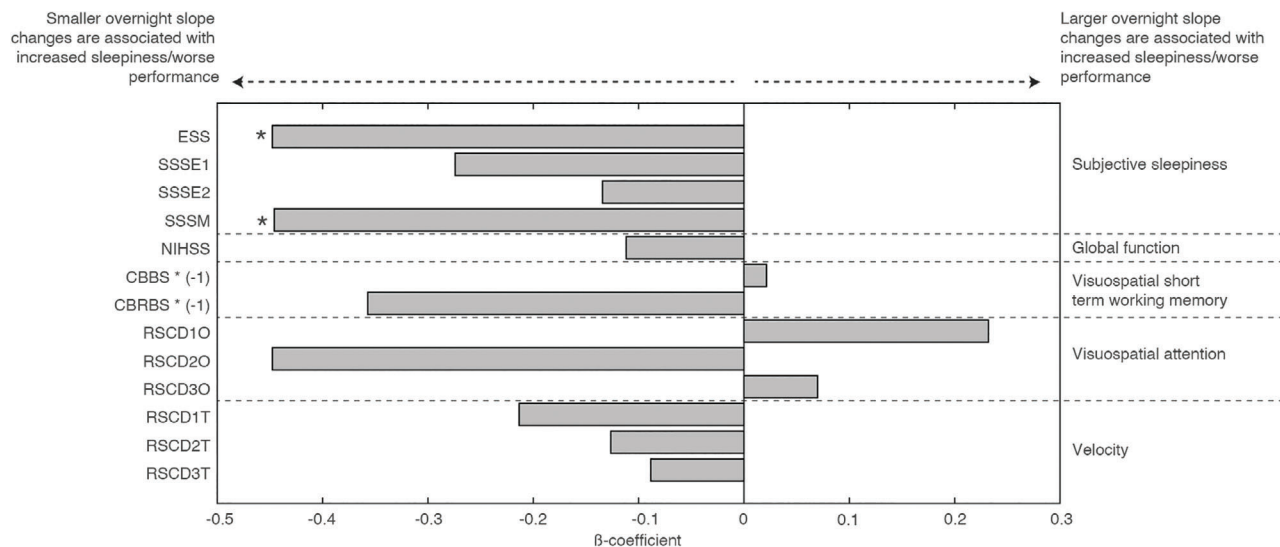
FIGURE 5: Association of overnight slope changes with subjective sleepiness and functional outcome measures across all patients. To test significant associations, linear mixed-effects models, with the subjective sleepiness or the functional outcome measure tested as dependent variable, age and overnight slope change (average across significant electrode cluster in Fig 4B) as fixed factors, and patient as random factor, were calculated. Negative standardized β coefficients indicate that reduced overnight slope changes were associated with increased subjective sleepiness or reduced performance. Significant associations are indicated with an asterisk (for SSSM: factor slope change $F[1, 22] = 6.07$, $p = 0.022$; factor age $p > 0.2$; for ESS: factor slope change $F[1, 19.9] = 4.34$, $p = 0.050$; factor age $p > 0.4$). Note that CBBS and CBRBS were multiplied by -1 to rate good performance in the same direction as for the other measures. Because for the NIHSS only the acute time point was available, a simple linear model was calculated (no random factor). Residual analysis of the linear mixed-effects model with RSCD1O as dependent variable indicated that the assumption of normally distributed residuals was violated and these results should be viewed with caution. CBBS = Corsi block-tapping block span; CBRBS = Corsi block-tapping reverse block span; ESS = Epworth Sleepiness Scale; NIHSS = National Institutes of Health Stroke Scale; RSCD1O = random shape cancellation difficulty level 1 omissions; RSCD1T = random shape cancellation difficulty level 1 time; RSCD2O = random shape cancellation difficulty level 2 omissions; RSCD2T = random shape cancellation difficulty level 2 time; RSCD3O = random shape cancellation difficulty level 3 omissions; RSCD3T = random shape cancellation difficulty level 3 time; SSSE1 = Stanford Sleepiness Scale in the evening before the motor and neurocognitive test batteries; SSSE2 = Stanford Sleepiness Scale in the evening before patients were allowed to sleep; SSSM = Stanford Sleepiness Scale in the morning before the motor and neurocognitive test batteries.

across the night, indicating that the reduction in neuronal synchronization is not directly linked to the reduction in synaptic renormalization. To sum up, both a reduction in attention during wakefulness and an impairment in synaptic renormalization during sleep may account for the reduced overnight slope changes in thalamic stroke patients. Eventually, both mechanisms might contribute and interact with each other to give rise to the reduced overnight slope changes.

From a clinical point of view, our results suggest that thalamic patients are more affected in terms of arousal and/or restoration during sleep than patients with other extrathalamic strokes with an overall comparable stroke severity. Furthermore, our data do not suggest a significant improvement of restorative sleep over time. This question remains, however, to be addressed more systematically by longitudinal comparisons in future studies. Although previous studies reported increased sleep needs and cognitive changes in paramedian thalamic stroke patients, these studies did not investigate the possibility of a direct relationship between sleep EEG changes and daytime sleepiness.[18,19] The current data suggest for the first time a link between

overnight slope changes and subjective daytime sleepiness. This difference may be related to the previous studies focusing on sleep architecture or SWA and not considering a more direct marker of sleep-dependent restoration. Thus, overnight slope changes might represent a useful marker for detecting arousal- or sleep-related impairments in stroke patients in addition to subjective sleepiness questionnaires, behavioral assessments, and actigraphy.[45] The lack of significant differences in other functional outcome measures between thalamic and extrathalamic patients may be explained by the absence of impairments in either of the groups, or alternatively, by the presence of a reduced performance in both groups. In the Corsi block-tapping task for visuospatial short-term working memory, both groups were on average below the norm, which is a block span of 6.2 for the forward tapping task, suggesting an impairment in both groups.[31] However, the norm was collected in younger individuals and with a slightly different methodology, which might also account for the reduced block span. In the random shape cancellation task for visuospatial attention, both groups seemed to perform well, considering that the threshold for diagnosing a visuospatial attention

impairment is >12.5% omissions[32] and on average only the thalamic group reached this threshold for the highest difficulty level. Although no group differences were found, reduced overnight slope changes tended to be associated with worse performance on the neurocognitive tests, suggesting that reduced sleep-dependent restoration might be at the core of impaired cognitive performance after stroke. This is in agreement with a previous study showing that poor functional outcome after thalamic stroke is predicted by increased sleep needs.[19] Moreover, hypersomnia may also increase the probability of institutionalization (increasing the economic burden) and long-term mortality. A retrospective study of 213 patients admitted to a stroke-specialized rehabilitation unit revealed that patients with hypersomnia were 10 times more likely to go to a nursing home.[46] Another study that followed stroke patients 2 years after the stroke found that always feeling tired was a predictor for institutionalization, dependency on help in daily activities, and death cases after 3 years.[47] Therefore, early recognition of insufficient restoration during sleep is important and might represent a new therapeutic window in the management of stroke patients.

Seven main limitations of our study should be mentioned. (1) Because the study did not include a healthy control group, it was not possible to determine whether the observed changes are due to alterations in the thalamic or the extrathalamic group, and the other way around, whether the lack of differences was driven by no impairments in either group or by impairments in both groups. Given that the slope of slow waves has been shown to decrease across the night in healthy controls but not in pathological conditions,[16,17,48,49] it seems likely that the reduction of overnight slope changes in thalamic stroke patients is pathological. (2) Patients were measured at a very acute time point (on average 2.7 days poststroke for the acute measurement). A general problem for studies in early poststroke patients is that sleep might not be impaired due to the brain damage per se but rather because of the unfavorable environment at the hospital (noise, light, touch, etc). In addition, sleep-disordered breathing is highly common in stroke patients (>50%) and might affect restoration during sleep.[45] However, this study corrected for these confounders as much as possible by comparing 2 patient groups, which were measured in the same clinical setting and did not differ in terms of sleep quality or AHI. (3) Patients were dichotomized into a thalamic and an extrathalamic group based on whether the thalamus was affected. This dichotomization may constitute a simplification, as more in-depth lesion topography might affect restoration during sleep differently. For example, paramedian thalamic stroke patients have previously been shown to display increased sleepiness after the

stroke.[18,19] Due to the low number of patients per thalamic region, a subgroup analysis was not feasible with respect to the sample size. Moreover, as most patients with thalamic strokes had associated extrathalamic lesions, it cannot fully be excluded that the observed findings are driven by the extrathalamic lesions. For example, lesion extension to the hypothalamus and/or the midbrain was observed in 6 of 12 thalamic patients. (4) The majority of extrathalamic stroke patients had left-sided lesions, whereas thalamic patients more often displayed right-sided lesions. Due to this high collinearity, it cannot fully be excluded that group differences were in part driven by laterality. There is evidence for a right hemispheric dominance in attentional functions and a resulting dysregulation of attention following right-sided lesions.[50] (5) Two night recordings were available for few patients, and therefore longitudinal comparisons of acute to subacute recordings were not possible. (6) Cognitive testing was only performed in patients who were awake and able to understand the tasks. Therefore, our functional outcome analysis was biased toward more mildly affected patients, making it more difficult to detect associations between overnight slope changes and functional outcome measures. Furthermore, due to several missing test sessions, evening and morning sessions were pooled, which prevented the analysis of sleep-related performance improvements from evening to morning sessions. (7) The thalamus has been shown to be important not only for the generation of slow waves, but also for the generation of sleep spindles, another prominent NREM sleep oscillation. Analysis of spindles (and how they are coupled to slow waves) was beyond the scope of this paper but was the focus of another recent paper in thalamic stroke patients.[43]

Taken together, our study suggests that restoration during sleep is impaired after thalamic stroke. Future studies are needed to further assess how this alteration affects functional outcome during early and later poststroke reorganization. To test a causal relationship between sleep and functional outcome measures, interventional studies, in which sleep is modulated, are needed. Finally, sleep modulation should be considered as a therapeutic option in patients suffering from hypersomnia after thalamic stroke in addition to other treatment strategies such as stimulant medication.[45]

Acknowledgments

Author Contributions

R.H., C.L.A.B., A.Me., N.C.H., and A.K.E.-M. contributed to conception and design of the study. V.J., J.J., A. Ma., A.Me., N.C.H., A.K.E.-M., and R.W. contributed to acquisition and analysis of data. V.J., J.J., A.Ma., R.W., R.H., and C.L.A.B. contributed to drafting a significant portion of the manuscript or figures.

Potential Conflicts of Interest

Nothing to report.

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