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Original Article

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Tonic-clonic seizures induce hypersomnia and suppress rapid eye movement sleep in mouse models of epilepsy

Ruizhi Wang[1,2], Sasa Teng[1,2], Matt Turanchik[3], Fenghua Zhen[4] and Yueqing Peng[1,2,*],

[1]Department of Neurology, Vagelos College of Physicians and Surgeons, Columbia University, New York, NY, USA,
[2]Department of Pathology and Cell Biology, Vagelos College of Physicians and Surgeons, Columbia University, New York, NY, USA,
[3]Columbia School of General Studies, Columbia University, New York, NY, USA and
[4]National Institute of Allergy and Infectious Diseases, Bethesda, MD, USA

[*]Corresponding author. Yueqing Peng, Department of Neurology, Vagelos College of Physicians and Surgeons, Columbia University, New York, NY 10032, USA. Email: yp2249@cumc.columbia.edu.

Abstract

The reciprocal relationship between sleep and epilepsy has been reported by numerous clinical studies. However, the underlying neural mechanisms are poorly understood. Animal models of epilepsy are powerful tools to tackle this question. A lagging research area is the understudied sleep in epilepsy models. Here, we characterize sleep architecture and its relationship with seizures in a mouse model of sleep-related hypermotor epilepsy, caused by mutation of *KCNT1*. We demonstrated that nocturnal tonic-clonic seizures induce more non-rapid eye movement (NREM) sleep but suppress rapid eye movement (REM) sleep, resulting in altered sleep architecture in this mouse model. Importantly, the seizure number is quantitatively anticorrelated with the amount of REM sleep. Strikingly, this modulation of NREM and REM sleep states can be repeated in another mouse model of epilepsy with diurnal tonic-clonic seizures. Together, our findings provide evidence from rodent models to substantiate the close interplay between sleep and epilepsy, which lays the ground for mechanistic studies.

Key words: epilepsy; tonic-clonic seizures; Sleep; EEG; Kcnt1

Statement of Significance

Clinical reports show that seizures can modulate subsequent sleep and wake states. In this study, we performed sleep analysis and its modulation by seizures in two mouse models of epilepsy, one with nocturnal seizures and the other with diurnal seizures. This study is the first to characterize sleep architecture in a *Kcnt1* mouse model of sleep-related hypermotor epilepsy. Then, in both models, tonic-clonic seizures induce hypersomnia and suppress rapid eye movement (REM) sleep, recapitulating the observations in patients with epilepsy. Importantly, the number of seizures is quantitatively anticorrelated with the amount of REM sleep. This study highlights the capability of tonic-clonic seizures in modulating brain states. Our findings in mouse models pave the way for mechanistic studies underlying sleep and epilepsy.

The interrelationship between sleep and epilepsy has long been recognized [1–5]. On one hand, wake and sleep states can modulate seizure occurrence [6]. For just two of the many diverse examples, seizures in patients with mesial temporal lobe epilepsy (MTLE) occur more frequently during wakefulness [7], while patients with frontal lobe epilepsy almost exclusively have seizures during non-rapid eye movement (NREM) sleep (previously called nocturnal frontal lobe epilepsy or NFLE, now called sleep-related hypermotor epilepsy or SHE) [8, 9]. Clinically, seizures occurring exclusively (>90%) from the sleep state account for a significant number—around 12% of those with epilepsy [2, 10]. Moreover, one-third of SHE patients are medically refractory and most have many seizures per month [11, 12].

On the other hand, both diurnal and nocturnal seizures affect sleep architectures [3]. Clinical studies reported that patients with epilepsy have worse sleep quality compared to controls [13]. In addition, they often display comorbid sleep disorders, including sleep apnea, insomnia, and parasomnias [14]. With the advances in human genetics and genomics, more genes involved in epilepsy have been identified [15–19], and corresponding mouse models have been generated [20–23]. Sleep alterations have been reported in several rodent models of epilepsy [24–26]. For instance, *Cntnap2*

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knockout rats exhibit motor seizures, hyperactivity, and abnormal sleep-wake patterns [26].

The familiar form of SHE has autosomal dominant inheritance (i.e. ADSHE) [27]. Human genetic studies have identified several genes associated with ADSHE, including *CHRNA4*, *CHRNB2*, *CHRNA2*, *KCNT1* [28], *DEPDC5*, *NPRL2*, and *CRH* [29]. Mutations in KCNT1 cause a range of epilepsies including ADSHE, early infantile epileptic encephalopathies, and malignant migrating focal seizures of infancy [30–32]. Notably, most patients with *Kcnt1* mutations have seizures that are drug-resistant [30]. Several mouse models of SHE have become available, notably mutations of *CHRNA4* [33, 34] and *CHRNB2* [35–37]. These models display abnormal excitability and are generally accompanied by disturbed sleep [29]. Although their wake and sleep patterns were analyzed based on the locomotor activity [35], to our knowledge, no electroencephalogram (EEG)-based sleep characterization has been performed. Recently, we have generated a mouse model of SHE with a missense mutation in *Kcnt1* (Y777H) [38]. In this *Kcnt1*^{Y777H} model, we demonstrated that homozygous mutant mice display spontaneous tonic-clonic seizures, mostly in the light phase [38]. Furthermore, interictal epileptiform discharges in *Kcnt1* mice are mostly localized to the frontal cortex [38]. These phenotypes recapitulate the signature features of those in KCNT1-associated ADSHE patients. Notably, Zhang et al. independently generated a similar Y777H mouse model, which exhibits anxious behavior [39]. However, no sleep analysis was performed.

In this study, we analyzed the relationship between sleep and seizures in two mouse models of epilepsy. In the *Kcnt1* mouse model, we first examined if seizures are modulated by sleep states. Next, we characterized sleep architecture in *Kcnt1* mice. Then, we examined if/how seizures modulate subsequent brain states. We found that spontaneous seizures in Kcnt1 mice predominately occur during NREM sleep, and subsequently induce more NREM sleep and suppress REM sleep, leading to sleep alterations. Finally, we repeated the correlation analysis between sleep and seizures in another mouse model of epilepsy, induced by altered neural activity in the dentate gyrus. Interestingly, the same modulation of seizures on sleep is confirmed in this different model. Taken together, our data in mouse models indicate that tonic-clonic seizures induce hyposomnia and suppress REM sleep.

Materials and Methods

Animals

All procedures were carried out in accordance with the US National Institute of Health (NIH) guidelines for the care and use of laboratory animals, and approved by the Animal Care and Use Committees of Columbia University. The following mouse lines were used in the current study: C57BL/6J (JAX 000664) and *Kcnt1*. The *Kcnt1*^{Y777H} mice were generated in the C57BL/6NJ (B6NJ) mouse strain (JAX stock # 005304) using CRISPR/Cas9 and an oligonucleotide donor sequence as part of the Jackson Laboratory Center for Precision Genetics program (JCPG, www.jax.org/research-and-faculty/research-centers/precision-genetics-center), and then maintained by backcrossing heterozygous males to WT C57BL/6J females. Both male and female *Kcnt1* homozygous, heterozygous, and WT mice (10–18 weeks at the time of surgery) were used. In the GCaMP6s-DG model, only male C57BL/6J (10–14 weeks at the time of surgery) were used.

The design of the study and the methods used are reported in accordance with ARRIVE guidelines (https://arriveguidelines.org/arrive-guidelines).

Surgical procedures

Mice were anesthetized with a mixture of ketamine and xylazine (100 mg kg^{-1} and 10 mg kg^{-1}, intraperitoneally), then placed on a stereotaxic frame with a closed loop heating system to maintain body temperature. After asepsis, the skin was incised to expose the skull and a small craniotomy (~0.5 mm in diameter) was made on the skull above the regions of interest. For the GCaMP6s-DG model, a solution containing ~200 nL viral construct (AAV9-CaMKII-GCaMP6s) was loaded into a pulled glass capillary and injected into the dorsal dentate gyrus (AP –1.9 mm, ML 1.4 mm, and DV 1.8 mm) using a nanoinjector (WPI). The DV coordinates are relative to the pial surface. For EEG and electromyogram (EMG) recordings, a reference screw was inserted into the skull on top of the cerebellum. EEG recordings were made from two screws on top of the cortex: (1) 1 mm from the midline and 1.5 mm anterior to the bregma and (2) 1 mm from the midline and 1.5 mm posterior to the bregma. Two EMG electrodes were bilaterally inserted into the neck musculature. EEG screws and EMG electrodes were connected to a PCB board which was soldered with a 5-position pin connector. All the implants were secured onto the skull with dental cement (Lang Dental Manufacturing). After surgery, the animals were returned to home-cage to recover for at least 2 weeks before any experiment. In GCaMP6s-DG and control mice, EEG/EMG data acquired during the 6th to 8th week following the viral injection were used in this study.

EEG recording and analysis

Mouse seizure and sleep behavior were monitored using EEG and EMG recording along with an infrared video camera at 30 frames per second. Recordings were performed for 24–48 hours (light on at 07:00 am and off at 07:00 pm) in a behavioral chamber inside a sound-attenuating cubicle (Med Associated Inc.). Animals were habituated in the chamber for at least 4 hours before recording. EEG and EMG signals were recorded, bandpass filtered at 0.5–500 Hz, and digitized at 1017 Hz with 32-channel amplifiers (TDT, PZ5 and RZ5D, or Neuralynx Digital Lynx 4S). For sleep analysis [25, 40], spectral analysis was carried out using fast Fourier transform (FFT) over a 5 s sliding window, sequentially shifted by 2 s increments (bins). Brain states were semi-automatically classified into wake, NREM sleep, and REM sleep states using a custom-written MATLAB program (wake: desynchronized EEG and high EMG activity; NREM: synchronized EEG with high-amplitude, delta frequency [0.5–4 Hz] activity and low EMG activity; REM: high power at theta frequencies [6–9 Hz] and low EMG activity). The semi-automated classification was validated manually by trained experimenters.

For seizure analysis, FFT of EEG was performed as described above. Then, the "seizure"-power (19–23 Hz) was calculated to extract seizure events based on a threshold of 2–3 standard deviations. We chose the 19–23 Hz band to detect seizures based on its clear separation from normal brain oscillatory activities [38, 41]. Algorithm-detected seizure events were further reviewed by trained experimenters. In Kcnt1 mice, we merged TS and GTCS into one event when their interval was shorter than 10 s.

Statistics

No statistical methods were used to predetermine sample size, and investigators were not blinded to group allocation. No method of randomization was used to determine how animals were allocated to experimental groups. Mice in which post hoc histological examination showed viral targeting or fiber implantation was in the wrong location were excluded from the analysis. Paired *t*-test,

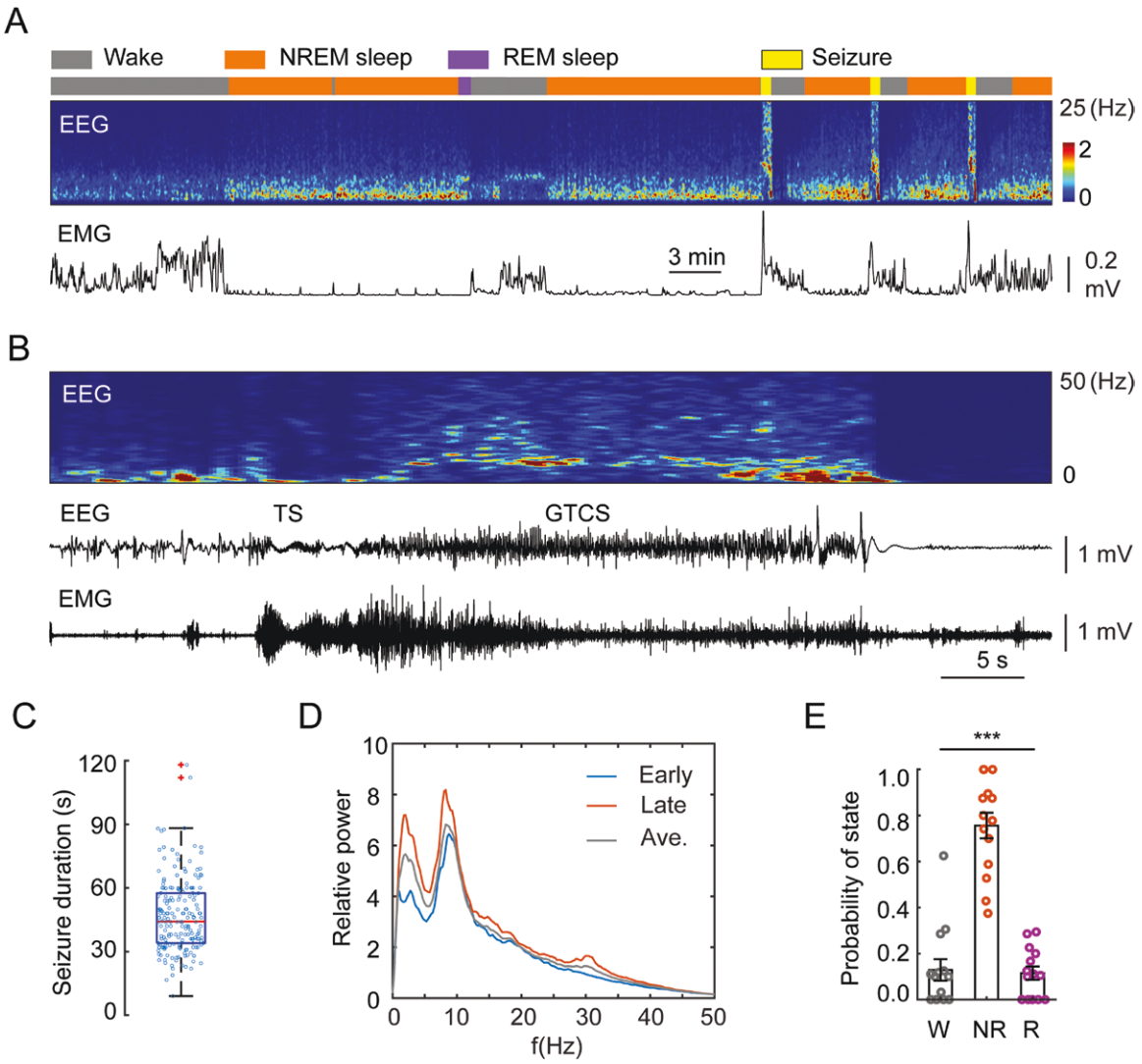

Figure 1. Seizures in *Kcnt1* mice occur during non-rapid eye movement (NREM) sleep. (A) Representative recording session showing seizures occurring during NREM sleep. From top to bottom: color-coded brain states, EEG power spectrogram (0–25 Hz), EMG amplitude in a *Kcnt1* homozygous mouse. (B) Representative example showing EEG spectrogram, EEG trace, and EMG trace during a combinatorial seizure event, including tonic seizure (TS) and generalized tonic-clonic seizure (GTCS). (C) Box plot showing the distribution of seizure durations in *Kcnt1* homozygous mice. The box plot indicates the range, interquartile range, and median (line inside the box). (D) EEG spectral power during early (the first half period, blue line) and late (the second half period, orange line) stages of seizures (195 evens used). The gray line indicates the averaged (Ave.) power during the whole seizure period. (E) Quantification of the probability of each brain state (W-wake, NR-NREM, and R-REM) prior to seizures in *Kcnt1* homozygous mice ($n = 14$ animals). Data are mean ± SEM. ***$p < 0.001$ (one-way ANOVA with post hoc Tukey HSD test, $p < 0.001$ between W and NR, and $p < 0.001$ between NR and R).

and one-way ANOVA were used and indicated in the respective figure legends. All analyses were performed in MATLAB. Data are presented as mean ± SEM.

Results

Seizures in the Kcnt1 *mouse model occur during NREM sleep*

To study epilepsy and sleep, we performed EEG and EMG recordings in *Kcnt1* mutant and control mice. Consistent with our previous study, we observe spontaneous tonic seizures (TS) and generalized tonic-clonic seizures (GTCS) in homozygous *Kcnt1* mice (Figure 1, A and B). TS mostly preceded GTCS but could also occur independently as isolated events. The duration of GTCS events varies between 10 s and 120 s with a median of 44 s (Figure 1C). The spectral power was dynamic during the seizure period, with a notable increase in the lower frequency range (overlapped with the delta range) in the late period (Figure 1, B and D). To investigate if seizures in *Kcnt1* mice are regulated by wake and sleep states, we classified wake and sleep states and then calculated the probability of the brain states prior to the seizure events. We found that seizures in *Kcnt1* mice predominantly occurred during NREM sleep, accounting for 76% of incidents compared to REM sleep and wakefulness (Figure 1E). This aligns with our earlier results showing that seizures were twice as likely to occur during the light cycles [38]. Together, these findings support our conclusion that *Kcnt1* homozygous mice exhibit epileptic traits resembling those of individuals with NFLE (or SHE).

Altered sleep architecture in Kcnt1 *mice*

To investigate the impact of seizures on sleep in *Kcnt1* mice, we performed sleep analysis in wild-type (WT), heterozygous, and homozygous mice. By analyzing the durations of wakefulness,

NREM sleep, and REM sleep across a 24-hour zeitgeber period, we observed significant alterations in sleep-wake patterns between light and dark phases in homozygous mice, compared to the WT and heterozygous mice (Figure 2A). Quantitation of the total duration shows that the homozygous mutant mice displayed decreased wake time, increased NREM sleep, and decreased REM sleep, compared to the WT and heterozygous mice (Figure 2B). Interestingly, the increased NREM sleep mostly happens in the dark cycles, whereas the decreased REM sleep mostly happens in the light cycles. Furthermore, no significant difference was observed between WT and heterozygous mice, which lack spontaneous seizures. We then break down the total duration to the bout number and bout durations for each wake state. Our analysis demonstrates that increased NREM sleep is mostly contributed by the increased bout durations but not the bout number, whereas the decreased REM sleep is due to the decreased bout numbers (Figure 2, C and D).

Next, we analyzed spectral analysis of EEG signals during sleep. Compared to WT and heterozygous mice, homozygous *Kcnt1* mice displayed a slight but not significant decrease in delta power during NREM sleep (Figure 3, A and B). Interestingly, the sigma power was significantly reduced in homozygous *Kcnt1* mice (Figure 3B). The sigma power is an indicator of sleep spindles, which are thought to play a role in the sleep-dependent memory process [42, 43]. During REM sleep, both homozygous and heterozygous *Kcnt1* mice exhibited significantly lower theta power, compared to that in WT mice (Figure 3, C and D). The decreased theta power in *Kcnt1* mice might be related to altered GABAergic neuron excitability and synaptic activity reported in both homozygous [38] and heterozygous *Kcnt1* mice [44]. Notably, homozygous mice also displayed significantly higher delta power during REM sleep (Figure 3D). Both spindles and theta oscillations during sleep play an important role in memory consolidation [45–48]. The decreased sigma and theta power during sleep in homozygous *Kcnt1* mice are in line with memory deficits reported previously [38].

Together, our data revealed altered sleep architecture in the Kcnt1mouse model.

Seizures in Kcnt1 *mice modulate brain states*

We reasoned that sleep alterations in *Kcnt1* mice were caused by seizure events. To investigate how/if seizures subsequently impact brain states, we aligned all detected seizure events and examined the brain states (i.e. wake, NREM sleep, and REM sleep) 30 minutes prior to and 30 minutes after the onset of seizures. Consistent with the previous analysis, the probability of NREM sleep is higher prior to the seizure events (Figure 4A). Immediately following the seizures, the brain state transitioned to wakefulness for a short period (1–5 minutes), then followed by a rebound of NREM sleep (Figure 4A). The NREM rebound is supported by the increased delta power following the seizures (Figure 4, B and C). This finding indicated that seizures cause acute disruption of NREM sleep, but subsequently induce more NREM sleep. Another interesting observation is the almost absence of REM sleep in the 15-minute period following the seizures (Figure 4, A and C). Then, REM sleep is partially recovered in the next 15 minutes (Figure 4C).

Seizures in Kcnt1 *mice suppress REM sleep*

To quantitatively study the relationship between sleep and seizures, we performed linear regression analysis between seizure numbers and different sleep metrics, including the total duration, bout numbers, and bout durations. Our analysis demonstrated a negative correlation between seizures and REM sleep. Both the total duration and bout numbers of REM sleep are significantly anticorrelated with seizure numbers (Figure 5, A and B). On the contrary, there are largely no correlations between seizures and wakefulness and NREM sleep in *Kcnt1* mice (Figure 5, A-C). This indicates that REM sleep is significantly compromised as the seizure rate increases.

Correlation between sleep and seizures in other mouse models of epilepsy

Next, we asked if the modulation effect of seizures on sleep can be generalized in other mouse models of epilepsy. Our previous study demonstrated that viral expression of GCaMP6s in the dentate gyrus induces tonic-clonic seizures [41]. These seizures mostly occur during wakefulness. In this study, we performed sleep analysis and its correlation with seizures in C57BL/6J mice unilaterally injected with AAV9-CaMKII-GCaMP6s (0.2 µL) and AAV9-CaMKII-GFP (0.2 µL) respectively in the dorsal dentate gyrus (termed as GCaMP6s-DG mice, Figure 6A). As discussed previously [41], part of the CA1 neurons was also affected due to the viral spread. EEG/EMG data acquired during the 6th to 8th week following the viral injection were used in this study, during which mice exhibited most spontaneous tonic-clonic seizures [41].

First, compared to GFP control mice, GCaMP6s-DG mice exhibited decreased wake time and REM sleep, and increased NREM sleep (Figure 6B). Similar to the findings in *Kcnt1* mice, the increased NREM sleep mostly occurred during the dark phase, whereas the decreased REM sleep largely occurred during the light phase (Figure 6B). A mild decrease in REM sleep time was also observed in the dark phase in GCaMP6s-DG mice.

Next, we aligned the seizure events and examined the brain states before and after seizures. Consistent with our previous report, GCaMP6s-induced seizures mostly occur during wakefulness, as indicated by the probability of brain states prior to the seizure onset (Figure 6C). Following the seizures, NREM sleep is dominant in the first 15 minutes, which is supported by the increased delta power (Figure 6, C and D). Similar to what was observed in *Kcnt1* mice, REM sleep was absent in the first 15 minutes following seizures, then gradually recovered in the next 15 minutes (Figure 6D). Together, our analysis confirmed the impact of seizures on the subsequent brain states.

Then, we performed linear regression analysis between seizure numbers and different sleep metrics. Similarly, we observed a negative correlation between seizures and REM sleep. Both the total duration and bout numbers of REM sleep (but not bout durations) are significantly anticorrelated with seizure numbers (Figure 7, A-C)), confirming that tonic-clonic seizures suppress REM sleep. In addition, the seizure numbers are negatively correlated with wake time (the total duration and the bout duration) and positively correlated with NREM sleep time (Figure 7, A and C). Together, the correlation analysis in the GCaMP6s-DG model confirmed the close relationship between seizures and sleep alterations.

Discussion

The close relationship between sleep and seizure has been reported in both human patients and animal models. In this study, we characterized tonic-clonic seizures and sleep architecture in a *Kcnt1* mouse model of SHE (or NFLE). We first demonstrate that sleep states regulate seizure occurrence. Then, we show that seizure events modulate subsequent brain states, by

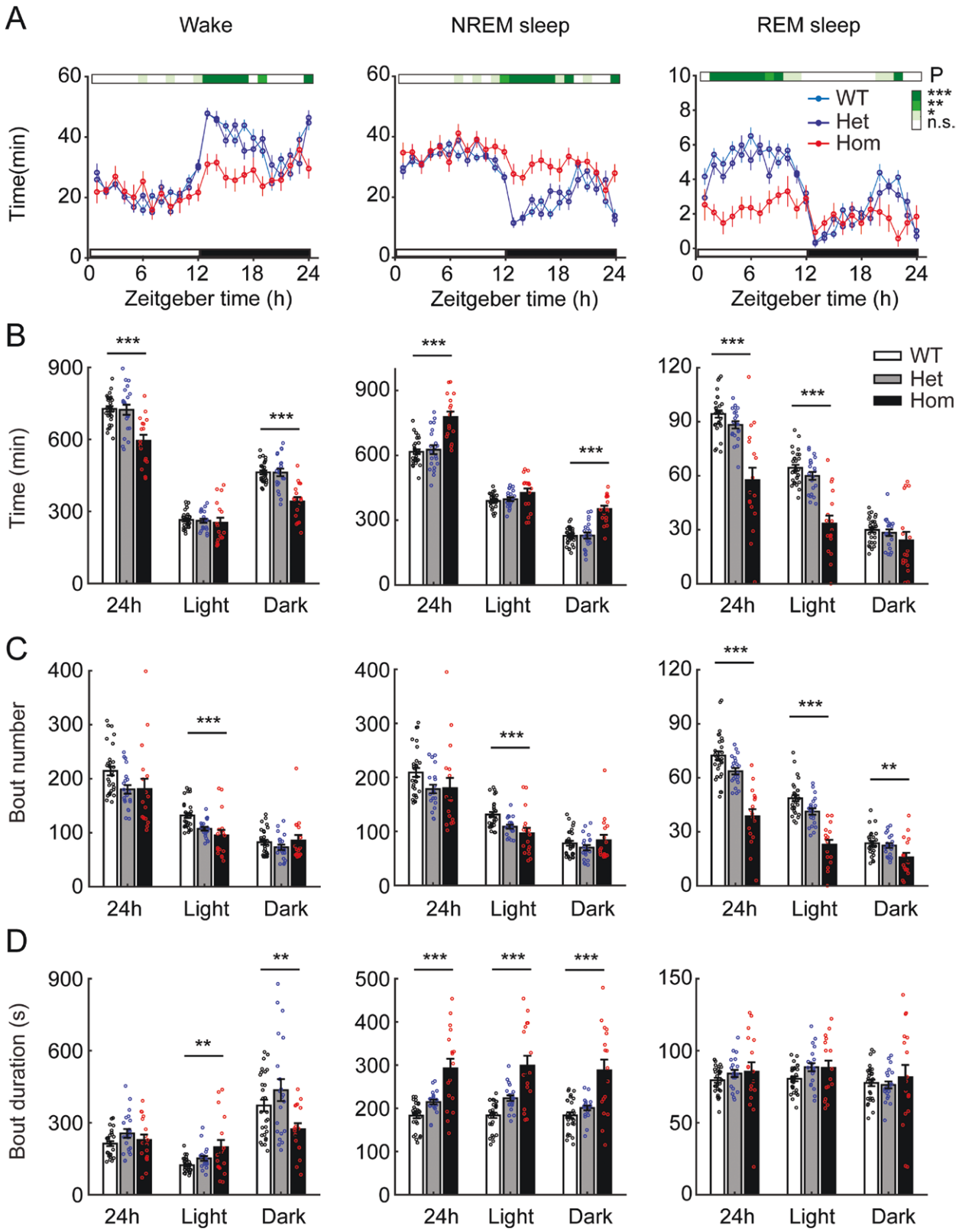

Figure 2. Sleep alterations in *Kcnt1* mice. (A) 24-hour zeitgeber time plots showing the total duration of wakefulness (left), non-rapid eye movement (NREM) sleep (middle), and rapid eye movement (REM) sleep time (right) per hour in *Kcnt1* wild-type (WT), heterozygous (Het), and homozygous (Hom) mice. The top bars indicate the statistical significance at each ZT timepoint (one-way ANOVA). (B) Quantification of total time spent in wake, NREM, and REM sleep across 24 hours, 12-hour light phase, and 12-hour dark phase. (C) Quantification of the number of bouts of wakefulness, NREM sleep, and REM sleep across 24 hours, light phase, and dark phase. (D) Quantification of bout duration of wakefulness, NREM sleep, and REM sleep across 24 hours, light phase, and dark phase. Data are mean ± SEM (n = 29 for WT, n = 22 for Het, and n = 17 for Hom). n.s. not significant. *p < 0.05, **p < 0.01, ***p < 0.001 (one-way ANOVA).

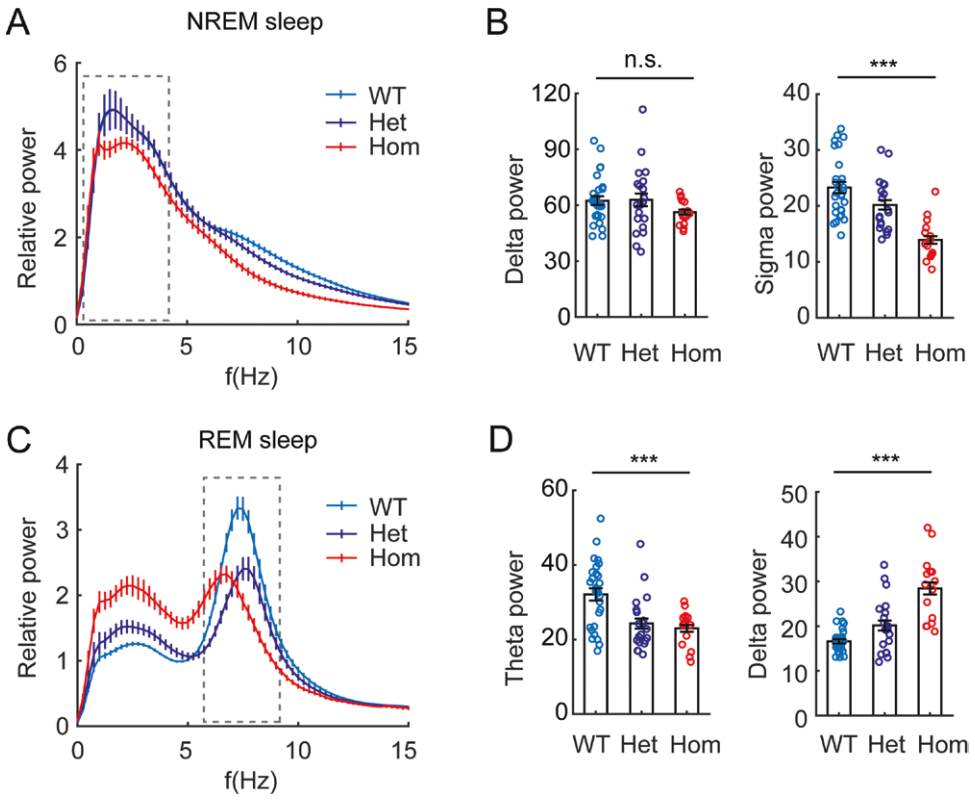

Figure 3. Spectral analysis of sleep states in *Kcnt1* mice. (A) Relative EEG power during non-rapid eye movement (NREM) sleep in *Kcnt1* wild-type (WT), heterozygous (Het), and homozygous (Hom) mice. (B) Quantification of delta power (left, 0.5–4 Hz) and sigma power (right, 9–15 Hz) during NREM sleep. n.s. not significant, ***$p < 0.001$ (one-way ANOVA with post hoc Tukey HSD test; for Sigma power, $p = 0.07$ between WT and Het, $p < 0.001$ between WT and Hom, and $p < 0.001$ between Het and Hom) (C) Relative EEG power during rapid eye movement (REM) sleep in WT, Het, and Hom mice. (D) Quantification of theta power (left, 6–9 Hz) and delta power (right, 0.5–4 Hz) during REM sleep. EEG power was normalized to the total power in each mouse. Data are mean ± SEM ($n = 29$ for WT, $n = 20$ for Het, and $n = 15$ for Hom). ***$p < 0.001$ (one-way ANOVA with post hoc Tukey HSD test; for theta power, $p < 0.01$ between WT and Het, $p = 0.001$ between WT and Hom, $p = 0.87$ between Het and Hom; for delta power, $p = 0.06$ between WT and Het, $p < 0.001$ between WT and Hom, and $p < 0.001$ between Het and Hom).

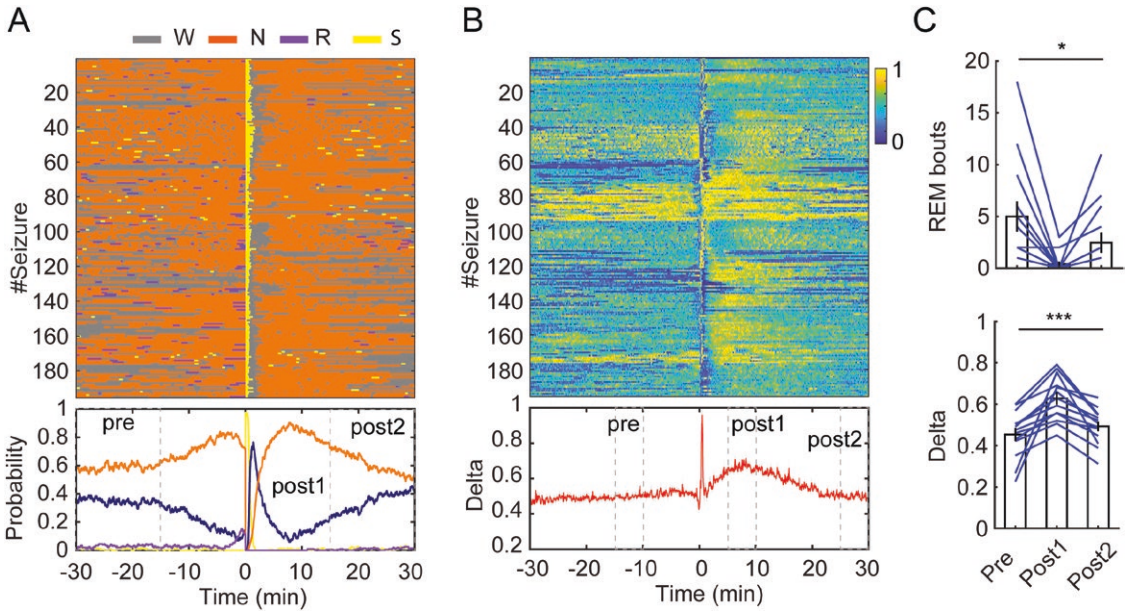

Figure 4. Seizures in *Kcnt1* mice modulate brain states. (A) Top, brain states (W-wake, N-NREM [non-rapid eye movement], R-REM [rapid eye movement], and S-seizure) aligned to the seizure onset in *Kcnt1* homozygous mice (195 evens from 13 animals). Bottom, quantification of the probability of each brain state during the pre-seizure (30 minutes) and post-seizure (30 minutes) periods. Time 0 indicates the seizure onset. (B) Top, representative relative delta power during the 1-hour window centered around the seizures. Bottom, quantification of the averaged delta power over the time. (C) Top, quantification of the number of REM sleep bouts during the pre-seizure (15 minutes, pre) and two post-seizure periods (15 minutes each, post1 and post2) marked in A. Bars are mean ± SEM. Bottom, quantification of the average of relative delta power during the pre-seizure (5 minutes, pre), and two post-seizure periods (5 minutes each, post1 and post2) marked in B. Bars are mean ± SEM. n. s. indicates not significant, *$p < 0.05$, ***$p < 0.001$ (one-way ANOVA with post hoc Tukey HSD test; for REM bouts, $p < 0.01$ between pre and post1, $p = 0.20$ between pre and post2, $p = 0.33$ between post1 and post2; for delta, $p < 0.001$ between pre and post1, $p = 0.60$ between pre and post2, and $p < 0.01$ between post1 and post2).

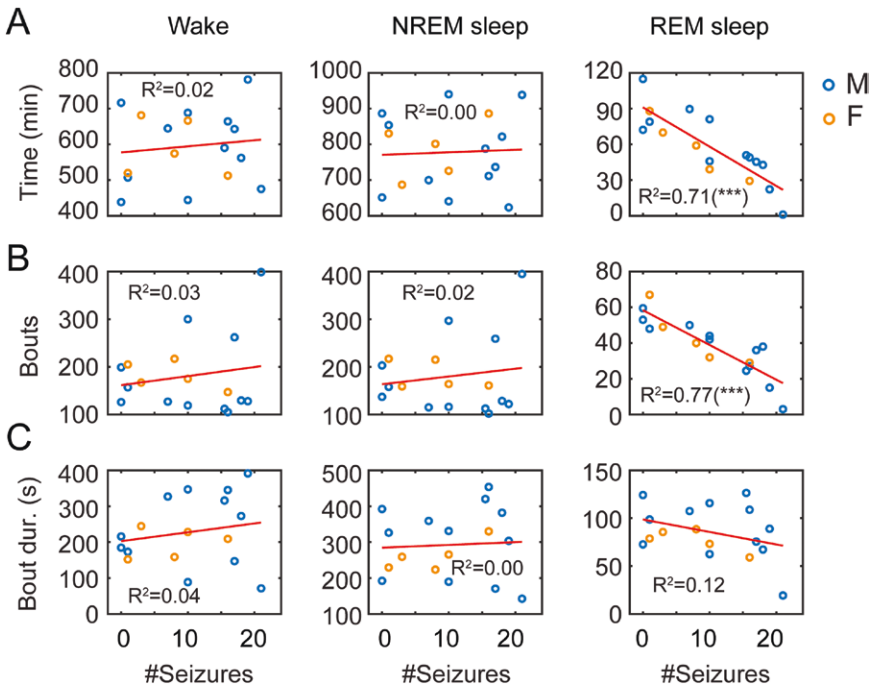

Figure 5. Correlation between sleep and seizures in *Kcnt1* mice. (A) Linear regression analysis of seizure numbers and the total duration of wakefulness (left, p = 0.64), non-rapid eye movement (NREM) sleep (middle, p = 0.85), and rapid eye movement (REM) sleep (right, p = 2.02e^{-5}) in *Kcnt1* homozygous mice (n = 17) including 12 male (M) and 5 female (F). (B) Linear regression analysis of seizure numbers and bout numbers (p = 0.5 for wake, p = 0.56 for NREM sleep, and p = 3.54e^{-6} for REM sleep). (C) Linear regression analysis of seizure numbers and bout durations (p = 0.46 for wake, p = 0.82 for NREM sleep, and p = 0.17 for REM sleep). Each data point indicates one mouse. R^2 for linear regression was included in each plot. ***p < 0.001.

promoting hypersomnia and suppressing REM sleep. The same modulation effect has been confirmed in another mouse model of epilepsy induced by viral expression of GCaMP6s in the dentate gyrus. Together, our studies in animal models provide more evidence to support the reciprocal relationship between sleep and seizure.

It's worth noting that the two models are very different in several key aspects, despite their similar seizure type, i.e. the tonic-clonic seizures. First, the *Kcnt1* model is a genetic model, caused by the missense mutation of a single gene [38], while the second model is viral induced, caused by dysfunction of hippocampal activity [41]. Second, the *Kcnt1* model displays nocturnal seizures, whereas the viral model displays diurnal seizures, mostly during the wake periods. Yet, seizures in both models subsequently promote more NREM sleep and suppress REM sleep. These findings highlight the capabilities of tonic-clonic seizures in regulating brain activity, potentially driven by shared downstream neural mechanisms.

There are some subtle differences in sleep modulation between the two models. *Kcnt1* mice often transitioned to a brief wake state immediately after a seizure event before entering prolonged NREM sleep (Figure 4A), whereas GCaMP6s-DG mice quickly transitioned into NREM sleep (Figure 6C). This discrepancy may be attributed to differences in pre-seizure brain states between the models. In *Kcnt1* mutant mice, seizures typically originate during NREM sleep, while in GCaMP6s-DG mice, seizures are preceded by prolonged neural excitation during wakefulness. Disruption of sleep in *Kcnt1* mice might require additional time for mice to transition back to NREM sleep.

Interestingly, the increased NREM sleep in *Kcnt1* homozygous mutant mice happened most during the dark phase, the active period in mice. In contrast, the incidence of seizures is ~2-fold higher during the light phase, compared to the dark phase [38]. These results suggest that seizures might compromise NREM sleep quality during the light phase, leading to compensatory sleep during the dark period, likely mimicking the excessive daytime sleepiness observed in many neurological diseases [49]. The increased NREM sleep in the dark phase also leads to the flatted circadian rhythm of sleep patterns in *Kcnt1* mutant mice, suggesting the interaction between circadian rhythm and epilepsy [50, 51]. In GCaMP6s-DG mice, the increased NREM sleep also occurred during the dark phase (Figure 6B), coinciding with more seizures during wakefulness, which suggests that the increased NREM sleep might be a direct, not compensatory effect of seizures.

Clinical studies in patients showed that both focal and generalized seizures rarely occur during REM sleep [52]. Consistent with this, we demonstrated that the probability of nocturnal seizures in *Kcnt1* mice is significantly lower during REM sleep, compared to that during NREM sleep. The argument is that REM sleep has a neuroprotective effect compared to other brain states, although the underlying neural mechanisms are unknown. Interestingly, our analysis also revealed that seizures suppress subsequent REM sleep and are strongly associated with the overall reduction of REM sleep, which is in line with clinical studies [4, 5, 53]. The decreased REM sleep mostly occurred during the light phase, when more seizures were present in *Kcnt1* mutant mice. This suggests a more direct modulation of the seizures on REM sleep. Indeed, our analysis revealed the absence of REM sleep during the first 15 minutes after a seizure event in both mouse models (Figures 4 and 6). Furthermore, the amount of REM sleep is highly anticorrelated with the number of seizures (Figures 5 and 7).

Despite the close relationship between sleep and epilepsy, our understanding of underlying neural mechanisms is incomplete. Key to this question will be the identification of the most relevant

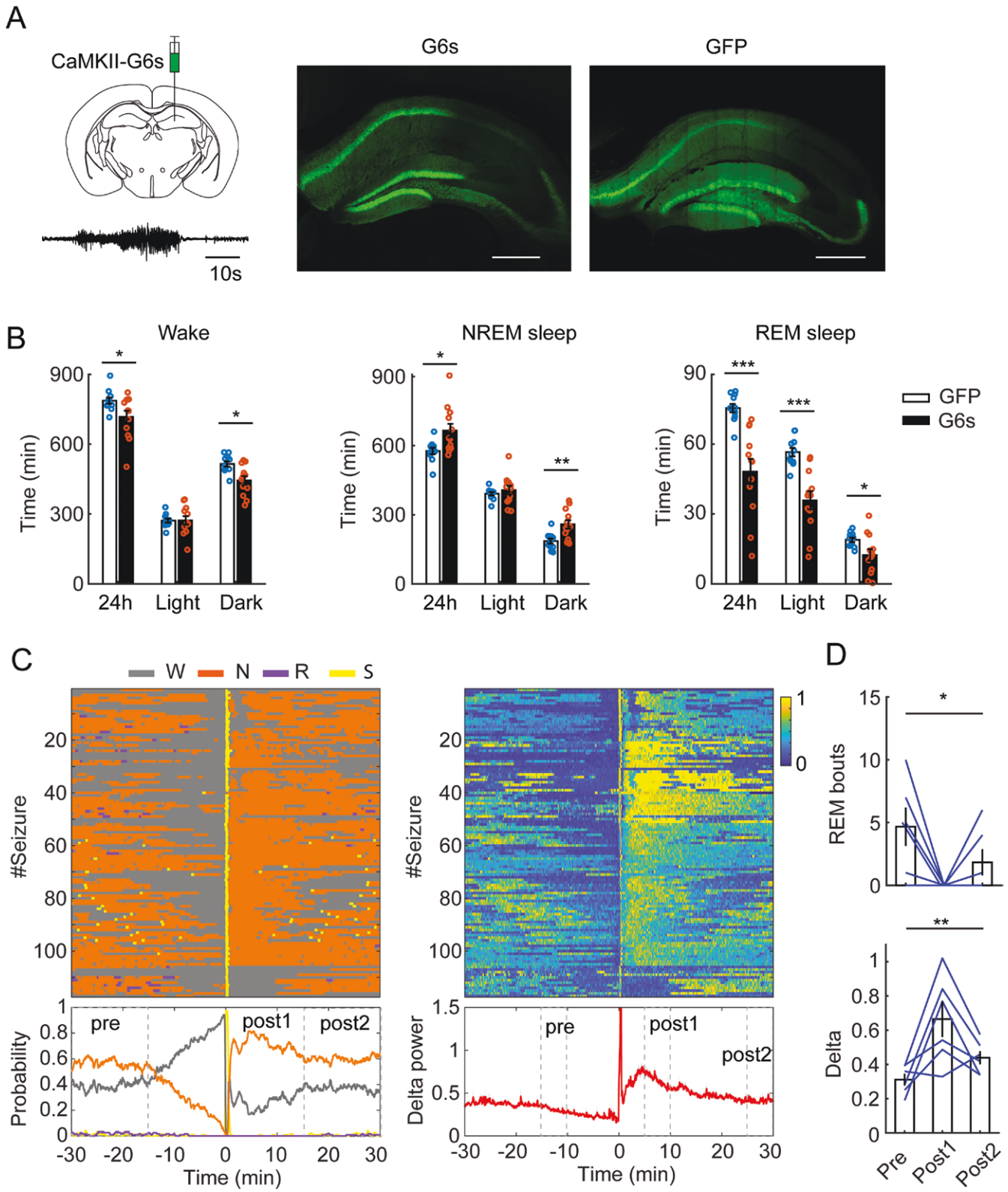

Figure 6. Modulation of brain states by seizures in GCaMP6s-DG mice. (A) Left top, schematic of experimental design showing viral injection of AAV9-CaMKII-GCaMP6s (G6s) or AAV9-CaMKII-GFP in the hippocampus. Left bottom, a representative EEG trace showing a seizure event. Right, representative fluorescent images showing viral expression of GCaMP6s (G6s) and GFP in the hippocampus. (B) Quantification of total time spent in wake, non-rapid eye movement (NREM), and rapid eye movement (REM) sleep across 24 hours, 12-hour light phase, and 12-hour dark phase in GCaMP6s ($n = 12$) and GFP ($n = 11$) mice. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ (paired t-test). (C) Left, brain states (W-wake, N-NREM, R-REM, and S-seizure) centered around seizure events and the probability of each brain states during the pre-seizure (30 minutes) and post-seizure (30 minutes) periods in GCaMP6s-DG mice. Time 0 indicates the seizure onset. Right, relative delta power spectrum of brain activity during the 1-hour window centered around the seizures. (D) Top, quantification of the number of REM sleep bouts during the pre-seizure (15 minutes, pre), and two post-seizure periods (15 minutes each, post1 and post2) marked in C left. Bottom, quantification of the average of relative delta power during the pre-seizure (5 minutes, pre), and two post-seizure periods (5 minutes each, post1 and post2) marked in C right. Data are mean ± SEM. n. s. not significant, *$p < 0.05$, **$p < 0.01$ (one-way ANOVA with post hoc Tukey HSD test; for REM bouts, $p < 0.05$ between pre and post1, $p = 0.18$ between pre and post2, $p = 0.46$ between post1 and post2; for delta, $p < 0.01$ between pre and post1, $p = 0.39$ between pre and post2, and $p = 0.08$ between post1 and post2).

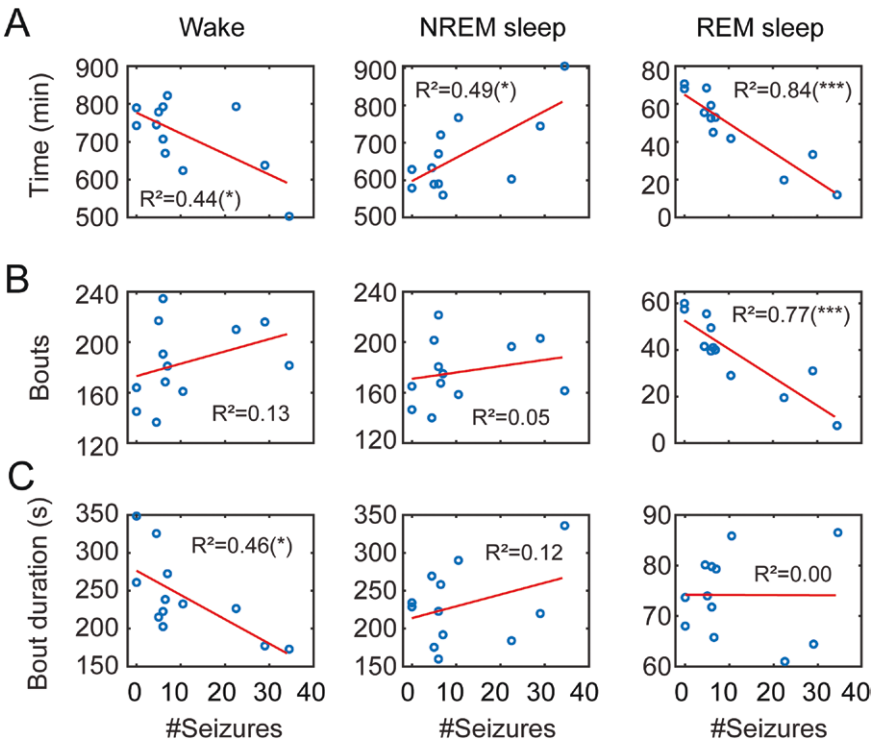

Figure 7. Correlation between sleep and seizures in GCaMP6s -DG mice. (A) Linear regression analysis of seizure numbers and the total duration of wakefulness (left, p = 0.0193), NREM sleep (middle, p = 0.0118), and REM sleep (right, p = 0.00003) in GCaMP6s-DG mice (*n* = 12). (B) Linear regression analysis of seizure numbers and bout numbers (p = 0.248 for wake, p = 0.474 for NREM sleep, and p = 0.0002 for REM sleep). (C) Linear regression analysis of seizure numbers and bout durations (p = 0.0152 for wake, p = 0.272 for NREM sleep, and p = 0.988 for REM sleep). Each data point indicates one mouse. R^2 for linear regression was included in each plot. ***p < 0.001.

brain regions and cell types, particularly those involved in sleep regulation. Huguenard and his colleagues have proposed that the sleep circuitry under pathological conditions can be hijacked to generate aberrant activity, such as epilepsy [54]. We believe that the *Kcnt1* mouse model offers a great opportunity for mechanistic studies to test this hypothesis.

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Author Contributions

Ruizhi Wang (Conceptualization [equal], Data curation [equal], Formal analysis [equal], Methodology [equal], Writing—original draft [equal]), Sasa Teng (Formal analysis [equal], Methodology [equal]), Matt Turanchik (Formal analysis [equal], Methodology [equal]), Fenghua Zhen (Conceptualization [equal], Methodology [equal]), and Yueqing Peng (Conceptualization [equal], Data curation [equal], Formal analysis [equal], Funding acquisition [equal], Investigation [equal], Methodology [equal], Project administration [equal], Software [equal], Writing—review & editing [equal])

Data Availability

Custom scripts for sleep and seizure analysis are available from the corresponding author upon reasonable request. All data supporting the findings of this study are available from the corresponding author upon reasonable request.

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