REM sleep is associated with distinct global cortical dynamics and controlled by occipital cortex

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The cerebral cortex is spontaneously active during sleep, yet it is unclear how this global cortical activity is spatiotemporally organized, and whether such activity not only reflects sleep states but also contributes to sleep state switching. Here we report that cortex-wide calcium imaging in mice revealed distinct sleep stage-dependent spatiotemporal patterns of global cortical activity, and modulation of such patterns could regulate sleep state switching. In particular, elevated activation in the occipital cortical regions (including the retrosplenial cortex and visual areas) became dominant during rapid-eye-movement (REM) sleep. Furthermore, such pontogeniculooccipital (PGO) wave-like activity was associated with transitions to REM sleep, and optogenetic inhibition of occipital activity strongly promoted deep sleep by suppressing the NREM-to-REM transition. Thus, whereas subcortical networks are critical for initiating and maintaining sleep and wakefulness states, distinct global cortical activity also plays an active role in controlling sleep states.

The brain comprises a highly interconnected, complex neural network. The overall dynamic changes of neural activity within this network are collectively referred to as “brain states”. Wakefulness and sleep are distinct brain states that, in mammals, are mainly defined by activity patterns of the thalamocortical system, as reflected by electroencephalogram (EEG) and motor activity. The different sleep stages are also observed in non-mammalian species. Distinct cortical activity associated with various sleep-wake stages is generally considered a reflection of each sleep stage and plays a limited role in sleep state switching, although emerging evidence supports an active role for the cortex in controlling sleep as well. However, from the perspective of network theory, the activity of key nodes within a complex network may have a broad impact on various properties of the network. Therefore, by providing many critical nodes of the brain network, the cerebral cortex may play an active role in regulating the dynamic change of brain states, including the transition between different sleep stages.

On the other hand, it is increasingly recognized that even simple behaviors may require coordinating neural activity in multiple brain regions. Consistent with this notion, sleep control has also been shown to involve a large number of brain regions that are distributed primarily in subcortical areas. It is thus important to obtain a global view of how ongoing activity in various brain regions contributes to the regulation and function of sleep. Recent developments in large-scale, high-speed recording, and manipulation of global neural dynamics at the mesoscale allow us to dissect the role of various cortical regions. In this study, we used “global neural activity” to denote the large-scale neural activity across large areas in the brain, for example, the entire dorsal part of the cortex or the whole brain.

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It is now generally appreciated that the brain is highly active during sleep\textsuperscript{16}. There are characteristic differences between global neural activity patterns during sleep and wakefulness, as revealed by EEG recording or functional magnetic resonance imaging (fMRI) recording in mammals, although with relatively low resolution\textsuperscript{2,17,18}, or by cellular resolution imaging in non-mammalian species\textsuperscript{4,19}. However, due to the considerable differences in the brain networks of different evolved animals, it is necessary to measure the global network dynamics of the mammalian brain during different sleep states with a high spatiotemporal resolution, and determine how these activity control sleep.

In the current study, we used mesoscale Ca\textsuperscript{2+} imaging to examine the global neural activity feature in sleep with a high spatiotemporal resolution from the entire dorsal cortex of mice, and uncovered an occipital activity pattern that is essential for promoting the transition from NREM to REM sleep. Our results demonstrate that dynamic cortical activity could play an active role in regulating sleep states.

**Results**

**Mesoscale Ca\textsuperscript{2+} imaging of cortical activity during sleep**

To determine whether there are specific global activity patterns that associate with different sleep states, we recorded the macroscopic neuronal activity dynamics with a high spatiotemporal resolution using widefield-of-view optical imaging in the mice. We measured Ca\textsuperscript{2+} activity from the entire dorsal part of the cortex from Thy1-GCaMP6s mice\textsuperscript{20} using a transparent skull preparation (mice with intact but optically transparent skulls)\textsuperscript{21,22}, when the mice were trained to sleep under the microscope (Fig. 1a and Supplementary Movie 1). The Thy1-GCaMP6s mice express a genetically encoded Ca\textsuperscript{2+} indicator, GCaMP6s\textsuperscript{23}, driven by the Thy1 promoter, providing stable access to pyramidal neurons’ activity in cortical layers 2/3 and layer 5\textsuperscript{2,14}. The current method allows the recording of Ca\textsuperscript{2+} activity from the motor, somatosensory and visual cortices, and some association areas\textsuperscript{2,12,24} (Fig. 1b, c). On the other hand, activity measured with mesoscale Ca\textsuperscript{2+} imaging reflects the summation of Ca\textsuperscript{2+} signals from a large number of neurons, so it may not be equivalent to electrophysiologically recorded population spiking activity. In this study, we used “cortical activity” to specifically refer to the Ca\textsuperscript{2+} signals obtained with widefield imaging.

During imaging, mice were head-fixed under the microscope with body and paws moving freely, and no external stimulus was applied. All imaging experiments were performed during the day with the lights on. EEG (from the left auditory cortex) and EMG (from the neck muscle) were recorded to determine the sleep-wake states of the mice. To

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**Fig. 1** Mesoscale Ca\textsuperscript{2+} imaging of the dorsal cortex during the sleep-wake cycle.

- **a** Schematic diagram depicting the mesoscale Ca\textsuperscript{2+} imaging from head-fixed mice.
- **b** Example field-of-view (time-averaged) of the dorsal cortex imaged through the transparent skull of a Thy1-GCaMP6s mouse. Scale, 1 mm. **c** Cortex atlas for alignment. MOs secondary motor cortex, MOp primary motor cortex, Ssb somatosensory cortex, barrel field, SSu somatosensory cortex, upper limb, SSI somatosensory cortex, lower limb, VIs primary visual cortex, VIs association visual cortex, RSPd dorsal retrosplenial cortex, RSPl lateral retrosplenial cortex. **d** Representative Ca\textsuperscript{2+} activity during different brain states. Scale (ΔF/Φ, z-score): Wake, −1.2 to 1.2; NREM, −1.2 to 1.2; REM, −2 to 2. Black bar, 1 mm. **e** Normalized activation across the recorded brain regions in different brain states. The averaged activity in each brain state was z-score normalized across the nine brain regions; thus, positive values mean more activation. n = 15 sessions from 5 mice. Data are mean ± SEM. Raw data for e are provided in a Source Data file.
score the brain states, we performed fast Fourier transforms (FFTs) on EEG to extract the $\delta$ ($0.5-4$ Hz) and $\theta$ ($6-10$ Hz) activity. Brain states were classified according to established criteria: Wakefulness, desynchronized EEG and high EMG activity; NREM, synchronized EEG with high $\delta$ activity and low EMG activity; REM, high EEG $\theta$ power and low EMG activity. The habituated mice spent the majority of time in sleep (NREM: $65\%$, Wake: $27\%$, REM: $8\%$; Supplementary Fig. 1), with diverse facial movements and occasionally gross body movements during wakefulness.

Various movements profoundly shape cortical activity, producing distinct patterns (Fig. 1d and Supplementary Movie 2). For example, we found that the facial movements (extracted from the face camera) were significantly associated with elevated Ca$^{2+}$ activity in the somatosensory cortex and motor cortex during wakefulness (Supplementary Fig. 2). On the other hand, we also observed highly structured activity patterns during sleep (Fig. 1d and Supplementary Movie 2), suggesting region-specific dynamic neuronal activations in sleep$^7$. To be noted, consistent with previous reports$^8$, the head-fixed mice in our recording typically slept with their eyes open, and the pupil size varied in different sleep states (Supplementary Fig. 3). The change in pupil size can have significant effects on cortical activity, especially in the visual cortex. However, multiple lines of our evidence suggest that changes in pupil size did not significantly contribute to the cortical activity observed in our recording: First, although both the pupil size and activity in the primary visual cortex (V1) showed oscillatory dynamics during NREM sleep, there was no positive correlation between these two signals; instead, they tended to be negatively correlated (Pearson’s $r = -0.41 \pm 0.041$, mean $\pm$ SEM; Supplementary Fig. 3a, b), with a phase difference close to zero ($0.50 \pm 0.85$ s, mean $\pm$ SEM; Supplementary Fig. 3c). This negative correlation suggests that both cortical activity and pupil reflect different aspects of brain state oscillations during NREM sleep. Second, during REM sleep, the pupil remains mostly constricted$^8$ however, V1 activity often showed large transient increases (Supplementary Fig. 3d, e). These results support that cortical activity during sleep is mainly spontaneous, unlike during wakefulness.

We began by analyzing the activity of each brain region during different sleep-wake states, and found three types of modulation that

![Figure 2](https://example.com/figure2.png)

**Fig. 2 | Anterior-posterior organization of cortical activity during sleep.**

a Regional activity correlation matrix during Wake (left), NREM (middle), and REM (right) in one example recording (~70 min). The pairwise correlation was computed using neural activity during Wake, NREM, or REM in each small patch (~10 x10 pixels) of the brain region. Brain regions with similar activity profiles were grouped together by applying a hierarchical clustering analysis. Scale (cc), -1 to 1. b Cortical networks revealed by the hierarchical clustering analysis. The dendrogram shows each grouped brain region (highlighted in yellow) with similar activity. c Regions of the somatic sensorimotor network and the medial network. Shown are regions of the two networks averaged from 15 recordings. Scale, 1 mm.
were strongly associated with functions and anatomical proximity of different brain regions (Supplementary Fig. 4): The motor cortex was more active during wakefulness and suppressed during REM sleep, in contrast to the visual cortex and the retrosplenial cortex (RSP) in the medial cortical network, which showed an opposing modulation pattern that was more active during REM sleep; The somatosensory cortex exhibited more activity during NREM sleep. Additionally, active wakefulness (with more facial movements) and quiet wakefulness were also associated with different cortical patterns (Supplementary Fig. 5).

Besides the region-specific modulation in discrete cortical regions, macroscopic imaging allows for identifying global activity patterns. We thus normalized activity in each brain state across the entire dorsal cortex and determined the relative activation of each cortical area. Consistent with the above result, the most active region during wakefulness was the motor cortex, while the most active region during REM sleep was the RSP. The NREM activity had a relatively uniform distribution across the dorsal cortex, except that the RSP showed the least activity (Fig. 1e and Supplementary Movie 2). This brain region-specific modulation suggests that different cortical regions may be specifically involved in regulating different sleep-wake states or implementing state-specific functions in the sleep-wake cycle.

Highly structured cortical activation patterns during sleep

The above analysis used time-averaged activity, which reflected the relative activation probability of each brain region during different brain states. Also, brain atlas-based quantification can be affected by the accuracy of atlas-matching procedures. We therefore examined cortical activity patterns in different sleep-wake states on a fine time scale and without prior compartmentation of the cortex.

We first performed a functional connectivity analysis, in which we computed the similarity of neural activity dynamics between small cortical subnetworks (extracted from the face camera) and found that the PC1REM was significantly higher than the PC2REM (Fig. 3b, Supplementary Movie 3; however, such correlation was not observed during NREM sleep, nor between the RSP and PC1REM, or quiet vs. active REM). We defined phasic and tonic activity changes during different sleep-wake states. In addition, the PCs exhibited prominent oscillations, similar to previous reports. Spectral analysis revealed strong power in the δ band (0.5–4 Hz) and slow oscillations (<1 Hz; Fig. 3f, g). However, although these oscillations showed a brain state-dependent manner, with the highest oscillation power during REM sleep (Supplementary Fig. 9a), they did not correlate with EEG δ or slow waves (Pearson’s r < 0.01, for all PCs) (Supplementary Fig. 9b, c).

Distinct occipital activity pattern during REM sleep

We next focused our analysis on cortical activity patterns during REM sleep. PCA (using only data in REM sleep) showed that REM activity was dominated by the first PC of REM sleep (PC1REM), which accounted for 27.5% ± 2.6% (mean ± SEM) of the total variance, much larger than other PCs (explained variance by PC2: 4.1–3.3; n = 15 recordings from 5 mice; Supplementary Fig. 10a). We thus only analyzed the PC1REM in all following sections.

The PC1REM primarily reflected activation of the occipital cortical network, especially the RSP (Fig. 4a, Supplementary Fig. 10b), consistent with previous reports. The PC1REM showed a large dynamic change in its coefficient (Fig. 4b and Supplementary Fig. 11) and often appeared as transient events. Further analysis showed that this intermittent activation is associated with REM sub-stages of REM sleep (tonic vs. phasic REM, or quiet vs. active REM). We defined phasic and tonic REM according to the presence of phasic facial movements (extracted from the face camera) and found that the PC1REM was significantly higher than the PC2REM (Wilcoxon signed rank test; Supplementary Fig. 12) during phasic REM, suggesting different REM stages exhibit distinct cortical activation patterns. In addition, the increased activation of the PC1REM also correlated with more general activation of the cortex, and it often occurred as a spreading activation pattern starting from the RSP (Fig. 4c, Supplementary Fig. 13, and Supplementary Movie 4 and 5).

We next further examined the dynamic change of cortical activation during REM sleep, particularly its relationship with other features associated with REM, such as EEG θ power and eye movements. The fluctuation of PC1REM moderately correlated with the EEG power ratio between the θ and δ band (Fig. 4d, e and Supplementary Movie 3; Pearson’s r = 0.41 ± 0.03, n = 4 mice), consistent with the fact that they both reflected activation of the cortex. The occurrence of burst eye movements, which were extracted from a video of the mouse pupil, was also associated with the increase of PC1REM (Fig. 4d, f and Supplementary Movie 3); however, such correlation was not observed during wakefulness (Supplementary Fig. 14).

Another feature of brain activity during REM sleep is the PGO waves, which originate in the pons and propagate through the lateral geniculate nucleus to the occipital cortex. The unique occipital activity we observed may represent the cortical activity of mouse PGO waves. We therefore examined this by recording local field potentials (LFP) from the pons during the mesoscale imaging (Fig. 4g). We identified the pontine waves (P-wave, the pontine part of the PGO waves) as large negative potential in the LFP and found that occipital activity significantly increased immediately after the P-wave (Fig. 4h, i, peak latency, 0.89 ± 0.07 s, mean ± SEM). This result suggests that mice also have PGO waves, and the structured occipital activity during REM sleep may represent the cortical activation of the PGO waves.
Occipital activity dynamic during NREM sleep

Although the occipital cortex was highly active during REM sleep, we noticed that the PC1REM-like activity pattern (REM-like pattern) also appeared in other brain states. We thus next analyzed the REM-like pattern during wakefulness and NREM by computing the similarity between the activity pattern of each imaging frame and the PC1REM (Fig. 5a). At the population level, there was no correlation between PC1REM and the activity pattern in both wakefulness and NREM (Pearson's r = -0.11 ± 0.01 and -0.046 ± 0.007 for Wake and NREM, respectively; mean ± SEM; P < 0.0001. Paired t-test, all the t-test used the current study is two-tailed; n = 15 recording from 5 mice; Fig. 5b). However, while cortical patterns during wakefulness were generally not similar with the PC1REM (except during the drowsy states when the pupil was constricted and lack of facial movements. Supplementary
Fig. 3 | Highly structured cortical activation patterns during the sleep-wake cycle. a Activation patterns in an example recording extracted using PCA, showing the most frequently detected five major PCs during the sleep-wake cycle. Scale (ΔF/F₀, z-score): PC1, −2 to 2; PC2−5, −1.2 to 1.2. Black bar, 1 mm. b Time course of EEG spectrum and the coefficient of PCs showing in panel a. Top to bottom, EEG power spectrogram, EMG (scale, 0.5 mV), coefficient of PCs (scale, 0.02 and 100 s). The brain states are color-coded; the same color code is used in all following figures. c Coefficient of the five major PCs in different brain states. The same color code was used for each PC, as shown in panel b. Each line represents data from one recording. *P < 0.05, **P < 0.001; Two-tailed Student’s paired t test or Wilcoxon signed-rank test. Data of PC1−PC5 were from 15, 15, 15, 13, and 14 sessions from 5 mice, respectively. In this and all subsequent figures, summary data are expressed as the mean ± SEM.

d An example trace showing the time course of the PC1 coefficient. The two blue boxes indicate two activity events. Scale: 2 s and 0.02. e Duration of the cortical activity events. n = 235 and 264 events for PC1 and PC2, respectively. f Time course of the PC1 spectrum and the normalized mean power (0−5 Hz) in a log scale. Time scale, 100 s. g Normalized power spectrum of the PCs. Shading is SEM. n = 15 recording from 5 mice. Raw data for c and exact P values are provided in a Source Data file.

Fig. 4 | Cortical activation patterns during REM sleep. a The first PC of REM activity. Scale (ΔF/F₀, z-score): −2 to 2. b Time course of PC1REM, showing a large dynamic change of the PC. Scale: EEG, 0.5 mV; coefficient of PC, scale, 0.01 and 10 s. An example showing the dynamic change of the spreading activity during REM sleep. Scale (ΔF/F₀, z-score): −1.5 to 1.5. d An example showing the PC1REM coefficient, EEG power ratio between 0 and 8 band, and eye movements in the horizontal direction. Scale: PCL, 0.02 and 10 s; EEG ratio, 1; eye movements, 10 pixels. The two blue boxes indicate two bouts of eye movements. e Correlation between PC1REM coefficient and EEG power ratio between 0 and 8 band. f Correlation between eye movements and PC1REM. PC1REM coefficient was aligned using the onset of eye movement. Each black trace represents averaged change of PC1REM coefficient from one recording (n = 9−29 events). The red trace is the group average (n = 4 mice). g Left, schematic diagram depicting LFP recording from the pons. Right, Examples of P-waves (Scale, 0.1 mV and 1 s) and associated cortical activity (Scale, ΔF/F₀, z-score: −1.5 to 1.5). h Occipital activity increased immediately after the P-waves. PC1REM coefficient aligned to the negative peak of p-waves detected from one recording (h, n = 33) or from all 5 mice (i, n = 199). Scale in h, ΔF/F₀ (z-score): −1.5 to 1.5. All black bars in the figure represent 1 mm. Raw data for e are provided in a Source Data file.
Fig. 15), the NREM activity frequently exhibited a high positive or negative correlation with the PC1REM in a transient manner (Fig. 5c), suggesting that cortical activity patterns during NREM oscillate between a REM-like state and an opposing state (REM-opponent state). Further analysis indicated that the occurrence of REM-like and REM-opponent cortical activity patterns showed a strong brain state-dependent manner — REM sleep was dominated by REM-like patterns, and wakefulness had more REM-opponent patterns (REM-like vs. REM-opponent: REM, 23:1; Wake, 1:3.2; NREM, 1:1.1) (Fig. 5d).

The REM-like state showed high activity in the medial network and low activity in the somatic sensorimotor network, while the REM-opponent state had opposing patterns with more activation in the somatic sensorimotor network (Fig. 5c and Supplementary Fig. 16). Indeed, these two cortical patterns were highly similar to the occipital network and anterolateral network revealed by our regional correlation analysis (Fig. 5e).

It has been recently found that EEG during NREM sleep exhibits infra-slow oscillations in the sigma band42,43. We next examined the relation between the sigma oscillations and the REM-like activity. We chose long NREM bouts with few micro-arousals and calculated EEG sigma power (Fig. 5g). Cross-correlation analysis revealed a significant positive correlation (Pearson’s $r = 0.47 \pm 0.02$; mean $\pm$ SEM) between the two signals (Fig. 5h, i), with no apparent difference in the peak time at the group level although the sigma oscillations may lead or lag the REM-like activity for a few seconds (Fig. 5h, j).
The infra-slow sigma oscillation is thought to represent a periodic increase of arousal level during NREM-REM transitions, which may lead to microarousal events. We thus next analyze the relation between the REM-like activity and microarousal events. We found that there were more REM-like states before microarousal events and more REM-opponent states during microarousal events. We thus next analyze the relation between the REM-like state and microarousal events. We found that there were more REM-like states immediately before the NREM-to-REM transition (preREM,

\[
\text{NREM} \rightarrow \text{REM} \rightarrow \text{NREM}
\]

and exact P values are provided in a Source Data file.

**Fig. 6 | Activity in the occipital cortex signifies REM sleep.** a Dynamic change of the REM-like state during NREM-to-REM transitions. Gray line, data from individual recordings. Red line, an average of 15 recordings from 5 mice. Shading is SEM. Blue line, a linear fit of the average data during preREM (the 20 s immediately before REM onset). b Quantification of P1REM dynamics during NREM-to-REM transitions. \( P = 0.036 \) for NREM vs. preREM; \( P < 0.0001 \) for NREM vs. REM; Two-tailed paired t-test; \( n = 15 \) recordings from 5 mice. Data are mean \pm SEM. c Diagram showing different NREM periods. d An example showing dynamic changes of P1REM before and after a REM bout. Scale, 0.5 and 100 s. e Quantification of P1REM before and after REM bouts. \( P = 0.011 \); Two-tailed paired t-test; \( n = 10 \) recording from 5 mice. f Diagram describing the definition of different periods of NREM before the NREM \( \rightarrow \) Wake or the NREM \( \rightarrow \) REM transitions. g Box-plot showing the quantification of REM-like brain state in different NREM periods. Box, 25-75% range and median line; Whisker, 1.5 interquartile range; Blue line, data fitting with a normal distribution model. \( F = 14.5 \); \( P = 0.41 \) for NW1 vs. NW2; \( P = 0.043 \) for NW2 vs. NR2; \( P < 0.001 \) for NW1 vs. NR2, one-way ANOVA with posthoc Tukey’s tests; \( n = 85 \) and 74 (15 recordings from 5 mice) for NREM \( \rightarrow \) Wake and NREM \( \rightarrow \) REM transitions, respectively. b Diagram describing the definition of different periods of NREM that are associated with the different propensities of transitioning into REM sleep. Box-plot showing the quantification of REM-like brain state in different NREM periods. The box-plot elements were same as g. \( F = 48 \); \( P = 0.48 \) and 0.98 for NREM1 vs. NREM2 and NREM1 vs. NREM4, respectively; \( P < 0.0001 \) for all other paired-wised comparisons; one-way ANOVA with posthoc Tukey’s tests; \( n = 81 \) REM bouts and 166 NREM bouts, 15 recordings from 5 mice. Raw data for b, e, g, i and exact P values are provided in a Source Data file.

**Occipital Activity Signifies NREM to REM Transition**

We have shown that cortical activity during REM sleep was dominated by occipital activation, and the NREM activity pattern oscillated between REM-like and REM-opponent states, raising a possibility that the occipital activation may signify the occurrence of REM sleep. To test this idea, we analyzed global cortical activity during NREM-to-REM transitions. We found that there were significantly more REM-like states immediately before the NREM-to-REM transition (preREM, defined as the 20-sec before the transition; \( P < 0.05 \); Paired t-test; Fig. 6a, b), and the occurrence of REM-like pattern increased linearly during preREM (Pearson’s \( r = 0.81 \), \( P = 0.0001 \); Fig. 6a). Additionally, the probability of REM-like states was significantly lower during the NREM period following prolonged REM bouts (\( P = 0.011 \); Paired t-test, \( n = 11 \) recordings from 7 mice) (Fig. 6c–e), suggesting that the REM-like activity pattern during NREM sleep may represent the propensity of brain state transition into REM sleep. Indeed, the occurrence of the REM-like state was associated with whether the brain would transit into wakefulness or REM sleep. There were significantly more REM-like states before the NREM-to-REM transitions (\( P < 0.05 \), one-way ANOVA with Tukey’s posthoc tests; Fig. 6f, g).

The association of REM-like activity patterns with the propensity of REM sleep was further supported by our additional analysis, in which we measured the probability of the REM-like state during different NREM periods that were associated with the different propensity of transitioning into REM sleep. We found that the cortical
The reduction in REM sleep was not because we could not identify REM sleep during the stimulation period—EEG signals for both REM and NREM sleep during the stimulation period showed characteristic θ and δ oscillations, respectively (Supplementary Fig. 20). Furthermore, optogenetic stimulation did not produce unnatural brain states, as the EEG spectrum (0.5–18 Hz) for both REM and NREM sleep during the stimulation period had no significant difference from that during the no-stimulation period (REM, P = 0.96; NREM, P = 0.68; Kolmogorov–Smirnov test; Fig. 7d).

In the optogenetic silencing experiments, we observed stimulus-induced reductions in REM sleep, which may be the result of reduced REM entry or reduced REM maintenance. Brain state transition analysis can distinguish these possibilities. For a given time point (5 s bin), brain state transition analysis determines the probability of the current brain state transitioning to other brain states. In this analysis, we compared the transition probability during the baseline and stimulation periods (Fig. 7e, f and Supplementary Fig. 21). Each bar in the figure represents the transition probability averaged in one minute. We found that the primary effect of the light stimulation was to decrease the transition from NREM to REM while stabilizing NREM sleep (increased NREM \rightarrow REM transition) (NREM \rightarrow REM, P < 0.001; NREM \rightarrow NREM, P > 0.001; Paired t-test; Fig. 7e). This result was consistent with our observation that the REM bout number was markedly decreased during the stimulation period (ChrimsonR: baseline, 26.2 ± 1.8; stimulation: 8.8 ± 1.4; P < 0.0001, Paired t-test; mCherry, baseline, 23.8 ± 3.1; stimulation: 18.4 ± 1.3; mean ± SEM; P = 0.16, Paired t-test; Supplementary Fig. 22). There was also a tendency for decreased REM consolidation and increased transition from REM to wakefulness (REM \rightarrow REM, P = 0.069; REM \rightarrow Wakefulness, P = 0.062; Paired t-test; Fig. 7e). Together, these results indicate that neural activity in the occipital cortex plays a role in controlling the NREM-to-REM transition.

We next tested whether neural activity in the occipital cortex can bidirectionally modulate the transition between NREM and REM. Since excessive activation of cortical excitatory neurons can cause seizure, we only expressed ChrimsonR (driven by CaMKII promoter) in a small region of the occipital cortex, the RSP (Fig. 7g). Light stimulation (638 nm, 0.3–2 mW at fiber tip, 20 Hz, 2 min/trial, randomly applied every 5–7 min) caused a significant increase of REM sleep (P < 0.0028, Paired t-test) and decrease of NREM sleep (P = 0.007, Paired t-test), and no detectable change in wakefulness was observed (P = 0.33, Paired t-test) (Fig. 7h). The main effect of the light stimulation was to increase the transition from NREM to REM and decrease the consolidation of NREM sleep (P < 0.001 for both transitions; Paired t-test; Fig. 7i), reverse mirroring the effect of inhibiting the occipital cortex.

Taken together, we have demonstrated that occipital activity signifies REM propensity and plays an active role in controlling REM sleep. An immediate intriguing question is whether inhibition of occipital activity leads to an increase in REM pressure since REM sleep is also homeostatically regulated46,47. In our optogenetic inhibition experiment, we did observe REM rebound shortly after the laser stimulation (Fig. 7b and Supplementary Fig. 19), suggesting the possibility of increased REM pressure by suppressing occipital activity. To further examine this, we performed a new experiment in which we inhibited occipital activity for a longer time (Laser on, 20 min; Laser off, 8–10 min) and with higher laser power (20 mW, to achieve more significant inhibition; Fig. 7j). We found that prolonged inhibition of occipital activity effectively suppressed REM sleep throughout the stimulation period—the percentage of REM sleep decreased to 3.5% compared with 10.5% before stimulation (P = 0.032, one-way ANOVA with posthoc Tukey’s test; Fig. 7j–l). Importantly, mice frequently entered into REM shortly after stimulation, resulting a substantial increase in the percentage of REM sleep (25.9%; P < 0.001, one-way ANOVA with posthoc Tukey’s test, compared with baseline; Fig. 7j–l). These results show that inhibition of occipital activity produces REM sleep pressure, suggesting that the occipital cortex plays a role in REM homeostasis.

Discussion

Using mesoscale Ca2+ imaging from the entire dorsal cerebral cortex in mice, we revealed highly structured cortical activity and distinct global activation patterns during the sleep wake-cycle, with more activation in the somatic sensorimotor network during wakefulness, and more active medial cortical network, especially the RSP, during REM sleep (Figs. 1e and 2–4). In particular, we uncovered a shared global cortical activity pattern for REM and NREM sleep control, as is illustrated by the diagram shown in Fig. 8. REM sleep is dominated by the cortical activity pattern with higher activation in the occipital cortex and low activity in the somatic sensorimotor cortex (the REM-like activity pattern), and such dominance gradually emerges during the NREM-to-REM transition. The cortical activity during NREM sleep oscillates between the REM-like pattern and the REM-opponent pattern. Moreover, the occurrence of the REM-like pattern signifies the propensity of brain state transition from NREM to REM sleep. Inhibition of the REM-like pattern by suppressing neural activity in the occipital cortex can retain the brain state in the REM-opponent state, thus preventing the transition from NREM to REM sleep and promoting NREM sleep (Fig. 7).

During sleep, although our body is relatively inactive, our brain is very active. Our direct imaging of global neural activity in the entire...
dorsal cortex with a high spatiotemporal resolution shed light on understanding the global organization of cortical activity patterns during sleep. Brain activity during REM sleep was thought to resemble that during wakefulness, at least at the EEG level. However, our measurement of global cortical activity suggests a fundamental difference between the two states, with a large difference in the activation patterns of the somatic sensorimotor network and the medial cortical network, and the cortical activity patterns during NREM sleep were more similar to the REM activity than that between wakefulness and REM.

Our results also showed that the cortical network during NREM sleep oscillates between the REM-like state and the REM-opponent state on a rapid time scale, demonstrating the heterogeneous cortical activity patterns during NREM sleep. Conceptually, our finding is consistent with the concept of “local sleep”, which refers to the locally generated neuronal ceasefire during wakefulness, especially.
when sleep pressure is high. While local sleep represents spatially localized micro-sleep and its occurrence is associated with increased sleep pressure, our finding indicates that transient REM-like states embed in NREM sleep, and the occurrence probability of such state is associated with transition probability from NREM to REM, resembling the “REM pressure” of the brain. Therefore, both studies emphasized that a specific brain state can have characteristic features of other brain states in the space or the time domain.

Neural activity during sleep has been proposed to associate with various brain functions, including memory consolidation32,33. Our mesoscale optical imaging of cortical activation provides new insights into how these activities are spatiotemporally organized in the dorsal cortex. The PGO wave-like activation of the RSP during REM sleep implicates its association with REM functions. In fact, RSP has been shown to be closely related to the memory process5. Our results offer a potential explanation of the RSP involvement in memory, which is also consistent with the memory role of the PGO waves39,40 and REM sleep39,40. The high activation of the occipital cortex during REM sleep is also consistent with a human EEG study, which shows that the ‘dreaming’ experience is tightly correlated with activation of the occipital “hot zone”.

The RSP is part of the brain default mode network (DMN), which is more active when a subject is not interacting with the outside world62. With this in mind, it is not difficult to understand that RSP is relatively inactive during the waking period, because the main purpose during wakefulness is to interact with the outside world. While interestingly, the RSP is also less active during NREM sleep but highly active during REM sleep, indicating that the highly active brain during REM sleep is more internally orientated.

The neural control of sleep-wake state switching has been thought to be a privilege of the subcortical network, especially the brainstem and hypothalamus8,39,63. The main role of the cortex in sleep-wake regulation is thought to generate homeostatic sleep pressure in an activity-dependent manner8,60,64, or to support arousal8,9 or promote sleep7 through top-down feedback connections to subcortical regions. For REM sleep control, it is generally accepted that the brainstem plays a central role, with significant modulation by the hypothalamus8,63,64,66,68. Our finding that activity in the medial cortical network is also required for REM sleep uncovers an unexpected role of the cortex in REM control. These results provide evidence supporting the control of the sleep-wake cycle by the fast cortical activity dynamics, and indicate that REM sleep, as a unique state of the entire brain network, requires coordinating neural activation within the whole-brain network. Conceptually, our results showed that the structured cortical activity during sleep is not just an epiphenomenon of various sleep-wake states, but also plays an active role in regulating sleep state switching.

Methods

Experimental model and subject details

All experimental procedures followed the National Institutes of Health guidelines and were approved by the Animal Care and Use Committee at the Institute of Neuroscience, Chinese Academy of Sciences. Both male and female mice (>8 weeks at the time of surgery) were used. Mice were housed in rooms (temperature: 23 ± 1°C; humidity: 50–70%) under a 12/12-hr light/dark cycle (light on at 7 a.m.) with ad libitum access to food and water. Mice with implants for widefield imaging or optogenetic manipulation were housed individually. Wild-type mice (C57BL/6) (six male) were purchased from institute-approved vendors (Shanghai Silaike or LingChang Experiment Animal Co., China); GAD2-ires-Cre (six male and ten female) and Thy1-GCaMP6s (eight male) mice (stock #: 010802 and 024275, respectively) were obtained from Jackson Laboratory.

Surgical procedures

For widefield imaging experiments, the Thy1-GCaMP6s mice were anesthetized with isoflurane (5% for induction; 1.5–2% for maintenance) and placed on a stereotaxic frame with a heating pad. A “transparent skull” was prepared for chronic optical imaging using a similar procedure described previously1. The scalp was removed, and the skull was cleaned using hydrogen peroxide solution. The entire dorsal part of the skull (except that above the olfactory bulb and the cerebellum) was thinned using a dental drill and covered with a thin
layer of cyanoacrylate glue (ZAP-A-GAP). Two stainless steel screws (ML 0.3 mm) were inserted into the skull above the auditory cortex (AP$−3.0$ mm, ML 4.6 mm) of both hemispheres, and another two screws were inserted into the skull above the olfactory bulb (AP 4.9 mm, ML 0.8 mm) and the cerebellum (AP$−6.0$ mm, ML 1.0 mm), respectively. A thin wire (stainless steel wire (0.1 mm in diameter) insulated with polyimide tubing (0.2 in diameter)) was attached to one screw above the auditory cortex to record EEG. Another two wires were inserted into the neck muscle to record EMG. The ground wire was attached to the screw above the cerebellum. Finally, a customized head-plate was cemented to contact the transparent skull, and the cap also served as a light-shielding cap during imaging. The dental cement used to secure the implant was mixed with carbon to minimize light leakage during imaging.

For pontine LFP recording, electrodes (FeNiCr wires, 50 $\mu$m in diameter, California Fine Wire) were implanted in the pons (AP$−5.4$ mm, ML 0.5 mm, DV 4.5 mm from the brain surface).

For optogenetic inhibition experiments, mice were prepared by using a similar surgical procedure as described above. To express the AAV virus, we made small craniotomies ($0.5$ mm in diameter) on top of the retrosplenial cortex (AP $−3.4$ mm, ML 0.7 mm, DV 0.5 mm from the cortical surface) and the visual cortex (injection site 1: AP $−3.1$ mm, ML 2.5 mm, DV 0.4 mm from the cortical surface; injection site 2: AP $−4.0$ mm, ML 2.2 mm, DV 0.4 mm from the cortical surface), and injected the virus ($0.3$ µl/injection site) using Nanoject II (Drummond Scientific) via a glass pipette (23 nl/injection; inter-injection interval, 15–30 s).

For the RSP activation experiments, AAV-CaMKII-Cre and AAV-FLEX-ChrimsonR were 1:1 mixed and injected ($0.3$ µl/injection site) using the following coordinate: AP $−3.3$ mm, ML 0.5 mm, DV 0.5 mm from the cortical surface.

The following AAV viruses were used in current study: In optogenetic inhibition experiments, AAV2/9-hSyn-FLEX-ChrimsonR-ttdTomato (Titer: $3.3\times10^{13}$ v.g./ml; Shanghai Taitool Bioscience Co., China); AAV2/9-EF1α-DIO-mCherry-WPRE-pA (Titer: $2.6\times10^{12}$ v.g./ml; BrainVTA., China). In optogenetic activation experiments, AAV2/9-hSyn-FLEX-ChrimsonR-ttdTomato (Titer: $5.0\times10^{12}$ v.g./ml; Shanghai Taitool Bioscience Co., China); AAV2/9-CaMKII-Cre (Titer: $2.23\times10^{12}$ v.g./ml; BrainVTA., China).

Widefield imaging
Imaging experiments were performed on head-fixed mice. Mice were habituated to the head-fixed apparatus starting one week after surgery. The head-fixed device consists of a head plate holder, a low-profile lightweight disc (made of carbon fiber), and an optical breadboard covered with bearing balls. During experiments, mice were placed into the disc and attached to the head plate holder, and they could easily move the disc around, thus exhibiting less stress. Each mouse was first handled for three days and then restrained to the head-fixed device daily. The duration of the restrain started from 10 min and gradually increased to 3–4 h until they could reach stable sleep. A camera was used to capture a facial video of the mouse during the habituation, and the procedure was stopped if there were signs of excessive stress.

During the imaging experiments, an infrared camera (Hikvision) with the illumination of 840 nm or 920 nm was used to capture a movie of the facial movements and pupils of the mice. The frame rate of the video was 25 Hz. The camera could also capture part of the excitation light from the macroscope, and we used this information to synchronize the behavior video with the Ca$^{2+}$ imaging data.

Imaging was performed with a custom-built fluorescence macroscope using parts from Olympus and Thorlabs. The macroscope consisted of a zoom body of the Olympus MVX10 microscope with a 2x objective lens (MVPLAPO 2XC), a GFP filter set (Thorlabs), excitation LEDs (470 nm and 405 nm, Thorlabs), and a CCD camera (Qimaging, Retiga R1). All other optomechanical components used to build the macroscope were purchased from Thorlabs. For imaging experiments with both 470 nm and 405 nm excitations, alternating 470 nm and 405 nm illuminations was controlled using an Arduino board triggered by the exposure-out of the camera. Images were acquired using Micro-manager software (NIH, ver: 1.4) with a frame rate of 10 Hz.

The light level in the recording box was ~80 lux, and the light level at the mouse eye was <10 lux (light is blocked by the imaging lens).

For imaging experiments with pontine LFP recording, LFP (high-pass filtered at 0.5 Hz and digitized at 1 kHz) were acquired using an RHD acquisition board from Intan Technologies.

Polysomnography recordings
For polysomnography recordings during widefield imaging, the EEG and EMG signals (high-pass filtered at 0.5 Hz and digitized at 1 kHz) were acquired using an RHD acquisition board and RHX software (Ver: 3.03) from Intan Technologies. The acquisition board was also used to record the exposure-out signal from the camera to synchronize the EEG/EMG signals with the imaging data.

For polysomnography recordings in optogenetic manipulation experiments, mice were transferred into recording cages, placed in a sound-attenuation box (80 × 100 × 120 cm), and connected to the amplifier (TDT system-3 amplifier RZ2 + PZ5) with a flexible recording cable via a commutator. EEG/EMG was high-pass filtered at 0.5 Hz and digitized at 1526 Hz. Mice were habituated for two days before recording. During recording, a large power LED (for optogenetic silencing experiments) or optical fibers (for optogenetic activation experiments) were attached to the head implant. The recording session started after 30 min and lasted for three hours.

Optogenetic inhibition
To achieve a large area of light illumination, we attached a high-power LED (567 nm, with a SinkPAD-II 20 mm base) (QUADICA) on the top of the 3D printed cap, and covered it with light-proof copper foil. The LED was powered using a T-Cube LED driver from Thorlabs, which was controlled by the TTL output of an RZ5 system (Tucker-Davis Technologies). The RZ5 system and OpenEx (Tucker-Davis Technologies, Ver: 2.31) were also used to record the EEG/EMG signals during the optogenetic manipulation experiments.

All experiments were performed during the daytime between 10 a.m. and 6 p.m., and each test session lasted for 3 h. For the 2-minute occipital cortex inhibition experiments, in each session, we applied 2-min of light stimulation (pulse duration: 5 ms; pulse interval: 45 ms; 21 trials/session) with a time interval of 5–7 min. For the occipital cortex inhibition experiments, the light power measured at the surface of the skull is 10 mW, which is equivalent to 0.004 mW when delivering light through an optical fiber with a diameter of 200 $\mu$m. Each mouse was tested for five sessions (1 session/day). For the 20-min occipital cortex inhibition experiments, we applied 20-min of light stimulation (pulse duration: 5 ms; pulse interval: 45 ms; 5 trials/session) with a time interval of 8–10 min in each session. The light power measured at the surface of the skull is 20 mW, equivalent to 0.008 mW when delivering light through an optical fiber with a diameter of 200 $\mu$m. Each mouse was tested for five sessions (1 session/day).

For the RSP activation experiments, optical fiber (diameter: 200 $\mu$m; N.A.: 0.37) was placed bilaterally on the top of the thinned skull above virus injection sites. The light power was measured at the tip of the optical fiber. Each mouse was tested for 5 or 8 sessions (1 session/day). In each session, we applied 2-minutes of light stimulation (pulse duration: 5 ms; pulse interval: 45 ms; 20 trials/session) with a time interval of 5–7 min. The light power measured at the surface of the skull is 0.3–2 mW.
Histology and immunohistochemistry
To verify the virus expression, mice were deeply anesthetized and immediately perfused using 0.1 M PBS, followed by 4% PFA. The brain tissues were removed and post-fixed overnight in 4% PFA before dehydration in a 30% sucrose solution. Brain samples were embedded with OCT compound (NEG-50, Thermo Scientific) and cut into 50-μm sections using a cryostat (HM525 NX, Thermo Scientific). Brain sections were washed in PBS and coverslipped with mounting media.

The fluorescence images were captured using an epifluorescence microscope (VS120, Olympus).

Analysis of widefield imaging data
**Preprocessing.** The imaging was performed using a camera binning of 4 × 4 or 2 × 2, and saved to a Tiff stack. The image stack was downsampled by a factor of 2 if 2 × 2 binning was used, thus, the final image used in all following analyses had a resolution close to 260 × 256. The image stack was converted to an unformatted binary file to facilitate file I/O in later processing. Previous reports showed that a registration step is required to correct motion-induced movements of the images. However, in our data, we found that the motion-induced movements were negligible as no obvious blurring was found in the time-averaged images (typically from 40,000 images recorded in about 1 h; see an example in Fig. 1b).

The imaging data were transformed into the change in fluorescence \( \Delta F/F_0 \) using a baseline computed by averaging all images in a recording (40,000 images). In a small fraction of our experiments, we found a slight decrease in the overall fluorescence intensity which was likely caused by bleaching (especially when the control excitation of 405 nm was used). In these cases, we computed the mean fluorescence intensity of each frame and fitted the change in the mean intensity using a second-order exponential function. The fitting result was then used to correct the slow signal decay caused by bleaching.

The global signal in the imaging data, which is signal fluctuations common to the whole brain, has been reported to associate with non-neuronal physiological artifacts, such as respiration and hemodynamics. We thus removed the global signal from the \( \Delta F/F_0 \) data via linear regression, and this process is termed global signal removal (GSR). The global signal in our imaging data had a high similarity with the first PCs when PCA was performed before GSR was implemented, and in such conditions, PCA showed a global activation, confirming that GSR removed global correlation in the imaging data. In order to minimize the influence of blood vessels (especially large veins), we generated a mask to remove large blood vessels in the imaging data. To generate the mask, we first enhanced blood vessels in the time-averaged image using a filter (https://github.com/timjerman/JermanEnhancementFilter/blob/master/vesselness2D.m), then created a mask using ‘imbinarize’, a build-in function in MATLAB, using the ‘adaptive’ method with ‘sensitivity’ = 0.5. Finally, the mask was superimposed with the standard deviation of the image stack, and manual ROIs were used to remove artifacts caused by remaining blood vessels. The mask and manual ROIs were merged to get the final mask.

The blood vessel mask was applied to the \( \Delta F/F_0 \) data before further processing was performed.

**Regional map generation.** In order to match the imaging data with an anatomical atlas, we registered the imaging data to a reference atlas (the Allen Mouse Brain Atlas). First, a flattened cortical map was generated from the reference atlas, and the map was manually trimmed to fit our imaging data by removing a fraction of the most posterior and lateral regions in the neocortex. Such an operation is commonly used in previous studies. We then merged small anatomy regions in the map and got the final reference regional map with nine brain regions, including the primary motor cortex (MoS), the secondary motor cortex (Mop), the somatosensory cortex, barrel field (SSB), the somatosensory cortex, upper limb (SSu), the somatosensory cortex, lower limb (SSI), the primary visual cortex (VISp), the association visual cortex (VISs), the dorsal retrosplenial cortex (RSpD), and the lateral retrosplenial cortex (RSPl). Only a small part of the auditory cortex can be imaged using the current method; we thus did not include the auditory cortex.

For each recording, we registered the reference map onto the imaging data using the registration package, ANTs (http://stnava.github.io/ANTS/). The registration was performed in three steps: 1) The time-averaged image was binarized using a combination of adaptive thresholding and manual ROIs; 2) The reference map was manually scaled to match the imaging data grossly. This step was performed to facilitate the following registration using ANTs. 3) Automatic, elastic registration was performed in ANTs using a similarity metric of neighborhood cross-correlation.

**Brain atlas-based quantification.** To quantify the \( \Delta F/F_0 \) for each brain region, we first averaged the \( \Delta F/F_0 \) for each brain state and then computed the mean \( \Delta F/F_0 \) in each brain region using a region mask after the removal of blood vessels (Supplementary Fig. 4). The results for each recording were then z-score normalized across the nine brain regions and generated plotting data for Fig. 1e.

A similar quantitation procedure was used to quantify the distribution of the PCs or activity pattern and generated plotting data for Supplementary Figs. 5b, 8c, 10b, and 16.

**Correlation matrix and hierarchical clustering.** We first divided the brain into a 16 × 16 grid (the size of each patch is 10 × 10 pixels) and computed all pairwise correlations using the ‘corrcov’ function in MATLAB to obtain a correlation matrix of neural activity in each brain state. We then used the ‘clustergram’ function in MATLAB with default parameters to perform hierarchical clustering, and grouped brain regions with similar activity dynamics. The regional cluster was generated using a distance of 5. Clusters containing fewer than four patches were merged into the nearest cluster.

To generate the anterolateral and occipital network, we divided cortical regions into two groups using hierarchical clustering and averaged the results from 15 recordings to get the map in Fig. 2c.

**PCA analysis.** PCA was applied to the blood vessels removed \( \Delta F/F_0 \) data to decompose the imaging data and extract activity patterns using the built-in function in MATLAB. For PCA across the entire recording, the imaging data were temporally down-sampled with a decimating factor of 2 or 5 to reduce the computational load. No down-sampling was used when PCA was used to analyze data during REM. The resulting PCs were then inpainted to fill regions removed by the blood vessel mask, using a built-in function ‘inpaintExemplar’ in MATLAB with ‘PatchSize’ = 5. The inpainted PCs were then spatially smoothed using an averaging filter of 5 × 5. Finally, a mask derived from the region map was applied to the PCs to remove pixels outside of the brain.

Because the PCs can be of either one of the polarities, we reversed the polarity of the major PCs (as well as the corresponding coefficient) in some cases, such that a positive coefficient always corresponded to an increase in the fluorescent signals.

**Analysis of PC events.** We first computed an adaptive baseline of the coefficient (sampling rate, 5Hz) of each PC using a moving average (20 s), detected signals above 1x standard deviation from the baseline, and used the peak of these signals to determine each event (from 2 s before to 2 s after the peaks). We then used the Gaussian function to fit each event and used the sigma of the Gaussian curve as the half-width of each event.

The distribution of the event width for each recording was used to generate Fig. 3e, and this distribution was then fitted using the Gaussian function to obtain a mean event duration for each recording.
Analysis of PC1REM-like pattern during NREM and Wakefulness. The cross-correlation (CC) between each image frame and PC1REM was calculated using the ‘corcoef’ function in MATLAB. The CC in each brain state was averaged to get data for Fig. 5b. The REM-like events and REM-opponent events were defined as image frames with a CC value >0.4 or <–0.4, respectively. The neocortical patterns in Fig. 5c were an average of 5 events as marked on the CC plot. The occurrence probability of the two events during the defined NREM periods was used to generate Fig. 5d.

The CC was also used to analyze the relation between PC1REM-like pattern and the infra-slow sigma oscillations or microarousals. EEG sigma oscillation (9–17 Hz) was computed according to previous work for manually selected NREM bouts with no microarousals. We calculated the EEG sigma power (9–17 Hz) every 1 s and then computed the correlation with smoothed CC (using a 10-s window). Microarousal events were identified manually as periods with a brief increase of EMG power during NREM sleep.

Correlation between PC1REM and EEG 9/5. We first computed a spectrogram for EEG using a fast Fourier transform (FFT) with a frequency resolution of 0.18 Hz, and calculated the ratio between the θ band (6–10 Hz) and δ band (0.5–4 Hz). We then averaged the ratio and the coefficients of PC1REM in each 5-s window and computed the correlation (Fig. 4e).

Correlation between PC1REM and eye movements. To extract the eye movements, we used the FaceMap software. There was a high correlation between eye movements in the horizontal direction and vertical direction; we thus only used the horizontal movements for all analyses. To detect bouts of eye movements, we first computed an adaptive baseline using a moving average of 500 points, and detected signals that were 1x standard deviation higher than the baseline. We then used the onset of eye movements to align the coefficients of PC1REM in each recording. The mean coefficient was z-score normalized and averaged across different animals (Fig. 4f).

To analyze the correlation between occipital activity and eye movements during wakefulness, we manually identified each eye movement event.

Detection of P-waves. P-waves were detected as large negative peaks according to previous work. We used the MATLAB function ‘findpeaks’ to extract events greater than 4x standard deviation of the pontine LFP during REM sleep. The detected events were used to align the coefficients of PC1REM in each recording. The z-score normalized coefficients were averaged across animals.

Detection of tonic REM vs. phasic REM. Tonic and phasic REM were defined based on the presence of muscle twitches according to previous work. We used the FaceMap software to extract facial movements and calculate the mean of the signal during REM. Phasic REM was defined as REM with greater than average facial movements, and tonic REM was defined as REM with less than average facial movements.

Brain states scoring. To score the brain states using EEG/EMG signals, we performed spectral analysis on the EEG using a fast Fourier transform (FFT) with a frequency resolution of 0.18 Hz. The brain states were scored every 5 s semi-automatically using a MATLAB GUI and validated manually by trained experimenters. Brain states classification was performed according to established criteria. Wakefulness was defined as desynchronized EEG and high EMG activity; NREM sleep was defined as synchronized EEG with high-amplitude δ activity (0.5–4 Hz) and low EMG activity; REM sleep was defined as high power at θ frequencies (6–10 Hz) and low EMG activity.

We also divided wakefulness into active and quiet wakefulness based on whether the facial movements of mice were greater or less than average movements. Please note that the current definition is different from the classical definition that uses body movements. We used facial movements because we only recorded a video of the face of the head-fixed mice.

NREM/REM EEG spectrum during light stimulation. We first computed a spectrogram for EEG using a fast Fourier transform (FFT) with a frequency resolution of 0.18 Hz. We then determined the NREM period during light stimulation and baseline (2 min immediately before light onset), computed the mean spectrum for stimulation and baseline period for each recording, and then averaged the result for each mouse. To avoid using data during the brain state transition period, we excluded the first 10 s and the last 10 s for each NREM bout. The average EEG spectrum for each mouse was then normalized using the mean value of the EEG spectrum between 0.5 and 18 Hz (18 Hz was chosen as the upper boundary because of the stimulation-induced artifact in 20 Hz in mice expressing CrimsonR).

Brain state transition analysis. Brain state transition analysis was performed according to procedures described previously. Briefly, for a given time point (5-s bin), we first determined the number of trials (x) that the mice were in a specific brain state, and then identified how many (y) of the x trials were transiting into each brain state in the next time point. The transition probability for each pair of brain states was then computed (y/x). In Fig. 7e, i and Supplementary Fig. 21, each bar represents the transition probability averaged across 12 consecutive bins. The baseline transition probabilities were averaged across all time bins within 300 s before laser onset.

Statistical tests

Statistic procedures. We performed a normality test on each dataset using the Shapiro-Wilk test. The parametric tests (two-tailed paired or unpaired Student’s t-tests) were used if the dataset was normally distributed (P < 0.05), otherwise, non-parametric tests (Wilcoxon signed-rank test or Wilcoxon rank-sum test) were used. For multiple group comparison, we used one-way or two-way ANOVA with post hoc Tukey’s test. All the statistical tests were two-tailed and performed in MATLAB (2019b) and OriginLab (OriginLab Corp., 2019b). The significance level was set at P < 0.05. For statistics for the optogenetic experiment, we used bootstrap procedures. For each experimental group with n mice (mi trials for mouse i), we resampled the data by randomly drawing mi trials for each mouse (random sampling with replacement) and calculated the mean across the n mice. The resampling was repeated for 100,000 iterations to get the final distribution. The confidence intervals were then extracted from the distribution of the resampled mean values. To test whether laser stimulation significantly modulated each brain state, we calculated the difference between the mean of the laser period and baseline for each bootstrap iteration. We then calculated a p-value from the resulting distribution.

Sample size. We did not perform a calculation on the sample size. We used a sample size comparable to studies using similar techniques and animal models.

Data exclusion criteria. Mice were excluded based on post hoc verification of the virus expression. No mouse was excluded for analysis. The investigators were not blinded to the genotypes or the experimental conditions of the animals.

Figure preparation. All figures were prepared using Adobe Illustrator (Adobe, CS6). Diagrams in Figs. 1a, 4g, and 7a were adapted using images from biorender.com (agreement #: CS24979GPX).
Reporting summary
Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability
The data supporting the findings of this study are included in the figures and supporting files. The raw data are available from the corresponding author upon request. Source data are provided with this paper.

Code availability
Custom codes are available on GitHub (https://github.com/xulabsleep/MesoscaleImagingInSleep).

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Author contributions

M.X. conceived and supervised the projects; Yan.W. performed all transparent skull surgery with help from Z.W. and X.L.; Y.H., X.L., and X.F. performed the mesoscale imaging; Z.W. performed the optogenetic experiments; M.X., X.F., Z.W., Y.H., X.L., Yan.W., S.Z., and W.P. analyzed the data; All authors contributed to data interpretation; M.X., S.Z., and Yin.W. wrote the manuscript with inputs from all other authors.

Competing interests

The authors declare no competing interests.
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