

Supplementary Information for

Parallel cholinergic circuit in oculomotor nucleus to control eye movements and REM sleep

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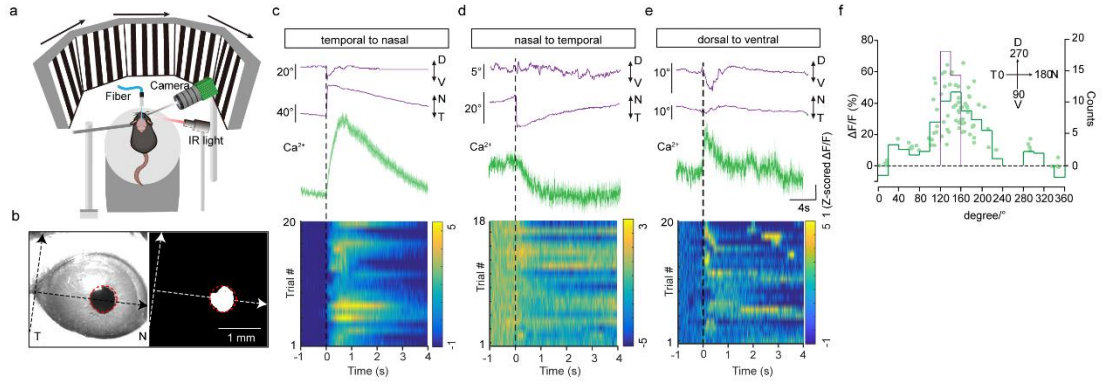
Supplementary Figures 1-24

Supplementary Table 1

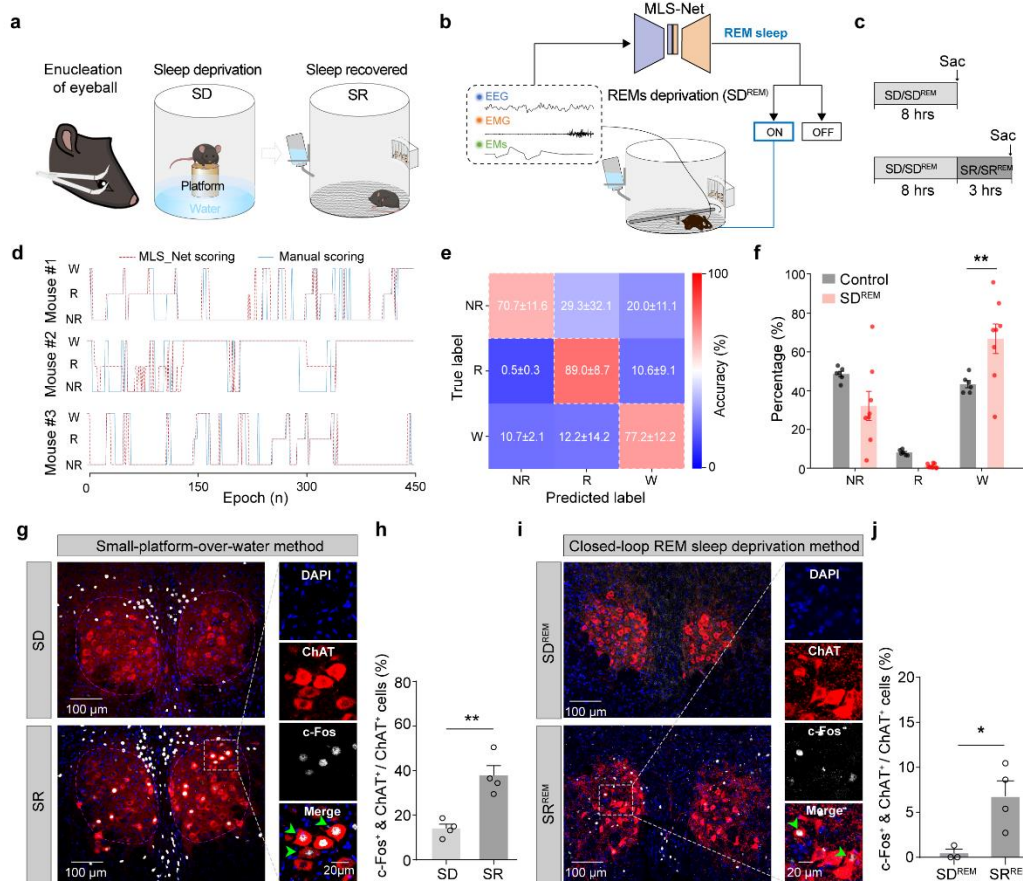
Supplementary Movies 1-6

Other Supplementary Material for this manuscript includes the following:

Source Data files

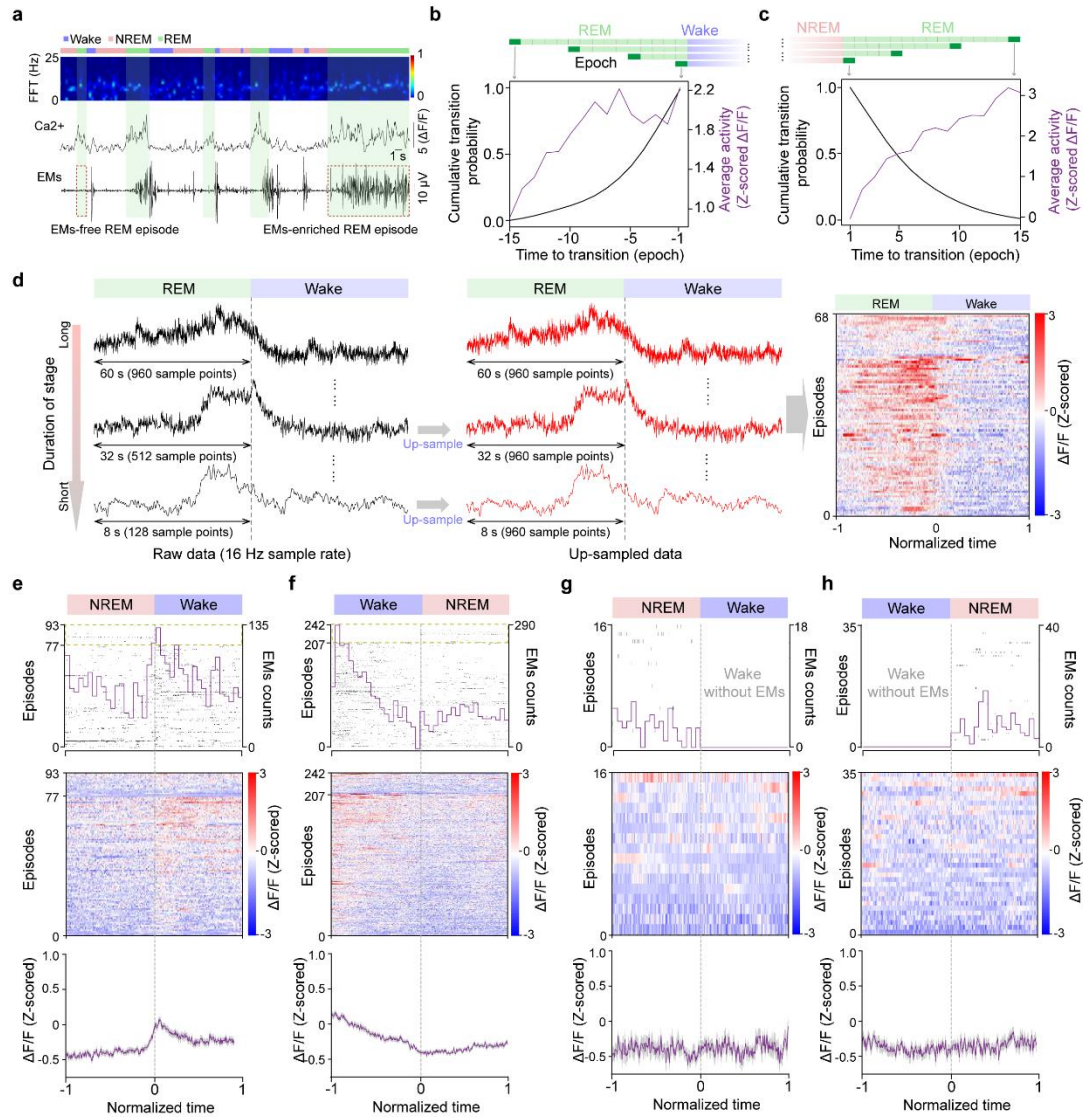


Supplementary Fig.1 | Activity of *nIII^{ChAT}* neurons in response to EMs in different direction. **a** Schematic of experimental setup for tracking EMs using optokinetic reflex (OKR) recording. Periodic drifting gratings were presented to awake head-fixed mice. **b** The representative image of EMs recorded by an infrared high-speed camera (IR light: infrared light). The pupil position of eye was analyzed by matlab. **c-e** Example of Ca^{2+} fluorescence in response to EMs at different angles induced by OKR in GCaMP6-tagged *nIII^{ChAT}* neurons. Upper: trace of EMs. Middle: calcium fluorescence signal. Lower: heatmap of Ca^{2+} fluorescence signals aligned to the onset of EMs ($n=3$ mice). Each row represents a single trial aligned to the stimulus. The black dashed line indicates the onset of the fast phase of EMs. **f** Scatter plotting between EMs degrees and corresponding Ca^{2+} fluorescence of GCaMP6-tagged ChAT-Cre mice. Left coordinate axis: the raw data of Ca^{2+} fluorescence in the scatter diagram and the average calcium signal at different angles was indicated by green line. Right coordinate axis: the probability density distribution of laser-evoked EMs at different angles in GCaMP6-tagged ChAT-Cre mice. Purple lines mean the probability of EMs. Inset: polar coordinate system. The bin size is 20° .



Supplementary Fig.2 | The c-Fos expression in nIII^{ChAT} neurons after sleep deprivation.

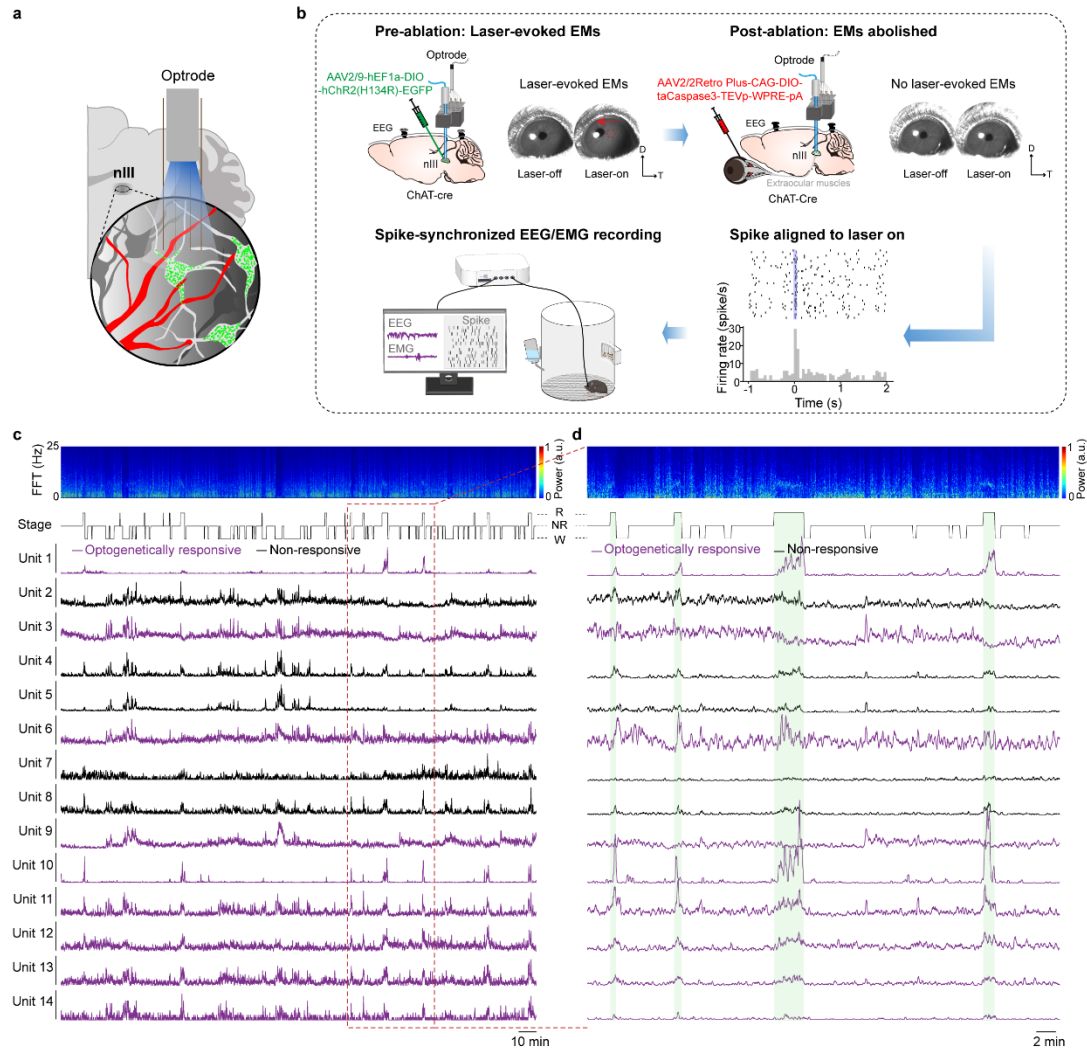
a The schematic of REM sleep deprivation (SD) and sleep recovered (SR) using small-platform-over-water method. **b** Schematic of REMs specific deprivation (SD^{REM}) and REMs specific rebound using a closed-loop sleep deprivation system. **c** The timeline of sleep deprivation and c-Fos screening. **d** The output hypnogram predicted by real-time autonomous sleep classifier compared with manual scoring. **e** The average confusion matrix comparing the true and predicted sleep labels for the input sleep-wake epochs on the MLS-Net model. **f** The percentage of different vigilance states for control and REM sleep deprivation mice using a closed-loop sleep deprivation system. N = 8 from SD^{REM} group, n = 6 from control group. Each dot indicates one individual mouse. Error bar represents the SEM across mice. Two-way ANOVA followed by Bonferroni post hoc analysis. **p<0.01. **g** Representative image of the c-Fos expression in the coronal sections of the nIII in the SD or SR mouse using small-platform-over-water method. Dashed box highlights the c-Fos (white) and ChAT (red) double-labeled neurons. **h** Percentages of c-Fos⁺/ChAT⁺ co-expressing neurons in the nIII region. Each dot indicates data from one mouse (n = 4 mice). Two-sided unpaired t-test. **p<0.01. **i** Representative image of the c-Fos expression in the coronal sections of the nIII in the SD^{REM} or SR^{REM} mouse using a closed-loop sleep deprivation system. Dashed box highlights the c-Fos (white) and ChAT (red) double-labeled neurons using small-platform-over-water method. **j** Percentages of c-Fos⁺/ChAT⁺ co-expressing neurons in the nIII region. Two-sided unpaired t-test. Each dot indicates data from one mouse (n = 3 for SD^{REM} and n=4 for SR^{REM} mice). *p<0.05. Data represent at least three biological replicates. All data and significance values for graphs f, h and j are shown in Supplementary Table 1.



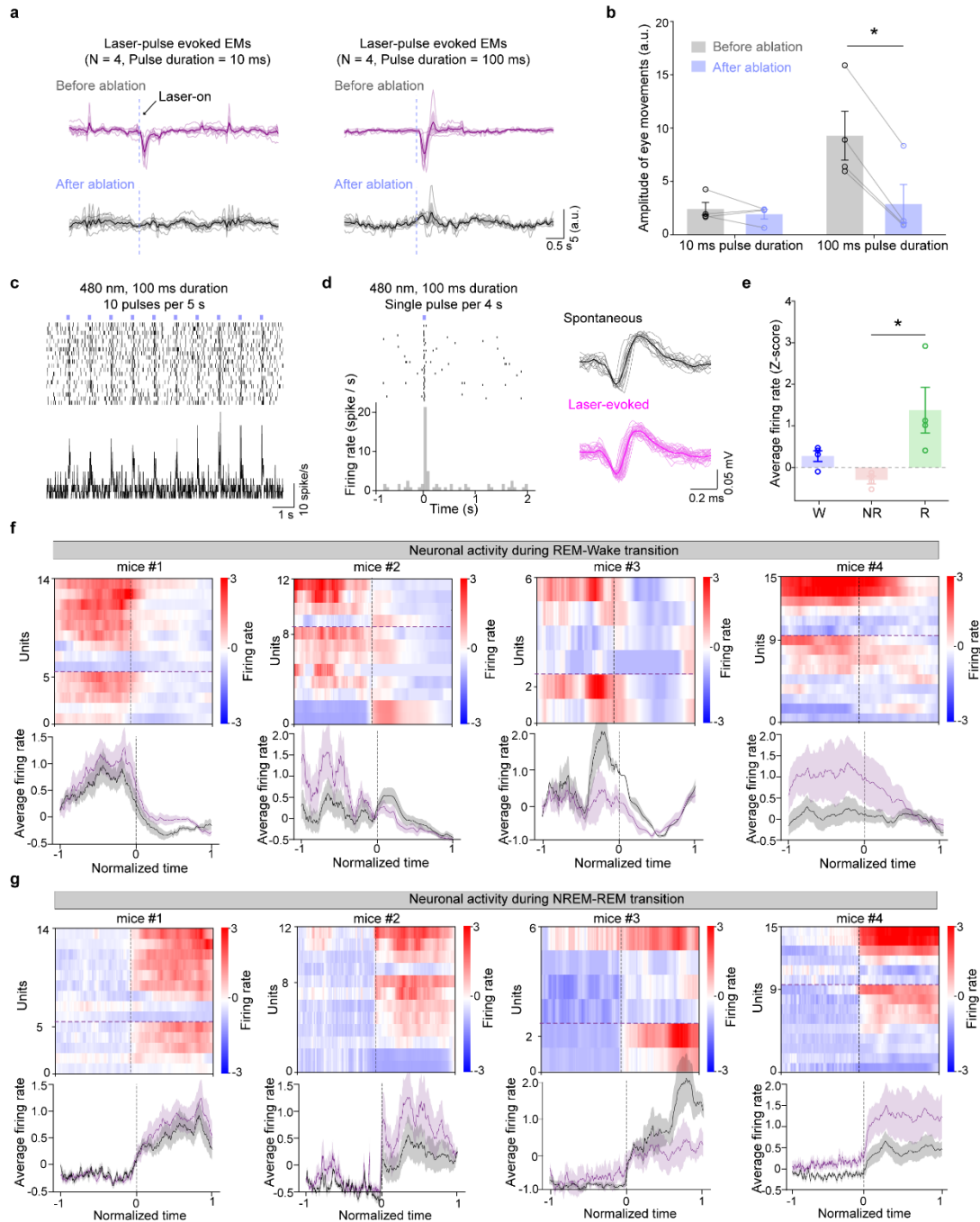
Supplementary Fig.3 | Activity of nIII^{ChAT} neurons during different vigilance transition.

a Representative recording of relative EEG power, EMG, EMs traces, and fluorescence traces of nIII^{ChAT} neurons across spontaneous sleep-wake cycles. Green-shaded areas represent REMs episodes, while red dashed boxes highlight EMs-free and EMs-enriched REM episodes. **b** The relationship between the average calcium signal, REM episode duration, and the subsequent REM-to-wake transition probability. Left: the cumulative probability of REM-to-wake transition. Right: average calcium signals during the first epoch of each REM episodes. The x-axis indicates the duration of REM episodes prior to the transition. **c** The relationship between the average calcium signal, REM episode duration, and the preceding NREM-to-REM transition probability. Left: the cumulative probability of NREM-to-REM transition. Right: average calcium signals during the last epoch of each REM episodes. The x-axis indicates the duration of REM episodes lag behind the transition. **d** Procedures for temporal normalization. Episodes of different durations were up-sampled to adjust the number of sampling points, thereby generating time-normalized traces of equal length. All trials were then temporally aligned to the transition point and visualized as a heatmap. **e** The distribution of EMs and calcium signals during the NREM-to-wake transition was analyzed across 93 episodes from 4

mice. Out of these 93 episodes, 16 episodes devoid of EMs during wake (EMs-free) are highlighted with a yellow dashed box. Upper: the raster plot shows events of EMs alongside normalized time from all trials aligned to transition time point and the histogram indicates the distribution of EMs. Middle: individual transitions with color coded fluorescence intensity. Y-axis shows the numbers of transitions episodes. Lower: the average neuronal activity across all the transitions. Dashed line indicates the transition time point. All episodes were sorted by averaged fluorescence intensity. The vertical dashed lines indicate the time point of states transition. **f** Same as **e**, shows the distribution of EMs and calcium signals during wake-to-NREM transition across 242 episodes from 4 mice. Out of these 242 episodes, 35 episodes devoid of EMs during wakefulness (EMs-free) are highlighted with a yellow dashed box. **g** Distribution of EMs and calcium signals during NREM-to-wake transition (EMs-free episodes) after taking out wakefulness episodes containing EMs. Upper: the raster plot shows events of EMs alongside normalized time from all trials aligned to transition time point and the histogram indicates the distribution of EMs. Middle: individual transitions with color coded fluorescence intensity. Lower: the average neuronal activity across all the transitions. **h** Same as **g**, shows the distribution of EMs and calcium signals during wake-to-NREM transition after taking out wake episodes containing EMs.

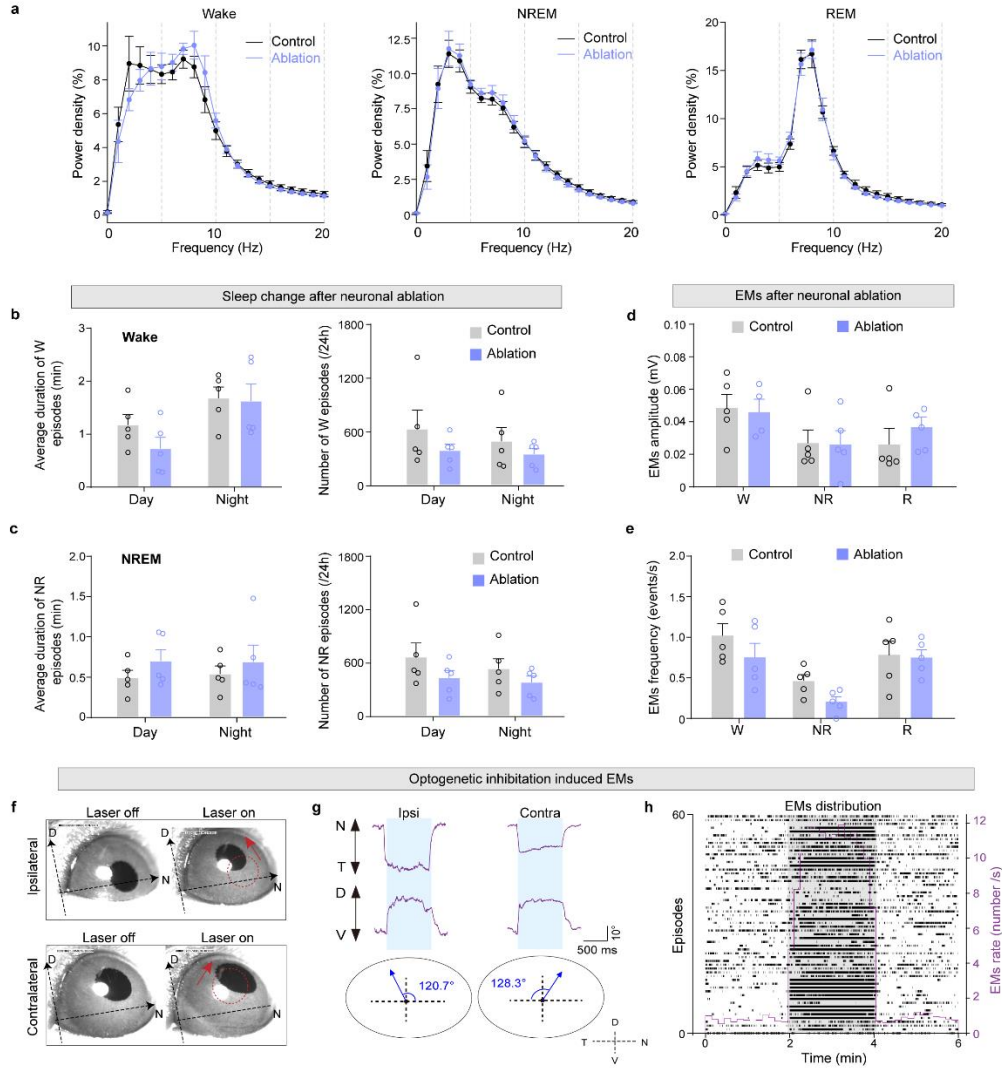


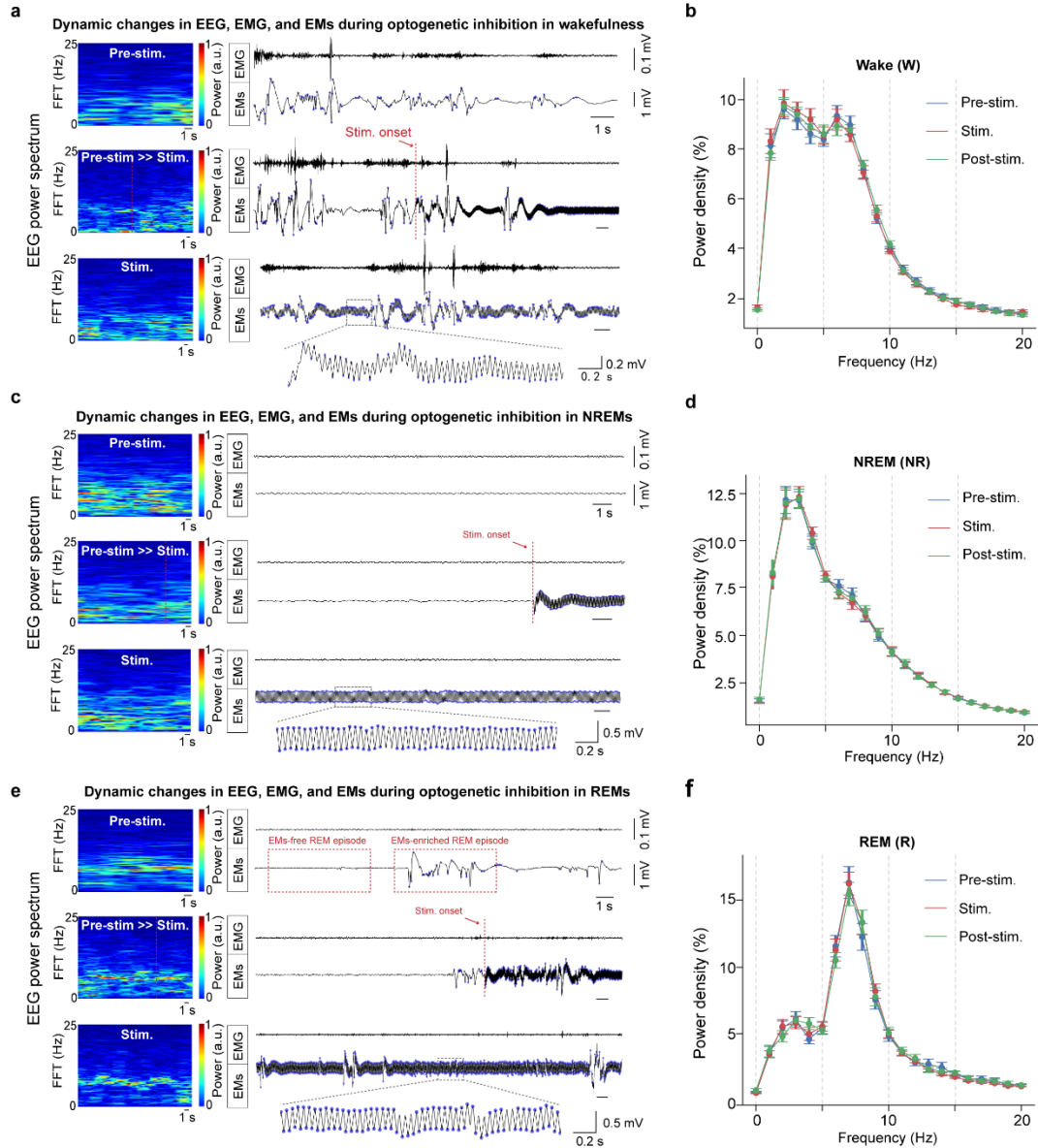
Supplementary Fig.4 | Optogenetics-assisted electrophysiological recording the activity of $nIII^{ChAT}$ neurons across sleep-wake cycle. **a** Schematic drawing of ChR2-tagged $nIII^{ChAT}$ neurons (green) using electrophysiology recording after co-injection of AAV2/2-Retro-DIO-tdCaspase3 (red) in the EOMs and AAV2/9-hEF1 α -DIO-hChR2-EGFP (green) in the nIII. **b** The schematic of the strategy to recording the activity of non-EOMs-projecting $nIII^{ChAT}$ neurons. The AAV2/9-hEF1 α -DIO-hChR2-EGFP was injected into the nIII followed by the optrode implantation. Once the laser-evoked EMs were recorded, the AAV2/2-Retro-DIO-tdCaspase3 was injected into the EOMs to ablate the EOMs-projecting $nIII^{ChAT}$ neurons and the evoked-EMs were disappeared. The spike and EEG/EMG were concurrently recorded to monitoring the activity of non-EOMs-projecting $nIII^{ChAT}$ neurons across sleep-wake cycle. **c** Representative firing rates recorded from multiple units across sleep-wake episodes. The purple trace indicates a unit that exhibits pulse-dependent responses during optogenetic stimulation. **d** Enlarged view of the red dashed time window shown in panel **c**.



Supplementary Fig.5 | Firing rate of the non-EOMs-projecting nIII^{ChAT} neurons during REM transition. **a** Average traces of laser-evoked EMs before (upper) and after (lower) ablation of EOM-projecting nIII^{ChAT} neurons. Left: 10 ms pulse duration at 2 mW; Right: 100 ms pulse duration at 2 mW. Each dot indicates data from one mouse (n = 4 mice). **b** Quantification of Laser-evoked EMs amplitude before and after cellular ablation. Each dot indicates one individual mouse. Error bar represents the SEM across mice. Two-way ANOVA followed by Bonferroni post hoc analysis. *p < 0.05. **c** Representative ChR2-tagged unit showing pulse-dependent firing rate across multiple laser-pulse stimulation. The purple boxes indicate the laser pulse (10 ms per pulse). **d** Left: raster plot (upper) and stimulus time histogram (50 bins, lower) for laser-evoked spikes of an example ChR2-tagged unit. The purple boxes indicate the laser pulse. Laser duration, 10 ms. 2 mW. Right: averaged waveform of

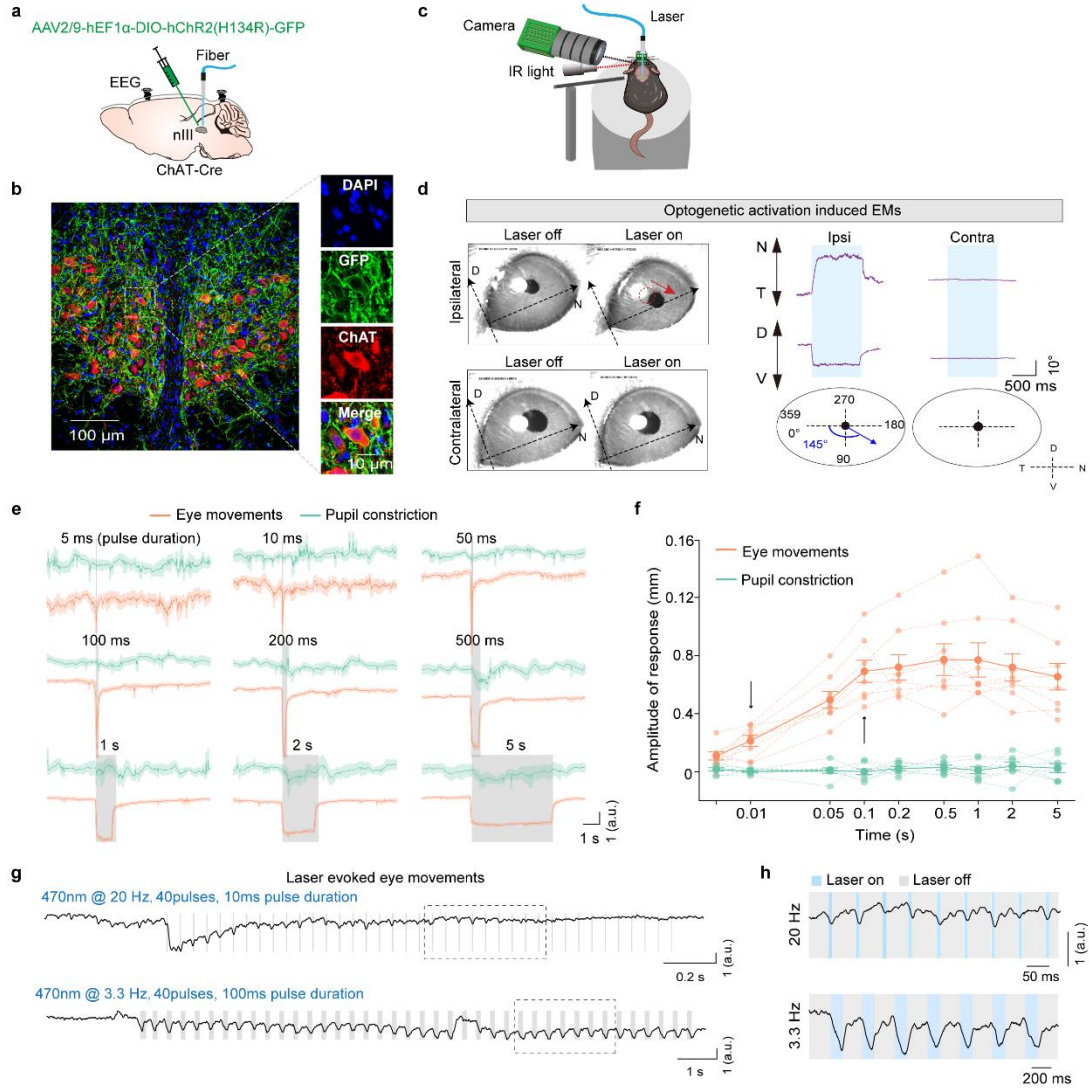
spontaneous (gray) and laser-evoked (purple) spike of an example unit. Each thin trace represents an individual spike, and the bold trace indicates the averaged waveform. **e** Quantification of the average fire rate of non-EOMs-projecting nIII^{ChAT} neurons cross different vigilance state episodes. Each dot represents one mouse (n=4 mice). One-way ANOVA followed by Tukey's post hoc analysis. Data are presented as mean $\pm$ SEM. *p < 0.05. **f** Upper: the heatmap show the fire rate of non-EOMs-projecting nIII^{ChAT} neurons during REM-to-wake (R-W) transition from 4 mice. The units above the horizontal dashed line are that exhibits pulse-dependent responses during optogenetic stimulation. The vertical dashed line indicates the time point of R-W transition. Lower: the average fire rate of selected units (purple, units above the horizontal dashed line in the heatmap) and non-selected units (gray) during R-W transition. The shielding indicates the 95% confidence intervals (CIs). **g** Upper: the heatmap show the fire rate of non-EOMs-projecting nIII^{ChAT} neurons during NREM-to-REM (NR-R) transition from 4 mice. The units above the horizontal dashed line are that exhibits pulse-dependent responses during optogenetic stimulation. The vertical dashed line indicates the time point of NR-R transition. Lower: the average fire rate of selected units (purple, units above the horizontal dashed line in the heatmap) and non-selected units (gray) during NR-R transition. The shielding indicates the 95% confidence intervals (CIs). Data represent at least three biological replicates. All data and significance values for graphs b and e are shown in Supplementary Table 1.





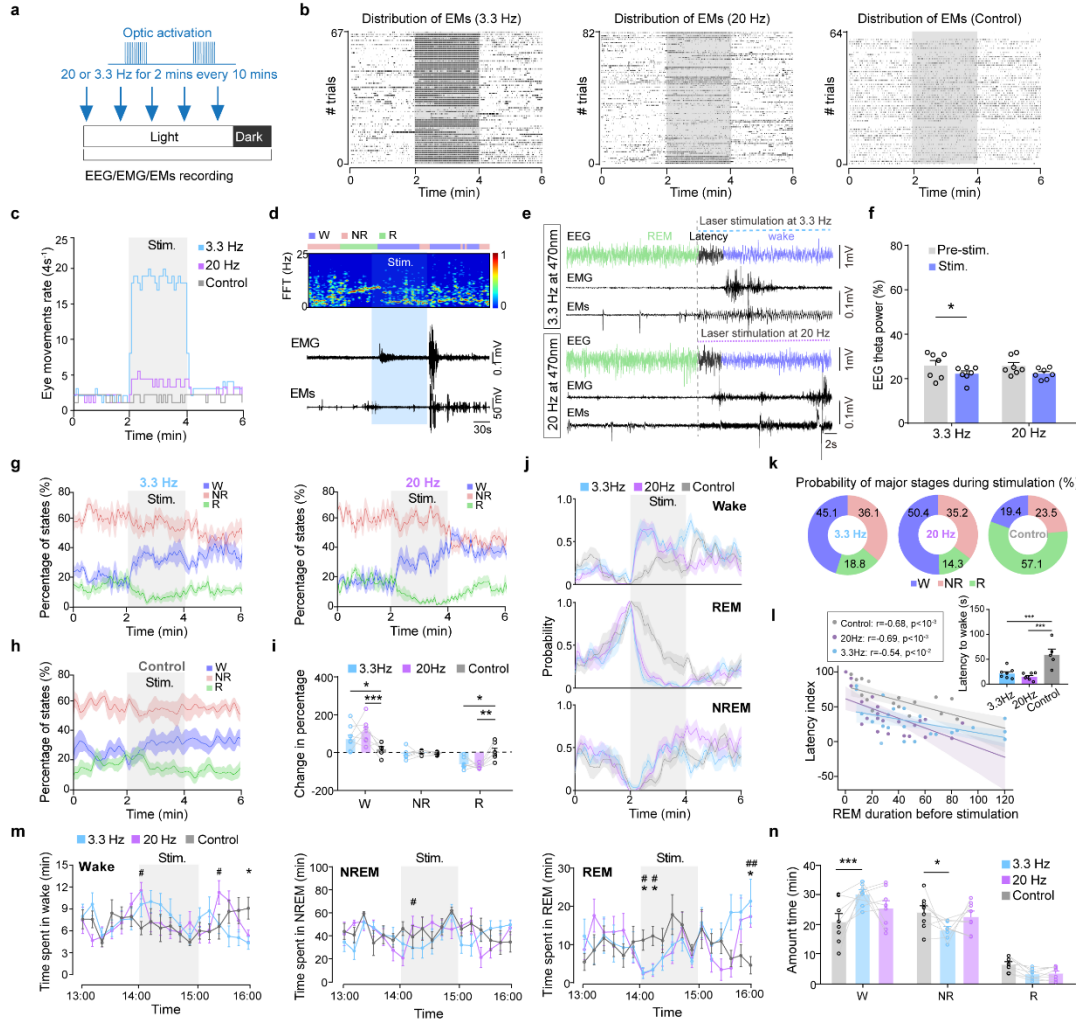
Supplementary Fig. 7 | Dynamic change of EMs, EEG and EMG during optogenetic inhibition. **a** Dynamic change in EEG power spectrum (left), EEG and EMs (right) during optogenetic inhibition of $nIII^{ChAT}$ neurons in wake episodes. The wake episodes are enriched with periodic EMs, and high amplitude of EMG. The optogenetic inhibition evoked sustained high-frequency EMs. The blue dots in the EMs trace indicate the automatically detected EMs event. **b** EEG power density during optogenetic inhibition of $nIII^{ChAT}$ neurons in wakefulness ($n = 8$). Two-way ANOVA revealed no significant differences. **c** Dynamic change in EEG power spectrum (left), EEG and EMs (right) during optogenetic inhibition in NREM sleep. The NREM episodes are characterized by a paucity of EMs and low amplitude of EMG. The optogenetic inhibition evoked sustained high-frequency EMs. The blue dots in the EMs trace indicate the automatically detected EMs event. **d** EEG power density during optogenetic inhibition of $nIII^{ChAT}$ neurons in NREM sleep ($n = 8$). Two-way ANOVA revealed no significant differences. **e** Dynamic change in EEG power spectrum (left), EEG and EMs (right) during optogenetic inhibition in REM sleep. The REM episodes are characterized by tonic or phasic EMs and low amplitude of EMG. The optogenetic inhibition evoked sustained high-frequency EMs. The

dashed red box indicates the EMs-free or EMs-enriched REM episodes. The blue dots in the EMs trace indicate the automatically detected EMs event. **f** EEG power density during optogenetic inhibition of nIII^{ChAT} neurons in REM sleep (n = 8). Two-way ANOVA revealed no significant differences.



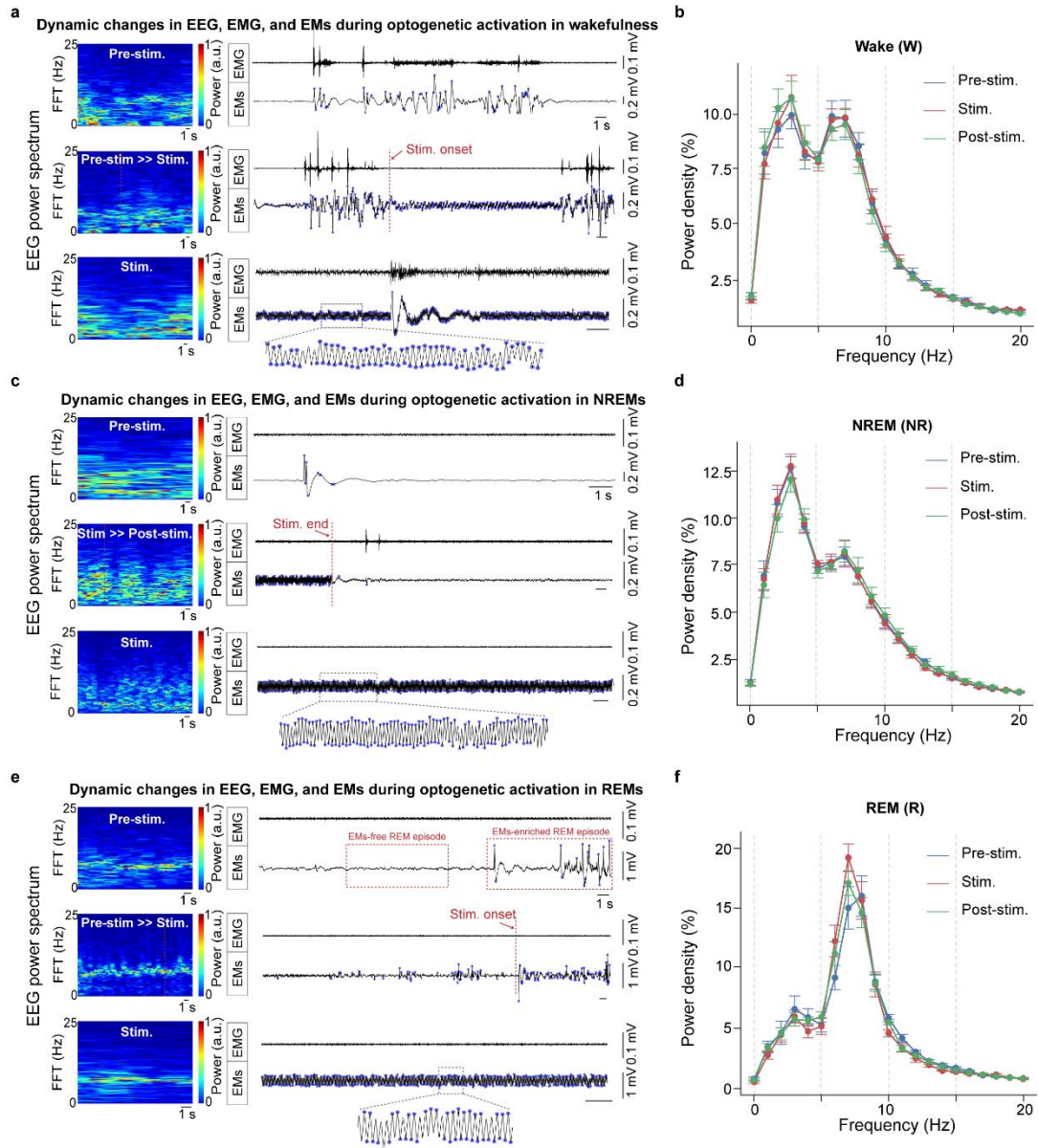
Supplementary Fig. 8 | Effect of activating nIII^{ChAT} neurons on the oculomotor and pupil constriction. **a** Schematic of viral injection in the nIII and implanting an optical fiber thereafter in ChAT-Cre mice. **b** Confocal image of viral expression of ChR2 within the nIII in ChAT-Cre mouse. Dashed box highlights the co-localization of ChR2 (green) and ChAT (red) within the nIII. **c** Experimental setup for EMs recording using IR camera in mice during optogenetic manipulation. **d** Left: The position of ipsilateral or contralateral eyeball before (laser off) and after (laser on) optogenetic activation of the nIII^{ChAT} neurons. Right: example and schematic diagram of ipsilateral or contralateral EMs traces induced by optogenetic activating the nIII^{ChAT} neurons (n = 3 mice). Blue area indicates the stimulation time window. D: dorsal, V: ventral, T: temporal, N: nasal. Laser duration, 1 s. 2 mW. **e** Dynamic change of ipsilateral EMs (orange) and pupil constriction (cyan) induced by optogenetic activation with varying durations (5 ms to 5 s) in ChAT-Cre mice. The gray shading area indicates the time window for laser stimulus. Orange and cyan shading indicates 95% confidence intervals (n = 8 mice). **f** Amplitude of laser-evoked EMs and pupil constriction induced by optogenetic activation with varying durations (5 ms to 5 s). Error bars represent SEM across mice in each states (n = 8 mice). **g** Traces of EMs induced by optogenetic activation of nIII^{ChAT} neurons using 20 Hz stimulation (10 ms pulse width) and 3.3 Hz stimulation (100 ms pulse width). **h** Enlarged view of the dashed

time window shown in panel **g**. The gray shielding indicates laser-off and the blue shielding indicates laser-on.



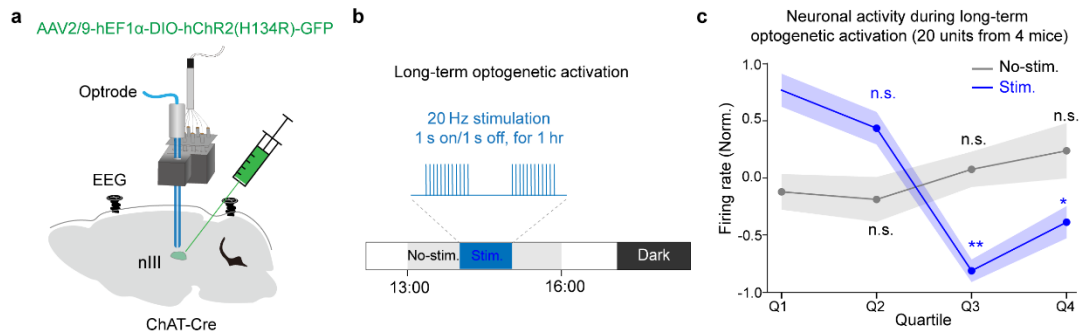
Supplementary Fig. 9 | Optical activation of nIII^{ChAT} neurons promotes REM-wake transition. **a** Schematic of optogenetic activation of overall nIII^{ChAT} neurons across the sleep-wake cycle using 3.3 Hz (100 ms pulses duration, 2 mW) and 20 Hz (10 ms duration, 2 mW) stimulation protocols. Each trial lasted 2 minutes, with 15–20 trials per session. Stimulation was applied either randomly across sleep stages (g-i) or specifically during REM sleep (j-k). Blue arrows indicate the timing of optogenetic stimulation trials. The light green shielding area indicates REM sleep period. **b** Raster plot for distribution of EMs from all trials aligned to onset of optical stimulation in Chr2-tagged nIII^{ChAT} neurons in ChAT-Cre mice. Shielding area indicates time window of optogenetic stimulation. **c** The average rate of EMs across trials during optogenetic activation. Gray shielding area indicated the stimulation coverage time window. **d** Typical example of sleep change with EEG spectrogram, EMG, and EM traces after optical stimulation in Chr2-tagged nIII^{ChAT} neurons in ChAT-Cre mice. Blue shielding area indicated the illumination coverage time window. **e** Representative EEG, EMG and EMs in response to 3.3 Hz (upper) or 20 Hz (lower) optogenetic activation during sleep. **f** Mean EEG power spectrum density in theta band (6-9 Hz) before and during optogenetic activation. Each dot indicates one mouse (n=7 mice). Error bar represents the SEM across mice. Two-way ANOVA followed by Bonferroni post hoc analysis. *p<0.05. **g** Percentage of wake, NREM, or REM states before, during, and after 488 nm optical activating overall nIII^{ChAT} neurons in

ChAT-Cre mice. Left: 3.3 Hz at 480 nm, n=8. Right: 20 Hz at 480 nm, n=8. The gray shielding indicates the time window of stimulation. **h** Percentage of wake, NREM, or REM states before, during, and after 598 nm optical activating nIII^{ChAT} neurons in ChAT-Cre mice (control, n=8 mice). The gray shielding area indicates laser stimulation window. The red, blue and green shading areas indicate 95% confidence intervals (CIs). **i** Percentage of sleep state changes during the stimulation window relative to the pre-stimulation window. Individual dot indicates one mouse (n=8 for 3.3 Hz, 20 Hz and control group). Each dot indicates one mouse (n=7 mice). Error bar represents the SEM across mice. One-way repeated-measures ANOVA followed by Tukey post hoc analysis. *p<0.05, **p<0.01, ***p<0.001. **j** The probabilities of different sleep architectures when optogenetic stimulation during REM sleep in ChR2 tagged ChAT-Cre mice. Dashed line indicates laser onset time. **k** The pie charts show changes in brain states during laser stimulation when specifically activating the nIII^{ChAT} neurons during REM sleep. **l** Correlation between latency index of R-W transition and the REM duration prior to transition. The histogram indicates the latency time to wakefulness when specific stimulation during REM sleep in ChR2 tagged ChAT-Cre mice (n=7 mouse in 3.3Hz and 20Hz, n=5 mouse in control). Each dot indicates one mouse (n=7 mice). Error bar represents the SEM across mice. One-way repeated-measures ANOVA followed by Tukey post hoc analysis. ***p<0.001. **m** Time course of wake (left), NREM sleep (middle), and REM sleep (right) during chronic optogenetic stimulation occurred at 14:00-15:00 every day (n=7). Two-way repeated-measures ANOVA followed by Bonferroni post hoc analysis. *p <0.05, **p <0.01 between 3.3 Hz and contro. #p <0.05, ##p <0.05 between 20 Hz and control. **n** Total amounts of each stage during chronic stimulation (n=7). Error bars represent SEM across mice. One-way ANOVA followed by Tukey post hoc analysis. *p<0.05, ***<0.001. Data represent at least three biological replicates. All data and significance values for graphs f, l, m and n are shown in Supplementary Table 1.

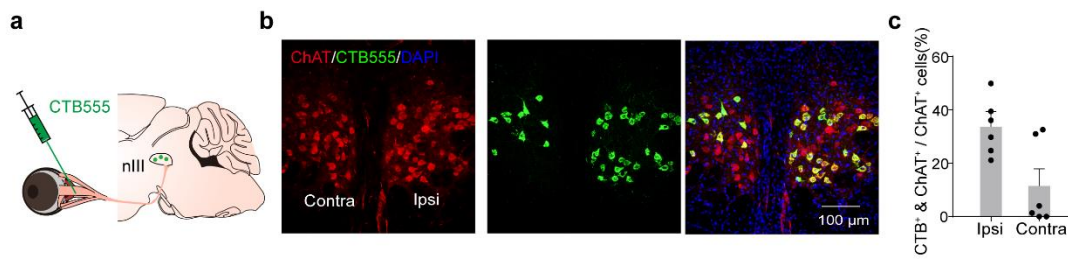


Supplementary Fig. 10 | Dynamic change of EMs, EEG and EMG during optogenetic activation. **a** Dynamic change in EEG power spectrum (left), EEG and EMs (right) during optogenetic activation of NIII^{ChAT} neurons in wake episodes. The wake episodes are enriched with periodic EMs, and high amplitude of EMG. The optogenetic activation evoked sustained high-frequency EMs. The blue dots in the EMs trace indicate the automatically detected EMs event. **b** EEG power density during optogenetic activation of NIII^{ChAT} neurons in wakefulness (n = 6). Two-way ANOVA revealed no significant differences. **c** Dynamic change in EEG power spectrum (left), EEG and EMs (right) during optogenetic inhibition in NREM sleep. The NREM episodes are characterized by a paucity of EMs and low amplitude of EMG. The optogenetic activation evoked sustained high-frequency EMs. The blue dots in the EMs trace indicate the automatically detected EMs event. **d** EEG power density during optogenetic activation of NIII^{ChAT} neurons in NREM sleep (n = 6). Two-way ANOVA revealed no significant differences. **e** Dynamic change in EEG power spectrum (left), EEG and EMs (right) during optogenetic activation in REM sleep. The REM episodes are characterized by tonic or phasic EMs and low amplitude of EMG. The optogenetic activation evoked sustained

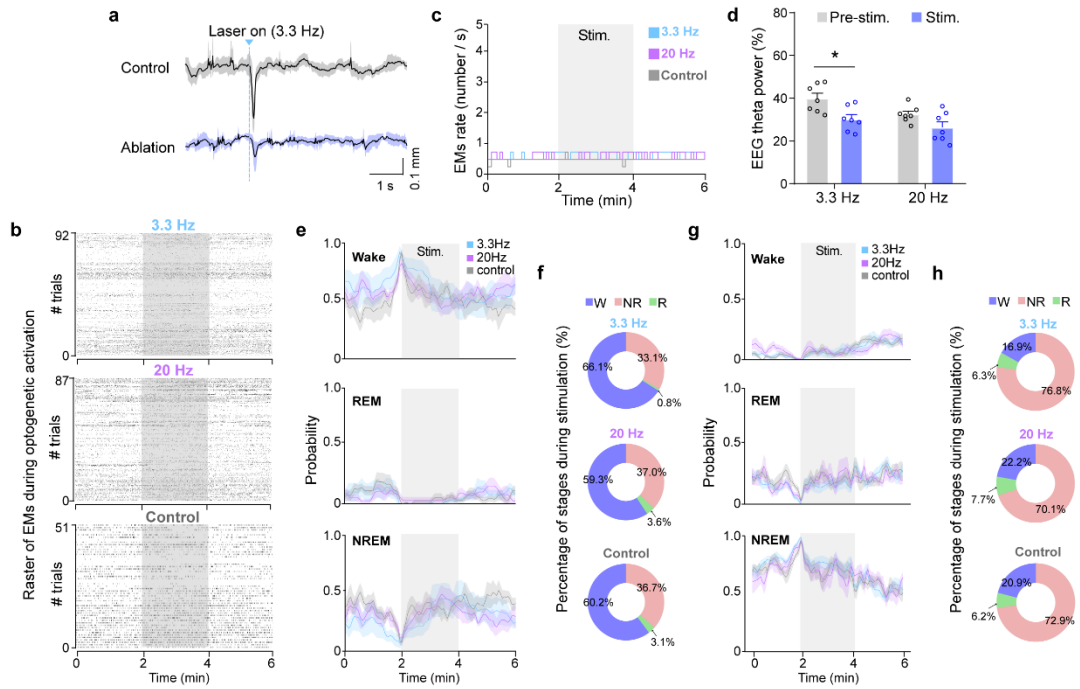
high-frequency EMs. The dashed red box indicates the EMs-free or EMs-enriched REM episodes. The blue dots in the EMs trace indicate the automatically detected EMs event. **f** EEG power density during optogenetic activation of NIII^{ChAT} neurons in REM sleep (n = 6). Two-way ANOVA revealed no significant differences.



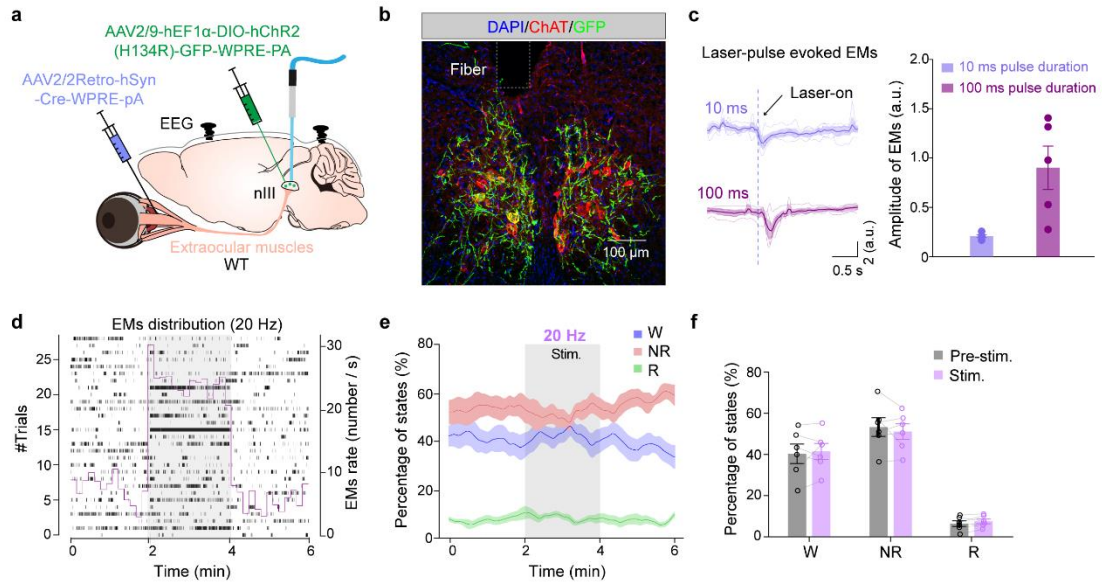
Supplementary Fig.11 | Temporal dynamics of nIII^{ChAT} neuronal firing during prolonged optogenetic stimulation. **a** Schematic of viral injection and fiber implantation. **b** Schematic of prolonged optogenetic activation protocol. Optogenetic stimulation was delivered at 20 Hz (10 ms pulse width), with each stimulation train lasting 1 sec followed by a 1 sec interval. This 1-s on/1-s off cycle was repeated continuously from 14:00 to 15:00 at light phase. **c** Temporal stationarity of ChR2-tagged nIII^{ChAT} neuron firing during 1 hr optogenetic stimulation (20 Hz, 10 ms pulse width, 2 mW; n = 20 units from 4 mice). The firing rates during stimulation were divided into four quartiles, and the average firing rate was calculated for each quartile. Spontaneous firing activity recorded 1 hr prior to stimulation served as the baseline control (No-stim.). Data are presented as mean $\pm$ SEM. Asterisks indicate statistical significance compared to the first quartile: * $p < 0.05$, ** $p < 0.01$. Two-sided paired t-test.



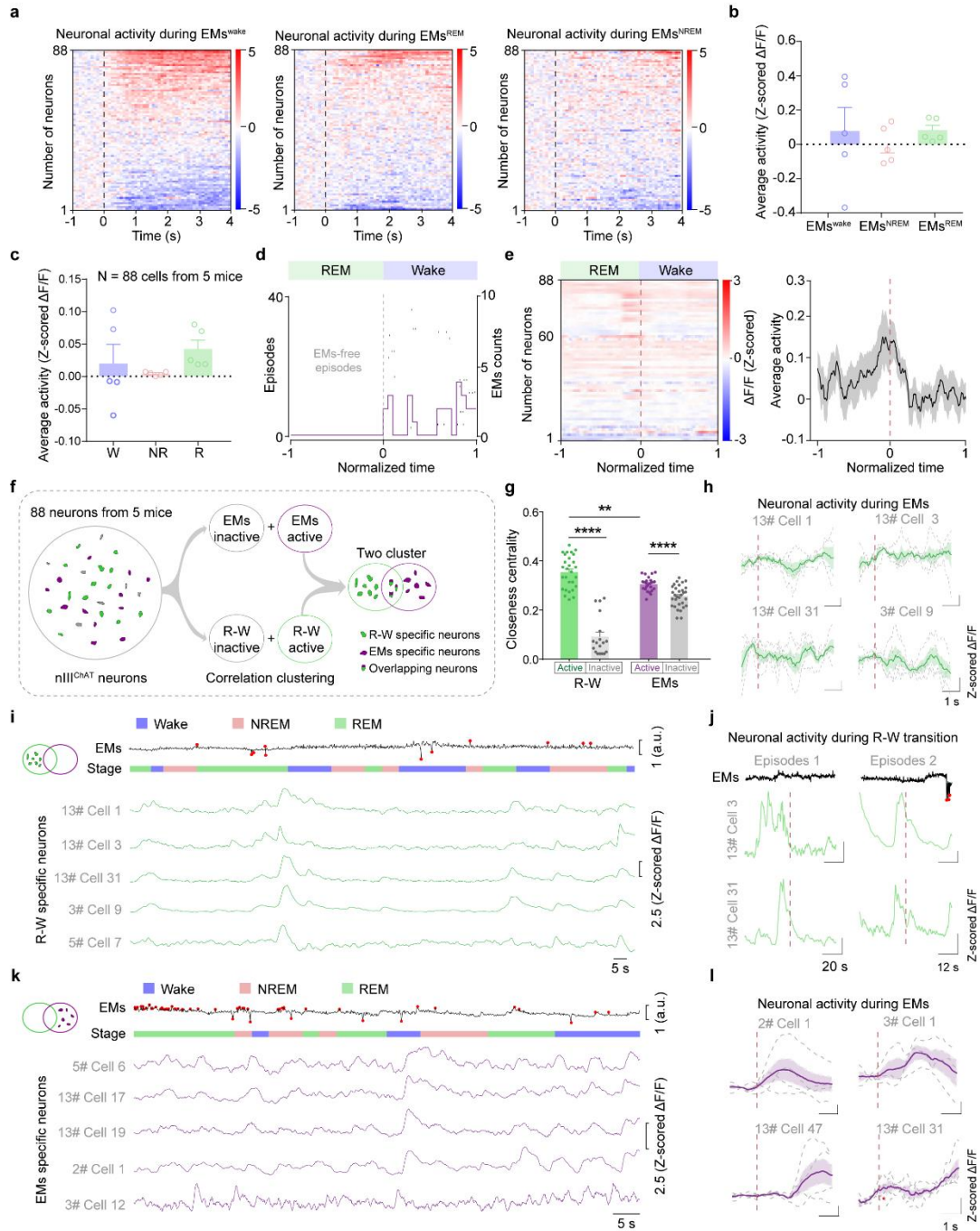
Supplementary Fig.12 | Proportion of EOMs-projecting neurons in the bilateral nIII regions. **a** Schematic of unilaterally injecting CTB555 into EOMs of C57 mice. **b** The confocal image of CTB555 (green) labelled ChAT⁺ (red) neurons in ipsilateral and contralateral nIII region. **c** Percentage of ChAT⁺ and CTB⁺ double-labeled neurons in ipsilateral and contralateral nIII region (Ipsi = 33.60 ± 5.37%, contra= 11.50 ± 6.65%).



Supplementary Fig.13 | Change of EMs and sleep architecture following optogenetic activating the non-EOMs-projecting nIII^{ChAT} neurons. **a** Laser-evoked EMs before and after genetic ablating the EOMs-projecting nIII^{ChAT} neurons in ChAT-Cre mice (n=4 mice). Shading indicates 95% confidence intervals (CIs). The blue dashed line indicates the onset of stimulus. Shading area indicates 95% confidence intervals (CIs). **b** Raster plot for events of EMs during optical stimulation in ChR2-tagged ChAT-Cre mice for 3.3 Hz (upper), 20 Hz (middle) and control group (lower). Shading area indicates time window of optical stimulus. **c** The average rate of EMs across trials during optogenetic activation after ablating the EOMs-projecting nIII^{ChAT} neurons in ChAT-Cre mice. The gray shading indicates laser stimulation window. **d** Mean EEG power spectrum density in theta band (6-9 Hz) before and during 3.3 Hz or 20 Hz, 480 nm optical stimulation (n=7 mice). Error bars represent SEM across mice. Two-way ANOVA followed by Bonferroni post hoc analysis. *p<0.05. **e** The probabilities of different vigilance states in ChAT-Cre mice when optogenetic activation during wake in ChR2-tagged nIII^{ChAT} neurons in ChAT-Cre mice. Lines represent the mean across mice, and shaded areas indicate 95% CIs. The gray shading indicates the time window of stimulation. **f** Pie charts showing changes in brain states during laser stimulation when specifically activating the nIII^{ChAT} neurons during wake. **g** Probabilities of different sleep state in ChAT-Cre mice when specifically activating the nIII^{ChAT} neurons during NREMs in ChR2-tagged ChAT-Cre mice. Lines represent the mean across mice, and shaded areas indicate 95% CIs. The gray shading indicates the time window of stimulation. **h** Pie charts showing changes in brain states during laser stimulation when specifically activating the nIII^{ChAT} neurons during NREMs. Data represent at least three biological replicates. All data and significance values for graphs d are shown in Supplementary Table 1.

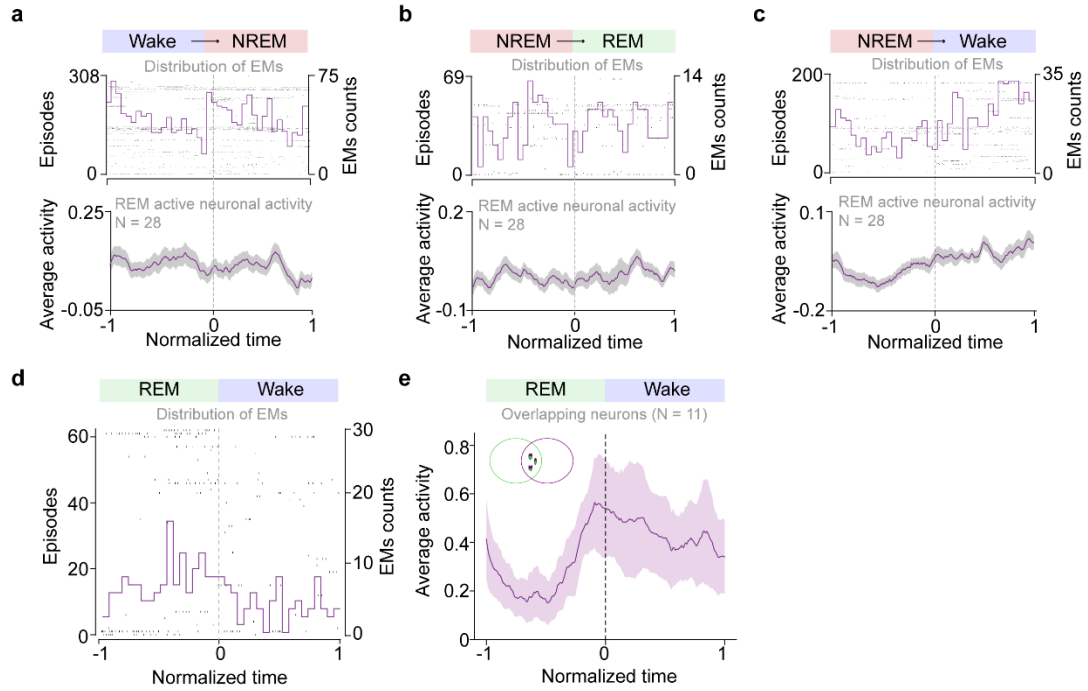


Supplementary Fig.14 | Effect of optogenetic activation of EOMs-projecting nIII^{ChAT} neurons on sleep. **a** Schematic of viral injection and fiber implantation in WT mice. **b** The confocal image of viral expression and location of fiber in the nIII. **c** Left: EMs trace induced by optogenetic activation of EOMs-projecting nIII^{ChAT} neurons. Right: quantification of laser-evoked EM amplitudes under 10 ms and 100 ms pulse durations. Each dot represents an individual mouse (n=5 mice). **d** Raster plot of EMs events during optogenetic activation of EOM-projecting nIII^{ChAT} neurons. Gray shading indicates the time window of optogenetic stimulation. **e** The fraction of wake, NREM, or REM states before, during and after laser stimulation (20 Hz, 480 nm) (n=6 mice). Lines represent the mean across mice, and shaded areas indicate 95% CIs. The gray shading indicates laser stimulation window. **f** The quantified probability of REMs, NREMs, and wake before (pre-stim.) and during (pre-stim.) laser stimulation (n = 6 mice). Error bars represent SEM across mice.

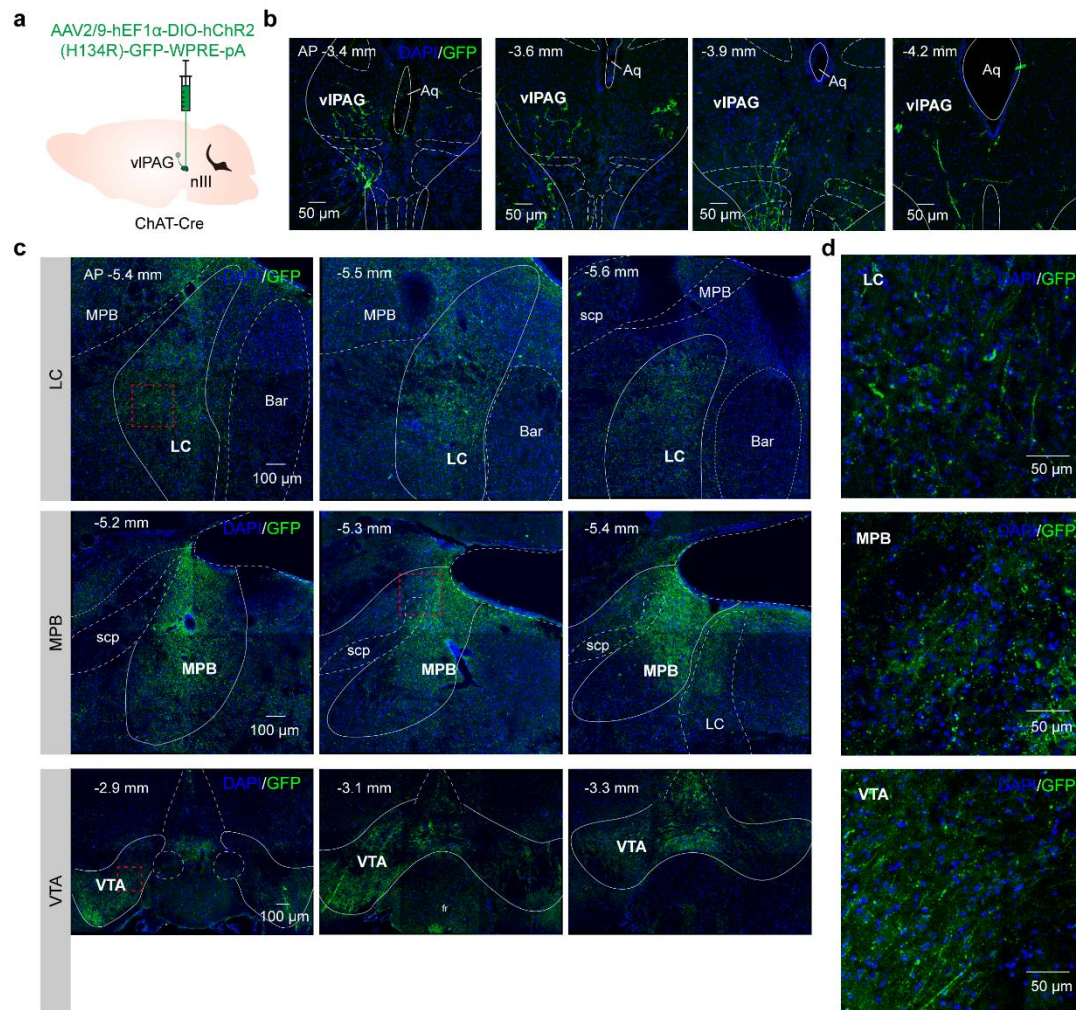


Supplementary Fig.15 | Differential activity patterns of $nIII^{ChAT}$ neurons during EMs and R-W transition. **a** The heatmap of neuronal activity aligned to onset of EMs in wake, REMs, and NREMs (n=88 from 5 mice). All cells were sorted by averaged fluorescence intensity. The vertical dashed lines indicate onset of EMs. **b** The average neuronal activity of $nIII^{ChAT}$ neurons corresponding to **a**. **c** Quantification of average activity of all $nIII^{ChAT}$ neurons during different brain state. Each dot represents one individual mouse (n=5 mice). One-way ANOVA followed bonferroni post hoc analysis between different states. **d** The distribution of EMs during R-W transition during which there is no EMs occurred in REM period. **e** Left: the activity heatmap for all 88 neurons aligned to all R-W transition episodes. Cells from 60-88 were the identified R-W active neurons. All cells are sorted by the average of activities. Right: the average activity of all cells during R-W transition. Time 0 represents the transition time point in R-W transition.

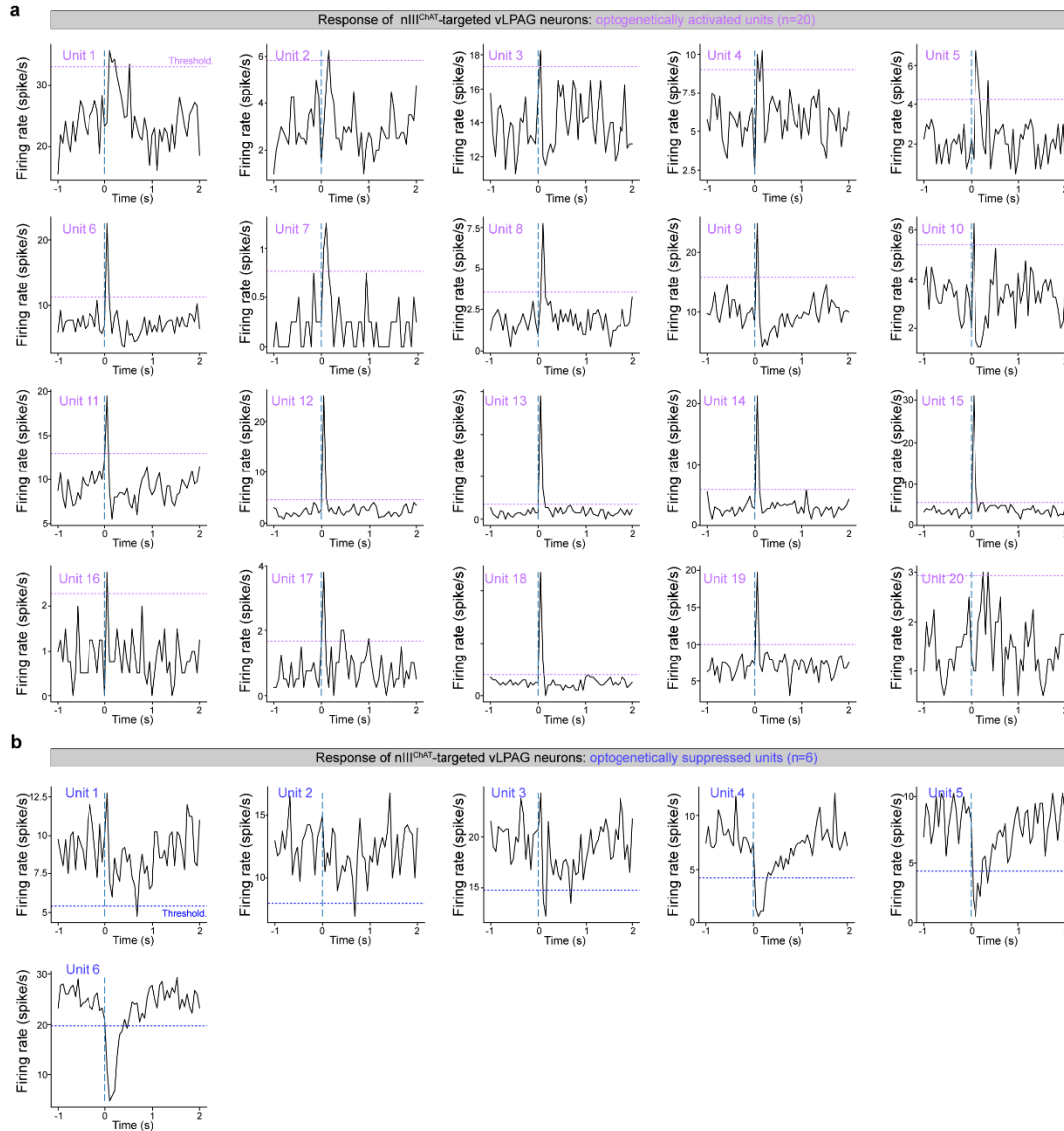
Lines represent the mean across mice, and shaded areas indicate 95% CIs. **f** The schematic of process to clustering the EMs active and R-W active neurons. **g** Comparison of the relative connectivity strength between R-W active and inactive neurons, EMs active neurons and EMs inactive neurons. Two-side unpaired t-test, ** $p < 0.01$, **** $p < 0.001$. Error bars represent SEM across mice. **h** The average calcium fluorescence of four representative R-W active neurons in response to EMs. The red dashed line indicates the onset of EMs. Lines represent the mean across mice, and shaded areas indicate 95% CIs. **i** $\Delta F/F$ trace of R-W active nIII^{ChAT} neurons across the sleep-wake cycle. **j** The average calcium fluorescence of four representative R-W active neurons during R-W transition. The red dashed line indicates the time point of transition. Lines represent the mean across mice, and shaded areas indicate 95% CIs. **k** $\Delta F/F$ trace of EMs-active nIII^{ChAT} neurons across the sleep-wake cycle, recorded concurrently with EMs signals (top). The red dots in the EMs trace indicate the events of EMs. **l** The average calcium fluorescence of four representative EMs-active neurons during EMs. The dashed line indicates the onset of EMs. Lines represent the mean across mice, and shaded areas indicate 95% CIs.



Supplementary Fig.16 | The distribution of EMs and average calcium signals of R-W transition-active $nIII^{CHAT}$ neuron across sleep-wake transitions. **a** The distribution of EMs (upper) and the average activity (lower) of all R-W active neurons ($n=28$) during W-NR transition. All modulated cells sorted by the average of activities. Each NREM and wake episode was temporally normalized. Lines represent the mean across mice, and shaded areas indicate 95% CIs. Time 0 represents the transition time point in W-NR transition. **b** The distribution of EMs (upper) and the average activity (lower) of all R-W active neurons ($n=28$) during NR-R transition. Each NREM and REM episode was temporally normalized. Time 0 represents the transition time point in NR-R transition. Lines represent the mean across mice, and shaded areas indicate 95% CIs. **c** The distribution of EMs (upper) and the average activity (lower) of all R-W active neurons ($n=28$) during NR-W transition. Time 0 represents the transition time point in NR-W transition. Lines represent the mean across mice, and shaded areas indicate 95% CIs. **d** The distribution of EMs during all R-W transition episodes. **e** The average activity of overlapping neurons during R-W transition. Lines represent the mean across mice, and shaded areas indicate 95% CIs. Time 0 represents the transition time point in R-W transition.

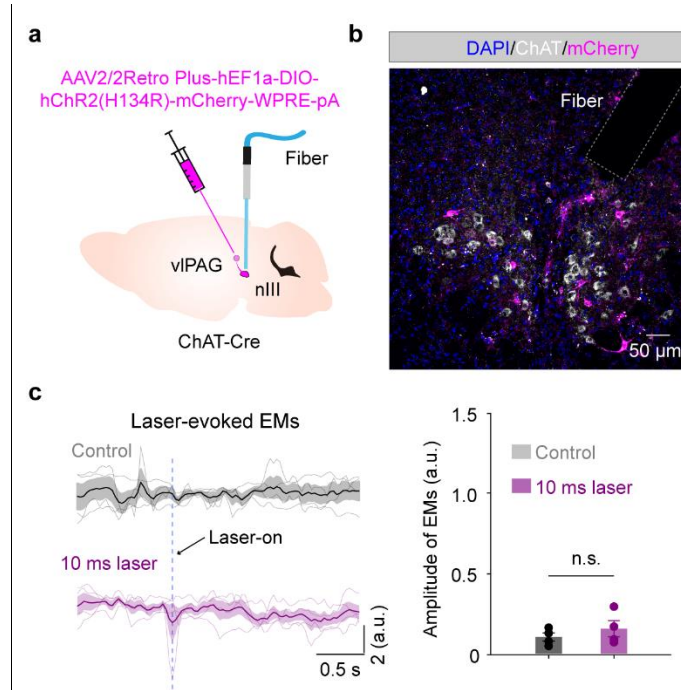


Supplementary Fig.17 | Axon terminals of nIII^{ChAT} neurons within the vIPAG, LC, MPB and VTA. a The schematic of virus injection in ChAT-Cre mice. **b** The distribution of nIII^{ChAT} neuronal terminal within the vIPAG along the rostral-caudal axis. **c** The distribution of nIII^{ChAT} neuronal terminal within the LC, MPB and VTA along the rostral-caudal axis. **d** Higher-magnification images of the LC, MPB, and VTA regions indicated by the red dashed boxes in (c).

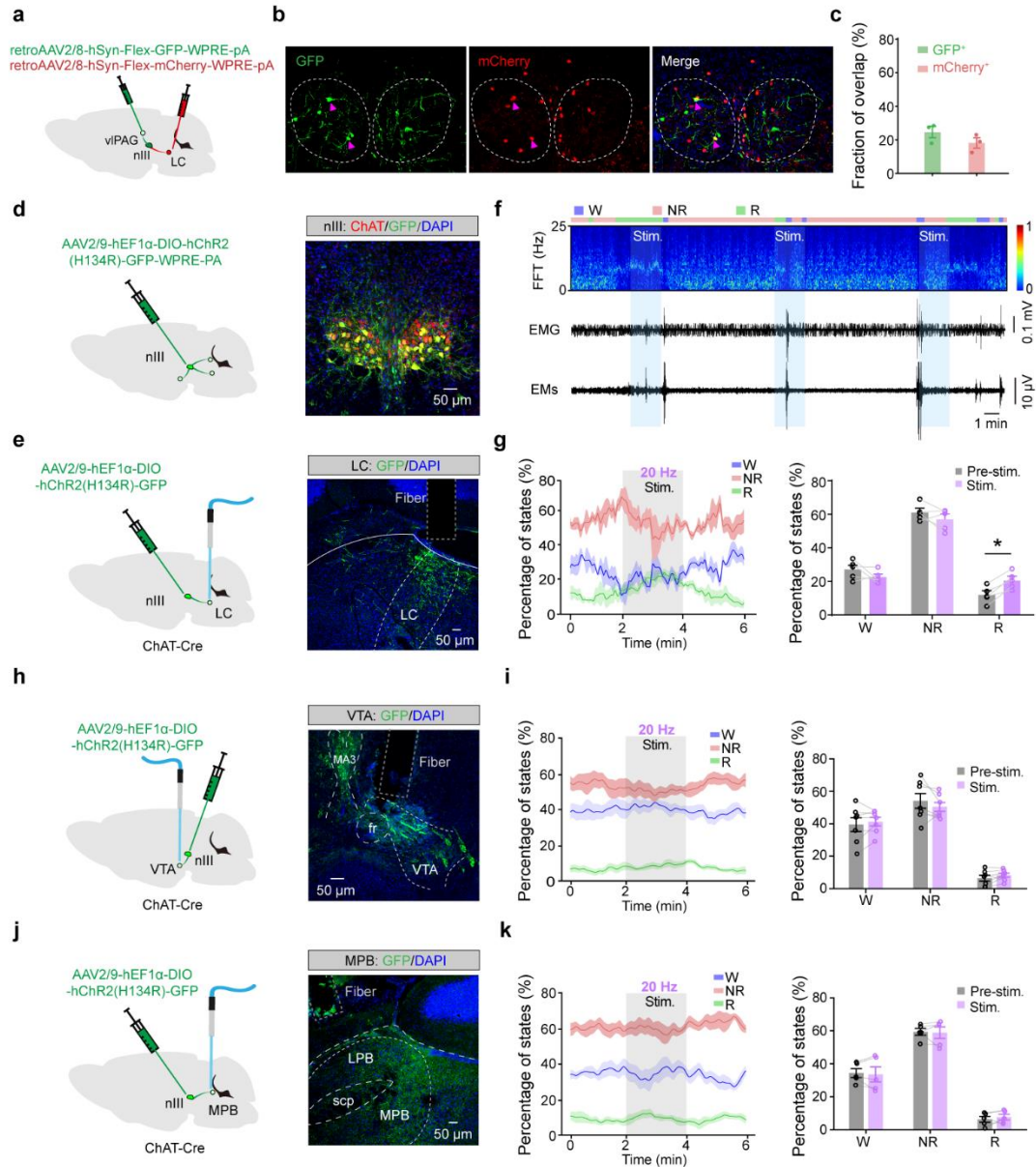


Supplementary Fig.18 | Laser-evoked response of nIII^{ChAT}-targeted vIPAG neurons. a

Units activated by optogenetic activating the nIII^{ChAT} neuronal terminal within the vIPAG. N = 20 units from 3 mice. The increased units were defined based on whether the post-stimulation peak firing rate exceeded a threshold of mean + 3 standard deviations (SD) of the baseline activity. Horizontal red dashed lines indicate response threshold. Vertical blue dashed lines indicate the onset of stimulus. Pulse duration, 10 ms. Pulse density, 2 mW. **b** Units suppressed by optogenetic activating the nIII^{ChAT} neuronal terminal within the vIPAG. N = 6 units from 3 mice. The decreased units were defined based on whether the post-stimulation peak firing rate below a threshold of mean - 3 standard deviations (SD) of the baseline activity. Horizontal blue dashed lines indicate response threshold. Vertical blue dashed lines indicate the onset of stimulus. Pulse duration, 10 ms. Pulse density, 2 mW.

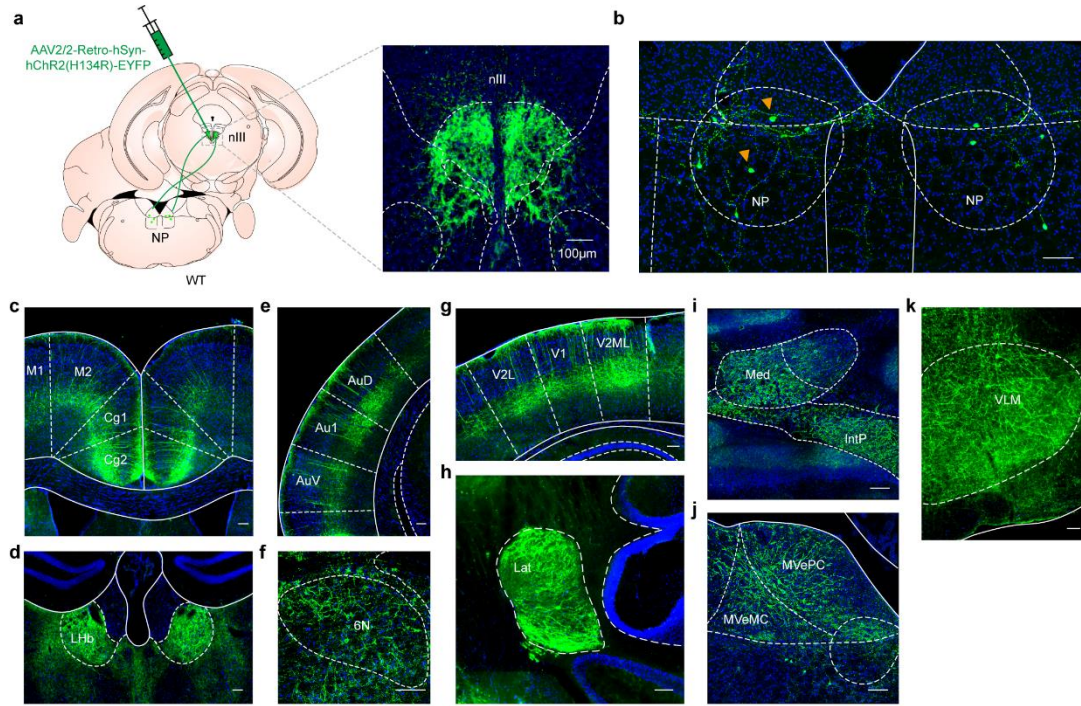


Supplementary Fig.19 | Effect of optogenetic activating vIPAG-projecting nIII^{ChAT} neurons on the EMs. **a** The schematic of virus injection and fiber implantation in the nIII. **b** Representative confocal image demonstrating overlap between ChR2 and ChAT expression in vIPAG-projecting nIII neurons. **c** Left: EMs trace induced by optogenetic activation of vIPAG-projecting nIII^{ChAT} neurons. Lines represent the mean across mice, and shaded areas indicate 95% CIs. Right: quantification of laser-evoked EMs amplitudes under 10 ms pulse durations compared to control group. Each dot represents an individual mouse. Two-sided paired t-test; n.s. indicates no significant difference.

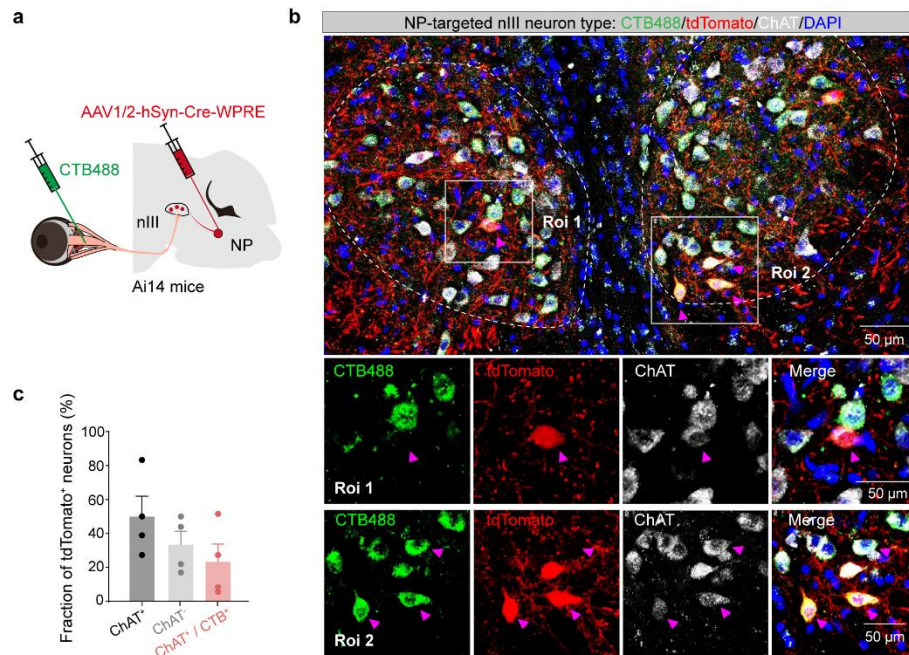


Supplementary Fig.20 | The effect of optogenetic activating the nIII^{ChAT}-targeted LC, VTA and MPB on sleep architecture. **a** The schematic of viral injection in ChAT-Cre mice. **b** Confocal image shows the vlPAG-projecting (GFP) and LC-projecting (mCherry) nIII^{ChAT} neuron. The arrows show the GFP and mCherry double labelling neurons. **c** Quantification of the proportion of double-labeled neurons among nIII^{ChAT} projections to both the LC and vlPAG. **d** Left: the schematic of viral injection in the nIII to label the efferent of nIII^{ChAT} neurons. Right: confocal image of the viral expression in injection site. **e** Left: the schematic of viral injection and fiber implantation in the LC. Right: the confocal image of the nIII^{ChAT} neuronal terminal and the fiber placement within the LC. The dashed box indicates the placement of fiber. **f** Typical example of sleep changes with EEG spectrogram, EMG, and EM traces during optical activating the nIII^{ChAT} neuronal terminal within the LC. Blue shielding areas indicate the time window of optogenetic activation. **g** Percentage (left) and quantification (right) of wake, NREM, or REM states before (pre-stim.), during (stim.), and after optogenetic activating the nIII^{ChAT} neurons terminal in the LC. Lines represent the mean across mice, and shaded areas indicate

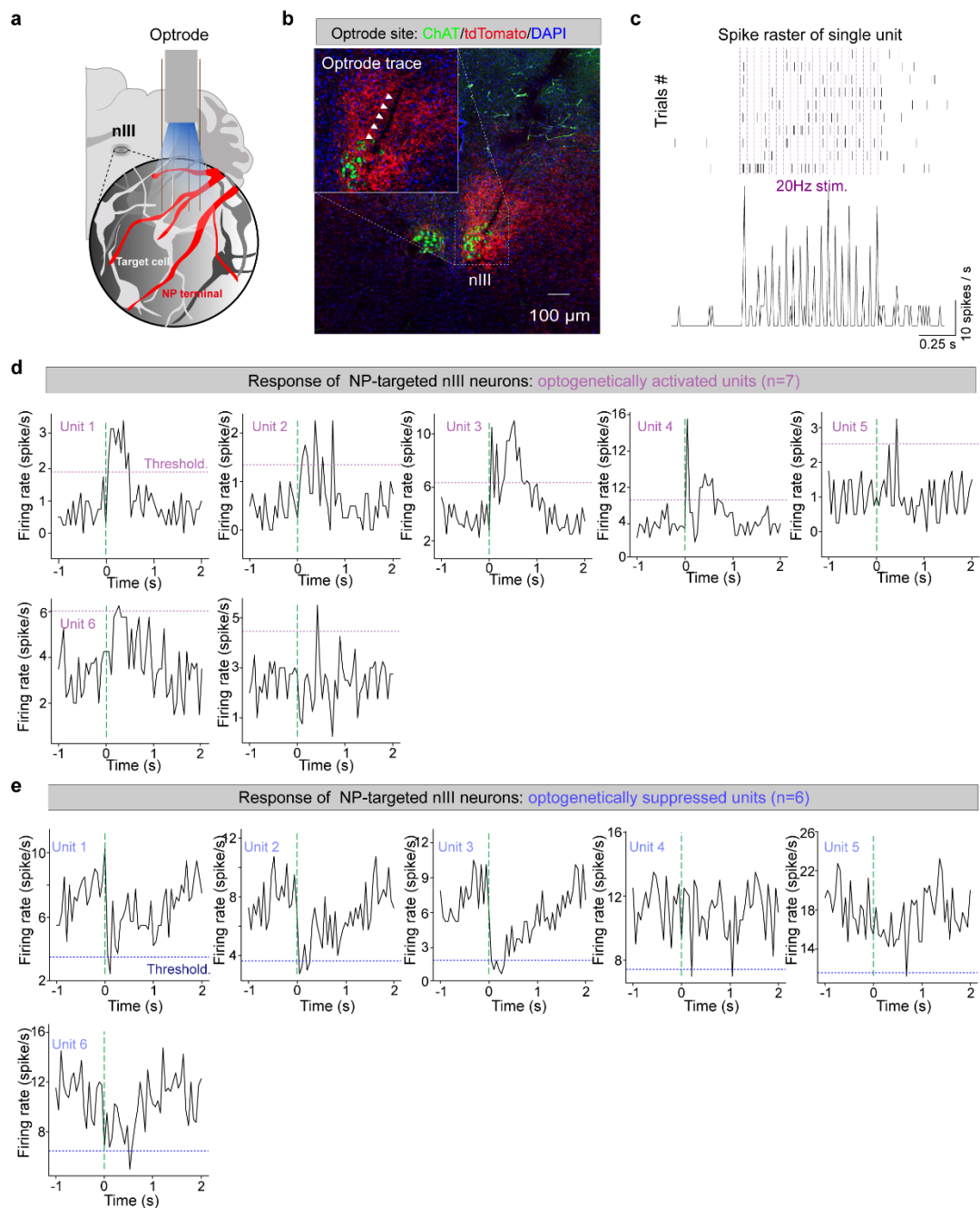
95% CIs. The gray shielding indicates the time window of stimulation. Error bars represent SEM across mice. ****p<0.01** for REM. Two-sided paired t-test. **h** The schematic of viral injection and fiber implantation in the VTA (left), the confocal image showing the nIII^{ChAT} neuronal terminal and the fiber placement within the VTA. The dashed box indicates the placement of fiber. **i** Percentage (left) and quantification (right) of wake, NREM, or REM states before (pre-stim.), during (stim.), and after optogenetic activating the nIII^{ChAT} neurons terminal in the VTA (n = 7). Lines represent the mean across mice, and shaded areas indicate 95% CIs. The gray shielding indicates the time window of stimulation. Error bars represent SEM across mice. **j** The schematic of viral injection and fiber implantation in the MPB (left), the confocal image showing the nIII^{ChAT} neuronal terminal and the fiber placement within the MPB. The dashed box indicates the placement of fiber. **k** Percentage (left) and quantification (right) of wake, NREM, or REM states before (pre-stim.), during (stim.), and after optogenetic activating the nIII^{ChAT} neurons terminal in the MPB (n=5). Lines represent the mean across mice, and shaded areas indicate 95% CIs. The gray shielding indicates the time window of stimulation. Error bars represent SEM across mice. Data represent at least three biological replicates. All data and significance values for graphs g are shown in Supplementary Table 1.



Supplementary Fig.21 | Primary long-range brain-wide inputs to nIII^{ChAT} neurons. **a** Left: the schematic of injecting retroAAV-EF1a-GFP virus into the nIII in ChAT-Cre mice. Right: the example image of viral expression in the nIII. Scale bar, 100 μ m. **b** The example image of retro-labeled neurons in the NP. **c** Primary motor cortex (M1), secondary motor cortex (M2), Cingulate cortex (Cg). **d** Lateral habenular nucleus (LHb). **e** Primary auditory cortex (Au1). **f** Root of abducens nerve (6N). **g** Primary visual cortex (V1), secondary visual cortex (V2). **h** Lateral (dentate) cerebellar nucleus (Lat). **i** Medial (fastigial) cerebellar nucleus (Med) and interposed cerebellar nucleus, posterior part (IntP). **j** Medial vestibular nucleus, parvicellular part (MVePC). **k** Ventrolateral medulla (VLM). Scaling bar, 100 μ m.

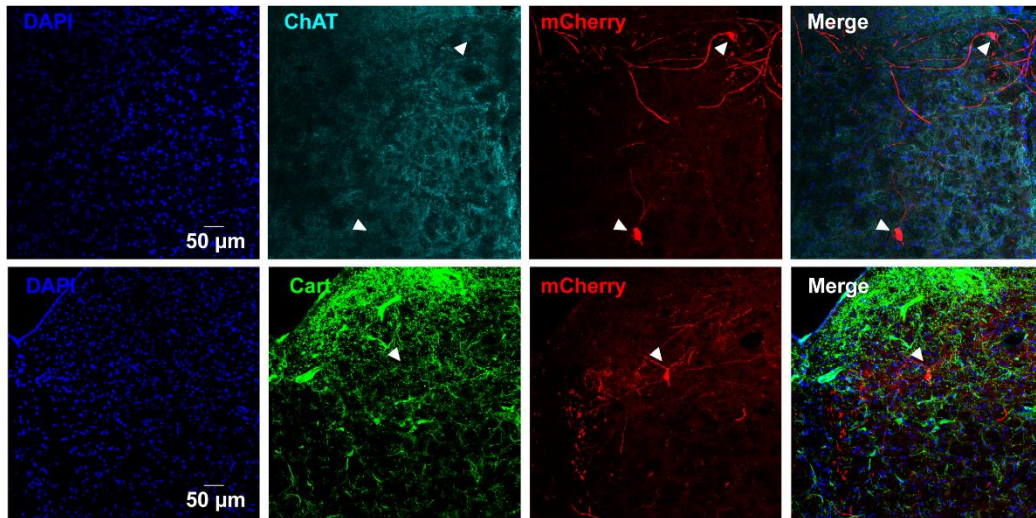


Supplementary Fig.22 | NP innervating two subpopulations of the nIII^{ChAT} neurons. **a** The schematic of strategy for bidirectional viral tracing. Injecting AAV1/2-hSyn-Cre-WPRE into the NP followed by CTB-488 into the EOMs in Ai14 mice. **b** Confocal image shows distribution of tdTomato (red), CTB488 (Green), and ChAT (white) positive neurons within the nIII. The arrows indicate co-labeled neurons. The ori 1 showing the tdTomato⁺/ChAT⁺ double-labeled neurons. The ori 2 showing CTB488⁺/tdTomato⁺/ChAT⁺ triple-labeled neurons. Scaling bar, 50 μ m. **c** The quantification of co-labeled neurons in the Ai14 mice. Each dot indicates one individual mouse (n=4 mice). Error bars represent SEM across mice.



Supplementary Fig.23 | Optogenetic-assisted electrophysiological recording of NP-targeted nIII neurons. **a** Schematic drawing of ChrimsonR-targeted nIII neurons using electrophysiology recording after co-injection of AAV2/9-hSyn-ChrimsonR-tdTomato (red) in the NP. **b** Confocal image showing the viral expression and fiber location within the nIII. The arrows in the magnified region show the trace of the electrode. **c** Representative ChrimsonR-targeted unit within the nIII showing pulse-dependent firing rate during continuous laser-pulse stimulation. Purple dashed lines indicate the laser pulse (20 Hz, 10 ms per pulse, lasting for 1 s). **d** Units activated by optogenetic activating the NP neuronal terminal within the nIII. N = 7 units from 3 mice. The increased units were defined based on whether the post-stimulation peak firing rate exceeded a threshold of mean + 3 standard deviations (SD) of the baseline activity.

Horizontal red dashed lines indicate response threshold. Vertical purple dashed lines indicate the onset of stimulus. Pulse duration, 10 ms. Pulse density, 2 mW. **e** Units suppressed by optogenetic activating the NP terminal within the nIII. N = 6 units from 3 mice. The decreased units were defined based on whether the post-stimulation peak firing rate below a threshold of mean - 3 standard deviations (SD) of the baseline activity. Horizontal blue dashed lines indicate response threshold. Vertical purple dashed lines indicate the onset of stimulus. Pulse duration, 10 ms. Pulse density, 2 mW.



Supplementary Fig.24 | Identification of NP neuronal subtypes by Immunostaining. Representative confocal image of NP in coronal section. ChAT, choline acetyltransferase. mCherry, fluorescent reporter carried by the rabies virus (RV). Cart, cocaine-and-amphetamine-regulated transcript. The white arrows indicate the mCherry positive neurons. Scale bar, 50 μ m.

1 **Supplementary Table 1:** Statistical detail information for figures.

Figure	Group	N value	Statistical methods	Test statistics
Fig. 1g	EMs-free wake episodes VS EMs-enriched wake episodes.	N = 4 mice	Two-way ANOVA followed by Tukey's multiple comparisons test	F (2, 27) = 20.66, q = 12.07, p < 0.0001.
Fig. 1g	Total wake episodes VS EMs-enriched wake episodes.			F (2, 27) = 20.66, q = 5.238, p = 0.0229.
Fig. 1g	Total NREM episodes VS EMs-enriched NREM episodes.			F (2, 27) = 20.66, q = 0.1198, p = 0.0002.
Fig. 1g	Total REM episodes VS EMs-enriched REM episodes.			F (2, 27) = 20.66, q = 9.595, p < 0.0001.
Fig. 1g	EMs-free REM episodes VS EMs-enriched REM episodes.			F (2, 27) = 20.66, q = 5.024, p = 0.0326.
Fig. 1g	EMs-free wake episodes VS EMs-free REM episodes.			F (2, 27) = 161.6, q = 14.83, p < 0.0001.
Fig. 1g	EMs-free NREM episodes VS EMs-free REM episodes.			F (2, 27) = 161.6, q = 17.49, p < 0.0001.
Fig. 2c	Control group VS Ablation group.	Control group: n = 3, Ablation group: n = 4.	Two-sided unpaired t-test.	t = 9.717, p = 0.0002.
Fig. 2f	N-T: Control group VS Ablation group.	Control group: n = 7, Ablation group: n = 5.	Two-way ANOVA followed by Tukey's multiple comparisons test	F (1, 20) = 4.512, q = 5.939, p = 0.0023.
Fig. 2f	T-N: Control group VS Ablation group.			F (1, 20) = 4.512, q = 2.173, p = 0.4354.
Fig. 2g, left	Wake time at 9 pm: Control group VS Ablation group.	Control group: n = 5, Ablation group: n = 5.	Two-way RM ANOVA followed by Bonferroni post hoc analysis.	F (1, 8) = 10.68, t = 3.378, p = 0.0212.
Fig. 2g, right	REM time at 9 pm: Control group VS Ablation group.			F(1, 8)=17.99, t=3.126, p=0.0491.

Fig. 2h, left	Wake time during 24 h phase: Control group VS Ablation group.	Control group: n = 5, Ablation group: n = 5.	Two-sided unpaired t-test	$t_8 = 3.267, p = 0.0114$.
Fig. 2h, left	Wake time during night phase: Control group VS Ablation group.	Control group: n = 5, Ablation group: n = 5.	Two-way ANOVA followed by Bonferroni post hoc analysis.	$F(1, 16) = 9.410, t = 2.254, p = 0.0771$.
Fig. 2h, right	REM time during 24 h phase: Control group VS Ablation group.	Control group: n = 5, Ablation group: n = 5.	Two-sided unpaired t-test	$t_8 = 4.241, p = 0.0028$.
Fig. 2h, right	REM time during night phase: Control group VS Ablation group.	Control group: n = 5, Ablation group: n = 5.	Two-way ANOVA followed by Bonferroni post hoc analysis.	$F(1, 16) = 19.84, t = 3.268, p = 0.0097$.
Fig. 2h, right	REM time during day phase: Control group VS Ablation group.	Control group: n = 5, Ablation group: n = 5.	Two-way ANOVA followed by Bonferroni post hoc analysis.	$F(1, 16) = 19.84, t = 3.031, p = 0.0159$.
Fig. 2i, histogram	REM episodes duration during night phase: Control group VS Ablation group.	Control group: n = 5, Ablation group: n = 5.	Two-way ANOVA followed by Bonferroni post hoc analysis.	$F(1, 16) = 15.4, t = 2.795, p = 0.0259$.
Fig. 2i, histogram	REM episodes duration during day phase: Control group VS Ablation group.			$F(1, 16) = 15.4, t = 2.754, p = 0.0282$.
Fig. 2i	REM episodes duration during day phase: Control group VS Ablation group.	Control group: n = 5, Ablation group: n = 5.	Two-sided Kolmogorov-Smirnov test	$p < 0.001$.
Fig. 2j	REM episodes number during hight phase: Control group VS Ablation group.	Control group: n = 5, Ablation group: n = 5.	Two-way ANOVA followed by Bonferroni post hoc analysis.	$F(1, 16) = 15.22, t = 3.289, p = 0.0092$.
Fig. 2j	REM episodes number during day phase: Control group VS Ablation group.			$F(1, 16) = 15.22, t = 2.229, p = 0.0794$.
Fig. 2k	R-W transition number: Control group VS Ablation group.	Control group: n = 5, Ablation group: n = 5.	Two-sided unpaired t-test	$t_8 = 3.052, p = 0.0158$.
Fig. 2p	REM sleep probability: Pre-stim. group VS Stim. Group.	Pre-stim. group: n = 6, Stim. group: n = 6.	Two-sided unpaired t-test	$t_5 = 3.826, p = 0.0123$.
Fig. 3c	ChAT ⁺ neurons density: Control group VS Ablation group.	Control group: n = 4, Ablation group: n = 4.	Two-sided unpaired t-test	$t_6 = 4.212, p = 0.0056$.

Fig. 3d	EMs amplitude: Control group VS Ablation group.	Control group: n = 7, Ablation group: n = 7.	Two-sided paired t-test	$t_6 = 4.250, p = 0.0054.$
Fig. 3l	Change of REM sleep: 3.3 Hz group VS Control group.	3.3 Hz group: n = 8, Control group: n = 8.	One-way RM ANOVA followed by Tukey post hoc analysis	$F(1.8, 12.7) = 8.311, q = 4.758, p = 0.0285.$
Fig. 3l	Change of REM sleep: 20 Hz group VS Control group.	20 Hz group: n = 8, Control group: n = 8.		$F(1.8, 12.7) = 8.311, q = 5.012, p = 0.0225.$
Fig. 3o	Latency: 3.3 Hz group VS Control group.	3.3 Hz group: n = 8, Control group: n = 8.	Two-way ANOVA followed by Tukey post hoc analysis.	$F(1.4, 9.4) = 13.66, q = 6.791, p = 0.0048.$
Fig. 3o	Latency: 20 Hz group VS Control group.	20 Hz group: n = 8, Control group: n = 8.		$F(1.35, 9.43) = 13.66, q = 4.950, p = 0.0238.$
Fig. 5k	Wake: Pre-stim. group VS Stim. Group.	20 Hz group: n = 5, Control group: n = 5.	Two-way ANOVA followed by Bonferroni post hoc analysis.	$F(2, 24) = 39.49, t = 7.208, p < 0.0001.$
Fig. 5k	NREM: Pre-stim. group VS Stim. Group.			$F(2, 24) = 39.49, t = 2.880, p = 0.0247.$
Fig. 5k	REM: Pre-stim. group VS Stim. Group.			$F(2, 24) = 39.49, t = 4.328, p = 0.0007.$
Fig. 6l	Control group VS 10 ms laser group.	N = 3 for both group.	Two-sided paired t-test	$t_2 = 4.362, p = 0.0488.$
Fig. 6p	NREM: Pre-stim. group VS Stim. Group.	20 Hz group: n = 5, Control group: n = 5.	Two-way ANOVA followed by Bonferroni post hoc analysis.	$F(2, 24) = 19.72, t = 4.610, p = 0.0003.$
Fig. 6p	REM: Pre-stim. group VS Stim. Group.			$F(2, 24) = 19.72, t = 4.250, p = 0.0008.$
Fig. S2f	Wake: Control group VS SR group.	Control group: n = 6, SR group: n = 8.	Two-way ANOVA followed by Bonferroni post hoc analysis.	$F(1, 36) = 3.2e-006, t = 3.197, p = 0.0087.$
Fig. S2h	SD group VS SR group.	SD group: n = 4, SR group: n = 4.	Two-sided unpaired t-test	$t_6 = 4.819, p = 0.0029.$
Fig. S2j	SD group VS SR group.	SD group: n = 3, SR group: n	Two-sided unpaired t-test	$t_5 = 3.071, p = 0.0278.$

		= 4.		
Fig. S5b	100 ms stimulation: Before ablation group VS After ablation group.	N=4 for both group.	Two-way ANOVA followed by Bonferroni post hoc analysis.	F (1, 12) = 5.181, t = 2.991, p = 0.0225.
Fig. S5e	Average firing rate of nIII ^{ChAT} neuron during Wake, NREM and REM sleep	N=4 for both group.	Two-way ANOVA followed by Tukey post hoc analysis.	F (2, 9) = 6.634, q = 5.065, p = 0.0147.
Fig. S9f	3.3 Hz: Pre-stim. group VS Stim. Group.	Pre-stim. group: n = 7, Stim. group: n = 7.	Two-way ANOVA followed by Bonferroni post hoc analysis.	F (1, 24) = 12.60, t = 3.077, p = 0.0103.
Fig. S9f	20 Hz: Pre-stim. group VS Stim. Group.	Pre-stim. group: n = 7, Stim. group: n = 7.		F (1, 24) = 12.60, q = 1.943, p = 0.1276.
Fig. S9i	Wake: 3.3 Hz group VS Control group.	3.3 Hz group: n = 8, Control group: n = 6.	One-way RM ANOVA followed by Tukey post hoc analysis	F (2, 54) = 0.1606, q = 2.283, p = 0.0217.
Fig. S9i	Wake: 20 Hz group VS Control group.	20 Hz group: n = 7, Control group: n = 6.		F (2, 54) = 0.1606, q = 3.890, p = 0.0003.
Fig. S9i	REM: 3.3 Hz group VS Control group.	3.3 Hz group: n = 8, Control group: n = 6.		F (2, 54) = 0.1606, q = 4.232, p = 0.0114
Fig. S9i	REM: 20 Hz group VS Control group.	20 Hz group: n = 7, Control group: n = 6.		F (2, 54) = 0.1606, q = 5.169, p = 0.0017.
Fig. S9l	Latency to wake: 3.3 Hz group VS Control group.	3.3 Hz group: n = 7, Control group: n = 5.	One-way RM ANOVA followed by Tukey post hoc analysis	F (2, 16) = 15.57, q = 6.416, p = 0.0009.
Fig. S9l	Latency to wake: 20 Hz group VS Control group.	3.3 Hz group: n = 7, Control group: n = 5.		F (2, 16) = 15.57, q = 7.477, p = 0.0002.
Fig. S9m, left	Wake time at 14:10 min: 20 Hz group VS Control group.	20 Hz group: n = 8, Control group: n = 8.	Two-way RM ANOVA followed by Bonferroni post hoc analysis	F (2, 21) = 0.4657, t = 2.592, p = 0.0198.
Fig. S9m, left	Wake time at 15:30 min: 20 Hz group VS Control group.	20 Hz group: n = 8, Control group: n = 8.		F (2, 21) = 0.4657, t = 2.369, p = 0.0367.
Fig. S9m,	Wake time at 15:30 min: 3.3 Hz group VS	3.3 Hz group: n = 8, Control		F (2, 21) = 0.4657,

left	Control group.	group: n = 8.		t = 2.4, p = 0.0338.
Fig. S9m, middle	NREM time at 14:20 min: 20 Hz group VS Control group.	20 Hz group: n = 8, Control group: n = 8.		F (2, 21) = 1.339, t = 2.893, p = 0.0237.
Fig. S9m, right	REM time at 14:20 min: 3.3 Hz group VS Control group.	3.3 Hz group: n = 8, Control group: n = 8.		F (2, 21) = 0.3222, t = 2.837, p = 0.0332.
Fig. S9m, right	REM time at 14:20 min: 20 Hz group VS Control group.	20 Hz group: n = 8, Control group: n = 8.		F (2, 21) = 0.3222, t = 2.925, p = 0.0285.
Fig. S9m, right	REM time at 14:30 min: 3.3 Hz group VS Control group.	3.3 Hz group: n = 8, Control group: n = 8.		F (2, 21) = 0.3222, t = 3.145, p = 0.0191.
Fig. S9m, right	REM time at 14:30 min: 20 Hz group VS Control group.	20 Hz group: n = 8, Control group: n = 8.		F (2, 21) = 0.3222, t = 2.988, p = 0.0221.
Fig. S9m, right	REM time at 16:00 min: 3.3 Hz group VS Control group.	3.3 Hz group: n = 8, Control group: n = 8.		F (2, 21) = 0.3222, t = 2.705, p = 0.0477.
Fig. S9m, right	REM time at 16:00 min: 20 Hz group VS Control group.	20 Hz group: n = 8, Control group: n = 8.		F (2, 21) = 0.3222, t = 3.551, p = 0.0067.
Fig. S9n	NREM amount time: 3.3 Hz group VS Control group.	3.3 Hz group: n = 7, Control group: n = 8.	One-way ANOVA followed by Tukey post hoc analysis	F (4, 61) = 6.246, q = 5.585, p = 0.0006.
Fig. S9n	NREM amount time: 3.3 Hz group VS Control group.	3.3 Hz group: n = 7, Control group: n = 8.		F (4, 61) = 3.62, q = 3.620, p = 0.0342.
Fig. S13d	3.3 Hz: Pre-stim. group VS Stim. Group.	Pre-stim. group: n = 7, Stim. group: n = 7.	Two-way ANOVA followed by Bonferroni post hoc analysis	F (1, 12) = 15.27, t = 3.077, p = 0.0103.
Fig. S19c	Laser-evoked EMs amplitude: Control group VS 10 ms laser group	Control group: n = 4; 10 ms laser group: n = 4.	Two-sided unpaired t-test	t ₃ = 1.687, p = 0.1903
Fig. S20g	REM: Pre-stim. group VS Stim. Group.	Pre-stim. group: n = 5, Stim. group: n = 5.	Two-way ANOVA followed by Bonferroni post hoc analysis	F (2, 24) = 5.280, t = 2.652, p = 0.0418.

3 **Supplementary Movies**

4 **Supplementary Movie 1.** EMs were induced by optogenetic activation of nIII^{ChAT} neurons at
5 3.3 Hz (100 ms pulse duration, 200 ms pulse width, 2 mW) (Affiliated to Supplementary Fig.8)

6 **Supplementary Movie 2.** EMs were induced by optogenetic activation of nIII^{ChAT} neurons at
7 20 Hz (10 ms pulse duration, 40 ms pulse width, 2 mW) (Affiliated to Supplementary Fig.8)

8 **Supplementary Movie 3.** Laser-evoked EMs (3.3 Hz, 100 ms pulse duration, 200 ms pulse
9 width, 2 mW) recorded prior to the ablation of EOMs-projecting nIII^{ChAT} neurons (Affiliated to
10 Fig.3)

11 **Supplementary Movie 4.** Laser-evoked EMs (3.3 Hz, 100 ms pulse duration, 200 ms pulse
12 width, 2 mW) recorded after the ablation of EOMs-projecting nIII^{ChAT} neurons (Affiliated to
13 Fig.3)

14 **Supplementary Movie 5.** In vivo single-cell calcium imaging of nIII^{ChAT} neurons across the
15 spontaneous sleep-wake cycle at 16 Hz sample rate (Affiliated to Fig.4)

16 **Supplementary Movie 6.** Optogenetic activation of nIII^{ChAT} neuronal terminals within the
17 vIPAG at 20 Hz resulted in the termination of REMs (10 ms pulse duration, 40 ms pulse width,
18 2 mW) (Affiliated to Fig.5)