**Supplementary Material for**

GABAergic Neurons in the Dorsal-intermediate Lateral Septum Regulate Sleep-wakefulness and Anesthesia in Mice

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**Supplemental Results**

**Video 1** Optogenetic activation of dorsal-intermediate lateral septum GABAergic neurons during non-REM sleep promotes wakefulness. Blue light (473 nm, 2-3 mW, 10 ms width at 20 Hz) was illuminated in the dorsal-intermediate lateral septum area in non-REM sleep state to activate ChR2-expressing dorsal-intermediate lateral septum GABAergic neurons.

**Supplementary Fig. 1.** Raw curve. **A** Representative EEG and EMG traces recorded from the same *Vgat-Cre* mouse during the awake state, non-REM sleep, and REM sleep. **B** Representative EEG and EMG traces recorded from the same *Vgat-Cre* mouse during the awake state, deep anesthesia (burst suppression), and anesthesia recovery periods. EEG: electroencephalogram; EMG: electromyography; non-REM: non-rapid eye movement; REM: rapid eye movement; Pre-anesth.: Pre-anesthesia; Anesth.: Anesthesia; Post-anesth.: Post- anesthesia.

**Supplementary Fig. 2.** Fluorescence signals aligned to wake state transitions. Upper panel, individual transitions with color coded fluorescence intensity (non-REM to wake; wake to non-REM; REM to wake; non-REM to REM). Middle panel, comparison of area under the curve (AUC) per second before (-30 to 0 s) and after (0 to 30 s) state transitions, Unpaired two-tailed Student’s *t* test: *t6* = 4.93, *P* = 0.003 (non-REM-wake); *t6* = 15.42, *P* < 0.001 (wake-non-REM); *t6* = 3.32, *P* = 0.016 (REM-wake); *t6* = 7.74, *P* < 0.001 (non-REM-REM). Lower panel, mean (the solid line) ± SD. (gray shading) showing the average calcium transients from all the transitions. non-REM: non-rapid eye movement; REM: rapid eye movement. \**P* < 0.05, \*\**P* < 0.01, \*\*\**P* < 0.001.

**Supplementary Fig. 3.** Optogenetic activation of lateral septum GABAergic neurons has no effect on REM sleep. **A** Representative EEG/EMG traces, heat map of EEG power spectra shows that acute photostimulation (20 Hz/30 s) applied during REM sleep has no change in a ChR2-eYFP mouse. **B** Mean latencies of REM to wake transitions following optogenetic stimulation at 20 Hz. *t8*= 0.15, *P* = 0.885 unpaired two-tailed Student’s *t* test. **C-D** Average of normalized spectral power distribution of relative cortical EEG power density in control animals (black trace) and ChR2-eYFP (blue trace) animals following optical activating LS GABAergic neurons during REM sleep. EEG: electroencephalogram; EMG: electromyography; non-REM: non-rapid eye movement; REM: rapid eye movement;Data represent mean ± SD.

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**Supplementary Fig. 4.** According to principal component analysis, neurons are divided into three categories. Dopaminergic neurons (red), GABAergic neurons (blue), Glutamatergic neurons (yellow).

**Supplementary Fig. 5.** Terminal calcium signal of the dorsal-intermediate lateral septum GABAergic neurons in ventral tegmental area across sleep-awake states. **A** Schemetic showing the virus injection into the dorsal-intermediate lateral septum and optical fiber implantation into the ventral tegmental area. **B** Typical image showing the GCaMP6s-labeled neuronal terminals in the ventral tegmental area and the location of the optical fiber. Scale bar represents 100 µm. **C** Representative EEG power spectrum, EEG-EMG traces, and raw fluorescence trace at distinct sleep-wake states. **D** Summary data showing the calcium signals during the awake state, non-REM sleep and REM sleep (n = 7 mice, 5 sessions per mouse, Kruskal-Wallis One Way ANOVA on ranks with Tukey’s post hoc comparison: H2 = 9.98, *P* < 0.01; *P* (non-REM-wake) = 0.035, *P* (non-REM-REM) < 0.001).EEG: electroencephalogram; EMG: electromyography; LS: lateral septum; VTA: ventral tegmental area. non-REM: non-rapid eye movement; REM: rapid eye movement. DAPI: 4',6-diamidino-2-phenylindole. Data represent mean ± SD. \**P* < 0.05, \*\*\**P* < 0.001.

******Supplementary Fig. 6.** Inhibition dorsal-intermediate lateral septum-ventral tegmental area GABAergic neurons during wake promotes non-REM-like sleep state and prolongs emergency time compare to control conditions. **A** Schematic of experimental preparation. AAV-DIO-NpHR-eYFP and AAV-DIO-EYFP (control) were infused in the dorsal-intermediate lateral septum of *Vgat-Cre* animals. Fibers were implanted in the ventral tegmental area. **B** Image showing NpHR-eYFP expressing neuronal axons in the ventral tegmental area and the placement of optical fiber. Scale bar represents 100 µm. **C** Representative EEG/EMG traces and EEG heat map of NpHR-eYFP. Animals showing changes in the EEG oscillation, EMG and correspondent heat map EEG power spectrum in response to local terminal optical silencing (yellow stimulation, 593 nm) circuit during wake. **D** Wake-to-sleep transitions following optogenetic stimulation at 20 Hz. U= 0.00 *P* = 0.004, Mann-Whitney rank sum test (two-tailed). **E-I** Normalized spectral power of the relative cortical EEG power of delta increased, theta, beta and gamma decreased under unpaired two-tailed *t* test. Delta: *t10*= 2.74, *P* = 0.021; Theta: *t10*= 2.74, *P* = 0.021; Beta: *t10*= 1.63, *P* =0.135; Gamma: *t10*=2.18, *P* = 0.054. **i** left: Protocol for optical stimulation and recording of emergence time. Laser stimulation was begun after turning off isoflurane and ended with recovery of righting. right: Optical inactivation of dorsal-intermediate lateral septum GABAergic neurons prolonged emergence time from 1.5% isoflurane anesthesia. U= 0.00 *P* = 0.004, Mann-Whitney rank sum test (two-tailed). EEG: electroencephalogram; EMG: electromyography; LS: lateral septum; VTA: ventral tegmental area; DAPI: 4',6-diamidino-2-phenylindole. Data represent mean ± SD. \**P* < 0.05, \*\**P* < 0.01.