

Supplementary figures and legends

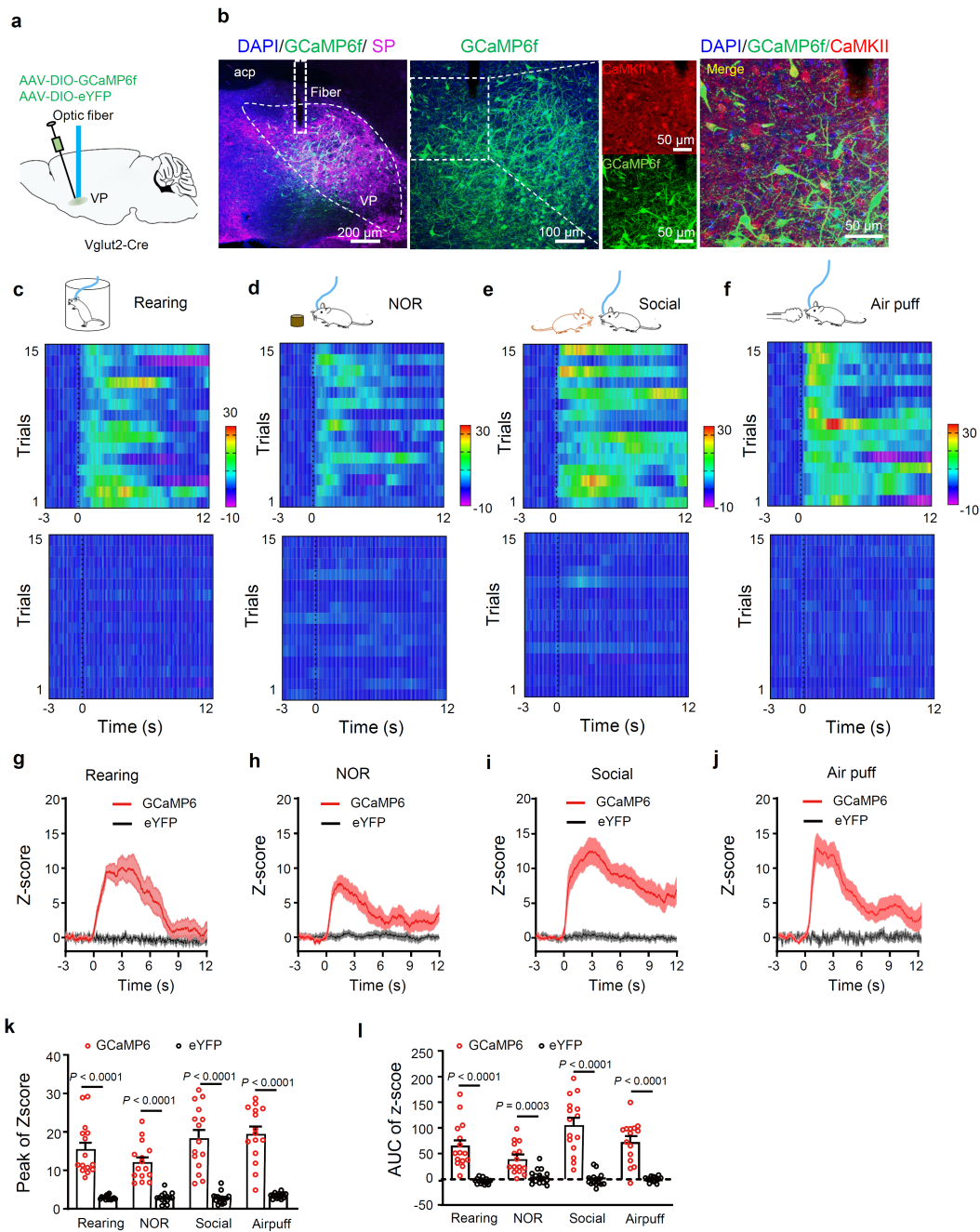


Fig 1 Ventral pallidal Glu neurons are activated by salient stimuli.

a AAV-EF1 α -DIO-GCaMP6 or AAV-EF1 α -DIO-eYFP was injected into the ventral pallidum (VP) of Vglut2-Cre mice to label glutamatergic (Glu) neurons. **b** Verification of virus expression in VP Glu neurons and the location of optical fiber. The VP was demarcated by higher level of Substance P (cyan). CaMKII: red; DAPI: blue; GCaMP6: green. **c–j** Heat map and averaged fluorescent signal in the VP in GCaMP6 and eYFP

mice while mice were rearing, recognizing novel object, encountering unfamiliar mice and receiving air puff. **k, l** The peak and area under the curve (AUC) of GCaMP6 ($n = 15$ trials from 5 GCaMP6 mice) and eYFP signals ($n = 15$ trials from 5 eYFP mice) during different behaviors. **k** Peak z-score. Rearing: $t = 7.10$, GCaMP6 vs eYFP; novel object recognition (NOR): $t = 7.05$, GCaMP6 vs eYFP; Social interaction: $t = 7.21$, GCaMP6 vs eYFP; Air puff: $t = 8.63$, GCaMP6 vs eYFP. **l** AUC. Rearing: $t = 8.63$, GCaMP6 vs eYFP; novel object recognition (NOR): $t = 4.14$, GCaMP6 vs eYFP; Social interaction: $t = 7.37$, GCaMP6 vs eYFP; Air puff: $t = 6.78$, GCaMP6 vs eYFP. Two-tailed t -tests in **k** and **l**. Mean $\pm$ SEM in (**g–l**). Source data are in Source Data.xlsx. Statistical results are in Supplementary Table 2.

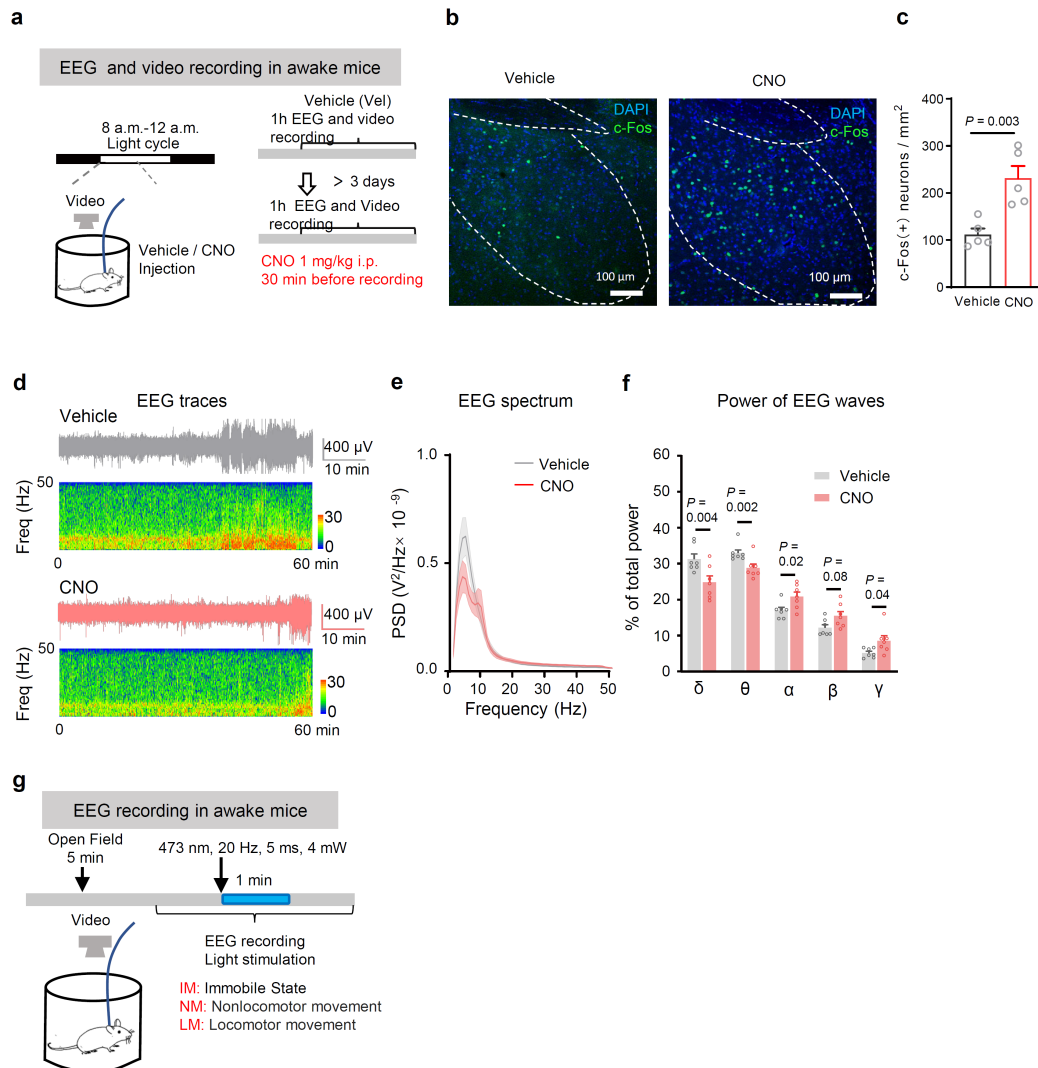


Fig. 2 Effects of chemogenetic activation of VP Glu neurons on EEG.

a Schematic diagram of chemogenetic activation of VP Glu neurons in mice subjected to EEG and video recordings to analyze movement and brain states. **b, c** Representative images and summary show that CNO activation of hM3Dq-expressing VP neurons increased c-Fos positive neurons in the VP. $t = 4.11$. Two-tailed unpaired t -test. $n = 5$ mice. **d** Representative EEG traces and corresponding power spectrum after intraperitoneal injection of Vehicle (above) and CNO (below). **e** Power spectrum analysis of 1 h EEG signals between 0.5 and 1.5 h after Vehicle and CNO injection. **f** % power of EEG waves at different frequency bands between 0.5 and 1.5 h after injection of Vehicle and CNO. δ : $t = 5.19$; θ : $t = 5.70$; α : $t = 3.42$; β : $t = 1.93$; γ : $t = 2.76$. **g** Optogenetic activation of VP Glu neurons and EEG recording during different movement states in mice. Mice were placed in the open field arena to adapt to the environment for 5 minutes before EEG recording and optogenetic stimulation. The blue line represents the time window of optogenetic stimulation (473 nm, 5 ms, 4 mW, 20 Hz). Two-tailed paired t -tests for **(d)**. $n = 7$ mice. Mean $\pm$ SEM in **(d, f, g)**. Source data are in Source Data.xlsx. Statistical results are in Supplementary Table 2.

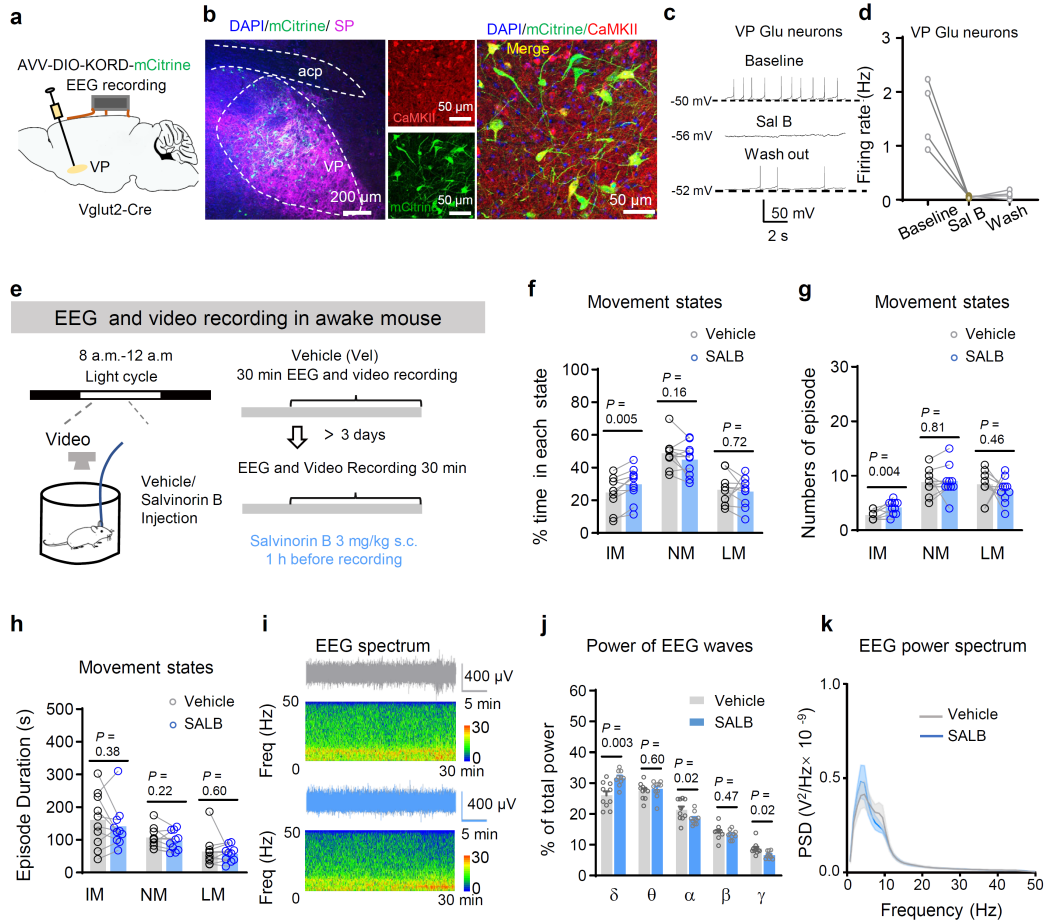


Fig 3 Effects of chemogenetic inhibition of VP Glu neurons on movement states and EEG waves in mice.

a Schematic diagram of virus injection and EEG recording. **b** Representative images showing virus expression in the VP. **c, d** Activation of KORD with salvininorin B (Sal B) inhibited firing in VP Glu neurons. **e** Schematic diagram of chemogenetic inhibition of VP Glu neurons in mice subjected to EEG and video recordings. **f** Time spent in each movement state between 1 and 1.5 h after Vehicle or Sal B injection. QS: $t = 3.65$; NM: $t = 1.51$; LM: $t = 0.36$; $n = 10$ mice. **g** Number of episodes. QS: $t = 3.77$; NM: $t = 0.25$; LM: $t = 0.78$; $n = 10$ mice. **h** Duration of each movement state. QS: $t = 0.93$; NM: $t = 1.33$; LM: $t = 0.55$; $n = 10$ mice. **i** Representative EEG traces and corresponding power spectra between 1 and 1.5 h after injection of Vehicle (above) and Sal B (below). **j** % power of waves 1 and 1.5 h after injection of Vehicle and Sal B. δ : $t = 4.02$; θ : $t = 0.54$; α : $t = 2.85$; β : $t = 0.75$; γ : $t = 2.87$. $n = 10$. **k** Power spectra of EEG signal between 0.5–1 h after Vehicle and Sal B injection.

Two-tailed paired *t*-test for (f–h, j). Mean $\pm$ SEM in (f–h, j, k). Source data are in Source Data.xlsx. Statistical results are in Supplementary Table 2.

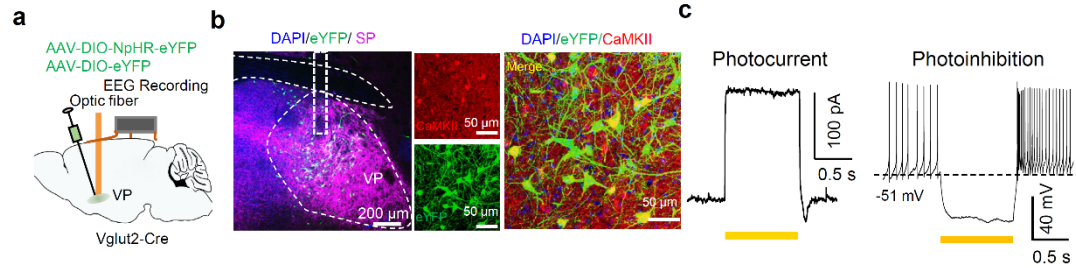


Fig 4 Optogenetic inhibition of VP Glu neurons.

a Schematic diagram of virus injection and optical fiber implantation for optogenetic inhibition. **b** Representative image of labeling of VP Glu neurons with AAV-EF1 α -DIO-NpHR-eYFP. **c, d** Representative traces showing that yellow light evoked an outward current and inhibited firing in a VP Glu neuron expressing NpHR-eYFP.

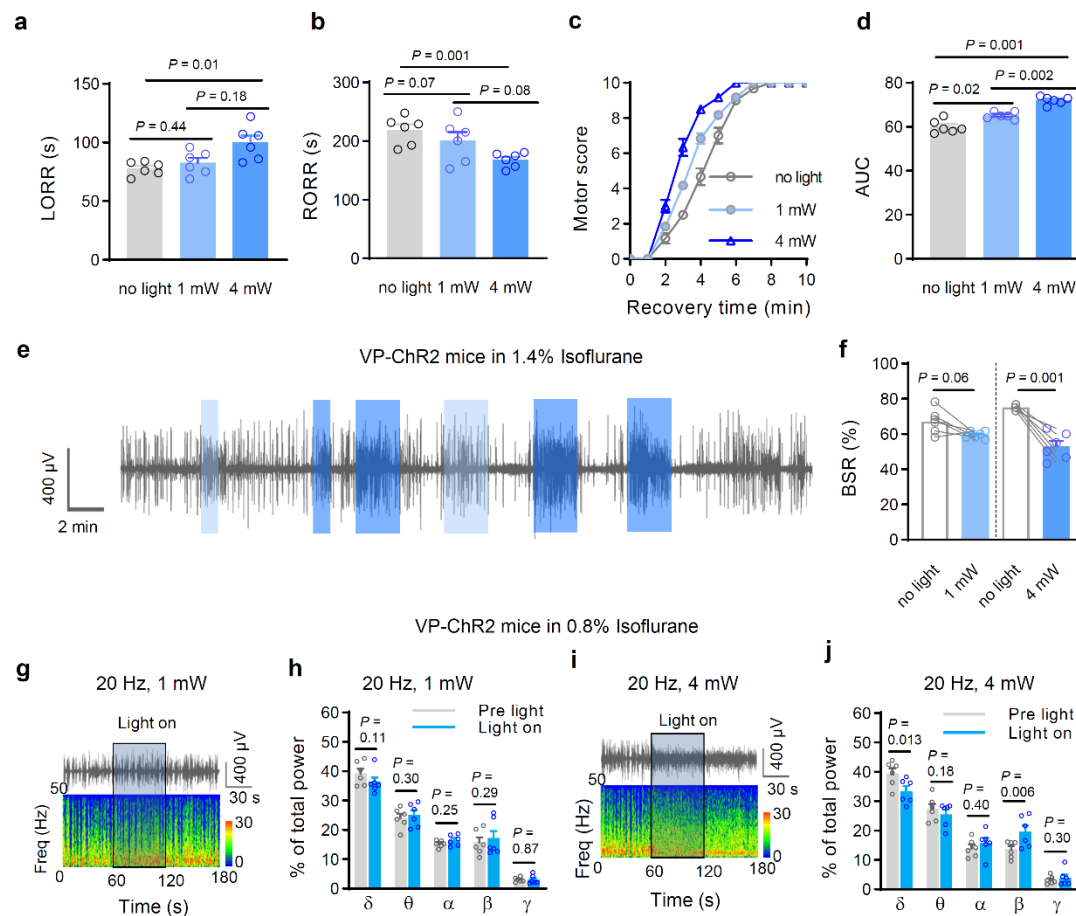


Fig 5 Optogenetic stimulation of VP Glu neurons regulates anesthesia depending on light-intensity.

Optogenetic stimulation of VP Glu neurons was performed and verified as Fig. 1i-l.

a, b Regulation of anesthesia induction and emergence by optogenetic stimulation of VP Glu neurons with 1 and 4 mW blue light (5 ms, 20 Hz, 2 min intervals between 2 min light episodes). LORR. $F_{(1.059, 5.296)} = 7.88$, $P = 0.03$; $q = 1.89$, 1 mW vs Control; $q = 6.39$, 4 mW vs Control. RORR. $F_{(1.071, 5.353)} = 19.11$, $P = 0.006$; $q = 4.21$, 1 mW vs Control; $q = 11.62$, 4 mW vs Control. One-way repeated measures ANOVA. **c, d** Motor score recovery after termination of isoflurane inhalation when mice received 1 and 4 mW blue light stimulation of VP Glu neurons (5 ms, 20 Hz, 2 min intervals between 2 min light episodes). AUC. $F_{(1.538, 7.689)} = 45.39$, $P < 0.0001$; $q = 5.98$, 1 mW vs Control; $q = 10.95$, 4 mW vs Control. **e, f** Effects of optogenetic stimulation on burst suppression ratio during general anesthesia with 1.4% isoflurane. 1 mW, $t = 2.49$, $df = 5$. 4 mW, $t = 6.92$, $df = 5$. **g-j** Effects of 1 mW and 4 mW blue light stimulation (5 ms, 4 mW, 20 Hz) on EEG in mice anesthetized with 0.8% isoflurane. h δ : $t = 1.93$; θ : $t = 1.16$; α : $t = 1.30$; β : $t = 1.19$; γ : $t = 0.17$. $n = 6$. j δ : $t = 3.67$; θ : $t = 1.57$; α : $t = 0.91$; β : $t = 4.60$; γ : $t = 1.15$. $n = 6$. One-way repeated measures ANOVAs in (**a, b, d**). Two-tailed paired t-tests in (**f, h, j**). Mean $\pm$ SEM in (**a-d, f, h, j**). Statistical results are in Supplementary Table 2. Source data are in Source Data.xlsx.

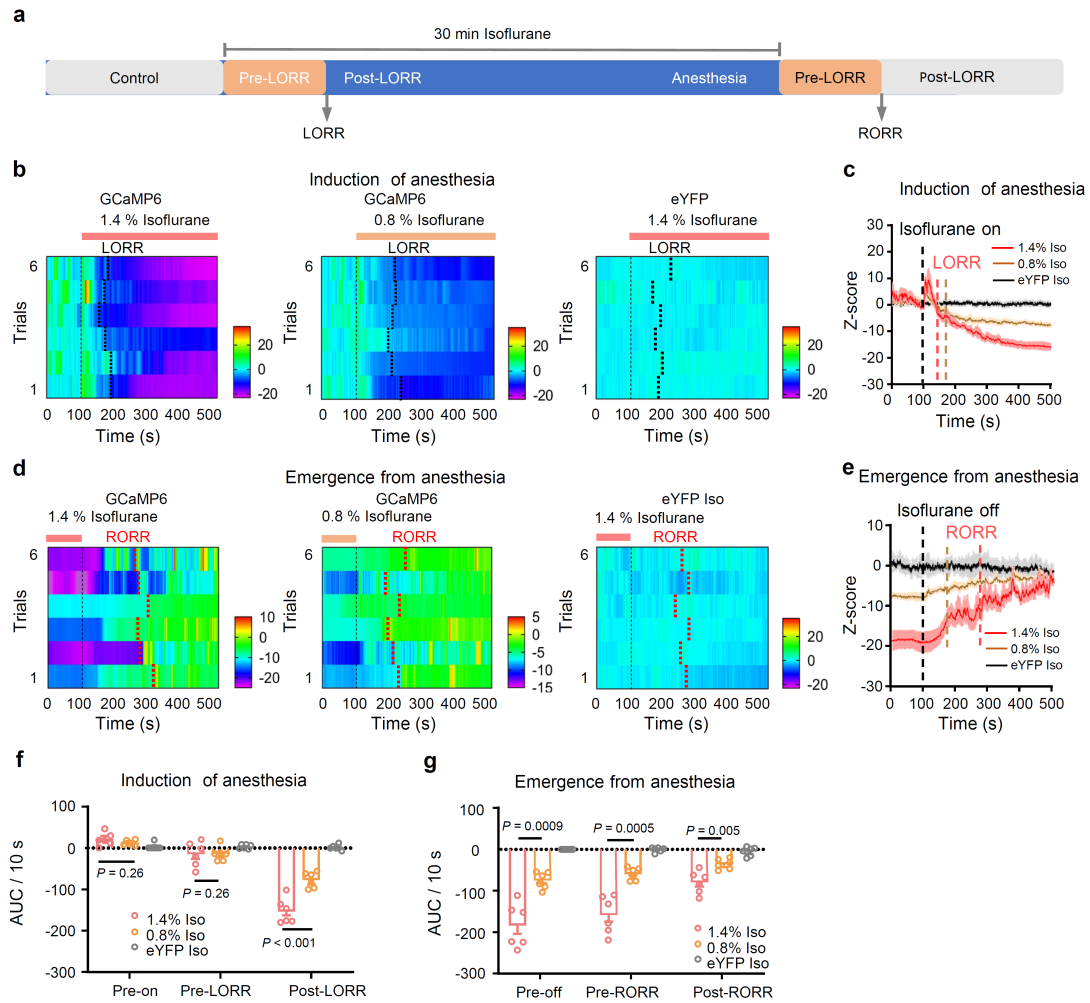


Fig 6 VP Glu neurons respond to isoflurane general anesthesia.

a Time line for fiber photometry recording of GCaMP6 signal in VP Glu neurons before, during, and after isoflurane general anesthesia. Induction of and emergence from anesthesia was identified with loss and recovery of righting reflex (LORR, RORR), respectively. **b, c** Heatmaps and averaged traces showing GCaMP6 signal in VP Glu neurons after inhalation of 1.4% and 0.8% isoflurane in GCaMP6 mice ($n = 6$) and eYFP signal in VP Glu neurons after inhalation of 1.4% isoflurane in eYFP mice ($n = 6$). **c** Black dashed line indicates when the mouse was placed into the induction box. Red and brown dashed lines respectively indicate LORR in 1.4% and 0.8% isoflurane. **d, e** Heatmaps and averaged traces of GCaMP6 signal in VP Glu neurons after termination of isoflurane after 30 min anesthesia in 1.4% and 0.8% isoflurane. **e** Black dashed line indicates when isoflurane was terminated. Red and brown dashed lines respectively indicate RORR after anesthesia in 1.4% and 0.8% isoflurane. **f, g**

Fluorescence intensity of GCaMP6 and eYFP before, during, and after 1.4% and 0.8% isoflurane inhalation and termination. **f** Isoflurane inhalation. Pre-on: $F_{(2, 15)} = 3.92$, $P = 0.04$, $t = 1.19$, 1.4% vs 0.8%. Pre-LORR: $F_{(2, 15)} = 1.49$, $P = 0.26$. Post-LORR: $F_{(2, 15)} = 79.27$, $P < 0.0001$; $t = 6.21$, 1.4% vs 0.8%. **g** Isoflurane termination following 30 min anesthesia. Pre-off: $F_{(2, 15)} = 46.94$, $P < 0.0001$; $t = 4.64$, 1.4% vs 0.8%. Pre-RORR: $F_{(2, 15)} = 48.78$, $P < 0.0001$; $t = 5.04$, 1.4% vs 0.8%. Post-RORR: $F_{(2, 15)} = 24.74$, $P < 0.0001$; $t = 3.60$, 1.4% vs 0.8%.

Pre-on: 100 s episode just before isoflurane inhalation. Pre-LORR: during isoflurane inhalation, but 100 s episode before LORR. Post-LORR: 100 s episode during anesthesia maintenance. Pre-off: 100 s episode before terminal of isoflurane. Pre-RORR: after isoflurane termination, but 100 s episode before RORR. Post-RORR: 100 s episode after RORR. One-way ANOVAs with Bonferroni tests for (**f**, **g**). $n = 6$ mice in each group. Mean $\pm$ SEM in (**c**, **e–j**). Source data are in Source Data.xlsx. Statistical results are in Supplementary Table 2.

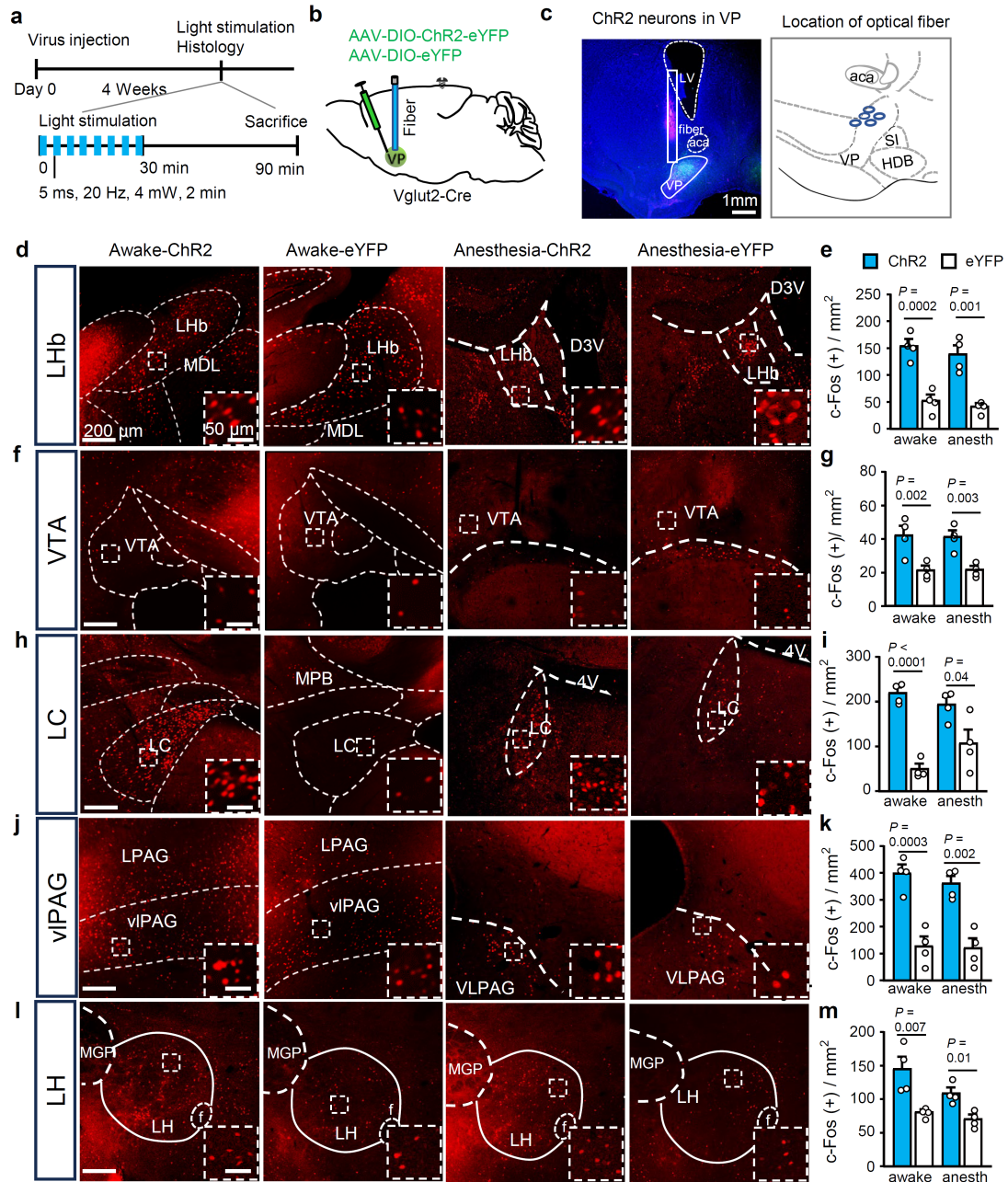


Fig 7 Stimulation of VP Glu neurons increase neuronal activity in several nuclei.

a Timeline of experiments for in vivo photostimulation of VP Glu neurons. **b** Vglut2-Cre mice received bilateral intracranial injection of AAV-EF1 α -DIO-ChR2-eYFP or AAV-EF1 α -DIO-eYFP and optical fiber implantation into the VP. **c** Representative image and summarized diagram showing expression of eYFP and locations of optical fiber implants in the VP. **d–m** Representative images and summary showing c-Fos(+) neurons in several nuclei after blue light illumination of the VP in ChR2 and eYFP mice. These nuclei include LHb (lateral habenula), VTA (ventral tegmental area), LC (locus

coeruleus), vIPAG (ventrolateral periaqueductal gray), and LH (lateral hypothalamus). **e** LHb. $t = 6.61$, awake ChR2 vs eYFP. $t = 5.81$, anesthetized ChR2 vs eYFP. **g** VTA. $t = 4.59$, awake ChR2 vs eYFP. $t = 4.65$, anesthetized ChR2 vs eYFP. **i** LC. $t = 8.24$, awake ChR2 vs eYFP. $t = 2.58$, anesthetized ChR2 vs eYFP. **k** vIPAG. $t = 6.11$, awake ChR2 vs eYFP. $t = 5.41$, anesthetized ChR2 vs eYFP. **m** LH. $t = 3.56$, awake ChR2 vs eYFP. $t = 3.94$, anesthetized ChR2 vs eYFP. $n = 4$ mice. Two-tailed t -tests in (**e**, **g**, **i**, **k**, **m**). Mean $\pm$ SEM in (**e**, **g**, **i**, **k**, **m**). Source data are in Source Data.xlsx. Statistical results are in Supplementary Table 2.

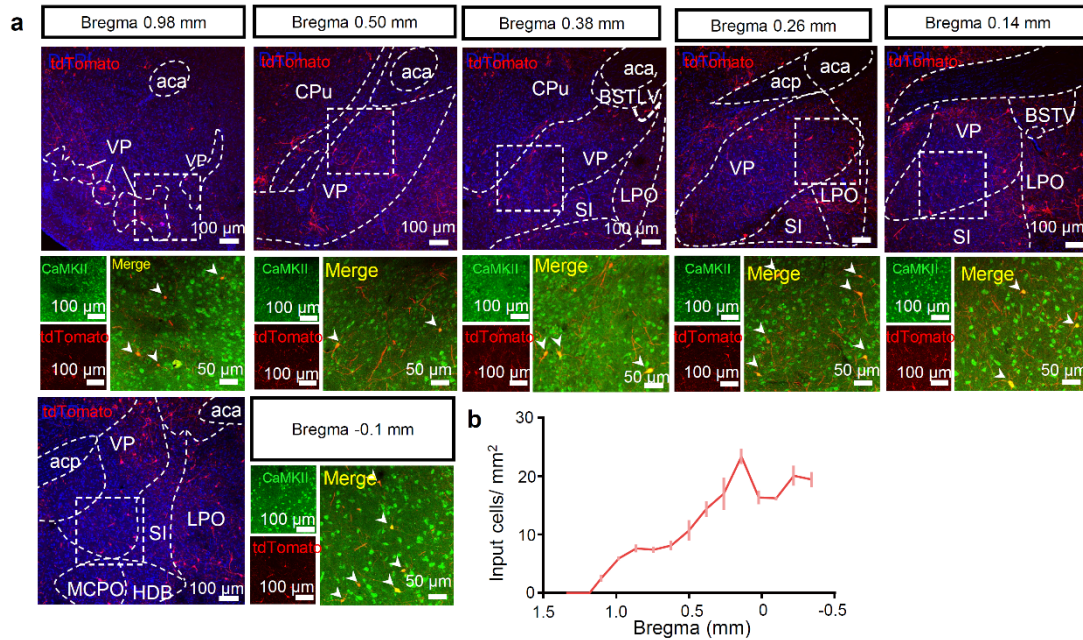


Fig 8 Retrograde labeling of VP neurons innervating LC NA neurons.

Rabies virus and helper viral vectors were injected into the LC of DBH-Cre mice as illustrated in Fig. 4k-m.

a Representative images of VP neurons innervating LC NA neurons labeled with rabies virus and helper viral vectors (AAV-EF1 α -DIO-TVA-GFP, AAV-EF1 α -DIO-RVG, RV-CVS-EnvA- Δ G-tdTomato) injected into the LC in DBH-Cre mice. Insets of each image show the overlapping of tdTomato-labeled neurons (red) and CaMKII-antibody-stained neurons (green). **b** Quantification ($n = 4$ mice) of tdTomato-labeled neurons in the VP in different bregma sections. Mean $\pm$ SEM in (**b**). Source data are in Source Data.xlsx. Statistical results are in Supplementary Table 2.

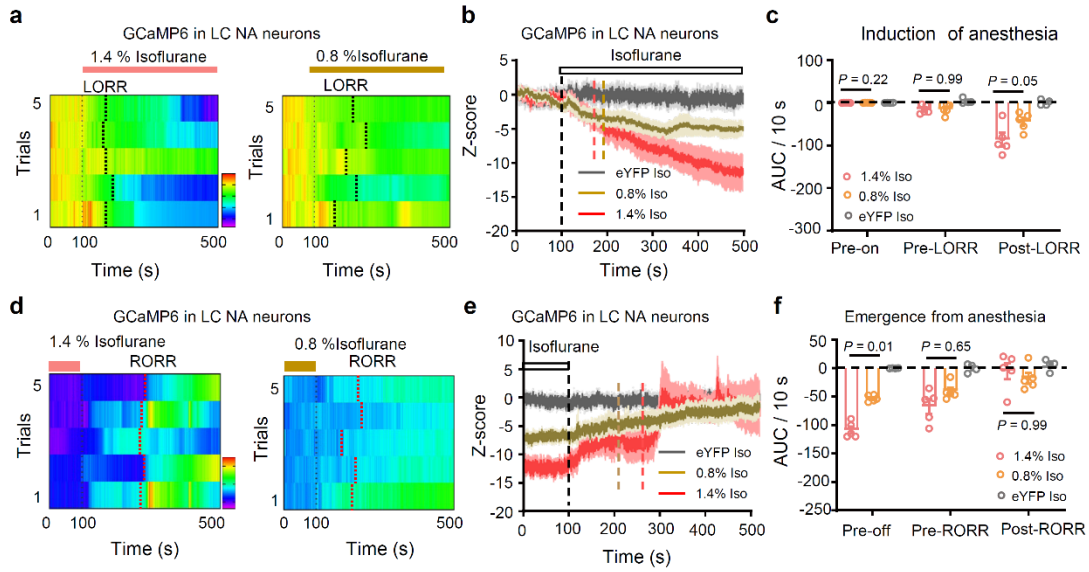


Fig 9 Activity of LC NA neurons innervated by VP Glu neurons alters during isoflurane anesthesia

Anterograde transsynaptic labeling of LC NA neurons innervated by VP neurons were transfected with calcium sensor (GCaMP6) as illustrated in Fig. 4k-m. Fiber photometry recording of GCaMP6 signal was conducted.

a–c Heat map, corresponding averaged fluorescence traces, and summary (area under curve, AUC) of GCaMP6 and eYFP signal in the LC before and during general anesthesia induction in 1.4% and 0.8% isoflurane. **b** Black dashed line indicates when the mouse was placed into the induction box. Red and brown dashed lines respectively indicate LORR in 1.4% and 0.8% isoflurane. **c** Pre-on: $F_{(2, 11)} = 1.38$, $P = 0.22$. Pre-LORR: $F_{(2, 11)} = 5.41$, $P = 0.02$; $t = 0.002$, 1.4% Iso vs 0.8% Iso. Post-LORR: $F_{(2, 11)} = 13.41$, $P = 0.001$; $t = 2.68$, 1.4% Iso vs 0.8% Iso. **d–f** Heat map, corresponding averaged fluorescence traces, and summary (AUC) of GCaMP6 and eYFP signal in the LC during general anesthesia maintenance with 1.4% and 0.8% isoflurane and emergence from anesthesia after termination of isoflurane inhalation. **e** Black dashed line indicates when isoflurane was terminated. Red and brown dashed lines respectively indicate RORR after anesthesia by 1.4% and 0.8% isoflurane. **f** Pre-off: $F_{(2, 11)} = 20.04$, $P = 0.001$; $t = 2.93$, 1.4% vs 0.8%. Pre-RORR: $F_{(2, 11)} = 6.91$, $P = 0.01$; $t = 1.29$, 1.4% vs 0.8%. Post-RORR: $F_{(2, 11)} = 0.75$, $P = 0.49$; $t = 0.02$, 1.4% vs 0.8%. One-way ANOVAs for (**c**, **f**).

$n = 5$ mice in each group. Mean $\pm$ SEM in (c, f). Source data are in Source Data.xlsx. Statistical results are in Supplementary Table 2.

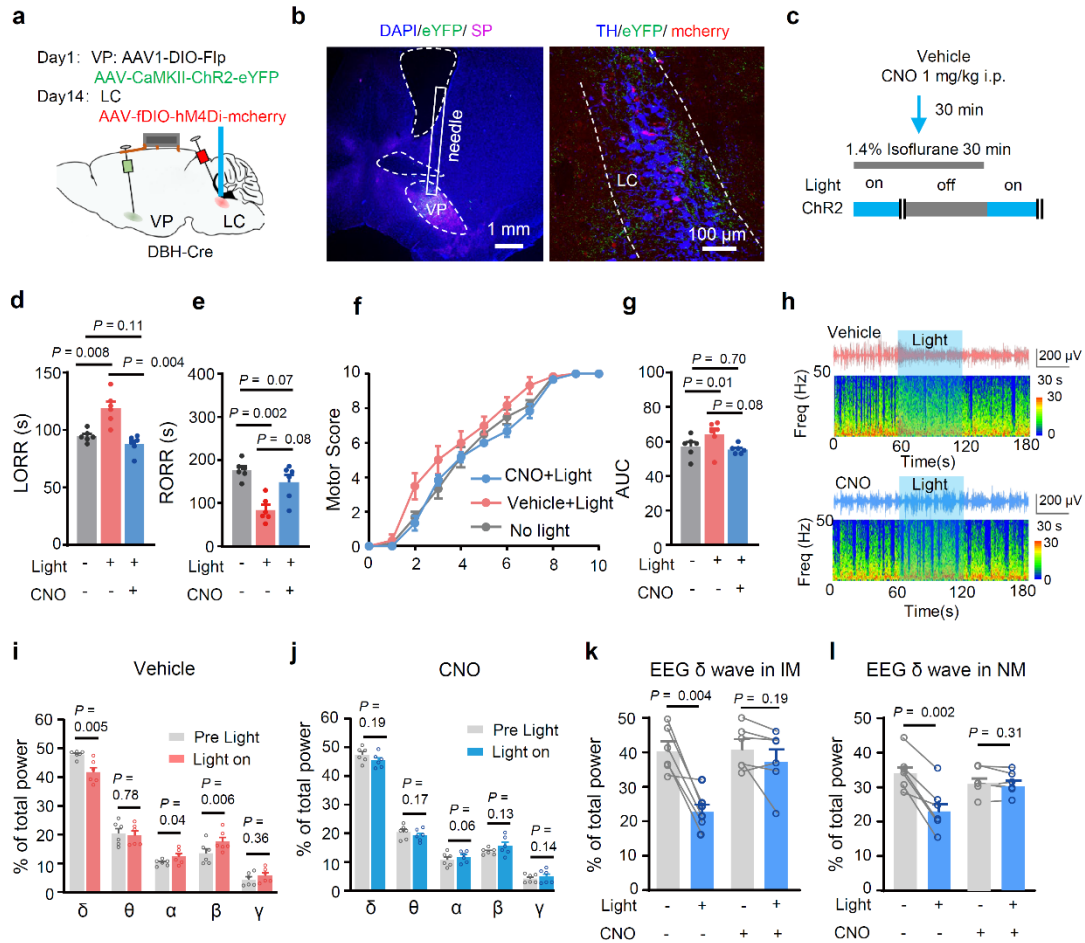


Fig 10 Optogenetic stimulation of the VP^{Glu}-LC projection induced cortical EEG changes through LC NA neurons in mice anesthetized with isoflurane.

a Schematic diagram of virus injection, fiber implantation and EEG recording. **b** Representative images of eYFP-labeled VP Glu neurons (left panel) and anterogradely labeled LC NA neurons innervated by VP Glu neurons (right panel). **c** Time line for intraperitoneal injection of vehicle and CNO and examination of anesthesia induction and emergence with 1.4% isoflurane with or without blue light illumination of the LC. **d, e** Time to LORR and RORR after intraperitoneal injection of Vehicle and CNO under blue light illumination in the LC and no light baseline. LORR: $F_{(1.610, 8.048)} = 22.75$, $P = 0.0007$, $n = 6$. RORR: $F_{(1.125, 5.624)} = 15.54$, $P = 0.008$, $n = 6$. **f, g** Time courses (mean $\pm$ SEM) and area under the curve (AUC) of the recovery of motor scores after Vehicle or

CNO injection under blue light illumination in the LC and no light baseline. AUC: $F_{(1.246, 6.228)} = 7.87$, $P = 0.03$, $n = 6$. **h** Representative EEG traces (above) and corresponding power spectra (below) before, during, and after optical stimulation after Vehicle or CNO injection under general anesthesia with 0.8 % isoflurane. **i, j** % power in different frequency bands before and during blue light illumination in mice subjected to intraperitoneal injection of Vehicle and CNO. Two-tailed paired t -test for each wave. $n = 6$. **k** % power of δ wave before and after optogenetic stimulation (blue light, 5 ms, 20 Hz, 4 mW) of the VP-LC in mice in IM state. **l** % power of δ wave before and after optogenetic stimulation (blue light, 5 ms, 20 Hz, 4 mW) of the VP-LC projection in mice in NM state.

One-way ANOVA with Bonferroni tests for **(d, e, g)**. Two-tailed paired t -test for **(i, j, m, n)**. Mean $\pm$ SEM in **(d–g, i–l)**. Source data are in Source Data.xlsx. Statistical results are in Supplementary Table 2.

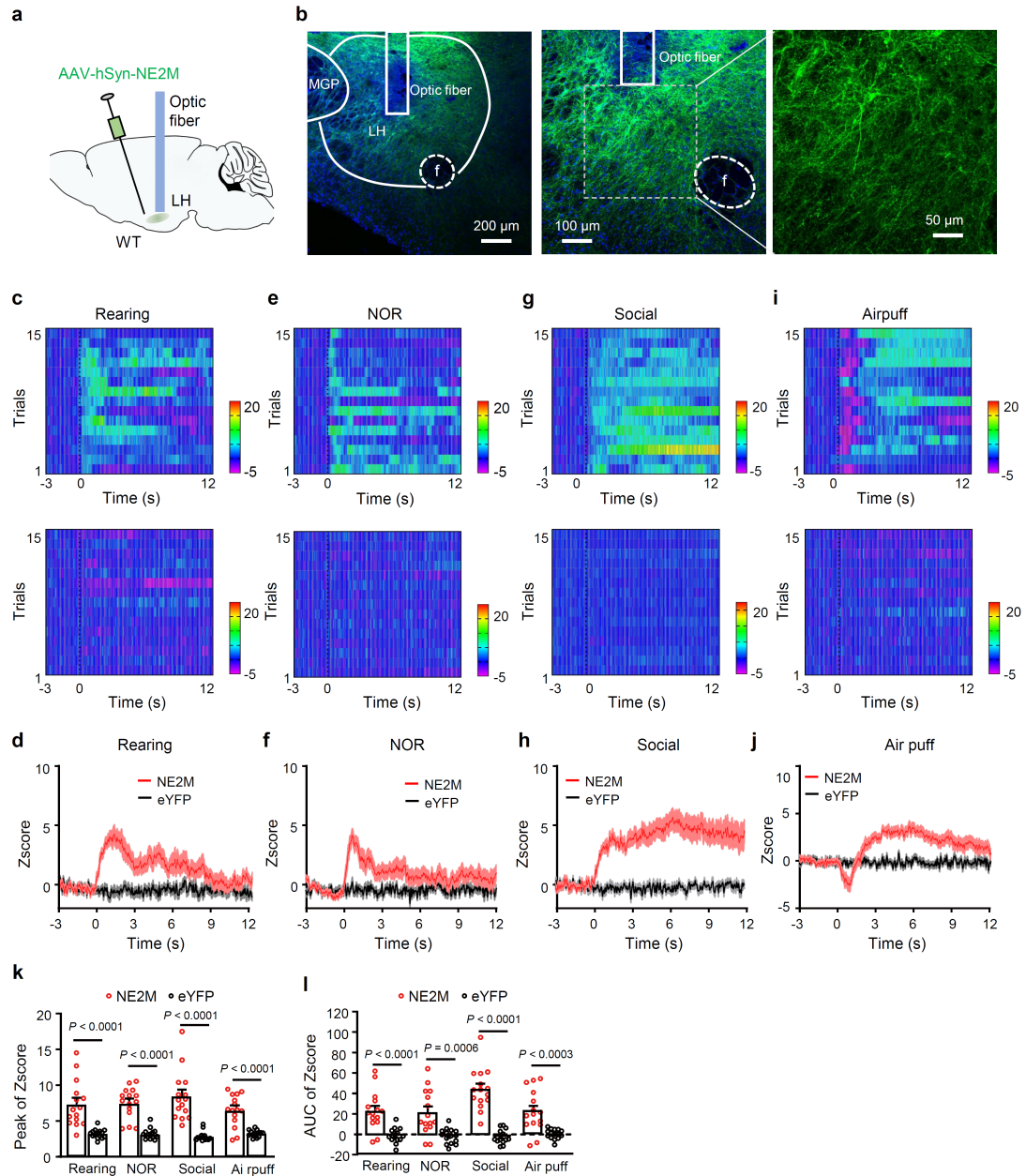


Fig 11 Release of norepinephrine in the LH is enhanced in response to salient stimuli.

a Schematic diagram for injection of AAV-hSyn-NE2M-WPRE-hGH-polyA or AAV-hSyn-eYFP into the LH of wild type C57BL6 mice to detect norepinephrine (NE) with fiber photometry. **b** Representative images of virus expression and location of optic fiber. (MGP: medial globus pallidus). **c-j** Heat map and averaged traces of fluorescent signal in the LH in NE2M and eYFP mice when the mice were rearing, recognizing novel object, interacting with unfamiliar mice, and were subjected to air puff. **k, l** The peak amplitudes and area under the curve (AUC) of fluorescence changes in response

to different behaviors in NE2M ($n = 15$ trials from 5 NE2M mice) and eYFP mice ($n = 15$ trials from 5 eYFP mice). Two-tailed unpaired t -test for z-score peak and AUC. Mean $\pm$ SEM in (d, f, h, j–l). Statistical results are in Supplementary Table 2. Source data are in Source Data.xlsx.

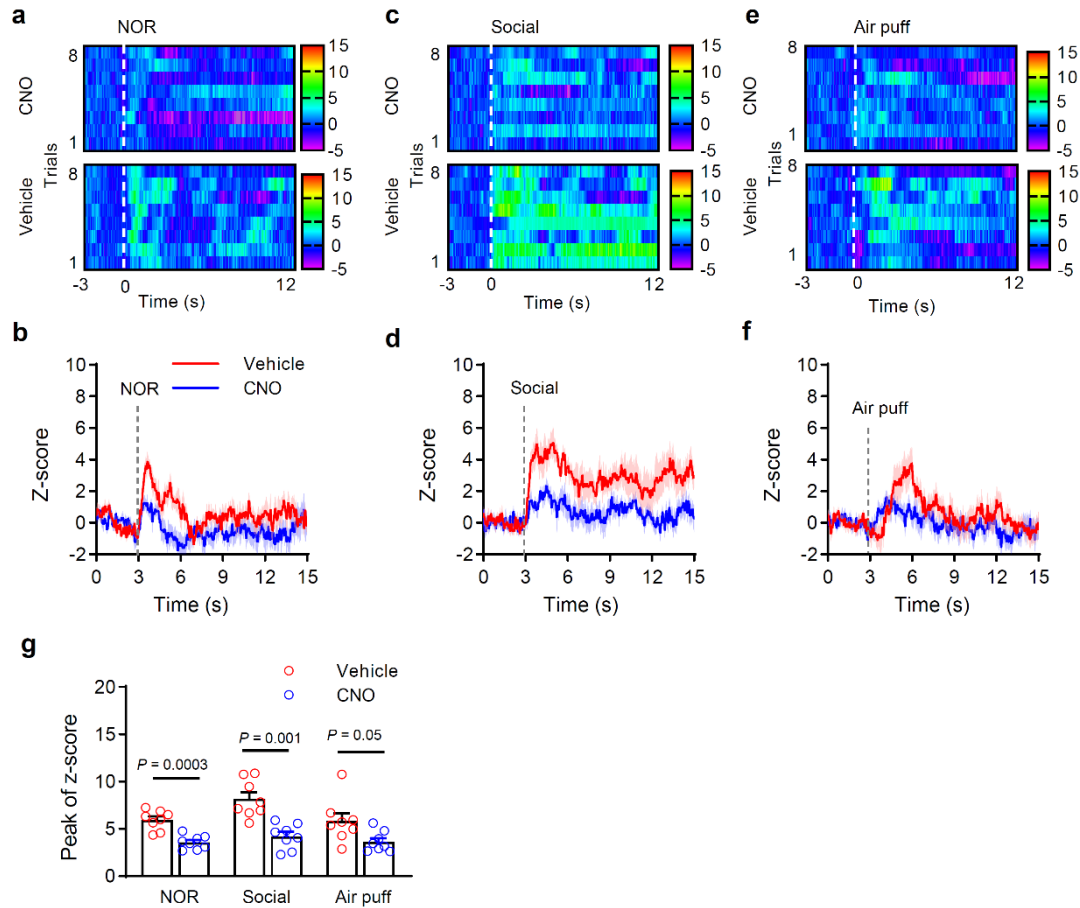


Fig 12 LC NA neurons innervated by VP Glu neurons control the release of norepinephrine in the LH evoked by salient stimuli.

Viral vector injection and optical fiber implantation were conducted in DBH-Cre mice according to Fig. 7e, f.

a–f Representative heat maps and summarized traces (Mean $\pm$ SEM) of NE2M signals in the LH when mice were performing novel object recognition (NOR), social interaction (Social), and were subjected to air puff onto the snout. **g** Summary of peak z-scores of NE2M fluctuations upon above salient stimuli. Two-tailed t -test. $n = 8$ NE2M mice. Mean $\pm$ SEM in (b, d, f, g). Source data are in Source Data.xlsx. Statistical

results are in Supplementary Table 2.

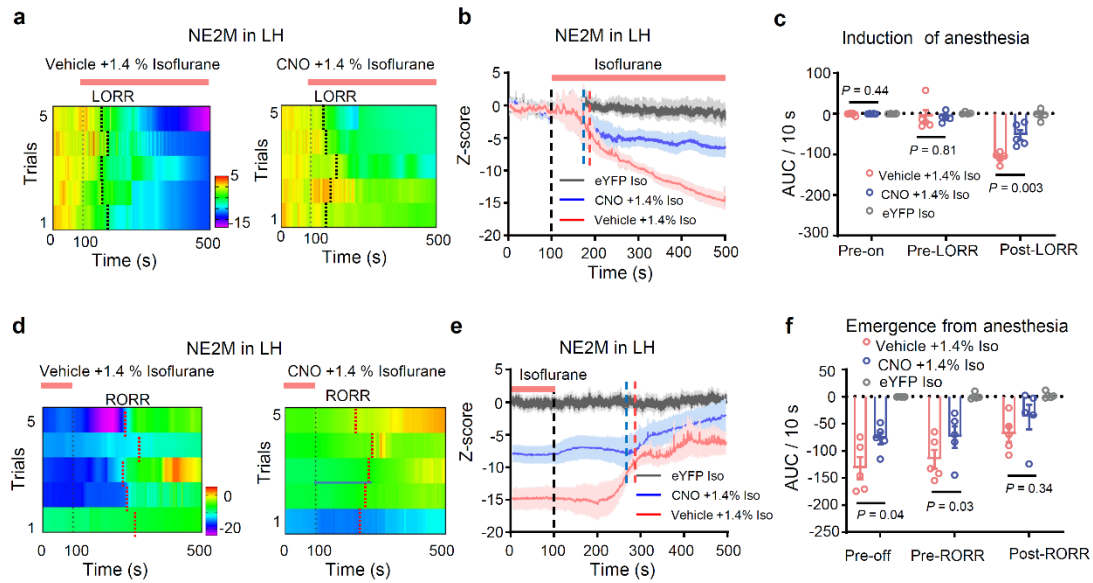


Fig 13 The release of norepinephrine in the LH during isoflurane anesthesia.

Viral vector injection and optical fiber implantation were conducted in DBH-Cre mice according to Fig. 7e, f.

Heat map, corresponding averaged fluorescence traces, and summary showing NE2M signal in the LH change in the process of induction (**a–c**) of and emergence (**d–f**) from anesthesia following inhalation of 1.4% isoflurane with intraperitoneal administration of vehicle or CNO. **b** Black dashed line indicates when the mouse was placed into the induction box. Red and blue dashed lines respectively indicate LORR by 1.4% isoflurane after vehicle and CNO i.p. injection. **c, f** The area under curves of NE2M and eYFP fluorescence traces before, during and after 1.4% isoflurane inhalation with vehicle or CNO administration. **c** Pre-on: $F_{(2, 11)} = 0.89$, $P = 0.44$. Pre-LORR: $F_{(2, 11)} = 0.22$, $P = 0.81$. Post-LORR: $F_{(2, 11)} = 31.65$, $P < 0.0001$; $t = 4.39$, $P = 0.003$, Vehicle vs CNO. **e** Black dashed line indicates when isoflurane was terminated. Red and blue dashed lines respectively indicate RORR after anesthesia by 1.4% after vehicle and CNO i.p. injection. **f** Pre-off: $F_{(2, 11)} = 19.71$, $P < 0.0001$; $t = 2.36$, $P = 0.04$, vehicle vs CNO. Pre-RORR: $F_{(2, 11)} = 17.95$, $P = 0.0003$; $t = 3.13$, $P = 0.03$, Vehicle vs CNO. Post-RORR: $t = 1.00$, $P = 0.34$, vehicle vs CNO. Mean $\pm$ SEM in (**b, c, e, f**). Source data are

in Source Data.xlsx. Statistical results are in Supplementary Table 2.

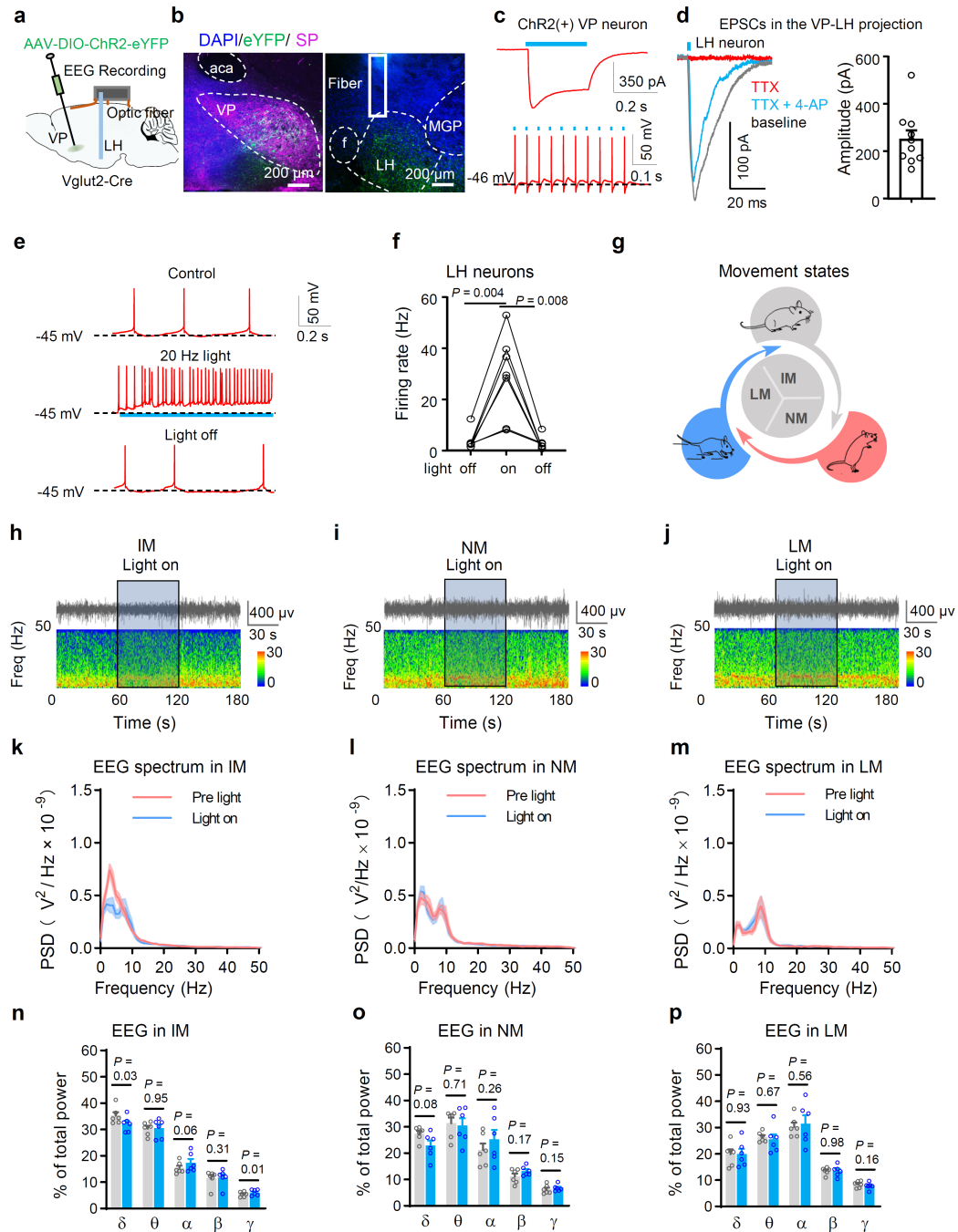


Fig 14 Stimulation of the VP^{Glu}-LH projection modulates brain arousal in freely behaving mice.

a Schematic diagram of viral injection, optical fiber implantation and EEG recording.

b Representative images for verifying virus expression in VP glutamatergic (Glu) neurons (green) and their projection (green) to the LH. The VP was delineated by Substance P-antibody staining (purple). An optical fiber was implanted in the LH. **c**

Blue-light-evoked inward current (0.3 s, 1 mW) and light-induced action potentials (2 ms, 1 mW, 20 Hz) were recorded via patch-clamp recording from a ChR2-positive neuron in the VP. **d** Representative traces and summary showing photo-EPSCs in LH neurons. $n = 10$ neurons from 2 mice. **e, f** Representative traces and summary showing that 20 Hz light stimulation (2 ms, 1 mW, 20 Hz, 1 s) of the VP^{Glu}-LH projection enhanced firing in LH neurons. Treatment: $F_{(1.006, 6.036)} = 21.14$, $P = 0.004$. $q = 6.60$, $P = 0.008$, control vs light. $n = 7$ neurons from 2 mice. One-way repeated measures ANOVA. **g** Diagram showing three movement states. **h–m** Representative EEG traces and corresponding power spectra in different movement states before and during blue light illumination of the VP^{Glu}-LH projection in ChR2 mice. **n–p** % power of EEG waves at different frequency bands in ChR2 mice in different movement states. $n = 6$. Two-tailed paired t -test. Mean $\pm$ SEM in (**d, k–p**). Source data are in Source Data.xlsx. Statistical results are in Supplementary Table 2.

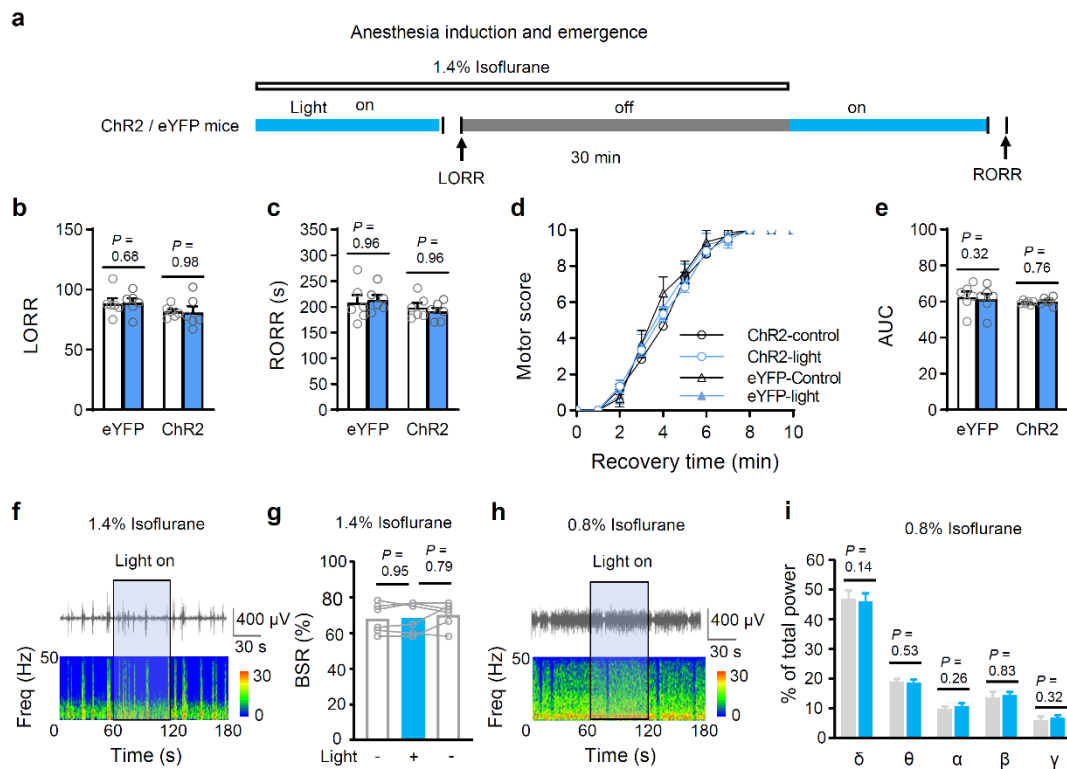


Fig 15 Stimulation of the VP^{Glu}-LH projection does not modulate anesthesia in mice.

Viral injection, optical fiber implantation and EEG recording were conducted as Fig. S14a.

a, Time to LORR and RORR in ChR2 and eYFP mice with (blue bars) or without (blank bars) blue light illumination in the LH. **b** LORR. Time. $F_{(1,10)} = 0.001$, $P = 0.98$, $n = 6$ mice in each group. **c** Time to RORR in ChR2 mice with (blue bars) or without (blank bars) blue light illumination in the LH. $F_{(1,10)} = 0.003$, $P = 0.96$, $n = 6$ mice in each group. **d, e** Motor function recovery without and with blue light illumination (5 ms, 4 mW, 20 Hz) (2 min on / off) in the LH in ChR2 mice. Time. $F_{(1, 10)} = 0.31$, $P = 0.59$. $n = 6$ mice. **f, g** Representative EEG traces, corresponding power spectrum and burst suppression ratio (BSR) in mice anesthetized with 1.4% isoflurane with blue light illumination in the LH. $F_{(1.161, 5.803)} = 0.43$, $P = 0.57$. $n = 6$ ChR2 mice. **h, j** Representative EEG traces, corresponding power spectrum and summary of % power of EEG signal in mice anesthetized with 0.8% isoflurane with and without blue light illumination in the LH in ChR2 mice. δ : $t = 1.71$, $P = 0.14$; θ : $t = 0.65$, $P = 0.53$; α : $t = 1.26$, $P = 0.26$; β : $t = 0.44$, $P = 0.83$; γ : $t = 1.11$, $P = 0.32$. $n = 6$ ChR2 mice. One-way repeated measures ANOVAs for **g**. Two-tailed paired t -test for **(b, c, e, i)**. Mean $\pm$ SEM in **(b–e, g, i)**. Source data are in Source Data.xlsx. Statistical results are in Supplementary Table 2.

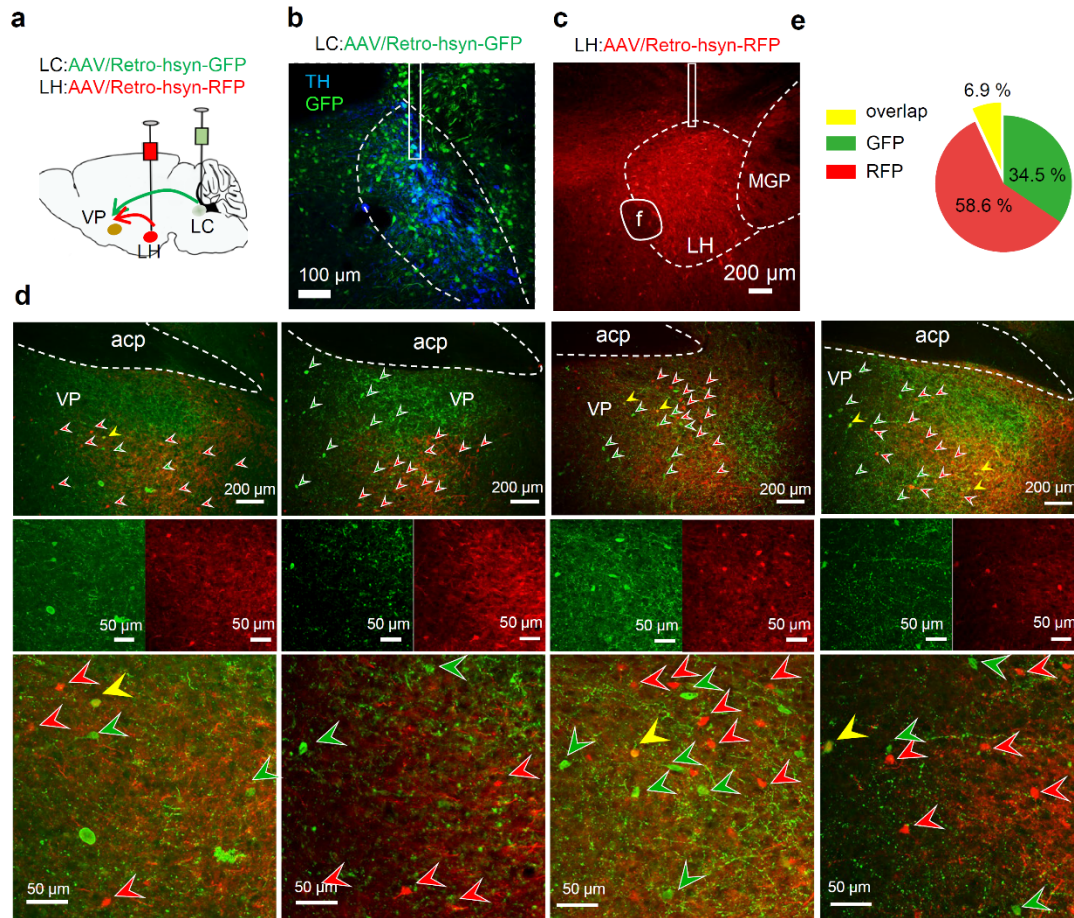


Fig 16 Retrograde labeling of VP neurons innervating the LC and LH.

a-c AAV retro-hSyn-GFP and AAV retro-hSyn-RFP were respectively injected into the LC and LH. 3 weeks later, the mice were sacrificed and coronal sections containing the LC, LH, and VP were imaged. Blue, TH. Green, GFP. Red, RFP. LC, locus coeruleus. LH, lateral hypothalamus. VP, ventral pallidum. **d** Representative images from 4 mice showing retrogradely labeled LC-(green) and LH-(red) projecting VP neurons. **e** Summary of overlapping of LC- and LH-projecting VP neurons. $n = 4$ mice. Source data are in Source Data.xlsx.

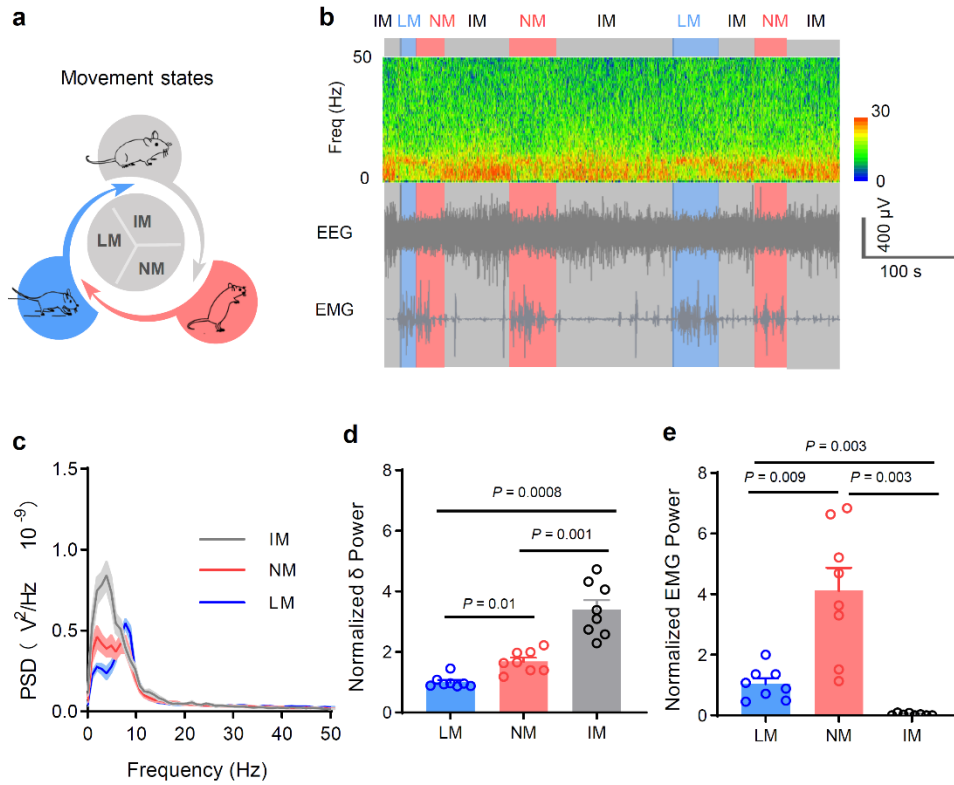


Fig 17 Electromyograph and EEG in mice during different movement states.

a Mice were placed in an open field arena and movement states were visually identified. Electromyograph and EEG signals were recorded. **b** Representative traces of EEG and EMG, and EEG spectrum in a mouse during IM (gray), LM (blue), and NM (red) states. **c** Averaged EEG spectrum in IM, LM, and NM. **d** Normalized power of delta wave in LM, NM, and IM. $F_{(1.234, 8.640)} = 41.40$, $P < 0.0001$; $t = 4.37$, $P = 0.01$, LM vs NM; $t = 6.78$, $P = 0.0008$, LM vs IM; $t = 6.40$, $P = 0.0001$, NM vs IM. **e** Normalized power of EMG in mice in LM, NM, and IM. $F_{(1.082, 7.577)} = 24.73$, $P < 0.0001$; $t = 4.43$, $P = 0.009$, LM vs NM; $t = 5.36$, $P = 0.003$, LM vs IM; $t = 5.50$, $P = 0.003$, NM vs IM. One-way repeated measures ANOVAs for (d, e). $n = 8$ mice. Mean $\pm$ SEM in (c–e). Source data are in Source Data.xlsx. Statistical results are in Supplementary Table 2.

Supplementary Table 1 Statistical results for main figures

Figures	Statistical methods	Statistics	P value
Figure 1			
Fig. 1f	Two-tailed paired t-tests CNO vs vehicle, $n = 7$, $df = 6$	% total time IM: $t = 3.33$ NM: $t = 0.64$ LM: $t = 2.65$	$P = 0.02$ $P = 0.54$ $P = 0.04$
Fig. 1g	Two-tailed paired t-tests CNO vs vehicle, $n = 7$, $df = 6$	Numbers of episode IM: $t = 1.16$ NM: $t = 5.68$ LM: $t = 5.12$	$P = 0.29$ $P = 0.001$ $P = 0.002$
Fig. 1h	Two-tailed paired t-tests CNO vs vehicle, $n = 7$, $df = 6$	Duration of episode IM: $t = 0.44$ NM: $t = 2.58$ LM: $t = 1.10$	$P = 0.68$ $P = 0.04$ $P = 0.32$
Fig. 1s	Two-tailed paired t-tests light vs baseline, $n = 7$, $df = 6$	IM δ : $t = 11.72$ θ : $t = 7.02$ α : $t = 7.09$ β : $t = 2.73$ γ : $t = 6.76$	$P < 0.0001$ $P = 0.0004$ $P = 0.0002$ $P = 0.03$ $P = 0.0005$
Fig. 1t	Two-tailed paired t-tests light vs baseline, $n = 7$, $df = 6$	NM δ : $t = 4.37$ θ : $t = 7.01$ α : $t = 5.73$ β : $t = 2.29$ γ : $t = 0.74$	$P = 0.003$ $P = 0.0002$ $P = 0.0007$ $P = 0.06$ $P = 0.48$
Fig. 1u	Two-tailed paired t-tests light vs baseline, $n = 7$, $df = 6$	LM δ : $t = 3.70$ θ : $t = 12.58$ α : $t = 4.29$ β : $t = 4.15$ γ : $t = 1.74$	$P = 0.008$ $P < 0.0001$ $P = 0.004$ $P = 0.004$ $P = 0.13$
Figure 2			
Fig 2g	Two-tailed paired t-tests light vs baseline, $n = 8$, $df = 7$	LORR eYFP: $t = 0.42$ ChR2: $t = 3.16$	$P = 0.69$ $P = 0.007$
Fig 2h	Two-tailed paired t-tests light vs baseline, $n = 8$, $df = 7$	LORR eYFP: $t = 0.57$ NpHR: $t = 2.42$	$P = 0.58$ $P = 0.03$
Fig 2i	Two-tailed paired t-tests	RORR	

	light vs baseline, $n = 8$, $df = 7$	eYFP: $t = 0.59$ ChR2: $t = 8.70$	$P = 0.57$ $P < 0.0001$
Fig 2j	Two-tailed paired t-tests light vs baseline, $n = 8$, $df = 7$	RORR eYFP: $t = 0.64$ NpHR: $t = 3.93$	$P = 0.53$ $P = 0.002$
Fig 2m	Two-tailed paired t-tests light vs baseline, $n = 8$, $df = 7$	Motor score eYFP: $t = 0.16$ ChR2: $t = 6.81$	$P = 0.87$ $P < 0.0001$
Fig 2o	Two-tailed paired t-tests light vs baseline, $n = 8$, $df = 7$	Motor score eYFP: $t = 0.08$ ChR2: $t = 5.39$	$P = 0.94$ $P < 0.0001$
Figure 3			
Fig 3c	One-way repeated measures ANOVAs	BSR, $n = 6$ eYFP: $F_{(1.029, 5.146)} = 0.09$ ChR2: $F_{(1.595, 7.975)} = 39.99$; $q = 10.25$, light vs baseline	$P = 0.78$ $P = 0.0001$ $P = 0.002$
Fig 3f	One-way repeated measures ANOVAs	BSR, $n = 6$ eYFP: $F_{(1.997, 9.986)} = 1.75$ NpHR: $F_{(1.667, 8.336)} = 15.61$; $q = 8.99$, light vs baseline	$P = 0.22$ $P = 0.002$ $P = 0.003$
Fig 3i	Two-tailed paired t-tests light vs baseline, $n = 10$, $df = 9$	ChR2, EEG waves, $n = 10$ δ : $t = 3.89$ θ : $t = 2.96$ α : $t = 0.90$ β : $t = 5.95$ γ : $t = 1.44$	$P = 0.004$ $P = 0.02$ $P = 0.39$ $P = 0.0002$ $P = 0.18$
Fig 3k	Two-tailed paired t-tests light vs baseline, $n = 6$, $df = 5$	eYFP, EEG waves, $n = 6$ δ : $t = 1.51$ θ : $t = 1.22$ α : $t = 1.53$ β : $t = 0.65$ γ : $t = 1.80$	$P = 0.21$ $P = 0.29$ $P = 0.20$ $P = 0.55$ $P = 0.15$
Fig 3n	Two-tailed paired t-tests light vs baseline, $n = 10$, $df = 9$	NpHR, EEG waves, $n = 10$ δ : $t = 3.22$ θ : $t = 1.64$ α : $t = 1.43$ β : $t = 3.42$ γ : $t = 3.02$	$P = 0.01$ $P = 0.14$ $P = 0.19$ $P = 0.008$ $P = 0.014$
Fig 3p	Two-tailed paired t-tests light vs baseline, $n = 6$, $df = 5$	eYFP, EEG waves, $n = 6$ δ : $t = 0.07$ θ : $t = 0.62$ α : $t = 0.69$ β : $t = 1.04$ γ : $t = 0.94$	$P = 0.95$ $P = 0.55$ $P = 0.51$ $P = 0.32$ $P = 0.37$

Figure 4			
Fig 4s	Two-tailed t-tests, $n = 15$ trial in 5 GCaMP6 mice, $n = 8$ in 4 eYFP mice, $df = 21$	Peak z-score Rearing: $t = 6.04$ NOR: $t = 4.52$ Social: $t = 5.13$ Air puff: $t = 4.84$	$P < 0.0001$ $P = 0.0002$ $P < 0.0001$ $P < 0.0001$
Fig 4t	Two-tailed t-tests, $n = 15$ trial in 5 GCaMP6 mice, $n = 8$ in 4 eYFP mice.	AUC Rearing: $t = 3.49$ NOR: $t = 4.13$ Social: $t = 5.52$ Air puff: $t = 2.68$	$P = 0.002$ $P = 0.0005$ $P < 0.0001$ $P = 0.014$
Figure 5			
Fig 5j	Two-tailed paired t-tests, light vs baseline, $n = 8$, $df = 7$	IM δ : $t = 4.0$ θ : $t = 2.16$ α : $t = 4.51$ β : $t = 2.50$ γ : $t = 3.06$	$P = 0.005$ $P = 0.07$ $P = 0.003$ $P = 0.04$ $P = 0.02$
Fig 5k	Two-tailed paired t-tests, light vs baseline, $n = 8$, $df = 7$	NM δ : $t = 1.36$ θ : $t = 2.17$ α : $t = 0.09$ β : $t = 0.87$ γ : $t = 0.60$	$P = 0.21$ $P = 0.07$ $P = 0.93$ $P = 0.41$ $P = 0.57$
Fig 5l	Two-tailed paired t-tests, light vs baseline, $n = 8$, $df = 7$	LM δ : $t = 3.06$ θ : $t = 2.27$ α : $t = 3.04$ β : $t = 0.71$ γ : $t = 0.93$	$P = 0.02$ $P = 0.06$ $P = 0.02$ $P = 0.50$ $P = 0.38$
Figure 6			
Fig 6f	Two-tailed paired t-tests, light vs baseline, $n = 6$, $df = 5$	LORR eYFP: $t = 1.02$ ChR2: $t = 6.41$	$P = 0.35$ $P = 0.001$
Fig 6g	Two-tailed paired t-tests, light vs baseline, $n = 6$, $df = 5$	RORR eYFP: $t = 0.32$ ChR2: $t = 2.94$	$P = 0.76$ $P = 0.03$
Fig 6i	Two-tailed paired t-tests, light vs baseline, $n = 6$, $df = 5$	AUC of motor score recovery eYFP: $t = 1.47$ ChR2: $t = 11.55$	$P = 0.20$ $P < 0.0001$
Fig 6j	Two-tailed paired t-tests, light vs baseline, $n = 6$, $df = 5$	LORR eYFP: $t = 0.34$ NpHR: $t = 3.30$	$P = 0.75$ $P = 0.02$

Fig 6k	Two-tailed paired t-tests, light vs baseline, $n = 6$, $df = 5$	RORR eYFP: $t = 0.32$ NpHR: $t = 2.62$	$P = 0.76$ $P = 0.04$
Fig 6m	Two-tailed paired t-tests, light vs baseline, $n = 6$, $df = 5$	AUC of motor score recovery eYFP: $t = 1.47$ NpHR: $t = 5.32$	$P = 0.20$ $P = 0.003$
Fig 6o	One-way repeated measures ANOVAs.	eYFP, $n = 6$ $F_{(1.614, 8.068)} = 0.34$ ChR2, $n = 6$ $F_{(1.923, 9.616)} = 0.49$	$P = 0.68$ $P = 0.63$
Fig 6q	One-way repeated measures ANOVAs	eYFP, $n = 6$ $F_{(1.133, 5.663)} = 0.0004$ NpHR, $n = 6$ $F_{(1.581, 7.907)} = 0.6540$	$P = 0.99$ $P = 0.51$
Fig 6s	Two-tailed paired t-tests, light vs baseline, $n = 6$ ChR2 mice, $df = 5$	EEG waves δ : $t = 4.63$ θ : $t = 0.09$ α : $t = 5.38$ β : $t = 2.97$ γ : $t = 1.08$	$P = 0.004$ $P = 0.93$ $P = 0.002$ $P = 0.02$ $P = 0.32$
Fig 6u	Two-tailed t-tests, light vs baseline, $n = 6$ NpHR mice, $df = 5$	EEG waves δ : $t = 4.71$ θ : $t = 2.49$ α : $t = 2.14$ β : $t = 3.07$ γ : $t = 0.03$	$P = 0.005$ $P = 0.05$ $P = 0.09$ $P = 0.03$ $P = 0.98$
Figure 7			
Fig 7i	Two-tailed t-tests, $n = 18$ trial in 6 NE2M mice before after CNO, $df = 34$.	Peak z-score, $t = 6.26$	$P < 0.0001$
Fig 7j	Two-tailed t-tests, $n = 18$ trial in 6 NE2M mice before vs after CNO, $df = 34$.	AUC of z-score curve, $t = 6.89$	$P < 0.0001$
Fig 7m	One-way repeated measures ANOVAs with Tukey tests and Geisser-Greenhouse correction.	$F_{(1.639, 19.66)} = 72.49$ $q = 22.03$, baseline vs light	$P < 0.0001$ $P = 0.04$
Figure 8			
Fig 8d	One-way repeated measures ANOVAs with Bonferroni tests and Geisser-Greenhouse correction, $n = 8$ hM3Dq mice.	% time in each state IM: $F_{(1.133, 7.934)} = 8.59$ $t = 4.51$, CNO vs baseline $t = 0.97$, CNO+Yohimbin vs baseline NM: $F_{(1.899, 13.30)} = 5.65$ $t = 2.80$, CNO vs baseline	$P = 0.02$ $P = 0.008$ $P = 0.99$ $P = 0.02$ $P = 0.08$

		$t = 0.10$, CNO+Yohimbin vs baseline LM: $F_{(1.389, 9.725)} = 2.41$	$P = 0.99$ $P = 0.15$
Fig 8e	One-way repeated measures ANOVAs, with Bonferroni tests and Geisser-Greenhouse correction, $n = 8$ hM3Dq mice.	Numbers of episode in each state IM: $F_{(1.463, 10.24)} = 2.88$ NM: $F_{(1.868, 13.08)} = 26.46$ $t = 7.67$, CNO vs baseline $t = 1.47$, CNO+Yohimbin vs baseline LM: $F_{(1.139, 7.973)} = 10.86$ $t = 3.36$, CNO vs baseline $t = 0.61$, CNO+Yohimbin vs baseline	$P = 0.11$ $P < 0.0001$ $P = 0.0004$ $P = 0.56$ $P = 0.01$ $P = 0.04$ $P = 0.99$
Fig 8f	One-way repeated measures ANOVAs with Bonferroni tests and Geisser-Greenhouse correction, $n = 8$ hM3Dq mice.	Duration in each state IM: $F_{(1.924, 13.47)} = 9.93$ $t = 2.29$, CNO vs baseline $t = 2.34$, CNO+Yohimbin vs baseline NM: $F_{(1.671, 11.70)} = 3.48$ LM: $F_{(1.766, 12.36)} = 4.15$ $t = 3.18$, CNO vs baseline $t = 2.23$, CNO+Yohimbin vs baseline	$P = 0.002$ $P = 0.17$ $P = 0.15$ $P = 0.07$ $P = 0.05$ $P = 0.046$ $P = 0.17$
Fig 8h	One-way repeated measures ANOVA with Bonferroni tests and Geisser-Greenhouse correction, $n = 6$ hM3Dq mice	EEG waves δ : $F_{(1.270, 6.351)} = 11.40$ $t = 3.97$, CNO vs baseline $t = 1.96$, CNO+yohimbin vs baseline θ : $F_{(1.702, 8.509)} = 0.64$ α : $F_{(1.514, 7.570)} = 12.41$ $t = 0.69$, CNO+yohimbin vs baseline $t = 5.14$, CNO vs baseline β : $F_{(1.207, 6.035)} = 3.19$ γ : $F_{(1.645, 8.223)} = 12.22$ $t = 4.06$, CNO vs baseline $t = 1.51$, CNO+yohimbine vs baseline	$P = 0.01$ $P = 0.03$ $P = 0.29$ $P = 0.64$ $P = 0.006$ $P = 0.89$ $P = 0.01$ $P = 0.12$ $P = 0.004$ $P = 0.03$ $P = 0.57$
Fig 8j	One-way repeated measures ANOVA with Bonferroni tests and Geisser-Greenhouse correction, $n = 6$ hM3Dq mice	LORR $F_{(1.245, 6.227)} = 12.05$ $t = 3.75$, CNO vs baseline $t = 1.61$, CNO+yohimbine vs baseline	$P = 0.01$ $P = 0.03$ $P = 0.34$
Fig 8k	One-way repeated measures ANOVA with Bonferroni tests and Geisser-Greenhouse correction, $n = 6$ hM3Dq mice	RORR $F_{(1.325, 6.626)} = 5.94$ $t = 3.48$, CNO vs baseline $t = 0.32$, CNO+yohimbine vs baseline	$P = 0.04$ $P = 0.035$ $P = 0.99$
Fig 8m	One-way repeated measures ANOVA with	AUC of motor score recovery $F_{(1.580, 7.900)} = 163.2$	$P < 0.0001$

	Bonferroni tests and Geisser-Greenhouse correction, $n = 6$ hM3Dq mice	$t = 13.78$, CNO vs baseline $t = 4.0$, CNO+yohimbine vs baseline	$P < 0.0001$ $P = 0.02$
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Supplementary Table 2 Statistical results for Supplementary figures

Figures	Statistical methods	Statistics	<i>P</i> value
Figure S1			
Fig. S1k	Two-tailed <i>t</i> -test, <i>n</i> = 15 trials from 5 GCaMP6 mice, <i>n</i> = 15 trials from 5 eYFP mice. <i>df</i> = 28	Peak of z-score Rearing: <i>t</i> = 7.10 novel object recognition (NOR): <i>t</i> = 7.05 Social interaction: <i>t</i> = 7.21 Air puff: <i>t</i> = 8.63	<i>P</i> < 0.0001 <i>P</i> < 0.0001 <i>P</i> < 0.0001 <i>P</i> < 0.0001
Fig. S1l	Two-tailed <i>t</i> -test, <i>n</i> = 15 trials from 5 GCaMP6 mice, <i>n</i> = 15 trials from 5 eYFP mice. <i>df</i> = 28	AUC of z-score Rearing: <i>t</i> = 8.63 NOR: <i>t</i> = 4.14 Social interaction: <i>t</i> = 7.37 Air puff: <i>t</i> = 6.78	<i>P</i> < 0.0001 <i>P</i> = 0.0003 <i>P</i> < 0.0001 <i>P</i> < 0.0001
Figure S2			
Fig. S2c	Two-tailed <i>t</i> -test, <i>n</i> = 5 eYFP mice without light in the VP, <i>n</i> = 5 Chr2 mice with VP light stimulation.	c-Fos(+) neuron density <i>t</i> = 4.11, <i>df</i> = 8.	<i>P</i> = 0.003
Fig. S2f	Two-tailed paired <i>t</i> -tests after vehicle and CNO administration, <i>n</i> = 7 hM3Dq mice. <i>df</i> = 6.	% of total EEG power δ : <i>t</i> = 5.19 θ : <i>t</i> = 5.70 α : <i>t</i> = 3.42 β : <i>t</i> = 1.93 γ : <i>t</i> = 2.76	<i>P</i> = 0.004 <i>P</i> = 0.002 <i>P</i> = 0.02 <i>P</i> = 0.08 <i>P</i> = 0.04
Figure S3			
Fig. S3f	Two-tailed paired <i>t</i> -test after vehicle and Salvinorin B administration, <i>n</i> = 10 KORD mice. <i>df</i> = 9.	% time in motor states IM: <i>t</i> = 3.65 NM: <i>t</i> = 1.51 LM: <i>t</i> = 0.36	<i>P</i> = 0.02 <i>P</i> = 0.16 <i>P</i> = 0.72
Fig. S3g	Two-tailed paired <i>t</i> -test after vehicle and Salvinorin B administration, <i>n</i> = 10 KORD mice. <i>df</i> = 9.	Number of episodes IM: <i>t</i> = 3.77 NM: <i>t</i> = 0.25 LM: <i>t</i> = 0.78	<i>P</i> = 0.004 <i>P</i> = 0.81 <i>P</i> = 0.46
Fig. S3h	Two-tailed paired <i>t</i> -test after vehicle and Salvinorin B administration, <i>n</i> = 10 KORD mice. <i>df</i> = 9.	Episode duration IM: <i>t</i> = 0.93 NM: <i>t</i> = 1.33 LM: <i>t</i> = 0.55	<i>P</i> = 0.38 <i>P</i> = 0.22 <i>P</i> = 0.60
Fig. S3j	Two-tailed paired <i>t</i> -tests after vehicle and Salvinorin	% of total EEG power δ : <i>t</i> = 4.02 θ : <i>t</i> = 0.54	<i>P</i> = 0.003 <i>P</i> = 0.60

	administration, n = 10 KORD mice. df = 9.	α : $t = 2.85$ β : $t = 0.75$ γ : $t = 2.87$	$P = 0.02$ $P = 0.47$ $P = 0.02$
Figure S5			
Fig. S5a	One-way repeated measures ANOVA with Tukey tests and Geisser-Greenhouse correction, n = 6 ChR2 mice. baseline, 1 mW, 4 mW light in the VP	LORR time $F_{(1.059, 5.296)} = 7.88$ 1 mW vs baseline: $q = 1.89$ 4 mW vs baseline: $q = 6.39$ 1 mW vs 4 mW: $q = 3.01$	$P = 0.03$ $P = 0.44$ $P = 0.01$ $P = 0.18$
Fig. S5b	One-way repeated measures ANOVA with Tukey tests and Geisser-Greenhouse correction, n = 6 ChR2 mice. baseline, 1 mW, 4 mW light in the VP	RORR time $F_{(1.071, 5.353)} = 19.11$ 1 mW vs baseline: $q = 4.21$ 4 mW vs baseline: $q = 11.62$ 1 mW vs 4 mW: $q = 4.02$	$P = 0.006$ $P = 0.07$ $P = 0.001$ $P = 0.08$
Fig. S5d	One-way repeated measures ANOVA with Tukey tests and Geisser-Greenhouse correction, n = 6 ChR2 mice. baseline, 1 mW, 4 mW light in the VP	AUC of motor recovery curve $F_{(1.538, 7.689)} = 45.39$ 1 mW vs baseline: $q = 5.98$ 4 mW vs baseline: $q = 10.95$ 1 mW vs 4 mW: $q = 10.22$	$P < 0.0001$ $P = 0.02$ $P = 0.001$ $P = 0.002$
Fig. S5f	Two-tailed paired t-test, n = 6 ChR2 mice. baseline, 1 mW, 4 mW light in the VP	Burst suppression ratio (BSR) 1 mW: $t = 2.49$ 4 mW: $t = 6.92$	$P = 0.06$ $P = 0.001$
Fig. S5h	Two-tailed paired t-test, n = 6 ChR2 mice. before and during 1 mW light in the VP. df = 5.	EEG waves δ : $t = 1.93$ θ : $t = 1.16$ α : $t = 1.30$ β : $t = 1.19$ γ : $t = 0.17$	$P = 0.11$ $P = 0.30$ $P = 0.25$ $P = 0.29$ $P = 0.87$
Fig. S5j	Two-tailed paired t-test, n = 6 ChR2 mice. before and during 4 mW light in the VP. df = 5.	EEG waves δ : $t = 3.67$ θ : $t = 1.57$ α : $t = 0.91$ β : $t = 4.60$ γ : $t = 1.15$	$P = 0.013$ $P = 0.18$ $P = 0.40$ $P = 0.006$ $P = 0.30$
Figure S6			
Fig. S6f	One-way ANOVAs in 6 GCaMP6 and 6 eYFP mice	fluorescence intensity of GCaMP6 and eYFP signal $Pre-On: F_{(2, 15)} = 3.92$	$P = 0.04$

	during isoflurane anesthesia induction	GCaMP : 0.8% vs 1.4%: $t = 1.19$ Pre-LORR: $F_{(2, 15)} = 1.49$ Post-LORR: $F_{(2, 15)} = 79.27$ GCaMP6: 0.8% vs 1.4%, $t = 6.21$	$P = 0.26$ $P = 0.26$ $P < 0.0001$ $P < 0.0001$
Fig. S6g	One-way ANOVAs in 6 GCaMP6 and 6 eYFP mice during isoflurane anesthesia emergence	fluorescence intensity of GCaMP6 and eYFP signal Pre-Off: $F_{(2, 15)} = 46.94$ GCaMP : 0.8% vs 1.4%: $t = 4.64$ Pre-RORR: $F_{(2, 15)} = 48.78$ GCaMP : 0.8% vs 1.4%: $t = 5.04$ Post-RORR: $F_{(2, 15)} = 24.74$ GCaMP6: 0.8% vs 1.4%, $t = 3.60$	$P < 0.0001$ $P = 0.0009$ $P < 0.0001$ $P = 0.0005$ $P < 0.0001$ $P = 0.005$
Figure S7			
Fig. S7e	Two-tailed unpaired t-test. $n = 4$ Chr2 mice without and with blue light in the VP, $df = 6$.	c-Foc(+) neurons in the LHb awake mice: $t = 6.61$ anesthetized mice: $t = 5.81$	$P = 0.0002$ $P = 0.001$
Fig. S7g	Two-tailed unpaired t-test. $n = 4$ Chr2 mice without and with blue light in the VP, $df = 6$.	c-Foc(+) neurons in the VTA awake mice: $t = 4.59$ anesthetized mice: $t = 4.65$	$P = 0.002$ $P = 0.003$
Fig. S7i	Two-tailed unpaired t-test. $n = 4$ Chr2 mice without and with blue light in the VP, $df = 6$.	c-Foc(+) neurons in the LC awake mice: $t = 8.24$ anesthetized mice: $t = 2.58$	$P < 0.0001$ $P = 0.04$
Fig. S7k	Two-tailed unpaired t-test. $n = 4$ Chr2 mice without and with blue light in the VP, $df = 6$.	c-Foc(+) neurons in the vIPAG awake mice: $t = 6.11$ anesthetized mice: $t = 5.41$	$P = 0.0003$ $P = 0.0015$
Fig. S7m	Two-tailed unpaired t-test. $n = 4$ Chr2 mice without and with blue light in the VP, $df = 6$.	c-Foc(+) neurons in the LH awake mice: $t = 3.56$ anesthetized mice: $t = 3.94$	$P = 0.01$ $P = 0.007$
Figure S9			
Fig. S9c	One-way ANOVAs in 5 GCaMP6 and 5 eYFP mice during isoflurane anesthesia induction	fluorescence intensity of GCaMP6 and eYFP signal in LC NA neurons Pre-On: $F_{(2, 15)} = 1.38$ Pre-LORR: $F_{(2, 15)} = 5.41$ GCaMP6: 0.8% vs 1.4%, $t = 0.002$ Post-LORR: $F_{(2, 15)} = 13.41$ GCaMP6: 0.8% vs 1.4%, $t = 2.68$	$P = 0.22$ $P = 0.02$ $P = 0.99$ $P = 0.001$ $P = 0.05$

Fig. S9f	One-way ANOVAs in 5 GCaMP6 and 5 eYFP mice during isoflurane anesthesia emergence	fluorescence intensity of GCaMP6 and eYFP signal in LC NA neurons Pre-Off: $F_{(2, 11)} = 20.04$ GCaMP : 0.8% vs 1.4%: $t = 2.93$ Pre-RORR: $F_{(2, 11)} = 6.91$ GCaMP : 0.8% vs 1.4%: $t = 1.29$ Post-RORR: $F_{(2, 11)} = 0.75$ GCaMP6: 0.8% vs 1.4%, $t = 0.02$	$P = 0.001$ $P = 0.01$ $P = 0.01$ $P = 0.65$ $P = 0.49$ $P = 0.99$
Figure S10			
Fig. S10d	One-way repeated measures ANOVA in 6 mice with stimulation of VP-LC projection and inhibition of LC NA neurons.	LORR ANOVA: $F_{(1.610, 8.048)} = 22.75$ Baseline vs light: $t = 4.26$ Baseline vs light+ CNO: $t = 1.91$	$P = 0.0007$ $P = 0.008$ $P = 0.11$
Fig. S10e	One-way repeated measures ANOVA in 6 mice with stimulation of VP-LC projection and inhibition of LC NA neurons.	RORR ANOVA: $F_{(1.125, 5.624)} = 15.54$ Baseline vs light: $t = 6.75$ Baseline vs light+ CNO: $t = 2.35$	$P = 0.008$ $P = 0.002$ $P = 0.07$
Fig. S10g	One-way repeated measures ANOVA in 6 mice with stimulation of VP-LC projection and inhibition of LC NA neurons.	AUC of motor recovery curve ANOVA: $F_{(1.246, 6.228)} = 7.87$ Baseline vs light: $t = 4.80$ Baseline vs light+ CNO: $t = 2.18$	$P = 0.03$ $P = 0.01$ $P = 0.44$
Fig. S10i	Two-tailed paired t-test, n = 6 ChR2 mice. before and during 4 mW light in the LC. df = 5.	EEG waves δ : $t = 3.57$ θ : $t = 0.28$ α : $t = 2.81$ β : $t = 4.65$ γ : $t = 0.96$	$P = 0.005$ $P = 0.78$ $P = 0.04$ $P = 0.006$ $P = 0.36$
Fig. S10j	Two-tailed paired t-test, n = 6 ChR2 mice. before and during 4 mW light in the LC after CNO injection. df = 5.	EEG waves δ : $t = 1.39$ θ : $t = 1.61$ α : $t = 2.44$ β : $t = 1.82$ γ : $t = 1.75$	$P = 0.19$ $P = 0.17$ $P = 0.06$ $P = 0.13$ $P = 0.14$
Fig. S10k	Two-tailed paired t-test in 6 mice with light stimulation of the VP without and with inhibition of LC NA neurons. df = 5	% of delta wave in total power when mice were in IM. Baseline vs light: $t = 5.11$ CNO vs light+ CNO: $t = 1.52$	$P = 0.004$ $P = 0.19$

Fig. S10l	Two-tailed paired t-test in 6 mice with light stimulation of the VP without and with inhibition of LC NA neurons. df = 5	% of delta wave in total power when mice were in NM. Baseline vs light: $t = 6.04$ CNO vs light+ CNO: $t = 1.13$	$P = 0.002$ $P = 0.31$
Figure S11			
Fig. S11k	Two-tailed t -test, $n = 15$ trials from 5 NE2M mice, $n = 15$ trials from 5 eYFP mice. df = 28	Peak of z-score Rearing: $t = 4.83$ NOR: $t = 7.38$ Social interaction: $t = 6.14$ Air puff: $t = 5.54$	$P < 0.0001$ $P < 0.0001$ $P < 0.0001$ $P < 0.0001$
Fig. S11l	Two-tailed t -test, $n = 15$ trials from 5 NE2M mice, $n = 15$ trials from 5 eYFP mice. df = 28	AUC of z-score Rearing: $t = 4.63$ NOR: $t = 3.88$ Social interaction: $t = 8.48$ Air puff: $t = 4.11$	$P < 0.0001$ $P = 0.0006$ $P < 0.0001$ $P = 0.0003$
Figure S12			
Fig. S11k	Two-tailed t -test, $n = 15$ trials from 8 NE2M mice before and during CNO. df = 28	Peak of z-score in the LH NOR: $t = 6.82$ Social interaction: $t = 5.29$ Air puff: $t = 2.32$	$P = 0.0003$ $P = 0.001$ $P = 0.05$
Figure S13			
Fig. S13c	One-way ANOVAs in 5 NE2M mice and 5 eYFP mice during isoflurane anesthesia induction	fluorescence intensity of NE2M and eYFP signal in the LH Pre-On: $F_{(2, 11)} = 0.89$ Pre-LORR: $F_{(2, 11)} = 0.22$ Post-LORR: $F_{(2, 11)} = 31.6$ GCaMP6: 0.8% vs 1.4%, $t = 4.39$	$P = 0.44$ $P = 0.81$ $P < 0.0001$ $P = 0.003$
Fig. S13f	One-way ANOVAs in 5 NE2M mice and 5 eYFP mice during isoflurane anesthesia emergence	fluorescence intensity of NE2M and eYFP signal in the LH Pre-Off: $F_{(2, 11)} = 19.71$ GCaMP : 0.8% vs 1.4%: $t = 2.36$ Pre-RORR: $F_{(2, 11)} = 17.95$ GCaMP6: 0.8% vs 1.4%: $t = 3.13$ Post-RORR: $F_{(2, 11)} = 3.34$ GCaMP6: 0.8% vs 1.4%, $t = 1.00$	$P < 0.0001$ $P = 0.04$ $P = 0.0003$ $P = 0.03$ $P = 0.07$ $P = 0.34$
Figure S14			
Fig. S14f	One-way repeated measures ANOVA with Tukey test and Geisser-Greenhouse correction in 7 neurons before, during, and	Firing rate ANOVA: $F_{(1.006, 6.036)} = 21.14$ Baseline vs light: $q = 6.60$	$P = 0.004$ $P = 0.008$

	after stimulation of VP-LH projection. df = 6		
Fig. S14n	Two-tailed paired t-test in VP-LH ChR2 mice. n = 6. df = 5.	EEG waves in IM δ : $t = 2.84$ θ : $t = 0.07$ α : $t = 2.32$ β : $t = 1.12$ γ : $t = 3.72$	$P = 0.03$ $P = 0.95$ $P = 0.06$ $P = 0.31$ $P = 0.01$
Fig. S14o	Two-tailed paired t-test in VP-LH ChR2 mice. n = 6. df = 5.	EEG waves in NM δ : $t = 2.20$ θ : $t = 0.40$ α : $t = 1.28$ β : $t = 1.60$ γ : $t = 1.69$	$P = 0.08$ $P = 0.71$ $P = 0.26$ $P = 0.17$ $P = 0.15$
Fig. S14p	Two-tailed paired t-test in VP-LH ChR2 mice. n = 6. df = 5.	EEG waves in LM δ : $t = 0.09$ θ : $t = 0.45$ α : $t = 0.63$ β : $t = 0.03$ γ : $t = 1.65$	$P = 0.93$ $P = 0.67$ $P = 0.56$ $P = 0.98$ $P = 0.16$
Figure S15			
Fig. S15b	Two-tailed paired t-test in VP-LH ChR2 mice. n = 6. df = 5.	LORR eYFP, baseline vs light: $t = 0.13$ ChR2, baseline vs light: $t = 0.16$	$P = 0.90$ $P = 0.88$
Fig. S15c	Two-tailed paired t-test in VP-LH ChR2 mice. n = 6. df = 5.	RORR eYFP, baseline vs light: $t = 0.48$ ChR2, baseline vs light: $t = 0.49$	$P = 0.65$ $P = 0.64$
Fig. S15e	Two-tailed paired t-test in VP-LH ChR2 mice. n = 6. df = 5.	AUC of motor recovery curve eYFP, baseline vs light: $t = 1.94$ ChR2, baseline vs light: $t = 1.00$	$P = 0.11$ $P = 0.36$
Fig. S15g	One-way repeated measures ANOVA in VP-LH ChR2 mice. n = 6.	BSR 1.4% isoflurane anesthesia $F_{(1.161, 5.803)} = 0.43$	$P = 0.57$
Fig. S15i	Two-tailed paired t-test in VP-LH ChR2 mice. n = 6. df = 5.	EEG waves in 0.8% isoflurane δ : $t = 1.71$ θ : $t = 0.65$ α : $t = 1.26$ β : $t = 0.44$ γ : $t = 1.11$	$P = 0.14$ $P = 0.53$ $P = 0.26$ $P = 0.83$ $P = 0.32$
Figure S17			

Fig. S17d	One-way repeated measures ANOVA with Bonferroni tests. n = 8 mice.	Delta wave in EEG ANOVA, $F_{(1.234, 8.640)} = 41.40$ $t = 4.37$, LM vs NM $t = 6.78$, LM vs IM $t = 6.40$, NM vs IM	$P < 0.0001$ $P = 0.01$ $P = 0.0008$ $P = 0.0001$
Fig. S17e	One-way repeated measures ANOVA with Bonferroni tests. n = 8 mice.	Power in EMG ANOVA, $F_{(1.082, 7.577)} = 24.73$ $t = 4.43$, LM vs NM $t = 5.36$, LM vs IM $t = 5.50$, NM vs IM	$P < 0.0001$ $P = 0.009$ $P = 0.003$ $P = 0.003$