Supplemental Information

A neural circuit from the paraventricular thalamus to the bed nucleus of the stria terminalis for the regulation of states of consciousness during sevoflurane anesthesia in mice

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**Figure S1.** (A) Representative images of c-Fos immunofluorescence in the paraventricular thalamus between the awake and sevoflurane anesthesia groups in female mice. Left: representative brain sections stained with c-Fos (green); scale bar, 200 μm. Right: magnified images; scale bar, 100 μm. D3V, dorsal 3rd ventricle. (B) Quantitative analysis of the intensity of c-Fos in the paraventricular thalamus. (Data are shown as the mean ± SD; \*\*\**P* < 0.001, n = 4.)



**Figure S2.** (A-B) Time to induction (A) and emergence (B) times with exposure to 2.4% sevoﬂurane (1 MAC) for 30 min after pretreatment with saline or CNO for male mice in the control group (AAV-CaMKIIa-mCherry) and the hM4D group (AAV-CaMKIIa-hM4D-mCherry). (Data are shown as the mean ± SD; \**P* < 0.05, \*\*\**P* < 0.001, \*\*\*\**P* < 0.0001, n = 12 in each group.) (C-D) Dose–response curves of the loss of the righting reﬂex (C) and the recovery of righting reflex (D) for male mice in the inhibition and control groups.

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**Figure S3.** (A-B) Time to induction (A) and emergence (B) times with exposure to 2.4% sevoﬂurane (1 MAC) for 30 min after pretreatment with saline or CNO for female mice in the control group (AAV-CaMKIIa-mCherry) and the hM4D group (AAV-CaMKIIa-hM4D-mCherry). (Data are shown as the mean ± SD; \**P* < 0.05, \*\*\**P* < 0.001, \*\*\*\**P* < 0.0001, n = 12 in each group.) (C-D) Dose–response curves of the loss of the righting reﬂex (C) and the recovery of the righting reflex (D) for female mice in the PVT inhibition and control groups.

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**Figure S4. Whole-brain mapping of direct outputs from vglut2+ neurons of the paraventricular thalamus.** Example of projections at eight coronal levels from vglut2+ neuron populations of the paraventricular thalamus. Scale bars: 1 mm. Cg, cingulate cortex; CPu, caudate putamen; NAcC, nucleus accumbens core; NAcsh, nucleus accumbens shell; BNST, bed nucleus of the stria terminalis; PVT, paraventricular thalamus; Rt, reticular thalamic nucleus; CEM, central amygdaloid nucleus, medial division; BLP, basolateral amygdaloid nucleus, posterior part; Fr, fasciculus retroflexus; ATg, anterior tegmental nucleus; MnR, median raphe nucleus.

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|  | Chemogenetic inhibition of paraventricular thalamus glutamatergic neurons in both male and female mice | | |
| mCherry-CNO | hM4D-saline | hM4D-CNO |
| MACLORR, % | 1.49 | 1.43 | 1.16 |
| MACLORR 95% CI | 1.46 to 1.53 | 1.40 to 1.46 | 1.12 to 1.20 |
| Hill’s Slope | 8.87 | 11.05 | 8.45 |
| Hill’s Slope 95% CI | 7.18 to 10.57 | 9.04 to 13.06 | 6.38 to 10.52 |
| MACRORR, % | 1.34 | 1.32 | 0.95 |
| MACRORR 95% CI | 1.29 to 1.40 | 1.29 to 1.34 | 0.86 to 1.03 |
| Hill’s Slope | -7.93 | -8.17 | -3.79 |
| Hill’s Slope 95% CI | -9.70 to -6.16 | -9.32 to -7.02 | -5.07 to -2.51 |

**Table S1. Table of best-fit values for sevoflurane dose–response curves after the inhibition of paraventricular thalamus glutamatergic neurons in both male and female mice, related to Figure 2F-G (n=20 for each group).** MACLORR and MACRORR along with the corresponding 95% confidence intervals (CIs) for the hM4D-CNO group and the control group (mCherry-CNO group and hM4D-saline group) under sevoflurane anesthesia. MACLORR, minimum alveolar concentration at which 50% of the mice lost their righting reflex; MACRORR,minimum alveolar concentration at which 50% of the mice recovered their righting reflex.

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|  | Chemogenetic inhibition of paraventricular thalamus glutamatergic neurons in male mice | | |
| mCherry-CNO | hM4D-saline | hM4D-CNO |
| MACLORR, % | 1.50 | 1.45 | 1.20 |
| MACLORR 95% CI | 1.44 to 1.57 | 1.44 to 1.46 | 1.18 to 1.21 |
| Hill’s Slope | 8.41 | 13.26 | 9.52 |
| Hill’s Slope 95% CI | 5.47 to 11.34 | 12.07 to 14.44 | 8.42 to 10.62 |
| MACRORR, % | 1.38 | 1.32 | 1.07 |
| MACRORR 95% CI | 1.34 to 1.42 | 1.29 to 1.36 | 0.94 to 1.22 |
| Hill’s Slope | -8.52 | -7.79 | -3.51 |
| Hill’s Slope 95% CI | -10.41 to -6.62 | -9.19 to -6.40 | -5.05 to -1.97 |

**Table S2. Table of best-fit values for sevoflurane dose–response curves** **after the inhibition of paraventricular thalamus glutamatergic neurons in male mice, related to Figure S3C-D (n=10 for each group).** MACLORR and MACRORR along with the corresponding 95% confidence intervals (CIs) for the hM4D-CNO group and the control group (mCherry-CNO group and hM4D-saline group) under sevoflurane anesthesia. MACLORR, minimum alveolar concentration at which 50% of the mice lost their righting reflex; MACRORR,minimum alveolar concentration at which 50% of the mice recovered their righting reflex.

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| --- | --- | --- | --- |
|  | Chemogenetic inhibition of paraventricular thalamus glutamatergic neurons in female mice | | |
| mCherry-CNO | hM4D-saline | hM4D-CNO |
| MACLORR, % | 1.48 | 1.40 | 1.11 |
| MACLORR 95% CI | 1.43 to 1.53 | 1.35 to 1.46 | 1.04 to 1.18 |
| Hill’s Slope | 9.39 | 9.75 | 7.23 |
| Hill’s Slope 95% CI | 6.75 to 12.03 | 6.62 to 12.89 | 4.25 to 10.21 |
| MACRORR, % | 1.30 | 1.32 | 0.86 |
| MACRORR 95% CI | 1.23 to 1.36 | 1.27 to 1.36 | 0.81 to 0.91 |
| Hill’s Slope | -7.39 | -8.54 | -4.32 |
| Hill’s Slope 95% CI | -9.95 to -4.82 | -10.91 to -6.17 | -5.33 to -3.30 |

**Table S3. Table of best-fit values for sevoflurane dose–response curves after the inhibition of paraventricular thalamus glutamatergic neurons in female mice, related to Figure S4C-D (n=10 for each group).** MACLORR and MACRORR along with the corresponding 95% confidence intervals (CIs) for the hM4D-CNO group and the control group (mCherry-CNO group and hM4D-saline group) under sevoflurane anesthesia. MACLORR, minimum alveolar concentration at which 50% of the mice lost their righting reflex; MACRORR, minimum alveolar concentration at which 50% of the mice recovered their righting reflex.

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|  | Chemogenetic inhibition of the paraventricular thalamus – bed nucleus of the stria terminalis pathway in male mice | | |
| mCherry-CNO | hM4D-saline | hM4D-CNO |
| MACLORR, % | 1.42 | 1.40 | 1.13 |
| MACLORR 95% CI | 1.38 to 1.47 | 1.36 to 1.44 | 1.09 to 1.17 |
| Hill’s Slope | 12.36 | 8.68 | 8.93 |
| Hill’s Slope 95% CI | 8.52 to 16.20 | 6.66 to 10.70 | 6.43 to 11.43 |
| MACRORR, % | 1.33 | 1.30 | 1.03 |
| MACRORR 95% CI | 1.28 to 1.39 | 1.26 to 1.35 | 0.97 to 1.09 |
| Hill’s Slope | -7.04 | -6.16 | -5.74 |
| Hill’s Slope 95% CI | -8.83 to -5.24 | -7.32 to -4.99 | -7.51 to -3.97 |

**Table S4. Table of best-fit values for sevoflurane dose–response curves after the inhibition of the paraventricular thalamus–bed nucleus of the stria terminalis pathway in male mice, related to Figure 7F-G (n=10 for each group).** MACLORR and MACRORR along with the corresponding 95% confidence intervals (CIs) for the hM4D-CNO group and the control group (mCherry-CNO group and hM4D-saline group) under sevoflurane anesthesia after the inhibition of paraventricular thalamus glutamatergic neurons. MACLORR, minimum alveolar concentration at which 50% of the mice lost their righting reflex; MACRORR,minimum alveolar concentration at which 50% of the mice recovered their righting reflex

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| --- | --- | --- | --- | --- | --- | --- | --- |
| ID | Group | Leg movement | Head movement | Tail  movement | Righting | Walking | Total score |
| 1 | ChR2-on | 2 | 2 | 2 | 2 | 2 | 10 |
| 2 | ChR2-on | 2 | 2 | 2 | 2 | 1 | 9 |
| 3 | ChR2-on | 2 | 2 | 2 | 2 | 0 | 8 |
| 4 | ChR2-on | 2 | 2 | 2 | 0 | 0 | 6 |
| 5 | ChR2-on | 2 | 2 | 2 | 2 | 2 | 10 |
| 6 | ChR2-on | 2 | 2 | 2 | 0 | 0 | 6 |
| 7 | ChR2-on | 2 | 2 | 2 | 2 | 1 | 9 |
| 8 | ChR2-on | 2 | 2 | 2 | 2 | 1 | 9 |
| 1 | ChR2-off | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | ChR2-off | 1 | 0 | 0 | 0 | 0 | 1 |
| 3 | ChR2-off | 1 | 0 | 0 | 0 | 0 | 1 |
| 4 | ChR2-off | 0 | 0 | 0 | 0 | 0 | 0 |
| 5 | ChR2-off | 1 | 1 | 0 | 0 | 0 | 2 |
| 6 | ChR2-off | 1 | 0 | 0 | 0 | 0 | 1 |
| 7 | ChR2-off | 1 | 0 | 0 | 0 | 0 | 1 |
| 8 | ChR2-off | 1 | 0 | 0 | 0 | 0 | 1 |
| 1 | mCherry-on | 1 | 1 | 0 | 0 | 0 | 2 |
| 2 | mCherry-on | 1 | 0 | 0 | 0 | 0 | 1 |
| 3 | mCherry-on | 1 | 1 | 0 | 0 | 0 | 2 |
| 4 | mCherry-on | 1 | 0 | 0 | 0 | 0 | 1 |
| 5 | mCherry-on | 1 | 0 | 0 | 0 | 0 | 1 |
| 6 | mCherry-on | 1 | 0 | 0 | 0 | 0 | 1 |

**Table S5. Arousal responses under sevoflurane continuous steady-state general anesthesia during optogenetic stimulation of the glutamatergic neurons of paraventricular thalamus in male mice, related to Figure 4F-H.** Behavioral responses, including spontaneous movements of the limbs, head, and tail, states of the righting reflex and walking, were scored during the 120-s acute optical stimulation of paraventricular thalamus glutamatergic neurons. The total score for each mouse was determined by the sum of all categories.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| ID | Group | Leg movement | Head movement | Tail  movement | Righting | Walking | Total score |
| 1 | ChR2-on | 2 | 2 | 2 | 0 | 0 | 6 |
| 2 | ChR2-on | 2 | 2 | 2 | 2 | 0 | 8 |
| 3 | ChR2-on | 2 | 2 | 2 | 2 | 1 | 9 |
| 4 | ChR2-on | 2 | 2 | 2 | 0 | 0 | 6 |
| 5 | ChR2-on | 2 | 2 | 2 | 2 | 1 | 9 |
| 6 | ChR2-on | 2 | 2 | 2 | 0 | 0 | 6 |
| 7 | ChR2-on | 2 | 2 | 2 | 2 | 1 | 9 |
| 8 | ChR2-on | 2 | 2 | 2 | 2 | 0 | 8 |
| 1 | ChR2-off | 1 | 0 | 0 | 0 | 0 | 1 |
| 2 | ChR2-off | 1 | 1 | 0 | 0 | 0 | 2 |
| 3 | ChR2-off | 1 | 1 | 0 | 0 | 0 | 2 |
| 4 | ChR2-off | 1 | 0 | 0 | 0 | 0 | 1 |
| 5 | ChR2-off | 1 | 0 | 0 | 0 | 0 | 1 |
| 6 | ChR2-off | 1 | 0 | 0 | 0 | 0 | 1 |
| 7 | ChR2-off | 1 | 0 | 0 | 0 | 0 | 1 |
| 8 | ChR2-off | 1 | 0 | 0 | 0 | 0 | 1 |
| 1 | mCherry-on | 1 | 0 | 0 | 0 | 0 | 1 |
| 2 | mCherry-on | 1 | 0 | 0 | 0 | 0 | 1 |
| 3 | mCherry-on | 1 | 0 | 0 | 0 | 0 | 1 |
| 4 | mCherry-on | 1 | 1 | 0 | 0 | 0 | 2 |
| 5 | mCherry-on | 1 | 1 | 0 | 0 | 0 | 2 |
| 6 | mCherry-on | 1 | 0 | 0 | 0 | 0 | 1 |

**Table S6. Arousal responses under sevoflurane continuous steady-state general anesthesia during optogenetic stimulation of the paraventricular thalamus–bed nucleus of the stria terminalis pathway in male mice, related to Figure 9F-H.** Behavioral responses, including spontaneous movements of the limbs, head, and tail, states of the righting reflex and walking, were scored during the 120-s acute optical stimulation of the paraventricular thalamus-bed nucleus of the stria terminalis pathway. The total score for each mouse was determined by the sum of all categories.