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Supplemental information

Ventrolateral periaqueductal gray GABAergic neurons promote arousal of sevoflurane anesthesia through cortico-midbrain circuit

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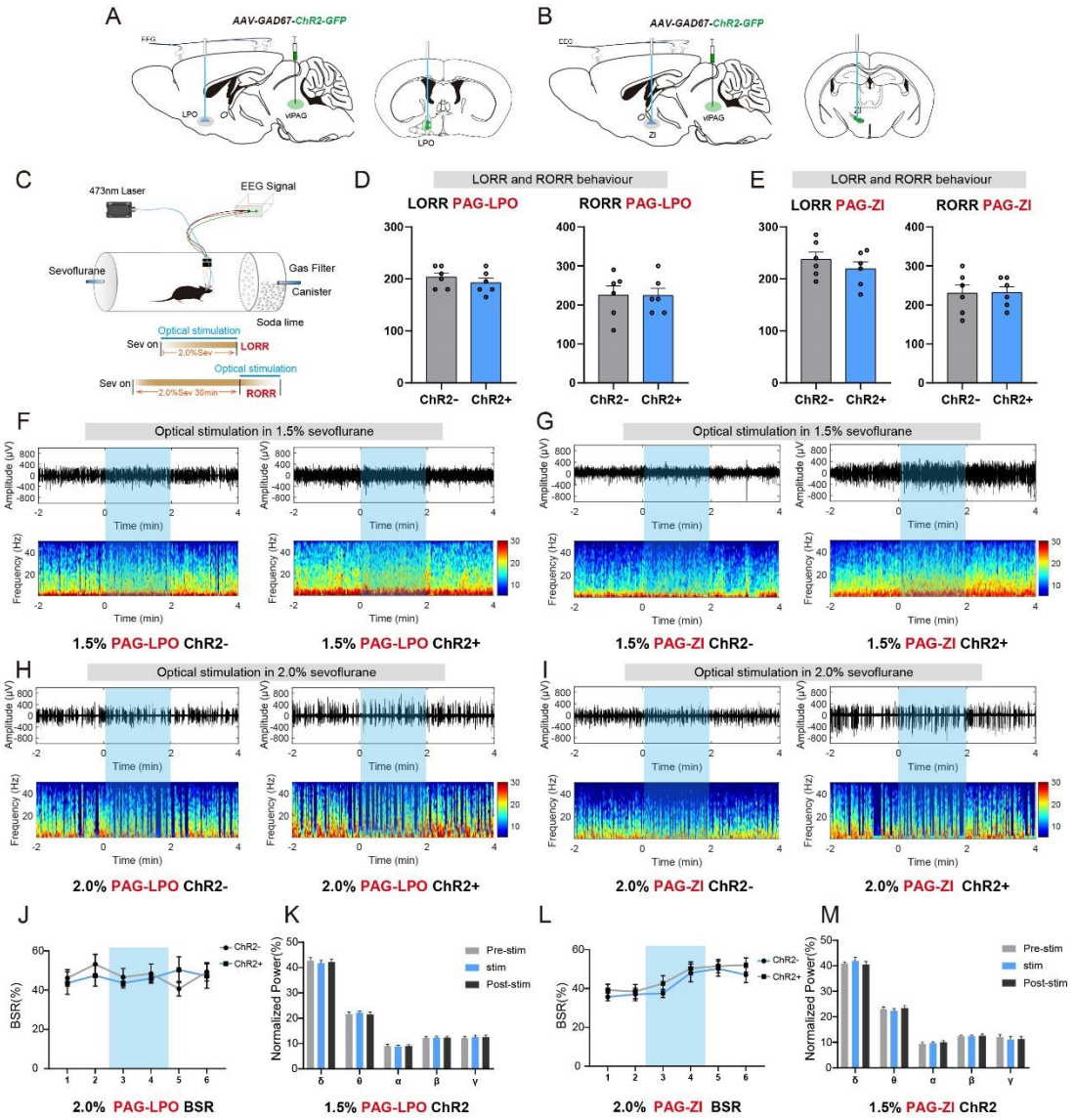

Figure S1. Optical stimulation of GABAergic terminals in the LPO and ZI did not affect sevoflurane anaesthesia, related to Figure 4. (a) Schematic diagram of optogenetic virus injection into the vlPAG and laser implant into LPO. (b) Schematic diagram of optogenetic virus injection into the vlPAG and laser implant into ZI. (c) Schematic of the EEG recording configuration and righting reflex detection. (d) Optogenetic activation of vlPAG^{GABA} projections in LPO has no significant effect on LORR and RORR time. (e) Optogenetic activation of vlPAG^{GABA} projections in ZI has no significant effect on the LORR and RORR times. (f-g) The representative EEG spectrum of the ChR2- group and the ChR2+ group under 1.5% sevoflurane anaesthesia. (h-i) The representative EEG spectrum of the ChR2- group and the ChR2+ group under 2.0% sevoflurane anaesthesia. (j-m) There were no significant difference in the BSR of EEG and the EEG spectrum between ChR2+ and ChR2- groups. Data are shown as the mean ± SEM, *P<0.05, **P<0.01, ***P<0.001.

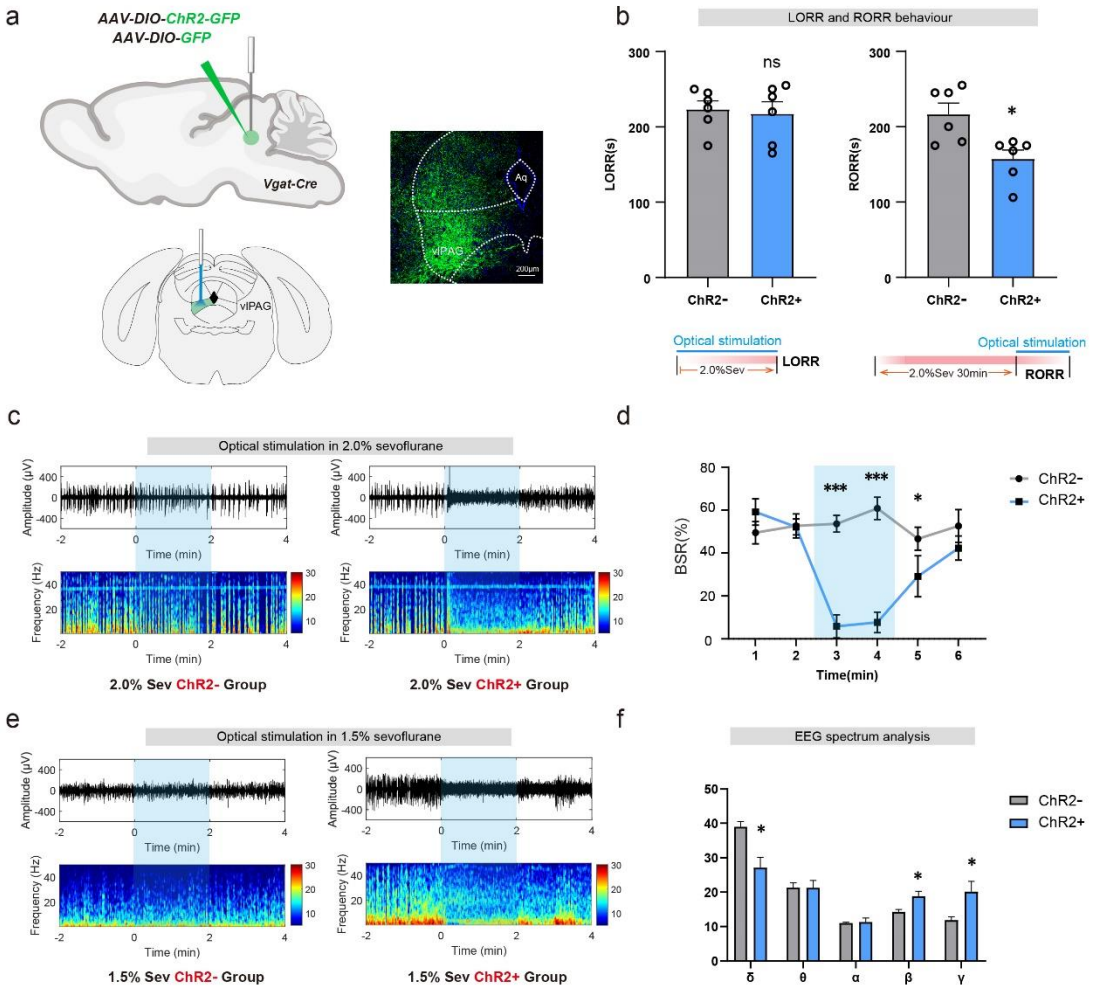

Figure S2. Optical activation vlPAG^{GABA} neurons promotes arousal from sevoflurane anaesthesia in Vgat-Cre mice, related to Figure 2. (a) Schematic diagram of optogenetic virus injection into the vlPAG and laser irradiation(left). Optogenetic virus (GFP, green) expression in vlPAG(right). (b) Optogenetic activation of vlPAG^{GABA} significantly shortened RORR time (right). (c) The representative EEG spectrum of the control group (ChR2- group) and the experimental group (ChR2+ group) under 2.0% sevoflurane anaesthesia. (d) At 20-22 minutes of light stimulation under 2.0% sevoflurane anaesthesia, the ChR2+ group demonstrated a significantly lower BSR of EEG compared to the ChR2- group. (e) The representative EEG spectrum of the ChR2- group and the ChR2+ group under 1.5% sevoflurane anaesthesia. (f) During 20-22 minutes of light stimulation under 1.5% anaesthesia, the ChR2+ group exhibited a decrease in the percentage of power in the δ band and a significant increase in the percentages of power in the β and γ bands. Data are shown as the mean ± SEM, *P<0.05, **P<0.01, ***P<0.001.

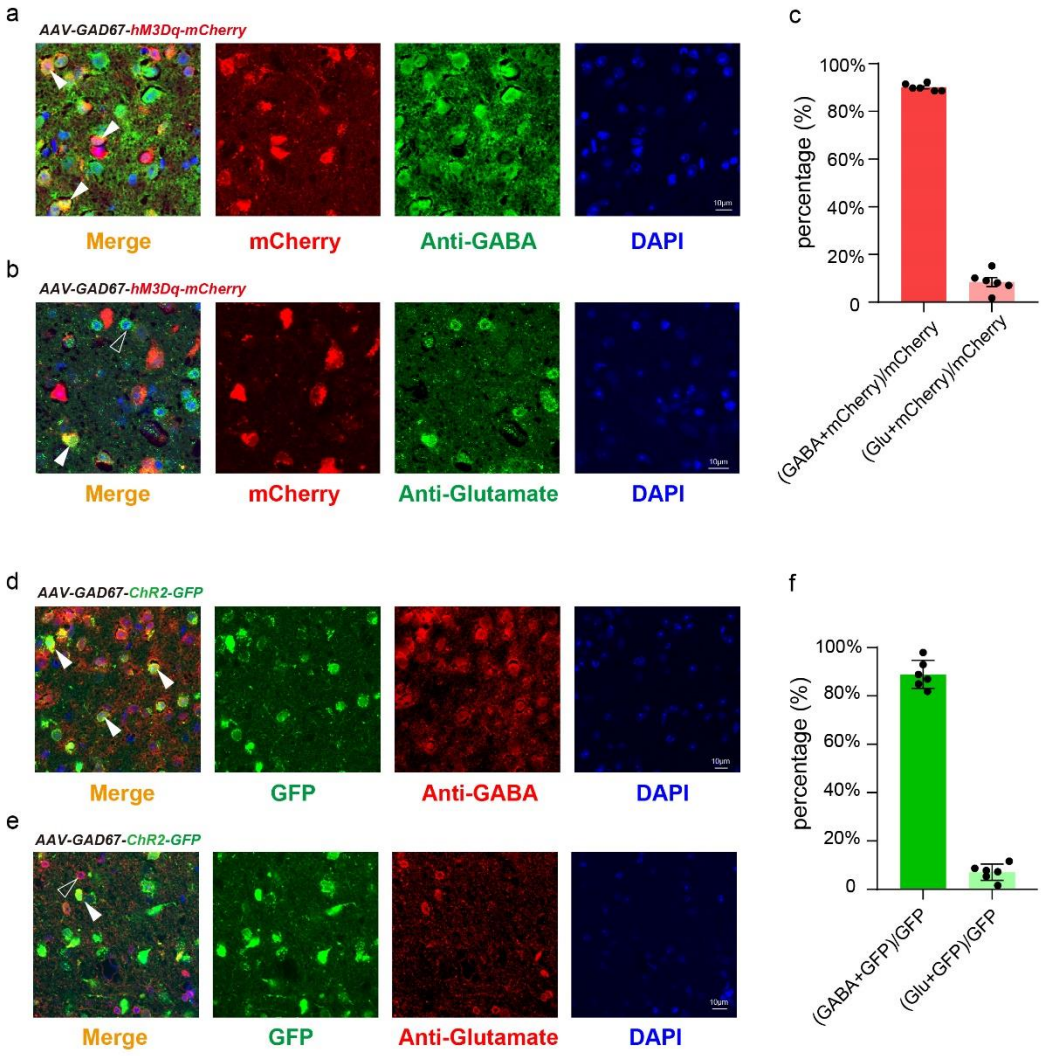

Figure S3. Verification of virus expression specificity of *GAD67* Promoter, related to Figure 1-4. (a) Co-labelled neurons of GABA and mCherry in mice injected with *rAAV2/9-GAD67-hM3Dq-mCherry* virus (solid arrow indicates co-labelled neuron, hollow arrow indicates GABAergic neuron). (b) Co-labelled neurons of Glutamate and mCherry in mice injected with *rAAV2/9-GAD67-hM3Dq-mCherry* virus (solid arrow indicates co-labelled neuron, hollow arrow indicates Glutaminergic neuron). (c) In mice injected with *rAAV2/9-GAD67-hM3Dq-mCherry* virus, 90.5% (90.5 ± 0.6%) of neurons expressing mCherry were GABAergic neurons, while 8.2% (8.2 ± 1.8%) were glutaminergic neurons. (d) Co-labelled neurons of GABA and GFP in mice injected with a *rAAV2/9-GAD67-ChR2-GFP* virus (solid arrow indicates co-labelled neuron, hollow arrow indicates GABAergic neuron). (e) Co-labelled neurons of glutamate and GFP in mice injected with a *rAAV2/9-GAD67-ChR2-GFP* virus (solid arrow indicates co-labelled neuron, hollow arrow indicates glutamate neuron). (f) In mice injected with *rAAV2/9-GAD67-ChR2-GFP* virus, 89% (89 ± 5.9%) of neurons expressing GFP were GABAergic neurons, while 7.2% (7.2 ± 3.4%) were glutaminergic neurons. Data are shown as the mean ± SEM.

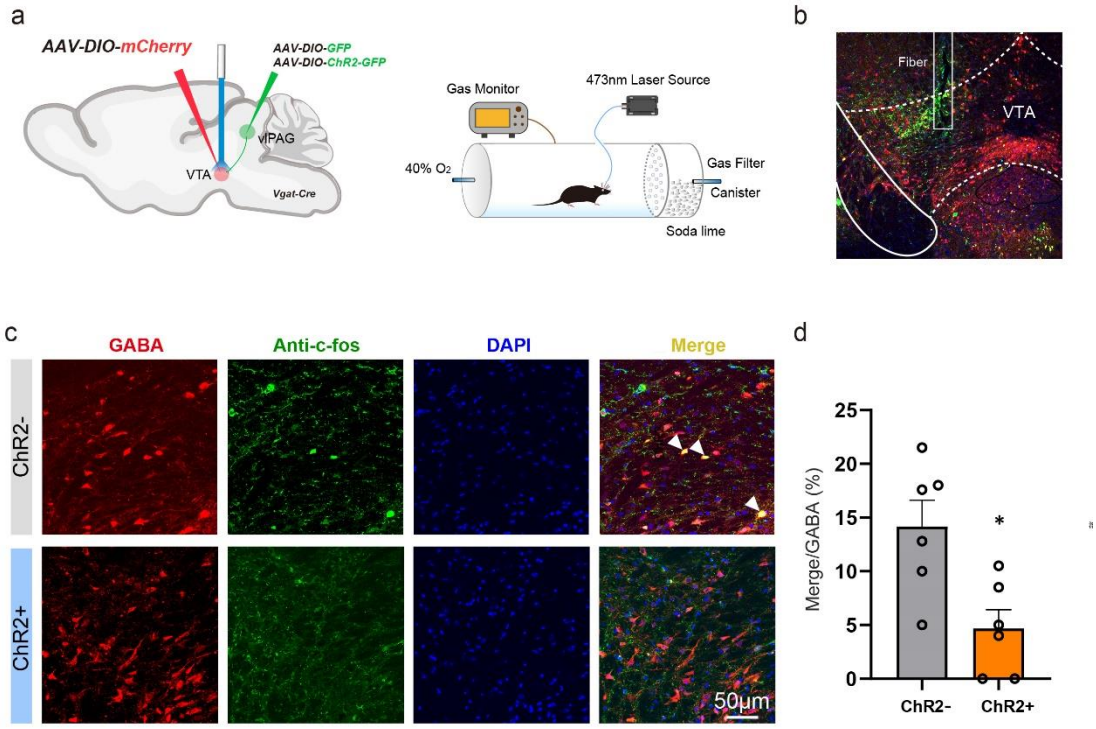

Figure S4. Optical activation of vlPAG^{GABA}-VTA projections can inhibit the activity of VTA^{GABA} neurons in Vgat-Cre mice, related to Figure 4. (a) Schematic diagram of virus injection and laser irradiation. (b) Fibre and fluorescence image of virus expression in VTA. (c) Immunofluorescence of c-fos (green) in VTA. (d) Significant inhibition of c-fos expression in VTA^{GABA} neurons activated by optogenetic. Data are shown as the mean ± SEM, *P < 0.05.

Table S1. Arousal behavioural scores of optical activation GABAergic neurons in the vlPAG, related to Figure 2.

Group	Leg movement	Head movement	Whisker movement	Righting	Walking	Total score
1-ChR2+	2	2	2	2	1	9
2-ChR2+	2	2	2	2	2	10
3-ChR2+	2	2	2	2	2	10
4-ChR2+	2	2	2	2	2	10
5-ChR2+	2	2	2	2	2	10
6-ChR2+	2	2	2	2	1	9
1-ChR2-	0	0	0	0	0	0
2-ChR2-	0	0	0	0	0	0
3-ChR2-	1	0	0	0	0	1
4-ChR2-	0	0	0	0	0	0
5-ChR2-	1	0	0	0	0	1
6-ChR2-	0	0	0	0	0	0

The arousal behavioural scores. Leg, head, and whisker movements, as well as the righting and walking status were scored for each animal. Comparison of the total scores of two groups, ChR2+ *vs* ChR2-, P=0.0022

Table S2. Arousal behavioural scores of optical stimulations of vlPAG GABAergic projections in the VTA, related to Figure 4.

Group	Leg movement	Head movement	Whisker movement	Righting	Walking	Total score
1-ChR2+	2	2	2	2	1	9
2-ChR2+	2	2	2	2	2	10
3-ChR2+	2	2	2	2	2	10
4-ChR2+	2	2	2	2	1	9
5-ChR2+	2	2	2	2	1	9
6-ChR2+	2	2	2	2	2	10
1-ChR2-	1	0	0	0	0	1
2-ChR2-	0	0	0	0	0	0
3-ChR2-	0	0	0	0	0	0
4-ChR2-	0	0	0	0	0	0
5-ChR2-	0	0	0	0	0	0
6-ChR2-	1	0	0	0	0	1

The arousal behavioural scores. Leg, head, and whisker movements, as well as the righting and walking status were scored for each animal. Comparison of the total scores of two groups, ChR2+ *vs* ChR2-, P=0.0022

Table S3. Arousal behavioural scores of optical stimulation mPFC-vlPAG pyramidal neurons, related to Figure 5.

Group	Leg movement	Head movement	Whisker movement	Righting	Walking	Total score
1-ChR2+	2	2	2	2	1	9
2-ChR2+	2	2	2	2	0	8
3-ChR2+	1	1	0	0	0	2
4-ChR2+	2	2	2	2	2	10
5-ChR2+	2	2	2	2	2	10
6-ChR2+	2	2	2	2	0	8
1-ChR2-	1	0	0	0	0	1
2-ChR2-	0	0	0	0	0	0
3-ChR2-	1	0	0	0	0	1
4-ChR2-	1	1	0	0	0	2
5-ChR2-	0	0	0	0	0	0
6-ChR2-	0	0	0	0	0	0

The arousal behavioural scores. Leg, head, and whisker movements, as well as the righting and walking status were scored for each animal. Comparison of the total scores of two groups, ChR2+ *vs* ChR2-, P=0.0022