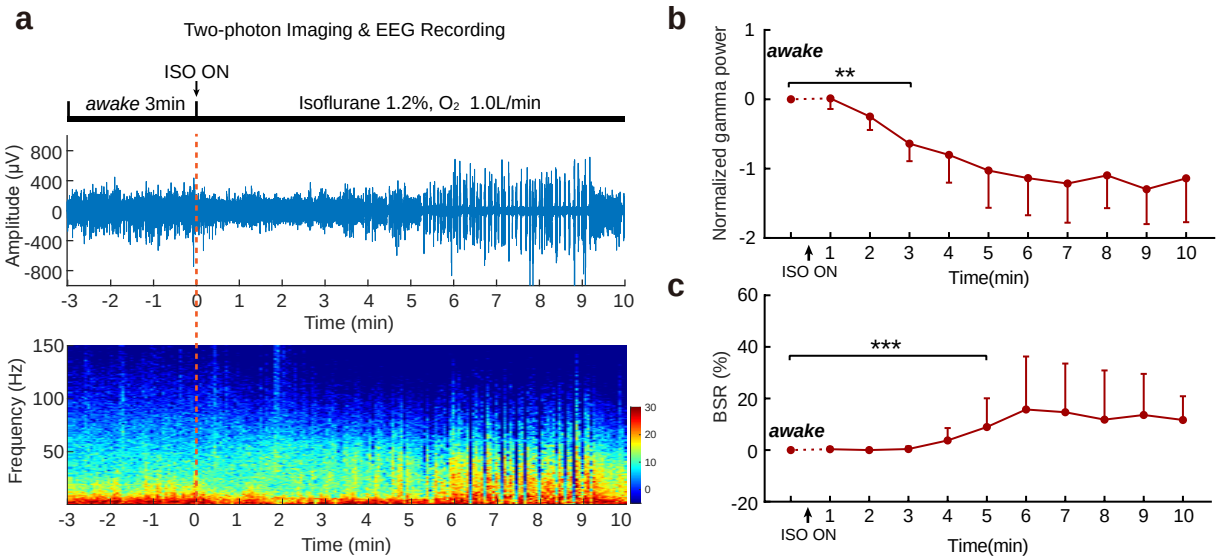


**Fig. S1** EEG recording showing that the volatile inhalation apparatus can induce stable unconsciousness under isoflurane anaesthesia. **a** A representative EEG raw trace (middle) and power spectrogram (bottom) during the imaging process (Up). **b** Normalized gamma power within 1 to 10 minutes after isoflurane administration (n=15 mice). Kruskal-Wallis one-way ANOVA with Dunn's multiple comparisons. **c** Burst suppression ratio (BSR) within 1 to 10 minutes after isoflurane administration. Kruskal-Wallis one-way ANOVA with Dunn's multiple comparisons. Data with error bars are presented as the mean ± SD, $^{**}p < 0.01$, $^{***}p < 0.001$.

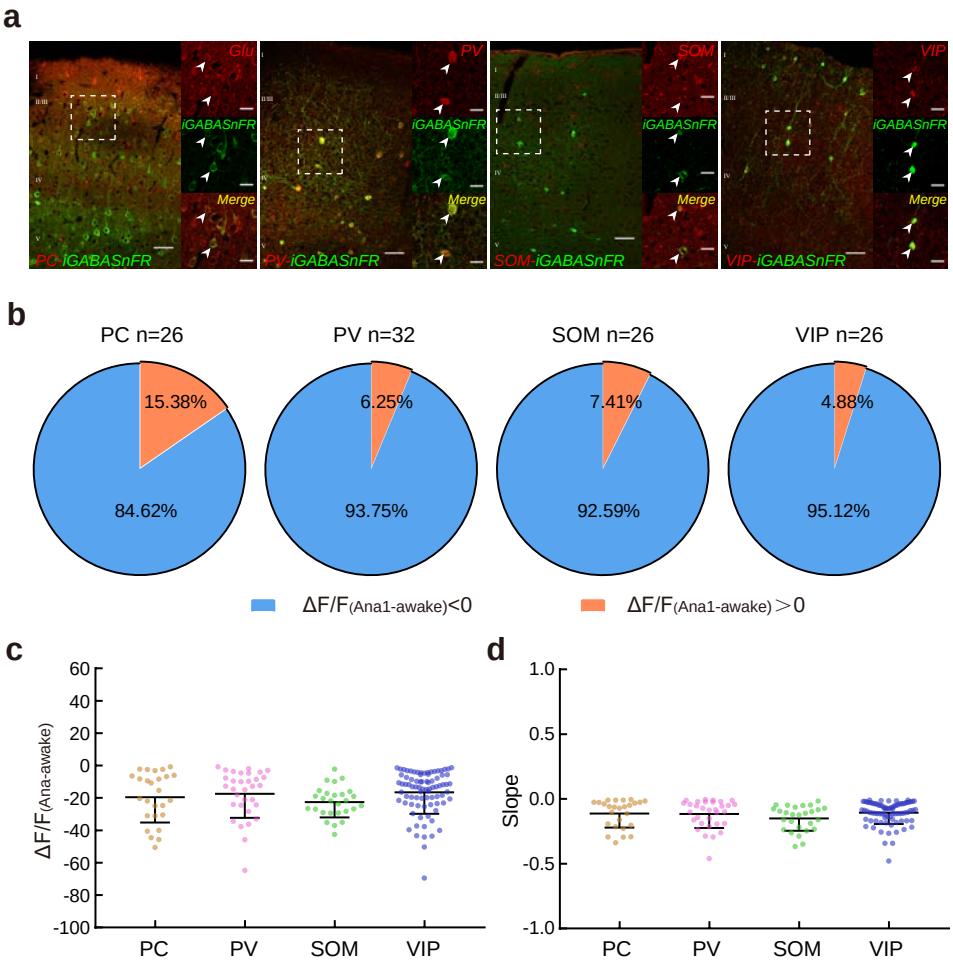


**Fig. S2** Rapid reduction in and undifferentiated maximal inhibitory effect of GABA transmission among cortical cell subtypes. **a** Coexpression of GCaMP6m (green) in PV+, SOM+, and VIP+ neurons (red) in layer 2/3 of the V1 cortex. Scale bars: 50 μm (left) and 20 μm (right). Arrowheads indicate coexpressing cells. **b** The percentage of ΔF/F(Ana-awake) > 0 within the pyramidal, PV, SOM, and VIP neurons. (**c, d**) The ΔF/F(Ana-awake) (**c**) and slope (**d**) of pyramidal, PV, SOM, and VIP neurons. Kruskal-Wallis one-way ANOVA with Dunn's multiple comparisons. Data with error bars are presented as the mean ± SD.

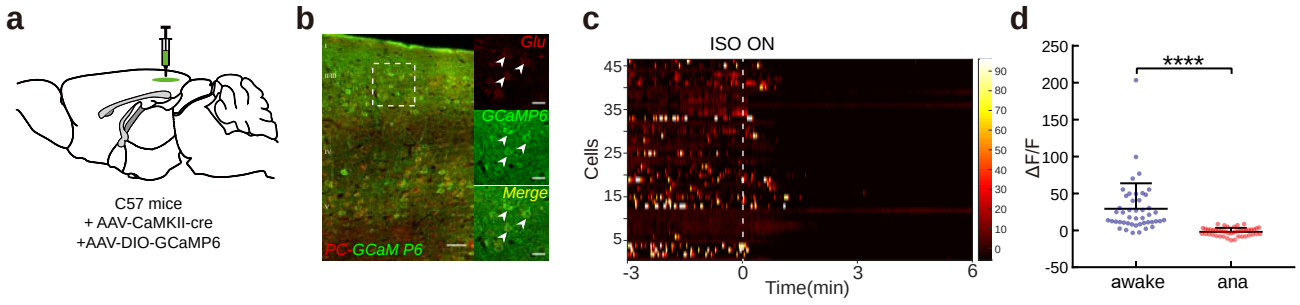


**Fig. S3** Decreased calcium activity of pyramidal cells. **a** Injection of AAV-CaMKII-cre and AAV-DIO-GCaMP6m virus into the V1 cortex of C57BL/6J mice. **b** Coexpression of GCaMP6m (green) and glutamate in the pyramidal cells (red) in layer 2/3 of the V1 cortex. Scale bars: 50 μm (left) and 20 μm (right). Arrowheads indicate cells with coexpression. **c** Heatmap of the calcium activity of pyramidal cells under isoflurane anaesthesia. White dashed line, administration of isoflurane. **d** ΔF/F difference in pyramidal neuronal calcium activity between the awake state and anaesthetized state (n=46 neurons from 5 mice). Paired two-tailed Wilcoxon test. Data with error bars are presented as the mean ± SD, ****$p < 0.0001$.

# Figure S4

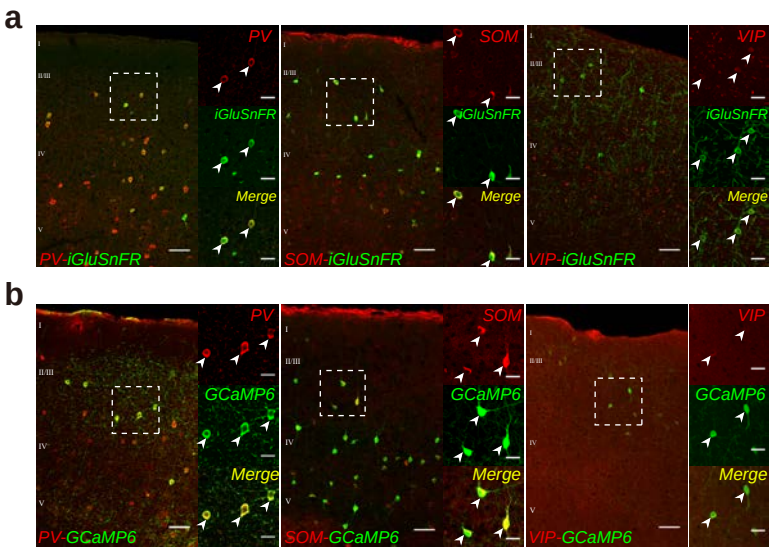


**Fig. S4** Expression of AAV-DIO-iGluSnFR and AAV-DIO-GCaMP6m in PV, SOM, and VIP interneurons. (**a, b**) Coexpression of GCaMP6m (**a**, green) and iGluSnFR (**b**, green) in PV+, SOM+, and VIP+ neurons (red) in layer 2/3 of the V1 cortex. Scale bars: 50 μm (left) and 20 μm (right). Arrowheads indicate coexpressing cells.