

Expanded View Figures

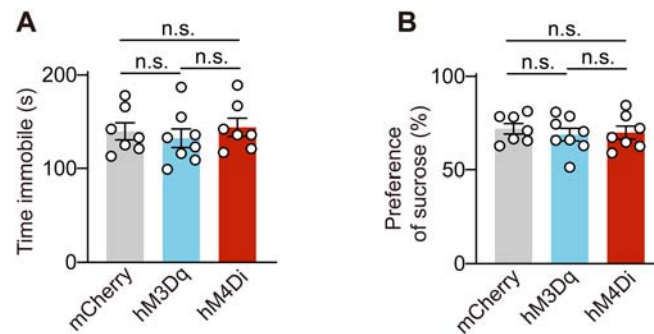


Figure EV1. MeATac1 neurons do not modulate depressive-like behaviors in male mice.

(A) The immobile time in the tail suspension test. (B) The preference score in the sucrose preference test. mCherry: $N = 7$; M3Dq: $N = 8$; hM4Di: $N = 7$. N animal number. All data are means $\pm$ s.e.m. n.s. not significant. See Table EV1 for detailed statistics. Source data are available online for this figure.

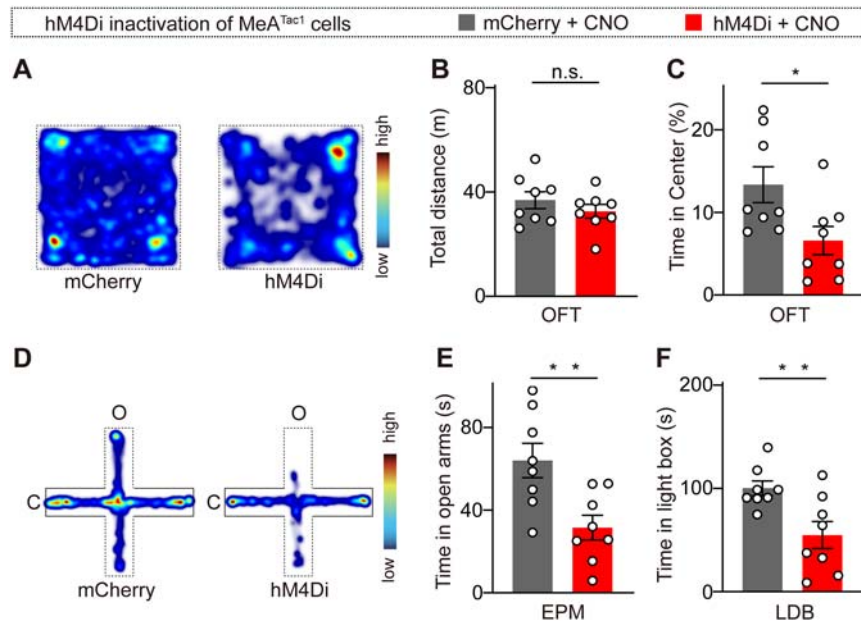


Figure EV2. MeATac1 neurons mediate anxiety-like behaviors in female mice.

(A) Heatmaps display time spent in different regions of the open field arena (warmer colors indicate more time). (B) Total distance traveled in the open field arena. $P = 0.3245$. mCherry: $N = 8$; hM4Di: $N = 8$. (C) Decreased time spent in the center of the open field arena was observed in hM4Di female mice. $P = 0.0275$. mCherry: $N = 8$; hM4Di: $N = 8$. (D) Heatmaps display time spent in different regions of the elevated plus maze (warmer colors indicate more time). (E) Decreased time spent in the open arms was observed in hM4Di female mice. $P = 0.0068$. mCherry: $N = 8$; hM4Di: $N = 8$. (F) Decreased time spent in the light box was observed in hM4Di female mice. $P = 0.0088$. mCherry: $N = 8$; hM4Di: $N = 8$. N animal number. Statistical significance was determined using an unpaired t test. All data are means $\pm$ s.e.m. * $P < 0.05$; ** $P < 0.01$; n.s. not significant. See Table EV1 for detailed statistics. Source data are available online for this figure.

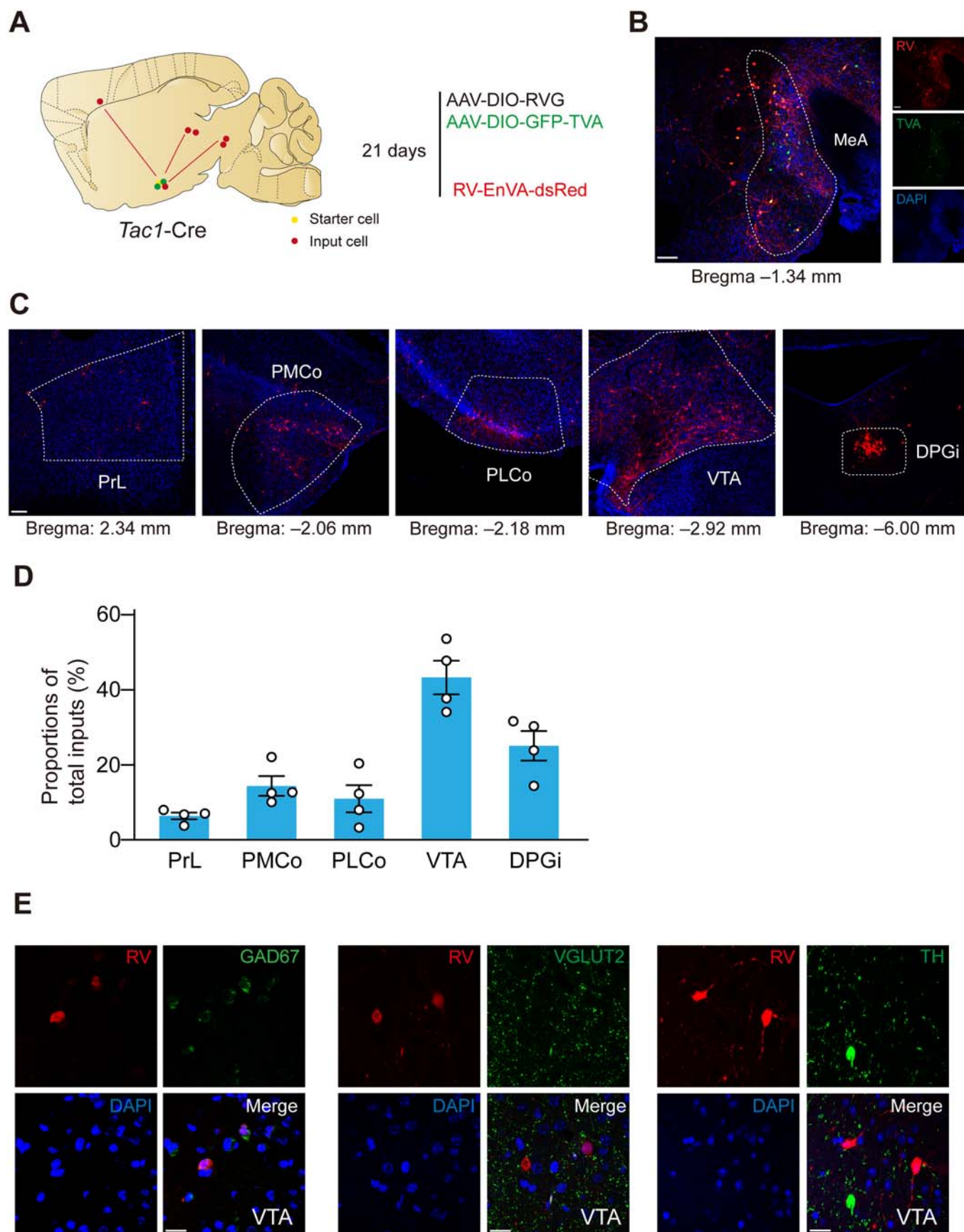


Figure EV3. Whole-brain inputs to Tac1 neurons in the MeA.

(A) Schematic of rabies-based cell type-specific monosynaptic tracing procedure. (B) Starter neurons with AAV-EF1 α -DIO-EGFP-T2A-TVA, AAV-EF1 α -DIO-oRVG and RV-ENVA- Δ G-mCherry. Scale bar, 100 μ m. (C) Representative images of tracing inputs from selected brain regions to MeATac1 neurons. PrL prelimbic cortex, PMCo posteromedial cortical amygdaloid area, PLCo posterolateral cortical amygdaloid area, VTA ventral tegmental area, DPGi dorsal paragigantocellular nucleus. Scale bar, 100 μ m. (D) Whole brain mapping quantitation of inputs to MeATac1 neurons. $N = 4$. N animal number. (E) Representative images of acute slices injected with RV-ENVA- Δ G-mCherry staining with antibodies against GAD67, VGLUT2 and TH. Scale bar, 10 μ m. Error bars represent s.e.m. Source data are available online for this figure.

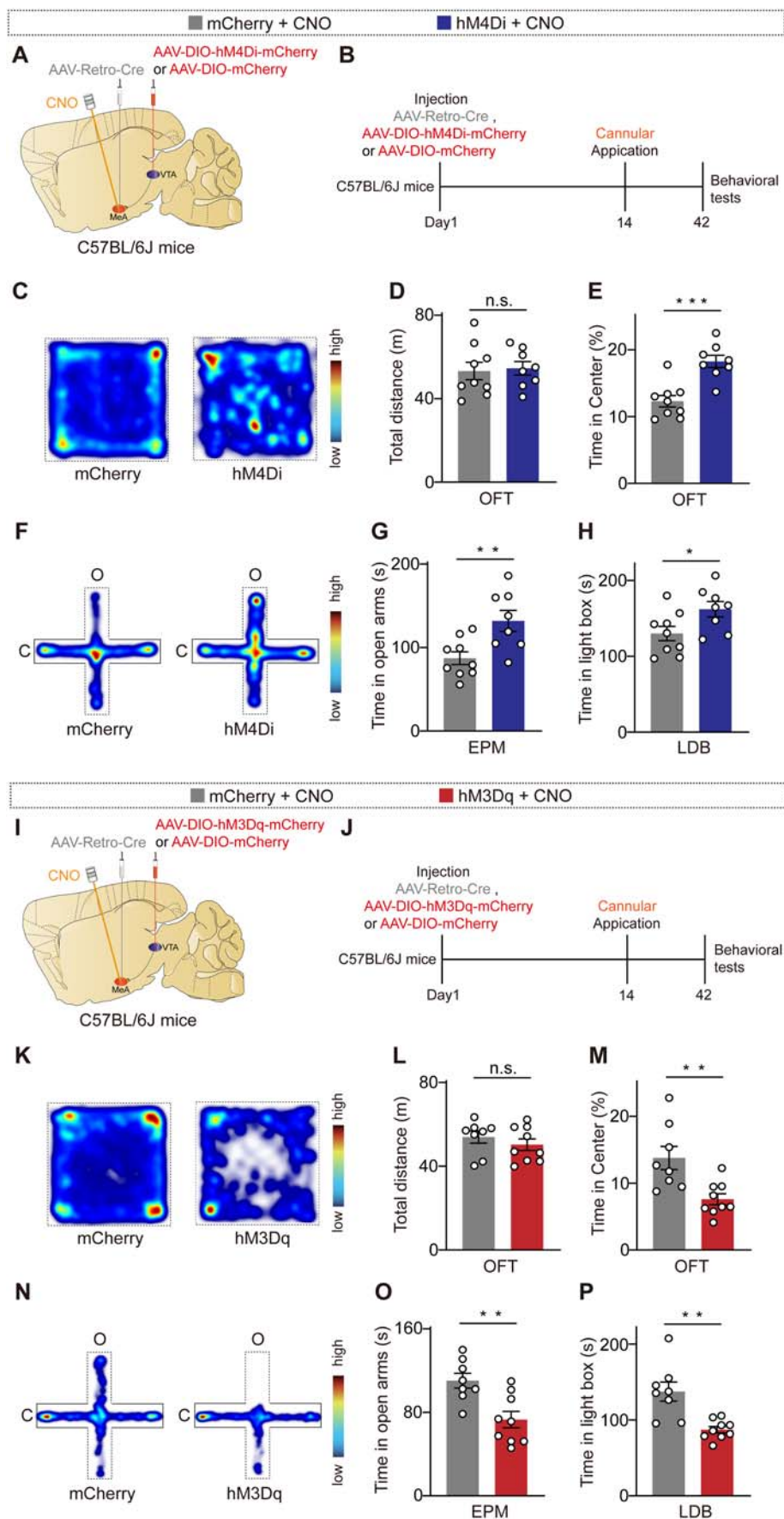



Figure EV4. The VTA→MeATac1 pathway modulates anxiety-like behaviors.

(A, B) Schematic of the strategies that were used to express AAV-Retro-Cre in the MeA and AAV-DIO-mCherry or AAV-DIO-hM4Di-mCherry in the VTA of C57BL/6 J mice. A cannula was then implanted into the MeA. (C) Heatmaps displaying the time spent in different regions of the open field arena (warmer colors indicate more time spent in a region). (D) Total distance traveled in the open field arena. $P = 0.8089$. mCherry: $N = 9$; hM3Dq: $N = 8$. (E) Time spent in the center of the arena. $P = 0.003$. mCherry: $N = 9$; hM3Dq: $N = 8$. (F) Heatmaps displaying the time spent in different regions of the elevated plus maze (warmer colors indicate more time spent in a region). (G) Time spent in the open arms. $P = 0.0068$. mCherry: $N = 9$; hM3Dq: $N = 8$. (H) Time spent in the light box. $P = 0.0345$. mCherry: $N = 9$; hM3Dq: $N = 8$. (I, J) Schematic of the strategies that were used to express AAV-Retro-Cre in the MeA and AAV-DIO-mCherry or AAV-DIO-hM3Dq-mCherry in the VTA of C57BL/6 J mice. A cannula was then implanted into the MeA. (K) Heatmaps displaying the time spent in different regions of the open field arena (warmer colors indicate more time spent in a region). (L) Total distance traveled in the open field arena. $P = 0.3819$. mCherry: $N = 8$; hM4Di: $N = 9$. (M) Time spent in the center of the arena. $P = 0.0047$. mCherry: $N = 8$; hM4Di: $N = 9$. (N) Heatmaps displaying the time spent in different regions of the elevated plus maze (warmer colors indicate more time spent in a region). (O) Time spent in the open arms. $P = 0.0032$. mCherry: $N = 8$; hM4Di: $N = 9$. (P) Time spent in the light box. $P = 0.0012$. mCherry: $N = 8$; hM4Di: $N = 9$. N animal number. Statistical significance was determined via an unpaired t test. All of the data are presented as the means $\pm$ s.e.m.s. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; n.s., not significant. See Table EV1 for detailed statistics. Source data are available online for this figure.

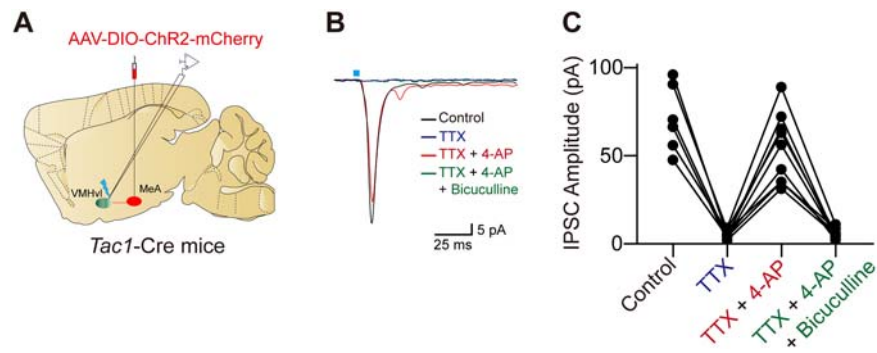


Figure EV5. Identifying the neurotransmission of MeATac1→VMHvl pathway.

(A) Schematic of optogenetic electrophysiological recording procedure. (B, C) Representative traces and quantification of the amplitudes. $n = 8$ cells from 4 animals. MeA medial amygdala. Source data are available online for this figure.

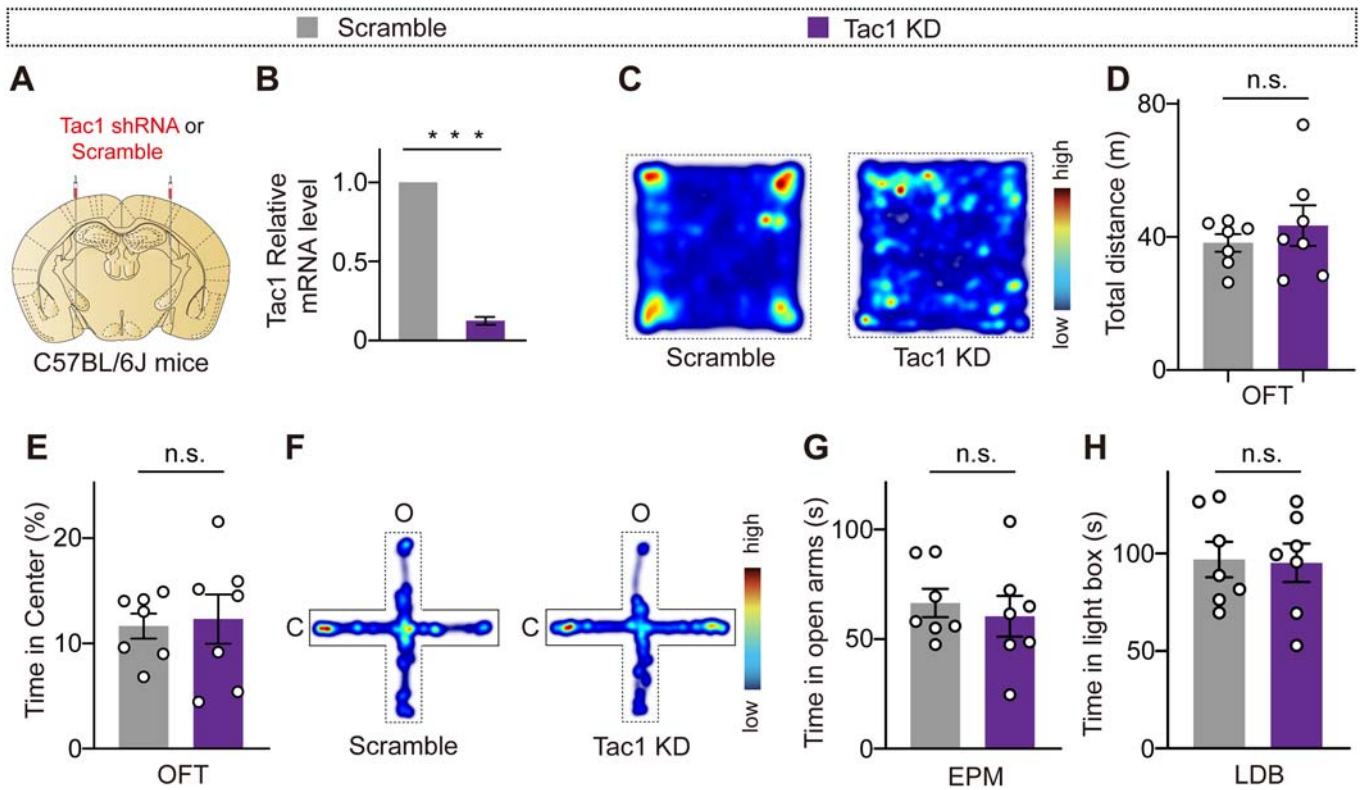


Figure EV6. Substance P does not affect anxiety-like behaviors in mice.

(A) Schematic of strategies used to express AAV-Tac1-shRNA and scramble shRNA in C57BL/6J mice. (B) Quantitative RT-PCR confirmed the reduction in Tac1 after knockdown. (C) Heatmaps display time spent in different regions of the open field arena (warmer colors indicate more time). (D) Total distance traveled in the open field arena. $P = 0.4478$. Scramble: $N = 7$; Knockdown: $N = 7$. (E) Time spent in the center of the open field arena. $P = 0.8068$. Scramble: $N = 7$; Knockdown: $N = 7$. (F) Heatmaps display time spent in different regions of the elevated plus maze (warmer colors indicate more time). (G) Time spent in the open arms. $P = 0.6055$. Scramble: $N = 7$; Knockdown: $N = 7$. (H) Time spent in the light box. $P = 0.8986$. Scramble: $N = 7$; Knockdown: $N = 7$. Statistical significance was determined using an unpaired t test. All data are means $\pm$ s.e.m. n.s., not significant. See Table EV1 for detailed statistics. Source data are available online for this figure.

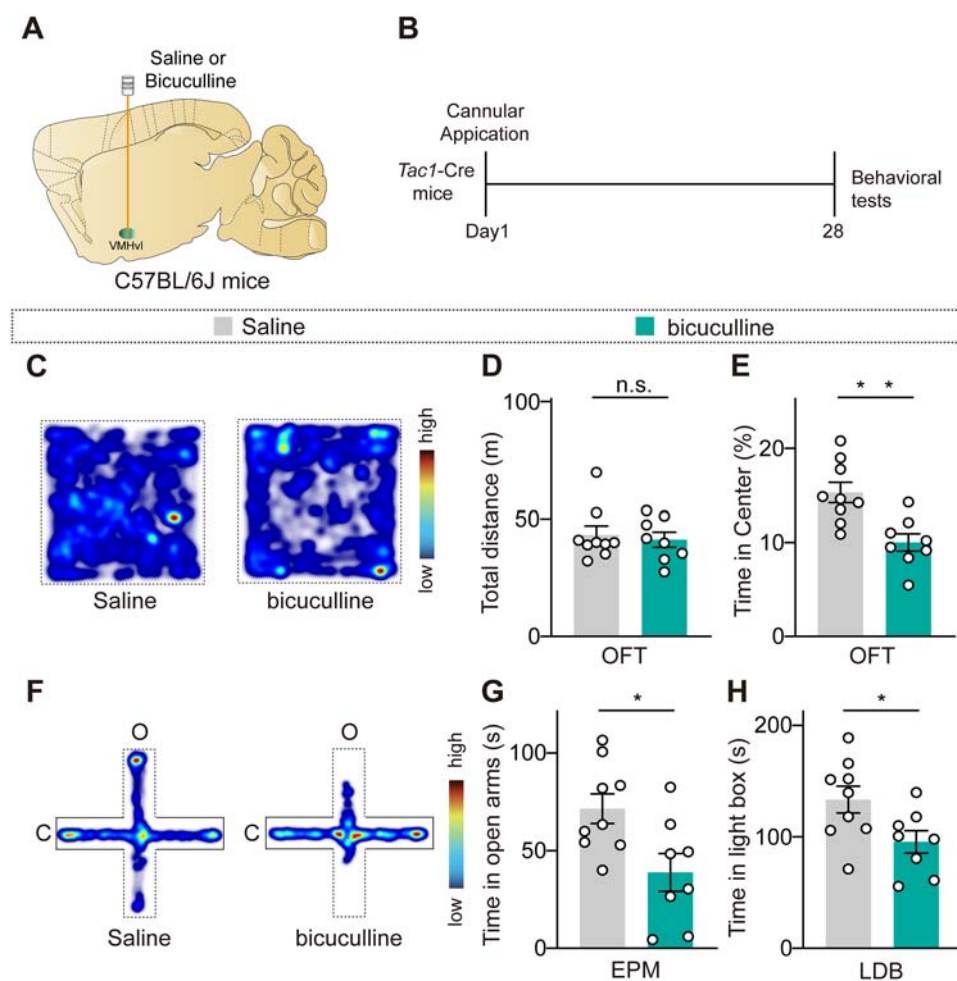


Figure EV7. GABA regulates anxiety-like behaviors in mice.

(A) Schematic of experimental strategies. (B) Experimental timeline. (C) Heatmaps display time spent in different regions of the open field arena (warmer colors indicate more time). (D) Total distance traveled in the open field arena. $P = 0.6904$. saline: $N = 9$; bicuculline: $N = 8$. (E) Time spent in the center of the arena. $P = 0.0024$. saline: $N = 9$; bicuculline: $N = 8$. (F) Heatmaps display time spent in different regions of the elevated plus maze (warmer colors indicate more time). (G) Time spent in the open arms. $P = 0.0171$. saline: $N = 9$; bicuculline: $N = 8$. (H) Time spent in the light box. $P = 0.0308$. saline: $N = 9$; bicuculline: $N = 8$. N animal number. Statistical significance was determined using an unpaired t test. All data are means $\pm$ s.e.m. * $P < 0.05$; ** $P < 0.01$; n.s., not significant. See Table EV1 for detailed statistics. Source data are available online for this figure.