

Expanded View Figures

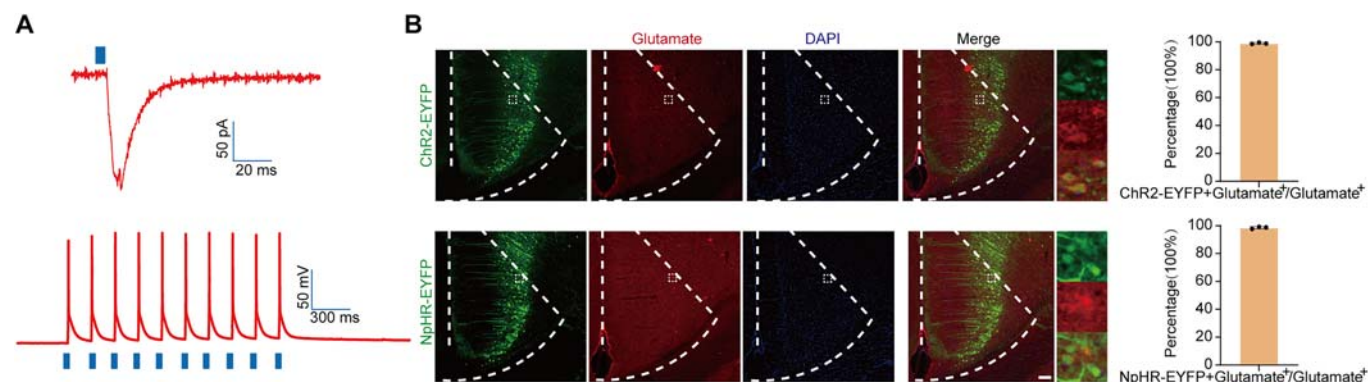


Figure EV1. Functional validation and cell-type specificity of optogenetic viral expression in ACC glutamatergic neurons.

(A) Ex vivo whole-cell patch-clamp recordings demonstrating the functional efficacy of ChR2 expressed in ACC glutamatergic neurons. Upper panel: Representative light-evoked inward photocurrent recorded from a ChR2-expressing neuron following brief blue light stimulation. Lower panel: Representative action potential firing induced by repetitive blue light pulses in a ChR2-expressing neuron. Scale bars: 50 pA, 20 ms (upper); 50 mV, 300 ms (lower). (B) Representative immunofluorescence images showing colocalization of ChR2-EYFP (upper panels) or NpHR-EYFP (lower panels) with the glutamatergic neuronal marker Glutamate in the ACC ($n = 3$ mice per group). Scale bar = 100 μ m. The data are represented as mean $\pm$ SEM.

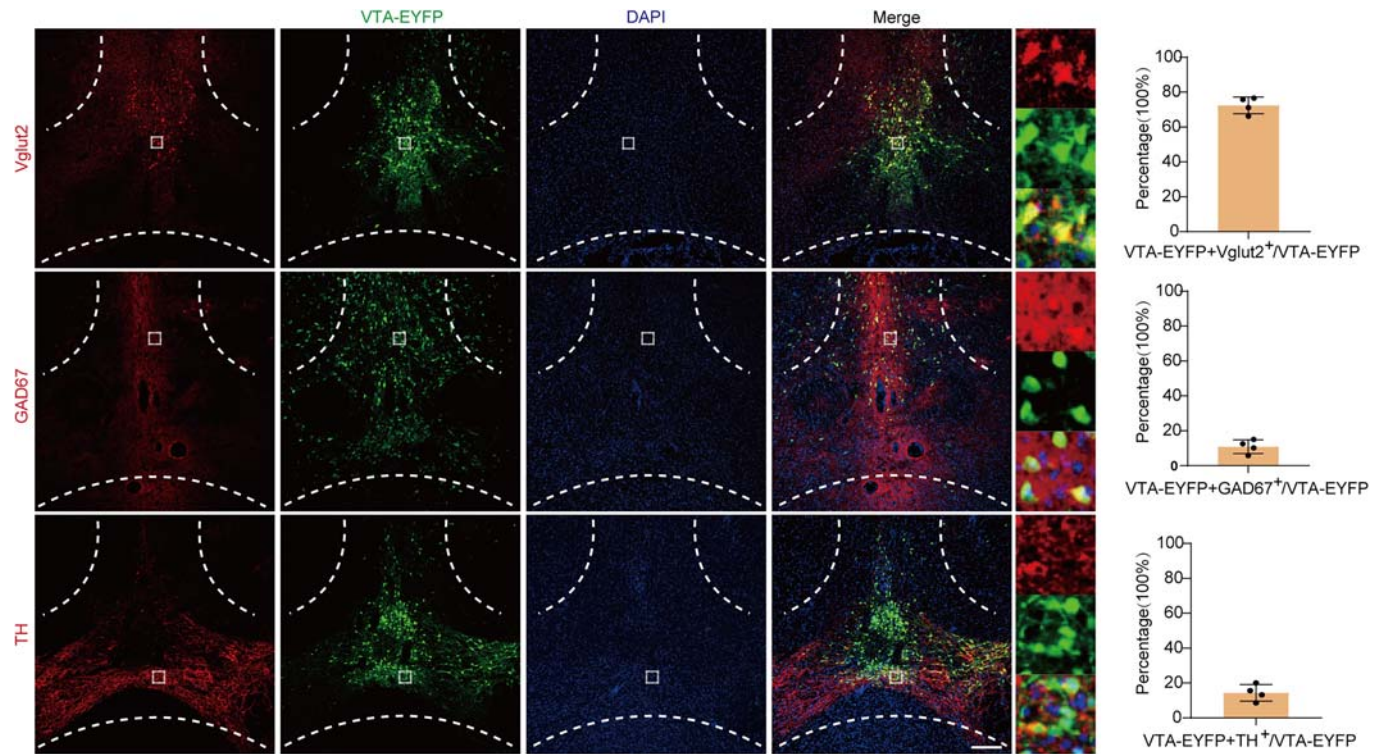


Figure EV2. Identification of the major postsynaptic neuronal population receiving ACC glutamatergic projections in the VTA.

A dual-virus anterograde tracing strategy was used to label ACC-derived EYFP-positive projections in the VTA, followed by immunofluorescence colocalization analysis with markers for the three major neuronal populations in the VTA: VGLUT2 (glutamatergic neurons, upper panels), GAD67 (GABAergic neurons, middle panels), and TH (dopaminergic neurons, lower panels) ($n = 4$ mice per group). Scale bar = 100 μ m. The data are represented as mean $\pm$ SEM.

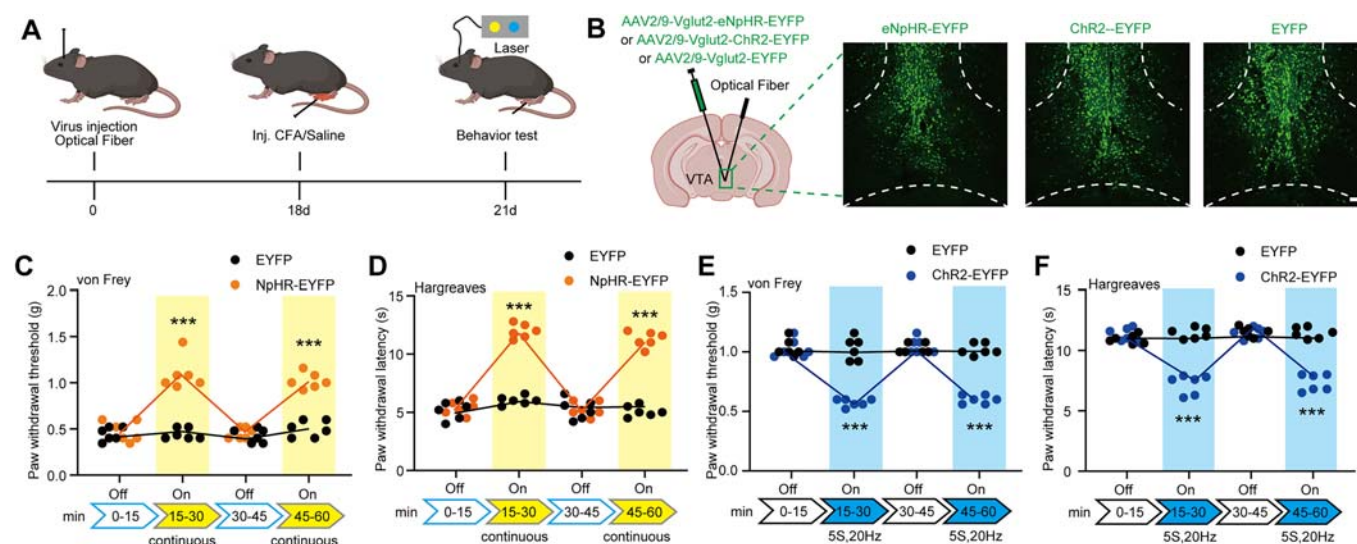


Figure EV3. Optogenetic manipulation of VTA glutamatergic neurons alters pain behavior.

(A) Experimental design for optogenetic manipulation of VTA glutamatergic neurons. (B) Representative images showing expression of eNpHR or ChR2 in VTA glutamatergic neurons. (C, D) Optogenetic inhibition of VTA glutamatergic neurons significantly increased PWT and PWL in CFA mice ($***P < 0.0001$, two-way ANOVA followed by Sidak's multiple-comparisons test, $n = 6$ mice per group). (E, F) Optogenetic activation of VTA glutamatergic neurons decreased PWT and PWL in CON mice ($***P < 0.0001$, two-way ANOVA followed by Sidak's multiple-comparisons test, $n = 6$ mice per group). Scale bar = 100 μ m. The data are represented as mean $\pm$ SEM.



Figure EV4. AK7 knockdown in ACC glutamatergic neurons attenuates CFA-induced mechanical and thermal hypersensitivity in female mice.

(A) Schematic illustration of stereotaxic viral delivery for selective knockdown of AK7 in ACC glutamatergic neurons of female mice. (B, C) Knockdown of AK7 significantly increased PWT and PWL in CFA mice ($***P < 0.0001$, one-way ANOVA with Tukey's multiple-comparisons test, $n = 6$ mice per group). The data are represented as mean $\pm$ SEM.