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Supplementary Materials for

An orbitofrontal cortex–anterior insular cortex circuit gates compulsive cocaine use

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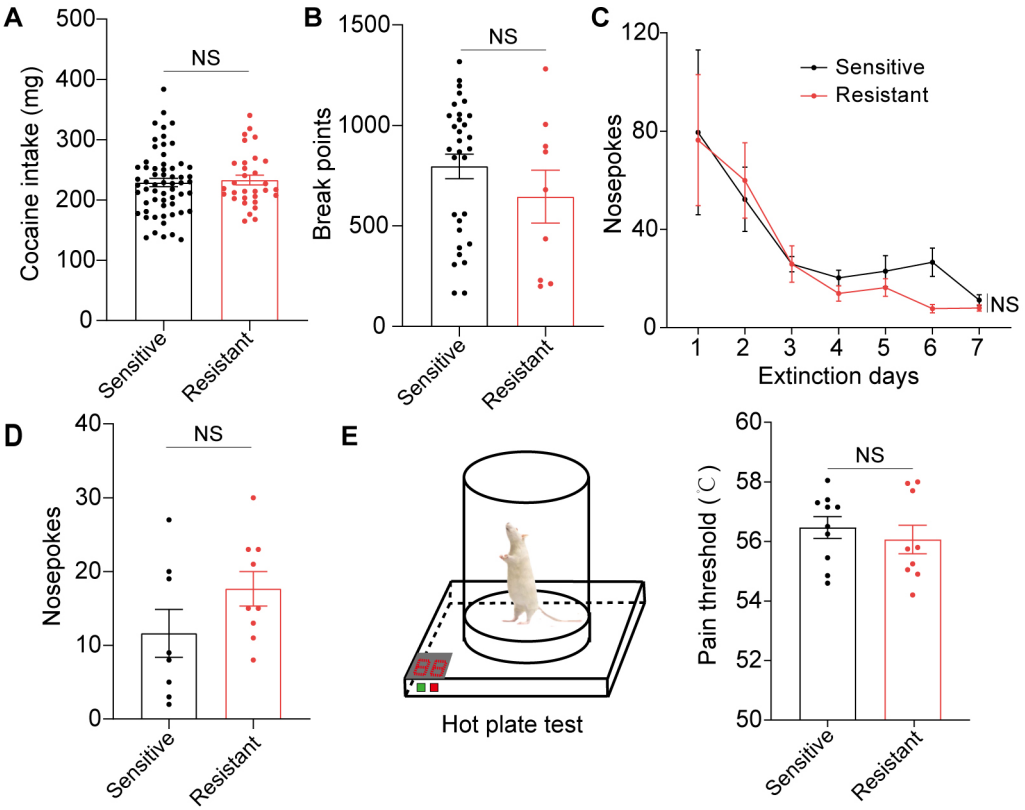

Figure S1. Similar cocaine self-administration, break points, cue-induced reinstatement and pain sensitivity between punishment-sensitive and punishment-resistant rats. (**A**) Total cocaine intake obtained from acquisition sessions. Unpaired *t* test, $t_{89} = 0.37$, $P = 0.709$, n = 60 and 31, respectively. (**B**) Break points obtained from sensitive and resistant rats. Unpaired *t* test, $t_{38} = 1.14$, $P = 0.263$, n = 31 and 9, respectively. (**C**) Nosepokes obtained from extinction sessions. Two-way ANOVA, $F_{1,15} = 0.25$, $P = 0.624$, post hoc analysis, not significant for sensitive vs resistant, n = 8 and 9, respectively. (**D**) Cue-induced reinstatement test. Unpaired *t* test, $t_{15} = 1.53$, $P = 0.146$, n = 8 and 9, respectively. (**E**) Pain threshold obtained from sensitive and resistant rats in hot plate test. Unpaired *t* test, $t_{17} = 0.67$, $P = 0.510$, n = 10 and 9, respectively. NS, not significant. Data are presented as mean values ± SEMs.

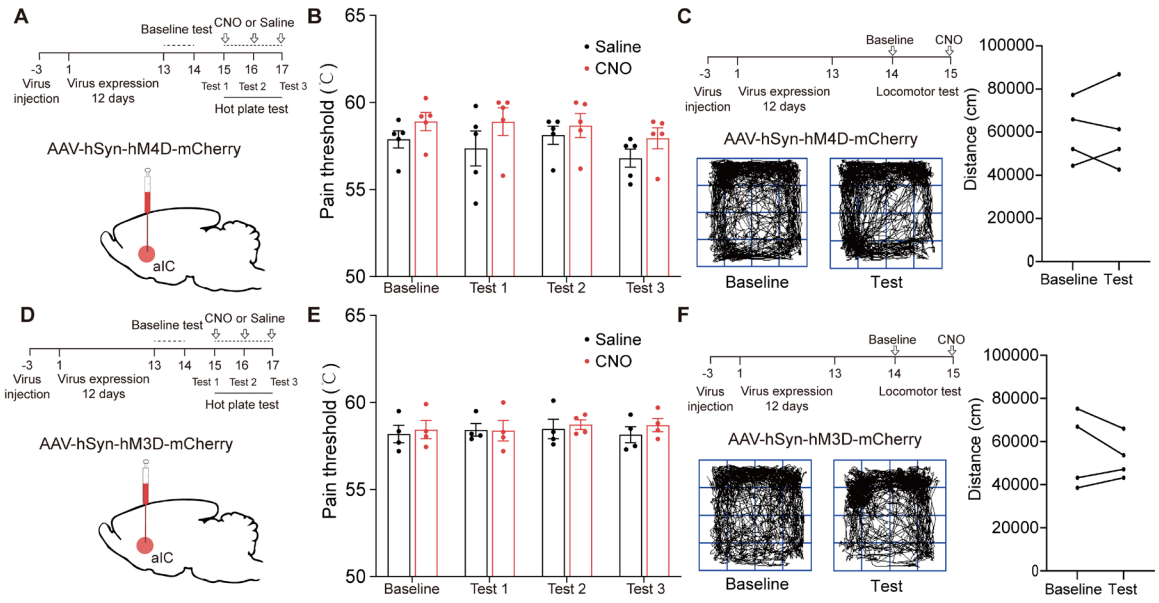

Figure S2. Pain threshold and locomotor activity were not affected by modulation of aIC activity. (**A**) Experimental timeline and schematic of viral infusion to analyze pain threshold after chemogenetic inhibition of aIC. (**B**) Pain threshold obtained from hot plate test after virus expression. Two-way ANOVA, $F_{1,8}$ = 1.71, P = 0.227, post hoc analysis, not significant for saline vs CNO group, n = 5 in each group. (**C**) Locomotor activity was not affected by hM4D-mediated inhibition of aIC. Paired t test, t_3 = 0.17, P = 0.8765, n = 4 in each group. (**D**) Experimental timeline and schematic of viral infusion to analyze pain threshold after chemogenetic activation of aIC. (**E**) Pain threshold obtained from hot plate test after virus expression. Two-way ANOVA, $F_{1,6}$ = 0.16, P = 0.702, post hoc analysis, not significant for saline vs CNO group, n = 4 in each group. (**F**) Locomotor activity was not affected by hM3D-mediated activation of aIC. Paired t test, t_3 = 0.76, P = 0.5018, n = 4 in each group. Data are presented as mean values ± SEMs.

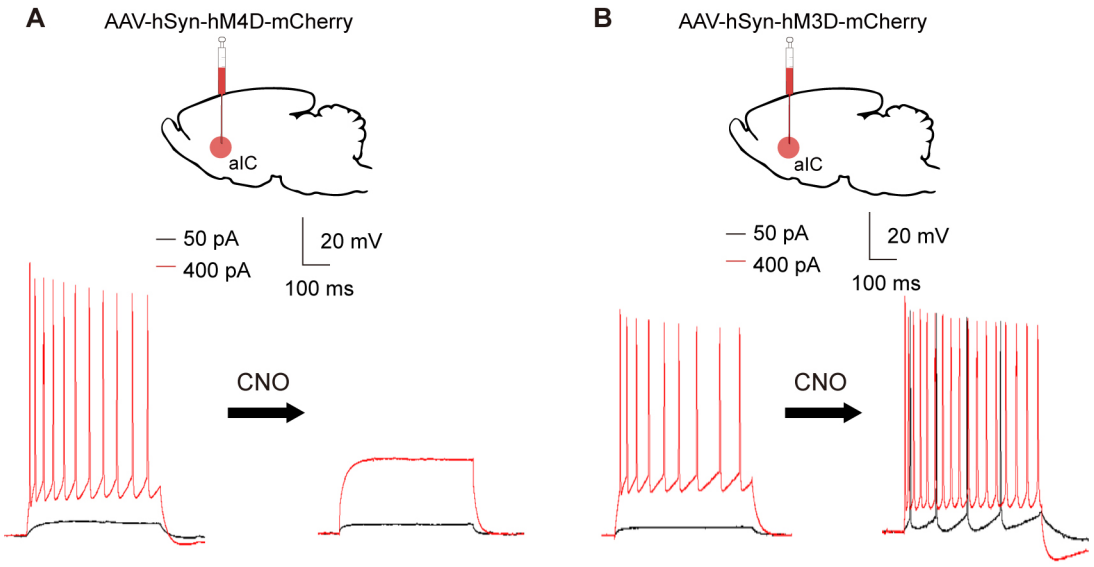

Figure S3. Electrophysiology-based verification of inhibitory and excitatory DREADD. (**A**) Inhibited hM4D-expressing aIC neurons 30 minutes after CNO (0.1 mM with ACSF) application and sample trace of evoked APs before (left) and after (right) CNO application. (**B**) Activated hM3D-expressing aIC neurons 30 minutes after CNO (0.1 mM with ACSF) application and sample trace of evoked APs before (left) and after (right) CNO application.

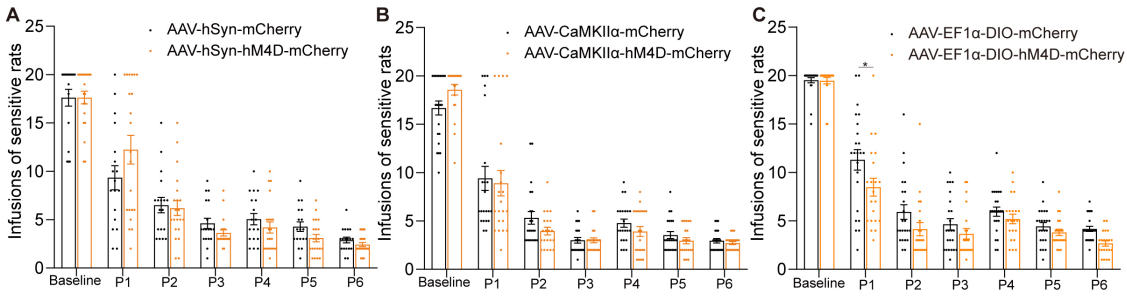

Figure S4. Inhibition of aIC neurons, aIC glutamatergic neurons or OFC-aIC projection did not affect cocaine use behavior in sensitive rats. (**A**) Cocaine infusions obtained from sensitive rats in AAV-mCherry and AAV-hM4D group after inhibition of aIC. Two-way ANOVA, $F_{1,37}$ = 0.05, P = 0.827, post hoc analysis, not significant for P1 to P6, n = 18 and 21, respectively. (**B**) Cocaine infusions obtained from sensitive rats in AAV-mCherry and AAV-hM4D group after inhibition of aIC glutamatergic neurons. Two-way ANOVA, $F_{1,41}$ = 0.28, P = 0.597, post hoc analysis, not significant for P1 to P6, n = 22 and 21, respectively. (**C**) Cocaine infusions obtained from sensitive rats in AAV-mCherry and AAV-hM4D group after inhibition of OFC-aIC projection. Two-way ANOVA, $F_{1,42}$ = 5.76, P = 0.021, post hoc analysis, $^{*}P$ = 0.013 for P1, not significant for P2 to P6, n = 23 and 21, respectively. Data are presented as mean values ± SEMs.

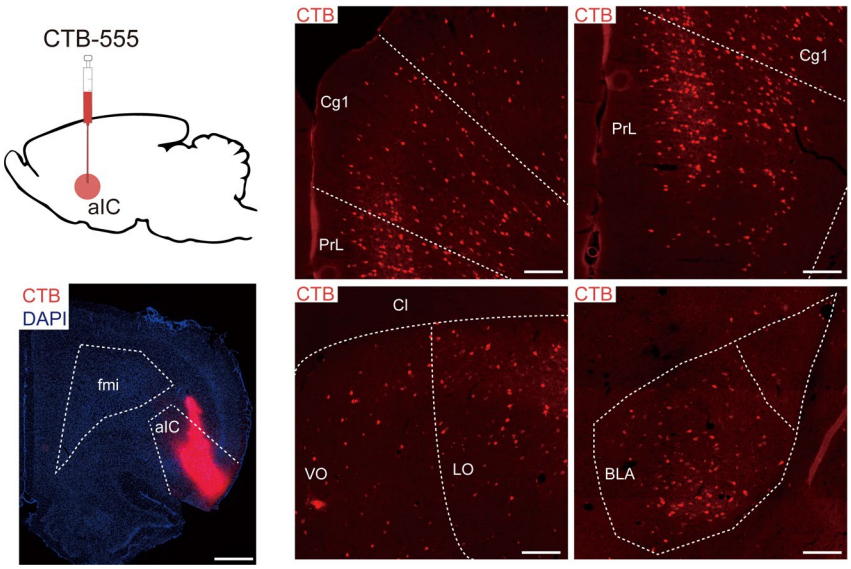

Figure S5. Retrograde tracing of aIC neurons by CTB-555. The aIC mainly received inputs from prelimbic cortex (PrL), cg1, ventral OFC (VO), lateral OFC (LO) and basolateral amygdala (BLA). Scale bars, 1 mm (left) and 150 μm (right).

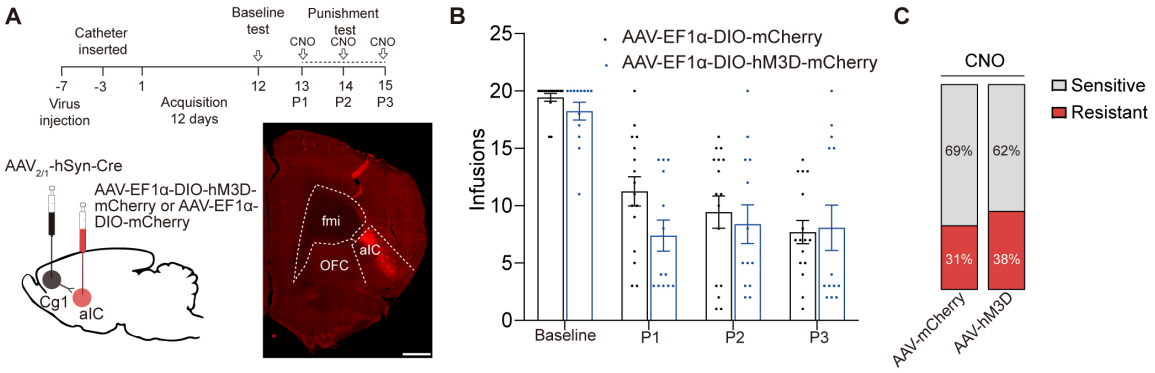

Figure S6. Activation of the Cg1-aIC circuit did not affect compulsive cocaine use. (**A**) Experimental timeline and schematic of viral infusion to analyze cocaine use behaviors after activation of Cg1-aIC circuit. (**B**) Cocaine infusions obtained from AAV-mCherry and AAV-hM3D group. Two-way ANOVA, $F_{1,27} = 0.95$, $P = 0.338$, post hoc analysis, not significant for AAV-mCherry vs AAV-hM3D, $n = 16$ and 13, respectively. (**C**) Percentage of sensitive and resistant rats after activation of Cg1-aIC circuit. Fisher's exact test, $P = 0.714$. Data are presented as mean values ± SEMs.

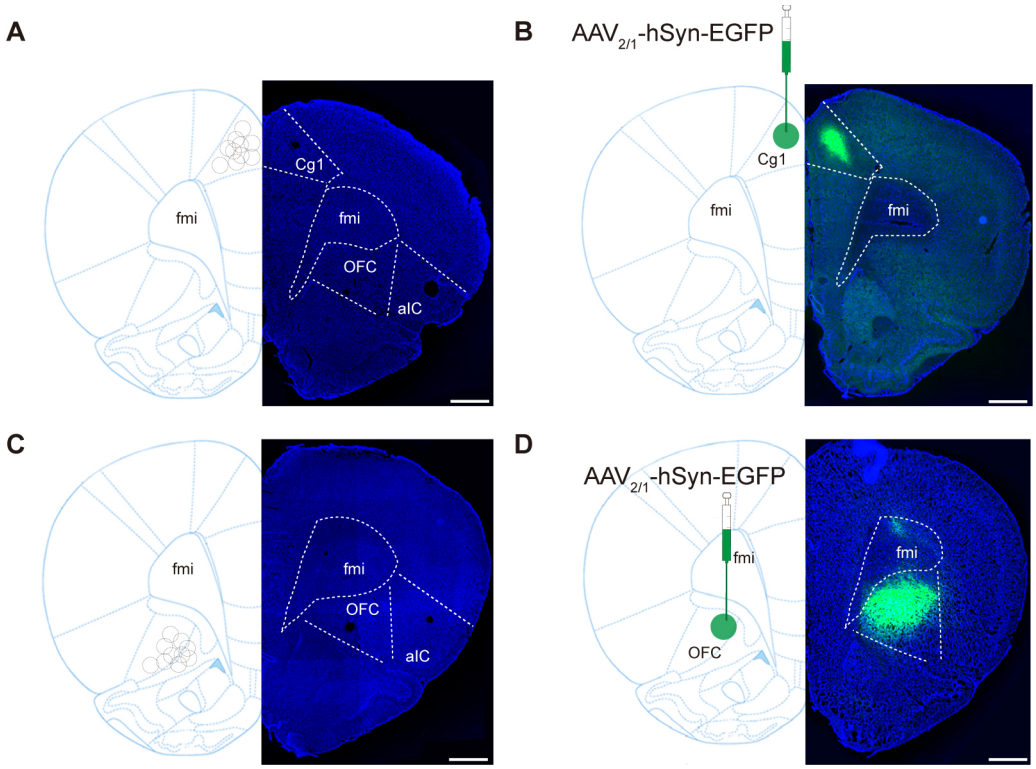

Figure S7. Infusion sites of virus. (**A**) Representative viral injection sites of Cg1-aIC labeling experiment (right) and schematic diagram of virus infusion sites in Cg1 from 10 rats (left). Scale bar, 1 mm. (**B**) The viral expression in Cg1 following infusion of $AAV_{1/2}$-hSyn-EGFP. Scale bar, 1 mm. (**C**) Representative viral injection sites of OFC-aIC labeling experiment (right) and schematic diagram of virus infusion sites in OFC from 10 rats (left). Scale bar, 1 mm. (**D**) The viral expression in OFC following infusion of $AAV_{1/2}$-hSyn-EGFP. Scale bar, 1 mm.

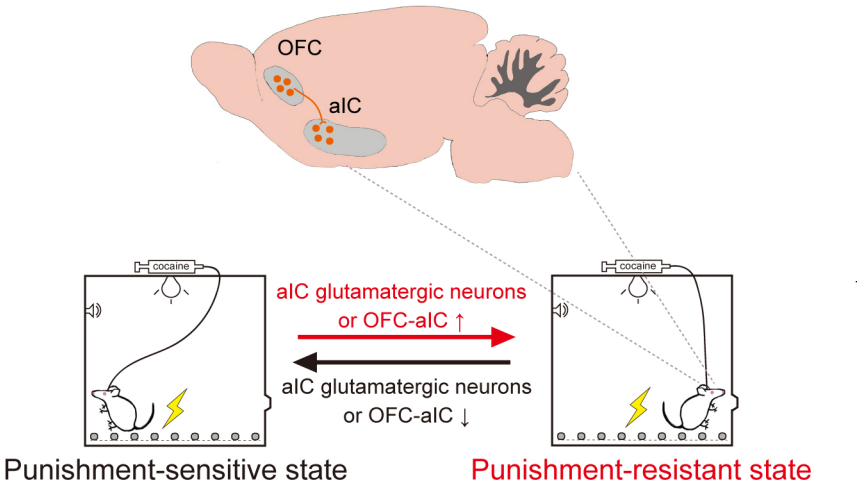

Figure S8. Summary scheme. Activation of aIC glutamatergic neurons or OFC-aIC circuit drove rats from a punishment-sensitive state to a punishment-resistant state, in which rats showed increased compulsive cocaine use behavior, while inhibition of these targets reversed this effect.