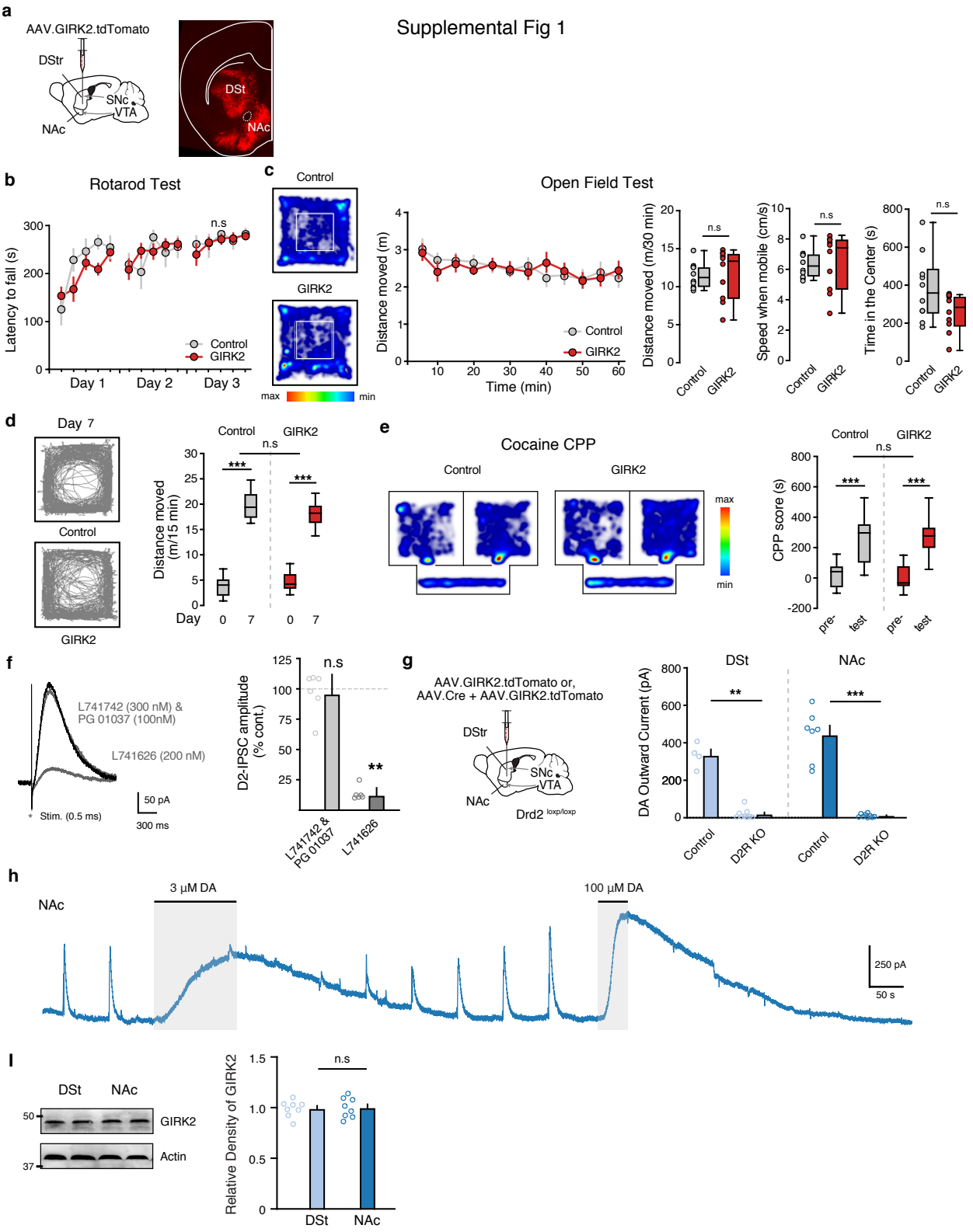
Cocaine Shifts Dopamine D2 Receptor Sensitivity to Gate Conditioned Behaviors

Sheng Gong [1,2], Nicholas Fayette [3], Jasper A Heinsbroek [3] & Christopher P Ford * [1]

1 - Department of Pharmacology, University of Colorado School of Medicine, Anschutz Medical Campus, Aurora, CO 80045, USA

2 - Department of Physiology and Biophysics, Case Western Reserve University, Cleveland, OH 44106, USA

3 - Department of Anesthesiology, University of Colorado School of Medicine, Anschutz Medical Campus, Aurora, CO 80045, USA



Supplemental Figure 1 (associated with Figure 1): GIRK2 controls and D2R controls.

a - tdTomato fluorescence in the DSt and NAc three weeks following injection of AAV.GIRK2.tdTomato.

b - Latency to fall measurements on the rotarod test of control wt mice and mice injected in the DSt and NAc with AAV.GIRK2.tdTomato (WT: N = 7; GIRK: N = 10; two-way RM ANOVA, subject (wt or GIRK) x time interaction $F_{14, 210} = 1.484$, $p > 0.05$; effect of subject $F_{1,15} = 0.426$, $p > 0.05$; effect of time $F_{14, 210} = 9.603$, $p < 0.001$).

c - Open field locomotor test comparing control wt mice and mice injected in the DSt and NAc with AAV.GIRK2.tdTomato (WT: N = 9; GIRK: N = 9; $p > 0.05$, Mann Whitney test).

d - Locomotor behavioral sensitization test comparing control wt mice and mice injected in the DSt and NAc with AAV.GIRK2.tdTomato (cocaine: 20 mg/kg; i.p. 7d) (wt: N = 11, $p < 0.001$; GIRK: N = 12, $p < 0.001$; Wilcoxon matched-paired signed rank test; wt vs. GIRK: two-way RM ANOVA, subject (wt or GIRK) x treatment (day 0 or 7) interaction $F_{1,21} = 0.846$, $p > 0.05$; effect of subject $F_{1,21} = 0.002$, $p > 0.05$; effect of treatment $F_{1,21} = 54.96$, $p < 0.001$).

e - Cocaine CPP (15 mg/kg; i.p. 5d) test comparing control wt mice and mice injected in the DSt and NAc with AAV.GIRK2.tdTomato (wt: N = 13, $p < 0.001$; GIRK: N = 12, $p < 0.001$; Wilcoxon matched-paired signed rank test; wt vs. GIRK: two-way RM ANOVA, subject (wt or GIRK) x treatment (pre or test) interaction $F_{1,23} = 0.278$, $p > 0.05$; effect of subject $F_{1,23} = 0.0002$, $p > 0.05$; effect of treatment $F_{1,23} = 66.20$, $p < 0.001$).

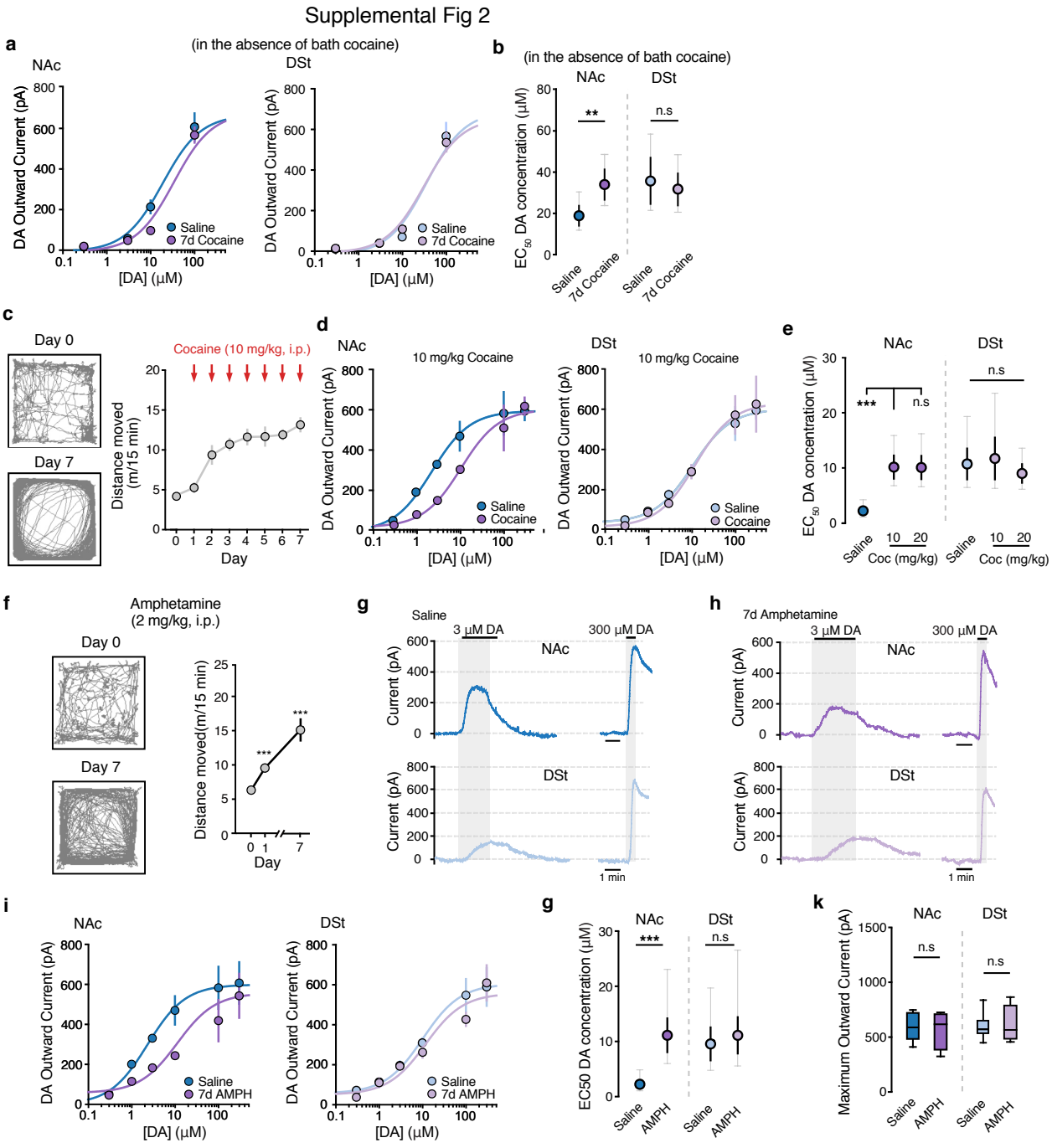
f - Representative D2-IPSCs from a GIRK2^{+} D2-MSN in control (black) and in the presence of the D4R antagonist L-741,742 (300 nM) & the D3R antagonist PG 01037 (100 nM) and in the presence of the D2R antagonist L-741,626 (200 nM). Right: summary data (L-741,742 & PG 01037: n=6, N = 3; $p > 0.05$; L-741,626: n = 6, N = 3; $p < 0.01$; Mann Whitney test).

g - Quantification of D2R mediated outward currents following bath application of 30 μM dopamine from GIRK2^{+} D2-MSNs from control (Drd2$^{flx/flx}$) and D2R KO mice (Drd2$^{flx/flx}$ + AAV.Cre) mice (DSt: Control: n = 4, N = 4; D2R KD: n = 9, N = 4; $p < 0.01$; NAc: Control: n = 7, N = 4; D2R KD: n = 9, N = 4; $p < 0.001$; Mann Whitney test).

h - Representative full trace of bath application of dopamine from a NAc D2-MSN.

i - Representative immunoblot images and quantification of GIRK2 expression levels in the NAc and DSt (N = 8, $p > 0.05$; Mann Whitney test)

Summary data are mean ± SEM. ns = $p > 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.



Supplemental Figure 2 (associated with Figure 1): Dopamine controls, effect of low dose cocaine and effect of amphetamine.

a - Dopamine concentration-response relationships for D2R mediated outward GIRK2 currents from D2-MSNs in the DSt and NAc from 7d saline or 7d cocaine treated mice in the absence of the cocaine in the bath.

b - EC_{50} values from (a) (NAc: 7d saline: n = 23, N = 5; 7d cocaine: n = 25, N = 5; t_8 = 3.824, $p < 0.01$; DSt: 7d saline: n = 25, N = 5; 7d cocaine: n = 18, N = 5; t_8 = 0.690, $p > 0.05$, two tailed unpaired t-test).

c - Locomotor behavioral sensitization induced by a low dose of cocaine (10 mg/kg; i.p. 7d) (N= 8, one-way ANOVA, cocaine effect $F_{2.397,\ 16.78} = 13.20$, $p < 0.001$).

d - Dopamine concentration-response relationships for D2R mediated outward GIRK2 currents from D2-MSNs in the DSt and NAc from low dose (10 mg/kg, i.p., 7d) cocaine treated mice.

e - EC_{50} values from (d) (NAc: n = 48, N = 7; $F_{2,19} = 47.73$, $p < 0.001$; DSt: n = 46, N = 7; $F_{2,19} = 3.02$, $p > 0.05$; one-way ANOVA; Sidak's post-test).

f - Locomotor behavioral sensitization induced by amphetamine (2 mg/kg; i.p. 7d) (N = 11, $F_{2,30} = 22.92$, $p < 0.001$, one-way ANOVA; Tukey's post-test).

g-h - Representative traces of D2R mediated outward currents following bath application of dopamine (DA) from the NAc and DSt comparing saline treated and amphetamine treated mice. All recordings were performed in the presence of cocaine (10 μM) to block uptake. Evoked D2-IPSCs blanked for clarity.

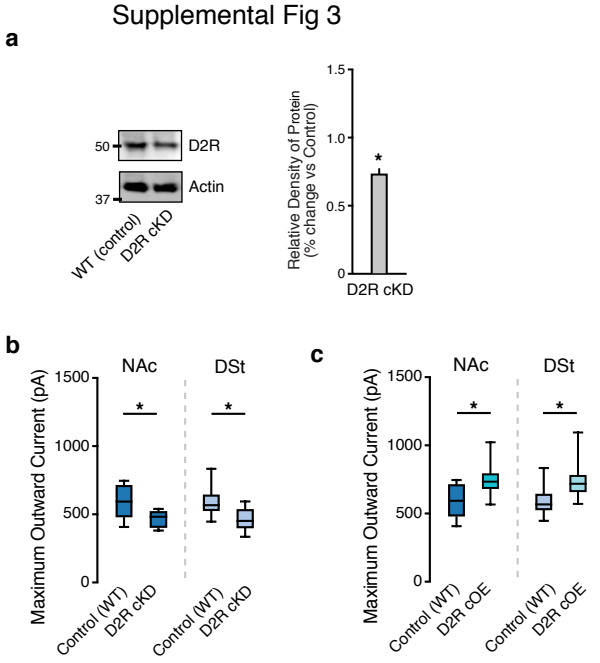
i - Dopamine concentration-response relationships for D2R mediated outward GIRK2 currents from D2-MSNs in the DSt and NAc in saline and amphetamine treated mice.

g - EC_{50} values from (i) (AMPH NAc: n = 51, N = 8; $t_{13} = 7.632$, $p < 0.001$; AMPH DSt: n = 45, N = 8; $t_{13} = 0.018$, $p = 0.986$; two tailed unpaired t-test).

k - Maximum outward currents evoked by 300 μM dopamine from (l-m) (AMPH NAc: n = 4, $t_{10} = 0.281$, $p = 0.785$; AMPH DSt: n = 5; $t_{11} = 0.008$, $p = 0.993$; two tailed unpaired t-test).

Summary data are mean ± SEM (grey traces represent 95% confidence intervals) or boxplots. ns = $p > 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

Supplemental Fig 3



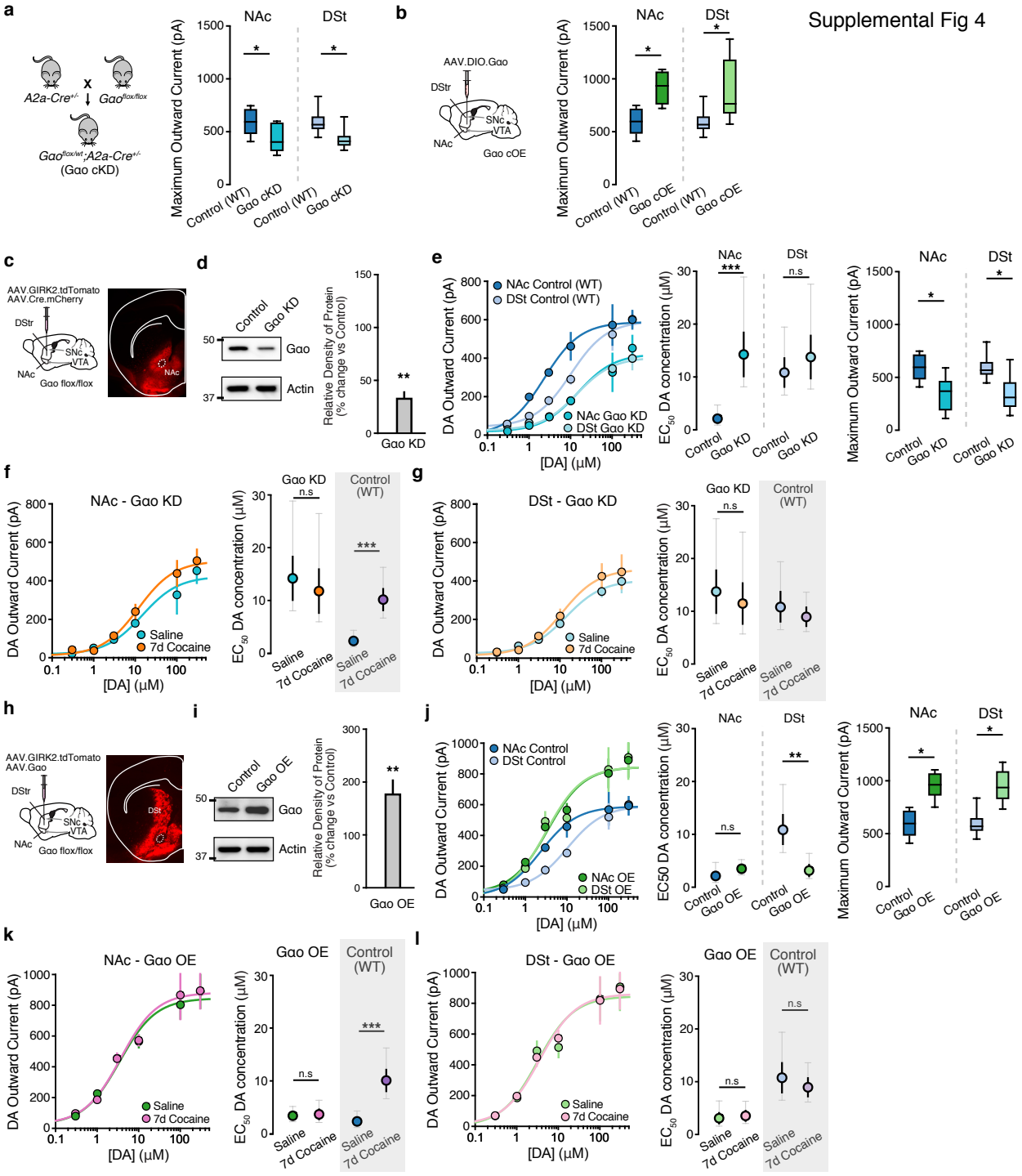
Supplemental Figure 3 (associated with Figure 2): D2R expression in D2R cKD mice and summary of maximum outward currents in D2R cKD and OE groups.

a - Representative immunoblot images and quantification of striatal D2R expression levels comparing wt and D2R cKD mice (N = 4, $p < 0.05$, Mann Whitney test).

b - Maximum outward currents evoked by 300 μM dopamine from comparing control and D2R cKD mice (NAc D2R cKD: n = 8, $p < 0.05$; DSt D2R cKD: n = 8, $p < 0.05$; Mann Whitney test).

c - Maximum outward currents evoked by 300 μM dopamine from comparing control and D2R cOE mice (NAc D2R cKD: n = 8, $p < 0.05$; DSt D2R cKD: n = 8, $p < 0.05$; Mann Whitney test).

Summary data are boxplots. * = $p < 0.05$.



Supplemental Figure 4 (associated with Figure 4): Maximum outward currents and results following non-cell type selective global viral mediated knockdown and overexpression of Gαo

a - Maximum outward currents evoked by 300 μM dopamine comparing control (WT) and Gαo cKD groups (n= 7-8, $p < 0.05$, Mann Whitney test).

b - Maximum outward currents evoked by 300 μM dopamine comparing control (WT) and Gαo cOE groups (n = 5, $p < 0.01$, Mann Whitney test).

c - tdTomato and mCherry fluorescence following injection of AAV.GIRK2.tdTomato and AAV.Cre.mCherry in the NAc in resulting Gαo KD mice (*$G\alpha o^{flx/flx}$*).

d - Representative western immunoblot and quantification of striatal Gαo levels comparing control and Gαo KD mice (N = 5, $p < 0.01$, Mann-Whitney test).

e - Dopamine D2R dose response curves, EC_{50} values (NAc Gαo KD: n = 46, N = 8; $t_{13} = 8.578$, $p < 0.001$; DSt Gαo KD: n = 44, N = 8; $t_{13} = 1.355$, $p = 0.199$; two tailed unpaired t-test), and maximum outward currents (NAc Gαo KD: n = 6, $p < 0.01$; DSt Gαo KD: n = 7, $p < 0.05$; Mann Whitney test) from control (WT) and Gαo KD groups.

f - g - Dopamine D2R dose response curves and EC_{50} values from NAc (Gαo KD cocaine: n = 37, N = 9, $t_{15} = 1.595$, $p > 0.05$; two tailed unpaired t-test) and DSt (Gαo KD cocaine: n = 39, N = 9, $t_{15} = 0.707$, $p > 0.05$; two tailed unpaired t-test) D2-MSNs from saline and 7-day cocaine treated $G\alpha_o$ KD groups. Control data from wild-type mice following 7-day saline and 7-day cocaine treatment taken from Figure 1 is reshown in boxes in grey.

h - tdTomato fluorescence following injection of AAV.GIRK2.tdTomato and AAV.Gao in the NAc and DSt in resulting Gαo OE mice (*$G\alpha o^{flx/flx}$*).

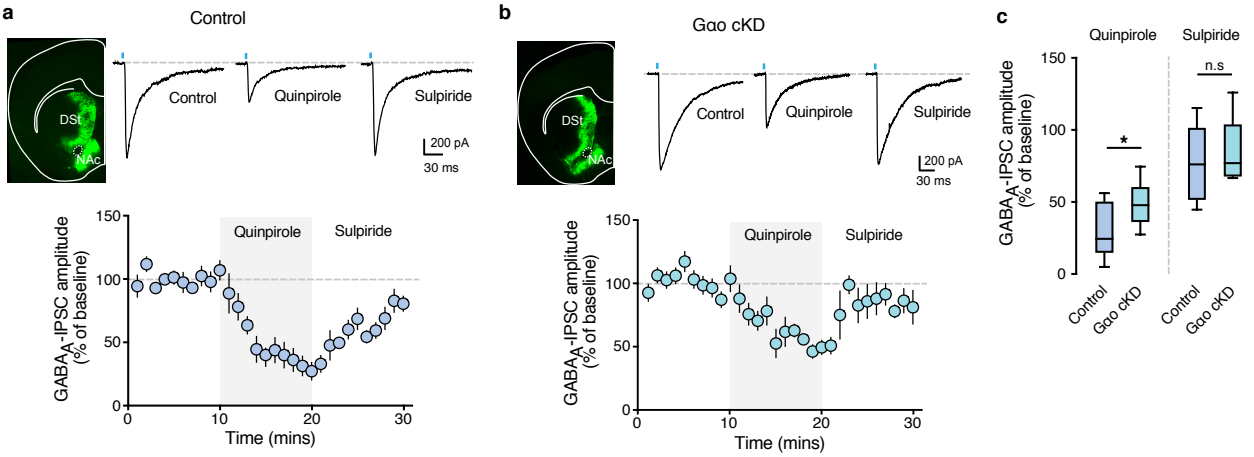
i - Representative western immunoblot and quantification of striatal Gαo levels comparing control and Gαo OE mice (N = 5, $p < 0.01$, Mann-Whitney test).

j - Dopamine D2R dose response curves, EC_{50} values (NAc Gαo OE: n = 52, N = 7, $t_{12} = 2.052$, $p > 0.05$; DSt Gαo OE: n = 54, N = 7, $t_{12} = 5.996$, $p < 0.001$; two tailed unpaired t-test), and maximum outward currents (NAc Gαo OE: n = 5, $p < 0.01$; DSt Gαo KD: n = 5, $p < 0.05$; Mann Whitney test) from control (WT) and Gαo OE groups.

k - l - Dopamine D2R dose response curves and EC_{50} values from NAc (Gαo OE cocaine: n = 54, N = 8; $t_{13} = 1.159$, $p > 0.05$; two tailed unpaired t-test) and DSt (Gαo OE cocaine: n = 57, N = 8; $t_{13} = 0.461$, $p > 0.05$; two tailed unpaired t-test) D2-MSNs from saline and 7-day cocaine treated $G\alpha_o$ OE groups. Control data from wild-type mice following 7-day saline and 7-day cocaine treatment taken from Figure 1 is reshown in boxes in grey.

Summary data are mean ± SEM (grey traces represent 95% confidence intervals) or boxplots. ns = $p > 0.05$, * = $p < 0.05$ ** = $p < 0.01$, *** = $p < 0.001$.

Supplemental Fig 5



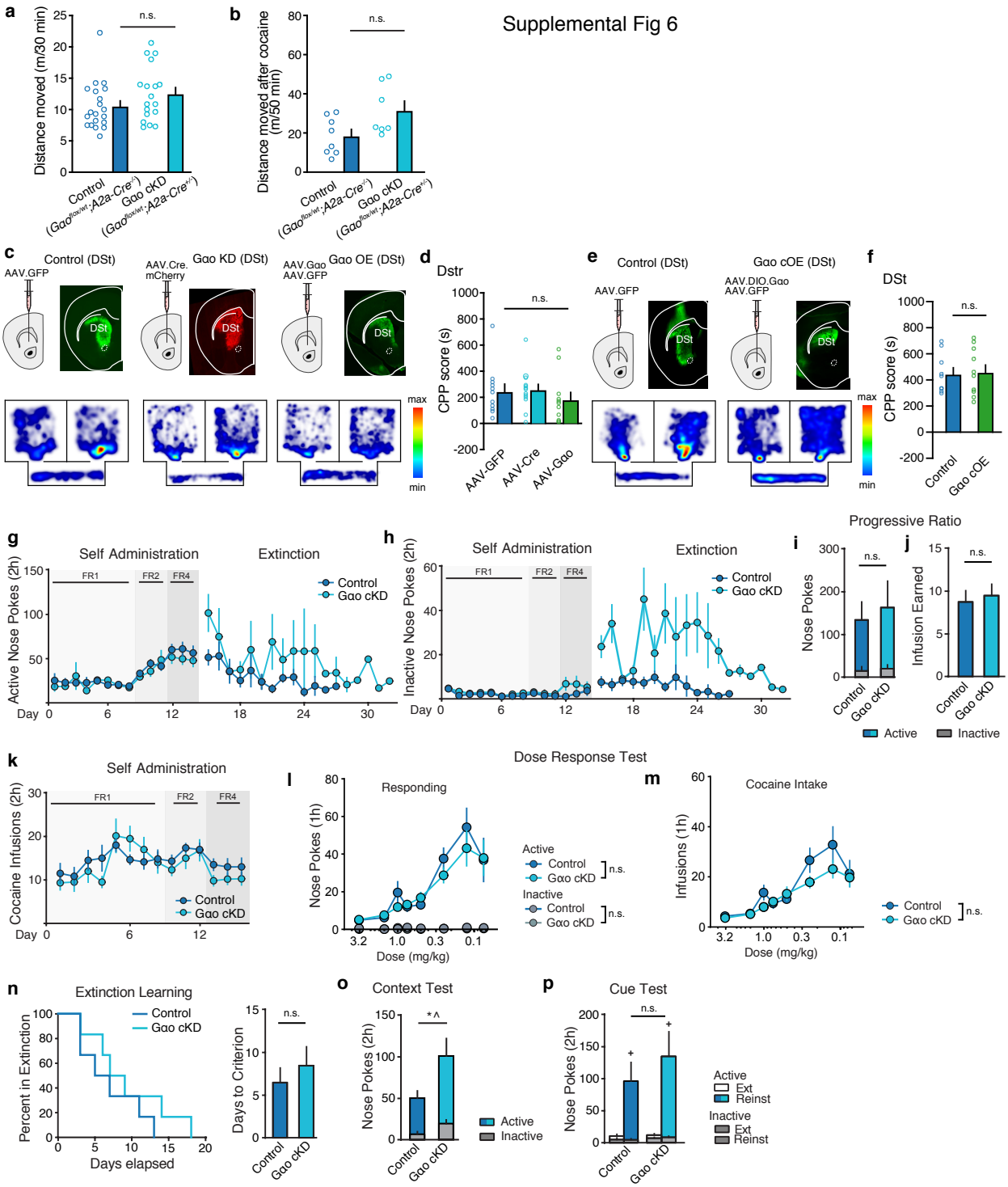
Supplemental Figure 5 (associated with Figure 5): Quinpirole D2-agonist controls

a - EYFP fluorescence in the NAc and DSt of control (WT) mice following AAV.EF1a.DIO.hChR2(H134R)-EYFP injection, representative $GABA_A$-IPSCs and quantification following the bath application of quinpirole and the reversal by sulpiride.

b - EYFP fluorescence in the NAc and DSt of Gαo cKD mice following AAV.EF1a.DIO.hChR2(H134R)-EYFP injection, representative $GABA_A$-IPSCs and quantification following the bath application of quinpirole and the reversal by sulpiride.

c - Quantification of bath application of quinpirole and sulpiride effect on $GABA_A$-IPSCs from (a-b) (n= 8, $p < 0.05$, Mann-Whitney test)

Summary data are boxplots. ns = $p > 0.05$, * = $p < 0.05$.



Supplemental Figure 6 (associated with Figure 6): DSt CPP data and supplemental IVSA data

a - Open field locomotor test comparing control (Gαo$^{flox/wt}$;A2a-Cre$^{-/-}$) mice and Gαo cKD (Gαo$^{flox/wt}$;A2a-Cre$^{+/-}$) mice (N = 18-20, $p > 0.05$; Mann-Whitney test).

b - Locomotor test in response to acute cocaine (20 mg/kg; i.p.) comparing control ($G\alpha o^{flox/wt}$;A2a-$Cre^{-/-}$) mice and Gao cKD ($G\alpha o^{flox/wt}$;A2a-$Cre^{+/-}$) mice (N = 7-8, $p > 0.05$; Mann-Whitney test)

c - Representative GFP and mCherry fluorescence and heat map illustrating time spent in chambers during CPP testing for control, $G\alpha_o$ DSt KD and $G\alpha_o$ DSt OE groups.

d - Quantification of CPP scores from each group (N = 11-13, $F_{2,32} = 0.620$, $p = 0.545$, one-way ANOVA; $p > 0.05$, Tukey's post-test).

e - Representative GFP fluorescence and heat map illustrating time spent in chambers during CPP testing for control, and $G\alpha_o$ DSt cOE groups.

f - Quantification of CPP scores from each group (N = 9-10, $p > 0.05$, Mann Whitney test).

g - Active port nose pokes during FR1, FR2, FR4 and extinction in control and Gαo cKD groups.

h - Inactive port nose pokes during FR1, FR2, FR4 and extinction in control and Gαo cKD groups.

i - Quantification of nose pokes during progressive ratio (active: N = 4, $t_6 = 0.398$, $p = 0.705$; inactive: N = 4, $t_6 = 0.458$, $p = 0.663$; two tailed unpaired t-test)

j - Quantification of infustions earned during progressive ratio (N = 4, $t_6 = 0.402$, $p = 0.702$; two tailed unpaired t-test).

k - Quantification of number of cocaine infusions over time during cocaine self-administration (N = 6, Mixed-model ANOVA time x genotype interaction: $F_{13,134} = 1.178$, $p > 0.05$, effect of time: $F_{13, 123} = 4.016$, $p < 0.05$, effect of genotype $F_{1,10} = 0.631$, $p > 0.05$.

l - Quantification of nose pokes for intravenous cocaine during the dose-response experiment (N = 7-8, Mixed-model ANOVA for active nose poke: time x genotype interaction: $F_{7,91} = 0.522$, $p > 0.05$, effect of dose: $F_{3, 34} = 16.07$, $p < 0.05$, effect of genotype $F_{1,13} = 0.569$, $p > 0.05$; inactive nose-poke: time x genotype interaction: $F_{7,91} = 1.109$, $p > 0.05$, effect of dose: $F_{3, 41} = 1.189$, $p > 0.05$, effect of genotype $F_{1,13} = 1.433$, $p > 0.05$).

m - Quantification of intravenous cocaine infusions during the dose-response experiment (N = 7-8, Mixed-model ANOVA: time x genotype interaction: $F_{7,91} = 1.154$, $p > 0.05$, effect of dose: $F_{3, 40} = 40.46$, $p < 0.05$, effect of genotype $F_{1,13} = 1.454$, $p > 0.05$).

n - Survival analysis of extinction learning in control and Gαo cKD groups (N = 6, $\chi^2 = 0.648$, $p = 0.421$; log-rank test)

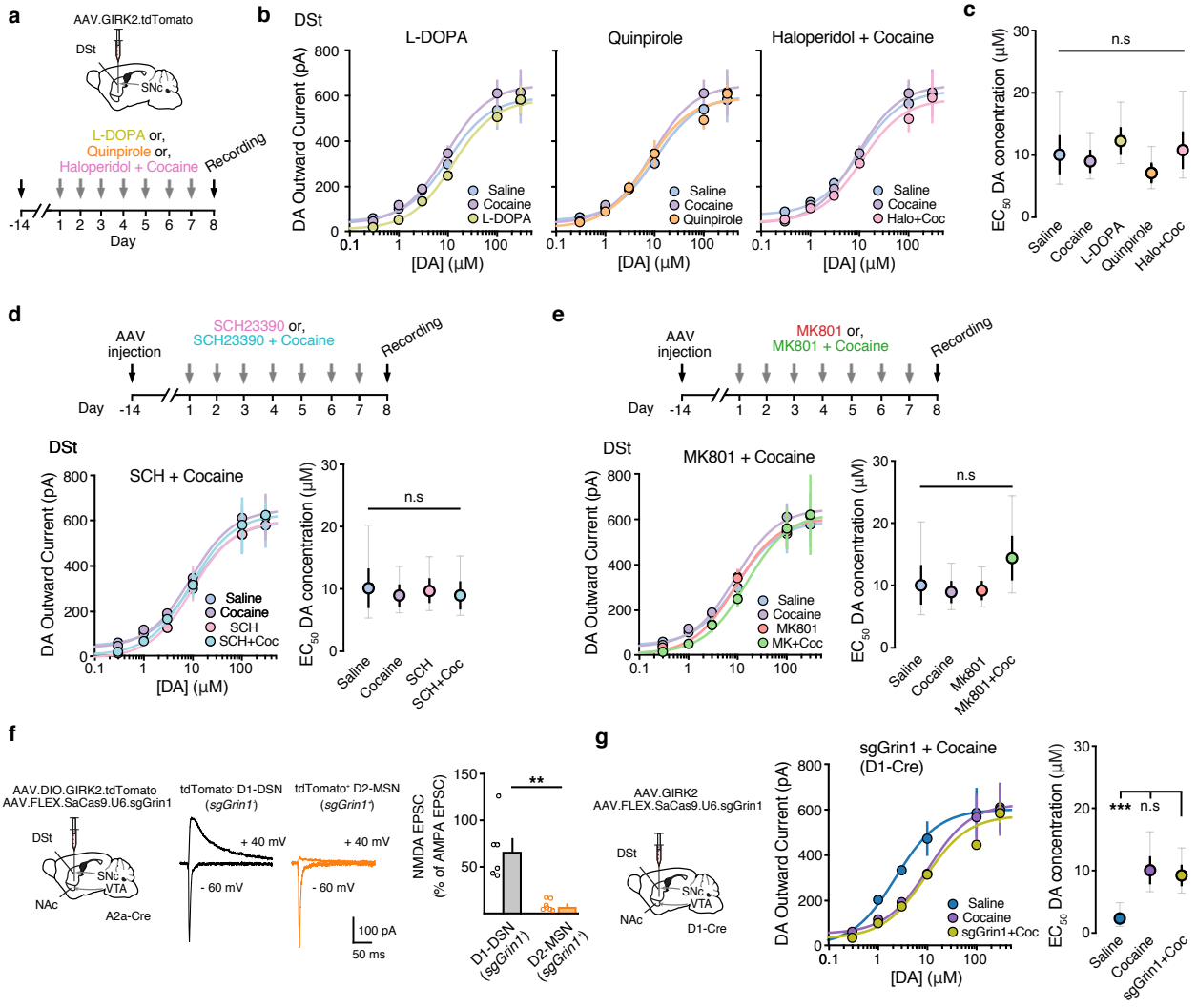
o - Quantification of active and inactive nose pokes for control and Gαo groups during post-abstinence context test (N = 6; active: $t_{10} = 2.249$, $p < 0.05$; inactive: $t_{10} = 2.462$, $p < 0.05$; two tailed unpaired t-test).

p - Quantification of cue-induced reinstatement test (N = 6; two-way ANOVA effect of reinstatement in both Gαo cKD and wt mice for active nose pokes: $F_{1,10} = 20.66$, $p <$

0.01; Sidak's post-test, $p < 0.05$; but not inactive nose pokes: $F_{1,10} = 0.021$, $p > 0.05$). No difference in reinstatement between Gαo cKD and wt mice (reinstatement x genotype interaction active nose pokes: $F_{1,10} = 0.634$, $p > 0.05$, effect of genotype: $F_{1,10} = 0.615$, $p > 0.05$; inactive nose pokes interaction: $F_{1,10} = 0.441$, $p > 0.05$, effect of genotype: $F_{1,10} = 2.519$, $p > 0.05$).

Summary data are mean ± SEM. ns = $p > 0.05$, * = $p < 0.05$ comparing active nose pokes between control and Gαo cKD, ^ = $p < 0.05$ comparing inactive nose pokes between control and Gαo cKD, + $p < 0.05$ comparing active nose pokes between extinction and reinstatement.

Supplemental Fig 7



Supplemental Figure 7 (associated with Figure 7): DSt data and D1-Cre data

a - Timeline of L-DOPA (100 mg/kg; i.p., 7 days), quinpirole (5 mg/kg; i.p. 7 days) and cocaine (20 mg/kg; i.p.; 7 days) preceded by a haloperidol (3 mg/kg, i.p. 7 days) groups.

b - Dopamine D2R dose response curves from DSt D2-MSNs comparing 7-day treatment of saline and cocaine (20 mg/kg) with L-DOPA (100 mg/kg; i.p., 7 days) (left), quinpirole (5 mg/kg; i.p. 7 days) (middle) and cocaine (20 mg/kg; i.p.; 7 days) preceded by a haloperidol (3 mg/kg, i.p. 7 days) (right) pretreatment.

c - EC_{50} values from (c) (n = 39-50, N = 8; $F_{4,32} = 1.883$, $p = 0.138$, one-way ANOVA; $p > 0.05$, Sidak's post-test).

d - Dopamine D2R dose response curves and EC_{50} values from DSt D2-MSNs comparing 7-day treatment of saline and cocaine (20 mg/kg) with cocaine (20 mg/kg; i.p.; 7 days) preceded by a SCH23390 (0.3 mg/kg, i.p. 7 days) pretreatment. Right: EC_{50} values (SCH23390 + cocaine: n = 46, N = 6; $F_{2,18} = 2.677$, $p = 0.096$, one-way ANOVA; $p > 0.05$, Sidak's post-test) and maximum outward currents evoked by 300 μM

dopamine (SCH23390 + cocaine: n = 6; $F_{2,16} = 0.059$, $p = 0.943$, one-way ANOVA; $p > 0.05$, Tukey's post-test).

e - Dopamine D2R dose response curves and EC_{50} values from DSt D2-MSNs comparing 7-day treatment of saline and cocaine (20 mg/kg) with cocaine (20 mg/kg; i.p.; 7 days) preceded by a MK801 (0.3 mg/kg, i.p. 7 days) pretreatment. Right: EC_{50} values (MK801 + cocaine: n = 43, N = 8; $F_{2,20} = 3.353$, $p = 0.055$, one-way ANOVA; $p > 0.05$, Sidak's post-test) and maximum outward currents evoked by 300 μM dopamine (MK801 + cocaine: n = 6; $F_{2,16} = 0.010$, $p = 0.990$, one-way ANOVA; $p > 0.05$, Tukey's post-test).

f - Left: Illustration of injection of AAV.FLEX.SaCas9.U6.sgGrin1 and AAV.DIO.GIRK2 into the NAc of A2a-Cre mice. Electrically evoked AMPA/NMDA currents from $tdTomato^-$ and $tdTomato^+$ putative D1- and D2-MSNs and quantification of NMDA currents (n = 6-7, N = 3; $p < 0.01$, Mann Whitney test).

g - Left: Illustration of injection of AAV.FLEX.SaCas9.U6.sgGrin1and AAV.GIRK2.tdTomato into the NAc of D1-Cre mice. Right: Dopamine D2R dose response curves and EC_{50} values from NAc D2-MSNs comparing 7-day treatment of saline and cocaine (20 mg/kg) treated mice with cocaine (20 mg/kg; i.p.; 7 days) treated mice expressing sgGrin1 (n = 54, N= 7; $F_{2,19} = 76.25$, $p < 0.001$, one-way ANOVA; Sidak's post-test).

Summary data are mean ± SEM (grey traces represent 95% confidence intervals). ns = $p > 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.