Supplemental Figure 1

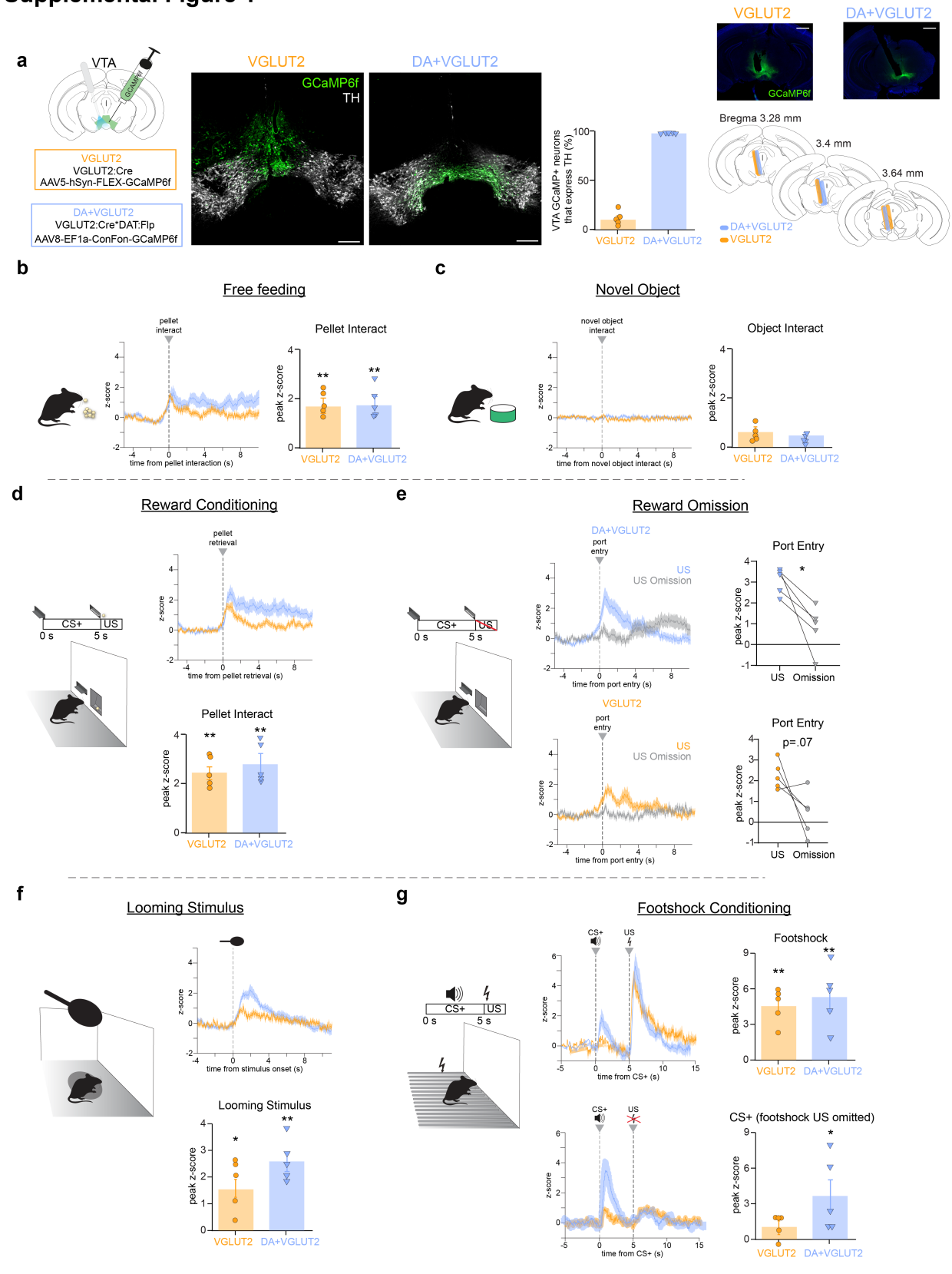

Figure S1: VTA glutamate and dopamine/glutamate neurons respond to rewarding and aversive stimuli, related to Figure 1. a) Illustration of injection into VTA. Cre-dependent GCaMP6f was expressed in VGLUT2:Cre mice to record from all VGLUT2-expressing VTA neurons (VGLUT2; n=5), or Cre plus Flp-dependent GCaMP6f was expressed in VGLUT2-Cre/DAT-Flp mice to record from only those VTA glutamate neurons that express the dopamine transporter, DAT (DA+VGLUT2; n=5). Expression of GCaMP6f (green) was restricted to TH+ neurons (white) in DAT-Flp/VGLUT2-Cre mice (n=5 mice), but only a small proportion of GCaMP6f labeled cells were TH+ in VTA of VGLUT2-Cre mice (n=5); scale bars=200 μm. Right shows placement map of optic fibers in VTA for each mouse (scale bars=500 μm). b) When mice were allowed to freely consume sucrose pellets in an open arena, both VTA VGLUT2 (orange trace; one-sample t-tests of peak z-score during 0-1 s time window after event, compared to a mean z-score of zero: t_4=7.7, p<0.01) and DA+VGLUT2 (blue trace; t_4=5.4, p<0.01) populations increased activity as mice approached and initiated consumption. Lines represent average trace of normalized dF/F (z-score), and shading depicts standard error. c) Motivational value appeared to be a crucial factor because approach and interaction with a novel neutrally valenced object failed to recruit either VTA population (one-sample t-tests: VGLUT2: t_4=0.09, p>0.05; DA+VGLUT2: t_4=1.9, p>0.05). d) In a Pavlovian conditioning assay where a lever extension conditioned stimulus (CS+) was paired with delivery of a sucrose pellet (unconditioned stimulus, US), entries into the magazine to retrieve the pellet also increased activity in VTA VGLUT2 (t_4=8.0, p<0.01) and DA+VGLUT2 populations (t_4=7.3, p<0.01). e) During a session when reward was omitted on 50% of trials, GCaMP activity was only increased upon entry when reward US was delivered but not when reward was omitted (grey trace) in DA+VGLUT2 (paired t-test: t_4=3.7, p<0.05) but not VGLUT2 population (t_4=2.4, p>0.05). f) In an open-field arena, a looming disk was presented overhead to simulate a threatening stimulus. Both VGLUT2 and DA+VGLUT2 populations responded to the looming disk (VTA VGLUT2: t_4=4.6, p<0.05; DA+VGLUT2: t_4=7.4, p<0.01). g) During a Pavlovian fear conditioning task, mice were exposed to a 5s tone (CS+) followed by 0.5s footshock (US). On the first day, both populations responded to the US footshock (VTA VGLUT2: t_4=6. 8, p<0.01; DA+VGLUT2: t_4=4.7, p<0.01). On the second day, when footshock was omitted and only CS+ tone was presented, the DA+VGLUT2 population showed a significant response to the CS+ (VTA VGLUT2: t_4=2.2, p>0.05; DA+VGLUT2: t_4=2.6, p<0.05). Data are represented as mean ± SEM. *p<0.05, **p<0.01, ***p<0.001

Supplemental Figure 2

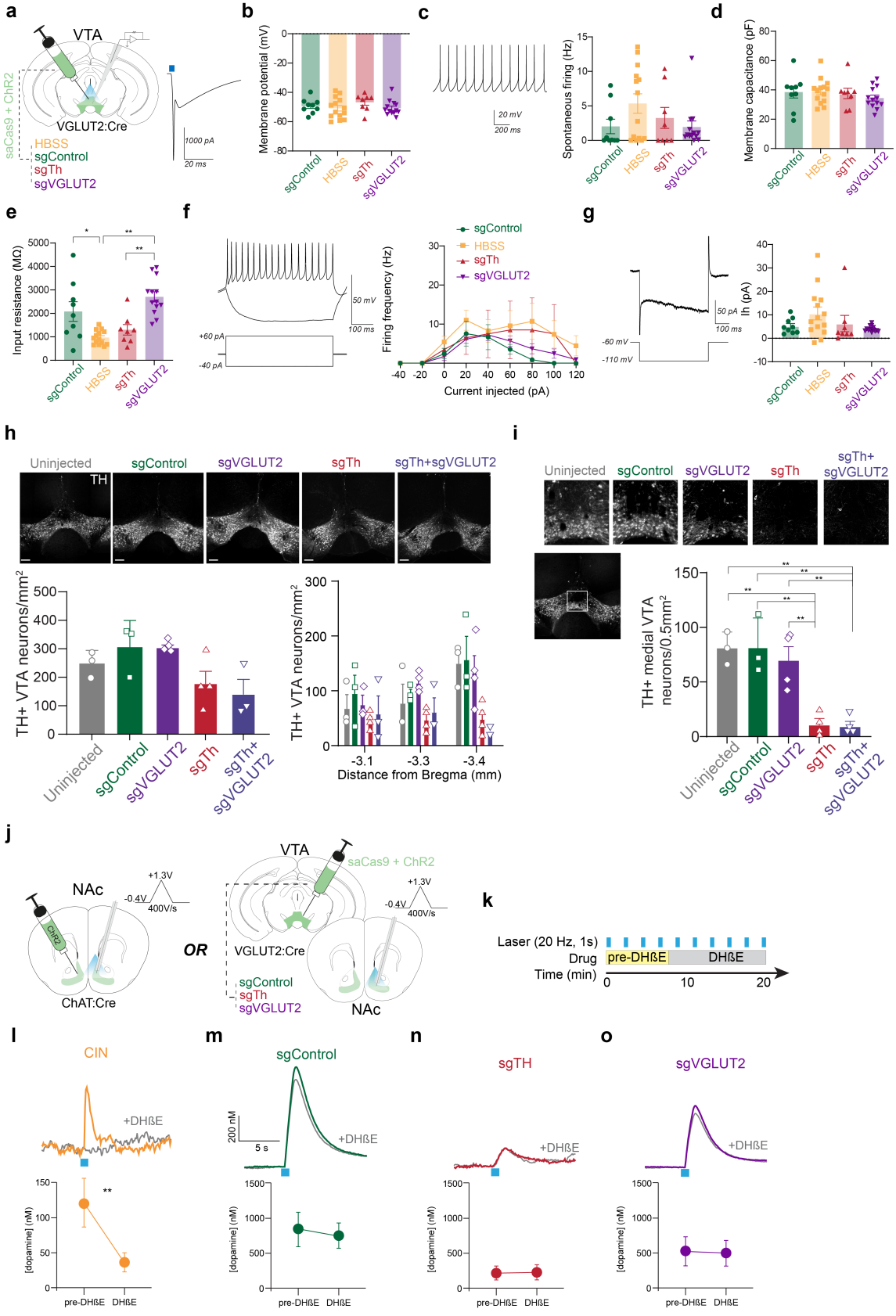

Figure S2: CRISPR-Cas9 vectors do not alter intrinsic properties or number of VTA DA neurons; and opto-evoked VTA→NAc dopamine co-release is not mediated by local acetylcholine release, related to Figure 1. a) Illustration of injections into VTA of either Hanks' buffered saline solution (HBSS) or one of three CRISPR/Cas9 AAVs, plus Cre-dependent ChR2:eYFP. Patch-clamp recordings were made in VTA neurons expressing ChR2:eYFP that displayed optogenetic-evoked photocurrent, inset example trace. b) Whole-cell, resting membrane potential did not differ between injection groups (one-way ANOVA: $F_{3,40}$=1.0, p>0.05) (sgControl: n=9 cells/3 mice; HBSS: n=14 cells/3 mice; sgTh: n=8 cells/2 mice; sgVGLUT2: n=13 cells/3 mice). c) Nor did spontaneous firing frequency ($F_{3,40}$=1.9, p>0.05) or (d) membrane capacitance ($F_{3,40}$=0.62, p>0.05). e) Input resistance was different across groups ($F_{3,40}$=12.9, p<0.0001), with sgControl higher than HBSS (Tukey multiple comparisons test: p<0.05), sgVGLUT2 higher than HBSS (p<0.01), and sgVGLUT2 higher than sgTh (p<0.01). f) Current-evoked firing was not different across groups ($F_{3,40}$=0.85, p>0.05). g) I_h current was not different across treatment groups ($F_{3,40}$=2.0, p>0.05).

h) TH expression in neurons (white) within rostral to caudal VTA was quantified by treatment group. TH+ neuron density was similar between un-injected mice (n= 3) and mice injected with either sgControl Cas9 AAV (n=3) (Dunnett's multiple comparisons test: q_{12}=0.96, p>0.05), sgVGLUT2 mice (n=4; q_{12}=0.68, p>0.05), sgTH mice (n=4; q_{12}=0.56, p>0.05), or sgTH+VGLUT2 mice (n=3; q_{12}=0.27, p>0.05). Right graph depicts density of TH+ neurons at rostral, middle, and caudal VTA locations. No changes were detected, even in the sgTH group, most likely because only a minority of VGLUT2-Cre neurons express TH, and these neurons are concentrated in medial VTA. Scale bars = 200 µm. i) TH+ neuron density within a 500 µm zone of medial VTA was similar between un-injected and sgControl mice (Tukey's multiple comparisons test: q_{13}=0.09, p>0.99). TH+ density was lower in sgTH compared to uninjected mice (Tukey's multiple comparisons test: q_{13}=6.9, p<0.01), sgControl mice (q_{13}=7.0, p<0.01), and to sgVGLUT2 mice (q_{13}=6.3, p<0.01). Similarly, TH+ density was lower among sgTH+sgVGLUT2 mice compared to uninjected mice (q_{13}=7.1, p<0.01), sgControl mice (q_{13}=7.2, p<0.01), and to sgVGLUT2 mice (q_{13}=6.4, p<0.01).

j) Illustration of injection strategies. Either a Cre-dependent ChR2 was injected into NAc shell of ChAT:Cre mice to target ChR2 to NAc cholinergic interneurons (CIN), or one of three saCas9 AAVs (sgControl, sgTh, or sgVGLUT2) plus a Cre-dependent ChR2 was injected into VTA of VGLUT2:Cre mice. Opto-evoked dopamine release was measured using carbon fiber electrodes placed in medial NAc shell applying a triangular waveform from -0.4 to 1.3V at a rate of 400V/s. k) Experimental design. Laser (1s at 20 Hz) was delivered every 2 min for a total of 4 baseline trials (pre-DHßE), after which the nicotinic receptor blocker DHßE was bath applied for 6 more trials (DHßE). l) 20 Hz laser stimulation of cholinergic interneurons evoked dopamine release, which was blocked by DHßE (n= 3 slices/2 mice; paired t-test: t_2=3.9, p<0.05) m-o) Opto-evoked dopamine release was not blocked by DHßE among sgControl (n= 12/4 mice; paired t-test: t_{11}=1.4, p>0.05), sgTh (n=11/4 mice; paired t-test: t_{10}=0.04, p>0.05), or sgVGLUT2 (n=15/4 mice; paired t-test: t_{14}=1.8, p>0.05) conditions. Data are represented as mean ± SEM. *p<0.05 **p<0.01

Supplemental Figure 3

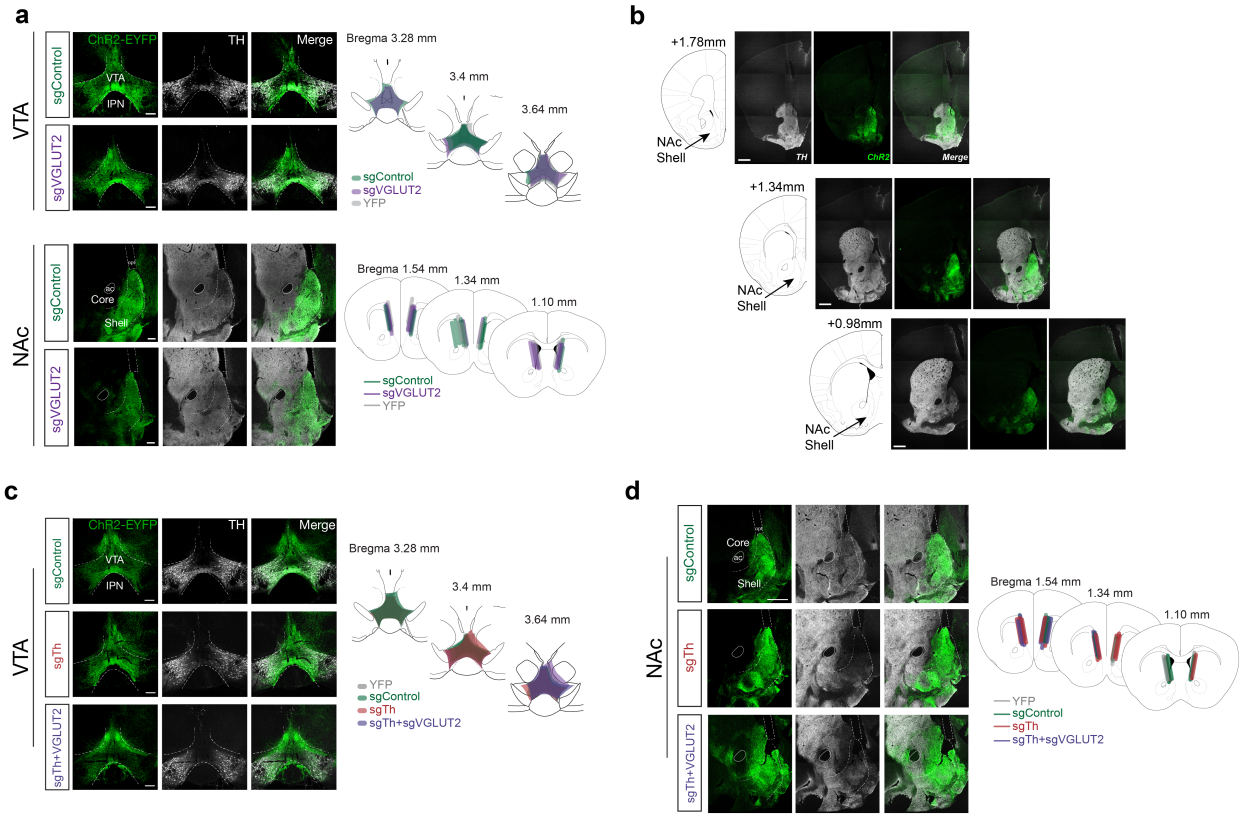

Figure S3: Opsin expression and map of optic fiber placements, related to Figures 2-4. a) Images of coronal sections showing ChR2 (ChR2-EYFP, green) and TH (white) expression in VTA and NAc of sgControl and sgVGLUT2 mice; relating to Figures 2 and 3. b) ChR2 expression in VTA VGLUT2→NAc terminals spans across rostral to caudal NAc medial shell. (c) Images of coronal sections showing ChR2 (ChR2-EYFP, green) and TH (white) expression in VTA and NAc (d) of sgControl, sgTh, and sgTh+VGLUT2 mice; relating to Figure 4. Illustrations on right show ChR2 spread throughout VTA for each mouse (a, c) and bilateral placements of optic fibers in NAc of each mouse (b, d). Scale bars=200 μm; anterior commissure (ac), optic fiber track (opt).

Supplemental Figure 4

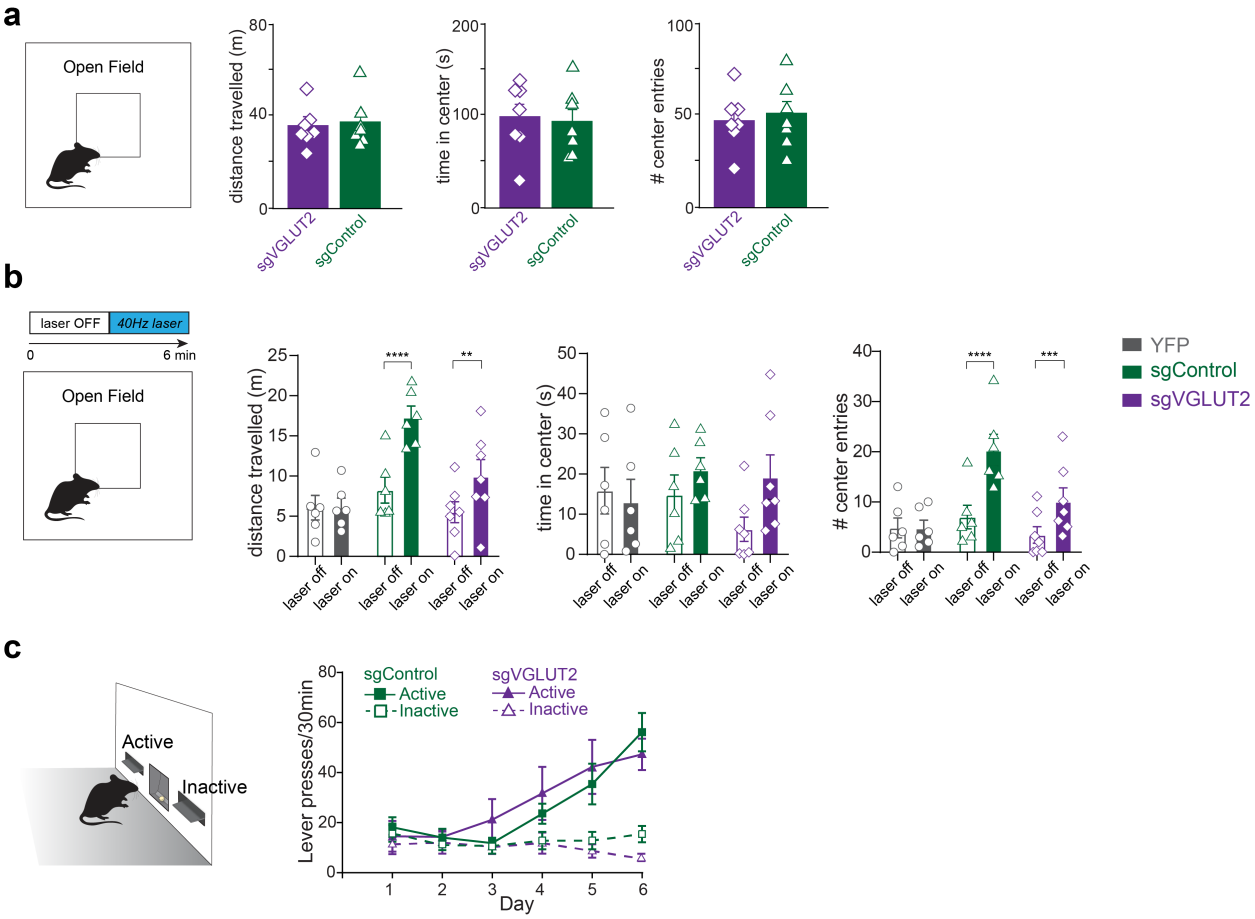

Figure S4: Supplemental behavioral measurements, related to Figures 2 and 3. a) Schematic of open-field test depicting center area (center square) and outside area. Distance travelled (unpaired t-test, t_{12}=0.42, p>0.05), time in center (t_{12}=0.15, p>0.05), and entries into center (t_{12}=0.27, p>0.05) were all similar between sgVGLUT2 (n=7) and sgControl (n=7) mice. b) Open-field assay when laser stimulation (473 nm, 40 Hz) was passively delivered during last 3 min of session. Distance travelled 2-way ANOVA showed interaction of treatment group and laser period: $F_{2,16}$=14.3, p<0.001), with distance travelled increased during laser period among both sgControl mice (n=7; Bonferroni multiple comparisons test: t=7.6, p<0.0001) and sgVGLUT2 mice (n=7; t=4.1 p<0.01), but similar among YFP mice (n=6; t=.08, p>0.05). While there was no effect of laser stimulation on time in center in any treatment group (2-way ANOVA, main effect of laser: $F_{1,16}$=3.1, p>0.05; laser x group interaction: $F_{2,16}$=2.2, p>0.05), a 2-way ANOVA showed interaction of laser x group for number of center entries ($F_{2,16}$=25.9, p<0.0001), with center entries increasing during laser period among both sgControl mice (Bonferroni multiple comparisons test: t=0, p<.0001) and sgVGLUT2 mice (t=5.5, p<0.01), but similar among YFP mice (t=.08, p>0.05). c) Schematic of instrumental sucrose self-administration assay. Presses at the active lever earned a 20 mg sucrose pellet (FR1 schedule) while inactive lever presses earned nothing. Active vs. inactive lever presses for sgControl (n=7) and sgVGLUT2 (n=7) mice across daily training sessions showed both groups discriminated between active and inactive levers at similar levels (main effect of group: $F_{2,17}$=0.81, p>0.05). Data are represented as mean ± SEM. *p<0.05, **p<0.01, ***p<0.001