Nucleus Accumbens Medium Spiny Neuron Subtypes Mediate Depression-Related Outcomes to Social Defeat Stress

Supplemental Information

Supplemental Experimental Procedures

Stereotaxic Surgery and Adeno-associated Viruses (AAVs)

Surgeries were performed as mentioned (see main text). Male wild type C57BL/6J mice (JAX, Bar Harbor, Maine) were used for AAV human synapsin promoter (hsyn) constructs, a promoter which specifically targets neurons. For stimulation experiments, C57BL/6J mice were injected in the nucleus accumbens (NAc) with AAV-hsyn-ChR2(H134)-EYFP, AAV-hsyn-ChETA$_A$-eYFP, AAV-hsyn-eYFP, or AAV-hsyn-eNpHR3.0 for whole NAc expression. For 10 Hz and some 130 Hz stimulations, (DIO)-ChR2(H134)-EYFP was injected into D1-Cre and D2-Cre mice. Mice were implanted with chronically implantable fibers (1) 1 day following social interaction (SI).

In vivo Electrophysiology

All *in vivo* electrophysiology was conducted in accordance with the guidelines set up by the Institutional Animal Care and Use Committee at the National Institute of Diabetes and Digestive and Kidney Diseases. D1-Cre mice were anesthetized with 0.5-1.5% isoflurane during surgery. Briefly, 1.5 µl of AAV-DIO-ChETA$_A$-EYFP was infused into the striatum (A/P: +0.8, Lat: +1.5, D/V: -2.7 mm from top of skull). One array of 32 Teflon-coated tungsten microwires (35 µm diameter) with an integrated 200 µm optic fiber was implanted unilaterally in striatum (A/P: +0.8, Lat: +1.5, D/V: -2.6 mm from top of skull). A stainless steel ground wire was also implanted under the skull.

On days of recordings, the animal was transported to the recording room and left in the home cage for recording. Recordings were 15 minutes long, during which 50 Hz LED light

pulses (2 mW from end of fiber) were administered at 50% duty cycles. For 50 Hz stimulation, the animal was stimulated twice per day for 5 days for 15 min at 50 Hz, and recorded on the 5th day. Signals from each microwire were recorded and amplified using commercial hardware and software (Plexon, Inc, Dallas, TX). Multi-unit activity during the stimulation was identified and average firing rates were determined using Neuroexplorer (Plexon, Inc).

***In vivo* Optogenetic Stimulation and Inhibition after Chronic Social Defeat Stress (CSDS)**

To assess the behavioral effect of a range of frequencies for repeated stimulation, C57BL/6J mice (JAX) injected with AAV-hsyn-ChR2(H134)-EYFP or AAV-hsyn-ChETA$_A$-eYFP were stimulated for 5 days with either 10 Hz (ChR2(H134)) or 20-130 Hz (ChETA$_A$) 473 nm blue light stimulation, 5 sec on/off cycles, 50% duty cycle, 3-5 mW from the end of the fiber tip. Two stimulations were performed each day (morning and evening) for 4 days and 1 stimulation session was performed before SI.

For acute optogenetic manipulation experiments, D1-Cre, D2-Cre, or C57BLJ/6 mice were stereotaxically injected bilaterally with either AAV-DIO-ChR2(H134)-EYFP, AAV-hsyn-ChETA$_A$-EYFP, or the halorhodopsin AAV-hsyn-eNpHR3.0-EYFP in the NAc prior to CSDS. Following CSDS, mice were tested in SI and, the next day, were implanted with bilateral chronically implantable fibers (see above). For stimulation, 473 nm blue light was delivered at frequencies of 10 Hz and 130 Hz (5 sec on/off cycles, 50% duty cycle, 3-5 mW from end of the fiber tip) during a second test. For inhibition, continuous green light (532 nm, 3-5 mW from fiber tip) was delivered through the fibers during a second SI test using a 532 nm DPSS laser (OEM Laser Systems).

Quantitative Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

NAc tissue punches were collected under a fluorescent dissecting scope after behavioral testing of mice that underwent optogenetic and pharmacogenetic manipulations. Tissue was

flash frozen on dry ice and stored at -80°C until further use. RNA was extracted using the E.Z.N.A. MicroElute Total RNA Kit (Omega, Norcross, GA) with a DNase digestion. RNA concentration was determined using a Nanodrop Spectrophotometer (Thermo Scientific, Waltman, MA). cDNA was synthesized from RNA using reverse transcriptase qScript cDNA Superscript kit (Quanta, Gaithersburg, MD). Quantitative PCRs using PerfeCTa SYBR Green FastMix (Quanta) were performed to measure differences in RNA expression. A $2^{-\Delta\Delta Ct}$ method, as previously described (2, 3) was used to analyze changes in RNA expression. GAPDH was used as a normalization control. The following primers were used:

caspase 1,

forward: ATGCCTGGTCTTGTGACTTGG, reverse: AATGTCCCGGGAAGAGGTAGA;

caspase 3,

forward: AGCTGTCAGGGAGACTCTCAT, reverse: TTGAGGTAGCTGCACTGTGG;

caspase 8,

forward: GCGTGAACTATGACGTGAGC, reverse: AAGCCATGTGAACTGTGGAGA;

caspase 9,

forward:CGAAGCTCTCATGGCTTGGA, reverse: ACTGCTCCACATTGCCCTAC;

neuronal nuclei (NeuN),

forward: CAGACACTGGGGAAGACCTG, reverse: CCTAGGAACCCCCTGTCTGG;

GAPDH,

forward: AGGTCGGTGTGAACGGATTTG, reverse: TGTAGACCATGTAGTTGAGGTCA

Supplemental Statistics

Students *t*-tests were used to compare changes in RNA expression between stimulated or inhibited groups and control groups and differences in medium spiny neuron (MSN) firing rates in Day 1 vs Day 5. All other statistics were performed as described in the main text or figure legends.

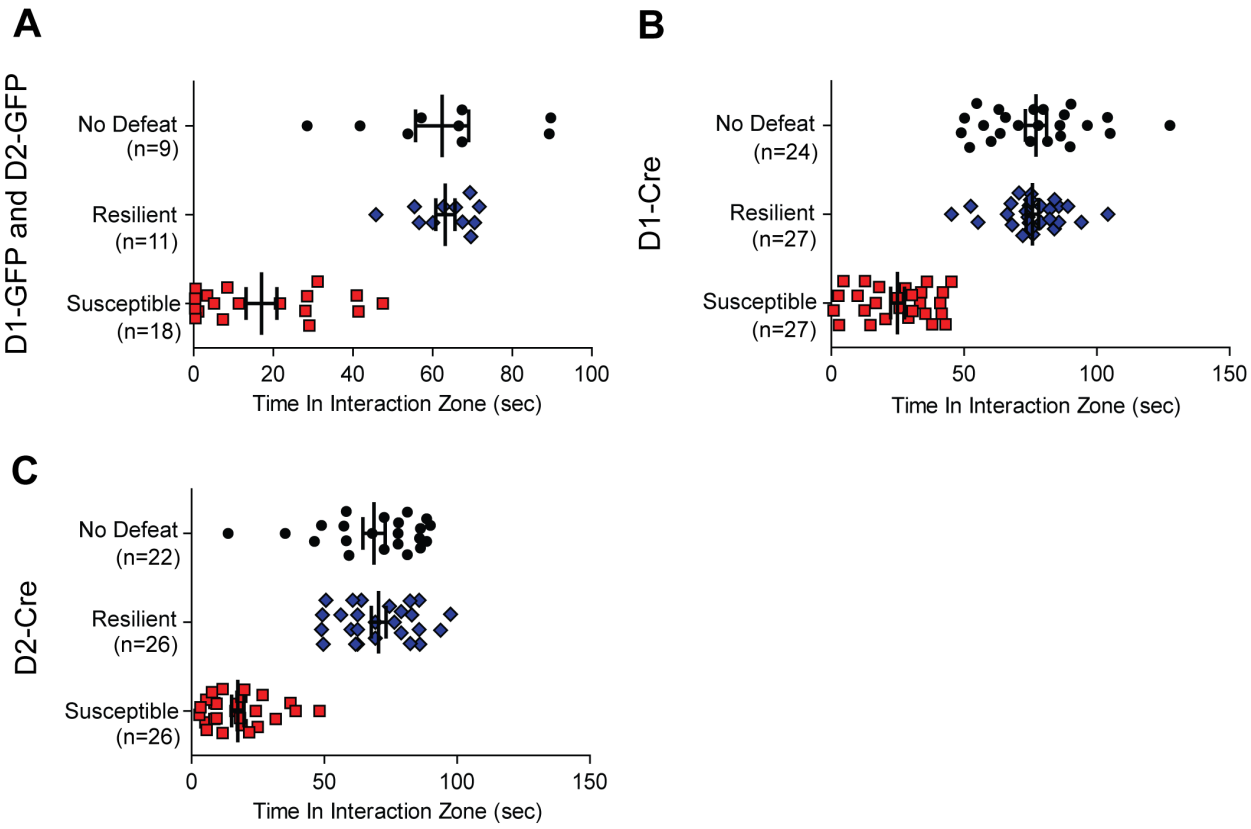

Figure S1. Distribution of Social Interaction Times following CSDS. (A-C) Time in the interaction zone with the target present was used to assay social interaction. Social interaction times are grouped by genotype and contain interaction times from all experiments. **(A)** Social interaction times of D1-GFP and D2-GFP mice used for electrophysiological recordings. **(B-C)** Social interaction times of D1-Cre and D2-Cre mice used for $ChETA_A$ stimulation and DREADDs inhibition experiments.

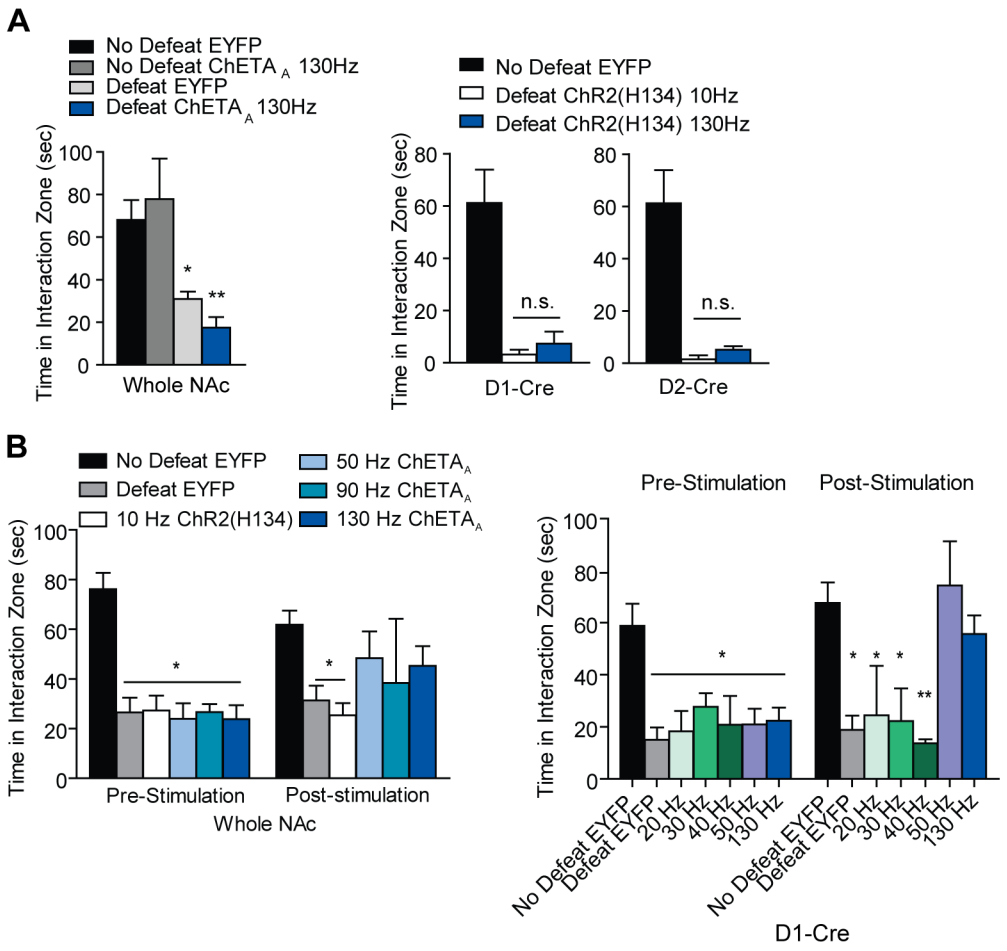

Figure S2. SI Outcomes to Acute and Repeated ChR2(H134) and ChETA$_A$ Blue Light Stimulation at Multiple Frequencies. (A) Susceptible mice receiving acute 130 Hz stimulation to the whole NAc display no alteration in social interaction (Two-way ANOVA no interaction $F_{1,16}$ = 0.21, p > 0.05, n = 4-6 per group). D1-Cre mice and D2-Cre mice remain susceptible after acute 10 Hz or 130 Hz stimulation of ChR2(H134) expressing D1-MSNs (One-way ANOVA $F_{2,12}$ = 11.01 , p < 0.01, defeat groups are not significantly different, n = 3-5 per group) or D2-MSNs (One-way ANOVA $F_{2,12}$ = 9.81, p < 0.01, defeat groups are not significantly different, n = 3-5 per group). **(B)** Stimulation frequencies ≥50 Hz in the whole NAc increased social interaction (Two-way repeated measures ANOVA significant interaction $F_{5,35}$ = 2.72, p < 0.05 with a main effect of stimulation $F_{5,35}$ = 10.67, p < 0.0001, n = 6-8 animals per group). In D1-MSNs, frequencies ≥50 Hz restores normal social interaction (Two-way repeated measures ANOVA with a significant interaction $F_{5,23}$ = 3.17, p < 0.05 and a main effect of pre- post-stimulation time $F_{1,23}$ = 5.25, p < 0.05 and a main effect of frequency of blue light stimulation $F_{5,23}$ = 3.51, p < 0.05). Bonferroni post-hoc test: n.s. non-significant, *p < 0.05, **p < 0.01. Error bars represent SEM.

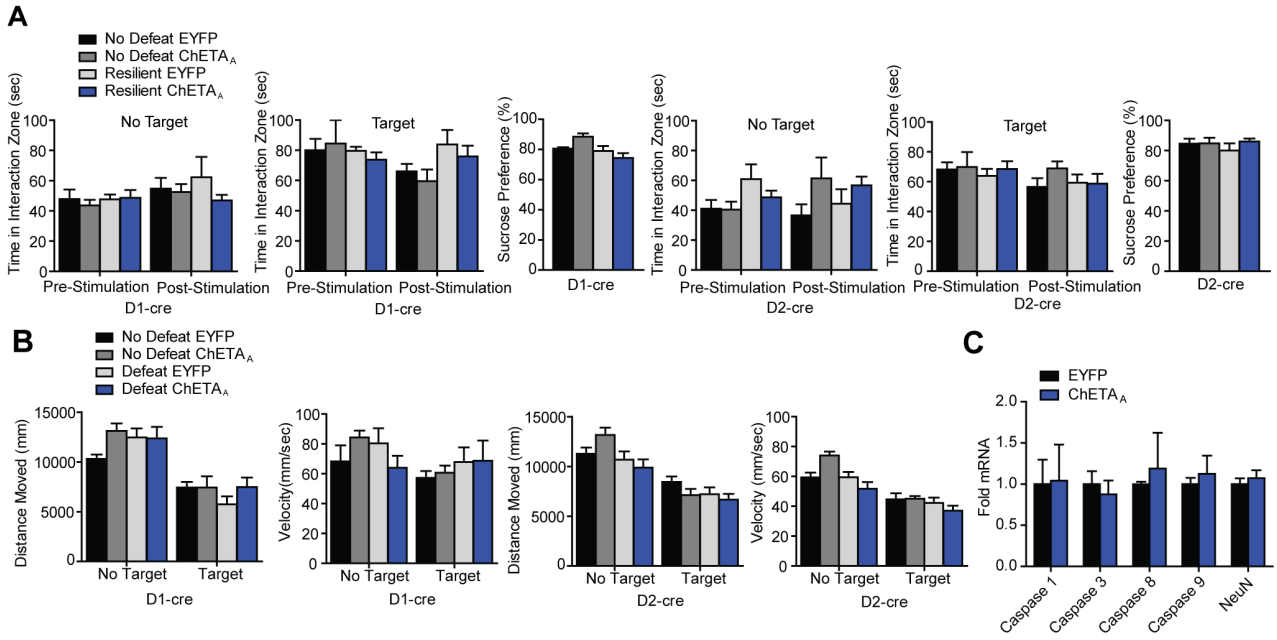

Figure S3. D1-Cre and D2-Cre Resilient Mice CSDS Behaviors, Locomotor Measures, and Cell Death Marker Analysis with ChETA$_A$ Stimulation. (A) Resilient D1-Cre mice display no change in target time (Two-way repeated measures ANOVA non-significant interaction $F_{3,21}$ = 1.82, $p > 0.05$, n = 4-6 animals per group), no target time (Two-way repeated measures ANOVA no interaction $F_{3,21}$ = 0.55, $p > 0.05$), or sucrose preference (Two-way ANOVA interaction $F_{1,21}$ = 4.12, $p > 0.05$) after repeated 50 Hz blue light stimulation of D1-MSNs. Resilient D2-Cre animals display no change in target time (Two-way repeated measures ANOVA non-significant interaction $F_{3,34}$ = 0.26, $p > 0.05$, n = 6-8 animals per group), no target time (Two-way repeated measures ANOVA no interaction $F_{3,19}$ = 1.825, $p > 0.05$), or sucrose preference (Two-way ANOVA no interaction $F_{1,18}$ = 0.002, $p > 0.05$) after repeated 50 Hz blue light stimulation of D2-MSNs. **(B)** In all D1-Cre SDS animals, no changes in distance (Two-way repeated measures ANOVA no interaction $F_{3,24}$ = 1.97, $p > 0.05$) or velocity (Two-way repeated measures ANOVA no interaction $F_{3,40}$ = 0.65, $p > 0.05$) are observed following blue light stimulation. In all D2-Cre SDS animals, no changes in distance (Two-way repeated measures ANOVA non-significant interaction $F_{2,22}$ = 0.22, $p > 0.05$) or velocity (Two-way repeated measures ANOVA non-significant interaction $F_{2,22}$ = 0.27, $p > 0.05$) are observed following blue light stimulation. There are no alterations in cell-death markers or the neuronal marker, NeuN, as shown by real-time quantitative RT-PCR analysis (means are not different between all EYFP and ChETA$_A$ pairs Students t-test $p > 0.05$).

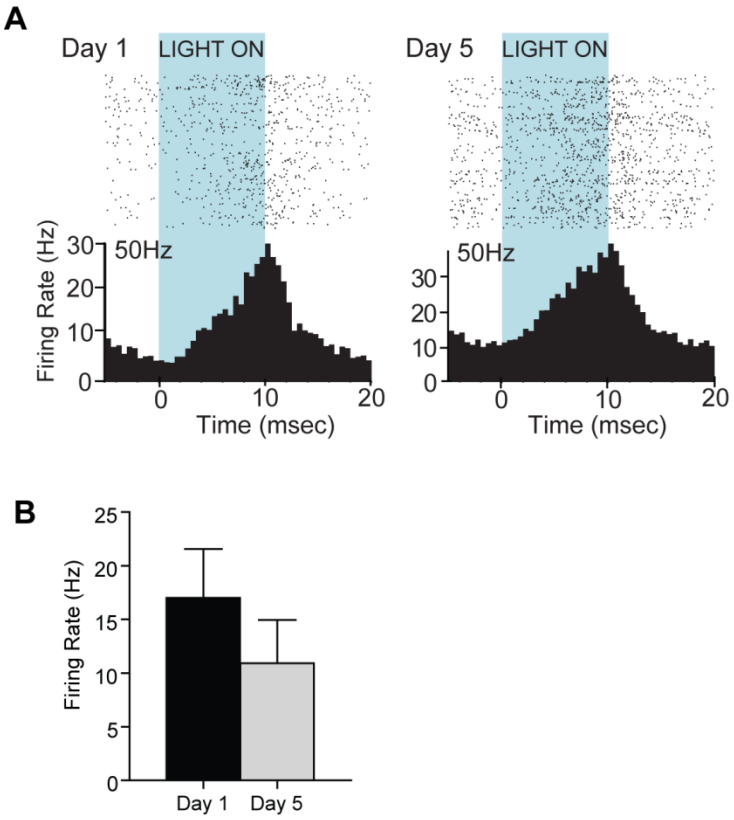

Figure S4. *In Vivo* MSN Responses to ChETA$_A$ Blue Light Stimulation. (A) Representative *in vivo* recordings display 1 light pulse in a train of 50 Hz light pulses. 50 Hz light pulses in D1-Cre mice demonstrate maximal population firing frequencies of ~30 Hz. **(B)** No difference was observed in *in vivo* multi-unit firing frequencies between Day 1 and Day 5 of stimulation (Students *t*-test, $p > 0.05$). Error bars are SEM.

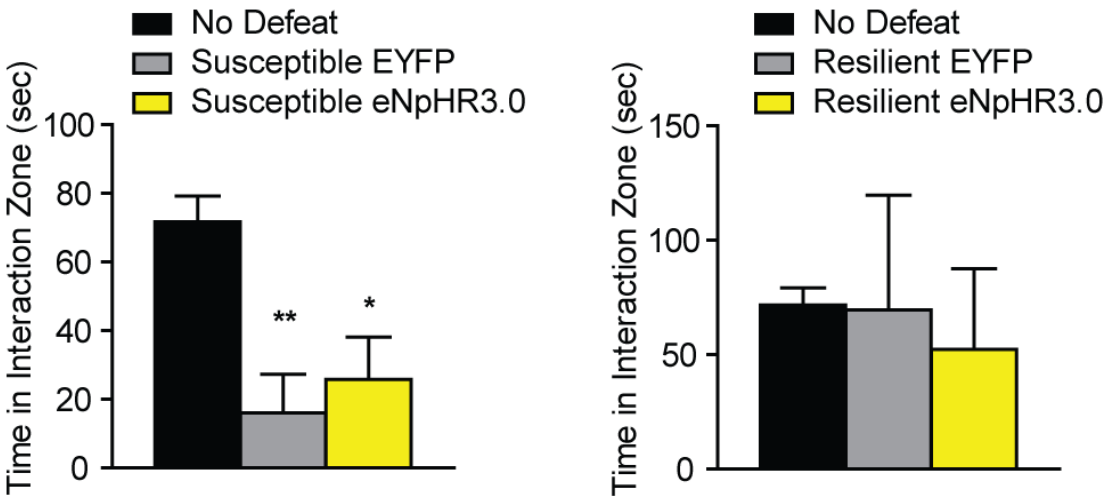

Figure S5. Acute Optogenetic Inhibition of NAc Cells in Susceptible and Resilient Mice Has No Effect on Social Interaction. No changes in social interaction are observed during inhibition of MSNs in susceptible animals (One-way ANOVA $F_{2,11}$ = 10.53, p < 0.01, defeat groups are not significantly different, n = 3-6 per group) or resilient animals (One-way ANOVA $F_{2,10}$ = 0.22, p > 0.05, n = 3-5 per group) during SI. Error bars are SEM.

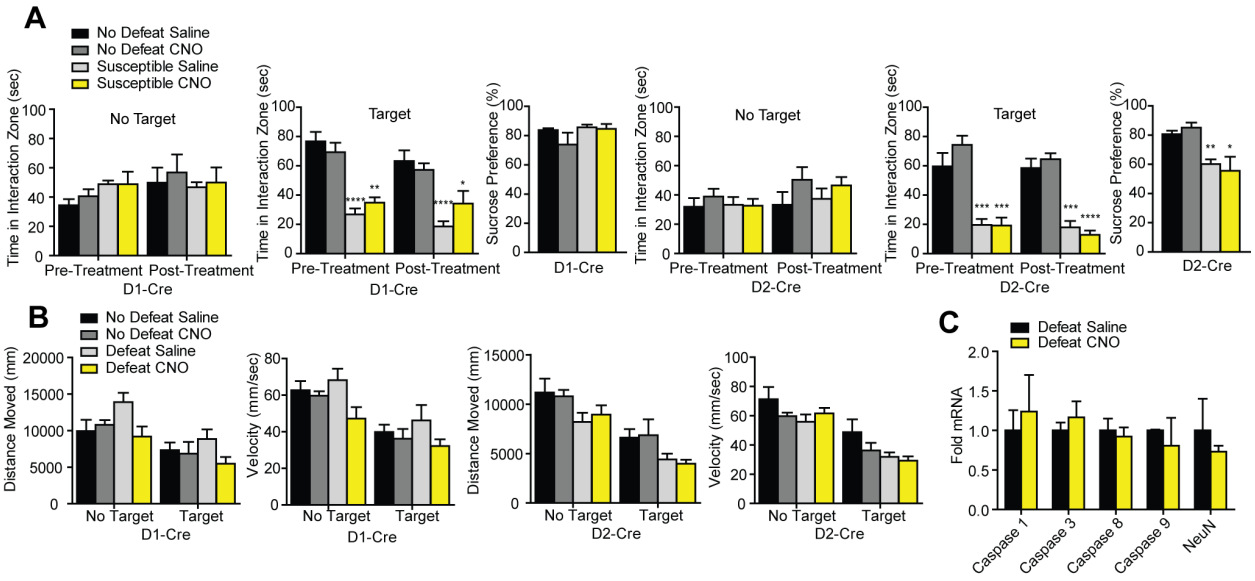

Figure S6. D1-Cre and D2-Cre Susceptible Mice CSDS Behaviors, Locomotor Measures, and Cell Death Analysis with Pharmacogenetic Inhibition. (A) Susceptible D1-Cre mice display no change in target time (Two-way repeated measures ANOVA no interaction $F_{3,23}$ = 0.39, $p > 0.05$, n = 4-8 animals per group), no target time (Two-way repeated measures ANOVA no interaction $F_{3,14}$ = 1.13, $p > 0.05$), or sucrose preference (Two-way ANOVA no interaction $F_{1,20}$ = 1.11, $p > 0.05$) after repeated injections of CNO. Susceptible D2-Cre mice display no changes in target time (Two-way repeated measures ANOVA no interaction $F_{3,22}$ = 0.41, $p > 0.05$, n = 4-8 animals per group), no target time (Two-way repeated measures ANOVA no interaction $F_{3,22}$ = 1.01, $p > 0.05$), or sucrose preference (Two-way ANOVA no interaction $F_{1,20}$ = 0.55, $p > 0.05$) after repeated injections of CNO. **(B)** In all D1-Cre CSDS mice, no change in distance (Two-way repeated measures ANOVA $F_{3,20}$ = 0.61, $p > 0.05$) or velocity (Two-way repeated measures ANOVA $F_{3,20}$ = 0.81, $p > 0.05$) are observed following CNO-mediated inhibition of MSN subtypes. In all D2-Cre CSDS mice, no change in distance (Two-way repeated measures ANOVA no interaction $F_{3,15}$ = 0.28, $p > 0.05$) or velocity (Two-way repeated measures ANOVA no interaction $F_{3,15}$ = 1.24, $p > 0.05$) are observed following CNO-mediated inhibition of MSN subtypes. **(C)** There are no alterations in cell-death markers as shown by real-time quantitative RT-PCR analysis (means are not different between all EYFP and $ChETA_A$ pairs Students t-test $p > 0.05$).

Supplemental References

1. Sparta DR, Stamatakis AM, Phillips JL, Hovelso N, van Zessen R, Stuber GD (2011): Construction of implantable optical fibers for long-term optogenetic manipulation of neural circuits. *Nat Protoc* 7:12-23.
2. Lobo MK, Covington HE,3rd, Chaudhury D, Friedman AK, Sun H, Damez-Werno D, *et al.* (2010): Cell type-specific loss of BDNF signaling mimics optogenetic control of cocaine reward. *Science* 330:385-390.
3. Chandra R, Lenz JD, Gancarz AM, Chaudhury D, Schroeder GL, Han MH, *et al.* (2013): Optogenetic inhibition of D1R containing nucleus accumbens neurons alters cocaine-mediated regulation of Tiam1. *Front Mol Neurosci* 6:13.