SUPPLEMENTAL FIGURES

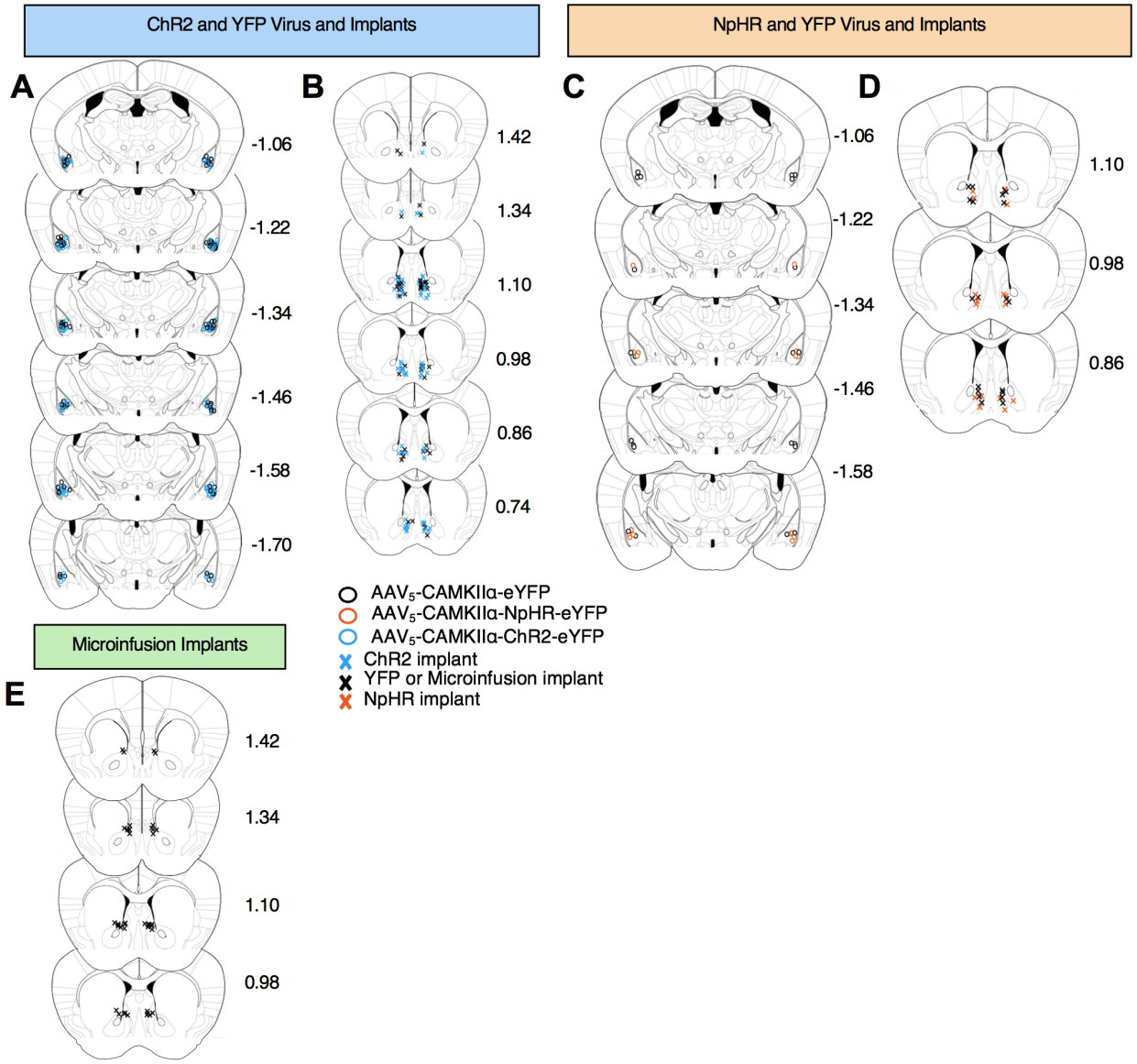

Figure S1. Histological verification of viral delivery and cannula placements. A) Location of ChR2 and YFP viral injections and **B)** unilateral fiber optic canula. **C)** Location of NpHR and YFP bilateral virus injections and **D)** bilateral fiber optic cannulas delivered to *Shank3B*$^{-/-}$ mice. **E)** Location of bilateral infusate cannulas in NAc of *Shank3B*$^{-/-}$ mice. Numbers represent Bregma coordinates in millimeters.

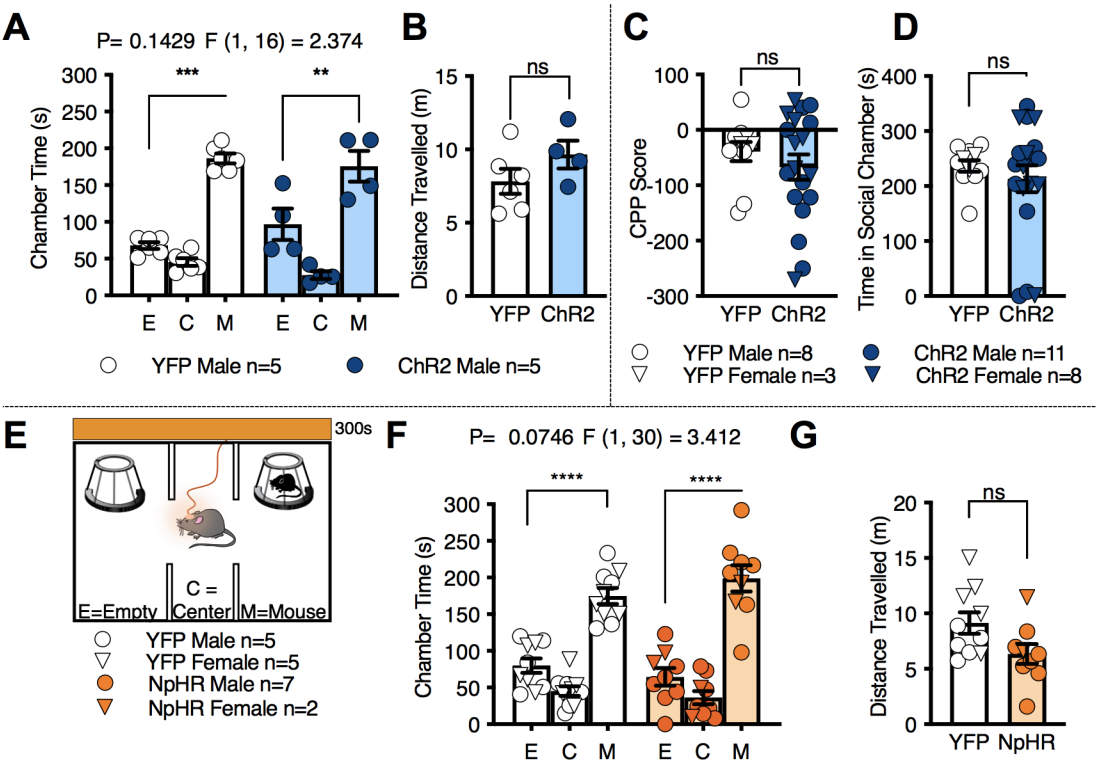

Figure S2: A-B) Pre-stimulation of blue light does not disrupt social interaction behavior in the three-chamber social interaction task. A) After 5-minute blue light pre-stimulation, mice expressing YFP and ChR2 both display significant preference for mouse-containing chamber (***p = 0.0002, **p = 0.0086). **B)** There was no effect of pre-stimulation on distance travelled (ns p = 0.1983); YFP n=5, ChR2 n=5 (A, B). **C-D) Unilateral stimulation during the Social CPP testing**. **C)** Unilateral blue light stimulation in the NAc does not cause any changes in CPP score (ns p = 0.3974) or **(D)** time in social-paired side (ns p = 0.4872) in the CPP task in ChR2-expressing animals compared to YFP-expressing animals; YFP n=11, ChR2 n=19 (C, D). **E-G) Inhibition of BLA-NAc circuit does not affect social preference. E)** Animals received constant orange light stimulation (10-13mW) for the duration of the 3-chamber social interaction task. **F)** Mice expressing YFP and NpHR both have a preference for the social chamber (YFP: ***p = 0.0002, NpHR: ***p < 0.0001) **G)** while distance travelled is not changed by NpHR or YFP expression (ns p = 0.0500); YFP n=10, NpHR n=9 (F, G). Data analyzed via Two-Way Mixed-Effects ANOVA with Sidak's multiple comparisons test (A, F) or unpaired two-tailed t-test (B-D, G). P and F values for Chamber x Virus Interaction shown in (A, F).

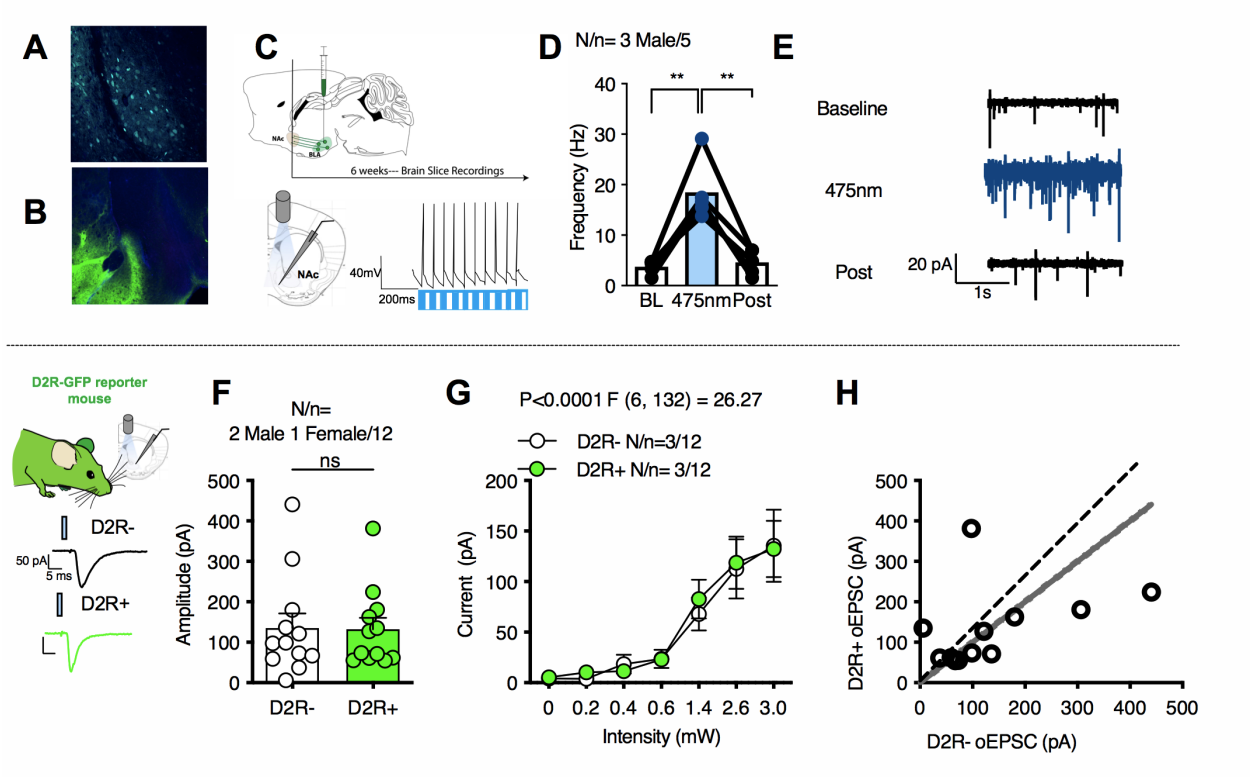

Figure S3: Blue light reliably stimulates BLA-NAc terminals projecting to dopamine D2 receptor positive (D2R+) and D2R negative (-) cell populations A) ChR2 (green) was delivered to BLA. **B)** Viral expression of the BLA terminals in the NAc. **C)** Schematic of whole-cell recording approach in the NAc while blue light (475nm) was delivered to stimulate BLA terminals. Blue light stimulation causes action potential firing in NAc neurons. **D**) Optogenetic stimulation of BLA terminals increases frequency of sEPSCs recorded from NAc neurons. (Baseline (BL)-475nm: ** p = 0.0051, 475nm-post-stimulation (Post): ** p = 0.0038); N=3. **E)** Sample current traces before, during, and after blue-light stimulation. **F-H**) D2R-GFP reporter mice were injected with ChR2 in the BLA, and whole-cell recordings were performed in the NAc in GFP+ (D2R+) or GFP- (D2R-) cells. There is no difference in **(F)** amplitude (ns p = 0.9492, N=3) or **(G)** optical EPSC input-output curves between BLA projections onto D2R- and D2R+ cells in the NAc. **H**) There was no bias of BLA inputs to D2+ or D2- neurons; D2+ N=3, D2- N=3 (G, H). Data analyzed data analyzed via unpaired, two-tailed t-test.

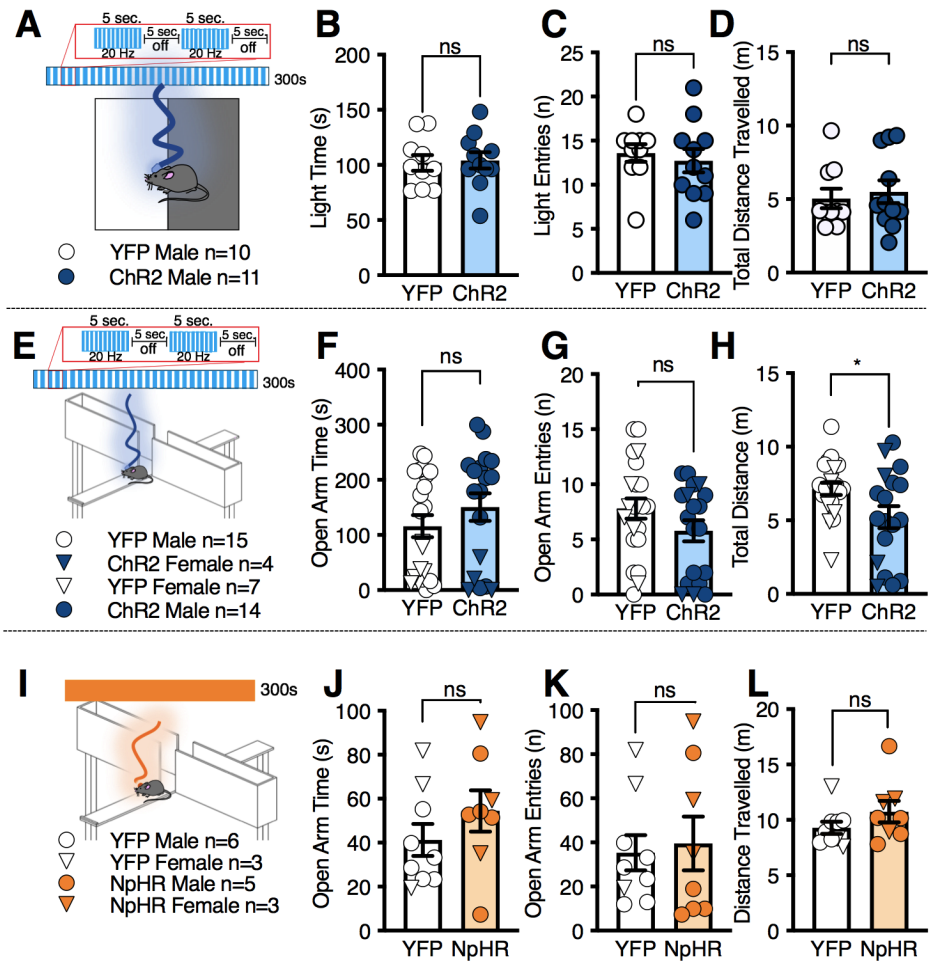

Figure S4: Activation and inhibition of BLA terminals in the NAc does not alter anxiety-like behavior. A) Schematic representing stimulation during light-dark box testing. **B)** ChR2-expression did not alter time in light chamber (ns p = 0.8346), **C)** number of light entries (ns p = 0.6105), or **D)** total distance travelled (ns p = 0.6598); YFP n= 10, ChR2 n=11 (B-D). **E)** Schematic of stimulation during elevated plus maze. **F)** Animals expressing ChR2 spent similar time in open-arms (ns p = 0.2779) and **G)** entered open arms a similar number of times compared to YFP-expressing animals (ns p = 0.1328). **H)** ChR2-expressing animals travelled less distance than YFP-expressing animals (*p = 0.0238); YFP n=22, ChR2 n=18 (F-H). **I)** Schematic showing optogenetic inhibition during elevated plus maze. **J)** Virus expression did not change open arm time (ns p = 0.2802), **K)** open arm entries (ns p = 0.7736), **L)** and did not alter distance traveled in the elevated plus maze (ns p = 0.2025); YFP n=9, NpHR n=8 (J-L). Data analyzed via unpaired two-tailed t-test.

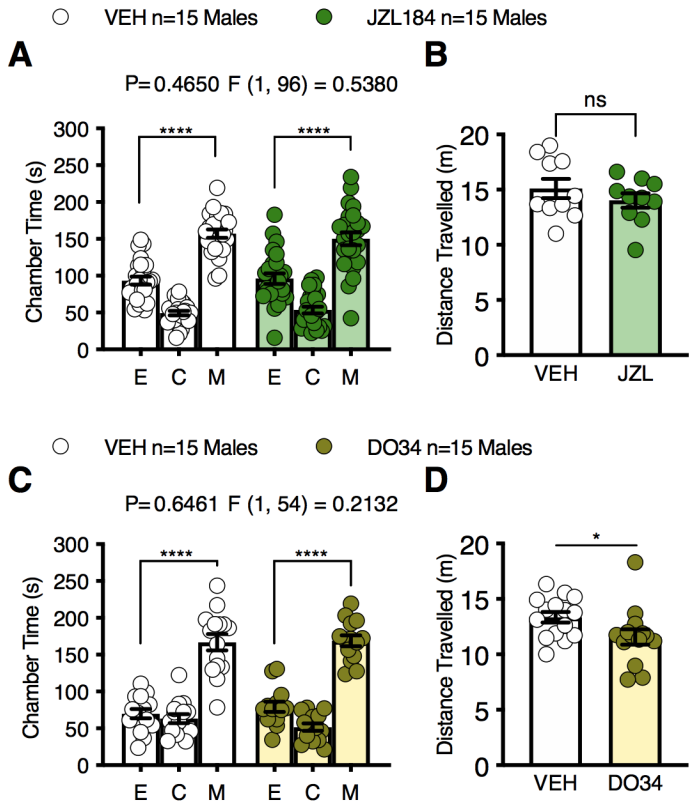

Figure S5: Pharmacologically modulating 2-AG levels does not impair SI in naïve mice. **A-B)** JZL184 (8mg/kg) was given 2 hours before the 3-chamber social interaction task. **A)** Both VEH and JZL184-treated animals have a preference for mouse chamber (****$p < 0.0001$) (VEH n= 15, JZL184 n=15) and **B)** do not exhibit differences in distance travelled (ns $p = 0.3314$). Distance travelled only collected from cohort 1 (VEH n=10, JZL184 n=10). **C-D)** DO34 50mg/kg was given 2 hours before the 3-chamber social interaction task. **C)** VEH and DO-34 treated animals have a preference for the social chamber (**** $p < 0.0001$). **D)** DO-34 pretreatment significantly decreases distance traveled relative to VEH treatment alone (*$p = 0.0404$); VEH n= 15, DO34 n=15 (C, D). Data analyzed via Two-Way Mixed-Effects ANOVA with Sidak's multiple comparisons test (A, C) or unpaired two-tailed t-test (B, D). P and F values for Chamber x Drug Interaction shown in (A, C).

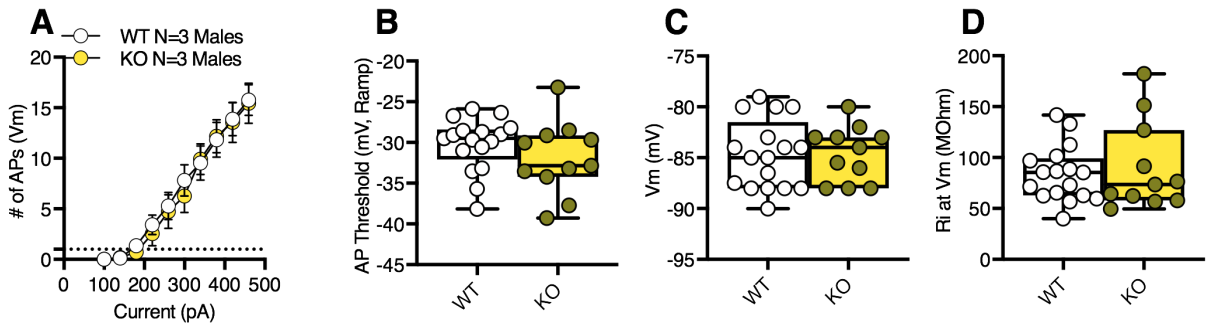

Figure S6: *Shank3B*$^{-/-}$ do not show changes in intrinsic neuronal properties in the NAc. A) *Shank3B*$^{-/-}$ (KO) do not show changes in intrinsic excitability as measured by the number of action potentials generated during successive current injections. *Shank3B*$^{-/-}$ exhibit **B)** a similar AP threshold, **C)** resting membrane potential (Vm) **D)** and input resistance (Ri) compared to WT animals; WT N=3, KO N=3 (A-D). Data analyzed via unpaired, two-tailed t-test. (B-D).

Supplemental Methods

Animals

Mice were housed on a 12:12 light-dark cycle with lights on at 06:00. All experiments were conducted during the light phase. Food and water were available *ad libitum*. Male and female C57/BL6J mice were used for all experiments. Breeding cages were given access to 5LOD chow (PicoLab®, 28.7% Protein, 13.4% Fat, 57.9% Carbohydrate) to improve the viability of litters. Upon weaning at P21-28, experimental animals were switched to standard chow. Wild-type male and female C57/BL6J were ordered from JAX (stock #00064) at 5 weeks of age, target mice for social behavior were ordered at 3 weeks of age and used in experiments at 4 weeks unless otherwise noted. Male and female *Shank3B*$^{+/-}$ mice were ordered from JAX laboratories (Stock number: 017688) and bred in house. Male and Female transgenic BAC *Drd1a-tdTomato* and BAC Drd2-EGFP mice were obtained from JAX laboratories on a C57BL/6J background.

For all animals, viral delivery was performed within 3 weeks of arrival, and delivery of fiber optic implant occurred 4 weeks after viral delivery surgery. Behavior testing for optogenetic studies occurred at least 6 weeks following virus surgery and ex vivo recordings were performed at least 4 weeks after surgery. Following implantation surgery, animals were single-housed. All experimental animals used were between 8-20 weeks old.

Drugs

JZL184 (8 mg kg^{-1}; AbCam, Cambridge, Massachusetts, USA) was prepared in dimethylsulfoxide (DMSO; Sigma-Aldrich, Milwaukee, WI, USA) and injected at a volume of 1 µl g^{-1} bodyweight. For microinfusion studies, JZL184 was prepared into 5 µg/µL DMSO. For electrophysiological recording, 2mM stock solution of JZL184 was prepared in DMSO and

added to 0.05% w/v Bovine Serum Albumin (BSA, Sigma-Aldich, St. Louis, MO, USA) containing ACSF to a final 1 μM concentration. DO34 (50 mg kg^{-1}) was synthesized as previously described (1), prepared in a 1:1:18 mixture of ethanol, kolliphor, and saline, and injected at a volume of 10 μl g^{-1} bodyweight. JZL184 was administered 1 hour before initiation of behavioral testing when combined with in vivo optogenetics **(Figure 5)**, while JZL184 and DO34 were administered 2 hours prior to behavior in naïve mice **(Figure S5)**. CP55,940 (Cayman Chemicals, Ann Arbor, Michigan, USA) and stocks were made at 10mM in DMSO and diluted 1:2000 (for a final concentration of 5μM) in ACSF with 0.05% w/v BSA to increase solubility. WIN55212-2 (Tocris Bio-Techne Corporation, Minneapolis, MN, USA) was dissolved in DMSO at 1mM and diluted into a final concentration of 1μM in ACSF. Ketoprofen, dexdomitodor, ketamine, and antisedan were obtained from Patterson Veterinary Supplies (Greeley, CO, USA).

Viruses

We used AAV5-CaMKIIa-hChR2(H134)-EYFP for optical excitation or AAV5-CaMKIIa-eNpHR3.0-EYFP for optical inhibition. AAV5-CaMKIIa-EYFP (250nL**,** UNC Vector Core, Chapel Hill, NC, USA) was used as a control in all studies.

Stereotaxic surgery

Male and female mice at 5–8 weeks old underwent unilateral or bilateral stereotaxic surgery, as indicated in the figures. Animals were anesthetized at 5% isoflurane, administered 10mg/kg ketoprofen (AlliVet, St. Hialeah, FL) as an analgesic before undergoing stereotaxic surgery. A motorized drill and 10uL microinjection syringe (Hamilton Co., Reno, NV) was guided via digital software (Neurostar Drill and Injection Robot, Tubingen, Germany) to each injection site. Animals were kept under constant 2.5% isoflurane anesthesia. For some ex vivo

electrophysiology studies **(Figure 2 E-G)** mice were anesthetized using a cocktail of ketamine (75 mg/kg) and dexdomitidor (0.5 mg/kg) and were revived using 0.5 mg/kg antisedam.

Viruses were infused into the basolateral amygdala (BLA) (AP: −1.25, ML: ±3.25, DV: 5.05) at a rate of 100 nl min^{-1}. The syringe was first lowered (0.20 mm s^{-1}) to 0.5 μm deeper than the injection site, after 5 seconds, it was raised to the injection site where it paused for 10 seconds before injecting. After the virus was infused, the syringe remained in place for 600 seconds before retracting.

At least 4 weeks following viral injection, mice underwent unilateral or bilateral fiber optic cannula implantation. Cannula implants were lowered to the ipsilateral nucleus accumbens (NAc) of the viral surgery or bilaterally (AP: 1.25, ML: ±0.55, DV: 4.10, +/- 10^{o} tilt for bilateral implants) (0.20mms^{-1}) using an XCL Cannula Holder (Thor labs, Newton, NJ, USA). For microinfusion studies, bilateral, stainless-steel infusion guide cannulas (26 gauge, cut to 3mm length, 2mm center to center distance, C235GS-5-2.0/SPC- Plastics One, Roanoke VA) were placed above the NAc (AP: 1.35, ML: ±1.00, DV: 3.00) and fitted with a dummy cannula (C235DCS-5/SPC, Plastics One, Roanoke VA) and dust cap (303DC/1 Plastics One, Roanoke VA). A bone screw (Plastics One, Roanoke VA) was placed in the skull to anchor the weight of the implant. Metabond (Parkell, Edgewood, NY) was coated across the skull, screw, and implant and allowed to dry for at least 5 minutes. Next, FujiCEM dental cement (Instech, Plymouth Meeting, PA) was applied and allowed to dry for at least 5 minutes. The skin was sutured around the implant (Ny-Superion Havel's, Cincinnati, OH) and mice were single-housed. Animals were treated with 10mg/kg ketoprofen (AlliVet, St. Hialeah, FL) for up to 3 days following surgery as needed. Animals recovered for at least one week prior to any experimental manipulation.

Ex Vivo Electrophysiology.

Mice were briefly anesthetized with isoflurane and transcardially perfused with ice-cold oxygenated (95% v/v O_2, 5% v/v CO_2) N-methyl-D-glucamine (NMDG) based ACSF comprised of (in mM): 93 NMDG, 2.5 KCl, 1.2 NaH_2PO_4, 30 $NaHCO_3$, 20 HEPES, 25 glucose, 5 Na-ascorbate, 3 Na-pyruvate, 5 N-acetylcyctine, 0.5 $CaCl_2 \cdot 4H_2O$ and 10 $MgSO_4 \cdot 7H_2O$. The brain was quickly removed and 250 μm coronal or parasagittal **(Figure 4 D-F)** slices of the NAc were cut using a Leica VT1000S vibratome (Leica Microsystems, Bannockburn, IL, USA) in the NMDG solution. Slices were incubated for 10-15 minutes at 32°C oxygenated NMDG-ACSF and stored at 24°C until recording in HEPES-based ACSF containing (in mM): 92 NaCl, 2.5 KCl, 1.2 NaH_2PO_4, 30 $NaHCO_3$, 20 HEPES, 25 glucose, 5 ascorbate, 3 Na-pyruvate, 5 N-acetylcyctine, 2 $CaCl_2 \cdot 4H_2O$ and 2 $MgSO_4 \cdot 7H_2O$. Recordings were performed in a submerged recording chamber during continuous perfusion of oxygenated ACSF containing (in mM): 113 NaCl, 2.5 KCl, 1.2 $MgSO_4 \cdot 7H2O$, 2.5 $CaCl_2 \cdot 2H2O$, 1 NaH_2PO_4, 26 $NaHCO_3$, 1 ascorbate, 3 Na-pyruvate and 20 glucose; at a flow rate of 2.5 - 3 ml/min. All drugs were dissolved in ACSF containing 1:2000 BSA (w/v) and ≤1:2000 DMSO (v/v). Slices were visualized using a Nikon microscope equipped with differential interference contrast video microscopy or Scientifica Slicescope Pro System (Scienifica, Clarksburg, NJ, USA) **(Figure 4D-F)**. Whole-cell current clamp recordings from NAc MSNs held at resting membrane potential were obtained under visual control using a 40x objective. 2 - 3 MΩ borosilicate glass pipettes were filled with high [K^+] based solution containing (in mM): 125 K^+-gluconate, 4 NaCl, 10 HEPES, 4 Mg-ATP, 0.3 Na-GTP, and 10 Na-phosphocreatine. Whole-cell voltage clamp recordings were performed with the same internal solution in the experiments shown in **(Figure 4 A-C)** and **(Figure S3)**. Pipettes were filled with a Cs^+ internal solution (in mM: 120 CsMeSO3, 15 CsCl, 8 NaCl, 10 HEPES, 0.2 EGTA, 10 TEA-Cl, 4.0 Mg2-

ATP, 0.3 Na2-GTP, 0.1 spermine, and 5.0 mM QX 314 bromide) for WIN-55 experiments **(Figure 4 D)**. Recording ACSF solution contained the $GABA_A$ receptor antagonist picrotoxin (50 μM; Abcam, Cambridge, MA) to pharmacologically isolate glutamatergic transmission. In the experiments where both glutamatergic and GABAergic currents were assessed in each cell **(Figure 4 G-I and Figure 9)** a cesium based internal solution was used consisting of (in mM): 120 CsOH (50%), 120 D-gluconic acid (50%), 2.8 NaCl, 5 TEA-Cl, 20 HEPES, 2.5 Mg-ATP, 0.25 Na-GTP. Spontaneous and optically evoked EPSCs and IPSCs were measured at -70mV and +13mV, respectively. Only cells with access resistance <20 MΩ were included. *Shank3B*$^{-/-}$ and WT data **(Figure 4 G-I and Figure 9)** was carried out by using single housed (1 week+) mice similar to the behavioral experiments.

Optical stimulation as well as data collection was coordinated using pClamp 10 (Molecular Devices, San Jose, CA, USA). Cell electrical properties were monitored using an Axopatch 500B Multiclamp amplifier and Axon Digidata 1550 low-noise data acquisition digitizer (Molecular Devices, San Jose, CA, USA). Responses were filtered at 2 kHz and digitized at 10 kHz. Optical stimulation of ChR2-expressing BLA terminals was achieved using a Thorlab 480nm LED system (Thor labs, Newtown, NJ, USA) **(Figure 4A-C, S3)**, CoolLED pE-100 480nm LED excitation system (CoolLED Ltd, Andover, UK)(**Figure 4D-F)** or a Mightex 455nm LED system (Mightex, Pleasanton, CA, USA) **(Figure 9)**. 2ms light pulses were applied through a 40x objective to excite ChR2+ terminals at 0.1 – 0.2 Hz. Light intensity was adjusted to evoke stable responses. Depolarization induced suppression of excitation (DSE) was examined under voltage-clamp condition and was evoked by depolarizing patched MSNs to +30mV for 10 seconds. DSE was classified as the first EPSC following the depolarizing pulse. Electrophysiology experiments were

analyzed using using Clampfit 10.4 (Molecular Devices, San Jose, CA, USA), Microsoft Excel, and Graphpad Prism v8.0 (Graphpad, San Diego, CA, USA).

Drug wash-on experiments were carried out after assessing a 7-10min stable baseline recording. Then, CP55,940 was applied during the entire session of the recordings, while WIN55212-2 was only applied during the initial 10min to allow assessment of long-term depression (LTD).

BAC transgenic mice expressing td-Tomato under the D1 receptor promoter or GFP under the D2 receptor promoter were used to identify the two major populations of direct and indirect pathway neurons of the striatum, respectively (as depicted in figures). Putative D1 receptor or D2 receptor neurons were identified by the presence or absence of fluorophore expression, as previously reported (2) and neighboring neurons were patch-clamped at ≤60μm apart within the same depth and plane of the slice. Input/output curves were generated by increasing the intensity of the applied light pulses and the maximal oEPSC amplitude was used to compare the excitatory input ratios between D2+ and D2- neurons **(Figure S3)**. In the experiments assessing the asynchronous release probabilities of the BLA-NAc pathway on D1+ and D1- MSNs, the frequency and amplitude of aEPSCs were analyzed within the 50ms-500ms time-window measured from the onset of the light stimulus **(Figure 4 B-C)**.

Cells were excluded from all analyses for any of the following reasons. 1: if the holding current dropped below -200 pA at any time during the recording. 2: if the access resistance was > 20 MΩ. 3: if the access resistance fluctuated by more than 20% throughout the recording. 4: There was no optogenetically-evoked response.

Histology and Imaging

After animals completed behavior experiments, brain tissue was collected in order to validate implant and viral placement. Mice were anesthetized using isoflurane (Abbott Labs, Chicago) and transcardially perfused with cold phosphate buffered saline (PBS, 10mL) followed by cold 4% paraformaldehyde in 0.1M phosphate buffer (PFA, 15-20mL). Brains were dissected and stored for at least two days at 4^{o}C in 4% PFA and then transferred to a 30% sucrose solution for at least 2 days. Brain tissue was cut at 100uM using a Leica CM3050 S cryostat (Leica Microsytems, Weitzlar, Germany) and transferred to an antifreeze, ethylene-glycol solution and stored in a -20^{0}C freezer.

For validation experiments, tissue was transferred to cell strainers in a bath of Tris-Buffered Saline for 10 minutes 6 times. Slices were then transferred to deionized water and mounted onto charged slides. Slides were mounted in VECTASHIELD + DAPI (Vector laboratories, Burlingame, CA, USA) and sealed with a clear nail polish. Images were collected using an upright Axio Imager M2 epifluorescent microscope at a 5x and 20x objective.

Behavior Testing

All behavioral experiments were recorded and analyzed using AnyMaze Behavioral tracking software (Stoelting, Wood Dale, IL).) with the exception of NAc microinfusion studies **(Figure 8**) in which Noldus EthoVision XT (Leesburg, VA USA). All animals for all experiments were single-housed, with the exception of the animals used in the grooming assay.

Cohorts:

We used 6 cohorts of animals in unilateral, WT, YFP/ChR2 experiments. With the exception of the bilateral social conditioned place preference (social CPP) assay, all animals were stimulated using unilateral stimulation at 20hz in a 5 seconds on 5 seconds off pattern at 10-13mW. Cohort 1 was sequentially tested in the 3-chamber SI (SI) test, elevated plus maze (EPM), and

light-dark box (LD). Cohort 2 was sequentially tested in 3-chamber SI test using 20Hz stimulation, and the EPM. Cohort 3 was first test in the 3-chamber SI test, using 20hz stimulation, the EPM, juvenile reciprocal SI, and social CPP. Cohort 4 was first run through real-time place-preference (RT-PP), and second run through the JZL-184 pretreatment experiment with 3-chamber social interaction test. Cohort 5 was first run through the JZL pretreatment 3-chamber SI test, sequentially followed by the ensure 3-chamber task. Finally, cohort 6 animals were run through juvenile SI, social CPP, and the ensure 3-chamber task. Additionally, we used 2 YFP/NpHR WT cohorts. Both cohorts were first run through the 3-chamber SI task, and subsequently run through the EPM. For our *Shank3B*$^{-/-}$ NpHR studies, we used 2 cohorts. Both cohorts were run through the 3-chamber SI task with light ON and with light OFF following two days later. For all in vivo optogenetic studies, further testing was completed at a minimum of 72 hours following previous stimulation.

For JZL-184 studies, we used three cohorts of *Shank3B*$^{-/-}$ and WT littermate control animals. For the 3-chamber SI studies, animals were only run through the 3-chamber SI task once and sacrificed afterwards. For grooming studies, we used 3 cohorts of animals. In a counter-balanced design, all animals were given either JZL-184 or VEH, tested, and then retested a minimum of 4-days later.

Finally, for our *Shank3B*$^{-/-}$ microinfusion experiments, we used 2 cohorts of animals. All animals were run through the 3-chamber SI task once and sacrificed for placement validation afterwards.

1) 3-chamber SI test: For in vivo optogenetic experiments, animals were first attached to a fiber optic cable. Mice were placed in the center chamber of a three-chamber arena, a 420mm x 565mm x 358mm box with 185mm x 420mm equal chambers made of 5mm thick, clear Plexiglas with two doors (120mm wide, 5mm thick, 358mm tall) connecting chambers from the middle

(Vanderbilt Machine Shop, Nashville TN). Inside the chamber were two empty, wire pencil cups (Organize-it, Rochester Hills, MI) with clear, plastic cups (10oz, 7.5cm diameter 10.4cm tall) with approximately 6oz of water to anchor the cups and prevent the test animal from climbing on the pencil cup. Mice were given a 10-minute habituation period to the apparatus, after which mice were returned to the center and guillotine doors were shut to contain the mouse in the center. When *Shank3B*$^{-/-}$ animals were used, *Shank3B*$^{-/-}$ and WT littermate controls were given a 5-minute habituation while restricted only to the center chamber, as previously described (3).

Next, a novel, sex-matched and juvenile (4-6 week-old) mouse was placed inside one pencil cup, and the doors were opened to begin the testing phase (5 minutes). *Shank3B*$^{-/-}$ and WT littermate controls were paired with age-matched, sex-matched targets. For in vivo optogenetic studies, stimulation was triggered to be delivered during the testing phase upon the opening of the doors, or prior to the habituation phase as indicated in the **(Figure S2)**. Time spent in close interaction (5 cm. perimeter around inverted cups) with the mouse-containing and empty cups and time spent in social, empty, and center chambers, were assessed and compared between the test and control groups. Sociability is defined in this assay as having a significant preference for the mouse chamber compared to the empty chamber. All animals were tested in 80-130 lux. Videos were coded post-hoc for time investigating each cup by two, blinded coders using Anvil 6.0 video annotation software (5). Time investigating represents time in which the test mouse is sniffing or interacting with the cup. Time investigating was averaged between two coders.

2) Ensure 3-Chamber Task: Animals were trained for 3 consecutive days in home cage to drink commercially available Ensure. 50mL conical tubes were filled with 30mL of Ensure, fitted with a sipper lid, and inserted into the home cage. Animals were exposed to the Ensure for 30 minutes. Animals that did not drink within 3 minutes on the third day were excluded from the study. On

test day, animals were placed in the 3-chamber apparatus (as described above, in 80-130 lux) containing two inverted, wire, pencil cups with clear plastic cups filled with water anchoring on top. Animals were habituated for 10 minutes and returned to the center chamber and guillotine doors shut. The Ensure sipper was affixed to an inverted pencil cup so the animals could easily reach and drink from the sipper. Next, stimulation was turned on (20Hz 5s on 5s off ~10mW) and animals were permitted to freely explore the apparatus for 5-minutes. Time in each chamber and distance travelled was recorded. Videos were coded post-hoc for time drinking from Ensure sipper bottle and time investigating (sniffing or interacting with) the empty cup by two, blinded coders using Anvil 6.0 video annotation software (5). Time investigating was averaged between two coders.

3) Juvenile Reciprocal Social Interaction Test: Animals were placed in a clean, novel amber cage (32.5cm x 19.3cm x 14cm) concurrently with a novel, sex-matched juvenile mouse (4 weeks old). Animals were permitted to freely interact for 10-minutes. For in vivo optogenetic studies, the test mouse received light stimulation via the HELIOS wireless optogenetic system (Plexon, Dallas TX, USA) for the duration of the test. Videos were recorded during the interaction and analyzed post-hoc by two independent blinded coders for behavioral activity using Anvil 6.0 video annotation research tool software (4). Videos were analyzed for the amount of time the test mouse was fleeing or withdrawing from target, sniffing target, self-grooming, following target, passively social, open exploring and immobility, as previously described (5). Animals tested in 80-130 lux.

4) Light-dark box testing: Animals were placed in a three-chamber apparatus (two 30cm x 15cm x 38cm chambers connected by a 10cm x 7.5cm x 38cm hallway) made of opaque, white Plexiglas, fitted with two removable clear plexiglass inserts. (Vanderbilt University Machine Shop, Nashville, TN). The “light” side was lined with white construction paper behind the clear Plexiglas

insert and brightly lit (>200lux) and the dark side was lined black construction paper and dimly lit (<20 lux). First, mice were attached to a fiber optic patch cable and placed in the center chamber, enclosed by two guillotine doors, and allowed to habituate for 5 minutes in the dark. Next, light for the light chamber was turned on and the doors to the light and dark sides were simultaneously lifted, triggering optogenetic stimulation and animals were tested for 5-minutes. Time spent in light, entries into the light side, and total distance traveled was quantified.

5) Elevated-plus maze: Animals were connected to a fiber optic patch cable and placed back into their home cage to habituate for 5 minutes in the dark (lux <10). Animals were then placed in the center of an elevated plus maze (San Diego Instruments, San Diego, CA, USA) and allowed to explore open arms (lux ~ 200) and closed arms (lux ~ 50). Stimulation was delivered through the entirety of the test. Time spent in open arms, entries into the open arms, and total distance traveled was quantified.

6) Social Conditioned Place Preference: We used a 3-chamber apparatus (two 30cm x 15cm x 38cm chambers connected by a 10cm x 7.5cm x 38cm hallway). One chamber was lined with dot-patterned construction paper, and the other outside chamber was lined with striped construction paper. Plastic floors with either a stippled pattern or a smooth pattern were inserted into the dots or smooth sides respectively. All chambers were lighted around 100 lux. First, animals were connected to a fiber optic patch cable and placed into the center chamber and allowed to habituate for 5 minutes in the dark, with doors enclosing the mouse in the center. The doors of both a side with a dot pattern and stippled floor and a side with a stripe pattern and smooth floor were simultaneously opened. The animals were given a 10-minute test period in order to exclude any bias preference for either side. Animals that had more than 65 percent bias (time in chamber/ (time in dot chamber plus stripe chamber) *100) for either chamber were excluded. The following day

mice were assigned in a counter balanced, unbiased manner in either the dots or stripe chamber with a novel, juvenile sex-matched mouse for 10 minutes. Immediately following the social exposure phase, animals were placed on the opposite chamber alone for 10 minutes. This was repeated the following day with the isolation phase occurring first, and on the third day of testing animals were exposed to the social stimuli first. Finally, on test day, animals were reconnected to the fiber optic cable and after 5-minute habituation in the center chamber, with doors closed to isolate the animal in the center. Next, in vivo optogenetic stimulation was turned on and the doors to social and empty chambers opened. Animals were able to freely move for 10 minutes. Data are recorded as CPP score (amount of time in social chamber in pretest minus the time in social chamber in post-test), amount of time in social chamber on post-test, and time in unassigned chamber on pre-test day.

7) Real-time place preference: We used the apparatus previously described in Social CPP. All chambers were lighted around 100 lux. First, a fiber optic patch-cable was attached, and animals were placed in the center chamber to habituate for 5 minutes in the dark (<10 lux) with doors enclosing the animal in the center. Next, the doors to the adjacent chambers were opened. When animals entered a randomly assigned chamber, light stimulation was delivered to the implant. Stimulation ended when animals left the stimulation chamber. Animals were tested for 5 minutes. Time in stimulation-assigned side and no stimulation side were quantified along with distance traveled in each chamber.

8) Grooming behavior assay: Animals were placed from home cage into separate clean, novel amber cages separated by dividers for 15 minutes. Videos were recorded during the interaction and analyzed post-hoc by two blinded coders for specified behavioral activity using Anvil 6.0

video annotation research tool software (5). Coding began after a 5-minute habituation period. Videos were scored for time self-grooming and averaged between coders.

In vivo optogenetic Stimulation:

Stimulation patterns were delivered from the PlexBright 4 Channel Optogenetic Controller and controlled by Radiant v2 Software (Plexon, Dallas TX). The optogenetics controller box was attached to the PlexBright Dual LED, rotatable commutator in which a blue (465 nm) or orange (620 nm) LED lights were affixed. PlexBright Optical Patch Cables (.5NA) were then attached to the commutator. Prior to studies, light power emitting from the patch cables was measured using the PlexBright Light Measurement Kit (Plexon, Dallas TX). For studies in which stimulation was triggered as a result of a distinct behavior (ie: RT-PP), we used the AnyMaze AMI2 box (AnyMaze Wood dale IL, USA) to trigger on and off stimulation. In blue-light stimulation studies, animals received 20Hz blue light stimulation (Plexon, Dallas, TX, USA) in a 5 second on 5 second off pattern at 10-13mW. For inhibition studies, animals received a constant orange light (~10mW). One week following implantation surgery, mice were habituated to dummy patch cables in 20-minute bins for 3 consecutive days. On test day, the patch cable was connected to fiber optic implants and animals were habituated for at least 5 minutes before the start of the test.

Microinfusion Studies:

After a 7-day surgery recovery period, animals were habituated for 3 consecutive days in which animals were restrained in increasing amounts (30s, 60s, 120s) and dummy cannula were replaced daily to prevent blocking and habituate animals to restraint. JZL184 was bilaterally infused into the NAc using a bilateral, 4mm cut length internal, infusion cannula (C235IS-5/SPC, Plastics One, Roanoke VA) at a dose of 0 or 5 μg/μL and at a volume of 0.2μL per hemisphere over 1 min as previously described (6). One additional minute was allowed for diffusion of drug

into the brain tissue. 30 minutes following infusion, animals were placed into the 3-chamber SI task.

Exclusion Criteria:

For all viral and implant studies, animals were excluded based on *a priori* standards. The injection site of all viral injections was identified by the presence of GFP or eYFP fluorescent marker. For all fiber optic and microinfusion implantations, location was determined by implantation track. Animals were excluded from all data sets if the viral expression or implantation was not in the targeted regions. For all in vivo studies the location of the viral expression and implantation is displayed in (**Figure S2)**. *Shank3B*$^{-/-}$ and WT controls were genotyped following sacrifice for confirmation. In all data sets, data were analyzed using Grubbs' outlier test (alpha = 0.05) and removed accordingly. If animals were excluded from a behavioral test for outlying data points, all data collected from the animal in the experiment were removed.

Statistics:

Data represented as means +/- SEM, and individual plot points overlaid on a mean bar graph. Statistical analysis conducted using Prism 8 (Graphpad, La Jolla, CA). Statistical tests and parameters are indicated in figure legends. Significance set at alpha=0.05.

Study Approval:

All studies were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and approved by the Vanderbilt University Institutional Animal Care and Use Committee.

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