Figure S1, Related to Figure 1

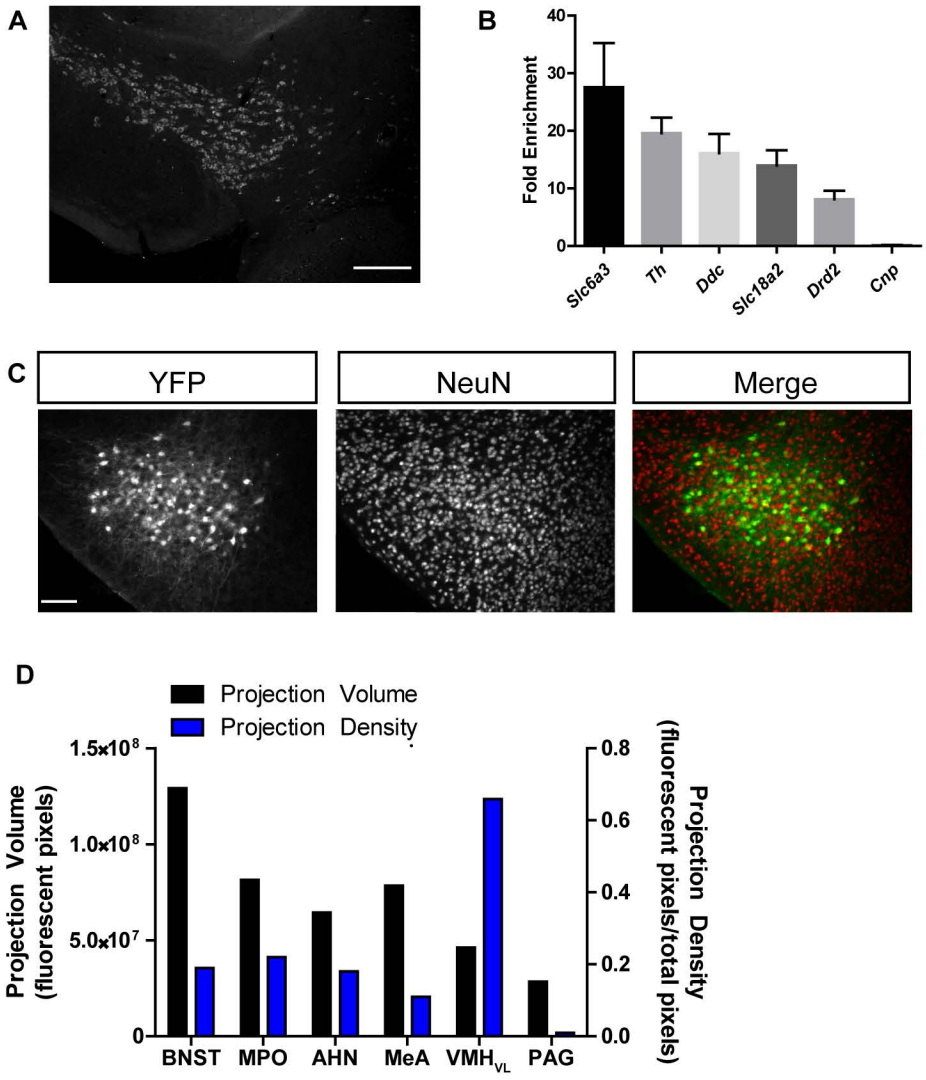

Figure S1.
(A) Immunohistochemistry for Rpl22-HA in DAT neurons of the VTA. Scale bar: 250 µm. (B) Fold enrichment (IP compared to input) of specific mRNAs isolated from VTA-DAT neurons. Data are represented as mean ± SEM. (C) Immunohistochemistry in the PMv for DAT neurons expressing YFP and the neuronal marker NeuN. Scale bar: 100 µm. (D) Quantification of DAT-$PM_V$ projections utilizing data from the Allen Brain Institute Connectivity Atlas. Projection Volume = total fluorescent pixels from labeled axon fibers in the indicated target structure. Projection Density = fluorescent pixels normalized to the total number of pixels in the target structure.

Figure S2, Related to Figure 2

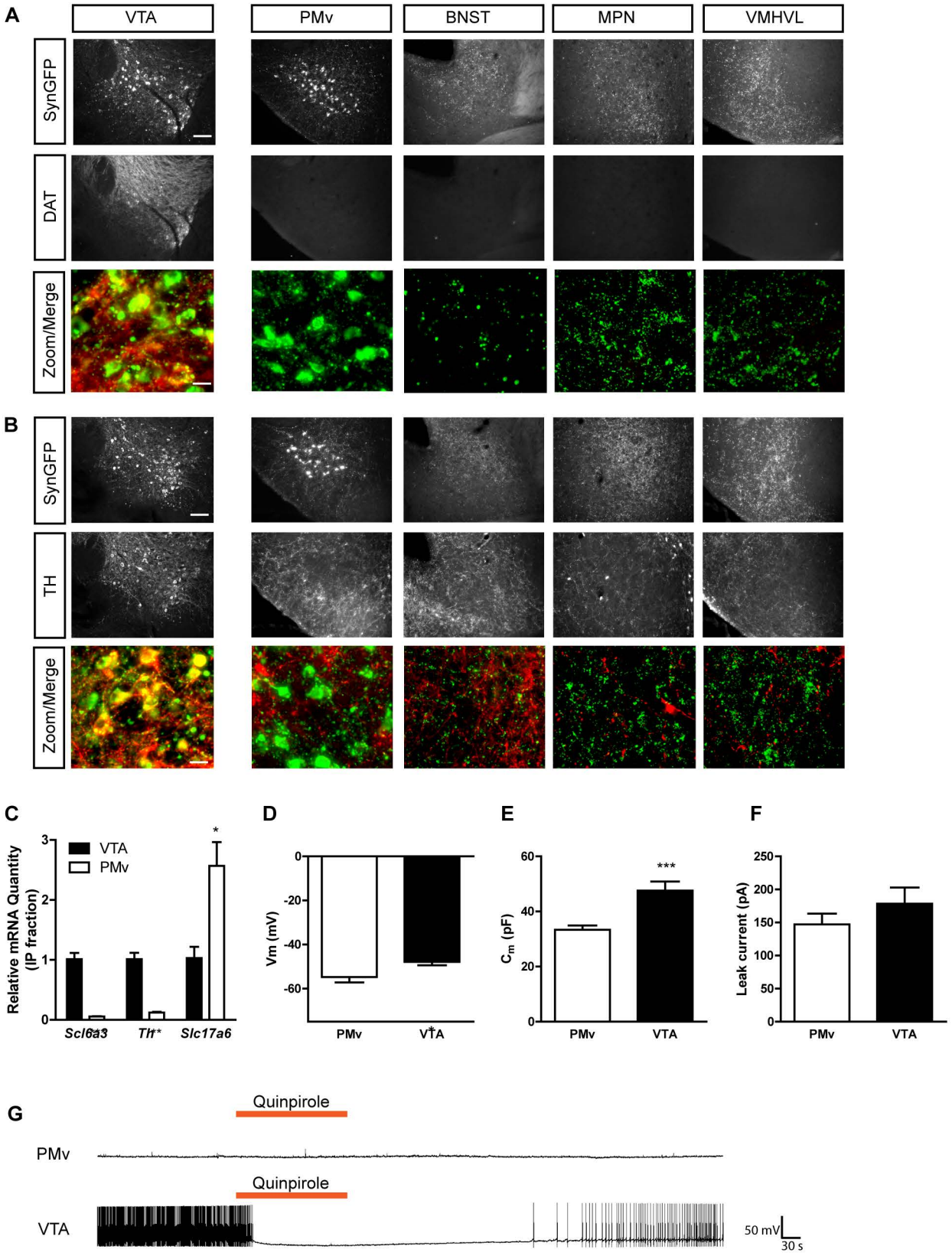

Figure S2.
Immunostaining for DAT (A) and TH (B) revealed no detectable protein in PMv-DAT cell bodies or projections, though staining was readily apparent in VTA cell bodies. TH protein was found in the vicinity of many synaptoGFP-labeled fibers in some areas, but no overlap was observed (see zoom and merge of representative regions). Scale bar: 100 µm; scale bar for zoom/merge: 10 µm. (C) Comparison of relative mRNA quantity for the indicated transcripts isolated from DAT-expressing neurons in the PMv or VTA. (D) Vm was significantly more depolarized in VTA-DAT neurons compared to PMv-DAT neurons. (E) Cm was significantly higher in VTA-DAT neruons compared to PMv-DAT neurons. (F) Leak current measured during hyperpolarizing voltage steps was unchanged. (G) Quinpirole hyperpolarized and reduced firing in VTA-DAT neurons, but had no noticable effect on PMv-DAT neurons. For all panels: Student's t test: *$p<0.05$, **$p<0.01$, ***$p<0.001$. Data are represented as mean ± SEM.

Figure S3, Related to Figure 3

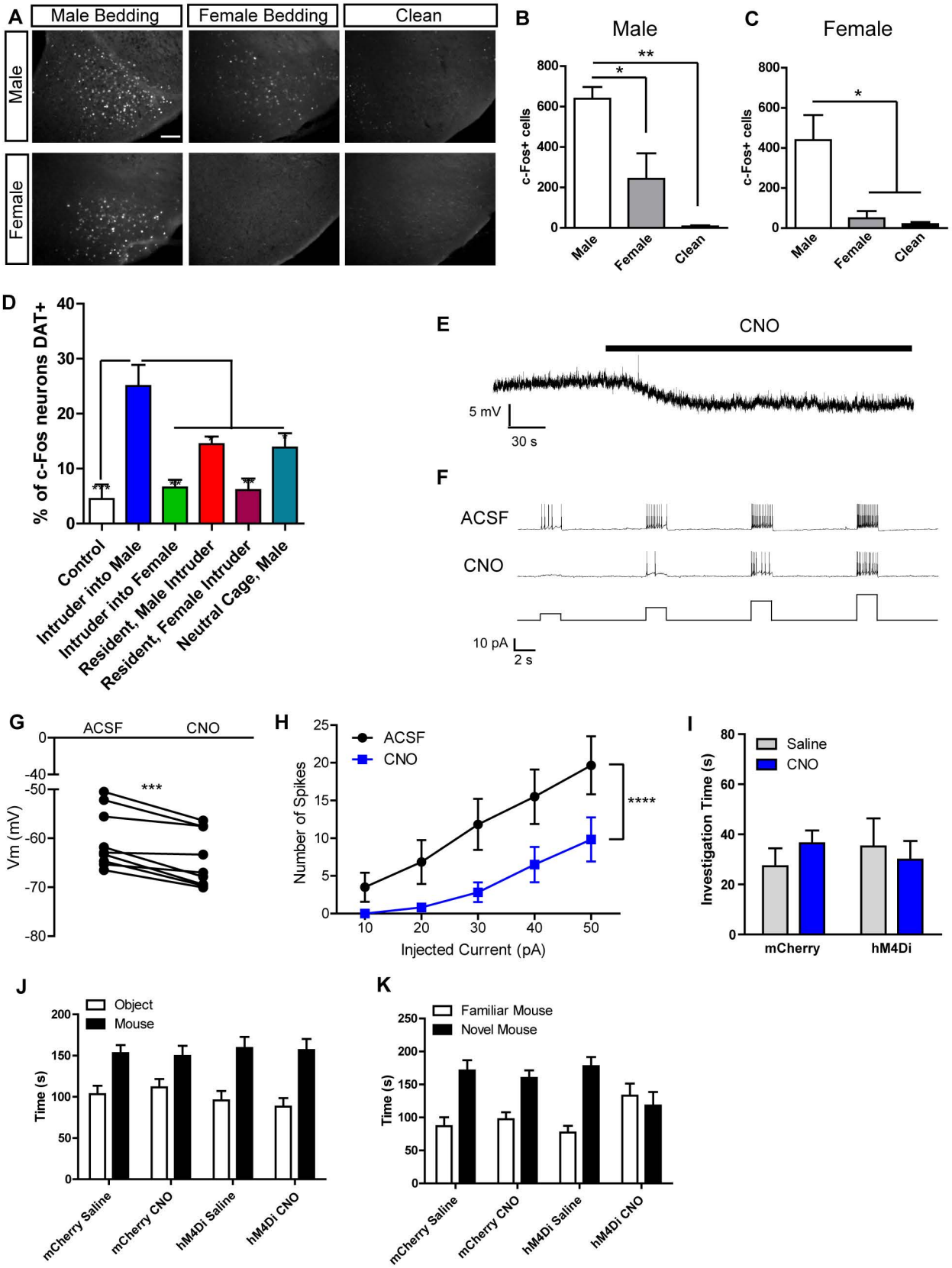

Figure S3.
(A) Immunostaining for Fos reveals activation of the PMv in male mice when exposed to male or female odorants (soiled bedding) and in female mice when exposed to male odorants. Scale bar: 100 µm. (B-C) Quantification of Fos-positive cells after odorant exposure. (n=3-4 mice/group; one-way ANOVA, Male: $F_{(2,6)}$ = 15.94, p<0.01, Female: $F_{(2,8)}$ =8.132, p<0.05). (D) The percent of Fos+ neurons also YFP labeled (DAT-Cre+) following social encounter. (n=3 mice/group; One-way ANOVA; $F_{(5,12)}$ = 9.829, p<0.001; for panels B-D: Tukey's Multiple Comparison Test: *p<0.05, **p<0.01, ***p<0.001). (E-F) Example traces showing hyperpolarization (E) and reduced excitability during current injection (F) following bath CNO application in hM4D-YFP-expressing PMv-DAT neurons. (G) Quantification of change in Vm following CNO application.(n=9 cells, paired t-test, ***p<0.001.) (H) Number of spikes elicited by 2 second current injection before and after CNO application. (n=6 cells, 2-way repeated measures ANOVA, $F_{(1,25)}$=51.35, ****p<0.0001.) (I) Inhibition of PMv-DAT neurons did not affect investigation time during a neutral cage encounter (n=6 mice/group). (J-K) Time spent in each chamber of the 3-chamber assay during Trial 1 (J) and Trial 2 (K). Data are represented as mean ± SEM.

Figure S4, Related to Figure 4

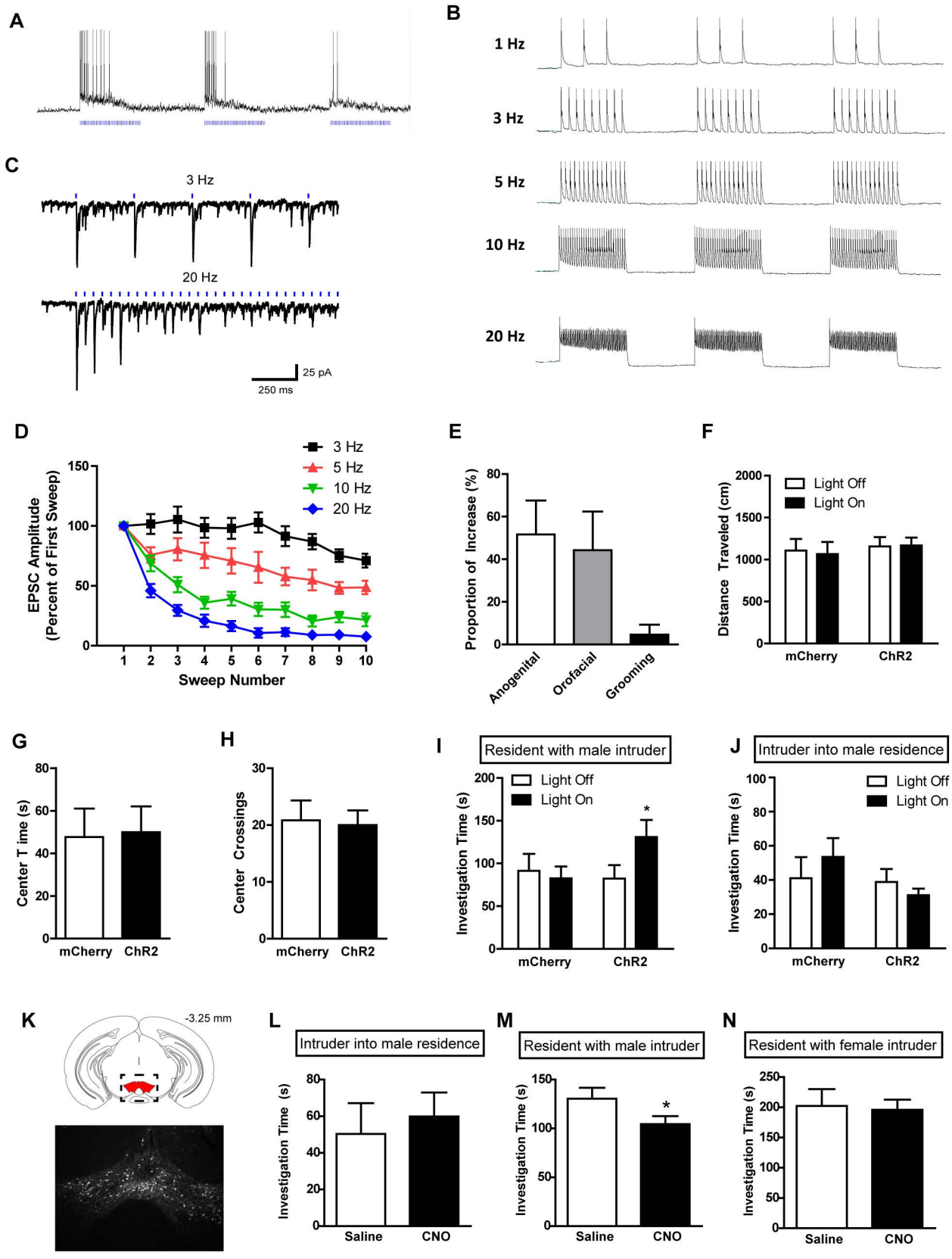

Figure S4.
(A) Example trace from a $VMH_{VL}$ neuron showing reduced excitation following repeated bouts of 20 Hz stimulation of incoming PMv-DAT fibers expressing ChR2. (B) Example traces of light-evoked action potentials in a PMv-DAT neuron at various frequencies. (C) Example traces from recordings in the $VMH_{VL}$ showing light-evoked EPSCs during the beginning of the first sweep at 3 or 20 Hz, illustrating significant rundown at the higher frequency. (D) Quantification of the average EPSC amplitude (first 5 events/sweep) across sweeps; each sweep was 3 seconds of light stimulation at the indicated frequency followed by 3 seconds of light off between sweeps (n=12 cells; 2-way repeated measures ANOVA Frequency x Sweep: $F_{(36, 440)}$=2.68, p<0.0001). (E) Proportion of the increased investigation time in ChR2 animals during the co-habitation assay that was attributed to each type of investigation. (F) Distance traveled was unaffected by light stimulation during the co-habitation assay. (G-H) Time spent in the center of an open field and number of center crossings were not affected by light stimulation in ChR2 vs control animals. (I) Light stimulation of PMv-DAT neurons expressing ChR2 during a standard resident-intruder assay increased investigation time by a resident animal compared to mCherry control (n=6 mice/group; 2-way repeated measures ANOVA Virus x Light: $F_{(1,10)}$=5.03, p<0.05. Bonferroni Multiple Comparisons *p<0.05). (J) Light stimulation of PMv-DAT neurons in intruder animals had no effect on investigation time. (K) Atlas image (top) and representative histology image (bottom) showing hM4Di-YFP expression in the VTA. Scale bar: 250 µm. (L) Social investigation of a resident male by an intruder male was unaffected by CNO/hM4Di inhibition of VTA-DAT neurons. (M) Social investigation of a male intruder by a resident male was decreased by VTA-DAT neuron inhibition (n=8 animals/group; Paired t test *p<0.05). (N) Social investigation of a female intruder was unaffected. Data are represented as mean ± SEM.

## Supplemental Experimental Procedures

### Mice

All procedures were approved and conducted in accordance to the guidelines of the Institutional Animal Care and Use Committee of the University of Washington. All mice were on a C57BL/6J background. Slc6a3Cre/+ (DAT-Cre) mice were as described (Zhuang et al., 2005). $Rpl22^{HA/+}$ (RiboTag) mice were as described (Sanz et al., 2009). $Slc17a6^{lox/lox}$ (vGlut2 lox) mice were as described (Hnasko et al., 2010); these mice were crossed with $Slc6a3^{icre/+}$ mice (DAT-ires-Cre; (Backman et al., 2006) Jackson Labs: B6.SJL-Slc6a3tm1.1(cre)Bkmn/J) to generate DAT-vGlut2 knockout animals ($Slc17a6^{lox/lox}$; $Slc6a3^{icre/+}$). Control mice were $Slc17a6^{lox/+}$; $Slc6a3^{icre/+}$. Mice were housed under a 12-hour light-dark cycle with ad libitum food and water access. Mice 8 weeks or older were used for all experiments except slice electrophysiology, where 5- to 8-week-old mice were used. See table below for age and weight ranges and housing conditions for all behavioral experiments. All interacting animals were wild-type C57BL/6J.

| Experiment(s) | Figure | Experimental Animal | | | Interacting Animal | | |
|---|---|---|---|---|---|---|---|
| | | Age Range | Weight Range | Housing Condition | Age Range | Weight Range | Housing Condition |
| Social Encounter Fos, Resident/Intruder | 3, 4 | 12-14 wks (Resident) | 27-29g | Single | 8-10 wks | 23-25g (male) 20-24g (female) | Grouped |
| | | 8-10 wks (Intruder) | 23-25g | Grouped | 12-14 wks (male), 10-12 wks (female) | 27-29g (male), 22-24g (female) | Single |
| 3 Chamber | 3 | 10-12 wks | 25-27g | Grouped | 8-10 wks | 23-25g | Grouped |
| Co-habitation, object, RTPP, open field | 4 | 10-12 wks | 25-27g | Grouped | 10-12 wks | 25-27g | Grouped |

### Viruses

All AAV viruses were produced in house with titers of 1-3 $x10^{12}$ particles per mL as described (Gore et al., 2013).

### Surgery

Mice were anesthetized with isoflurane before injection of AAV. Coordinates for the $PM_V$ were intentionally rostral to avoid infecting DAT cells in the midbrain (from bregma in mm, A-P: -1.9,

M-L: ±0.5, D-V: -5.4). Coordinates for the VTA were deliberately caudal to avoid infecting DAT cells in the PMV (from bregma in mm, A-P: -3.75, M-L: ±0.5, D-V: -4.5). For in vivo ChR2 experiments mice were injected unilaterally and implanted with fiber optic cannulae manufactured in house as described (Sparta et al., 2012) (from bregma in mm, A-P: -2.3, M-L: -+0.5, D-V: -5.0). Recovery was allowed for 2 weeks prior to behavioral testing or slice electrophysiology. Correct injection sites were confirmed using immunohistochemistry on brain tissue sections collected after behavioral testing. Only mice with correct targeting were included.

**Immunohistochemistry**

Primary antibodies were against GFP (mouse, 1:1000, Invitrogen A11120; or rabbit, 1:1000, Invitrogen A11122), c-Fos (rabbit, 1:2000, Millipore ABE457) TH (mouse, 1:1000, Millipore MAB318), DAT (rat, 1:1000, Millipore, MAB369), HA (mouse, 1:1000, ABM G036), or NeuN (rabbit, 1:2000, AbCam 177487). Secondary antibodies were conjugated to DyLight488 or CY3 (1:250, Jackson Immunolabs). All staining was done on free-floating sections (overnight primary incubation at 4°C), with the exception of DAT,TH, and HA staining, for which sections were mounted on slides and boiled for 20 min in antigen retrieval solution (10 mM sodium citrate, 0.05% Tween 20, pH 6.0) prior to staining (48-hr primary incubation at 4°C).

**RiboTag**

Immunoprecipitation was performed as described previously (Sanz et al., 2009). Briefly, 1 mm x 1 mm punches of PMV and VTA were removed and flash-frozen. Tissue from 2-3 animals was pooled and homogenized and incubated with 5 µl of anti-HA antibody (Covance) coupled to 200 µl of magnetic beads (Pierce) overnight at 4°C. Following elution from magnetic beads, RNA from both immunoprecipitated (IP) samples and input samples was obtained using the RNeasy micro kit (Qiagen) according to manufacturer's directions. Total RNA was quantified using a Ribogreen RNA assay kit (Invitrogen). cDNA was generated using oligo dT primers from equal amounts of starting RNA, according to manufacturer protocol (Invitrogen). For qRT-PCR analysis, TaqMan (Applied Biosystems) primers were used to detect gene expression levels. Relative expression values were obtained using the comparative CT method and normalized to Actb mRNA levels. Fold enrichment was calculated as the IP versus input ratio and represented the amount of the transcript in the targeted cell type (IP) when compared to equal amounts of RNA from the input.

**Projection analysis**

Projection data was obtained from the Allen Institute for Brain Science Mouse Connectivity Atlas Application Programming Interface (Oh et al., 2014). Total fluorescent pixel count and projection density (fluorescent pixel count/ total pixel count) for each defined target region in *Slc6a3*$^{Cre/+}$ mice injected in the $PM_V$ with AAV1-FLEX-EGFP were queried using Python scripts created with the aid of Allen Institute staff. Detailed Python scripts available upon request.

**Electrophysiology**

Whole-cell recordings were made using an Axopatch 700B amplifier (Molecular Devices) with filtering at 1 KHz using 4-6 MΩ electrodes filled with an internal solution containing (in mM): 130 K-gluconate, 10 HEPES, 5 NaCl, 1 EGTA, 5 Mg-ATP, 0.5 Na-GTP, pH 7.3, 280 mOsm. Coronal brain slices (250 µm) were prepared from 5-8 week old mice in an ice slush solution containing (in mM): 92 NMDG, 2.5 KCl, 1.25 $NaH_2PO_4$, 30 $NaHCO_3$, 20 HEPES, 25 glucose, 2 thiouria, 5 Na-ascorbate, 3 Na-pyruvate, 0.5 $CaCl_2$, 10 $MgSO_4$, pH 7.3-7.4 (Ting et al., 2014). Slices recovered for ~12 min in the same solution at 32°C and then were transferred to a room temperature solution including (in mM): 92 NaCl, 2.5 KCl, 1.25 $NaH_2PO_4$, 30 $NaHCO_3$, 20 HEPES, 25 glucose, 2 thiouria, 5 Na-ascorbate, 3 Na-pyruvate, 2 $CaCl_2$, 2 $MgSO_4$. Slices recovered for an additional 45 min. All solutions were continually bubbled with $O_2/CO_2$, and all recordings were made in ACSF at 32°C continually perfused over slices at a rate of ~2 ml/min. Light-evoked synaptic transmission was induced with 5-ms light pulses delivered from an optic fiber placed directly in the bath. $I_h$ currents were induced by 2-s hyperpolarizing voltage steps from -70 mV to -120 mV. SK currents were induced by depolarizing voltage steps from -70 to 0 mV. Capacitance measurements were calculated by Clampex software using 5 mV hyperpolarizing steps.

**Voltammetry**

Slices were prepared as described above. Fast-scan cyclic voltammetry was performed using carbon-fiber microelectrodes encased in fused-silica capillary tubing (Polymicro Technologies) as described (Clark et al., 2010). 5-ms light stimuli were delivered as described for electrophysiology. A variety of stimulus parameters were used in an attempt to measure dopamine release (5 to 100 stimuli at frequencies of 5 to 30 Hz). Example trace of successful release in the NAc from VTA fibers was elicited with 5 stimuli at 20 Hz.

**Fos Induction**

For odorant induction of Fos experimental animals were singly housed and were transferred to a clean cage each day for 3 days prior to the experiment in order to acclimate the mice to cage changes. To generate soiled bedding a single wild-type male or female animal was housed in a cage with no bedding changes for one week. These animals were removed immediately prior to the experiment. Experimental animals were transferred to a cage containing soiled bedding and were perfused after 90 min.

For social encounter Fos experiments mice were assigned to the resident, intruder, or control condition. Resident animals were singly housed for at least two weeks (final 7 days with no cage change), while intruder and control mice were group housed. Animals experienced a 20-min social encounter with an appropriately matched resident or intruder animal, and were euthanized and perfused 90 min following the start of the encounter. Control animals remained in their home cage. For the neutral cage encounter, “resident” and “intruder” animals were housed and treated as described above, but the encounter took place in a clean cage. The “resident” animal was placed in the cage first, followed by the “intruder” animal within one minute.

Fos-positive neurons were identified and counted automatically using ImageJ software. Virally transduced DAT neurons and Fos-positive DAT neurons were counted by hand by an experienced investigator blind to condition.

**Behavior**

For resident/intruder encounters, resident mice were singly housed for at least two weeks (final 7 days with no cage change), were sexually experienced, and were 3-4 weeks older than intruder animals, which were group housed. Female intruders were not staged for sexual receptivity. Encounters took place during the light cycle. Saline or CNO (1 mg/kg) was administered intraperitoneally 40 min prior to the start of the encounter. Each mouse received saline and CNO on subsequent days (order of administration was counterbalanced across groups) and encountered a different resident or intruder mouse on each day. Encounters lasted for 10 min, and videos were hand scored by an experienced investigator blind to treatment. Social behaviors scored included anogenital sniffing, oronasal sniffing, following, and grooming. Overt sexual and aggressive behaviors (chasing, mounting, cornering, tail rattle, and fighting)

were not seen in intruder mice and were rarely seen in resident mice, and were not included in the scoring of “social investigation.”

For the 3-chamber assay mice were administered saline or CNO (counterbalanced) 40 min prior to the start of the experiment. The arena was a white Plexiglas box (60 cm x 30 cm x 30 cm) divided into three equal sized chambers with clear Plexiglas dividers, each with a doorway allowing the mice to freely pass between chambers. Mice were given 10 min to explore the empty arena, then were briefly removed and returned to their home cage while the novel object (empty wire pencil cup) was introduced to one chamber and the first mouse (contained in a wire pencil cup) was introduced to the opposite chamber. The experimental animal was returned to the arena for a 10-min exploration and then briefly removed again while the novel mouse was added, before a final 10-min exploration. The first 5 min of each exploration period was scored for the time spent in each chamber.

For home cage social encounters mice implanted with fiber optics were housed with a single littermate during recovery from surgery. After the implanted mouse was connected to the fiber optic cable they were allowed a 10 minute habituation period, which was not scored, followed by a 5-min baseline period and 5 min of light stimulation (3 Hz, 5 ms, 3 s on, 3 s off). For object exploration the littermate was removed and an object (50 mL conical tube lid) was placed in the cage for at least 3 hr prior to testing.

For the real-time place preference assay mice were placed in a two-chambered arena with partial walls dividing the two sides that allowed for passage of the fiber optic cable. One side of the arena had horizontal black and white stripes, while the other side had vertical stripes. One side was randomly assigned to be paired with light stimulation, while the other was unpaired. The assay lasted for 20 min.

**Statistics**

All statistical analyses were performed using Prism software (GraphPad). For comparison of two groups an unpaired Student’s t test was used, except where noted. For comparison of multiple groups a one-way ANOVA was used, followed by Tukey’s post hoc analysis. For comparison of two or more groups across treatment condition or time a two-way repeated measure ANOVA was used, followed by Bonferroni post-hoc analysis.

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