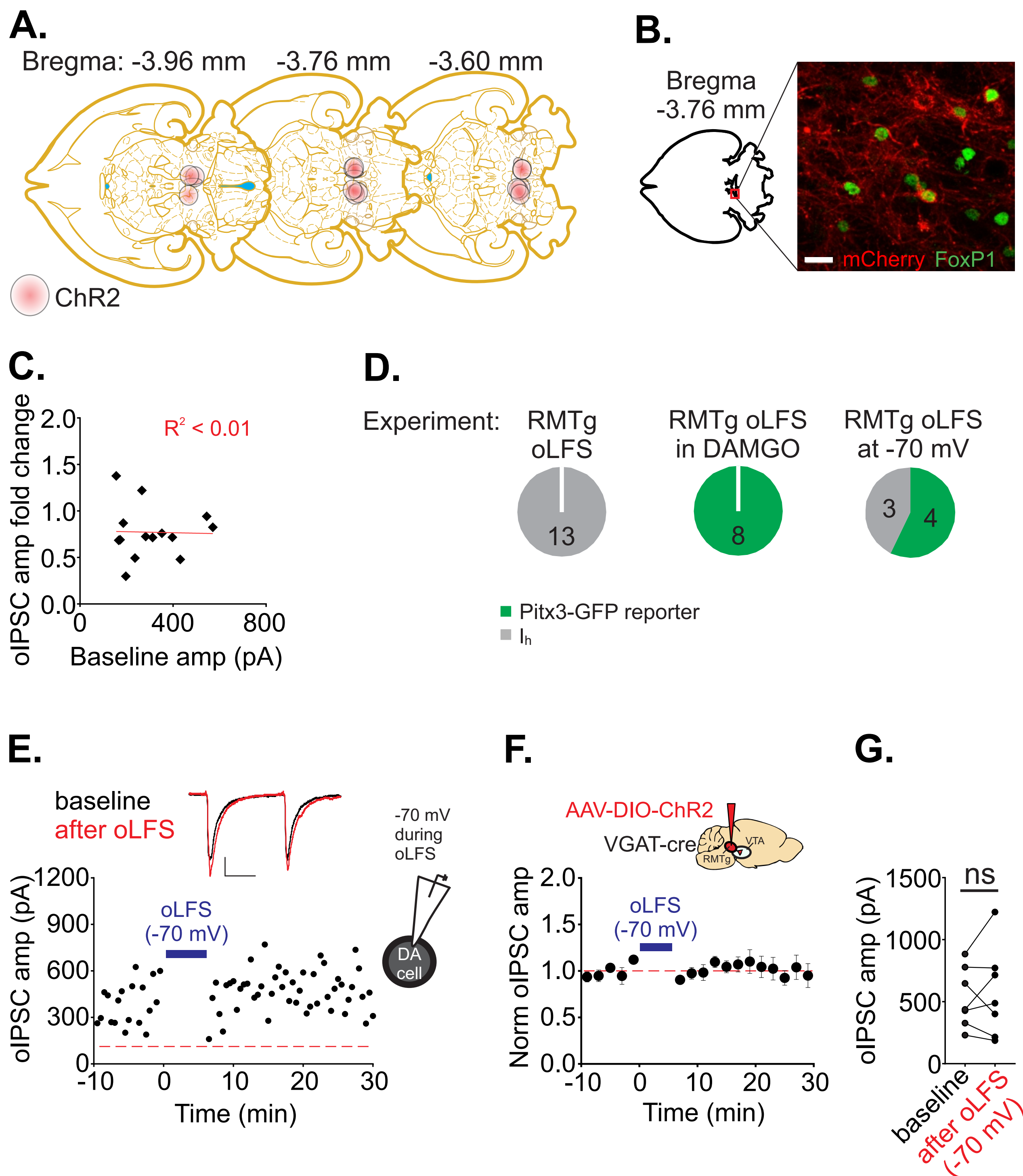


Figure S1. Relating to Figure 1.
A. Injection site maps of ChR2 expression in the RMTg for LTD experiment (horizontal sections). Atlas images adapted with permission (Franklin, 2013). **B.** Immunostain of FoxP1 overlapping with ChR2 expression in the RMTg (Lahti et al., 2016). Calibration, 20 μm. **C.** LTD fold change plotted against magnitude of the baseline amplitude. **D.** Chart illustrating the total numbers of genetically identified dopamine cells for each experiment. **E.** Representative experiment showing RMTg oIPSCs before and after oLFS when the cell was voltage-clamped at -70 mV throughout. Inset: oIPSCs during baseline (black trace) and 10-20 min after oLFS (red trace); calibration, 20 ms, 100 pA. **F.** Time course of LTD; averaged oIPSC amplitudes before and after oLFS (n = 7 cells, 6 mice). **G.** Mean oIPSC amplitudes for each cell during a 10 min baseline period and at 10-20 min after oLFS (Baseline: 533 ± 91pA, 10-20 min after oLFS: 571 ± 138pA; $p = 0.64$, paired t-test. Data are represented as mean ± SEM.

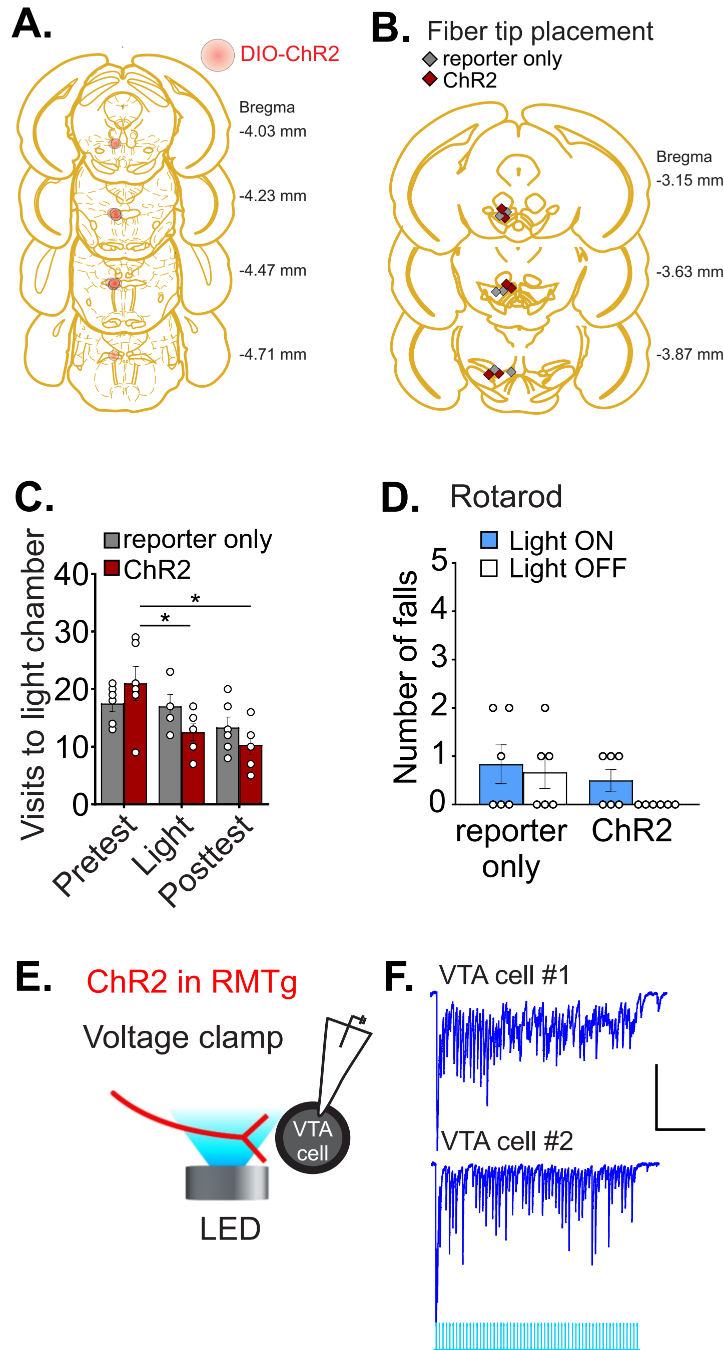
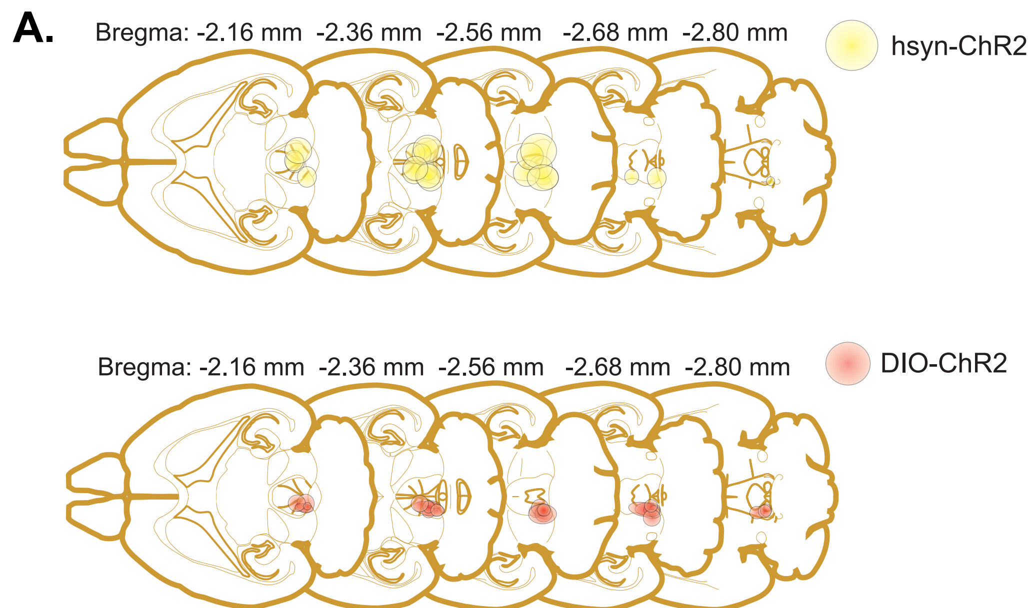
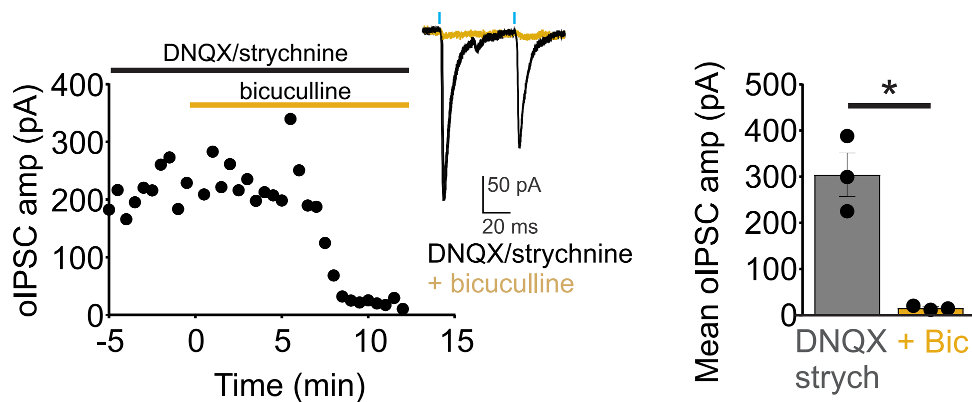
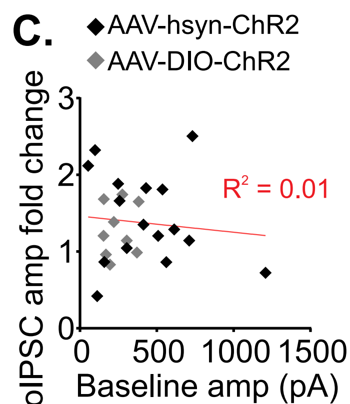


Figure S2. Relating to Figure 2.
A. Injection site maps of ChR2 expression in the RMTg for behavioral experiments (coronal sections). **B.** Location of optical fiber tip in the VTA for all behaviorally-tested mice. **C.** Number of visits to the light chamber decreased in ChR2-expressing mice compared to reporter-only mice on the light test and the posttest days (n = 6 mice/group). **D.** Performance on a rotarod did not differ between groups in light ON or light OFF conditions (F (1, 20) = 3.10, p = 0.09, n = 6 mice/group). **E.** Diagram of experiment in F. **F.** Example traces in voltage-clamp from two different dopamine neurons from different animals when RMTg-labeled GABAergic afferents were stimulated optically at 60Hz; calibration, 250 ms, 500 pA. On average, 60 Hz light stimulation of RMTg afferents evoked 33 ± 8 IPSCs per second IPSCs in individual VTA cells. Data are represented as mean ± SEM.







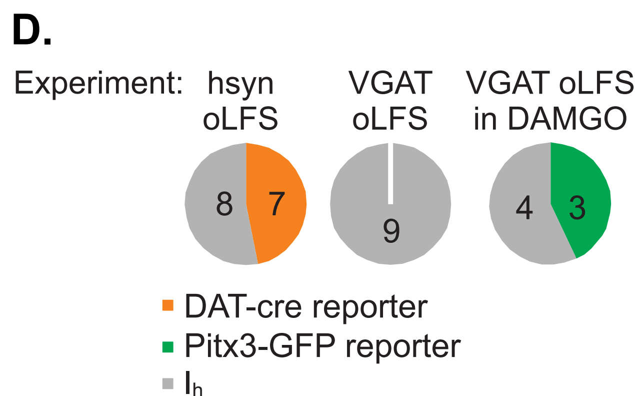
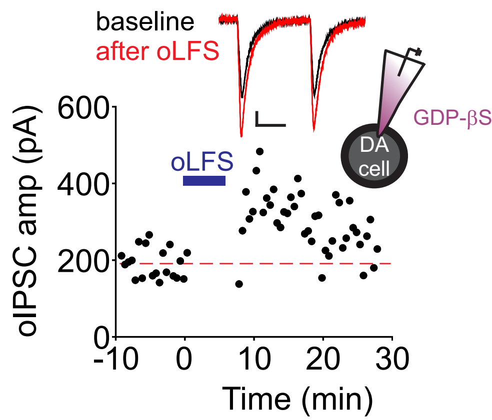
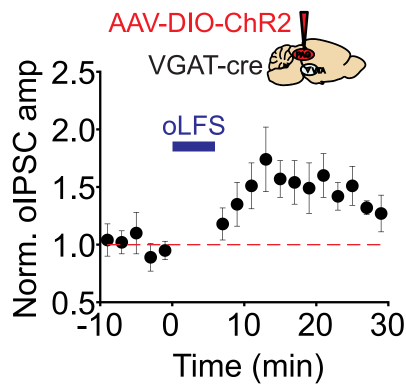
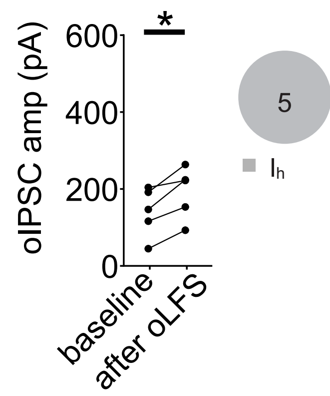
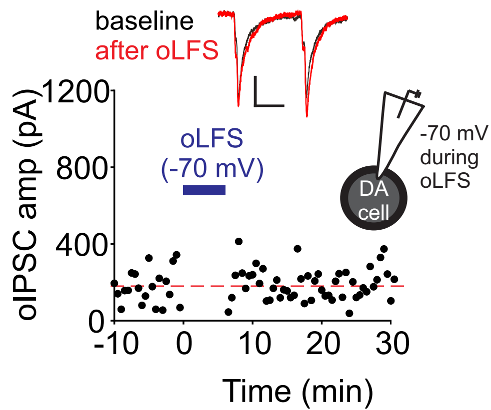


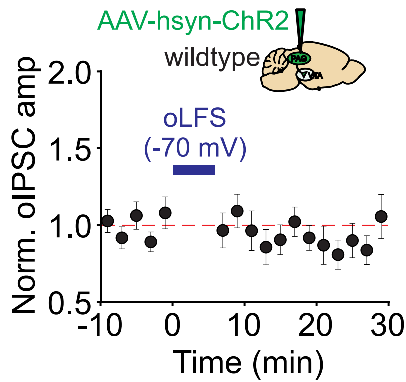
Figure S3. Relating to Figure 3.
A. Injection site maps of ChR2 expression in the PAG for LTP experiments (horizontal sections). **B.** Voltage-clamp recording from a VTA cell showing PAG oIPSCs in DNQX and strychnine before and after bath application of bicuculline (left and middle panels) and average amplitudes in similar experiments (n = 3 cells, right panel). **C.** oLFS LTP fold change from PAG afferents to VTA neurons plotted against the baseline amplitude. **D.** Chart of total numbers of genetically identified dopamine cells used in each experiment.
Data are represented as mean ± SEM.

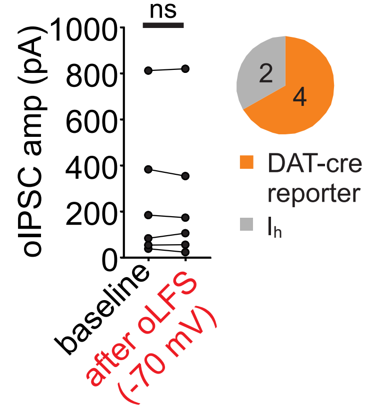


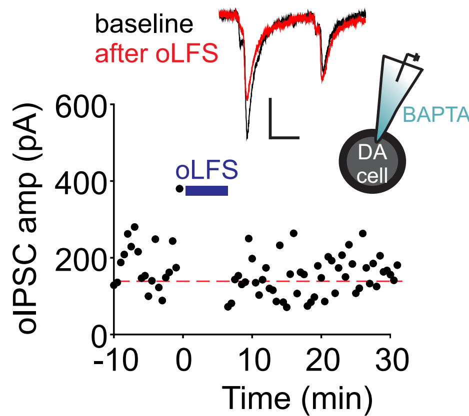


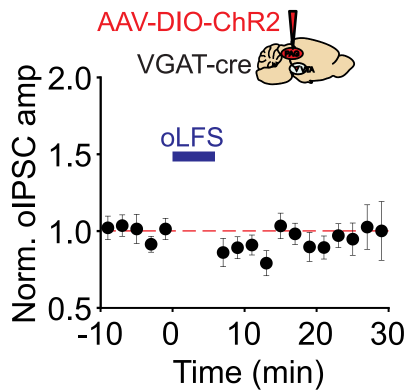


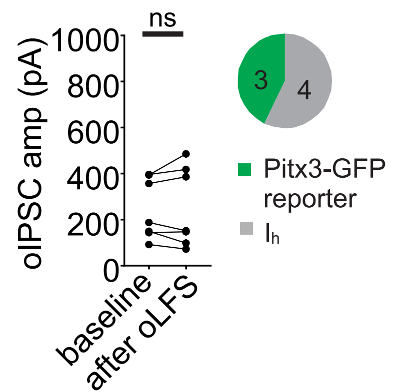












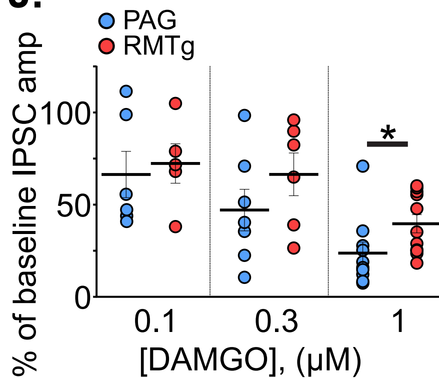


Figure S4, relating to Figure 3

Figure S4. Relating to Figure 3.
A. Representative experiment showing LTP after oLFS of PAG afferents recorded in a VTA cell with 1 mM GDP-βS included in the pipette solution. Inset: oIPSCs during baseline (black trace) and 10-20 min after oLFS (red trace). Calibration for insets: 50 pA, 20 ms. **B.** Time course of LTP in cells with 1 mM GDP-βS; averaged oIPSC amplitudes before and after oLFS (n = 5 cells, 4 mice). **C.** Left, mean oIPSC amplitudes for each cell during a 10 min baseline period and at 10-20 min after oLFS with 1 mM GDP-βS (baseline: 141 ± 29pA, 10-20 min after oLFS: 191 ± 90; p = 0.01, paired t-test). Right, chart showing that I_h was the criterion used for all of these experiments. **D.** Representative experiment in a VTA cell voltage-clamped at -70 mV throughout. Inset: oIPSCs during baseline (black trace) and 10-20 min after oLFS (red trace). **E.** Time course of oLFS at -70 mV; averaged oIPSC amplitudes before and after oLFS (n = 6 cells, 4 mice). **F.** Left, mean oIPSC amplitudes for each cell during a 10 min baseline period and at 10-20 min after oLFS without depolarization (baseline: 259 ± 122pA, 10-20 min after oLFS: 255 ± 122 pA, p = 0.614, paired t-test). Right, chart of numbers of genetically identified dopamine cells in this experiment. **G.** Representative experiment showing PAG oIPSCs before and after oLFS recorded in a VTA cell with 30 mM BAPTA in the pipette solution. Inset: oIPSCs during baseline (black trace) and 10-20 min after oLFS (red trace). **H.** Time course of LTP in cells with 30 mM BAPTA; averaged oIPSC amplitudes before and after oLFS (n = 7 cells, 5 mice). **I.** Left, mean oIPSC amplitudes for each cell during a 10 min baseline period and at 10-20 min after oLFS with 30 mM BAPTA (baseline: 246 ± 50pA, 10-20 min after oLFS: 251 ± 65pA; p = 0.765, paired t-test). Right, chart of numbers of genetically identified dopamine cells in this experiment. **J.** DAMGO dose-response curve with for RMTg (red) and PAG (blue) oIPSCs recorded in VTA cells. At the highest dose (1 μM), PAG inputs are depressed more than RMTg inputs onto VTA cells (p = 0.0366, unpaired t-test, PAG: n = 23 cells, 13 mice; RMTg: n = 22 cells, 14 mice). Data are represented as mean ± SEM; calibration, 20 ms, 100 pA.

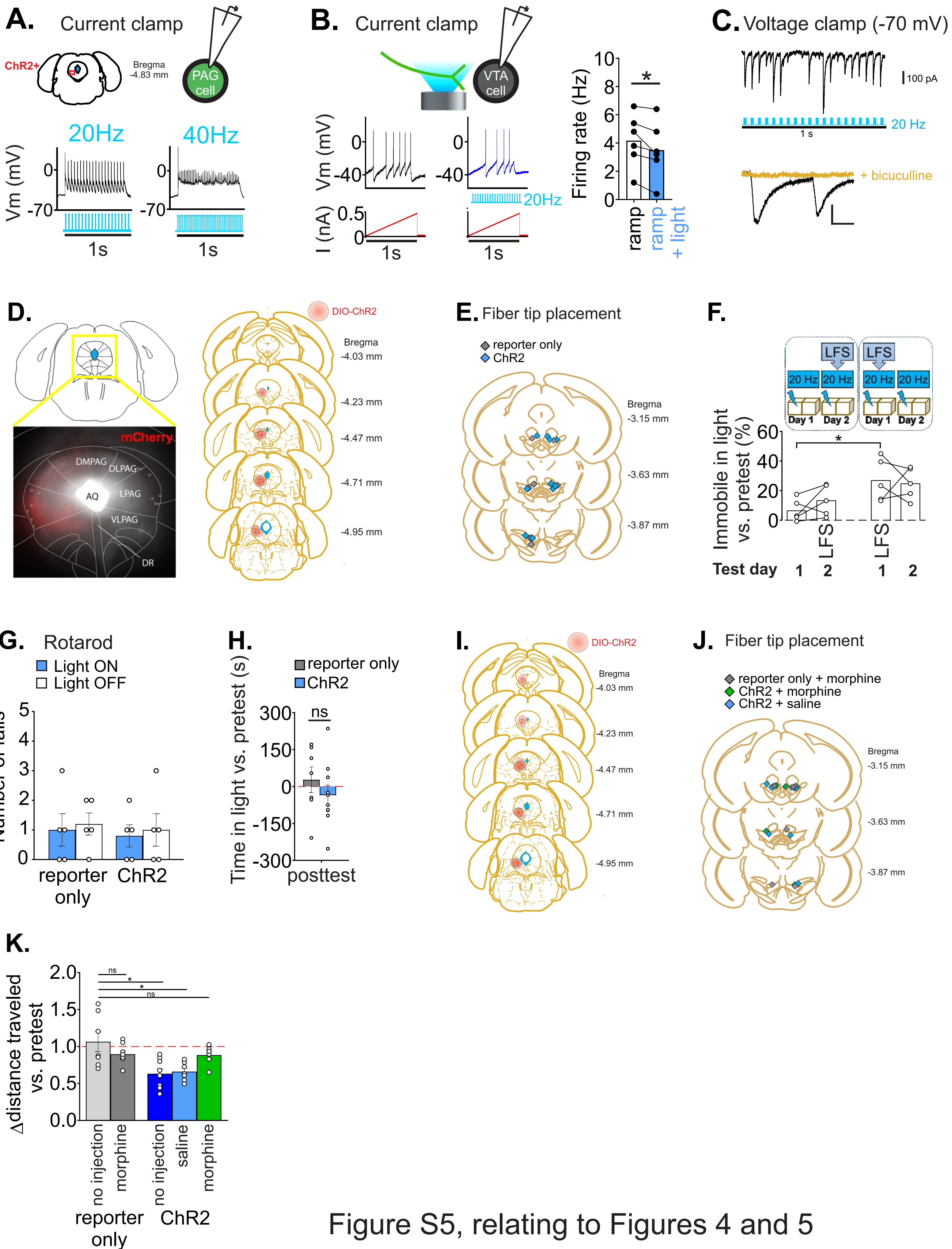


Figure S5, relating to Figures 4 and 5

Figure S5. Relating to Figures 4-5.
A. Representative action potential firing recorded in a $VGAT^+$, $Chr2^+$ cell in the PAG in response to 20 or 40 Hz trains of LED pulses. **B.** 20 Hz stimulation of PAG oIPSCs in a VTA dopamine cell reduces evoked firing. Left panel, representative current clamp recording from a VTA neuron receiving alternating ramps of current injection without light stimulation (bottom left) and with concurrent 20 Hz light stimulation of PAG oIPSCs (bottom middle). Right panel, mean firing rate without and with trains of light (Baseline 4.2 ± 0.8 Hz, 10-20 min after oLFS 3.5 ± 0.8 Hz; p = 0.022, n = 6 cells, 4 mice; paired t-test). **C.** Top, example of GABAergic synaptic currents recorded in a VTA cell during optical stimulation of PAG terminals at 20 Hz. Calibration, 100 pA, 200 ms. Bottom, representative voltage clamp recording of PAG oIPSCs recorded using a potassium gluconate pipette solution in a VTA cell; oIPSCs are blocked with the $GABA_AR$ antagonist, bicuculline. Calibration: 25 pA, 20 ms. **D.** Injection site maps of ChR2 expression in the PAG, and **E.** location of optical fiber tip in the VTA for drug-free (no morphine) RTPP experiments, coronal sections. **F.** Data from Figure 4D immobility tests were re-analyzed using a between-subjects metric to determine if test order might mask an effect of 1 Hz oLFS to PAG afferents. Mice receiving 1 Hz oLFS prior to the first 20 Hz light test exhibited more immobility (left side of bar graph) than mice that first received 20 Hz light alone (F(3, 16) = 3.80, p = 0.031), suggesting the possibility that LFS preconditioning enhanced the immobility phenotype. **G.** Performance on a rotarod did not differ between PAG ChR2-expressing and reporter-only groups in light ON or light OFF conditions (F(1, 8) = 0.108, p = 0.75, n = 5 mice/group). **H.** Time spent in the light-paired chamber vs. pretest during a post-test conducted 24h after the second light test. **I.** Injection site maps of ChR2 expression in the PAG, and **J.** location of optical fiber tip in the VTA for RTPP with morphine, coronal sections. **K.** Distance traveled normalized to pretest values for all groups reported in Figures 4 and 5.
Data are represented as mean ± SEM.