Supplementary Materials

Differential control of learning and anxiety along the dorso-ventral axis of the dentate gyrus

Authors:
Mazen A. Kheirbek, Liam J. Drew, Nesha S. Burghardt, Daniel O. Costantini, Lindsay Tannenholz, Susanne E. Ahmari, Hongkui Zeng, André A. Fenton and René Hen

Extended Experimental Procedures

Mice and Stereotactic Surgery

ROSA26-CAG-stop^{flox}-ChR2(H134R)- tdTomato (Ai27), ROSA26-CAG-stop^{flox}- eNpHR3.0-YFP (Ai39) and POMC-Cre were generated as previously described(Madisen et al., 2012; McHugh et al., 2007). Breeding Ai27 and Ai39 homozygous mice with POMC-Cre heterozygotes generated male POMC-ChR2 and POMC-eNpHR3.0 heterozygote experimental and ChR2 heterozygote and eNpHR3.0 heterozygote littermate single transgenic control mice. Mice were housed 3-5/cage on a 12 h (6 a.m. to 6 p.m.) light–dark colony room at 22 °C and had free access to food and water. Male mice were surgically implanted with fiber optic cannulas at 8-10 weeks of age using published protocols (41), and behavioral experiments commenced >3 wks after surgery to allow for recovery. For surgical implantation, mice were anesthetized with 100mg/kg ketamine and 10mg/kg xylazine, and placed in a stereotaxic frame (Stoelting). Mice were implanted bilaterally with chronically dwelling optical fibers targeted to the dentate gyrus (dorsal implants: +/-1mm ML +/-1.5mm AP, -1.7mm DV, ventral implants: +/-2.5mm ML +/-3.7mm AP, -2mm DV, intermediate implants: +/-2 mm ML +/-2.9mm AP -1.9mm DV). Optical fibers were secured with anchoring screws and dental cement. After surgery, mice were returned to their home cage and monitored until recovery from surgery. >3 weeks after surgery, mice were habituated to being attached to the patch cable by handling and attaching to the cable via a zirconia sleeve, and allowed to explore a novel cage for 15 min with no light for two consecutive days. All experiments were approved by the IACUC at Columbia University and the New York State Psychiatric Institute.

Construction of Optical Fibers

We employed use of published techniques for the construction of chronically dwelling optical fibers and patch cables for behavioral procedures (Sparta et al., 2012). Briefly, a 200um core, 0.37 numerical aperture (NA) multimode fiber (ThorLabs) was threaded through and glued with epoxy to a 230um core stainless steel or zirconia multimode ferrule (Fiber Instrument Sales and Precision Fiber Products), polished and cut at ~4mm for implantation. They were then were tested for light output (~80-90% light recovery) and clean scoring of the fiber, and then each implant was numbered and the percent light recovery was noted for calibration of output for behavioral experiments. Optical patch cables were generated the same way, with the free end (~1m from ferrule) connected to a multimode FC ferrule assembly for connecting to a 1X2 Optical rotary joint (Doric lenses). The other end of the rotary joint was connected via a patch cable to either a 100mw 593.5 or 473nm laser diode (OEM laser systems) via a non-contact style laser to fiber coupler (OZ optics).

Slice electrophysiology

Brains were taken from 12-14 week old mice following halothane anesthesia and decapitation. Brains were chilled in ice-cold dissection solution (in mM: sucrose 195, NaCl 10, KCl 2.5, NaH_2PO_4 1, $NaHCO_3$ 25, glucose 10, $MgCl_2$ 6, $CaCl_2$ 0.5) prior to removal of the cerebellum and the anterior portion of the brain and then cutting of 350 μm horizontal slices on a Leica VT1000 vibratome. Slices were recovered in an intermediate solution (in mM: sucrose 70, NaCl 80, KCl

2.5, NaH_2PO_4 1, $NaHCO_3$ 25, Glucose 10, $MgCl_2$ 4, $CaCl_2$ 2) in a submerged chamber at 37 °C for 45 min and then at room temperature until use in ACSF (in mM: NaCl 124, KCl 2.5, NaH_2PO_4 1, $NaHCO_3$ 25, Glucose 20, $MgCl_2$ 1, $CaCl_2$ 2). Whole-cell patch clamp recordings were made in ACSF at 31–32 °C using borosillicate glass pipettes (initial resistance 5–6 MΩ) filled with an internal solution that contained (in mM): $KMeSO_4$ 130, KCl 10, HEPES 10, NaCl 9, EGTA 0.1, MgATP 4, Na_2GTP 0.3, phosphocreatine 10. Junction potentials were not corrected for. Voltage-clamp recordings were made at a holding potential of -65 mV and current clamp recordings at the cell's resting potential.

Light was delivered via a cleaved 200 um core, 0.37NA fiber optic (~10-15mW at tip of optic) held at approximately 30° to the slice surface with its tip ~150 μm from the recorded cell. DG GCs were recorded from at random. For recordings from POMC-ChR2 mice, functional channel expression was confirmed with 1 sec blue light pulses and then responses to 10 Hz stimulation for 2 sec using pulse durations of 5, 10 and 20 msec were recorded. In a subset of cells, it was confirmed that ChR2 responded to 10 Hz stimulation over a 3 minute period (data not shown). In POMC-eNpHR3.0 mice, functional pump expression was checked using a 1 sec yellow light pulse pulse and then three stimulation paradigms were used to assay the suppression of action potential generation: 1) light was delivered for 800 msec and incremental depolarizing current injections of 500 were given during illumination 2) 1.5 sec incremental depolarizing current injections were delivered and yellow light was given for the middle 0.5 sec 3) 250 msec 150 pA current injections were given at 1 Hz for 20 sec without light and then throughout a 3 min yellow light pulse and for 20 sec after light. All protocols were repeated 3–6 times (twice for 3 min illuminations) for each cell and the average taken to represent that cell's response.

Behavioral experiments

Open field test

Mice were quickly attached to the fiber optic patch cables (bilaterally) via a zirconia sleeve, then placed in an open-field chamber 22.1" wide x 22.1" long x 15.83" high (Kinder Scientific) with high lux illumination (600lux). Sessions lasted for 15 min consisting of three 5 min epochs: light off, light on, and light off. In eNpHR3.0 experiments, the patch cables were interfaced to an FC/PC rotary joint (Doric lenses), which was attached on the other end to a 593.5 nm laser diode that was controlled by a Master-8 stimulator (AMPI). During the light on epoch, yellow light was provided for the full 5 min at a light power of 15mw at the tip of the implanted fiber optic. For ChR2 experiments, the hardware configuration was identical to the eNpHR3.0 experiments with the exception that illumination was provided by a 473nm laser diode (OEM). During the light on epoch, mice received blue light illumination for the full 5 min at 10hz, 20 ms pulses at a light power of 8 mw at the tip of the implanted fiber (a stimulation parameter that did not elicit seizures in POMC-ChR2 mice). Data was collected and analyzed with *MotorMonitor* software, and total distance traveled and percent of that distance traveled in the center of the arena was documented.

Elevated plus maze

Hardware configuration and experimental protocols for EPM were identical to OFT (15min session, 5min light off/on). Mice were placed in the closed arm of the open field and allowed to explore the maze. Sessions were videotaped, and the videos were analyzed for time spent in closed arms, open arms and center of the maze using *TopScan* software (Clever Sys).

Home cage exploration

Hardware configuration was identical to OFT and EPM. Mice were singly housed for 1 week before being brought into a novel testing room, the cage top removed and mice were attached to the fiber optic cables. Mice explored their home cage under low lux (16-20 lux), and were

videotaped from above. Total distance traveled during the 3 light epochs was analyzed maze using *TopScan* software (Clever Sys).

Social interaction test

We have used a modified version of a published technique for the analysis of social approach behavior in mice (4). Briefly, mice were attached to fiber optic patch cables, and placed in the open field arena which included novel male mouse placed on one wall under a wire pencil cup. The enclosure allowed for sniffing and investigating the new mouse, without direct interaction. Sessions were videotaped, and an investigator blind to mouse genotyped analyzed the number of approaches and time investigating the novel mouse.

Novel object investigation

Mice were attached to the fiber optic patch cables, then were placed in a storage container (Sterilite, 45 × 30 × 30 cm) with woodchip bedding, and videotaped from above at a light intensity of 16-20 lux. One object was placed in the center of the arena (a white ceramic shoe), and mice were allowed to explore for 15min (5min light off, 5 min light on, 5 min light off). An experimenter blind to the treatment condition analyzed videotapes, and total approaches, and investigation time was measured.

Fear conditioning

Hardware configuration and light intensity was identical to OFT and EPM, stimulation epochs are presented in the text. Conditioning took place in Coulbourn Instruments fear conditioning boxes that contained one clear plexiglass wall, three aluminum walls and a stainless steel grid as a floor. Mice were brought in to the testing room in a novel cage, attached to the fiber optic patch cables then placed in fear conditioning boxes. The training session began with the onset of the houselight and fan, and anise scent was placed under the grid floor. In this one-trial contextual fear conditioning protocol, mice received light stimulations as described in the text, and 180 s after placement of the mouse in the training context and onset of houselight and fan, mice received single 2-s foot shock of 0.75 mA. All freezing was measured before the single footshock. The mouse was taken out 15 s after termination of the foot shock and returned to its home cage. The grid and the waste tray were cleaned with Sanicloths between runs. Mice were recorded by digital video cameras mounted above the conditioning chamber, and were scored for freezing by an investigator blind to the genotype of the animal. For cued fear conditioning, mice were trained in the same context as in contextual fear conditioning, except that a 20s, 80db, 2 kHz pure tone was provided as the discrete cue CS, and a 2s footshock that co-terminated with the tone was provided. 24hr later, mice were tested for cued fear in a novel context, in which the conditioning chamber was altered, the stainless steel grid floor was covered with a plastic panel and novel cage bedding, the chamber walls were covered and made circular using plastic inserts, the house fan and lights were turned off, and a mild lemon scent was placed below the floor. The chamber door was left ajar during testing. Mice were brought into the testing room in white transport buckets. Mice were given the tone, and an investigator blind to genotype scored freezing before the first tone presentation and during tone presentations as a measure of cued fear.

Active place avoidance

We tested active place avoidance using methods previously described (Burghardt et al., 2012). Briefly, mice were attached to fiber optic cables and placed on a circular (40 cm diameter) platform that rotated clockwise at a speed of 1 rpm. The rotating platform was exposed to the room environment with multiple visual cues, including a black curtain, a white piece of cardboard, a black and white stripped piece of paper, and a cream colored cloth. A shock zone was defined within a 60° region of the stationary room. Entrance into the shock zone resulted in a brief constant current footshock (500ms, 60Hz, 0.2mA) that was scrambled across pairs of parallel rods located on the platform floor. If the mouse remained in the shock zone, it received additional

shocks of the same intensity and duration every 1.5 seconds. The position of the mouse was tracked by PC-based software that analyzed images from an overhead camera and delivered shocks appropriately (Tracker, Bio-Signal Group Corp., Brooklyn, NY). Mice were trained for 3 sessions (30 min session, sessions 1 and 3, light on, session 2 light off). In the conflict trial, the shock zone was flipped to the opposite quadrant and mice were tested for 20 minutes with the light on. Active place avoidance was measured as the number of times a mouse entered the shock zone, which was computed by Track Analysis software (Bio-Signal Group Corp., Brooklyn, NY), and time in place heat maps were generated as previously described (Burghardt et al., 2012).

Immunohistochemistry

For all cFos induction experiments, mice were separated and singly housed for at least 24hr before tested for light effects on cFos induction. Mice were placed in a novel arena, and then stimulated with light. For ChR2 experiments mice received 5 min of stimulation (10 Hz, 20ms pulses, 8mw light power) then returned to their home cage. For eNpHR3.0 experiments, mice received 20min of constant yellow light stimulation (15mw), then returned to their home cage. All mice were transcardially perfused with 4% paraformaldehyde 90min after the onset of light stimulation. Brains were postfixed overnight at 4°C, cryoprotected in 30% sucrose, then coronal serial sections (35 μm) of the entire DG were taken. Sections were blocked in 10% Normal donkey serum (NDS), then incubated in primary antibody overnight at 4° (1:5000, Rabbit anti-cFos, Calbiochem PC38). Fluorescent labeled secondary antibodies were used 1:400 (donkey-anti Rabbit Alexa-flour 488 and donkey anti rabbit Cy3 (Jackson and Invitrogen). For eNpHR mediated inhibition, Experimenters blind to genotype counted (at 20X) cFos+ cells in every 6th section throughout the DG. The 12 sections containing the entire DG were divided into the most anterior 1/3rd (dorsal sections), the middle 1/3rd (intermediate sections) and the posterior 1/3rd (ventral sections). For counting induction of cFos after ChR2 mediated stimulation, due to density of induction, an automated counting technique was used to get a measure or percent of the DG recruited by stimulation. Sections stained for cFos and the cell nuclei stain Hoechst 33342, and sections across the dorsal-ventral DG and CA3 were photographed, and imported into ImageJ. The Image-based Tool for Counting Nuclei (ITCN) plug-in (http://www.bioimage.ucsb.edu/automatic-nuclei-counter-plug-in-for-imagej) was used to count total cell number (Hoechst 33342) in the DG GCL and CA3 and all cells expressing cFos. All sections were imaged with identical exposure times, and parameters for the ITCN were set at: width: 12, minimum distance: 6, and threshold: 0.5). As with eNpHR3.0 experiments, 12 sections of DG and CA3 (every 6th section, 35um sections) from each mouse were imaged from most anterior to most posterior, and divided into the most anterior 1/3rd (dorsal sections), the middle 1/3rd (intermediate sections) and the posterior 1/3rd (ventral sections)

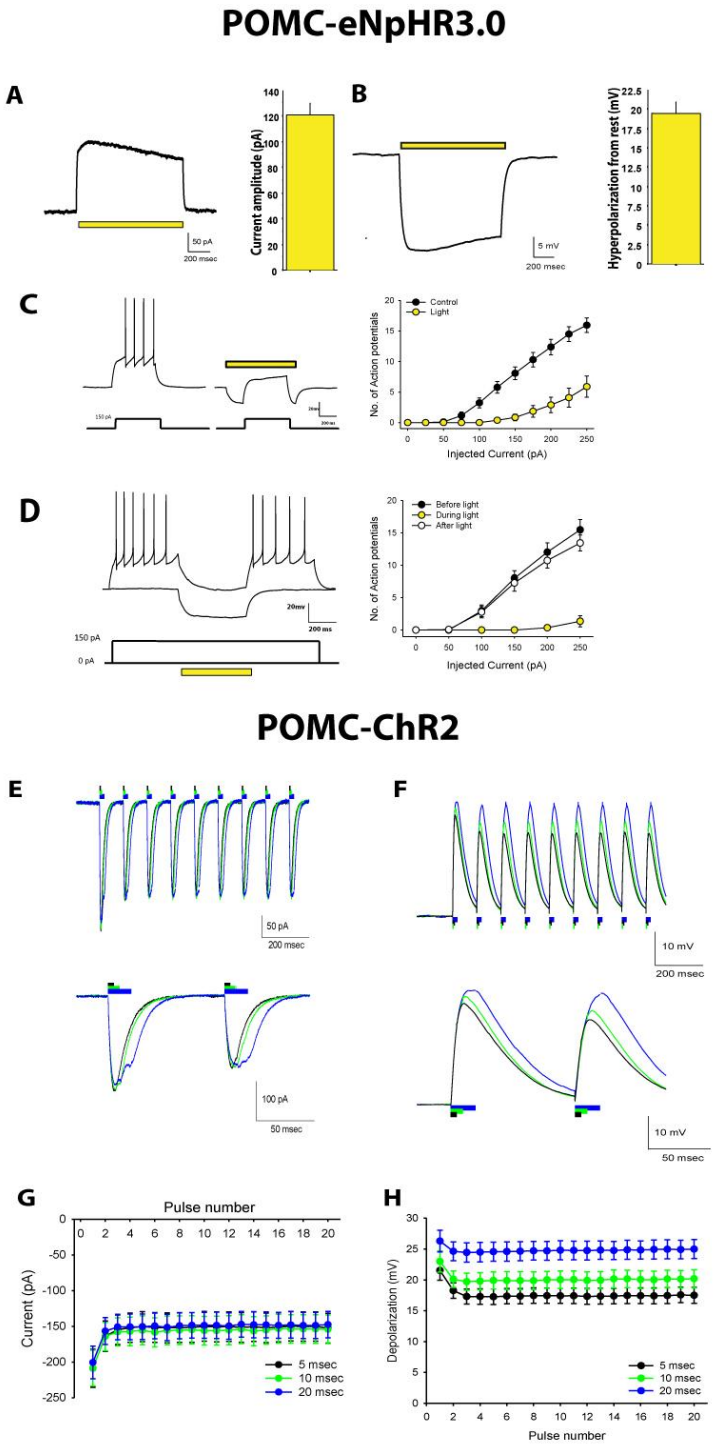

Figure S1. Acute modulation of DG GC firing by eNpHR3.0 and ChR2 A-D) POMC-eNpHR3.0 **A)** Left, Voltage-clamp recording from a GC in a brain slice showing an outward current evoked by a 1s pulse of 593.5 nm light at a holding potential of -65 mV. Right, average current amplitude (n = 18). **B)** Left, Current-clamp recording showing robust GC hyperpolarization from resting potential. Right, average hyperpolarization (n = 18). **(C)** 500 msec depolarizing current injections evoked fewer action potentials when delivered during yellow light illumination. Left, example traces from same cell showing 150 pA current injection with and without light. Right, quantification of spiking suppression by light (n = 13, 2 way, repeated

measures ANOVA, light effect, $F_{(1,12)} = 120.692$, $p < 0.001$, light x current step effect, $F_{(9,108)} = 40.731$, $p < 0.001$). **(D)** Yellow light can inhibit spiking already initiated by current injection. Light was delivered for the middle 500 msec of a 1.5 sec depolarizing current injection. Left, example traces of illumination with and without a 150 pA current injection. Right, quantification of spiking suppression by light (n = 9, 2 way repeated measures ANOVA, light effect, before vs during: $F_{(1,8)} = 119.471$, $p < 0.001$, during vs after: $F_{(1,8)} = 167.125$, $p < 0.001$). **E-H)** POMC-ChR2 **E)** Example traces of inward currents, recorded in voltage-clamp, evoked by 10 Hz stimulation using 5, 10 and 20 msec pulses; top, 9 pulses and, bottom, first two on expanded time scale. Currents followed the train with high fidelity. **F)** Average current amplitudes show that currents rapidly reached steady state and that pulse duration did not significantly affect current amplitude (n = 11, 2 way, repeated measures ANOVA, effect, $F_{(2,14)} = 167.125 p < 0.001$, light x current step effect, $F_{(2,20)} = 3.025$ $p = 0.071$). **G)** Example traces (same cell as in A) of light-evoked potentials, recorded in current-clamp, evoked by 10 Hz stimulation using 5, 10 and 20 msec pulses. Top, first 9 stimuli and bottom first two at higher resolution. **H)** Average amplitudes show that currents quickly reached steady state and were larger in response to longer light pulses (n = 8, 2 way, repeated measures ANOVA, effect, pulse length effect, $F_{(2,14)} = 167.125$, $p < 0.001$).

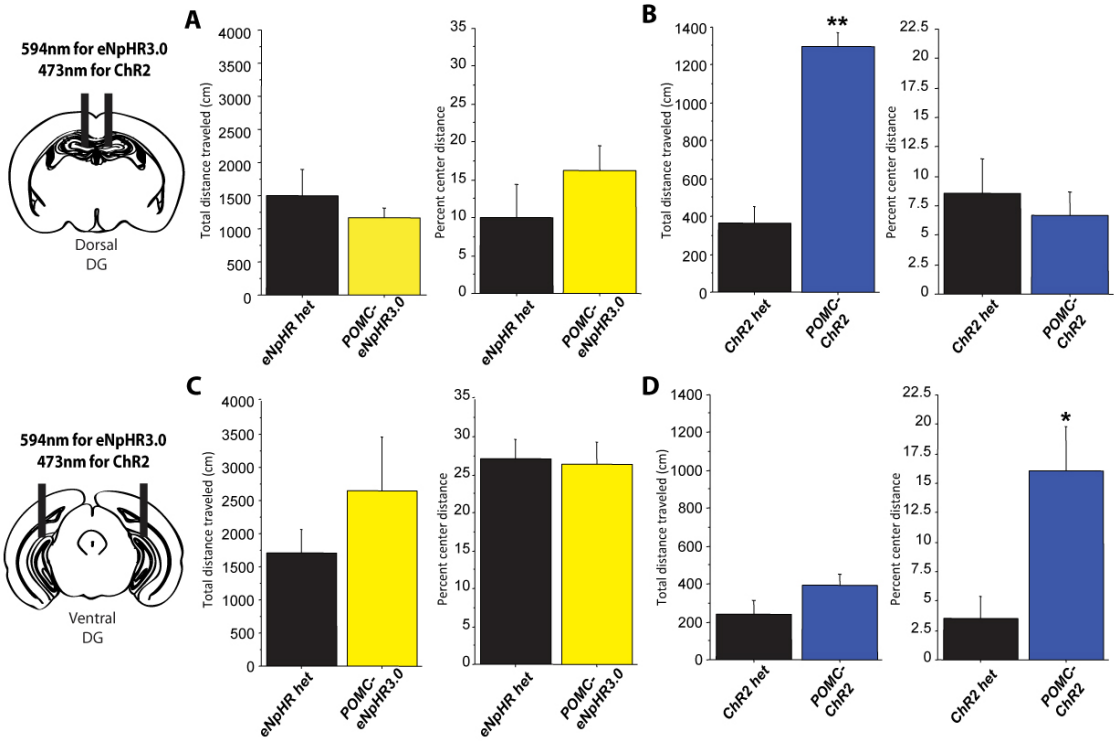

Figure S2. Behavior for mice used in cFos analysis. POMC-ChR2, POMC-eNpHR3.0 and respective littermate controls were implanted in either the dorsal or the ventral DG, and allowed to explore a novel environment (open field box, MedAssociates) for 5 minutes (POMC-ChR2) or 20 minutes (POMC-eNpHR3.0) while receiving light stimulation as described in the methods. **A)** Inhibition of the dorsal DG did not impact behavior (distance traveled, t_6=0.8, p=0.5, percent center distance t_6=-1.2, p=0.3). **B)** Stimulation of the dorsal DG increased exploration, but not percent distance traveled in center of the arena (distance traveled t_5=-8.2, p<0.01, percent center distance t_6=-0.5, p=0.6). **C)** Inhibition of the ventral DG did not impact behavior (distance traveled, t_7=-1.2, p=0.3, percent center distance t_7=-0.2, p=0.9). **D)** Stimulation of the ventral DG did not impact exploration, but increased percent distance traveled in the center of the arena (distance traveled, t_3=1.4, p=0.2, percent center distance t_3=3.4, p<0.5).

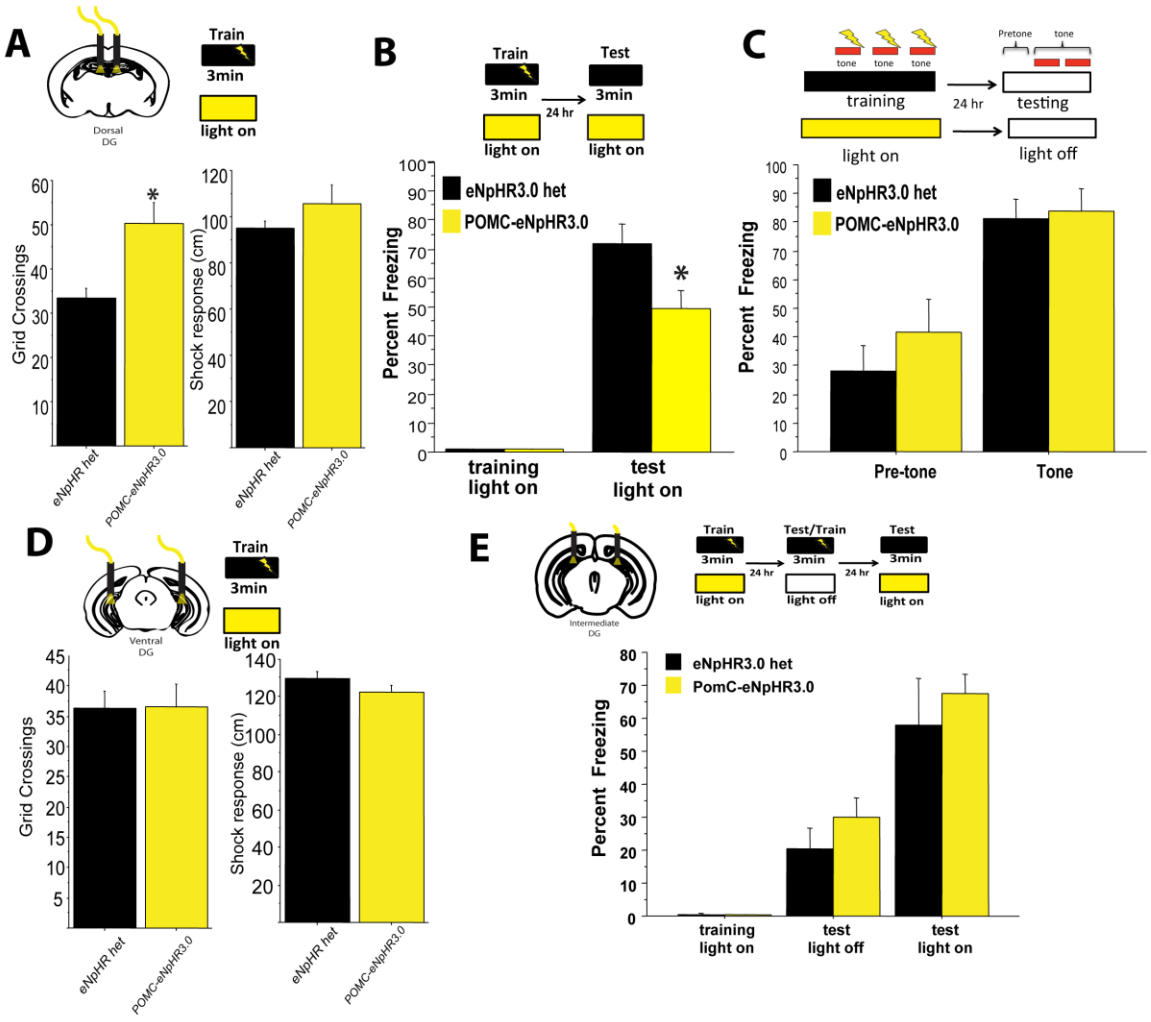

Figure S3. Optogenetic inhibition of the DG during fear conditioning. A) POMC-eNpHR3.0 mice illuminated in the dorsal DG explored the conditioning chamber significantly more that littermate controls prior to the footshock, but responded similarly to the shock (exploration, t_{11}=-3.2, p<0.05, shock response t_{11}=-1.1, p=0.3. **B)** POMC-eNpHR3.0 mice froze significantly less to the training context when light was given during both training and retrieval (n=6-8/geno, repeated measures ANOVA, geno effect $F_{(1,12)}$= 5.6, p<0.05, session effect $F_{(1,12)}$= 154.3, p=<0.0001, genotype X session interaction $F_{(1,12)}$= 5.5, p<0.05, t-test on retrieval day t_{12}=2.4, p<0.05) **C)** Cued fear conditioning during local suppression of dorsal DG activity (n=7-8/geno, repeated measures ANOVA, geno effect $F_{(1,13)}$= 0.66, p=0.4, tone effect $F_{(1,13)}$= 46.5, p=<0.0001, genotypeXtone interaction $F_{(1,13)}$= 0.59, p=0.5). **D)** POMC-eNpHR3.0 mice did not differ from controls in exploration of the conditioning chamber before the shock or in response to the shock when illumination was provided to the ventral DG (exploration, t_{10}=-0.1, p=0.9, shock response t_{10}=-1.5, p=0.9) **E)** Contextual encoding and retrieval during local suppression of the intermediate DG. Light did not impact either of these measures. Light on during training, n=6-9/geno repeated measure ANOVA genotype X training $F_{(1.13)}$=1.2, p=0.3, light on during retrieval t_{13}=-.7, p=0.5)

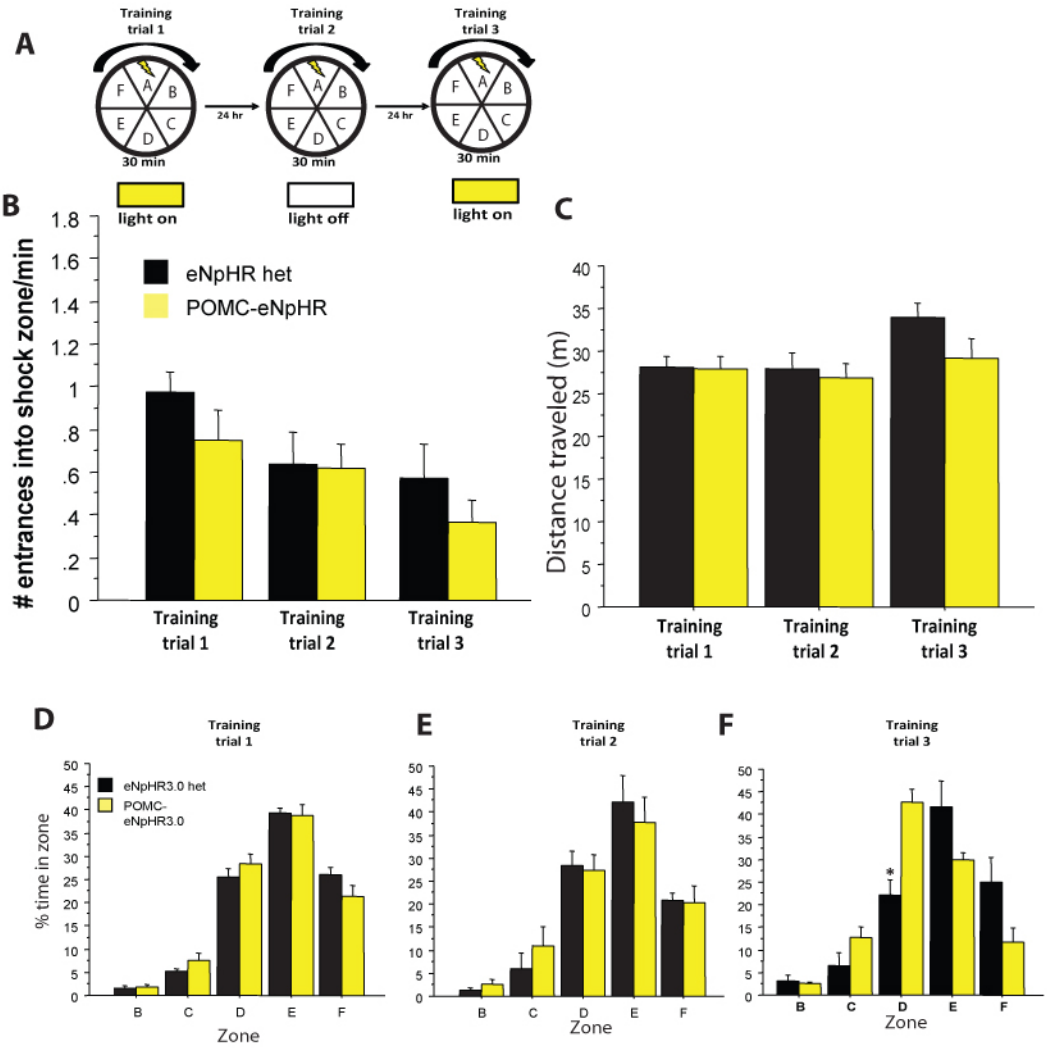

Figure S4. Optical inhibition of the DG does not impact exploration, encoding or retrieval of spatial information during early acquisition of active place avoidance. A) Training protocol. Mice were trained with light on to avoid the shock zone, then twenty-four hours later tested/trained with light off, then retrained with light on. **B)** Both groups of mice avoided the shock zone at a similar rate during all phases of training (repeated measures ANOVA, genotype effect $F_{(1,9)}$=1.1, p=0.3, trial effect, $F_{(2,18)}$= 7.3, p<0.01, trial X genotype interaction $F_{(2,18)}$=0.7, p=0.5). **C)** Optogenetic inhibition did not impact exploration of the arena during any phase of training (t-test, trial 1, t_9=0.1, p=0.9, trial 2, t_9=0.4, p=0.7, trial 3, t_9=1.6, p=0.1). **D)** Optogenetic inhibition did not impact distribution of time spent in each zone of the arena in trial 1, genotypes did not differ in light-off conditions in trial 2, but there was a modest effect in trial 3 in zone D. (repeated measures ANOVA, trial 1 zone X genotype interaction $F_{(5,45)}$=1.2, p=0.3, trial 2 zone X genotype interaction $F_{(5,45)}$=0.3, p=0.9, trial 3 zone X genotype interaction $F_{(5,45)}$=7.7, p<.05, t-test on zone D, t_9=-4, p<0.05).

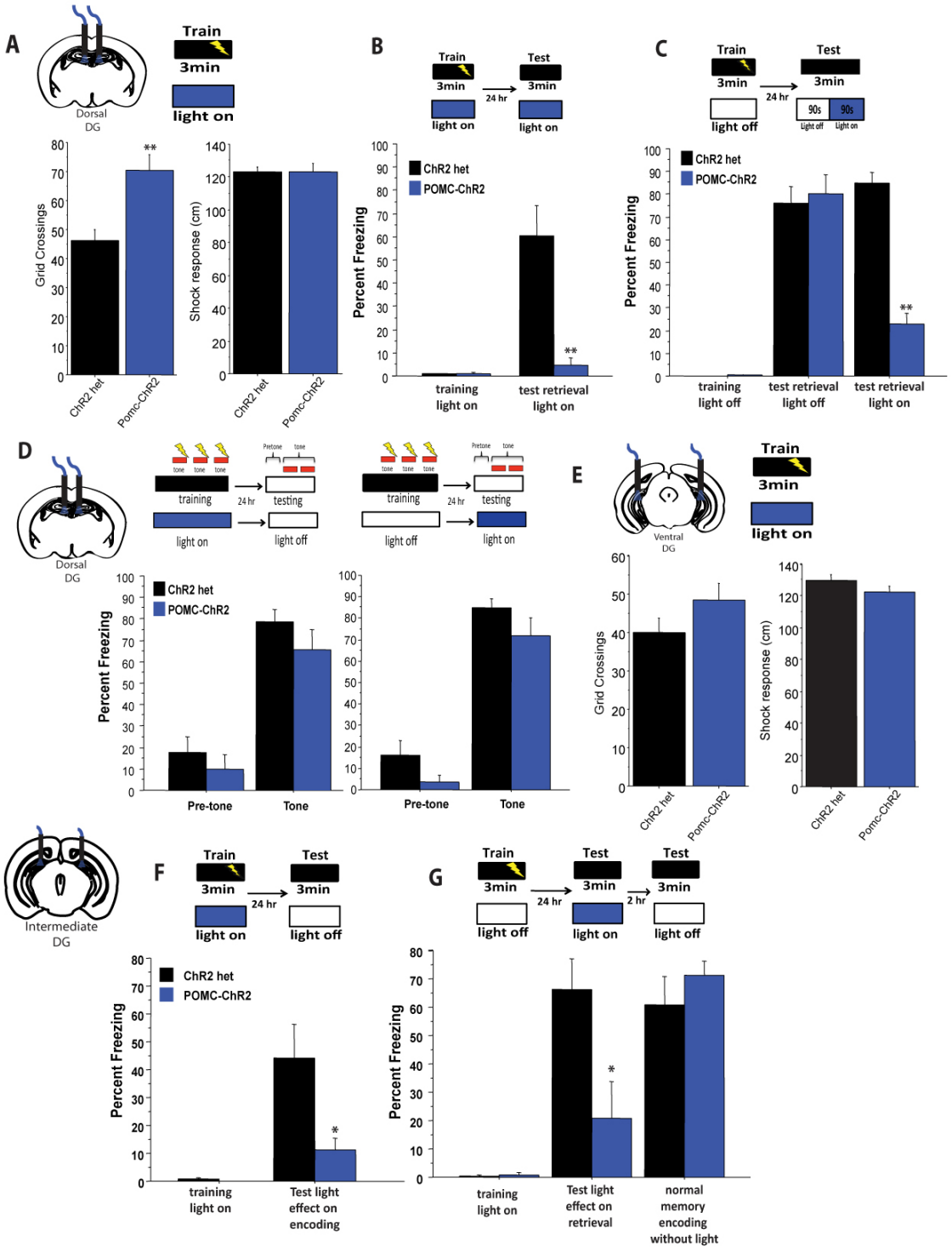

Figure S5. Optogenetic stimulation of the DG during fear conditioning. A) POMC-ChR2 mice mice illuminated in the dorsal DG explored the conditioning chamber significantly more that littermate controls prior to the footshock, but responded similarly to the shock (exploration, t_{14}=-3.7, p<0.01, shock response t_{14}=-0.002, p=0.99. **B)** POMC-ChR2 mice froze significantly less to the training context when light was given during both training and retrieval (n=6/geno, repeated measures ANOVA, geno effect $F_{(1,10)}$= 17.8, p<0.01, session effect $F_{(1,10)}$= 24.6,

p=<0.001, genotype X session interaction $F_{(1,10)}$= 17.9, p<0.01, t-test on retrieval day t_{10}=4.2, p<0.01) **C)** POMC-ChR2 mice and their single transgenic littermate controls were implanted in the dorsal DG, and trained in CFC with the light off, then tested for 90s light off, 90s light on. Blue light illumination of the dorsal DG blocked the expression contextual fear, as POMC-ChR2 mice ceased freezing with the onset of stimulation (repeated measures ANOVA, genotype effect $F_{(1,14)}$=13.5, p=0.003, light effect, $F_{(1,14)}$= 29.2, p<0.0001, light X genotype interaction $F_{(1,14)}$=52.7, p<0.0001, t-test on test day t_{14}=6, p<0.0001. **D)** POMC-ChR2 mice and littermate controls were trained in a cued fear conditioning protocol, with light stimulation provided either during acquisition or retrieval. *Left* Light on during acquisition, (n=4-7/geno repeated measures ANOVA, genotype effect $F_{(1,9)}$=1.2, p=0.29, tone effect, $F_{(1,9)}$= 134.9, p<0.0001, tone X genotype interaction $F_{(1,9)}$=0.22 p=0.6. *Right* Light on during expression $F_{(1,9)}$=2.9, p=0.12, tone effect, $F_{(1,9)}$= 196.8, p<0.0001, tone X genotype interaction $F_{(1,9)}$=0.007 p=0.9). **E)** POMC-ChR2 mice did not differ from controls in exploration of the conditioning chamber before the shock or in response to the shock when illumination was provided to the ventral DG (exploration, t_{16}=1.5, p=0.16, shock response t_{16}=1.6, p=0.12) **F-G**) Optogenetic stimulation of the intermediate DG can acutely block the encoding or retrieval of contextual fear. POMC-ChR2 mice and their single transgenic littermate controls were implanted in the intermediate portion of the DG, and trained in CFC with light on during either encoding (**F**) or retrieval (**G**). Light on during training impaired encoding (n=6/geno, repeated measures ANOVA, genotype effect $F_{(1,10)}$=6.9, p=0.02, training X genotype interaction $F_{(1,10)}$=6.4, p=0.03, t-test on test day t_{10}=6, p<0.02.), light on during testing blocked the retrieval, (n=5-6/geno, repeated measures ANOVA, genotype effect $F_{(1,9)}$=6.8, p=0.03, training X genotype interaction $F_{(1,10)}$=6.7, p=0.03, t-test on test day t_9=2.6, p=0.02.), yet did not permanently erase the memory, as it was intact 2hr later when tested with light off(t_9=-1, p=0.3). All error bars are +/- SEM. *p<0.05, **p<0.01

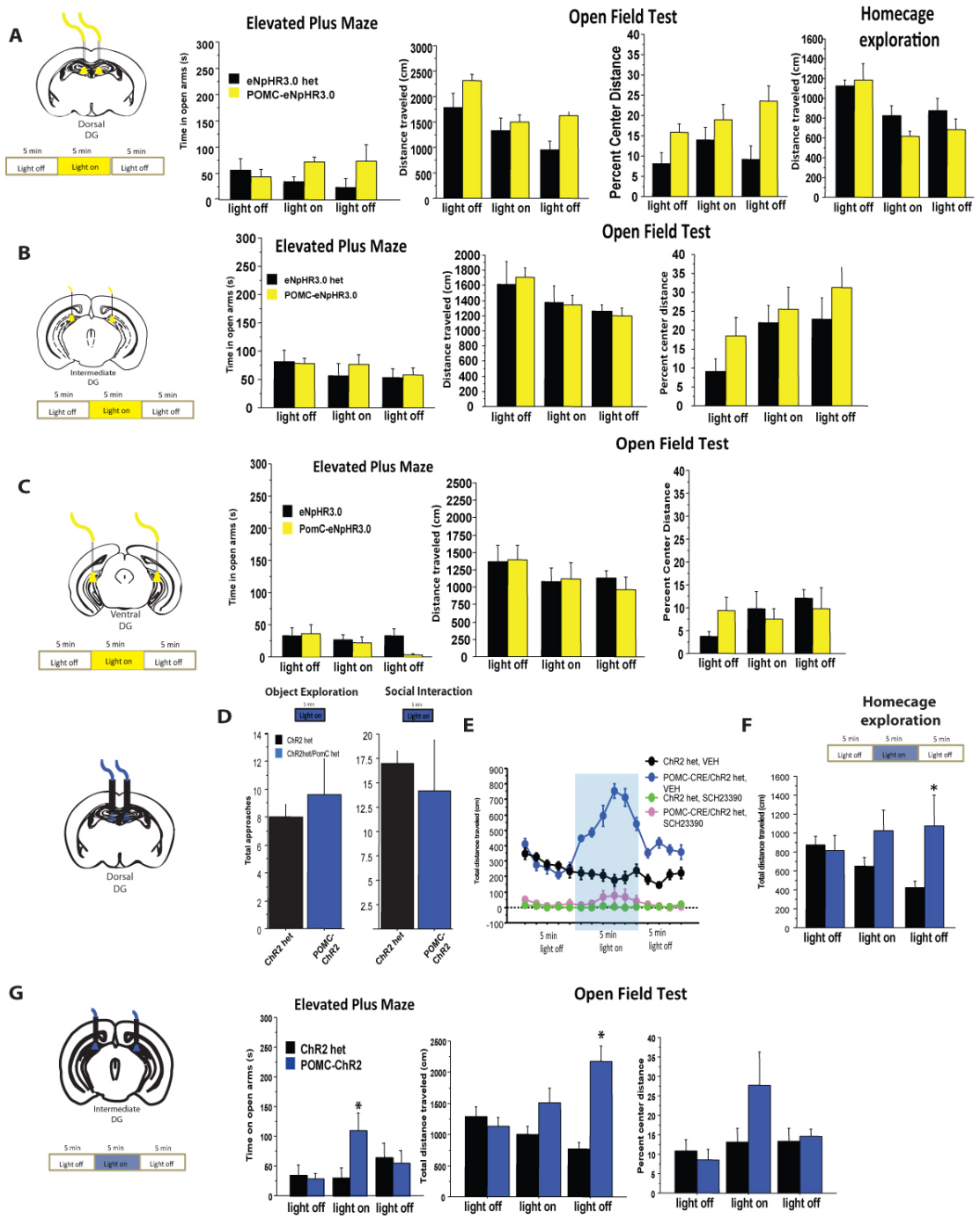

Figure S6. Optogenetic modulation of the DG during anxiety and exploration tests. A-C) POMC-eNpHR3.0 **A)** Dorsal DG. Yellow light illumination of the dorsal DG did not influence time spent in open arms of the elevated plus maze (n=6-7/geno, repeated measure ANOVA genotype X light epoch interaction $F_{(2,22)}$=2.3, p=0.1), or total distance traveled or percent distance traveled in center of the open field (total distance, repeated measure ANOVA genotype X light epoch interaction $F_{(2,22)}$=3, p=0.1, percent center distance genotype X light epoch interaction $F_{(2,22)}$=2.5, p=0.1), or home cage exploration when tested in a novel room (n=5-8/geno, repeated measure ANOVA genotype X light epoch interaction $F_{(2,22)}$=1.1, p=0.4). **B)** Optogenetic inhibition of the intermediate DG DG did not impact anxiety measures (n=6-9/geno, time in open

arms of EPM, repeated measure ANOVA genotype X light epoch interaction $F_{(2,26)}$=0.6, p=0.5, total distance traveled in OFT, genotype X light epoch interaction $F_{(2,26)}$=0.4, p=0.8, percent center distance in OFT, genotype X light epoch interaction $F_{(2,26)}$=0.8, p=0.4. **C)** Optogenetic inhibition of the ventral DG did not impact anxiety measures (n=6/geno time in open arms of EPM, repeated measure ANOVA genotype X light epoch interaction $F_{(2,20)}$=1.5, p=0.2, total distance traveled in OFT, genotype X light epoch interaction $F_{(2,20)}$=0.8, p=0.5, percent center distance in OFT, genotype X light epoch interaction $F_{(2,20)}$=2.1, p=0.14. **D)** Optical stimulation of the dorsal DG in POMC-ChR2 mice and littermate controls did not impact exploration of a novel object or social interaction with a novel mouse (object exploration, t_7=-0.5, p=0.6, social interaction, t_7=-0.4, p=0.7). **E**) Optogenetic enhancement of exploration in dorsal DG stimulated POMC-ChR2 mice is absent in mice treated with D1 antagonist SCH-23390 (n=7-9/geno, 0.03mg/kg, repeated measures ANOVA, genotype effect $F_{(1,27)}$=443, p<0.001, drug effect $F_{(1,27)}$=57, p<0.001 drug X genotype interaction $F_{(1,27)}$=34, p<0.001, drug Xgenotype X time $F_{(14,378)}$=12.9, p<0.001. **F)** Optical stimulation of the dorsal DG in POMC-ChR2 mice and littermate controls impacted home cage exploration when tested in a novel room, but impaired habituation (repeated measures ANOVA, genotype effect $F_{(1,9)}$=2.8, p=0.13, training X genotype interaction $F_{(2,18)}$=9.5, p<0.05, t-test light on epoch t_9=1.9, p=0.1, light off epoch 3 t_9=2.5, p=0.03, time effect, ChR2 het, $F_{(2,9)}$=6.7, p<0.01, POMC-ChR2 $F_{(2,9)}$=0.3, p=0.7. **G)** POMC-ChR2 mice and their single transgenic littermate controls were implanted in the intermediate portion of the DG and tested for blue light effects on anxiety-like behavior and exploration. Blue light stimulation of the intermediate DG increased entries into the open arms of the EPM (n= 7/geno, geno effect $F_{(1,12)}$=0.9, p=0.4 geno X light epoch interaction $F_{(2,24)}$=5, p=0.01). In the OFT, blue light stimulation produced both an increase in exploration, as well as an decrease in anxiety, manifested as an increase in percent distance traveled in the center of the arena. (n=10-11/geno. Total distance traveled: repeated measures ANOVA genotype effect $F_{(1,19)}$=9.8, p=0.005, light X genotype interaction $F_{(2,38)}$=15.6, p<0.001. Percent center distance repeated measures ANOVA genotype effect $F_{(1,19)}$=0.9, p=0.4, light X genotype interaction $F_{(2,38)}$=3.6, p<0.05. All error bars are +/- SEM. *p<0.05.

Supplementary Video 1. Optogenetic stimulation of the ventral DG in a POMC-ChR2 mouse leads to an acute reduction in anxiety. Video played at 8X speed.