Supplemental Experimental Procedures

Histological processing: Mice were killed by transcardial perfusion with 13ml PBS, followed by 10ml 4% paraformaldehyde. Brains were extracted and coronal sections of the BLA (100μm) were cut on a vibratome. The slices were labeled with the following primary antibodies: goat anti-GFP (Abcam), rabbit anti-DsRed (Clontech), rabbit anti-c-fos (Santa Cruz) and goat anti-c-fos (Santa Cruz). The following fluorophore-conjugated secondary antibodies were used: Alexa 488 donkey anti-goat (Invitrogen), Alexa 594 donkey anti-rabbit (Invitrogen). Slices were counterstained with DAPI (Vector Laboratories). All images were taken using a Zeiss LSM-710 confocal microscope system.

***In situ* hybridization:** *In situ* hybridization was conducted by the *in situ* hybridization core at UNC Neuroscience Center. catFISH experiments were conducted as described previously (Guzowski and Worley, 2001). Briefly, we utilized an intronic c-fos probe to detect nuclear localized intronic RNA present 5 minutes after US exposure, and an exonic c-fos probe to detect cytoplasmic exonic RNA present 45 minutes after US exposure. This provides 2 time-points at which to label active cells using only endogenous c-fos activation. The c-fos exonic probe spans the first 2 exons of the *c-fos* gene. The fluorescein-labeled c-fos exonic probe was detected using a horseradish peroxidase-conjugated anti-fluorescien antibody (Roche) on 20μm frozen sections. The signal was amplified using DNP-conjugated tyramide (Perkin Elmer) and subsequently visualized using Alexa 488-conjugated anti-DNP antibody (Life Technologies). The c-fos intronic probe was a kind gift from Dr D. Lin and contains the entire first intron of the *c-fos* gene (Lin et al., 2011). The digoxigenin-labeled c-fos intronic probe was detected using an

alkaline phosphatase-conjugated anti-DIG antibody (Roche) on 20μm frozen sections. The signal was amplified and detected using the HNPP Fast Red system (Roche).

Cell counts: To quantify the number of cells expressing fluorophores and c-fos, we acquired images from a single z plane across 3 adjacent slices. The mean background intensity of each image was subtracted and DAPI, c-fos, EYFP and mCherry positive nuclei were counted manually (ImageJ). All histological procedures were conducted by an individual who was blind to the experimental condition. For cell counts, N refers to number of animals.

Data analysis: Statistical significance was assessed using *t*-tests or analysis of variance (ANOVA), followed by post-hoc tests (Bonferroni test for difference between means, unless otherwise stated) when applicable, using α tests. Data were analyzed using Microsoft Excel with the Statplus plugin. All error bars are ± standard error of the mean (s.e.m.).

Conditioned place preference: A biased design for conditioned place preference was used, in which a positive valence of nicotine was tested by its ability to increase the time spent in the initially non-preferred chamber. The apparatus consisted of a rectangular chamber split into 2 compartments (120×165×200mm per compartment) connected by a 50×50mm opening. Each compartment had distinct olfactory (1% acetephenone or 2% octanol), tactile (rough or smooth flooring) and visual (vertical or no stripes on walls) cues. Singly housed animals were removed from their homecage and placed in the center of the conditioning apparatus and allowed to explore for 10 minutes (pre-test). After the pre-test an initial compartment preference for each mouse was recorded. The following day animals were assigned to either saline or nicotine groups. Animals in the nicotine

group were given an i.p. injection of nicotine and confined to their initially non-preferred compartment for 20 minutes. Animals in the saline group were given an i.p. injection of saline and confined to their initially non-preferred compartment for 20 minutes. 5 hours later, animals of both groups were given an i.p. injection of saline and confined to their initially preferred compartment for 20 minutes. The following day animals were placed in the center of the conditioning apparatus and allowed to explore for 10 minutes to determine any change in compartment preference as a result of conditioning. The time spent in each compartment was scored manually by individuals who were blind to the experimental conditions.

***In vivo* electrophysiology:** Animals were injected with virus expressing c-fos:ChR2-EYFP-2A-nCherry. Nine days later animals were treated with either footshock or nicotine to induce ChR2 expression. Eighteen hours later animals were anaesthetized with urethane (1800mg/kg) and placed in a stereotactic system with 1% oxygen delivery throughout the recording. An optrode consisting of 16 stereotrodes (25μm Formvar-coated tungsten microwire (California Fine Wire)) glued to a 200μm optical fiber (0.37 NA, Thor labs), with the tip of the stereotrodes extending 300-500μm beyond the tip of the fiber, was used for simultaneous optical stimulation and extracellular recordings. The optical fiber was connected to a 473nm laser (Shanghai Laser and Optics Century), which was controlled by a stimulator (Master-8, A.M.P.I.). The power intensity of light emitted from the optrode was adjusted to 18mW prior to recordings. The optrode was lowered to the BLA. Light pulses of 100ms were delivered at 0.1Hz at recording sites throughout the BLA. After light-responsive cells were detected two types of optical stimulation were delivered: 100ms pulses delivered every 10s (100-120 sweeps), and bursts of 200, 10ms

light pulses at 20Hz delivered every 40s (3-5 sweeps). Recordings were obtained via a unitary gain head-stage preamplifier attached to a fine wire cable (Neuralynx). Multi- and single-unit recordings were obtained by lowering stereotrodes through the BLA in 100μm steps from -4.2 to -4.7 DV. Signals were amplified, bandpass filtered and acquired by a Digital Lynx SX programmable amplifier (Neuralynx) on a personal computer running Cheetah data acquisition software (Neuralynx). Spikes were bandpass filtered (600-6000 Hz) and recorded at 32 kHz with 30μV threshold. Single units were clustered using Klustakwik (by Ken Harris, http://klustakwik.sourceforge.net/), using the first two principal components, energy, and peak of the action potentials. Data were analyzed with custom software written in Matlab. The response latency of single units to light pulses was quantified as the most likely first spike time across all sweeps, corresponding with the onset of the light-evoked response.

***Ex vivo* slice electrophysiology:** Acute brain slices were made from mice 18 hours after shock or nicotine induction. Mice were anesthetized with isoflurane and transcardially perfused with ice-cold artificial cerebrospinal fluid (aCSF, in mM: 125 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 25 $NaHCO_3$, 20 glucose, 2 $CaCl_2$, 1 $MgCl_2$, 2 Na-pyruvate; equilibrated with 95% O_2 and 5% CO_2). After perfusion, brains were removed and submerged in an ice-old, isotonic solution for sectioning (in mM: 10 NaCl, 2.5 KCl, 0.5 $CaCl_2$, 7 $MgSO_4$, 1.25 NaH_2PO_4, 25 $NaHCO_3$, 10 glucose, and 195 sucrose; equilibrated with 95% O_2 and 5% CO_2). Coronal sections (350μm) of the forebrain were cut with a vibrating microtome (VT1200, Leica), and were immediately placed into aCSF. Slices were incubated in aCSF at 35°C for 25 minutes, and then maintained at room temperature until transfer to the recording chamber beneath an upright microscope (Olympus Optical) with a 40X

objective (LUMPLFLN 40XW, 0.8 N.A.). Slices were perfused with fresh aCSF at 33-35°C during all recordings. Glass patch microelectrodes (2-5 MΩ) contained (in mM): 135 $KMeSO_4$, 5 KCl, 2 NaCl, 10 HEPES, 10 Na_2-phosphocreatine, 5 MgATP, 0.4 Na_2GTP, 0.2 EGTA, and 0.2% biocytin. The BLA was located under differential interference contrast (DIC) microscopy and cells expressing EYFP were located under epifluorescent illumination with a 470nm LED source (pE-100, CoolLED) at low power, and a 520Δ50 BP emission filter (Chroma). Optogenetic stimulation was with the same 470nm LED source (14.7 mW) or a 595nm LED (9.6 mW), delivered through the objective. Voltage-clamp and current-clamp responses were recorded with a Multiclamp 700A amplifier (Molecular Devices), low-pass filtered at 2-4 kHz, and digitized at 20 kHz (Digidata 1440A, Instrutech). Series resistance upon whole-cell configuration was typically 10-12 MΩ, and cells that had initial series resistance ≥ 15 MΩ or for which series resistance increased by ≥ 5 MΩ during the course of experiment were excluded from analysis. In voltage-clamp experiments, series resistance was compensated with the correction circuit (≥95%) of the amplifier. Microelectrode voltage drop (bridge) was compensated at the beginning of each current-clamp experiment with the automatic feature of the amplifier. We did not correct for liquid junction potential. Data were collected and analyzed using Axograph X and Matlab (Mathworks). For halorhodopsin experiments, current was injected to generate spike trains 50 pA above rheobase (minimum current to induce firing), with rheobase first determined for each cell by a ramp protocol (Δ400pA/s). This ensured that all cells were perithreshold. Small amounts of current were injected to hold all cells at the same resting voltage of -70mV. All light OFF vs. light ON data were collected from interleaved trials.

Assay for physiologic responses to optical stimulation: Respiration and heart rate were measured using a pulse oximeter (MouseOx, Starr Life Sciences) connected to a computer that was equipped with MouseOx software. Recordings were made using a collar sensor. Mice were shaved around the neck and acclimated to the collar sensor for 30 minutes. Heart rate (beats per minute) was reported as a moving average across 5 heartbeats. Breath rate (breaths per minute) was reported as a moving average across 10 breath cycles. Eighteen hours after footshock or nicotine treatment animals were prepared for assessment of their response to optical stimulation. Stylets were removed from the guide cannulae and a flat-cut 300μm diameter fiber-optic cable, coupled to a 473nm laser (Shanghai Laser and Optics Century) outside of the operant chamber, was inserted through the guide cannula and positioned directly above the BLA. Immediately before this the power of the laser was adjusted to 18mW. The collar sensor was then attached. Mice were placed into a clean home cage and were acclimated to the cage for 10 minutes. After this, heart and respiration rate were recorded for 3 minutes to establish a baseline. Animals then received three 3 minute presentations of optical stimulation (18mW, 20Hz, 20% duty cycle). Heart and respiration rate were computed as the percent difference in rate during optical stimulation compared to baseline.

Assay for freezing response to optical stimulation: Mice were placed into an illuminated Med-Associates operant chamber equipped with nosepoke portals, audio stimulus generator, infra-red light source and a house light (the testing chamber), and behavior was recorded using a modified web cam capable of detecting infra-red light (Logitech). Upon initiation of a 500 second session the house light was dimmed and animals received 5 presentations of 10 seconds of optical stimulation. Upon completion

of the session the house light was illuminated and animals were returned to their home cage. All stimuli were presented and all timestamp data recorded using Med-PC software. Freezing behavior was defined as the cessation of all movements except those caused by respiration and was scored manually by individuals who were blind to the experimental conditions.

Self-administration assay: Upon initiation of a 60 minute session the house light was dimmed and nosepoke entries into the active and inactive nosepoke portals, detected by breakage of an infrared beam across each portal, were separately recorded. Entry into the active portal resulted in 5 seconds of optical stimulation (18mW, 20Hz, 20% duty cycle). Entry into the inactive portal had no consequence. Upon completion of the session, the house light was illuminated and animals were returned to their home cages.

Fear conditioning assay: Upon initiation of a 1000 second training session the house-light was dimmed and animals receiving paired training received 10 randomly presented 10-second 1.5kHz tones, all of which coterminated with 2 seconds of optical stimulation (18mW, 20Hz, 20% duty cycle). Animals receiving unpaired training received 10 randomly presented 10-second 1.5kHz tones and 10 randomly presented 2 seconds of optical stimulation. Upon completion of the training session the house light was illuminated and the animals were returned to their home cage. 3 hours later animals received a second training session identical to the first. 3 hours after this, animals were exposed to a 500 second test session in which they received 5 presentations of the 10-second CS. Upon completion of the test session the house light was illuminated and animals were returned to their home cage. Freezing behavior during the training and test sessions was recorded.

Context assay: Animals were removed from their homecage and placed into a standard Med-Associates operant chamber equipped with a grid floor and aversive stimulator. Animals received 20 1.5mA footshocks over 10 minutes before being returned to their homecage. The following day animals were injected and cannulated as described previously. 9 days later animals were removed from their homecage and returned to the footshock chamber. Upon initiation of a 500 second session the house light was dimmed. After 500 seconds of context exposure, the house light was illuminated and animals were returned to their homecage. Freezing behavior was scored during the first 2 minutes of exposure to context. 18 hours later animals were assayed for their response to optical stimulation.

Odor assay: Animals were placed into a Med Associates operant chamber that had air passing through it at 1 liter/minute, with a vacuum removing air at an equal rate. Acetephenone (1%) and octanol (2%) were dissolved in mineral oil and their entry into the chamber was controlled manually. Over a 1000 second session animals received 10 presentations of 10 seconds of acetephenone that always coterminated with 2 seconds of optical stimulation (18mW, 20Hz, 20% duty cycle), and 10 presentations of 10 seconds of octanol, randomly interleaved. When the session was complete animals were returned to their home cage. 3 hours later animals received a second identical training session. 3 hours after this, animals were placed into the center of a 3-compartment chamber (Med Associates) comprising a central compartment with opaque walls and 2 extreme compartments with Perspex walls. Animals were left to explore the chamber for 5 minutes. Animals were then removed and the two odors were presented from opposite ends of the chamber at a rate of 1 liter/minute. Animals were returned to the central

compartment and left to explore the chamber for a further 5 minutes. Behavior was recorded using a camcorder (Sony) and the amount of time spent in each compartment during the 'without odor' and 'with odor' epochs was scored manually by individuals who were blind to the experimental conditions.

CS assay: Animals were removed from their homecage and placed into a standard Med-Associates operant chamber equipped with a grid floor and aversive stimulator. Upon initiation of a 1000 second training session the house-light was dimmed and animals receiving paired training received 10 randomly presented 10-second 1.5kHz tones, all of which coterminated with 2 seconds of 1.5mA footshock. Animals receiving unpaired training received 10 randomly presented 10-second 1.5kHz tones and 10 randomly presented 2 seconds of 1.5mA footshock. Upon completion of the training session the house light was illuminated and the animals were returned to their home cage. 3 hours later animals received a second training session identical to the first. The following day animals were injected and cannulated as described previously. 9 days later animals were removed from their homecage and placed into the testing chamber. Upon initiation of a 500 second session the house light was dimmed and animals received 5 presentations of the 10-second 1.5kHz tone. Upon completion of the session the house light was illuminated and animals were returned to their homecage. 18 hours later animals were either assayed for their response to optical stimulation or placed into the self-administration assay.

Assay for effect of optical inhibition on response to auditory CS: Animals were removed from their homecage and placed into a standard Med-Associates operant chamber equipped with a grid floor and aversive stimulator. Upon initiation of a 1000

second training session the house-light was dimmed and animals received 10 randomly presented 10-second 1.5kHz tones, all of which coterminated with 2 seconds of 1.5mA footshock. Upon completion of the training session the house light was illuminated and the animals were returned to their home cage. 3 hours later animals received a second training session identical to the first. The following day animals were bilaterally injected with lentivirus expressing c-fos:NpHR-EYFP and bilaterally cannulated 250μm above the BLA. 9 days later animals were treated with either footshock or nicotine as previously described. 18 hours later animals were prepared for assessment of their response to the CS in the presence and absence of optical inhibition. Stylets were removed from the guide cannulae and a flat-cut 300μm diameter fiber-optic cable, coupled to a 593nm laser (Shanghai Laser and Optics Century) outside of the operant chamber, was inserted through each guide cannula and positioned directly above each BLA. Immediately before this the power of each laser was adjusted to 10mW. Mice were then placed into the testing chamber. Upon initiation of a 500 second session the house light was dimmed, the lasers were turned on and animals received 5 presentations of the 10-second 1.5kHz tone. Upon completion of the session the house light was illuminated and lasers were turned off. Immediately following this a second 500 second session was initiated. The house light was dimmed and the animals received 5 10 second presentations of the 1.5kHz tone. Upon completion of the session, the house light was illuminated and animals were returned to their homecage.

Assay for effect of optical inhibition on response to olfactory CS: Animals were placed into a Med Associates operant chamber that had air passing through it at 1 liter/minute, with a vacuum removing air at an equal rate. Acetephenone (1%) and

octanol (2%) were dissolved in mineral oil and their entry into the chamber was controlled manually. Over a 1000 second session animals received 10 presentations of 10 seconds of acetephenone that always coterminated with 2 seconds of 1.5mA footshock, and 10 presentations of 10 seconds of octanol, randomly interleaved. When the session was complete animals were returned to their home cage. 3 hours later animals received a second identical training session. The following day animals were bilaterally injected with lentivirus expressing c-fos:NpHR-EYFP and bilaterally cannulated 250μm above the BLA. 9 days later animals were treated with either footshock or nicotine as previously described. 18 hours later animals were prepared for assessment of their response to the CS in the presence and absence of optical inhibition. Stylets were removed from the guide cannulae and a flat-cut 300μm diameter fiber-optic cable, coupled to a 593nm laser (Shanghai Laser and Optics Century), was inserted through each guide cannula and positioned directly above each BLA. Immediately before this the power of each laser was adjusted to 10mW. Animals were then placed into the center of a 3-compartment chamber. Animals were left to explore the chamber for 5 minutes. Animals were then removed and the two odors were presented from opposite ends of the chamber at a rate of 1 liter/minute and the lasers were turned on. Animals were returned to the central compartment and left to explore the chamber for 5 minutes. Animals were then removed and the lasers were turned off. Animals were returned to the central compartment and left to explore the chamber for a further 5 minutes. Behavior was recorded using a camcorder (Sony) and the amount of time spent in each compartment during the 'without yellow light' and 'with yellow light' odor epochs was scored manually by individuals who were blind to the experimental conditions.

Supplemental References

Lin, D., Boyle, M.P., Dollar, P., Lee, H., Lein, E.S., Perona, P., and Anderson, D.J. (2011). Functional identification of an aggression locus in the mouse hypothalamus. Nature *470*, 221-226.