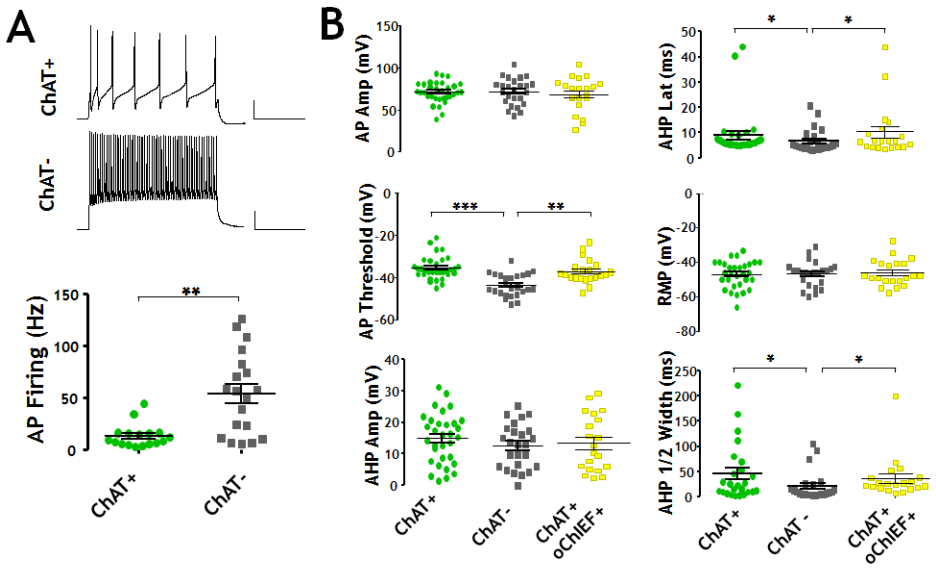


Figure S1. Related to Figure 1. Characterization of cholinergic vs non-cholinergic neurons in the NBM and assessment of electrical properties in *ChAT*+ neurons +/- oChIEF expression.

A: Top, example firing patterns of cholinergic (ChAT+) and non-cholinergic (ChAT-) neurons in the NBM in response to a 500 msec x 5 pA current injection (Calibration:15 mV x 200 ms). **Bottom:** ChAT- neurons had significantly higher firing rates than ChAT+ neurons (n = 20 ChAT + vs 19 ChAT- neurons $p<0.01$). All data (1 point / neuron) are shown in scatter plot format, lines show the mean ± S.E.M.

B: Comparison of various neuronal parameters among cholinergic neurons (ChAT+, green, n=29), non-cholinergic neurons (ChAT-, grey, n=26), and oChIEF expressing cholinergic neurons (ChAT+/oChIEF+, yellow, n=20) in the nucleus basalis. Cholinergic neurons had significantly higher action potential threshold, after-hyperpolarization latency and after-hyperpolarization half-width than non-cholinergic neurons. None of the parameters measured were different in ChAT+ neurons with vs without oChIEF expression (*$p<0.05$, **$p<0.01$, ***$p<0.005$).

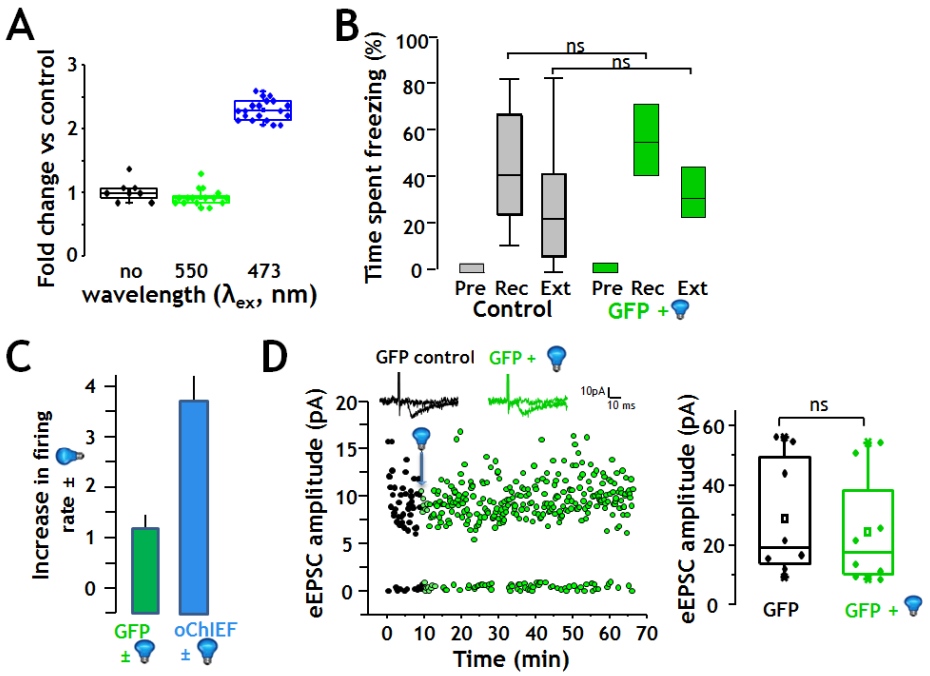


Figure S2. Related to Figure 1, 2, 3, 6 and 7. Illumination *per se* has no effect on neuronal excitability, fear conditioning or synaptic transmission in either *in vivo* or *ex vivo* studies.

A: Illumination controls for *ex vivo* recording in NBM: oChIEF expressing NBM neurons are exclusively depolarized by 473nm wavelength blue light. In *ex vivo* slice recording, NBM neurons expressing oChIEF were stimulated with 473nm (blue), 550nm (green symbols), or no light (black). Green light induced depolarization similar to baseline (no light) levels, while blue light induced about 2.5 fold larger depolarizations (n = 10).

B: Illumination controls for behavioral testing: Mice expressing a viral vector encoded GFP underwent fear conditioning, recall testing and extinction training as in Figure 2. A subset of these animals received illumination of the BLA for 2.5 minutes with 473 nm light to mimic the longest illumination in experiments with optogenetic manipulation of behavior (n = 4). There was no difference between the control group and the laser exposed group on training, recall or retention of extinction learning (Control vs GFP recall, p= 0 .392; control Ext vs GFP Ext, p= 0.633; Student's t-test).

C. Illumination controls for *in vivo* extracellular recording from BLA neurons: *ChAT-Cre* mice were infected with floxed AAV vectors encoding GFP alone or oChIEF. Cholinergic terminal fields were illuminated with the same stimulus paradigm as in **Figure 3**. In mice expressing oChIEF in cholinergic neurons of the NBM, 473 nm illumination evoked a 4 fold increase in BLA neuron firing: no increase in firing rate was observed in response to the same stimulation pattern in mice expressing GFP in cholinergic neurons of the NBM. Data are represented as mean ± SEM.

D: Illumination control for *ex vivo* recording of glutamatergic transmission: Blue light (473 nm, 100 pulses, 10 Hz) had no effect on evoked transmission in *ChAT* tau-eGFP mice. **Left bottom:** In the representative cell shown, neither the amplitude nor the success rate of eEPSCs was changed by blue light illumination. **Left top:** Sample trace from the same cell. **Right**: Box and scatter plots of pooled data from ex vivo recordings from 8 *ChAT* tau-eGFP mice. eEPSC amplitude was not affected by blue light illumination (Paired Wilcoxon Signed Rank Test, p=0.2936, n = 8).

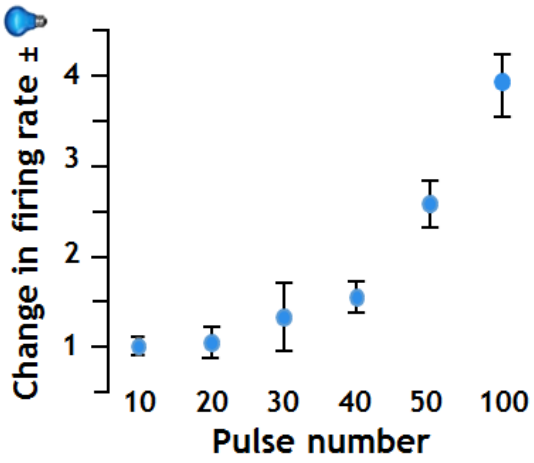


Figure S3. Related to Figure 2 and 3. Number of laser pulses delivered, rather than frequency of delivery, determines responsiveness *in vivo*.

1ms duration laser pulses were delivered at 10 Hz to the BLA of animals expressing oChIEF in cholinergic terminal fields. The number of pulses was varied and the ratio between the firing rate obtained post opto-stimulation to pre opto-stimulation was quantified. The degree of firing rate change in response to optical stimulation increased as the number of pulses delivered increased. The degree of firing rate change induced by delivery of 100 pulses was similar whether these pulses were delivered at 10 Hz or 20 Hz.

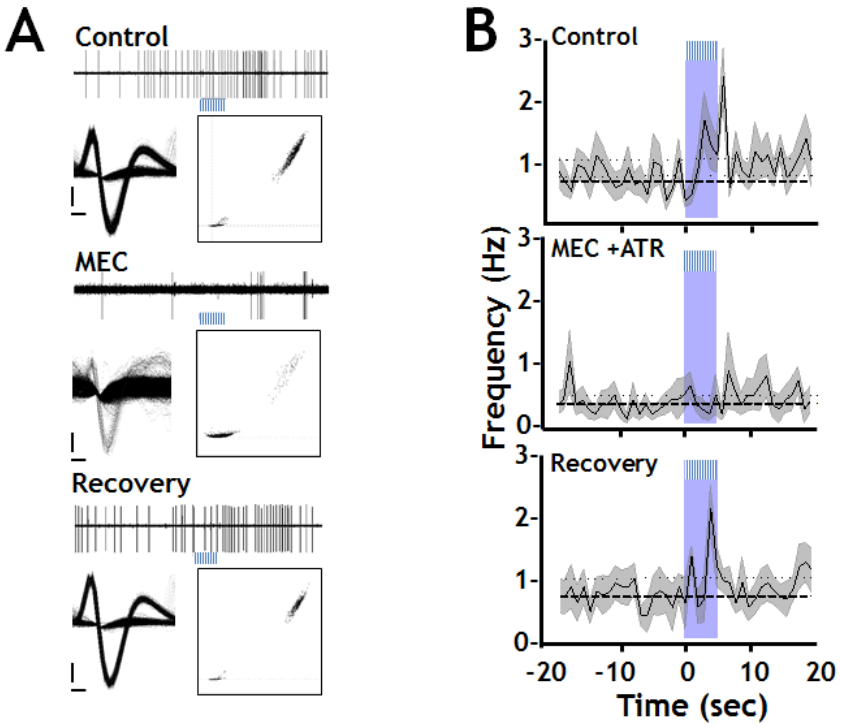


Figure S4. Related to Figure 3. Spike waveform & PCA before, during and after drug or ACSF injectrode mediated local perfusion

A: Extracellular recordings (spike amplitude: 200mV, optical stimulation duration: 5 s), sorted waveforms and plot of principle component 1 vs principle component 2 of the sorted waveforms are shown for a representative unit recorded with ACSF (control, top), during perfusion of mecamylamine (MEC, middle) and after washout of the drug with ACSF (Recovery, bottom). The consistency of the waveform shape, quantified by the consistency of its principle components, indicates that this is indeed the same unit recorded under all three conditions. Timing of optical stimulation on the raw data traces are indicated by blue hash marks. Calibration for inset: 500mV x 0.2msec.

B: Population frequency vs peri-stimulus time plots of 19 units recorded before, during and after photo-activation of cholinergic terminal fields in the BLA under control conditions (top, control), in the presence of both AChR antagonists (middle, mecamylamine (MEC) + atropine (ATR) at 10 µM and 200 nM respectively) and after saline washout of the antagonists (bottom, Recovery). These data are the same as those shown in figure 3B, but they have not been smoothed with the beta spline.

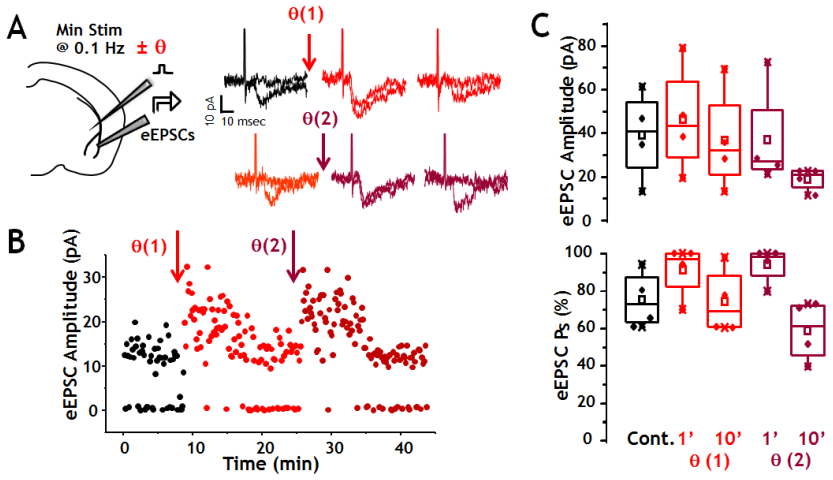


Figure S5. Related to Figure 6. Repetitive θ burst stimulation of cortical afferents does not induce LTP

A: Left: Schematic of configuration for voltage clamp recording from putative BLA principal neurons with electrical stimulation of cortical inputs under control (0.1 Hz) conditions before and after patterned **θ** burst stimulation (4 x 50 Hz bursts with 200 ms inter burst intervals). Repetitive electrical **θ** burst stimulation was delivered to the external capsule in *ChAT-Cre* mice without oChIEF expression. **Right:** sample recordings of single electrical evoked responses of cortico-BLA synapses before **θ** (black), after **θ** alone (red), and after a second **θ** burst (deep red). A single **θ** burst alone briefly increased the eEPSC amplitude and success rate. Repetitive **θ** burst stimulation did not evoke sustained potentiation (calibration: 10 pA x 10 msec).

B: Plot of evoked EPSCs (eEPSCs) obtained from a representative BLA neuron in response to 0.1 Hz stimulation before (black), after **θ** burst stimulation alone (red), and after repetitive **θ** burst (deep red) is shown. Electrical stimulations of the external capsule that did not elicit a post synaptic response (i.e. failures) are indicated by amplitudes of 0 pA. The number of failures was briefly reduced after **θ** burst stimulation alone. This same pattern of short term plasticity was observed after a second θ burst; i.e a second θ burst, unlike photo stimulation + θ did not further potentiate the synaptic transmission.

C: Box and scatter plot presentation of population data of eEPSC amplitude (**upper**) and eEPSC success probability (**lower**) before patterned stimulation (control, shown in black), after **θ** burst stimulation (red), and after repeated **θ** burst stimulation (deep red) calculated over the first 1 minute following stimulation (1') and the first 10 minutes following stimulation (10'). A single **θ** burst stimulation alone induced a brief increase in amplitude (Paired Wilcoxon Signed Rank Test, p =0.10035, n = 4) that returned to control within 10 minutes. Repeated **θ** burst stimulation did not elicit a sustained increase in eEPSC amplitude (Wilcoxon Signed Rank Test, p = 0.85513, n = 4). Success rate was briefly increased by a single **θ** burst alone (WSR, p = 0.10035, n = 4). Repeated **θ** burst stimulation did not elicit a sustained increase in Probability of success (Wilcoxon Signed Rank Test, p = 0.58388, n =4).

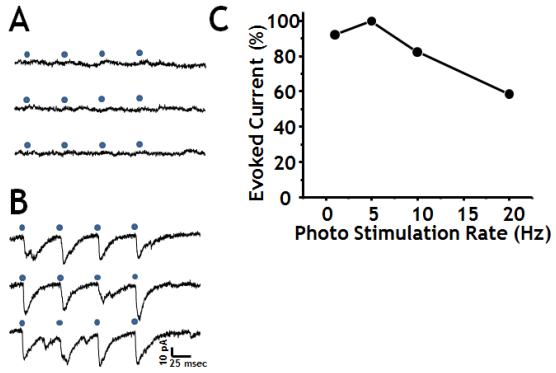
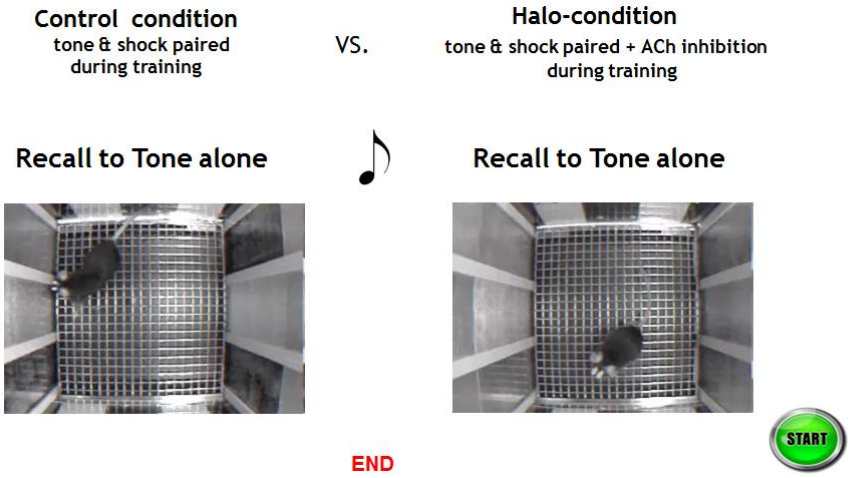


Figure S6. Related to Figure 5. Photo-stimulation of cholinergic terminal fields elicited direct inward currents in less than 1% of recordings.

A: Representative voltage clamp recordings in BLA principal neurons: blue dots indicate the time of individual flashes of 473 nm blue light. This recording, typical of the vast majority of those obtained, is devoid of detectable change in steady state current.

B: In only 3 cases did individual light flashes evoked direct inward current responses in BLA principal neurons. In these 3 cases, consistent postsynaptic responses were elicited without decrement for 100 repeat flashes at 1 - 10 Hz.

C: Increasing the frequency of stimulation above 10 Hz resulted in a decline in the number of successful evoked responses.



Supplemental Video (Related to Figure 2)

Videos of two mice during the test "recall" trial to tone alone are shown. The example on the left is a control mouse (optogenetically labeled, implanted but without light exposure during training). The example on the right is a Halo experimental mouse (identically labeled, implanted and exposed to 590 nm light only during the training period). Activation of the "start" button in slide mode initiates both videos together and the appearance of the sound symbol indicates the onset of the tone alone. The video continues until "END" appear.

Training Paradigms Tested +/- photo-stimulation						
CONDITIONS	Animals tested	Shock intensity / duration	Duration of tone	Tone/ shock pairings	Photo-stimulation parameters (Hz/ stim dur. /flash dur/λ)	Outcome relative to final protocol
T 1	3	0.2 mA / 2 sec	2 sec	4	no light (sham op)	Less robust learning
T 2	3	0.5 mA / 2 sec	2 sec	4	no light (sham op)	Somewhat more variable
T 3	2	0.8 mA / 2 sec	2 sec	4	no light (sham op)	Equivalent
Bl 1	36	0.8 mA /2 sec	30 sec	1	no light vs 20 / 5s/ 5 msec/ 473 (B)	Equivalent
Bl 2	22	0.5 mA / 2 sec	30 sec	1	no light vs 20 / 5 s - 2.5'/5 msec/473 (B)	Sham op (GFP)
Bl 3 = Final	67	0.8 mA / 2 sec	30 sec	3	no light vs 20 / 5s/ 5 msec/ 473 (B) &/or drug	Final protocol (473λ)
Y1	16	0.8 mA / 2 sec	30 sec	3	no light vs 2.5min/590 (Y)	Equivalent
Y2 = Final	40	0.8 mA / 2 sec	30 sec	1	no light vs 2.5min/590	Final protocol (590λ)

Retention of Extinction (RoE) Paradigms Tested					
CONDITIONS	Animals tested	Tone exposure (trials per day /duration/ days)	Sequence of delivery	Photo-stimulation parameters (Hz/ stim dur. /flash dur/λ)	Outcome relative to final protocol
RoE 1	20	4 / 2 sec / 7 days	Every 2 min over 10 min	no light	Less retention of extinction learning
RoE 2	32	10 / 30 sec / 7 days	Every 2 min over 20 min	no light	Equivalent
Final RoE Protocol	129	10 / 30 sec / 5- 7 days	Pseudorandom over 20 min	no light	Final Protocol

Supplemental Table S1 (Related to Figure 2)

A. Parameter space examined for behavioral training ± OPTO-stimulation: 4 times of 2 sec tone pairing with 2 sec, 0.8 mA foot shock induces an equivalent fear memory to the final protocol (final condition), while weaker shock, 0.2 or 0.5 mA, shock induced less robust memory (T1-T3). A single paring of a 30 sec tone with a 2 sec, 0.8 mA foot shock induces equivalent fear learning and recall (Bl 1).

B. Parameter space examined for assays of retention of extinction. Once the mice learned the tone-shock pairing, they need as many as 10 times tone-alone trials to show any within-day extinction (final protocol). A shorter time exposure (4 times of 2 sec exposure per day) does not induce any within-day extinction (RoE 1). After multiple days of tone delivery (10 x/day) the freezing level is equivalent to that seen with the final protocol (RoE2).
Bl 1, Y2 and Final RoE protocols were used in the experiments shown in Fig 2.

Supplemental Experimental procedures

Viral delivery

For viral infections, mice were anesthetized with 0.01 ml per 10 g body weight of a 9:1 ketamine (100mg/ml): xylazine (20mg/ml) cocktail (Baller Schein and Lloyd Laboratory, Iowa, USA respectively) positioned in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA) and injected with virus using a 26 gauge microsyringe (Hamilton, USA). For *in vivo* electrophysiological experiments, 0.25 µl virus was delivered to 3 injection sites bilaterally: Anterior/Posterior (from Bregma) -0.46 mm, -0.7mm and -0.94 mm; Medial/Lateral (from Bregma) ±1.2 mm, ±1.7 mm, and ±1.7 mm; Dorsal/Ventral (from dura) -3.85, -3.7, and -3.6. In all other experiments, 0.5 µl of virus was injected bilaterally to the central coordinate only. A subset of mice used for behavioral experiments were injected with an alternate viral vector encoding eGFP only (AAV9-CAG-DIO-eGFP), to control for the effects of illumination in the absence of light activated ion channels. After injection, craniotomy holes were covered with styptic powder (Kwik stop, ARC Laboratory), the scalp was repositioned using 3M Vetbond tissue adhesive and ketarolac tromethamine (30 mg/ml, from Hospira, Inc., Lake Forest, IL) was administered subcutaneously at a dose of 0.1 mg per 20 g of animal body weight.

Optogenetic targeting of NBM cholinergic neurons:

For the quantification of oChIEF-positive neurons in the NBM (**Fig 1B, C, D**), six ChAT-Cre x *tau*GFP mice were injected with the AAV construct as above. Two weeks post injection, mice were anesthetized with the ketamine/xylazine cocktail and transcardially perfused with 4% paraformaldehyde solution. Brains were extirpated, post-fixed overnight in the same fixative, and then transferred to phosphate buffered saline solution. Coronal brain slices (100 µm thick) were prepared using a vibratome (series 1000, Ted Pella, Inc, Redding, CA) and low magnification (10X) z-stack images were obtained by optically scanning at 5 µm/slice using a Laser scanning confocal microscope (Olympus FV1000). Matching brain slices and regions of interest (ROIs) within the NBM were chosen from each mouse based on anatomical landmarks. An ROI of 400 x 400 µm was divided into quadrants and evaluated in each slice. The number of single labeled *tau*GFP (green) and oChIEF-tdTomato (red) cells was determined, and the percentage of doubly labeled (*tau*GFP + oChIEF-tdTomato, yellow) cells was calculated in each ROI.

The use of oChIEF as the optogenetic label of choice for photo-stimulation of cholinergic inputs was made based on an in depth comparison of electrical vs. photo-stimulated cholinergic NBM neurons and assessment of the firing properties of cholinergic vs. non-cholinergic neurons in the NBM (Fig S1). These studies revealed that: (1) photo-stimulation and direct electrical stimulation of oChIEF neurons elicited equivalent firing patterns at rheobase, at maximum stimulation intensity and over a frequency range of 1- 20 Hz; and that (2) the firing properties of oChIEF expressing cholinergic NBM neurons were equivalent to oChIEF negative, cholinergic NBM neurons, but distinct from non-cholinergic neurons in the NBM (**Fig 1** & **Fig S1**). Earlier experiments testing firing patterns of channel rhodopsin 2 labeled cholinergic neurons indicated less faithful recapitulation of normal firing properties and motivated our switch to oChIEF for the current study (Cruikshank et al. 2012, Cruikshank et al. 2010, Grybko et al. 2011).

Fiber Optic Implantation

Mice used in behavioral testing underwent delivery of the optogenetic construct as above and implantation of bilateral fiber optics with magnetic connectors (Doric Lenses) targeted to the dorsal surface of the BLA (A/P -

1.6 mm; M/L -3.10 mm; and D/V -4.5 mm) in a single surgical procedure. The fiber-optic implants and connectors were secured to the skull with dental cement(Metabond).

Fear Conditioning, recall testing and retention of extinction learning

Initially we tested several variables in terms of both conditioned fear learning and extinction (Table S1). Based on these pilot studies we selected the following paradigms. Two weeks after virus injection/fiber-optic implantation in *ChAT-Cre* mice, the mice were handled daily for 5 days, and allowed to accommodate to fiber optic attachment and tethering in preparation for behavioral training. All training and assessments were done experimenter blinded as to condition. On the training day, mice were connected bilaterally via their fiber-optic implants to two, 200 μm diameter fiber-optics, connected to a 2-channel optical rotary joint, connected to a fiber-coupled laser, which was adjusted to produce 10-12 mW total power at the fiber tip, and were place in a fear conditioning chamber (Coulbourn Instruments). In mice expressing oChIEF (and in GFP controls), a 473 nm laser was used; in mice expressing eNpHR3.0, a 590 nm laser was used (Shanghai Dream Laser Corp). After being allowed to freely explore the chamber, a 30 s, 80 dB, 5 kHz tone was played, which co-terminated with a 2-second foot shock (0.5-0.8 mA). Two behavioral groups of littermates comprised the control group, which received no laser stimulation (or laser stimulation at an inappropriate wave length), and an opto-stimulated group which received laser light stimulation as follows: (a) oChIEF expressing, *ChAT-Cre* mice: 5 sec of laser-light, pulsed at 10-20 Hz x 5 msec pulse duration, co-terminating with the tone/shock pairing. The pairing was repeated 3 times, with 1.5 minutes between the end of the pairing and the onset of the next tone/shock/laser pairing (b) eNpHR3.0 expressing mice and AAV9-CAG-DIO-eGFP transduced mice: 2.5 minutes laser-light stimulation that began at the onset of the tone. All mice were allowed 2 minutes to freely explore the chamber after the termination of the final tone/shock pairing, and were then removed to their home cage. Chambers were thoroughly cleaned with 70% ethanol, and chamber floors were cleaned with 70% ethanol and rinsed with water and dried before each trial.

A test of the extent of fear learning (recall) was administered 24 hours after the completion of the training. The test protocol for recall was the same for all mice, in all experiments regardless of training conditions. To assay the response to cue alone, the contextual features of the test chambers were altered: the texture of the floors was changed and a mild vinegar-based cleaning solution (7th Generation, Fresh Scent) was used instead of ethanol. Mice were placed in the chambers (not connected to optical fibers) and allowed 2 minutes to freely explore the chamber. The test of the extent of fear learning was assayed as the percent time freezing to a single tone alone, identical to the tone used for training.

Following the test of the extent of fear learning, we began the process of extinction training. The final retention of extinction learning paradigm used a series of 10 tones, played over 20 minutes in a pseudo-random order with a minimum of 30 s and maximum of 2 minutes between tones. This order was shuffled on each extinction day. After the series of 10 tones, mice were allowed 2 minutes to freely explore before being removed to their home cages.

Freezing, defined as no movement (except for breathing) for a bout of at least 1 second, was measured using an automated video-based system (Actimetrics FreezeFrame). Experiments were binned in 30s intervals. Freezing was measured only during the first tone. The automated video analysis was cross-checked visually. For training days, freezing data was binned in 30 second increments. In oChIEF expressing mice, a two-way ANOVA was used to compare differences in freezing between groups and across time. In eNpHR3.0 expressing mice, a two-way ANOVA was used to compare differences in freezing between groups and across time bins before (opto light off) the tone/shock pairing, and after (opto light on) the tone/shock pairing.

For recall, freezing in each control group (no activation of either oChIEF or halorhodospin) were compared between experimental cohorts. Each control cohort displayed equivalent variance and no significant difference in freezing bouts were seen (4 cohorts of control vs. oChIEF ($F(3,29) = 1.74$, $p=.181$; 4 cohorts of control vs Halo stimulated animals ($F(3,21)= 1.895$, $p=.161$). Data presented in Figure 2B and 2C are derived from 2 cohorts (n=33) that underwent a 3 tone-shock pairing training protocol (Table S1). Data presented in Figure 2E and 2F are derived from 3 cohorts (n=40) that underwent a single tone-shock pairing training protocol. The effect of tone-shock pairings on fear memory recall (first tone exposure, 24 hr post-training) was compared within each experimental condition. The effect of oChIEF or halorhodopsin activation assessed by comparing freezing between controls and laser light exposed groups (Mann-Whitney Rank Sum). Statistical outliers were not eliminated from data analysis and are included in box plots as symbols beyond the 99% data/whisker boundaries.

The effect of extinction training on fear memory was assessed by quantifying the retention of extinction memories 24 hr following the prior extinction training session (Alberini and Chen 2012, Choi et al. 2010, Heinrichs et al. 2013, Kutlu and Gould 2014, Menezes et al. 2015, Radiske et al. 2015, Young et al. 2015). We chose this measure because (a) within session extinction learning is unreliable for cue conditioned mice and (b) it is the most relevant to understanding parameters that might influence the reversal of pathological fear learning (Anderson and Insel 2006, Quirk and Mueller 2008). For post-test extinction trials in both oChIEF and Halo experiments, mixed-model two- way repeated measures ANOVA was used with Sidak *post-hoc* multiple comparisons.

Pharmacology in Behavior

A subset of mice used in behavioral testing underwent implantation of bilateral cannulas for drug delivery targeted to the BLA (A/P -1.6 mm; M/L -3.10 mm; and D/V -5.0 mm) and were allowed several weeks to recover. They then underwent handling as described above. Immediately prior to behavioral training, 0.5 µl of pharmacological agent was administered simultaneously bilaterally at a rate of 0.125 µl every 10 s. Mice were administered either saline, a cocktail of both atropine (1µM) and Mecamylamine (20 µM) or atropine alone (20 µM). To limit the amount of clearance of the drug, the normal 2 minutes acclimation period before the first tone/shock pairing was cut to 1 minute. Recall and extinction learning were conducted as above (Table S1 Bl 3 / Final RoE).

Fiber optic and infusion cannula relocation

After behavior tests, mice were anesthetized with the ketamine/xylazine cocktail and transcardially perfused with 4% paraformaldehyde solution. Brains were extirpated, post-fixed overnight in the same fixative, and then transferred to phosphate buffered saline solution. Coronal brain slices (100 µm thick) were prepared using a vibratome (series 1000, Ted Pella, Inc, Redding, CA). Using a stereoscope (Zeiss SteREO Discovery V20, Zeiss AxioCam Mrm, San Francisco, CA), low magnification images under white light were taken for track relocation, epi-fluorescence images were taken for viral expression check. Track of fiber optic and/or infusion cannula were found and relocated onto adjusted mouse brain atlas. Animals with the end of cannula track above or in the BLA area on at least one side of the brain, and with good expression of virus in BLA were used for further analysis.

***In vivo* electrophysiological recording**

In vivo single unit extracellular recordings were collected using 1 MΩ tungsten electrodes (A-M System, USA). The electrode was placed inside an injectrode (Alpha Omega, USA), which was attached to a flow controller system (BASi, USA) and positioned into BLA (A/P -1.65, M/L -3.32, D/V -4.6 mm from dura). The injectrode

assembly was coupled with a 200 µm diameter optical fiber connected to a 473 nm laser (Shanghai Dream Laser Corp).

In preliminary studies with *in vivo* recording we tested a range of stimulation protocols that included assays of light evoked responses at 5, 10, 15 or 20 Hz, and using stimulation duration of 5 msec and a a range of light intensities (Fig 1). We also examined the effects of stimulating with 10, 20, 50, 100 pulses at 10 or 20 Hz (Fig S3). From these studies we concluded that 100 pulses at ~1 mW/mm2 was optimal (e.g. 10 x10 Hz bursts or 5x 20 Hz bursts with a stimulus duration of 5 msecs; Fig 1; Fig S3 (Munoz et al. 2014)). Based on these tests we next tested a subset of protocols in the *ex vivo* preps. Because of the slightly lower fidelity of NBM stimulation in *ex vivo* preps at 20 Hz and the limitations of the shutter for light exposure in the *ex vivo* configuration, we settled on a 10 Hz x 5 msec stimulus duration for 10 seconds as our standard stimulation paradigm in the *ex vivo* studies. Both the dynamics and the extent of modulation of excitability are similar with these stimulation parameters *in vivo* and *ex vivo*.

In all of the experiments presented, photo-stimulation of the cholinergic terminal fields *in vivo* was identical to those used in the behavioral training paradigm and was controlled by Spike2 version 6.1 software. Simultaneous single unit extracellular recordings were acquired as represented in Figure 3A. I*n vivo* recordings were acquired at 10 KHz using Spike2 and a CED Power 1401 data board, and were pre-amplified by 1000 times with the aid of a head stage attached to an A-M Systems amplifier (model 1700). Recorded spike trains were then sorted offline using a combination of waveform template matching and cluster analysis based on the primary components (see Supp Fig 4, Plexon software) and further analyzed using Neuroexplorer version 4.1 (Nex Technologies, MA, USA). The vast majority of units recorded from BLA exhibited the profile typically associated with BLA principal neurons, referred to as regular spiking BLA neurons (Duvarci and Pare 2014). These had low firing rates and their spike profiles had relatively long peak to trough durations (see Fig 3A, inset, black trace). A subset of units recorded from BLA exhibited the faster and higher firing rate profile that is typical of interneurons (see Fig 3A, inset, grey trace; (Duvarci and Pare 2014)). These fast spiking units were excluded from further analysis based on their ambiguous cell type.

Population peri stimulus time histograms (PSTH) represent pooled data from 22 BLA units (n=19; **Fig 3B**; n = 3 **Fig 3E**) and were smoothed using a beta-spline (non-smoothed results are shown in Fig S4). Mean ± SEM analyses of firing rates were performed for at least 20 second before and after optogenetic stimulation and significance determined by t test (Fig **3C**). For assay of temporal dynamics (**Fig 3D**), a cumulative plot of spike counts vs time for all cells (n=19) was determined. The pooled cumulative distribution is plotted ± S.E.M and the fit to the pooled data is shown as dotted lines.

In vivo pharmacology

Pharmacological agents were delivered locally to the BLA *in vivo* through the injectrode. Artificial CSF was delivered during recording of baseline and initial response to optical stimulation, followed by four microliters of a cocktail of 0.5 µM atropine and/or 10 -20 µM mecamylamine (Tocris) delivered at a rate of 1 µl / min. Before and after completion of drug infusion, ACSF was delivered via the injectrode into the same area for 30 minutes - 1 hour to serve as vehicle control and/or to wash out the drug (Fig 3 and Fig S4).

Ex vivo slice preparation

Coronal brain slices were prepared from injected mice 3 weeks after surgery. Animals were anesthetized with a ketamine/xylazine cocktail (100 mg/kg ketamine + 6 mg xylazine/kg; i.p.) and then transcardially perfused with a sucrose-based solution (details below). After decapitation, the brain was transferred quickly into a sucrose-

based cutting solution bubbled with 95% O_2 and 5% CO_2 and maintained at ~3°C. This solution contained (in mM): sucrose 230; KCl 2.5; $MgSO_4$ 10; $CaCl_2$ 0.5; NaH_2PO_4 1.25; $NaHCO_3$ 26; glucose 10 and pyruvate 1.5. Coronal brain slices (300μm) were prepared using a Leica VT1000S vibratome (Leica, Inc). Slices were equilibrated with a mixture of oxygenated artificial cerebrospinal fluid (aCSF) and sucrose-based cutting solution at room temperature (24-26°C) for at least 1 hour prior to transfer to the recording chamber. Pyruvate (0.15–0.75 mM) was added to reduce oxidative damage and enhance survival. With this protocol, slices are first incubated in a mixture of 50% cutting solution with pyruvate and 50% aCSF (in mM): sucrose 115; NaCl 63; KCl 2.5; NaH_2PO_4 1.25; $MgSO_4$ 5; $CaCl_2$1.25; $MgCl_2$ 1; $NaHCO_3$ 26; glucose 10; and sodium pyruvate 0.75 at 35°C for 30 min and then transferred to a mixture of 10% cutting solution and 90% aCSF (in mM): sucrose 23; NaCl 113.4; KCl 2.5; NaH_2PO_4 1.25; $MgSO_4$ 1; $CaCl_2$ 1.85; $MgCl_2$ 1.8; $NaHCO_3$ 26; glucose 10; and sodium pyruvate 0.15 at 35°C for 1–4 h prior to recording. The slices were continuously superfused with aCSF at a rate of 2ml/min containing (in mM); NaCl 126, KCl 2.5, NaH_2PO_4 1.25, $NaHCO_3$ 26, $CaCl_2$ 2, $MgCl_2$ 2 and glucose 10 bubbled with 95% O_2 and 5% CO_2 at room temperature.

***Ex vivo* Electrophysiological recording**

Brain slices were placed on the stage of an upright, infrared-differential interference contrast microscope (Olympus BX51WI, Olympus Optical). BLA pyramidal neurons were visualized with a 40X water-immersion objective by infrared microscopy (COHU 4915 camera, COHU, Inc). Patch electrodes with a resistance of 4–6 MΩ were pulled with a laser based micropipette puller (P-2000, Sutter Instrument Company). Signals were recorded with a Multi Clamp 700A amplifier and pClamp10 software (Molecular Devices, Inc). The pipette solution contained (in mM) 130 K-gluconate, 2 KCl, 2 $MgCl_2$, 10 HEPES, 0.5 EGTA, 1 ATP and 0.2 GTP (pH=7.3). All recordings included in this study are from rapidly activating and strongly accommodating, putative principal neurons in the BLA (Jiang and Role 2008). The following criteria were applied for inclusion of recorded cells in the study; failure to meet all of these criteria resulted in exclusion from the sample population: (a) seal resistance maintained throughout the recording period at >5 GΩ; (b) holding current in whole cell clamp configuration remained within 10% of the initial value and was ≤ 100 pA; (c) Series resistance (R_S) measured at 10 min intervals remained stable at < ±10% of initial value throughout the course of the experiment.

To examine transmission at cortical-amygdala inputs, EPSCs were evoked by field stimulation with a concentric bipolar stimulation electrode (FHC, Inc) placed in the external capsule (M/L 3.2, D/V 3.8 mm). A 0.1 Hz single stimulation (duration: 1 ms, intensity: 10-30 μA) was delivered via the Master-8 stimulator (A.M.P.I., Inc). For minimal stimulation, the stimulation strength was adjusted to trigger EPSCs in ~50% of the trials, which was typically equivalent to activation at ~10-15% of the maximum response amplitude (Jiang et al. 2013). The theta burst stimulation pattern (θ) used was four 2 ms pulses of depolarization at 50 Hz, repeated 10 times at a 5 Hz inter-bust interval.

Tetrodotoxin (1μM) resistant, glutamatergic EPSCs in BLA pyramidal neurons were monitored in voltage clamp configuration at a holding potential of -60mV. Bicuculline (10 μM) was continuously present in aCSF to isolate TTX resistant glutamatergic synaptic transmission. Data were filtered at 2 kHz by Multi Clamp 700A and analyzed using Clampfit10 (Axon instruments, Inc) and Mini Analysis 6.0 (Synaptosoft, Inc).

To examine action potential firing frequency, BLA principal neurons were recorded in a current clamp configuration after forming a giga-ohm seal. Membrane potentials were clamped at -60 or -50 mV by injecting 0-~50 pA current through the recording electrode. Cells that maintained steady membrane potentials for at least 5 mins were included in the analysis.

Optogenetic stimulation *ex vivo*

oChIEF was activated with a train of light flashes delivered through the 40x microscope objective. The light source was an Olympus x-cite 120Q lamp (Olympus) gated with a TTL controlled shutter (LAMBDA SC, Sutter Instrument). The filter cube contained an HQ480/40x excitation filter, a Q505lp bypass filter and an HQ535/50m emission filter (Chroma Technology). The fluorescence illumination intensity delivered at the brain slices was adjusted to 1-3 mW/mm^2, measured with a PM100D optical power and energy meter (THORLABS). In the NBM cholinergic neurons were identified by tdTomato fluorescence and light flashes were delivered at 1 Hz, 5 Hz, 10 Hz, 20 Hz, and 30 Hz. In the BLA we recorded from principal neurons located in the zone of maximal oChIEF-tdTomato expression and light flashes were typically delivered at 10Hz.

Drug application *ex vivo*

Drugs were stored as concentrated stock aliquots and diluted in aCSF for each experiment. EPSCs were recorded following local superfusion at a rate of 2 ml/min. All drugs were delivered in bath via superfusion at the following concentrations: TTX 1 μM, bicuculline 10 μM, MEC 10 μM (all from Tocris), and atropine 500 nM (Sigma).

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