It has been proposed that memories are encoded by modification of synaptic strengths through cellular mechanisms such as long-term potentiation (LTP) and long-term depression (LTD)\(^1\). However, the causal link between these synaptic processes and memory has been difficult to demonstrate\(^2\). Here we show that fear conditioning\(^3\), a type of associative memory, can be inactivated and reactivated by LTD and LTP, respectively. We began by conditioning an animal to associate a foot shock with optogenetic stimulation of auditory inputs targeting the amygdala, a brain region known to be essential for fear conditioning\(^4\). Subsequent optogenetic delivery of LTD conditioning to the auditory input inactivates memory of the shock. Then subsequent optogenetic delivery of LTP conditioning to the auditory input reactivates memory of the shock. Thus, we have engineered inactivation and reactivation of a memory using LTD and LTP, supporting a causal link between these synaptic processes and memory.

To examine the relation between synaptic plasticity and memory, we used cued-fear conditioning\(^5\) in rats, wherein a neutral conditioned stimulus (CS), such as a tone, when paired with an aversive unconditioned stimulus (US), results in a tone-driven conditioned response (CR) indicating memory of the aversive stimulus. Temporally (but not non-temporally) pairing a tone with a shock led to a robust CR (reduced lever pressing to a previously learned cued lever-press task\(^6\); Extended Data Fig. 1) during subsequent testing with a tone alone\(^6,7\) (Fig. 1a). To investigate the synaptic basis underlying this associative memory, we replaced a tone with optogenetic stimulation of neural inputs to the lateral amygdala originating from auditory nuclei. We injected an adeno-associated virus (AAV) expressing a variant of the light-activated channel ChR2, oChIEF, that can respond faithfully to 50–100 Hz stimuli\(^8\), into the medial geniculate nucleus and auditory cortex (Extended Data Fig. 2).

After the channel reached axonal terminals in the lateral amygdala (Extended Data Fig. 3), a cannula permitting light delivery was placed targeting the dorsal tip of the lateral amygdala (Extended Data Fig. 4). An optical CS alone (a 2 min 10 Hz train of 2 ms pulses, see Methods) had no effect on lever pressing (Extended Data Fig. 5). However, temporally (but not non-temporally) pairing the optical CS with a foot shock (see Methods) led to a CR (Fig. 1b) that was sensitive to extinction (see below) and blocked by NMDA receptor inhibition during conditioning (Extended Data Fig. 6), indicating the generation of an associative memory.

To examine if LTD occurred after pairing optical CS with foot shock\(^5,6\) (Fig. 1d), we prepared amygdala brain slices from animals receiving unpaired, paired or no conditioning, and measured the AMPA receptor component (A) and NMDA receptor component (N) of the optically driven synaptic response (Fig. 1c). The A/N ratio increased in animals receiving paired conditioning indicating that LTD had occurred\(^9,10\) at optically driven inputs to lateral amygdala neurons.

Can memories be inactivated? If LTD occurred at the optically driven synapse onto the lateral amygdala, and this LTD contributes to the memory, reversing LTD with LTD protocol (see Methods). One day later, animals were tested with optical CS and displayed no CR, indicating inactivation of the memory of the shock by LTD (Fig. 2a, b, f). Next we examined if memories can be reactivated. To these animals we delivered an optical LTP protocol (see Methods). One day later, animals displayed a CR...
Figure 2 | LTD inactivates and LTP reactivates memory. a–e. A single group of rats (n = 12) was tested for CR two days following paired conditioning of ODI and shock (a). Graphs as in Fig. 1. After testing, animals were delivered an optical LTD protocol and tested for CR one day later (b). After testing, animals were delivered an optical LTP protocol and tested for CR one day later (c). After testing, animals were delivered another optical LTD protocol and tested for CR one day later (d). After testing, animals were delivered another optical LTP and tested for CR one day later (e). f. Normalized lever presses one minute into optical CS after different protocols (as indicated). g. Cellular models of synaptic modifications occurring in the lateral amygdala that may contribute to behavioural responses following LTD (left) or LTP (right) protocols delivered to ODI.

(Fig. 2c, f), suggesting reactivation of the memory. Synapses are capable of undergoing multiple rounds of bidirectional plasticity. We thus delivered a second optical LTD protocol; the next day animals produced no CR (Fig. 2d, f), indicating re-inactivation of the memory. Subsequent optical LTP conditioning recovered the CR (Fig. 2e, f and Extended Data Fig. 7) indicating reactivation of the memory. The behavioural effects of LTD and LTP conditioning were rapid and long lasting (Extended Data Fig. 8). These experiments suggest that a necessary component of the optical CS-triggered memory of the shock can be inactivated by LTD and reactivated by LTP.

In the experiments described above, LTP may be restoring a memory of the shock or merely potentiating random inputs that are sufficient to drive lateral amygdala neurons that produce fear and reduce lever pressing. Thus, we examined if generation of a CR by an LTP protocol requires prior optical CS-shock pairing. Indeed, an LTP protocol produced a CR only in animals that had previously received optical CS-shock conditioning (Fig. 3). These results support the view that LTP reactivates the memory that was formed by optical CS-shock pairing and inactivated by LTD.

To confirm that the test and conditioning stimuli were producing the expected synaptic effects, we conducted in vivo recordings in the lateral amygdala of anaesthetised rats expressing oChIEF in auditory regions (see Methods). Brief light pulses at the recording site produced in vivo field responses (that resembled optically evoked responses in amygdala brain slices; Extended Data Fig. 9), which were not affected by optical CS, depressed by optical LTD conditioning and potentiated by optical LTP conditioning (Fig. 4 and Extended Data Fig. 10). These results confirm that the synaptic stimulation conditioning protocols used to perturb behaviour modify synapses in the expected manner.

To examine further the relationship between these synaptic stimulation conditioning protocols and memory processes, we tested the effects of these protocols on auditory cued-fear conditioning. In two groups of naive animals, we infected unilaterally auditory regions with AAV-oChIEF, and pharmacologically ablated the contralateral amygdala (see Methods). One group of animals received tone paired with shock, which led to a tone-evoked CR (Fig. 5a, d). A second group of animals received the same tone paired with shock conditioning, immediately followed by an optical LTD protocol. This second group showed significantly reduced tone-evoked CR (Fig. 5b, d); subsequent tone conditioning without an optical LTD protocol produced a tone-evoked CR (Fig. 5c, d). This result is consistent with a memory model in which tone conditioning induces LTP at auditory inputs to the lateral amygdala and that subsequent LTD at these synapses reverses LTP and thereby inactivates the memory.

Next we examined extinction, a process whereby repeated exposure to a CS (in the absence of a shock (US)) leads to a reduced CR. We asked if optical LTP reverses extinction of tone conditioning. Animals received tone conditioning and an extinction protocol (see Methods), which removed the CR (Fig. 5e). Delivery of an optical LTP protocol did not restore the CR (Fig. 5f, g), consistent with the view that extinction is not a weakening of synapses potentiated during paired conditioning. Similarly, animals receiving paired optical CS-shock conditioning produced a CR that could be removed by repeated exposure to optical CS (see Methods) and optical LTP did not recover the CR (Fig. 5h–k), again demonstrating that extinction is not LTD.

Prior studies examining the relation between LTP, LTD and memory have employed pharmacological (for example, ref. 15) or genetic (for example, refs 16, 17) manipulations to perturb and demonstrate parallels between cellular and behavioural processes. Other studies have measured randomly sampled sites in regions required for memory formation to detect changes in biochemistry and synaptic transmission following memory formation14–21. However, selective perturbation of synapses that are employed to form a memory was not possible in these studies. Here by optogenetically isolating a neural input that can be used to form an associative memory, we can selectively manipulate synapses driven by this input and assess directly the relationship between cellular and behavioural processes.

Formation of an associative memory produced LTP at the lateral amygdala optogenetic input, as indicated by an increased A/N. Such LTP appears to be required as delivery of an LTD conditioning stimulus that can reverse LTP effectively removed the ability of the optogenetic
input to elicit the memory. Furthermore, subsequent delivery of an LTP conditioning stimulus to the optogenetic neural input restored the CR. Our data support the view that LTP had reactivated the memory of the aversive stimulus, because delivery of an LTP protocol without prior formation of the memory did not evoke a CR. Our findings demonstrate that memories of aversive events formed through activation of selected inputs can be turned off and on by conditioning protocols that produce bidirectional synaptic plasticity at those inputs, strengthening the causal relation between synaptic plasticity and memory formation22.

It is notable that optical LTP in naive animals did not produce a CR; whereas in these animals, optical LTP did produce a CR after optical CS-shock pairing and optical LTD. This result suggests that non-specific inputs can be turned off and on by conditioning protocols that produce bidirectional synaptic plasticity at those inputs, strengthening the causal relation between synaptic plasticity and memory formation22.

Figure 3 | LTP produces conditioned response only after prior paired conditioning. a–f, A naive group of animals (n = 4) was tested for CR one day after LTD protocol (a), one day after subsequent LTP protocol (b), one day after subsequent paired optical CS-shock conditioning (c), one day after subsequent LTD protocol (d) and one day after subsequent LTP protocol (e). f, Graph of normalized lever presses one minute into optical CS one day following indicated protocols. g–k, A separate naive group of animals (n = 5) was tested for CR one day after LTP protocol (g), one day after paired optical CS-shock conditioning (h), one day after subsequent LTD protocol (i) and one day after subsequent LTP protocol (j). k, Graph shows normalized lever presses one minute into optical CS one day following indicated protocols. Note that CR is seen following LTP protocol only after prior paired conditioning. It is notable that optical LTP in naive animals did not produce a CR; whereas in these animals, optical LTP did produce a CR after optical CS-shock pairing and optical LTD. This result suggests that non-specific

Figure 4 | In vivo electrophysiological responses to 10 Hz, LTD and LTP protocols. a–c, Left, in vivo field response (average of 20 responses) in lateral amygdala to single optical stimulus before (black) and after (red) indicated conditioning protocol. Plot of individual experiment (middle) or average of 10 experiments recorded from 10 rats (right) of field EPSP slope (normalized to baseline period) before and after indicated stimulation. Average baseline normalized value 30–40 min following conditioning: 10 Hz, 102.2 ± 5%; 1 Hz, 82 ± 8%; 100 Hz, 118 ± 9%. Scale bars, 1 mV, 10 ms.
potentiation of auditory inputs to the lateral amygdala is not sufficient to produce a CR. It may be that specific potentiation onto a subset of inputs, presumably those neurons also activated by the foot shock, is necessary to produce a CR. Furthermore, the pairing of optical CS with shock probably produces additional modifications (not produced by optical LTP alone) that may be required to produce a CR23–26. Thus, LTP is used to form neuronal assemblies that represent a memory.

Our studies complement recent studies that have used optogenetics to examine how neuronal assemblies can represent a memory27–29. In those studies synaptic mechanisms were not examined. Our studies suggest that LTP is used to form neuronal assemblies that represent a memory. Furthermore, our findings predict that LTD could be used to disassemble them and thereby inactivate a memory.

METHODS SUMMARY

Surgery. AAV expressing a variant of the light-activated channel ChR2, oChIEF16, was injected into the auditory nuclei of 6–8-week-old rats. Then 3–4 weeks later an optic fibre cannula was placed above the dorsal tip of the lateral amygdala (dLA).

Behaviour. Rats were trained to associate lever press for a reward and tested for a CR during the lever press task. Tone conditioning protocol consisted of 10 pairs of 20 s tones co-terminated with 500 ms of 0.5 mA foot shock. Optical conditioning was as above, except that each tone was replaced with 1 s of 10 Hz blue light. Optical plasticity induction. LTD was induced with 900 2 ms pulses of light delivered at 1 Hz. LTP was induced with 5 trains of light, each train containing 100 2 ms pulses, delivered at 100 Hz, with 3 min inter-train intervals.

During all behavioural manipulations the light intensity remained the same for each animal.

In vitro recording. Acute slices were prepared from rats expressing AAV-oChIEF in the auditory nuclei. Extracellular field potentials (fEPSPs) or excitatory postsynaptic current (EPSC) responses were obtained from the dLA by optical stimulation of the auditory projections.

In vivo recording. Rats expressing AAV-oChIEF in auditory nuclei were anaesthetized and a recording glass pipette was placed in the dLA. fEPSPs were evoked using an optic fibre placed above the recording site.

Analysis. A CR was measured as the reduction in the frequency of lever presses during the CS (2 min of tone or 10 Hz light stimulation). fEPSP initial slope and EPSC amplitude were measured.

All values indicate mean ± s.e.m. Student’s paired and non-paired t-tests were used with P < 0.05 considered as significant. All behavioural data were reanalysed with Wilcoxon rank-sum test which produced similar significance values as the t-test.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Author Contributions S.N. and R.M. designed the experiments and wrote the manuscript. S.N., R.F. and R.M. analysed the data. S.N., R.F. and C.D.P. performed the experiments. J.Y.L. and R.Y.T. provided the oChIEF-tdTomato construct.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to R.M. (rmalinow@ucsd.edu).
METHODS

Subject. Male Spargue-Dawley rats, aged 6–8 weeks for virus injection and cannula placement and 10–12 weeks for behavioural and electrophysiological studies, were housed two per cage and kept on a 12/12 h light–dark cycle (lights on/off at 7:00/19:00). The behavioural studies were done during daylight. All procedures involving animals were approved by the Institutional Animal Care and Use Committee of the University of California, San Diego.

Virus. We used a ChR variant, named oChIEF, which is a mammalian codon optimized version of ChIEF10,30 with the same properties except that it has stronger expression in mammalian cells and has an additional N-terminal amino acid residue. Expression was driven by the neuron-specific synapsin promoter30.

Surgery. Male Spargue-Dawley rats, aged 6–8 weeks, were anaesthetized with isoflurane for stereotactic injection of AAV-oChIEF into the medial geniculate nucleus (AP: −5.1 mm and −5.7 mm; ML: 2.9 mm; DV: −5.5 to −6.7 mm) and the auditory cortex (AP: −5.7 mm; ML: 4.8 mm with a 20° angle; DV: −4.5 to −5.7 mm). A total of 0.4–0.5 μl of virus was injected over a 10–15 min period. At the end of the injection, the pipette remained at the site for 5 min to allow for diffusion of the virus.

An optic fibre cannula (Doric Lenses) was implanted just above the dorsal tip of the lateral amygdala (AP: −3.3 to −3.5 mm; ML: 4.2 mm; DV: −7 mm with a 7° angle) and secured to the skull with screws and dental cement. Rats were injected with 5 μg per kg carprofen (NSAID) after surgery.

Excitotoxic lesion. Rats aged 6–8 weeks were anaesthetized with isoflurane for stereotactic injection of N-methyl-D-aspartate (NMDA) into one amygdala (AP: −3 mm; ML: 4.2 mm; DV: −7 to −8 mm with a 7° angle). 0.5 μl of NMDA (20 mg ml−1) was injected over a 10–15 min period31. At the end of the injection, the pipette remained at the site for 5 min to allow for diffusion of the solution.

Behavioural assays

Training. Rats were trained to associate lever press for a reward (40 μl of 10% sucrose per lever press). During the training period rats were kept on a restricted water schedule (2 h daily of water ad libitum). Training context was a modular operant test chamber (12.5 × 10 × 13 inches) with a stainless steel floor and open roof located in a sound attenuating cubicle (Med Associates, St. Albans, VT). The test chamber was equipped with a retractable response lever, a liquid dispenser receptacle and a light above the dispenser that signalled when liquid was injected into the dispenser. The consumption of liquid was detected by a head entry detector in the receptacle; each successive liquid reward was subsequently followed by a 15 s delay after head removal from the receptacle. The system was controlled and the data collected through a MED-SYST-16 interface, which was controlled by MED-PC IV software running on a PC. Rats were initially trained to associate the reward with the light above the dispenser receptacle. In a 45 min session, rats with at least 60 head entries into the receptacle were selected for lever press training.

Lever-press training was conducted in the same context as above, but this time rats had to press a lever to receive the liquid. The level press turned the light above the receptacle on, which in the previous training session they had associated with liquid in the receptacle. Rats with a minimum of 6 responses per min in the first 10 min of the training session were selected for conditioning.

Tone conditioning. The conditioning chamber was a box (12 × 10.5 × 13 inches) with an electrified grid floor (Couilbourn Instruments, Allentown, PA) within a larger sound-attenuating box. Rats had full access to water 24 h before conditioning. Conditioning protocol consisted of 10 trials of 20 s tone (tone volume 80 dB), with randomized intervals (average interval duration 3 min). In the paired group tones were co-terminated with a 0.5 ± 0.5 mA footshock (or a single 20 s tone co-terminated with a 1 ± 0.5 mA footshock for mild conditioning, Fig. 5). In unpaired group tones and shocks were separated by at least 1 min. Paired and unpaired groups received equal number of tones (CS) and shocks (US) in the same context; however, only in the paired group did tone and shock coincide. The next day, conditioned rats were placed into the test chamber to measure the effect of CS on their lever presses (for details, see the section on testing).

Optical conditioning. Rats were placed into the conditioning chamber and were attached to an optic fibre patch cord connected to a 473 nm solid-state laser diode (OEM Laser Systems) with 15–20 mW of output from the 200 μm fibre. They were allowed to explore the chamber for 3 min before the conditioning. Optical conditioning was 10-trains of blue light (10 pulses of 10 Hz, 2 ms duration) applied at randomized intervals with an average of 3 min apart. For paired conditioning, the light stimulus co-terminated with 0.5 ± 0.5 mA footshock; in unpaired conditioning, the light and shock were separated by a minimum of 1 min. Paired and unpaired groups received equal number of light stimuli and shocks in the same context; however, only in the paired group did light and shock coincide. The delivery of shock and light was controlled by a pulse generator (Master 8; AMPI, Jerusalem, Israel). After the conditioning rats remained in the box for additional three minutes before returning to their home cage.

Testing. After the conditioning, rats were water restricted for 24 h before they were tested for lever press testing. Testing was done in the same context as training except that the floor was a plastic sheet with white and red strips. Testing was a 7 min session in which rats had to press a lever to receive the liquid (10% sucrose). Rats were attached to the optic fibre patch cord, placed into the chamber, and allowed to explore the environment for 5 min before having access to the lever. The testing session, in which rats had free access to the lever, was a 3 min period of no light, followed by two minutes of light on (10 Hz of pulses with 2 ms duration), and 2 min of no light. At the end of the session rats were returned to their home cage. Only rats that in two consecutive days showed consistent reduction (>30%) in the lever press during the light-on period were used for further behavioural phases. Those which failed the test were examined histologically to locate the position of cannula and viral injection (Extended Data Fig. 4).

Tone-conditioned rats were tested in the same way except that they received 2 min of tone instead of light stimulation.

LTD induction. Within one hour following testing, rats were placed in a separate context, a translucent plastic container (22.5 × 15 × 12 inches), attached to the optic fibre patch cord and allowed to explore the environment for 3 min before LTD induction. Optical LTD was induced with 900 pulses of light, each 2 ms, at 1 Hz. After the induction rats remained in the chamber for 3 additional minutes before returning to their home cage.

During all behavioural assays the light intensity remained the same for each animal. At the end of the experiment, animals were perfused and the location of the optic fibre was verified.

Systemic injection of MK801. Rats were anaesthetized with isoflurane for 5 min before being given an intraperitoneal injection of MK801 (ref. 32) (0.2 mg per kg) in sterile saline. The conditioning protocol was administered 30 min following injection.

Perfusion, slicing and imaging. Prior to perfusion, rats were administered a ketamine/dexdomitor (75 and 5 mg per kg respectively) mixture by intraperitoneal injection. Rats were then transcardially perfused with ~150 ml of saline followed by ~150 ml of 4% paraformaldehyde in 0.1 M phosphate buffer solution (PB, pH 7.4). Brains were then fixed overnight in the same solution and rinsed and stored in 0.1 M PB for slicing.

Brains were sliced coronally in 150 μm sections using a vibratome sectioning system and stored in PB. Slices were imaged using an Olympus XYX10 epifluorescent microscope to verify AAV-oChIEF-tomato expression in the MGN, auditory cortex, and their projections to the dorsal lateral amygdala. Additionally, appropriate positioning of the optic fibre cannula over the lateral amygdala was verified.

In vitro recording. For extracellular field potential recordings, acute slices (as described in ref. 33) were prepared from 3–4-month-old rats expressing AAV-oChIEF in the medial geniculate nucleus and/or auditory cortex. Extracellular field potentials were recorded with Axopatch-1D amplifiers (Axon Instruments) in dorsal tip of the lateral amygdala with glass electrodes (1–2 MΩ) filled with the perfusion solution. The auditory projection to the lateral amygdala was evoked by optical stimulation above the recording site. To measure AMPA-R field potential, 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline-2,3-dione (NBQX) (10 μM) was added at the end of the experiments. Data were acquired and analysed using custom software written in Igor Pro (WaveMetrics). The perfusion solution contained: 119 mM NaCl, 2.5 mM KCl, 2 mM CaCl2, 1 mM MgCl2, 26 mM NaHCO3, 1 mM NaH2PO4, 11 mM glucose (pH 7.4), and gassed with 5% CO2/95% O2.

For whole-cell recording, acute slices (as described in refs 34–36) were prepared from 3–4-month-old rats expressing AAV-oChIEF in the medial geniculate nucleus and/or auditory cortex. Whole-cell recordings were obtained from individual cells in dorsal tip of the lateral amygdala using glass pipettes (3–4 MΩ) filled with internal solution containing, in mM, cesium methanesulfonate 115, CsGlc 20, HEPES 10, MgCl2 2.5, Na2ATP 4, NaGTP 0.4, sodium phosphocreatine 10, and EGTA 0.6, at pH 7.25. External perfusion consisted of artificial cerebrospinal fluid (ACSF), containing 119 mM NaCl, 2.5 mM KCl, 26 mM NaHCO3, 1 mM NaH2PO4, 11 mM glucose, supplemented with 1 mM MgCl2, and 2 mM CaCl2, 100 μM picrotoxin and 1 mM Sodium t-ascorbate. Synaptic responses were evoked every 10 s by stimulating auditory projections to the lateral amygdala using 2 ms of blue light generated by the epi fluorescence microscope and passed through the ×60 objective lenses placed immediately above the recorded cell. The AMPA/NMDA ratio was calculated as the ratio of peak current at −60 mV to the current at +40 mV, 50 ms after stimulus; both values subtracted from the current at 0 mV.

In vivo recording. Four weeks after injection of AAV-oChIEF-tomato into auditory regions (8 animals were injected in both MGN and auditory cortex; 2 animals were injected only in the auditory cortex; results were pooled), rats were anaesthetized with a set of three injections of 700 μl urethane (330 mg ml−1) given at 10 min
intervals 2 h before the recording\textsuperscript{27} and then mounted on a custom-made stereotaxic frame with an adjustable angle, to hold the head in a fixed position during the recording. The body temperature was regulated by a heating pad. Using aseptic surgical tools the skull was exposed and a hole (\( \sim 3 \text{ mm} \)) was made, centred at \(-3.3 \text{ mm AP}\) and \(4.2 \text{ mm ML}\). The recording electrode was a glass pipet (4–5 M\( \Omega \)) filled with 0.9\% NaCl. The recording electrode was connected to a Axopatch-1D amplifier. The signal was amplified (\( \times 1,000 \)), filtered (2K Hz) and digitized at 10 kHz using an Instrutech A/D interface. Data were acquired and analysed using custom software written in Igor Pro (Wavemetrics).

For optical stimulation, the optic fibre was glued to the glass pipet so that the tip of the fibre was 500 \( \mu \text{m} \) above the tip of the glass pipet to form an optrode. The optic fibre was connected to a 473 nm solid-state laser diode (OEM Laser Systems). The parameters for the optical stimulation were identical to those used during behaviour (2 ms duration, 15–20 mW intensity). The optrode was slowly lowered in at a 7° angle following the start of stimulation. After establishing a stable baseline of at least 30 min (stimulation frequency 0.033 Hz) at the recording site (DV: \(-7\) to \(-7.5\)), 2 min of 10 Hz stimulation was evoked, which was followed by 40 min of 0.033 Hz stimulation. Subsequent LTD and LTP, with the same parameters used in the behavioural assay, were induced 40 min apart. Electrode resistance and light intensity were monitored before and immediately after the recordings to ensure that there was no change in the course of recording. All animals were perfused after the recordings and the position of the recording site verified.

**Analysis.** The number of lever presses were binned for each minute and normalized to the 2-min period before light stimulation. Suppression ratio was measured by dividing the number of lever presses during the first minute of conditioning stimulus (tone or optical stimulation) by that immediately preceding the stimulus.

To minimize the voltage dependent conductance component, the initial slope of field excitatory postsynaptic potentials\textsuperscript{31} were measured using a custom written MATLAB program.

Excitatory postsynaptic current amplitude was measured by averaging a fixed 3 ms window covering the peak amplitude and subtracting from an average current window before stimulation.

All values given in the text and figures indicate mean \( \pm \) s.e.m. Student’s paired and non-paired \( t \)-tests were used with \( P < 0.05 \) considered as significant. All behavioural data were also analysed with the Wilcoxon rank-sum test (MATLAB statistic toolbox) and yielded the same significance values as the \( t \)-test.

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Extended Data Figure 1 | Freezing correlates well with reduction in lever presses to previously learned task. Plot of per cent freezing versus per cent reduction in lever presses to previously learned task. Best fit line indicates significant positive correlation ($R^2 = 0.4; P < 0.01; F$-test). Data includes results from 3 manipulations (paired optical CS-shock conditioning, optical LTD and optical LTP). The per cent change in lever presses to previously learned task (60% ± 9%) was significantly greater than change in per cent freezing (20% ± 5%; $n = 21; P < 0.001$, paired Student’s $t$-test).
Extended Data Figure 2 | *In vivo* optically evoked synaptic responses in lateral amygdala. Field responses to 10 Hz (top) and 100 Hz optical stimulation (middle, bottom), obtained from animal infected with AAV-oChIEF in auditory regions four weeks before recording. Note that the responses follow stimulation faithfully.
Extended Data Figure 3 | Expression of oChIEF in auditory regions reaches lateral amygdala. a, b, Diagram (left) and epifluorescent image (right) of coronal section of rat brain indicating areas expressing AAV-oChIEF-tdTomato 3–4 weeks after *in vivo* injection in auditory cortex (a) and medial geniculate nucleus (b). c, Axonal expression of AAV-oChIEF-tdTomato in lateral amygdala (dashed white line); approximate placement of cannula and light (blue) indicated. Scale bars, 500 μm.
Extended Data Figure 4 | Optic fibre locations in representative group of rats used in the behavioural assays. Histologically assessed optic fibre tip location for rats which responded (blue circles; upper panel, right, is one example) or did not respond (orange circles; lower panel, right, is one example) to optical conditioning. The arrow on the panels shows the location of the tip of optic fibre. Lateral amygdala is indicated by dashed line. Note that the ventricle opened during tissue sectioning in the lower image. Scale bars, 500 μm.
**Extended Data Figure 5 |** The 10 Hz test protocol does not produce CR. Test for CR (blue) in naive animals ($n = 8$), as measured by changes in lever presses normalized to baseline period. Subsequent delivery of paired optical CS and shock produced CR in these animals (not shown). Each point represents data collected over 1 min.
Extended Data Figure 6 | Systemic NMDA receptor blockade during conditioning blocks ODI-induced conditioned response. a, Animals \((n = 5)\) were injected with MK801 (see Methods) and given optical CS paired with foot shock and subsequently tested one day later for CR. b, The same group of animals was then given optical CS paired with foot shock (in the absence of MK801) and subsequently tested one day later for CR. c, MK801 significantly blocked conditioning.
Extended Data Figure 7 | LTD and LTP remove and reactivate memory.
a–e, Data from an individual rat, measuring lever presses per minute before, during (blue) and after optical CS, one day after paired conditioning of optical CS and shock (a), one day after subsequent optical LTD protocol (b), one day after subsequent optical LTD protocol (c), one day after subsequent second optical LTD protocol (d) and one day after subsequent second optical LTP protocol (e). f, Graph of lever presses during first minute into optical CS one day after delivery of indicated conditioning protocols.
Extended Data Figure 8 | The effects of LTD and LTP are rapid and long-lasting. a, Animals (n = 5) were tested for CR one day following pairing of optical CS with shock. b, c, Within one hour of testing, animals received optical LTD protocol and were tested for CR 20 min (b) and three days (c) later. d, e, Following day three, testing animals received optical LTP protocol and were tested for CR 20 min (d) and three days (e) later. f, Graph of normalized lever presses for the first minute of optical CS following indicated protocols.
Extended Data Figure 9 | Optically evoked in vivo and in vitro stimuli produce similar electrophysiological responses. Animals were injected in vivo with AAV-oChIEF-tdTomato in auditory regions 4 weeks before recordings. Left, in vivo electrophysiological response obtained from glass electrode placed in lateral amygdala and evoked by light pulse delivered through fibre optic cable placed 500 µm above tip of glass electrode. Right, in vitro brain slice electrophysiological response obtained from glass electrode placed in lateral amygdala and evoked by light pulse delivered through fibre optic cable placed above the brain slice. Black trace is before and red trace after bath application of 10 µM NBQX. Scale bars, 1 mV, 10 ms.
Extended Data Figure 10 | LTD reverses LTP and LTP reverses LTD of in vivo optical responses in amygdala. a, Plot of baseline normalized fEPSP in vivo optically evoked responses (n = 5 from 5 rats) following optical LTP (100 Hz) and optical LTD (1 Hz). b, Same as a for a separate group of recordings (n = 5) following optical LTD (1 Hz) and optical LTP (100 Hz). All comparisons to baseline period.