Materials and Methods

Animals. Male or female adolescent Sprague-Dawley rats (postnatal day 20–25) were housed on a 12-hour light/dark cycle with *ad libitum* access to water and food. Procedures were performed in strict compliance with the animal use and care guidelines of Cold Spring Harbor Laboratory.

Infection of lateral amygdala neurons in vivo. Genes of interest (GFP; GFP fused to the N-terminus of the AMPA receptor subunit GluR1flop and GFP fused to the N-terminus of the C-terminal portion of the GluR1 subunit [(809–889); see (S1)] were cloned by using standard methods into a HSV-amplicon vector and verified by sequencing. Virus vectors were generated as described elsewhere (S2, S3). Animals were anaesthetized with Ketamine/Medetomidine (2 mg Ketamine HCl and 0.016 mg Medetomidine HCl / 50 g rat) and positioned in a stereotaxic apparatus. Injections of viral solutions (3–9 injection sites; 100–200 nl per injection) were delivered with a glass micropipette through a skull window (2–3 mm²) by pressure application (5–12 psi, controlled by a Picospritzer II, General Valve, Fairfield, NJ, USA). The injections were performed within the following stereotaxic coordinates: –2.2 mm to –3.9 mm from Bregma; 4.5 mm to 5.3 mm lateral from midline, and 5.7 mm vertical from cortical surface. Subsequently the skull and skin were repositioned and maintained with cyanacrylate glue. Infections with test or control vectors were delivered to animals from the same litter. During procedures, animals were kept on a heating pad and were brought back into their home cages after regaining movement. Animals infected with the plasticity-tag vector were kept in individual cages to avoid possible fearful experiences. Before behavioral training, we waited 14 to 20 hours for expression of cytosolic localized GluR1-C-tail-GFP and GFP and 36 hours for expression and processing of the membrane protein GluR1-GFP.

Electrophysiology. Animals were anesthetized with Ketamine/Medetomidine, decapitated and the brains quickly removed and chilled in ice-cold dissection buffer (110.0 mM choline chloride, 25.0 mM NaHCO₃, 1.25 mM NaH₂PO₄, 2.5 mM KCl, 0.5 mM CaCl₂, 7.0 mM MgCl₂, 25.0 mM glucose, 11.6 mM ascorbic acid, 3.1 mM pyruvic acid; gassed with 95%O₂/5%CO₂). Coronal slices (300 µm) were cut in dissection buffer using a VT-1000 S vibratome (Leica, Nussloch, Germany) and subsequently transferred to a storage chamber containing artificial cerebrospinal fluid (ACSF; 118 mM NaCl, 2.5 mM KCl, 26.2 mM NaHCO₃, 1 mM NaH₂PO₄, 20 mM Glucose, 4 mM MgCl₂, 4 mM CaCl₂; 22°–25°C; pH 7.4; gassed with 95%O₂/5%CO₂). After at least 1 hr of recovery time slices were transferred to the recording chamber and were constantly perfused with ACSF maintained at 24°C. Patch-clamp whole-cell recordings of lateral amygdala neurons were obtained under IR-DIC visualization with Axopatch–1D amplifiers (Axon
Instruments, Foster City, CA). For basic voltage-clamp experiments we filled patch pipettes (3–6 MΩ) with 135 mM CsMeSO₄, 20 mM TEA-Cl, 2 mM MgCl₂, 10 mM Hepes, 10 mM EGTA, 5 mM QX-314, pH 7.25. In experiments investigating long-term synaptic plasticity we used 115 CsMeSO₄, 20 CsCl, 10 HEPES, 2.5 MgCl₂, 4 Na₂ATP, 0.4 Na₃GTP, 10 Na-phosphocreatine, 0.6 EGTA, pH 7.25. For current-clamp recordings pipettes were filled with 130 mM K-Gluconate, 5 mM KCl, 10 mM Hepes, 2.5 mM MgCl₂, 4 mM Na₂ATP, 0.4 mM Na₃GTP, 10 mM Na-phosphocreatine, 0.6 mM EGTA at pH 7.25. Liquid junction potential was not corrected.

We targeted large, pyramidal-like somata, thus selecting for spiny glutamatergic principal neurons, which make up the majority of neurons in the lateral amygdala. Firing patterns obtained in current-clamp recordings also have been consistent with previous data for principal neurons (S4). Synaptic responses were evoked by electrical stimulation of fibers in the ventral striatum just medial to the dorsal lateral amygdala with bipolar Platinum/Iridium electrodes (Frederick Haer & Co., Bowdoinham, ME). These fibers originate in the auditory thalamus and project to neurons in the lateral amygdala (S5). We reasoned that possible stimulation of fibers from sources other than the auditory thalamus would lead to an underestimation of learning-induced effects.

In experiments investigating synaptic incorporation of recombinant receptors AMPA receptor-mediated transmission was pharmacologically isolated by addition of 100 µM Picrotoxin and 50 µM D,L-AP5 to the ACSF. We recorded AMPA PSCs at −60, 0, and +40 mV holding potential and 25 to 40 consecutive responses from each holding potential were averaged. If possible, two synaptic pathways onto the same postsynaptic cell have been acquired by stimulation of two bundles of fibers in the ventral striatum. Half of the electrophysiological data from paired or unpaired rats was acquired and analyzed with the experimenter blinded to the protocol used for conditioning. No significant differences between blinded and non-blinded data were detected and therefore pooled.

In experiments analyzing long-term synaptic plasticity slices were maintained in ACSF with the following composition: 115 NaCl, 3.3 KCl, 25.5 NaHCO₃, 1.2 NaH₂PO₄, 5 lactic acid, 25 glucose, 1 MgSO₄, 2 CaCl₂, 100 µM Picrotoxin, equilibrated with 95% O₂/5% CO₂ (S6). In a subset of experiments (6 from 15 non-infected neurons; 4 from 12 infected neurons) standard ACSF was used as described above. In order to monitor synaptic transmission we evoked AMPA PSCs at two synaptic pathways by interleaved stimulation of two fiber bundles at 0.33 to 0.1Hz and recorded at −60 mV holding potential in voltage-clamp mode. LTP was induced at one pathway by a pairing protocol consisting of presynaptic fiber stimulation at 3Hz for 3 min paired with postsynaptic depolarization to 0 mV holding potential. Experiments were excluded from analysis if unpaired control pathways displayed changes in transmission by more than 50%.

All data were reported as mean ± SEM and significance level was set at \( P<0.05 \). Statistical differences were determined by unpaired two-tailed t-test for unpaired data or paired two-tailed t-test for paired data, unless otherwise stated. If necessary, data was log-normalized prior to testing.

**Behavioral training and analysis.** All animals were handled and habituated to the shocking chamber, testing chamber, and the conditional acoustic stimulus before entering...
the experimental schedule. Conditioning was performed in a Habitest chamber (30 cm x 26 cm x 28 cm) with an electrifiable grid floor (Coulbourn Instruments, Allentown, PA) within a larger sound attenuated cabinet. During conditioning the cabinet was illuminated and the behavior was captured with an infrared PC-6EX2 CCD-camera (Supercircuits, Liberty Hill, TX) and stored on a personal computer. Delivery of the shock and the tone was controlled by custom written software in Matlab (MathWorks, Natick, MA). Tones (either 0.5 s white noise bursts at 1Hz for 20 s at ~80 dB for HSV-GluR1 infected rats or 5kHz pure tones 20 s continuously at ~80 dB for the plasticity-block vector and the infection-control vector infected rats) were delivered via a RP2 real time processor (TDT, Alachua, FL) and a tweeter (Radio Shack, Fort Worth, TX) connected to a P1000 amplifier (Hafler, Tempe, Az). Each conditioning session was performed with experimental and control animals in parallel. Conditioning with HSV-GluR1 infected animals was done with 10 tones, on randomized intervals, on average 3 min apart. In the paired group tones were co-terminated with a 0.5 s 1 mA footshock, in the unpaired group tones and shocks were separated by at least 1 min. Animals infected with the plasticity-block vector or the infection-control vector were conditioned with a single pairing of a tone, co-terminated with a 0.5 s 0.5 mA footshock. Memory retention tests were performed in darkness in a different shaped plastic container (20 cm x 20 cm x 28 cm) and behavior was recorded during 1 min of silence and 1 min of tone presentation. The fraction of time spent freezing (defined as complete cessation of all movements except breathing) was scored post hoc with the experimenter blinded to the vector that was used for infection.

**Histology.** After the last behavioral testing session all animals were anaesthetized, decapitated and the brains quickly chilled in ice-cold phosphate-buffered saline (PBS). The brains were trimmed to small blocks containing the amygdala and were subsequently immersion fixed in PBS containing 4% paraformaldehyde and 4% sucrose overnight at 4°C. After fixation 10–13 coronal slices (200 µm) containing the amygdala were cut and mounted on cover glasses. Transmitted light and epifluorescence images were taken from each section with a Spot CCD camera (Diagnostic Instruments, Sterling Heights, MI) mounted on an Axiophot microscope (Zeiss, Oberkochen, Germany). We focused our analysis on the lateral amygdala, as described by Paxinos and Watson (57), because of its essential function in tone-cued fear conditioning (55, 58). Therefore, we defined the lateral amygdala as an area of interest based on the transmitted light picture using custom written scripts in Matlab (MathWorks, Natick, MA). Based on the fluorescence images the fraction of pixels with fluorescence values higher than two standard deviations of background was calculated for all slices from each animal. This provided a measure for the area within the lateral amygdala that was infected for all animals. In order to obtain an estimate of the fraction of neurons infected within a given lateral amygdala we furthermore needed to estimate the efficiency of infection at the level of individual cells within an area showing green fluorescence. Three slices from a GFP expressing and two slices from a GluR1-C-tail-GFP expressing animal were labeled immunohistochemically with a primary antibody against the neuron-specific nuclear marker protein NeuN (Chemicon, Temecula, CA) and a secondary antibody conjugated with fluorescent marker Alexa594 (Molecular Probes, Eugene, OR). GFP fluorescence differed markedly between randomly chosen somata within the area of injection and showed a clearly bimodal distribution, thus indicating a good discrimination between
infected and non-infected neurons. We found that within an infection site on average 58% of the NeuN-labeled neurons showed GFP fluorescence (range: 48–67%). Thus, the value obtained for fraction of infected area was multiplied by 0.58 to establish the fraction of infected neurons in the lateral amygdala.
**Supplemental Figure S1:**

**Fig. S1:** Viral infection with amplicon vectors does not alter basic electrophysiological properties. Mean resting potential \( t\) test, \( P = 0.88 \) \( (A) \), mean input resistance \( t\) test, \( P = 0.18 \) \( (B) \), and input/output relationship (KS test, \( P = 0.99 \) \( (C) \) of infected (green bars) and non-infected (black bars) neurons showed no statistically significant differences (n.s.). Error bars are SEM.

**Supplemental Figure S2:**

**Fig. S2:** Histological analysis of infection efficacy. \( (A) \) Distribution of green fluorescence intensities of neuronal somata identified in the red channel of double labelling experiments. The bimodal nature of the distribution indicates good discrimination of infected and non-infected neurons. \( (B \) and \( C \)) Lower magnification, transmitted light \( (B) \) and epifluorescence \( (C) \) images of same slice
as (Fig. 5B). Red line circumscribes lateral amygdala. **(D)** Estimation of infection size by thresholding of (C) (see methods for details).

**References and Notes**


