Supporting Information

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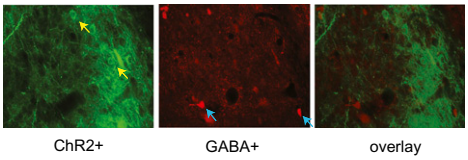

Fig. S1. ChR2 is expressed at low levels in LA GABAergic neurons. Immunolabeled ChR2$^+$ cells (green, *Left*), GABA$^+$ cells (red, *Middle*), and an overlay of the two (*Right*), with examples of single-labeled ChR2$^+$ (blue) and GABA$^+$ (white) cells. Individual cell examples are indicated by yellow (ChR2/YFP$^+$ cells) and blue (GABA$^+$ cells) arrows.

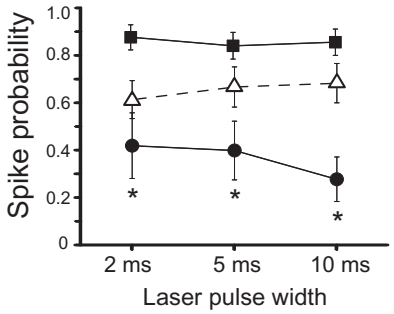

Fig. S2. Parametric analysis of laser stimulation of LA neurons with different stimulation frequencies (20 Hz and 50 Hz) and laser pulse durations (10, 5, and 2 ms). The spike probability (*y* axis) of each single laser pulse (at different pulse durations, *x* axis) within the 1-s stimulation period of 20 Hz (open triangles) or 50 Hz (circles) or to a single laser pulse (squares) to produce an action potential in the 9 cells that received all stimulation parameters (only 9 of the 15 total cells received all types of stimulation). * indicates significant differences between 50-Hz stimulation and both 20-Hz and single-pulse stimulation.

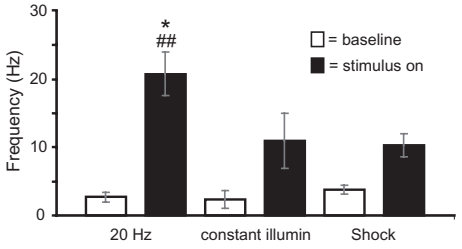

Fig. S3. Comparison of neural responses evoked by 20-Hz laser stimulation (20 Hz; n = 15 cells), constant laser illumination (constant Illumin; n = 9 cells), and eyelid shock (shock; 2 ms, 2.5-mA shocks at 6.66 Hz; n = 25 cells) during a 1-s stimulation period. Firing rate (frequency, *y* axis) during a 1-s baseline period before stimulus onset (white columns) and during the 1 s in which the stimulus was on (black columns). A repeated-measures ANOVA revealed a significant interaction ($F_{2,46}$ = 5.83, P = 0.006) between stimulus (baseline and stimulus on) and group (20 Hz, constant illumination and eyelid shock), and post hoc comparisons demonstrated that evoked firing rate during the 20-Hz laser stimulation on period was significantly higher than firing rate during both the constant laser stimulation (*P = 0.005) and the eyelid shock ($^{\#\#}P$ = 0.008). The eyelid shock dataset was gathered using tetrode recordings in awake, behaving rats that had previously undergone conditioning and were receiving random, unpredicted shocks interspersed with paired shocks. The data presented here is the response to the random, unpredicted shocks, exclusively. Single-unit recordings were obtained using a 32-channel data acquisition system (Neuralynx). Offline cluster cutting was performed manually using Neuralynx SpikeSort 3D software. To be included in the study, spike trains had to exhibit a refractory period of at least 1 ms and a mean spike amplitude of at least 80 μV over background noise of ±20 μV. The experimenter visually inspected spike waveforms and cluster boundaries to make sure that they remained stable throughout the recording session for cells to be included in the data analysis. For data analysis, peristimulus time histograms (PSTH) were constructed as described in *Materials and Methods*, except that spike counts during a 1-s bin before stimulus onset and a corresponding 1-s bin during the stimulus period for each trial was calculated. Baseline firing rate and stimulus-evoked firing rate for each cell were then determined by averaging across all baseline and stimulus on trials to give frequency during the two conditions for individual cells. Baseline and stimulus-evoked firing rates for the population were then calculated by averaging across all cells.

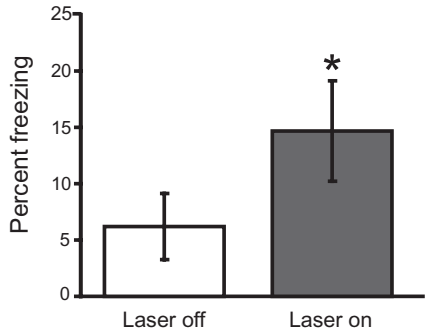

Fig. S4. Laser stimulation produced a freezing unconditioned response. To avoid any freezing elicited by the CS, freezing (*y* axis) was measured in the ChR2/unpaired group (i.e., they received laser stimulations uncoupled from the auditory CS, *n* = 6) during the 2-s "laser on" period and during a corresponding baseline period before laser onset ("laser off"). *Significant difference between stimulus on and off conditions. Behavioral ratings were collected as described in *Materials and Methods* during the 2-s laser stimulation period and the 2-s period preceding laser onset.

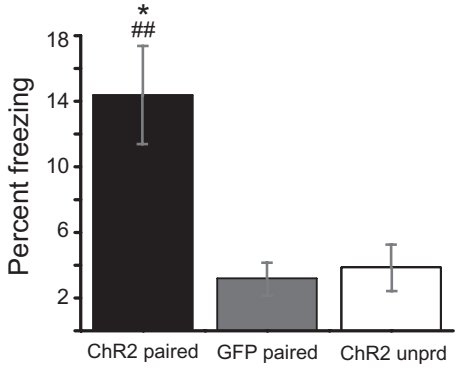

Fig. S5. Optical stimulation as an US (using a 2-s constant illumination US) produced behavioral fear conditioning, percent freezing (*y* axis) during the LTM test in the ChR2/paired (black column, *n* = 8), GFP/paired (gray column, *n* = 8), and ChR2/unpaired (white column, *n* = 8) for the different groups (*x* axis). A one-way ANOVA comparing CS-evoked freezing by group (ChR2/paired vs. GFP/paired vs. ChR2/paired) found a significant main effect ($F_{2,21}$ = 9.81, *P* = 0.001) and post hoc analyses revealed that the ChR2/paired group froze significantly more than both the GFP/paired (*P* = 0.002) and the ChR2/unpaired (*P* = 0.001) groups. * and # indicate significant differences between the ChR2/paired group and the GFP/paired and ChR2/unpaired groups.

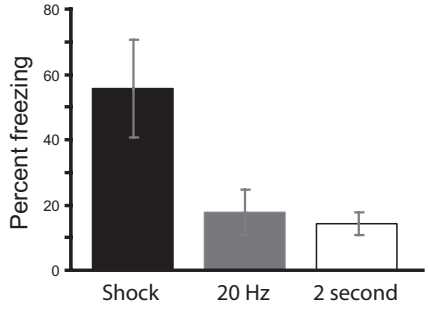

Fig. S6. Comparison of shock vs. laser US-induced fear conditioning. Percent freezing (*y* axis) during the LTM test in the eyelid shock US conditioned group (shock, black column, *n* = 6), 20 Hz laser stimulation US conditioned group (20 Hz, gray column, same as ChR2/paired in Fig. 3*B*) and 2-s laser stimulation US conditioned group (2 s, white column, same as ChR2/paired in Fig. S5).