Supporting Information

Shin et al. 10.1073/pnas.1009803107

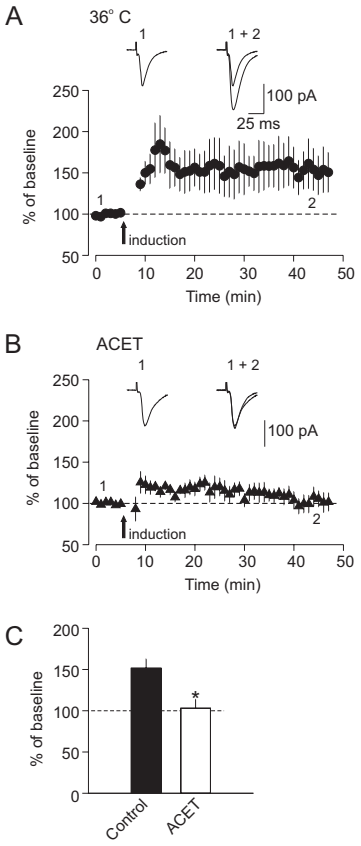

Fig. S1. Presynaptic long-term potentiation ("pre-LTP") in thalamic input could be induced at physiological temperatures and blocked by the glutamate receptor (GluR5)-specific antagonist (S)-1-(2-Amino-2-carboxyethyl)-3-(2-carboxy-5-phenylthiophene-3-yl-methyl)-5-methylpyrimidine-2,4-dione (ACET). (*A*) Normal pre-LTP at thalamo-amygdala synapses was observed at 35–36 °C (n = 6). The magnitudes of pre-LTP at 35–36 °C and room temperature were not significantly different (P = 0.9). (*Insets*) Averaged excitatory postsynaptic currents (EPSCs) before (1) and after (2) the induction of LTP. (*B*) Pre-LTP in thalamic input was blocked in the presence of the specific antagonist of GluR5 subunit-containing kainate receptors ACET (0.5 μM; (n = 4, P = 0.8 vs. baseline). (*Insets*) Averaged EPSCs before (1) and after (2) the delivery of LTP-inducing stimulation. (*C*) Summary graph of LTP experiments: control (n = 8, same data as in Fig. 1*H*); ACET (0.5 μM) (n = 4, P = 0.01 vs. control LTP). Error bars indicate SEM.

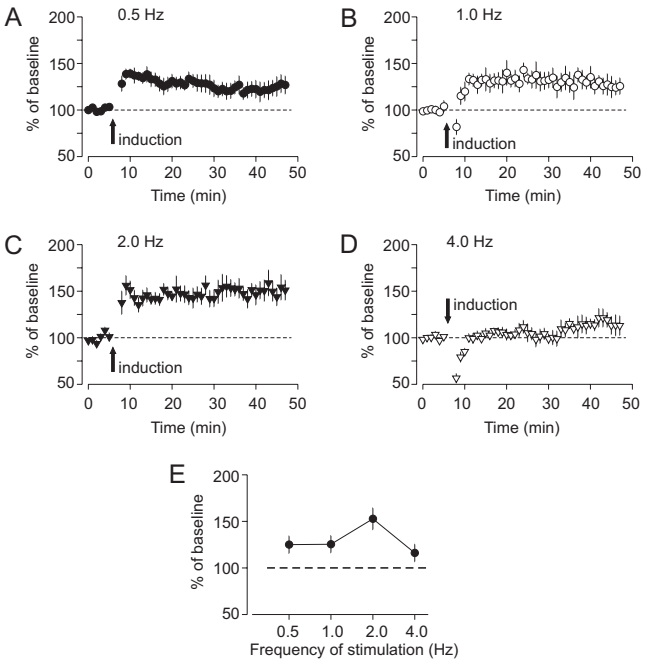

Fig. S2. Dependence of the induction of pre-LTP on the frequency of presynaptic stimulation. (*A–D*) Summary graphs of the experiments in thalamic input when pre-LTP–inducing stimulation was delivered at four different frequencies (*A*: 0.5 Hz, $n = 6$, $P = 0.17$ vs. baseline; *B*: 1.0 Hz, $n = 5$, $P = 0.024$ vs. baseline; *C*: 2.0 Hz, $n = 8$, $P = 0.0004$ vs. baseline; *D*: 4 Hz, $n = 5$, $P = 0.12$ vs. baseline) at a holding potential of −70 mV. The graph in *C* shows the same data as in Fig. 1*D*. (*E*) The magnitude of pre-LTP in thalamic input at four different frequencies of presynaptic stimulation, expressed as the percentage change relative to the baseline value when the same number of presynaptic pulses (240 paired pulses at 50-ms interpulse interval) was delivered at four different frequencies. Thus, the machinery of pre-LTP in thalamic input might be tuned to detect the frequencies of incoming signals in the range of 1–2 Hz. This, in fact, is consistent with the low firing rates of the auditory thalamic areas implicated in fear conditioning.

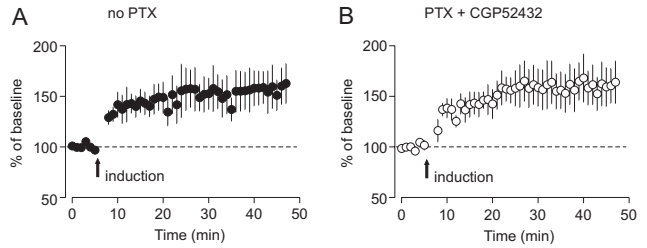

Fig. S3. Inducibility of pre-LTP is not sensitive to changes in GABA-mediated inhibition. (*A*) Normal pre-LTP at thalamo-amygdala synapses was observed without picrotoxin (PTX) in the external solution ($n = 6$; *t* test, $P = 0.78$ for the magnitude of pre-LTP without PTX vs. pre-LTP in the presence of PTX, as shown in Fig. 1*H*; control). (*B*) The induction of pre-LTP in thalamic input was unaffected by the selective $GABA_B$ receptor antagonist CGP52432 (10 μM; $n = 5$; *t* test, $P = 0.7$ for the magnitude of pre-LTP in the presence of CGP52432 vs. control pre-LTP, shown in Fig. 1*H*).

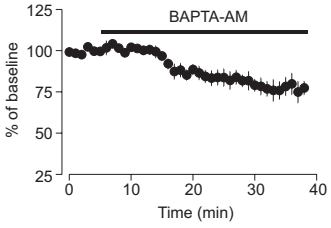

Fig. S4. The effect of exogenously applied 1,2-Bis(2-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid tetrakis(acetoxymethyl ester) (BAPTA-AM) on synaptic transmission in thalamic input. BAPTA-AM was applied in a concentration of 50 μM. The EPSC amplitude was suppressed by 23.8 ± 5.3% ($n = 6$) at 30 min after switching to the BAPTA-AM–containing solution. Because slices were treated with BAPTA-AM–containing solution for more than 30 min before recording, the pre-LTP experiments (Fig. 1 *F* and *H*) were performed with already stabilized baseline EPSCs. Error bars indicate SEM.

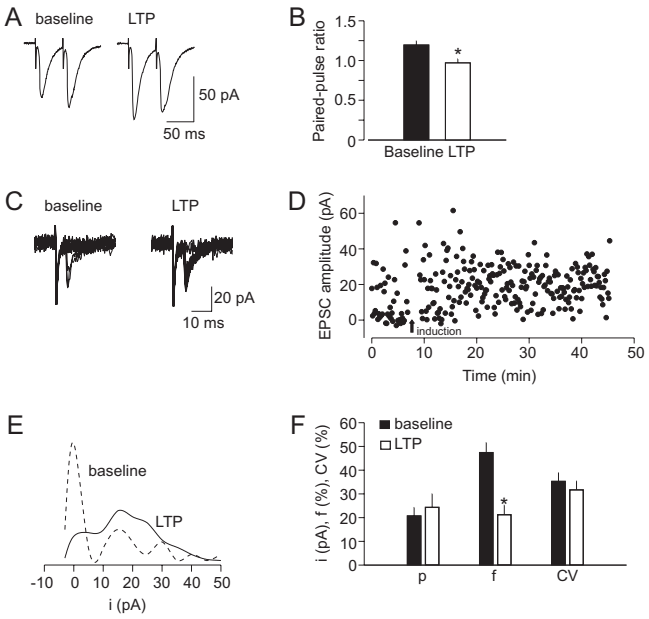

Fig. S5. The form of LTP in thalamic input (induced without postsynaptic depolarization) is presynaptically expressed. (*A*) Paired-pulse facilitation (PPF) assayed with 50-ms interpulse interval before (*Left*) and after (*Right*) the induction of LTP. (*B*) Summary PPF data ($n = 8$; $P < 0.01$ for LTP vs. baseline). (*C*) Superimposed successive EPSCs evoked with minimal stimulation in thalamic input under baseline conditions (*Left*) and after the induction of LTP (*Right*). (*D*) Amplitude of individual EPSCs during the course of LTP experiment. The LTP induction protocol was delivered at the arrow. The unitary EPSCs were evoked one time every 6 s. (*E*) Superimposed density estimate plots of unitary EPSCs recorded before and after the induction of LTP. (*F*) Summary plots for EPSC data before and after LTP was induced ($n = 7$; p, potency; paired *t* test, $P = 0.25$ for LTP vs. baseline; f, fraction of failures; paired *t* test, $P < 0.01$ for LTP vs. baseline; CV, coefficient of variation of EPSC successes; $P = 0.51$ for LTP vs. baseline).

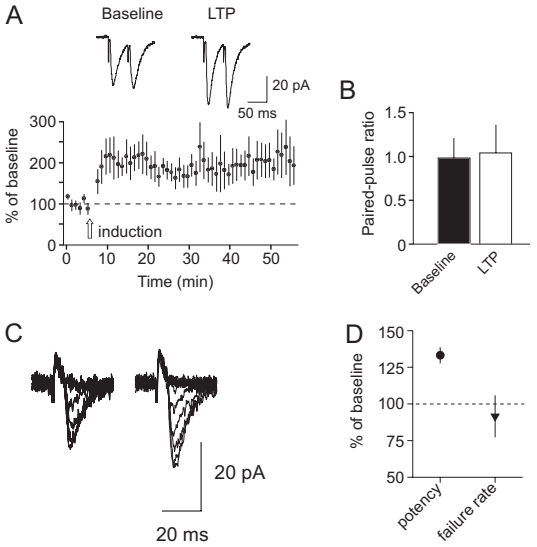

Fig. S6. LTP in thalamic input, induced by pairing of presynaptic stimulation with postsynaptic depolarization, is postsynaptically expressed. (*A*) LTP in thalamic input induced by the delivery of 240 paired presynaptic pulses (50-ms interpulse interval) at 2 Hz frequency at a holding potential of +30 mV ($n = 7$; $P = 0.0048$ vs. baseline at 30 min postinduction). (*Insets*) Averaged paired EPSCs (50-ms interpulse interval) before (*Left*) and after (*Right*) the induction of LTP. (*B*) Summary of PPF data. PPF values obtained during baseline recording were not different from PPF values after the induction of LTP ($n = 7$; paired *t* test, $P = 0.43$). (*C*) Superimposed EPSCs evoked with minimal stimulation in thalamic input under baseline conditions (*Left*) and after the induction of LTP with the same protocol as in *A*. The unitary EPSCs were evoked one time every 6 s. (*D*) Summary plots for the unitary EPSC data before and after LTP was induced are expressed as the percentage change relative to the baseline value ($n = 8$). The increase in potency (p) after the induction of LTP was highly significant (paired *t* test, $P = 0.001$ for LTP vs. baseline), whereas the failure rate (f) remained unchanged (paired *t* test, $P = 0.3$ for LTP vs. baseline). Data points show mean ± SEM. This confirms that the expression of LTP in thalamic input, induced by pairing of low-frequency presynaptic stimulation and postsynaptic depolarization, might be postsynaptic, because it was not associated with increases in probability of release.

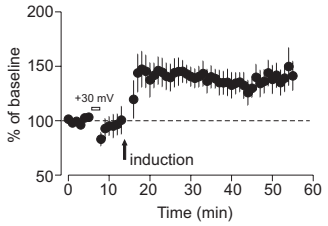

Fig. S7. Depolarization to +30 mV alone did not influence the inducibility of pre-LTP. Normal pre-LTP in thalamic input to the lateral nucleus of the amygdala (LA) without BAPTA in the pipette solution was observed when pre-LTP–inducing stimulation was delivered after depolarization of postsynaptic neuron to +30 mV for 2 min alone (n = 6; t test, P = 0.56 vs. control pre-LTP).

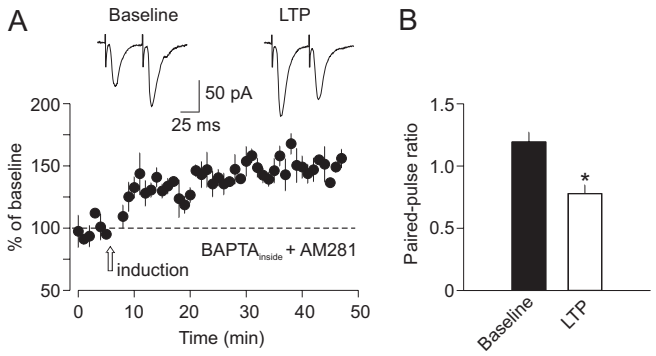

Fig. S8. LTP, induced with postsynaptic LTP ("post-LTP") protocol in the presence of BAPTA in the pipette solution and AM281 in the bath, is presynaptically expressed. (*A*) Summary graph of the experiments where LTP in thalamic input was induced in the presence of 20 mM BAPTA in the intrapipette solution and the cannabinoid type 1 (CB1) receptor antagonist AM281 (0.5 μM) in the external medium by delivering 240 paired pulses (50-ms interpulse interval) at 2 Hz frequency at a holding potential of +30 mV (post-LTP induction protocol; n = 5). (*Insets*) Averaged paired EPSCs before (*Left*) and after (*Right*) the induction of LTP. (*B*) PPF was occluded after the induction of LTP under these conditions (n = 5; paired t test, P = 0.015 for baseline PPF vs. PPF after the induction of LTP).

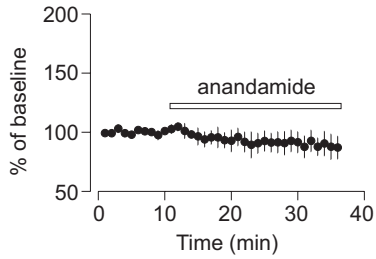

Fig. S9. Exogenously applied anandamide had no significant effect on baseline synaptic transmission in thalamic input to the LA. Anandamide was applied in a concentration of 0.5 μM (n = 4). Error bars indicate SEM.