Supporting material – Retrieval-specific endocytosis of GluA2-AMPARs underlies adaptive reconsolidation of contextual fear; Rao-Ruiz et al.

Figures and legends

Supplementary Fig. S1 Behavioral analysis of retrieval tests. (a) Experimental design with 4 groups, in which mice –24 h prior to a retrieval session– were exposed to the context only (no shock: NS-R, n=8), or received a shock (US-R, n=8) or an immediate shock (IS-R, n=8) in the same context, or in a shock in a different context (US-RCB, n=6) with US of 0.7 mA, 2 s during training (T) as described previously1. (b) During retrieval (RT1; day 2), only the animals that received the shock at the end of the training exhibited a fear response when compared with animals that received either no shock, an immediate shock –and hence no learning– or a retrieval session in an unrelated context –and hence no recall of the memory– (F(3,29)=36.14). (c) Experimental design with 2 groups, in which mice –24 h prior to a retrieval session– were tested for retrieval and were exposed to systemic injections for saline (Sal) and anisomycin (Ani) 30 min before retrieval (n=8 mice per group). (b) In RT1 no effect of Ani is observed since both groups exhibited similar freezing. In retrieval test 2 (RT2) on day 3, animals that received Ani exhibit impaired expression of contextual fear memory due to inhibition of protein synthesis and reconsolidation (day 3, F(1,15)=13.15), as shown before by others2. All data points show mean±SEM. Significant p-values are indicated.
Supporting material – Retrieval-specific endocytosis of GluA2-AMPARs underlies adaptive reconsolidation of contextual fear; Rao-Ruiz et al.

Supplementary Fig. S2 Examples of blots and input. Representative blots from figures of the main text (main text figure number is indicated on the left side); approximate MW indicated, all ~100 kDa) and the coomassie gels as input control to compare the total protein amount from each sample3,4 is shown for immuno-detection of GluA1-3. No shock with or without retrieval (NS-R, NS-NR), Shock with or without retrieval (US-R, US-NR), 4 and 7 h are indicated as time after retrieval when deviating from the 1 h time point; retrieval in context B 24 h after shock exposure (US-RCB); retrieval in training context 24 h after an immediate shock (IS-R); NS-S, surface expression in NS sample; NS-H, expression in homogenate in NS sample; US-S surface expression in US sample; US-H, expression in homogenate in US sample; 3A, after dorsohippocampal injection of the GluA2 control peptide (GluA23A); 3Y, after dorsohippocampal injection of the GluA2 regulated endocytosis blocking peptide (GluA23Y).
Supplementary Fig. S3 Preventing regulated endocytosis of GluA2-containing receptors blocks retrieval-induced down-regulation. (a) Experimental design with 2 main groups, in which mice –24 h prior to the absence or presence of a retrieval session– were exposed to the context only (NS-NR and NS-R), or received a shock in the same context (US-NR or US-R). Experimental design with 2 groups (NS; shock: S), and timeline for intervention with the GluA23Y and GluA23A peptides to prevent endocytosis, and collection of dorsal hippocampi for proteomics (n=4 samples/condition). (b) Quantification of synaptic AMPA receptor subunits (% to NS-values of the 3Y sample). Representative blots are shown (approximate MW indicated, all ~100 kDa), as well as the coomassie gels used as input control. The sample NS-3Y is shown twice, as it was used as control for separate immunoblots. Preventing endocytosis of GluA2 containing receptors prevented retrieval-induced down-regulation of GluA2 ($F_{(2,11)}=8.67$) and GluA3 ($F_{(2,11)}=12.61$). All data points show mean±SEM, significant p-values are indicated.
Supplementary Fig. S4 Frequency distributions of AMPAR-dependent mEPSCs. (a) Experimental design with 2 main groups, in which mice –24 h prior to the absence or presence of a retrieval session– were exposed to the context only (NS-NR and NS-R), or received a shock in the same context (US-NR or US-R). Timeline for intervention (dorsohippocampal injections of 3A or 3Y), testing in a retrieval test (RT1) and electrophysiological analysis (1 or 7 h after RT1) is indicated. (b) Group data of averaged mean frequencies of events from ex vitro slice physiology (NS-NR, n=6; US-NR, n=6; NS-R 1 h, n=4; US-R 1 h, n=4; US-R 7 h, n=10; US-R 7 h, n=8; US-3A-R 7 h, n=4; US-3Y-R, n=5; number of cells is indicated) (c–f) Overlapping cumulative frequencies of inter-event intervals of individual events comparing NS and S from each set of experimental groups (see labels) show no significant differences (Kolmogorov-Smirnov test). For the 1 h and 7 h time points (c–e) the line of the US-NR cumulative frequency (thick gray line) is indicated for comparison. Data (b) show mean±SEM.
Supporting material – Retrieval-specific endocytosis of GluA2-AMPARs underlies adaptive reconsolidation of contextual fear; Rao-Ruiz et al.

Supplementary Fig. S5 Effect of TAT-GluA23Y endocytosis blocking peptide on baseline activity of mice. (a) Experimental design with 2 groups that both did not receive an shock during training on day 1, and timeline for intervention (dorsohippocampal injections of GluA23A or GluA23Y) and testing in a retrieval test (RT; NS-3A-R: n=10, NS-3Y-R: n=11). (b) Blocking regulated AMPAR-mediated endocytosis with GluA23Y had no influence on exploration (left) or total activity (right) compared with control GluA23A peptide as measured in the first retrieval test. Hence it has no effect on baseline activity in accordance with others.5 (c) Experimental design with 2 groups that both received an shock (US) during training on day 1, and timeline for intervention (dorsohippocampal injection of 3A or saline) and testing (US-3A-R: n=10, US-Sal-R: n=8). (d) The control peptide GluA23A had no effect on freezing. All data points show mean±SEM.
Supporting material – Retrieval-specific endocytosis of GluA2-AMPARs underlies adaptive reconsolidation of contextual fear; Rao-Ruiz et al.

Supplementary Fig. S6 Acquisition of extinction effects during reconsolidation-update. (a) Experimental design for the effect and timing of a pre-extinction retrieval session on reconsolidation-update, and timeline for testing (R-E2 h: n=10, NR-E: n=10, R-E24 h: n=8). Ext1–10 indicates a 30-min extinction session divided into 10 bins of 3 min intervals. (b) All groups acquired extinction similarly and reached the same levels of freezing in the last 3 min (Ext10) of the 30-min session. (c) Experimental design for the effect of blocking regulated AMPAR-endocytosis by dorsohippocampal injections for the GluA2_{3Y} and control GluA2_{3A} peptide on reconsolidation-update, and timeline for intervention and testing (n=5 mice per condition). (d) All groups acquired extinction similarly and reached the same levels of freezing in the last 3 min (Ext10) of the 30 min session. It should be noted that only in the first 3 min of the extinction session, the same effect was observed as in a second retrieval session of 3 min (Ext1: F(1,9)=10.01) (c.f. Fig. 5). Therefore, there is no difference in extinction, but only a temporal enhancement of fear due to reconsolidation. All data points shown are mean±SEM, significant p-values are indicated.
Supplementary Fig. S7 Schematic of synaptic plasticity mechanisms modulating fear after a single retrieval trial. Once consolidated, fear memories can be recalled and expressed during a non-reinforced retrieval session. This short retrieval of contextual memory returns the hippocampus-dependent memory into a labile yet dynamic state, during which synapses can be reorganized. Besides memory restabilization, the adaptive nature of reconsolidation allows for modification of memory strength and content. An extinction session given within this window of lability leads to a reinterpretation of the context as being safe, resulting in a long-term attenuation of conditioned contextual fear response. At the molecular level, we find a direct, significant and temporally defined role for glutamate receptor-based synaptic plasticity as neural mechanism that inhibits memory strengthening while underlying the reinterpretation of persistent fear memory content during adaptive reconsolidation. Colored lines represent the temporal regulation of specific glutamate receptor subunits.

References