Supporting Information

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SI Methods

Immediate Shock Protocol. Rats in the immediate shock condition received a single 0.7 mA (0.5 s) footshock immediately upon entry to the fear-conditioning chamber. Two minutes later, rats received a single 20-s tone, and were removed from the chamber after an additional 3 min. Rats in the paired condition received an identical procedure, except that the shock occurred after 2 min in the chamber and overlapped with the last 0.5 s of the tone. Rats in the context condition were place in the chamber but received neither tone nor shock. All rats except the naive rats spent a total duration of 5 min in the training chamber, followed by 60 min in the home cage before decapitation and brain removal.

Western Blots. Amygdale were rapidly dissected (<3 min following killing) from rats following experimental treatment. Freshly extracted amygdale were added to prechilled tubes on dry ice and immediately flash frozen. Soluble protein extracts were prepared by homogenizing tissue samples in ice-cold buffer [in mM: 50 Tris-HCl, pH 7.5; 150 KCl; 1 DTT; 1 EDTA; 1× complete protease inhibitor mixture III; 1× phosphatase inhibitor mixture I; 1× phosphatase inhibitor mixture II (Sigma)]. Protein concentration was measured by means of the BCA assay (Pierce). Aliquots of protein (15–20 μg) were subjected to SDS/PAGE (4–12% Tris-Bis or 3–8% Tris-Acetate), transferred to a nylon membrane, and processed for overnight incubation with primary antibodies (see below), followed by secondary antibodies. Membranes were washed and proteins were detected enhanced chemiluminescence reagent (ECL+; GE Healthcare) and visualized using a Kodak 4000MM imager to obtain pixel density values for the band of interest (Carestream). All images were obtained using maximum sensitivity settings with no binning (0–65 K signal range). No images analyzed presented saturating signals for the bands of interest (>65 K grayscale value). Band density values were normalized to one of the following: β-actin, GAPDH, or eIF4G (immunoprecipitation: eIF4G).

Antibodies. The following antibodies were used in this study: anti-eIF4E monoclonal mouse antibody (1:1,000; Abgent), anti-eIF4E (1:1,000; Cell Signaling Technology), anti-eIF4G1 polyclonal rabbit antibody (1:100; Bethyl laboratories), anti-eIF4G1 polyclonal mouse antibody (1:1,000; R&D Systems), anti-GAPDH rabbit polyclonal antibody (1:1,000; Chemicon), 582 anti–N-terminal eI4FG (poly clonal rabbit, 1:5,000), 586 anti–C-terminal eIF4G (polyclonal rabbit, 1:5,000) [For description of antibodies 582 and 586, see Lloyd et al (1) and Byrd et al (2)]), anti-puromycin (mouse 12D10, 1:5,000) [for information about this antibody, see Schmidt et al. (3)], antiphospho (T202/Y204) ERK (1/2) rabbit polyclonal (1:5,000; Cell Signaling Technology), anti-ERK(1/2) rabbit polyclonal (1:5,000; Cell Signaling Technology), antiphospho(T180/Y182) p38 MAPK (1:5,000; Cell Signaling Technology), anti-p38 MAPK rabbit polyclonal (1:5,000; Cell Signaling), antiphospho (T183/Y185) SAPK/JNK rabbit polyclonal (1:1,000; Cell signaling Technology), and anti-SAPK/JNK rabbit polyclonal (1:3,000, Cell signaling Technology). Secondary antibodies used: goat anti-rabbit IgG-HRP (1:10,000; Promega), goat anti-mouse IgG-HRP (1:10,000; Promega).

1. Lloyd RE, Jense HG, Ehrenfeld E (1987) Restriction of translation of capped mRNA in vitro as a model for poliovirus-induced inhibition of host cell protein synthesis: relationship to p220 cleavage. *J Virol* 61:2480–2488.
2. Byrd MP, Zamora M, Lloyd RE (2002) Generation of multiple isoforms of eukaryotic translation initiation factor 4GI by use of alternate translation initiation codons. *Mol Cell Biol* 22:4499–4511.
3. Schmidt EK, Clavarino G, Ceppi M, Pierre P (2009) SUnSET, a nonradioactive method to monitor protein synthesis. *Nat Methods* 6:275–277.

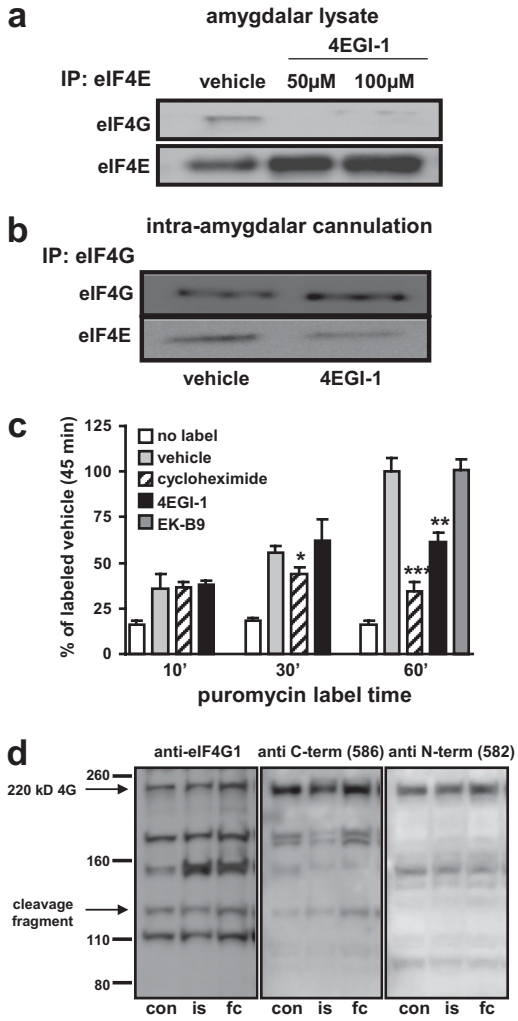

Fig. S1. Small molecule inhibitor 4EGI-1 blocks eIF4E/eIF4G interactions and protein synthesis in the amygdala as detected by SUnSET. (*A*) The formation of eIF4F is blocked by in amygdalar lysates incubated with 4EGI-1. Only immunoprecipitated protein is shown; total levels of the proteins did not change. (*B*) The eIF4E–eIF4G interactions are blocked in the lateral amygdala of rats following infusion of 4EGI-1 via a cannula. This observation was confirmed with three independent experiments. (*C*) Small molecule inhibitor 4EGI-1 blocks protein synthesis in the amygdala. Rat coronal slices containing lateral amygdala (LA) were incubated with protein synthesis inhibitors and then were labeled with subinhibitory doses of puromycin. Protein synthesized during the puromycin labeling time was visualized with an antipuromycin antibody. Cycloheximide (CHX) concentration was 20 μg/mL, 4EGI-1 and EK-B9 concentrations were 200 μM. No label, $n = 2$ for all times. 10′ puromycin label: vehicle (veh), $n = 3$; CHX, $n = 3$; 4EGI-1, $n = 3$. 30′ puromycin label: veh, $n = 3$; chx, $n = 3$; 4EGI-1, $n = 4$. 60′ puromycin label: veh, $n = 3$; CHX, $n = 4$; 4EGI-1, $n = 4$; EK-B9, $n = 4$. All statistical comparisons were made against the vehicle-treated sample in each time category: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, ANOVA. (*D*) Confirmation of eIF4G1 cleavage fragments using N- and C-terminal–specific antibodies to eIF4G1. The 220 kDa active isoform of eIF4G is recognized by all antibodies used. The C-terminal 586 antibody recognizes the cleavage fragment quantified in Fig. 3*D*. Behavior conditions: context exposure only (con), immediate shock (is), fear-conditioned (fc). Blot panels: (*Left*) amygdalar lysate probed with a mouse polyclonal anti-eIF4G1 antibody (Abnova); (*Center*) The same blot probed with a C-terminal eIF4G (586) antibody. (*Right*) The same blot probed with an N-terminal eIF4G (582) antibody. Blots were checked for efficient stripping before reprobing.

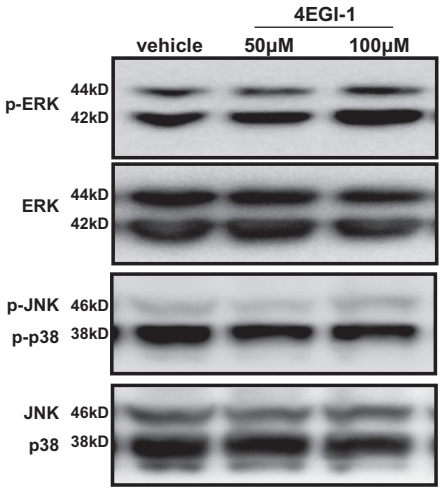

Fig. S2. Incubation with 4EGI-1 does not activate stress kinases. Coronal slices containing amygdala were incubated with vehicle, 50, or 100 μM 4EGI-1. Levels of phosphorylated stress kinases did not change after 45 min of incubation with 4EGI-1. Phospho-ERK (T202/Y204), phospho p38 MAPK (T180/Y182), and phospho-JNK/SAPK (T183/Y185) were assayed. The same results were obtained after three independent experiments.

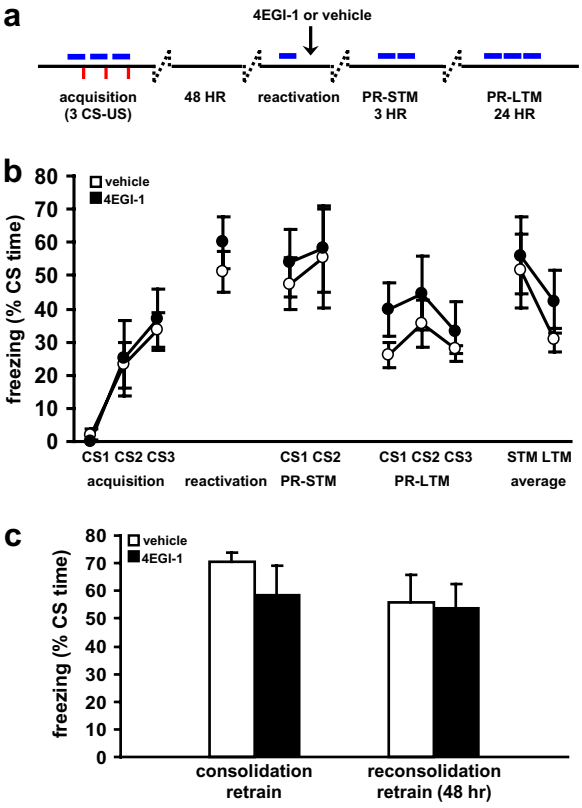

Fig. S3. Small molecule inhibitor 4EGI-1 does not impair postreactivation short-term memory (STM) or long-term memory (LTM) after a 48-h consolidation period. (*A*) Behavior and drug infusion schematic for consolidation experiments. (*B*) Small molecule inhibitor 4EGI-1 attenuates LTM, but neither STM nor LTM after reconditioning. (*C*) Behavior and drug infusion schematic for reconsolidation experiments [reconsolidation: vehicle (V), $n = 4$; drug (D), $n = 6$ ($P > 0.05$), ANOVA]. (*C*) 4EGI-1 does not permanently alter the function of the LA. Previously treated rats retrained in the absence of 4EGI-1 acquire fear memory normally (Consolidation, vehicle, $n = 6$; 4EGI-1, $n = 12$. Reconsolidation vehicle, $n = 4$; 4EGI-1., $n = 6$; $P > 0.05$, ANOVA).

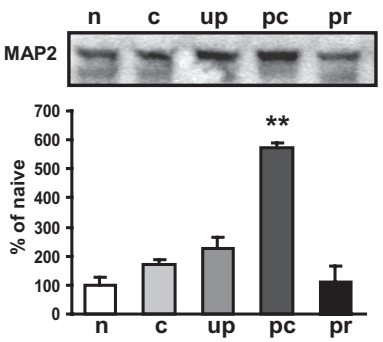

Fig. S4. Expression of MAP2 following consolidation and reconsolidation. MAP2 expression increases after fear conditioning, but not after memory reactivation. Tissue was harvested 15 min after each behavioral manipulation. $^{**}P < 0.05$, Student's *t* test. Naive (n), context-only (c), unpaired (up), postconditioning (pc), and postreactivation (pr). $n = 5–6$ for all conditions.

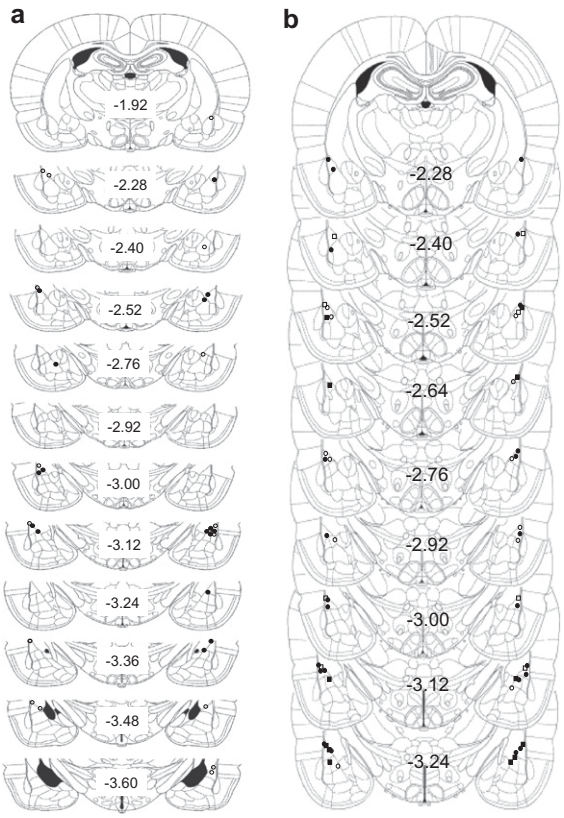

Fig. S5. Schematic representation of cannulation position for animals used in behavioral experiments. (*A*) Cannula emplacement for 24-h reconsolidation behavioral experiments. Cannula placements in the LA were histologically determined following behavioral experiments. Correct placements are denoted by symbols on coronal sections of the rat brain. Numbers indicate bregma coordinates (mm). Vehicle (●), 4EGI-1 (○). Drug (D)/*R* = 6 Vehicle (V)/*R* = 6. Rats that displayed infection or inaccurate cannulation emplacement were excluded from the data analyzed (excluded rats, $n = 4$ consolidation; $n = 4$ reconsolidation). (*B*) Schematic representation of cannulation position for animals used in Cannula emplacement for 24-h consolidation and 48-h reconsolidation behavioral experiments. Cannula placements in the LA were histologically determined following behavioral experiments. Correct placements are denoted by symbols on coronal sections of the rat brain. Numbers indicate Bregma coordinates (mm). ●, 4EGI-1/consolidation; ○, vehicle/consolidation; ■, 4EGI-1/reconsolidation; □, vehicle/reconsolidation. D/C = 12; V/C = 6; D/*R* = 6; V/*R* = 6. Rats that displayed infection or inaccurate cannulation emplacement were excluded from the data analyzed (excluded rats, $n = 3$ for consolidation; $n = 4$ for reconsolidation).