Supplementary Information for "Synaptic retinoic acid receptor signaling mediates mTOR-dependent metaplasticity that controls hippocampal learning" by Hsu et al.,

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Mouse monoclonal anti-β-actin (1:10,000)	Millipore	Cat#MAB1501
mouse anti-mTOR (1:1,000)	Cell Signaling Technology	Cat#4517S
Rabbit polyclonal anti-phospho-mTOR (Ser2448) (1:1,000)	Cell Signaling Technology	Cat#2971S
rabbit anti-phosphor-S6 ribosomal protein (Ser235/236) (1:1,000)	Cell Signaling Technology	Cat#2211S
mouse anti-S6 ribosomal protein (54D2) (1:1,000)	Cell Signaling Technology	Cat#2317S
rabbit anti-phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (1:1,000)	Cell Signaling Technology	Cat#9101S
mouse anti-p44/42 MAPK (Erk1/2) (L34F12) (1:2,000)	Cell Signaling Technology	Cat#4696S
rabbit anti-phospho-Akt (Ser473) (1:800)	Cell Signaling Technology	Cat#9271S
mouse anti-Akt (pan) (40D4) (1:2,000)	Cell Signaling Technology	Cat#2920S
rabbit anti-GluA1 (1:1,000)	Synaptic Systems	Cat#182003
mouse anti-GluA2 (1:1,000)	Millipore	Cat#MAB397
mouse anti-PSD-95 (1:2,000)	Invitrogen	Cat#MA1-046
rabbit anti-SAP97 (1:1,000)	Invitrogen	Cat#PA1-741
donkey anti mouse - IR DYE 800CW (1:10,000)	Licor Odyssey	Cat#926-32212
donkey anti rabbit - IR DYE 680 LT (1:10,000)	Licor Odyssey	Cat#926-68023

www.pnas.org/cgi/doi/10.1073/pnas.1820690116

rabbit anti-c-Fos (1:500)	Millipore	Cat#ABE457
Goat anti-Rabbit IgG, Alexa Fluor 594 (1:500)	Thermo Fisher Scientific	Cat# A-11037
Chemicals, Peptides, and Recombinant Proteins		
Rapamycin	LC Laboratories	Cat#R-5000
Experimental Models: Organisms/Strains		
The RARα floxed mouse (C57BL/6 background)	Drs. Pierre Chambon and Norbert Ghyselinck (IGBMC, Strasbourg, France) (Chapellier et al., 2002, Sarti et al., 2012)	N/A
B6.Cg-Tg(CaMKIIα-Cre)T29-1Stl/J	The Jackson Laboratory	Cat#005359
Recombinant DNA		
Plasmid: Cag-Cre-EGFP	Ref (1)	N/A
Plasmid: Cag-mCre-EGFP	Ref (1)	N/A
Plasmid: pHelper	Ref (2)	N/A
Plasmid: pRC-DJ	Ref (2)	N/A
Software and Algorithms		
Image Studio 5.2.5	LI-COR Bioscience	https://www.licor.com/bio/products/software/image_studio_lite/
Prism 6	GraphPad	https://www.graphpad.com/scientific-software/prism/
Viewer III tracking system	Biobserve	http://www.biobserve.com/behavioralresearch/products/viewer/
Freezeview	Coulbourn Instruments	https://www.coulbourn.com/product_p/act-100a.htm

METHODS

RARα conditional knockout mouse

The RARα floxed mouse (C57BL/6 background) was a generous gift from Dr. Pierre Chambon and Norbert Ghyselinck (IGBNC, Strasbourg, France) (3). Breeding colonies were maintained in the animal facility at Stanford Medical School following standard procedures approved by the Stanford University APLAC. The homozygous RARα floxed mice were bilaterally stereotaxic injected with adeno-associated viruses (AAVs) expressing Cre recombinase or a truncated and inactive version of Cre (mCre). Mice were group housed with littermates and maintained under 12/12 hr daylight cycle. Animal experiments were conducted following protocols approved by Institutional Animal Care and Use Committee (IACUC) at Stanford University.

AAV preparation and stereotaxic Injection

AAV-DJ virus, which shows high *in vivo* infection efficiency (2) was used for *in vivo* injections. AAV vectors containing CMV-actin-globin promoter either with wild-type active Cre-recombinases (Cre) or with truncated-inactive Cre recombinase as control (mCre) both fused to EGFP were constructed as previously described (1). AAV vectors were co-transfected with pHelper and pRC-DJ into HEK293 cells. 72 hours post-transfection, cells were collected, lysed and ran onto iodixanol gradient for 2 hours at the speed of 400,000g. The fraction of 40% iodixanol gradient was collected, washed and purified with 100,000 MWCO tube filter. The titer of AAVs were measured in HEK293 cells and adjusted to around 2.5-5 x 10^9 infectious AAV particle/µl for stereotaxic injections. Six- to seven-week-old homozygous RARα floxed mice were bilaterally injected into the hippocampal CA1 at two sites with a glass pipette at a flow speed of 0.15µl/min. Coordinates used for stereotactic injections were AP -1.95mm, ML ± 1.25mm, DV - 1.2mm and DV -1.35mm. The sites at DV-1.2mm and DV -1.35mm both received 0.75 µl of viral injections. Injected animals were subjected to one of the behavior assays or electrophysiological analysis four weeks after viral infection.

Acute brain slice electrophysiology

Ten- to eleven-week-old mice were anesthetized and the brains were rapidly removed. Transverse hippocampal slices (300µm) were cut in ice-cold cutting solution containing (in mM): 228 sucrose, 26 $NaHCO_3$, 11 D-glucose, 2.5 KCl, 1 NaH_2PO_4, 7 $MgSO_4$ and 0.5 $CaCl_2$ saturated with 95%O_2/ 5%CO_2. Slices were then transferred to a holding chamber containing artificial cerebrospinal fluid (ACSF) composed of (in mM) 119 NaCl, 26 $NaHCO_3$, 11 D-glucose, 2.5 KCl, 1 NaH_2PO_4, 1.3 $MgSO_4$ and 2.5 $CaCl_2$. The slices were allowed to recover at 32°C for 30 min and then moved to room temperature for a further 1 hour. During recordings, the slices were placed in a recording chamber constantly perfused (1ml/min) with oxygenated ACSF (heated at 27℃-29℃).

Whole-cell voltage-clamp recordings were obtained from GFP positive CA1 pyramidal neurons in acute hippocampal slices. EPSCs were recorded with glass pipettes (3-4 MΩ) filled with an internal solution containing (in mM) 135 $CsMeSO_4$, 8 NaCl, 10 HEPES, 0.25 EGTA, 2 $MgCl_2$, 4 Mg-ATP, 0.3 Na_3-GTP, and 5 phosphocreatine (pH 7.3) (~300 mOsm). IPSCs were recorded with an internal solution containing (in mM): 40 CsCl, 90 K-Gluconate, 1.8 NaCl, 3.5 KCl, 1.7 $MgCl_2$, 10 HEPES, 0.05 EGTA, 2 Mg-ATP, and 0.4 Na_3-GTP., 10 phosphocreatine. mEPSCs and mIPSCs were recorded in 0.5 µM tetrodotoxin (TTX) plus either 50 µM picrotoxin (mEPSCs) or 10 µM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; mIPSCs) and 50 µM D-(-)-2-amino-5-phosphonopentanoic acid (D-APV; mIPSCs), respectively. Evoked AMPA- and NMDA-mediated currents were recorded in the presence of picrotoxin (50uM). Randomly selected 100 mEPSCs from each cell were pooled to generate the cumulative probability plots for mEPSC amplitude.

Evoked responses in CA1 pyramidal neurons were achieved by placing a concentric bipolar stimulation electrode in stratum radiatum. Synaptic E/I ratio was measured by recording with an internal solution containing (in mM): 143 $CsMeSO_4$, 10 HEPES, 0.25 EGTA, 0.5 $MgCl_2$, 4 Mg-ATP, 0.3 Na_3-GTP, and 5 phosphocreatine (pH 7.3) (~300 mOsm). The reversal potentials for EPSCs and IPSCs were validated with I-V recordings in the respective antagonists for $GABA_A$ and AMPA receptors. Neurons were first clamped at -80mV (reversal potential of IPSC) to record EPSCs, and then clamped at -50mV for dual PSCs. 50 μM APV was included in ACSF. The EPSCs at -50 mV (scaled from -80 mV recordings) were subtracted from the biphasic (EPSC and IPSC) components measured at -50mV to generate pure IPSCs component at -50 mV (4). All EPSC and IPSC traces were averaged over 15-40 consecutive trials.

For LTP and LTD experiments, AMPAR EPSCs were recorded in voltage clamp mode at -70 mV while afferent inputs were stimulated at 0.1 Hz. LTP was induced by 1 or 4 trains of high frequency stimulation (100 Hz, 1 s) separated by 10 s, while the cells were depolarized to 0 mV. LTD was induced by 1Hz 900-pulse trains while cells were held at -40 mV. For voltage-pulse LTP, cells were depolarized 20 times from -70 mV to +10 mV for 1 s in the absence of stimulation but in the presence of AP5. The magnitudes of LTP and LTD were calculated based on the EPSC values 41-45 minutes or 26-30 minutes after the beginning of the induction protocol, respectively. All data are presented as means ± SEMs. All electrophysiological recordings were carried out with Multiclamp 700B amplifiers (Axon Instruments) and analysis was completed in Clampfit (Axon), Excel (Microsoft), and Prism (GraphPad).

Immunoblotting and immunocytochemistry

The following antibodies were used for immunoblotting experiments: mouse anti-β-actin (Millipore MAB1501, 1:10,000,), rabbit anti-phospho-mTOR (Ser2448) (Cell Signaling Technology 2971S, 1:1,000), mouse anti-mTOR (Cell Signaling Technology 4517S, 1:1,000), rabbit anti-phosphor-S6 ribosomal protein (Ser235/236) (Cell Signaling Technology 2211S, 1:1,000), mouse anti-S6 ribosomal protein (54D2)(Cell Signaling Technology 2317S, 1:1,000), rabbit anti-phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (Cell Signaling Technology 9101S, 1:1,000), mouse anti-p44/42 MAPK (Erk1/2) (L34F12) (Cell Signaling Technology 4696S, 1:2,000), rabbit anti-phospho-Akt (Ser473) (Cell Signaling Technology 9271S, 1:800), mouse anti-Akt (pan) (40D4) (Cell Signaling Technology 2920S, 1:2,000), rabbit anti-GluA1 (Synaptic Systems 182003, 1:1,000), mouse anti-GluA2 (EMD Millipore MAB397, 1:1,000), donkey anti Mouse - IR DYE 800CW (Licor Odyssey Cat# 926-32212. 1:10,000) and donkey anti rabbit - IR DYE 680 LT (Licor Odyssey Cat# 926-68023, 1:10,000). The following antibodies were used for immunocytochemistry: rabbit anti-c-Fos (Millipore ABE457, 1:500), and Goat anti-Rabbit IgG, Alexa Fluor 594 (Thermo Fisher Scientific A-11037, 1:500).

For immunoblotting, hippocampal slices were rapidly dissected from 3-month-old CaMKII:RARα floxed mice, submerged in chilled sucrose cutting solution (as previously described in acute brain slice electrophysiology). Hippocampal CA1 regions were further dissected out from the slices and were homogenized in cold lysis buffer [50 mM Tris, 150 mM NaCl, 5 mM EDTA, 1 mM EGTA, 1 mM PMSF, 10 mM NaF, and 1 mM Na_3VO_4 , pH = 7.5, with 1:1000 protease inhibitor mixture (Sigma)]. Denatured proteins were separated by SDS-PAGE using 4–20% Precise Protein Gels (Pierce), and transferred onto nitrocellulose membranes. Membranes were then blocked with 5% bovine serum albumin (BSA) dissolved in Tris-buffered saline (TBS) containing 0.1% Tween 20 (TBST) at room temperature for 1 h and reacted overnight with antibodies at 4°C. Membranes were washed three times with TBST for 5 min each and incubated with Odyssey infrared-conjugated secondary antibodies in 5% BSA in TBST for 1 hr at room temperature. Following three times of 10 min washes with TBST, membranes were scanned and analyzed quantitatively

using an Odyssey infrared imaging system (LI-COR Biosciences). Data were calculated as fold of control and expressed as means ± SEM from at least 3 independent experiments. GluA1 and GluA2 signals were normalized to actin intensity. Phospho-mTOR and phospho-S6 signals were normalized to total mTOR and S6 signals, respectively. Phospho-Erk1/2 and phospho-Akt signals were normalized to total Erk1/2 and Akt signals, respectively.

For immunocytochemistry, brains were perfused in ice-cold 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS) and post-fixed overnight. Coronal slices were sectioned (30 μm) using a cryostat. Free-floating sections were washed in PBS for 10 mins and incubated for 1 hr with blocking buffer containing 5% normal donkey serum, 0.3% Triton X-100 in PBS at room temperature shaking. Sections were washed with PBS three times and incubated overnight (4°C) with anti-c-Fos antibody. After three times of 10 min washes in PBS, sections were reacted with a secondary antibody, Alexa Fluor 594-conjugated goat anti-rabbit IgG, for 2 hrs at room temperature shaking. Following three times washing with PBS, sections were mounted with Vectashield mounting medium (Vector Laboratories, H-1500). And the images were taken using Nikon A1R Si confocal microscope.

Rapamycin injection

Mice received rapamycin (LC Laboratories) intraperitoneally injection (5 mg/kg) once daily starting at day 1 of enriched environment experience for 10 days. Vehicle control was 100% DMSO at 2 ml/kg and did not exceed 50 μl per injection (5). Mice were subjected to biochemical, behavioral or electrophysiological experiments 24 hours after receiving the last injection.

Behavioral experiments

Each animal was subjected to only one behavioral test.

Enriched environment

Enriched environment (EE) consists a Habitrail Ovo hamster cage (Hagen Inc. Canada) with running wheels installed. The cage can be extended and modified in different ways with a plastic connector provided by the manufacture. Three to four objects were positioned into the cage at a time including paper tubes, 1.5 ml test tubes, 50 ml conical tubes, marbles, absorbent bedding, various hamster toys. The positions of the objects were changed daily and one of the objects was replaced with a new one from day to day. Mouse had free access to water and food inside EE. Mice from 2 to 3 home cages were group-housed together in one EE chamber for 4 to 6 hours per day for 10 to 11 days.

Elevated plus maze

An elevated plus maze (Stoelting Co. IL) is composed of two open arms and two closed arms that extend from a central square area and elevate to a height of 50cm above floor level. The mouse was placed in the center of the maze facing an open arm and left to freely explore the maze for 10 min. The amounts of the time spent in the open vs. closed arms were recorded by Viewer III tracking system (BIOBSERVE). Data were analyzed with Student's *t*-test.

Water T-maze

A water T-maze was custom made out of clear Plexiglas. The dimension of each arm is 30 cm x 11 cm x 20 cm. Depth of opaque water (with nonfat dry milk) was kept at 13 cm. On the habituation day, mice were allowed to freely explore by swimming in the maze for 1 minute starting from the stem arm. No platform was present. Their first choices of arm entry (either left or right) were recorded.

During training day 1-3, a 7cm x 9cm platform was submerged 1 cm below the water surface and placed in the arm opposite to the choice arm recorded on habituation day for each mouse. The mouse was placed into the maze from the stem arm, and allowed to explore for 1 min. If the mouse located the platform within 1 minute, it would be allowed to remain on the platform for 5 seconds before being returned to home cage. If locating the platform was unsuccessful, the mouse would be guided to the platform and allowed to remain on the platform for 10 seconds before returning to home cage. Each mouse was given 10 trials per day and the percentage of correct entries was calculated (excluding the first trial on the first day). All mice reached 80% or more of correct entries by day 3. Reversal learning started on day 4, when the platform was moved to the opposite arm. Training rules identical to the first three training days were applied. Reversal training was stopped on the day when the group average met the criteria of 80% or more correct entries.

Open field

Individual mouse was placed inside an open field chamber (29.5cm x 23.5cm) for 10 minutes. Time spent in the center area (14.75cm x 11.75cm) and the track length of each animal were recorded. The data were collected using Viewer III tracking system (BIOBSERVE).

Alternating Y-maze

Spatial working memory was assessed by measuring spontaneous alternation in a plastic Y-maze (Stoelting Co. IL). Individual mouse was placed in the Y-maze from the stem arm while facing towards the center and let freely explore for 10 minutes. The sequence of entries into each arm were recorded by a camera above the maze and analyzed by Viewer III tracking system (BIOBSERVE). A spontaneous alternation performance (%SAP) was calculated by successive entries into each of the three arms on overlapping triplet sets to all possible alternations (total arm entries − 2) × 100%.

Three chamber social interaction test

Social approach behavior was tested in a 3 chamber apparatus custom made out of clear Plexiglas as previously described (6). The dimension of each chamber was 20cm x 30cm x30cm and the subject mouse was first habituated in the center chamber for 5 minutes followed by another 5 minutes of free exploration in the three chambers. A novel male mouse of same species at the comparable age was placed in one side chamber (social chamber) and a novel object was placed in the other side chamber (non-social chamber). Both the novel mouse and the novel object were placed inside an upside-down wire mesh can. A filled plastic water bottle was placed on top of each of the wire mesh can to weigh it down and to confine the locations of the novel mouse and the object so as to prevent the possible movement made from the subject mouse. The locations of the novel object and the novel mouse were alternated across subject mice to prevent spatial preference (if any). The time durations each subject mouse spent in each chamber were recorded and analyzed with the Viewer III tracking system (BIOBSERVE)

Fear Conditioning

The fear conditioning chamber (Coulbourn Instruments, PA) was cleaned between each trial with 10% ethanol to provide a background odor, and the chamber was located inside a sound attenuating cubicle (Coulbourn Instruments, PA). A ventilation fan provided a background noise at ~55dB. The mice were allowed to freely explore the chamber for two minutes then received three tone-foot shock pairings separated by 1 minute no-tone intervals. The 85-dB 2-kHz tone presented for 30 seconds and a 2-s 0.75mA foot shock was delivered and co-terminated with the tone. The mice remained in the training chamber for another 30 seconds before being returned to their home cages. In the contextual memory test, mice were placed back in the chamber where

they received foot shocks for 5 minutes. The altered-context and the tone test were performed in a different chamber which was identical to the conditioning chamber but modified by replacing the metal grid with plastic sheet, white metal side walls with red strips decorated plastic walls, and background odor with vanilla. The ventilation fan was turned off to change background noise. Mice were placed inside the altered chamber for 5 minutes before a 1 minute tone (85dB, 2kHz) was delivered. The behavior of the mice was recorded and freezing percentage was analyzed with Freezeview software (Coulbourn Instruments). Motionless bouts lasting more than 1 second were measured as freeze.

Barnes maze

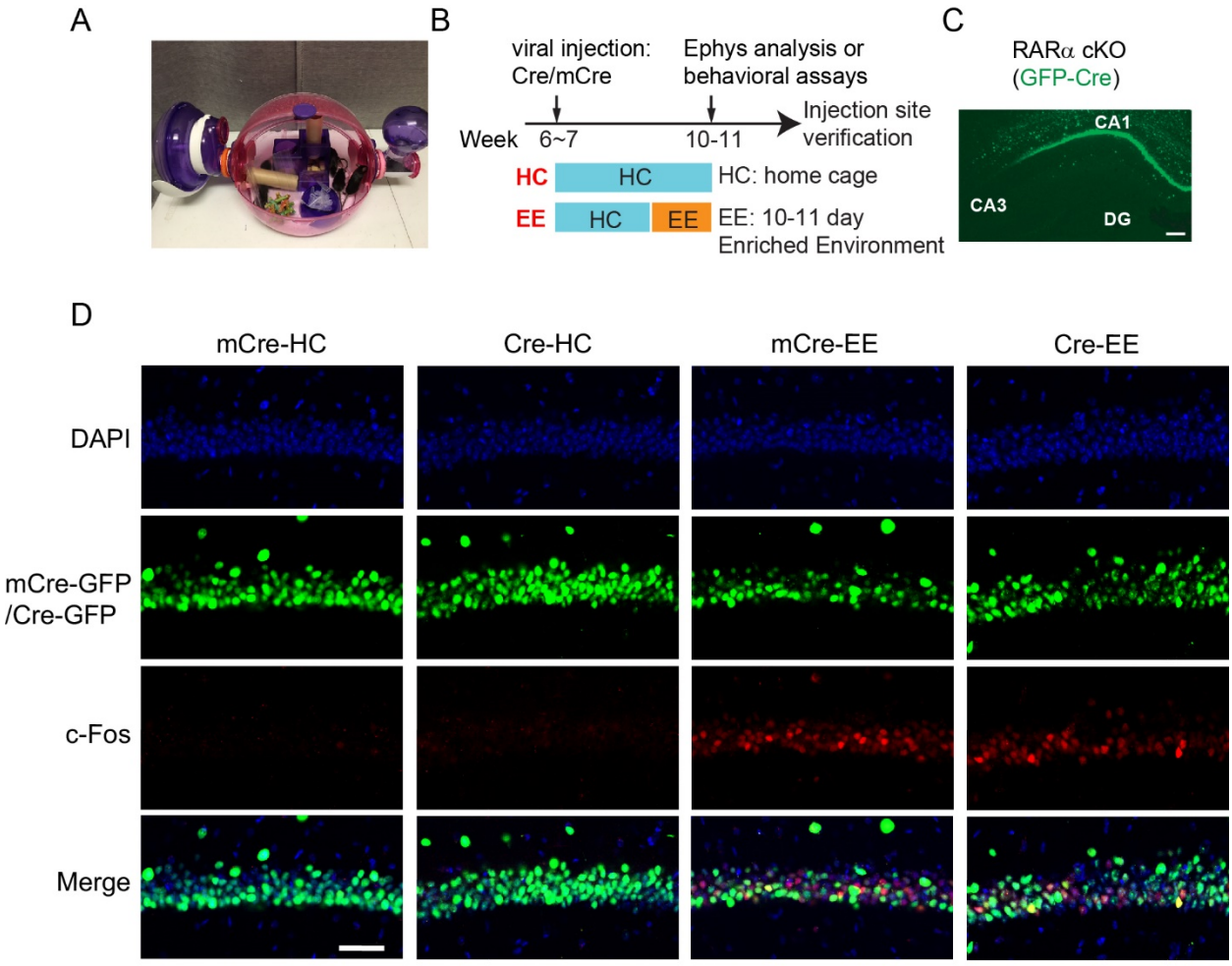
Barnes maze test was performed based on a published protocol (7). During the habituation period, mice were placed in a start chamber in the center of the maze for 10 s. Then the chamber was removed and the light turn on, mice was gently guided to enter the escape box. Light was turned off upon mouse entering the box and remaining inside the box for 2 min. During the learning session, mice were held inside the start chamber for 10 s before being released to freely explore the maze with the light on for 3 min. Mice were allowed to stay inside the box for 1 min after entering. Training consisted 4 learning sessions per day for 2 days. The 90 s probe test was performed twenty-four hours after the last training session with the same procedure except the target hole covered. Reversal learning started 24 hours after the first probe test, where the original target hole was blocked and a new target hole on the opposite side of the platform opened. Reversal learning also consisted eight training sessions (4 per day). Reversal probe test was performed twenty-four hours after the last reversal learning session.

Statistical analysis

Data analysis was performed using GraphPad Prism 6 software. Data were first tested for normality using Shapiro-Wilk test. Datasets passed normality were subjected to parametric statistic tests (ANOVA or student's t-test). If the conditions for parametric tests were not met, non-parametric tests with Bonferroni corrections (when multiple comparisons are required) were used.

References

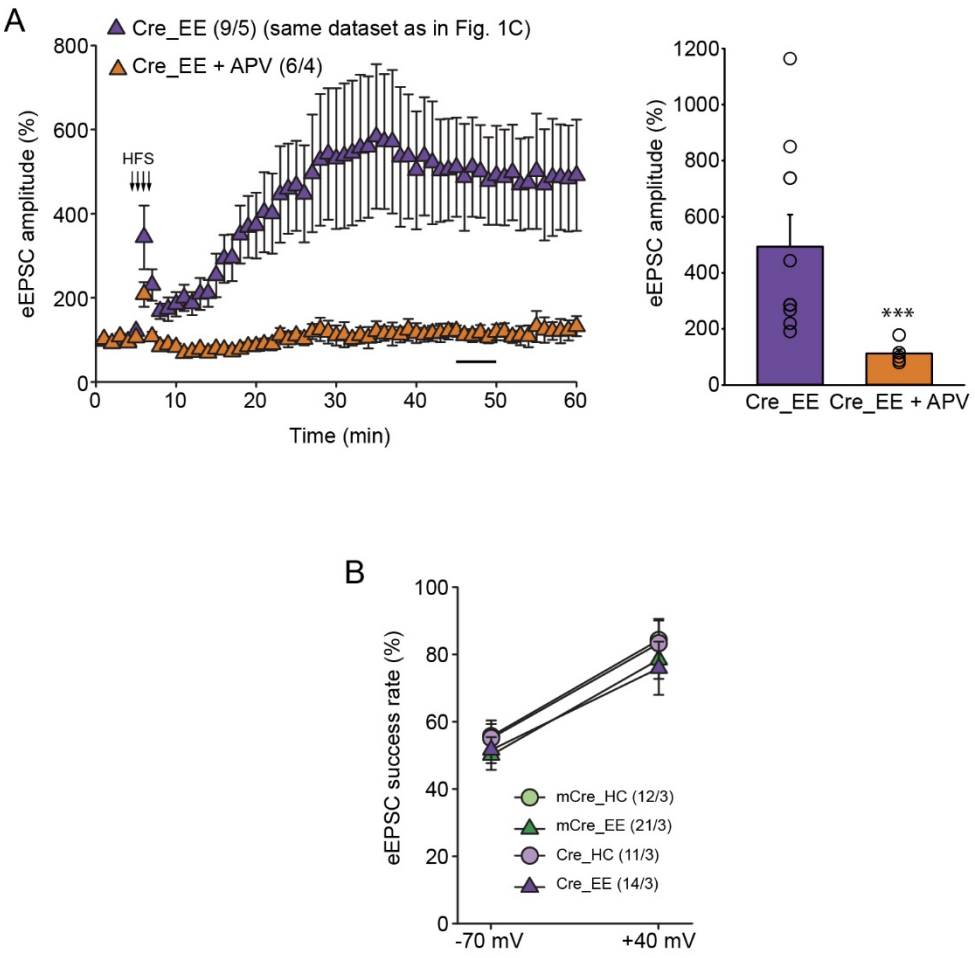
1. Kaeser PS*, et al.* (2011) RIM proteins tether Ca2+ channels to presynaptic active zones via a direct PDZ-domain interaction. (Translated from eng) *Cell* 144(2):282-295 (in eng).
2. Xu W*, et al.* (2012) Distinct neuronal coding schemes in memory revealed by selective erasure of fast synchronous synaptic transmission. (Translated from eng) *Neuron* 73(5):990-1001 (in eng).
3. Chapellier B*, et al.* (2002) A conditional floxed (loxP-flanked) allele for the retinoic acid receptor alpha (RARalpha) gene. *Genesis* 32(2):87-90.
4. Gabernet L, Jadhav SP, Feldman DE, Carandini M, & Scanziani M (2005) Somatosensory integration controlled by dynamic thalamocortical feed-forward inhibition. (Translated from eng) *Neuron* 48(2):315-327 (in eng).
5. Ehninger D*, et al.* (2008) Reversal of learning deficits in a Tsc2+/- mouse model of tuberous sclerosis. (Translated from eng) *Nat Med* 14(8):843-848 (in eng).
6. Liang J*, et al.* (2015) Conditional knockout of Nlgn2 in the adult medial prefrontal cortex (mPFC) induces delayed loss of inhibitory synapses. (Translated from eng) *Mol Psychiatry* 20(7):793 (in eng).
7. Sunyer B, Patil S, Höger H, & Lubec G (2007) Barnes maze, a useful task to assess spatial reference memory in the mice.



Hsu et al., Figure S1

Supplementary **Fig. 1. Normal activation of hippocampal CA1 neurons by enriched environment in CA1-RARα KO mice**

(*A*) A picture of the enrichment environment (EE) apparatus showing multiple sensory, motor and social enrichment. (*B*) A diagram of the experimental paradigm. (*C*) An example image of a hippocampal section showing CA1-specific AAV-mediated expression of GFP-Cre. (*D*) Immunocytochemistry for c-Fos expression was performed one hour after 4 hours of EE. Both mCre-injected WT and Cre-injected RARα KO CA1 neurons showed robust c-Fos activation. Scale bar: 50 μm.



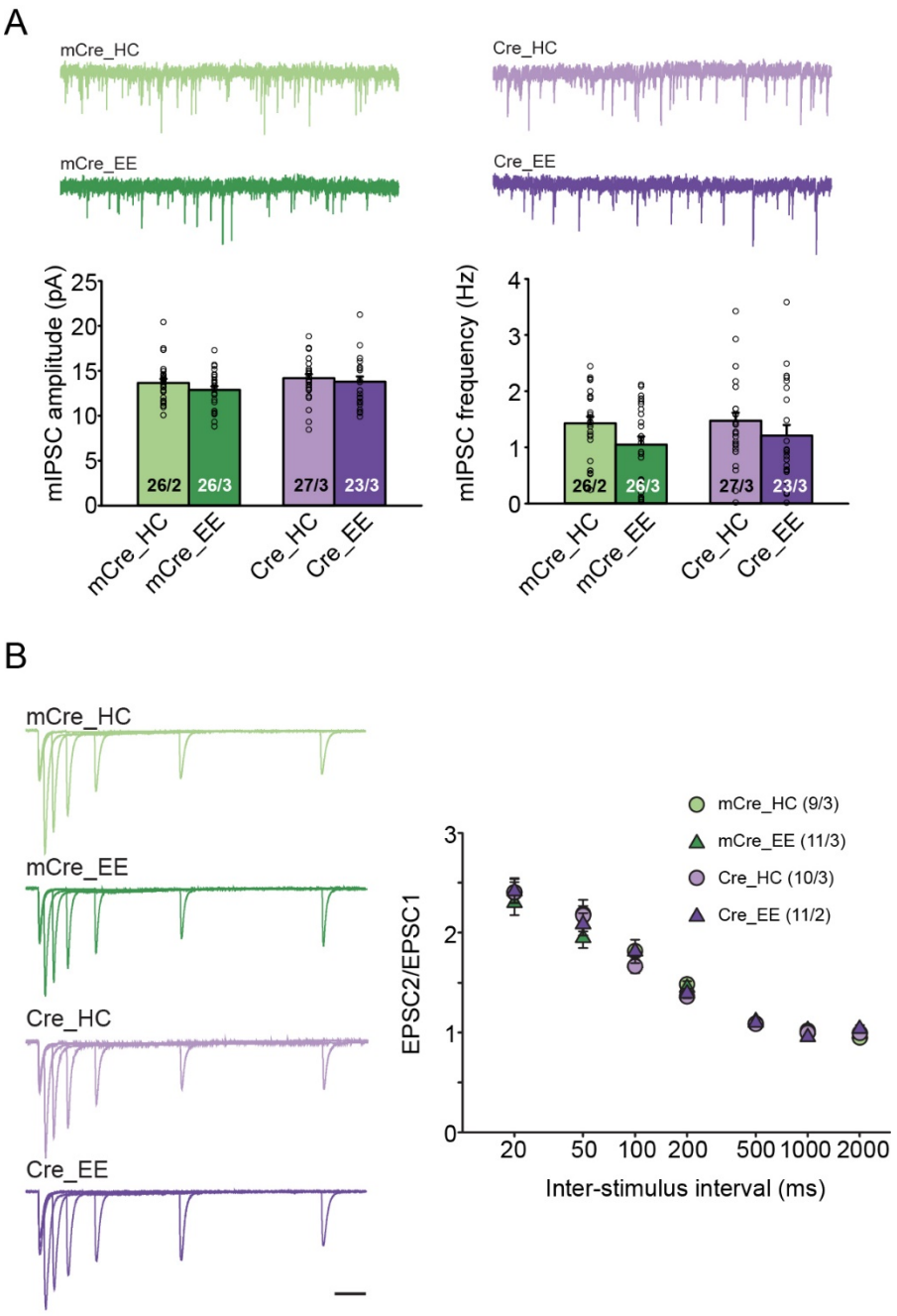
Hsu et al., Figure S2

Supplementary **Fig. 2. Additional properties of SC-CA1 synapses**

(*A*) The enhanced LTP observed in the CA1-RARα KO mice with enriched environment (EE) experience is NMDAR-dependent. LTP at SC-CA1 synapse was induced using standard 4x100Hz high-frequency trains (HFS). NMDAR antagonist D-APV (50 μM) was included in the ACSF. Magnitude of potentiation was measured at 41-45 min post HFS (right) (two tailed Mann-Whitney U test, ***, $p < 0.001$;). Note data from the Cre_EE group is the same as shown in Fig. 1C as these two experiments were done on the same days in interleaved slices.

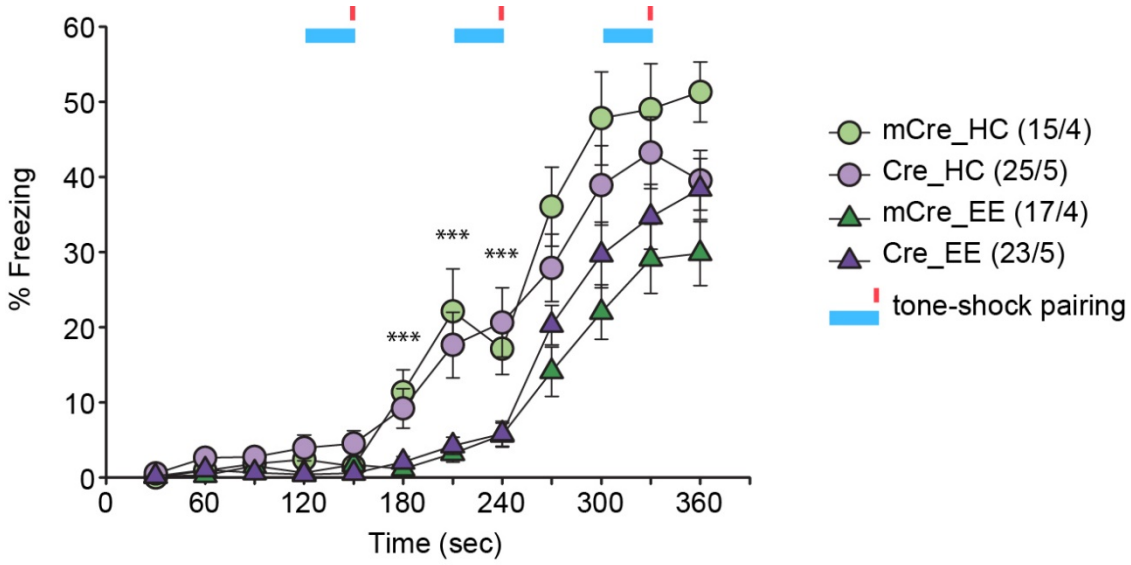
(*B*) RARα deletion or EE experience does not promote silent synapse formation. Presence of silent synapses was evaluated by measurement of eEPSC success rate under minimum stimulation at -70 mV and +40 mV. Although there is a general increase in success rate from -70 mV to +40 mV, there is no difference between any of the groups.

All graphs represent mean ± S.E.M..



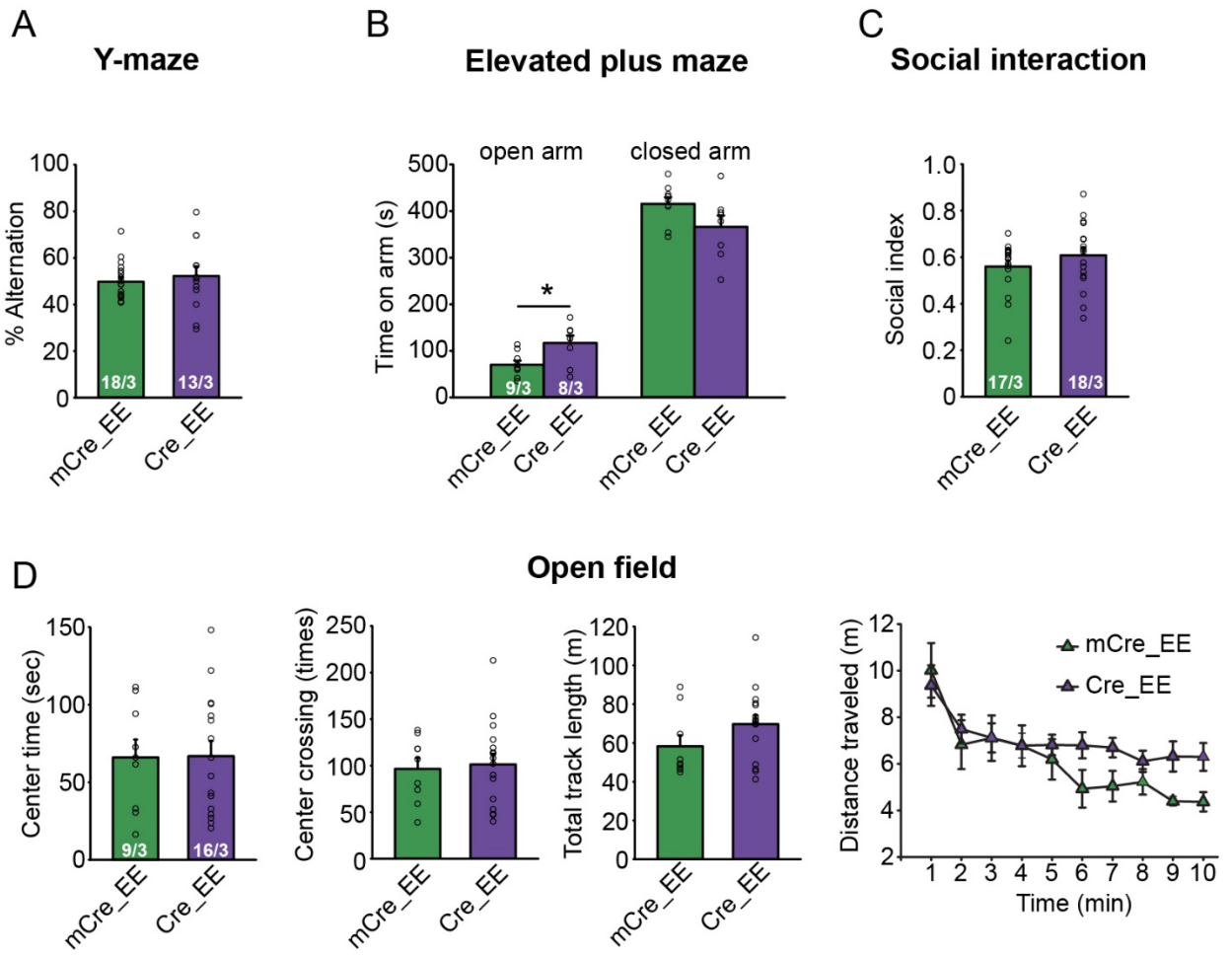
Hsu et al., Figure S3

Supplementary **Fig. 3. Characterization of Inhibitory Synaptic Transmission and Paired-pulse Ratio of Excitatory Synaptic Transmission**
(*A*) Representative traces (top) and quantification (bottom) of mIPSCs recorded from CA1 pyramidal neurons. Scale bars: 20 pA, 1 s.
(*B*) Paired-pulse ratio was measured at varying intervals in hippocampus from wildtype (mCre) and CA1-RARα KO (Cre) mice exposed to home cage or EE. Scale bar: 100 ms.
All graphs represent mean ± S.E.M..



Hsu et al., Figure S4

Supplementary **Fig. 4. RARα Deletion or Enriched Environment Experience Does Not Enhance Unconditioned Response During Fear Conditioning Training**
Three pairs of tone (30 s, blue bar) and foot shock (2 s, red bar) were delivered with a 1.5 min interval. Freezing before, during and after each tone-shock pair was quantified. While CA1-RARα KO experienced in home cage did not differ in its unconditioned responses when compared to wildtype mice, EE experience seemed to reduce the unconditioned responses to the first tone-shock pair in both wildtype and RARα KO groups (Statistical analysis was performed using a two-way repeated-measures ANOVA, group factor (genotype + experience) $F(3,76)=8.762$ $P<0.0001$, time factor $F(11,836)=161.0$ $p<0.0001$, interaction $F(33,836)=3.492$ $P<0.0001$,Tukey *post hoc* test, mCre_HC vs. mCre_EE ***, $p < 0.001$).
All graphs represent mean ± S.E.M..



Hsu et al., Figure S5

Supplementary **Fig. 5. Normal Anxiety Level and Working Memory in CA1-RARα KO Mice with Enriched Environment Experience**

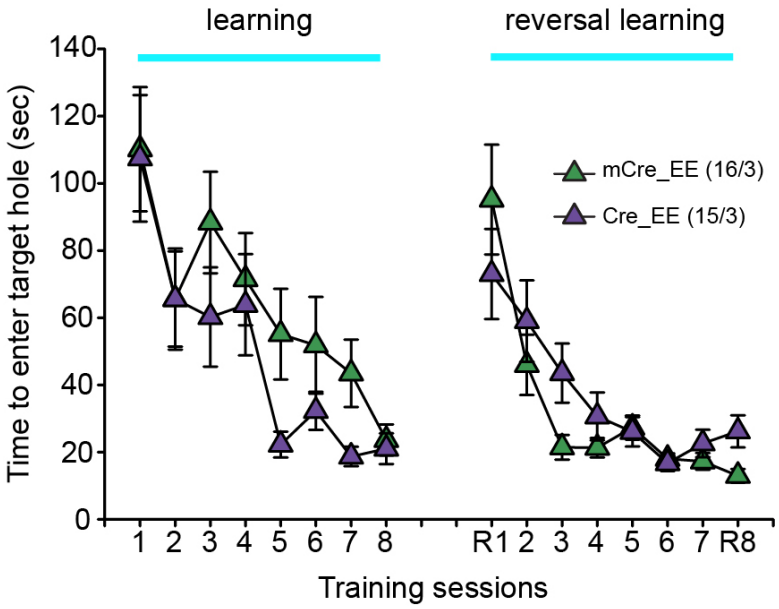
(*A*) Quantification of percent spontaneous alteration performance in the Y-maze.

(*B*) Quantification of time spent on the open vs. closed arm in the elevated plus maze (two tailed student's t-test, t=2.662 df=15, *, $p < 0.05$;).

(*C*) Quantification of social interaction in the three-chamber social test.

(*D*) Quantification of locomotion activity (time spent in the center area, number of center crossing, total track length and travel distance over time) in the open field test.

All graphs represent mean ± S.E.M..

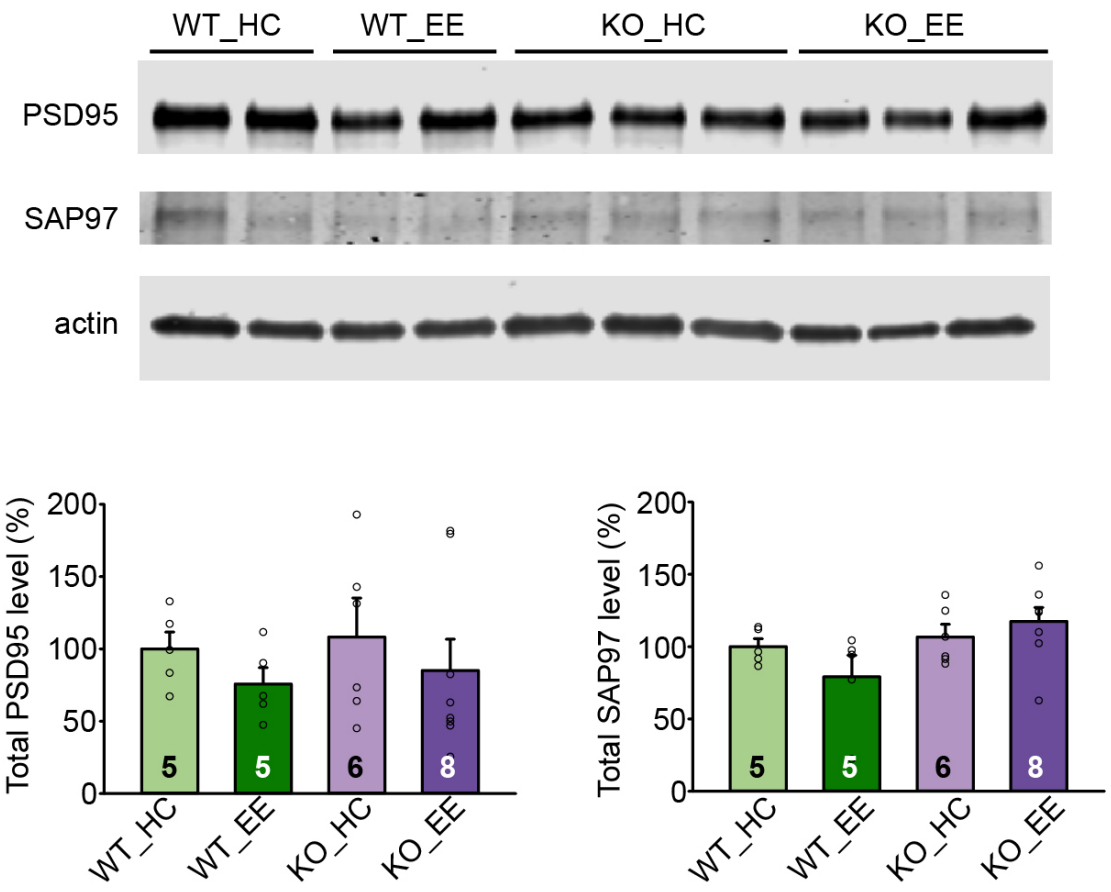


Hsu et al., Figure S6

Supplementary **Fig. 6. Performance of Enriched Environment-experienced WT and CA1-RARα KO Mice, Measured as Time to Enter Target Hole, During Initial Learning and Reversal Learning Session**

Mice went through total 8 training sessions (4 sessions per day) for both learning and reversal learning phases, which were spaced 48 hours apart.

All graphs represent mean ± S.E.M..



Hsu et al., Figure S7

Supplementary **Fig. 7. Expression of Postsynaptic Scaffold Proteins Is not Perturbed by RARα Deletion in Home Cage or Enriched Environment-experienced Mice**
Immunoblot analysis of total PSD95 and SAP97 expression was performed with CA1 tissues of home cage and EE-experienced WT and CaMKII-Cre RARα KO mice.
All graphs represent mean ± S.E.M..