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Persistent modifications of hippocampal synaptic function during remote spatial memory

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Abstract

A widely accepted notion for a process underlying memory formation is that learning changes the efficacy of synapses by the mechanism of synaptic plasticity. While there is compelling evidence of changes in synaptic efficacy observed after learning, demonstration of persistent synaptic changes accompanying memory has been elusive. We report that acquisition of a hippocampus and long-term potentiation dependent memory for places persistently changes the function of CA1 synapses. Using extracellular recordings we measured CA3-CA1 and EC-CA1 synaptic responses and found robust changes in the CA3-CA1 pathway after memory training. Crucially, these changes in synaptic function lasted at least a month and coincided with the persistence of long-term place memories; the changes were only observed in animals that expressed robust memory, and not in animals with poor memory recall. Interestingly, our findings were observed at the level of populations of synapses; suggesting that memory formation recruits widespread synaptic circuits and persistently reorganizes their function to store information.

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Author Contributions: AP performed and analyzed the electrophysiological experiments, carried out the statistical analyses and wrote an initial draft of the manuscript; EW performed and analyzed the behavioral experiments. AP, EW, AAF, JMA designed and interpreted the experiments. AAF and JMA wrote the manuscript with contributions from AP and EW.

INTRODUCTION

The synaptic plasticity and memory hypothesis is recognized as the most influential proposition for the mechanisms that underlie learning and memory. It asserts that the neural activity that underlies experience changes the efficacy of appropriate synapses to create and store memory reviews: (Martin and Morris, 2002; Neves et al., 2008). To support the hypothesis, an extensive body of work has focused on investigating the functional and mechanistic properties of synaptic plasticity elicited by artificial manipulations and how these properties correlate with altered learning and memory expression in, typically, genetically manipulated animals (Frick et al., 2004; Malenka and Bear, 2004; Abraham, 2008; Mayford, 2014; Takeuchi et al., 2014). Indeed, selective modifications of gene expression or protein activity have provided tremendous insight into the molecular mechanisms that underlie synaptic plasticity and to a lesser extent, learning and memory processes (Malenka and Bear, 2004). In contrast, relatively few studies have investigated whether learning changes synaptic function and whether these changes maintain with the persistence of memory reviews: (Mayford, 2014; Takeuchi et al., 2014).

There is compelling evidence of changes in neural and synaptic function in neocortex with sensory stimulation (Barth and Poulet, 2012; Wen et al., 2013) and dendritic spine structure changes in prefrontal cortex and hippocampus with environmental enrichment (Kozorovitskiy et al., 2005; Makara et al., 2009), however, it has been difficult to reliably show that persistent memory storage is accompanied by persistent changes in synaptic function. In the hippocampus, the structure that is central to our concepts of memory for places and events (Garner et al., 2012; Mayford, 2014; Takeuchi et al., 2014), and even procedural learning (Micheau et al., 2004), recent studies show evidence of changed hippocampal neural and synaptic function after the acquisition of a hippocampus-dependent memory (Gruart et al., 2006; Whitlock et al., 2006; Matsuo et al., 2008; McKay et al., 2013; Park et al., 2015).While these studies support the synaptic plasticity and memory hypothesis, there still is an absence of direct evidence that persistent hippocampal synaptic function accompanies long-term hippocampus-dependent memory.

Detecting a memory trace in hippocampus synapses has long been a subject of intense investigation because it is a key prediction of the synaptic plasticity and memory hypothesis. Prior work showed that learning can change hippocampus excitability (Oh et al., 2003; McKay et al., 2013) as well as synapses (Green et al., 1990; Sacchetti et al., 2001; Gruart et al., 2006; Whitlock et al., 2006) but the memory persisted much longer than the changes in synapse function (Sacchetti et al., 2001; Whitlock et al., 2006). This discrepancy in duration has raised the question of whether the experience-driven synaptic alterations were indeed due to memory storage, and not instead due to transient changes in synaptic homeostasis (Kirkwood et al., 1996; Turrigiano and Nelson, 2000), or other confounding features of the experience (Moser et al., 1993). Hence, demonstration that changes in synaptic circuit function during learning persist with memory still is an ongoing challenge for the synaptic plasticity and memory hypothesis. In particular, morphological changes of putative learning-recruited CA1 synapses have been reported to last only three days after contextual fear conditioning (Matsuo et al., 2008). Enhancement of the post-synaptic response induced during passive avoidance learning could only be observed for a few hours (Whitlock et al.,

2006). Encouragingly, changes in CA1 synaptic function have been observed up to seven days after contextual fear conditioning, although the memory could be expressed for at least a month (Sacchetti et al., 2001). In addition to the technical challenge, the lack of correspondence between the persistence of synaptic plasticity changes and the persistence of memory may also constitute an important conceptual challenge for the synaptic plasticity and memory hypothesis. It is largely assumed by the community that synaptic changes should persist with memory; however a demonstration of this is lacking.

Here we report the use of a robust experimental system to investigate memory associated functional changes in hippocampus CA1 synaptic inputs that mediate spatial information. Our findings demonstrate that long-term traces of a spatial experience can be detected as persistent modifications in the function of the CA1 hippocampal circuitry lasting at least a month. Remarkably, these changes in synaptic function coincided with the persistence of long-term place memories; the changes were only observed in animals that expressed robust memory, and not in animals with poor memory recall. Notably, these changes were detected in the extracellular synaptic potentials recorded from the CA1 region of *ex vivo* slices, indicating a widespread change in the function of the CA1 synaptic network with memory. We speculate that widespread synaptic circuit changes at the level of hippocampal microcircuits include the embedding of explicit memory information at a particular set of synapses within a broader synaptic network that contains related information to which the newly acquired memory is associated.

MATERIALS AND METHODS

Behavior

All procedures were performed in compliance with the Institutional Animal Care and Use Committee of the State University of New York, Downstate Medical Center and New York University. C57BL/6 male mice (3–4 months old) were trained in a hippocampus-dependent two-frame active place avoidance task. The place avoidance system consisted of a 40-cm diameter arena with a parallel rod floor that could rotate at 1 rpm. The position of the animal was tracked using PC-based software (Tracker, Bio-Signal Group Corp., Brooklyn, NY) that analyzed 30-Hz digital video images from an overhead camera. Mice in the trained condition learned the “Room+Arena−” task variant. Place avoidance of a 60° zone was reinforced by a constant current foot shock (60 Hz, 500ms, 0.2mA) that was scrambled (5-poles) across pairs of the floor rods. Rotation of the arena would carry the mouse into the shock zone unless the animal actively avoided the zone. Entering the shock zone for more than 500 ms triggered shock. Additional shocks occurred every 1.5 seconds until the animal left the shock zone. Measures of place avoidance were computed by TrackAnalysis software (Bio-Signal Group Corp., Brooklyn, NY). The behavioral protocol began with a 10-min session with shock off to habituate the mice to the rotating arena. Twelve training trials followed, with three trials occurring each day across a period of four days. Within a day, the mice were returned to the home cage for 40-min between trials. Except for the shock being always off, the conditions were identical for pretraining and retention sessions, as well as for the control mice (untrained mice). The conditions were identical for the yoked group of mice except that these mice received the same time series of shocks as a mouse from the trained group.

The times that shocks were delivered to the trained mice over the 4-day training period were recorded and used to deliver shocks to the yoked group of mice (yoked-group) who could not avoid or otherwise control the delivery of shock. Typically, the number of shocks trained mice received rapidly decreased over the training period due to place avoidance learning. On Day 1, mice experienced the bulk of shocks during the first trial (20–25 shocks, most of them during the first 5 min); then the number of shocks reduces in half during the second trial of Day 1, to only a few shocks in the third and last trial of Day 1. As learning progressed from Day 2 to Day 4, trained mice received very few to no shocks. For yoked-conditioning, we replayed the precise timing of the shock sequence from a trained mouse so that the yoked mouse received the identical time order of shocks. Hence, the yoked group of mice is exposed to the rotating arena and receives shocks just like the trained mice, but the shocks are uncorrelated to a specific location and cannot be avoided.

Long-term and remote memory was tested either 24 h or 30 days after the final training session with the shock off, respectively.

Electrophysiology

All procedures were performed in compliance with the Institutional Animal Care and Use Committee of the State University of New York, Downstate Medical Center. One or thirty days after the last training day, mice were tested for retention memory and then returned to their home-cage for 20 min (some mice were not memory tested but they were handled at the same times as the mice that had the memory retention test). Then, mice were transferred into an induction (anesthetizing) chamber and kept there to habituate to the chamber for 10 minutes. Next, mice were deeply anesthetized with vaporized Isoflurane (5% in 100% oxygen) for 3 min, and immediately euthanized by rapid decapitation. Transverse slices (400 μm) from the right dorsal hippocampus were obtained for *ex vivo* electrophysiology experiments. Slices were cut (Neurolab tissue chopper) in ice cold artificial cerebrospinal fluid (ACSF containing: (mM) 119 NaCl, 4.0 KCl, 1.5 MgSO4, 2.5 CaCl2, 26.2 NaHCO3, 1 NaH2PO4 and 11 Glucose saturated with 95% O2, 5% CO2) and then warmed in oxygenated ACSF to 35°C for 45 min. Slices were thereafter allowed to equilibrate for at least 60 min in oxygenated ACSF at room temperature. For experiments, slices were immersed in a submerged recording chamber subfused with oxygenated ACSF at 35–36°C. Field excitatory postsynaptic potentials (fEPSP) from the Stratum Radiatum (SR, CA3-CA1 input) and the Stratum Lacunosum Moleculare (SLM, EC-CA1 input) and population spikes (pop-Spikes) from the Stratum Pyramidale of the CA1 area were obtained via stimulation with bipolar electrodes (FHC & Co, ME, USA), the stimulus pulse duration was 50 μs and was kept the same for all electrophysiological studies, and recording with borosilicate glass pipettes (5–10 MΩ) (Sutter Inst. Co., Novato, CA. USA) filled with ACSF solution. Two slices from the same animal were placed and simultaneously stimulated/recorded within the same recording chamber. To examine the functional changes associated with active place avoidance memory, we characterized 1) synaptic transmission, 2) paired-pulse facilitation (PPF), 3) synaptic plasticity (potentiation and depression), and 4) pop-Spike generation. The electrophysiologist was blind to the behavioral conditions.

1. Synaptic transmission: Stimulus-response relationships between input voltage stimulation and fEPSP slope amplitude were generated at increasing voltage

stimulations at both CA3-CA1 and EC-CA1 inputs. To examine differences between the curves from the distinct groups of mice, we identified the fEPSP slope value that we estimate to represent most accurately the rising phase of the sigmoidal (or exponential) nature of curve. This measure is the most appropriate to examine differences between groups of mice compared to other measures that may be confounded by the nature of our examination in population of synapses. In addition, repeated measures two-way ANOVA analyses validated this examination (Supplementary Table).

2. Paired-pulse facilitation (PPF): For PPF, we examined the magnitude of the second post-synaptic response relative to the first post-synaptic response observed for a pair of stimuli at the 50, 100 and 200 ms time intervals. For the PPF analysis, we particularly focus on examining the differences between groups of mice at the 50 ms time interval. We chose this time interval because post-synaptic responses at 50 ms are strongly correlated with presynaptic release probability, rather than other features of the synaptic response (Schulz et al., 1995). The lower the facilitation of the second response, the higher is the estimated release probability of the tested synaptic population (Schulz et al., 1995). Additionally, repeated measures two-way ANOVA analyses validated this examination and found no differences between groups at 100 or 200 ms intervals (Supplementary Table).

3. Synaptic plasticity: For synaptic potentiation and depression experiments the test pulse intensity was set at approximately 40% of the maximum fEPSP slope (given by the stimulus-response curves). Baseline test sampling was 0.017 Hz. Synaptic potentiation was induced by high frequency stimulation (HFS) consisting of a single 1-s 100 Hz train. Synaptic depression was induced by one 15-min train of paired-pulse (50 ms) low frequency stimulation (PP-1Hz). To report changes in the expression of synaptic potentiation or depression, we selected the time period of 30–45 min post stimulus to describe the late expression of each form of synaptic plasticity. For some studies, analyses at shorter time periods (10–15 min, early expression) were also carried out. Also, we carried out examination of the induction envelopes for potentiation (HFS) and depression (PP-LFS) -inducing stimulation.

4. Population spikes (pop-Spikes): Pop-spikes were recorded in the *stratum pyramidale* of the CA1 neurons in response to Schaffer collateral stimulation (CA3-CA1 input). One stimulation and one recording electrode were placed at the *stratum radiatum* of CA1 area (CA3-CA1 input). A second recording electrode was placed at the *stratum pyramidale*, just superior to the location of the first recording electrode (as it optimizes the possibility of following the dendritic projections from the recorded synapses to their cell bodies). This configuration allowed simultaneous recording of post-synaptic responses (fEPSP) and population spikes (pop-Spike) elicited by increasing the input voltage stimuli to Schaffer-collateral afferents to CA1 synapses. Hence, for a given input voltage there will be two functional responses, the fEPSP and the pop-Spike. A fEPSP slope to pop-Spike amplitude relationship was generated by

matching the averaged fEPSP slope and pop-Spike amplitude values linked by the same input voltage stimulation. To generate the fEPSP slope to pop-Spike probability relationship (shown in Figure 5A), we first surveyed each single data-set at each given input voltage stimulation (hence, at each fEPSP slope amplitude), and defined for each unique data set whether or not a pop-Spike was observed (we considered pop-Spikes events larger than 0.5 mV –twice above noise– and that occurred after the onset of the fEPSP). With this information, we calculated the fraction of observed pop-Spikes across data-sets for a given fEPSP slope value.

Drugs

To examine the role of fast inhibitory drive, in some experiments we bath applied the $GABA_A$ receptor blocker picrotoxin (100 μM; Sigma, USA) to the slices.

Data acquisition

Electrophysiological data were acquired with PClamp software (Molecular Devices, Sunnyvale, CA. USA). Data analysis was performed with Origin 8.0 software (Microcal Software Inc., Northampton, MA. USA).

Statistics

The Microcal Origin Statistical tool was used to determine statistical significance between groups using two-tailed Student's t tests, one-way or two-way ANOVAs as appropriate. The data in the figures are averages ± standard errors of the mean. To validate our analyses we also carried out repeated measures two-way ANOVA analyzes for all of our main findings from stimulus-response relationships and PPF data sets (Supplementary Table). The repeated measures two-way ANOVA analysis validates our findings.

RESULTS

Changes in CA1 synaptic function detected one day after active place avoidance training

We trained mice in a hippocampus- and LTP maintenance-dependent active place avoidance task (Cimadevilla et al., 2001; Pastalkova et al., 2006; Serrano et al., 2008). To induce a long-term spatial memory, mice were given spatial memory training to avoid a shock zone for four consecutive days (trained mice). During pretraining with the shock off, all mice moved throughout the arena. Control mice (henceforth untrained mice) were exposed to the same environment for an identical amount of time but were not shocked. Whereas untrained mice continued this exploratory behavior throughout the subsequent sessions, the trained mice rapidly learned to avoid the shock zone (Figure 1A,B). Twenty-four hours after the last training or control session, mice were euthanized and slices from the dorsal portion of the right hippocampus were prepared to investigate how the memory training and control experiences modified the Schaffer collateral/commissural-Stratum Radiatum (CA3-CA1) and temporoammonic-Stratum-Lacunosum Moleculare (Entorhinal Cortex EC-CA1) synaptic pathways. At the CA3-CA1 input, a day after training, compared to the untrained group, the trained mice showed increased synaptic transmission of the evoked field excitatory postsynaptic (fEPSP) response (Figure 1C), enhanced presynaptic function

measured by paired-pulse facilitation of the fEPSP response (Figure 1D) and decreased synaptic potentiation of the fEPSP response induced by 100-Hz high frequency stimulation (HFS) (Figure 1E). Similar trends were observed at the EC-CA1 input, although they did not reach statistical significance (Figure 1F–H); which may indicate that memory training has a weaker impact at the EC-CA1 input relative to the CA3-CA1 input. However, we cannot exclude the possibility that putative memory associated changes at this input may be difficult to unveil because the EC-CA1 population responses are relatively small.

Altogether, these observations suggest that long-term place avoidance memory is associated with functional changes that persist at least a day in a population of CA1 synapses.

Experience-induced CA1 synaptic changes parallel one-day memory retention

We then examined whether these experience-dependent changes in synaptic function paralleled the expression of long-term memory. Mice received the same control exposure and memory training as described above. But in this case, twenty-four hours after the final training session memory retention was tested with the shock off (Figure 2A). Half an hour after the end of the memory retention test, slices from each group of mice were prepared. Measurements of synaptic function at the CA3-CA1 input revealed essentially the same pattern of changes that were observed when retention was not tested 24 h after training (Figure 2B–D and compare with Figure 1C–E). Similar to experiments without the retention test, at the EC-CA1 input, synaptic transmission and synaptic potentiation maintained the same trend between the trained and untrained groups after the retention test (Supplemental Figure 1A,C). A statistically significant change in presynaptic function was also observed between the trained and untrained groups (Supplemental Figure 1B).

The observation of similar modifications with or without returning to the training environment and expression of memory recall a day after training indicates that the observed changes in CA1 synaptic function reflect memory storage rather than the retrieval experience, itself.

Changed CA1 synaptic function associates with place memory and not with the foot-shock experience alone

Place avoidance training recruits a number of structures and systems that altogether contribute to the formation and consolidation of a memory trace (Cimadevilla et al., 2001; Vafaei et al., 2007; Serrano et al., 2008; Wesierska et al., 2009; Park et al., 2015). In order to identify the contribution of the aversive foot-shock to the observed changes in CA1 synapses, we investigated the relationship between CA3-CA1 synaptic changes and the number of shocks during training. We found that the changes in synaptic function were not related to the number of shocks during training (Figure 3A–C). Similarly, changes in CA3-CA1 synaptic transmission and synaptic potentiation were also not observed in mice that received unavoidable “yoked” shocks that were delivered in the same time sequences that individual mice received in the course of memory training (Figure 3D–G). Because the mice in the trained and yoked groups received the virtually identical physical experience of the training environment, this demonstrates that neither foot shock itself, nor the physical

experience of training is sufficient to cause either the conditioned behavior or the changes in synaptic function that are observed after place avoidance training.

CA1 synaptic changes persist for at least one month and parallel memory retention

Our prior observations suggest that the training-related changes in CA1 synaptic function are associated with memory processes. Next, we investigated whether the changes in synaptic function persisted beyond a day, consistent with the persistence of remote spatial long-term memory. Place avoidance memory depends on hippocampus LTP for at least a month (Pastalkova et al., 2006). Mice were given either memory or control training and then retention with the shock off was tested 30 days after the final training session (Figure 4A). The trained mice avoided entering the shock zone on the retention test, but memory performance was bimodally distributed within the group (Figure 4A, bottom left; ratio of the two peaks R = 0.95, Kurtosis = −2.1). Based on the time to first entrance, we categorized trained mice into two subgroups: those with poor memory retention (gray, <300 s) and those with robust memory retention (red, >300 s) (Figure 4A, bottom right). The animals with poor memory retention had CA3-CA1 synaptic function measurements that were indistinguishable from the untrained mice (Figure 4B–D; gray color data sets). In contrast, the mice with good memory retention showed enhanced synaptic transmission and decreased potentiation of the CA3-CA1 input, similar to the trained group 1-day after memory training (Figure 4B,D; red color data sets), in which memory retention was uniformly strong. We did not detect changed presynaptic function at the CA3-CA1 input after 1 month (Figure 4C). While a trend in synaptic function change was seen one day after training at the EC-CA1 input, no distinguishable trend was observed between the trained and untrained groups one month after training (Supplemental Figure 2A–C). These findings indicate that persistence of memory is associated with persistent functional modifications of the hippocampus CA1 synaptic circuitry.

Modified population spike properties after place avoidance memory formation

Our last series of experiments were focused on characterizing the properties of the memory-associated changes in CA1 synaptic function one day after memory training. First, we characterized the relationship between the enhanced synaptic transmission at the CA3-CA1 input and the generation of population spikes of CA1 pyramidal neurons one day after memory training. We found that the trained group showed a greater reliability of inducing a CA1 population spike driven by CA3-CA1 synaptic responses compared to the untrained group (Figure 5A), as well as a lowered threshold for synaptic responses to generate a population spike (Figure 5B). This enhancement, however, did not translate into significant differences between the groups in the maximum amplitude or average number of population spikes elicited by CA3-CA1 synaptic responses (Figure 5C), or in the frequency-driven (10–100 Hz) modulation of population spike generation (Figure 5D). Thus, memory training modified hippocampus CA1 output, making CA3-CA1 synapses more reliable in activating populations of neurons without significantly affecting population spike amplitude, an estimate of population size, or affecting frequency-driven population excitability. This is consistent with the idea that memory formation affects the likelihood of neural responses by selectively modifying the coupling between synaptic strength and spike formation without changing overall neuronal excitability (Buzsaki et al., 2002). Such changes may increase the

reliability and coordination of action potentials within neural ensembles to signal information during memory tasks (Kelemen and Fenton, 2010; Singer et al., 2010; Dragoi and Tonegawa, 2011; Kelemen and Fenton, 2013).

Bidirectional rescaling of synaptic plasticity in the CA3-CA1 synaptic input after place avoidance memory formation

To determine the effect of place avoidance memory formation on the direction of change in synaptic plasticity we extended our investigation to the analysis of synaptic depression. The memory-associated changes in CA1 synaptic function were examined one day after memory training. The reduced expression of synaptic potentiation was accompanied by enhanced expression of synaptic depression (Figure 6A), indicating a metaplastic shift in the CA3-CA1 synapse after memory training. This type of metaplastic shift was also observed in the neocortex after motor skill learning (Rioult-Pedotti et al., 2000) or sensory stimulation (Clem et al., 2008). This change was not accompanied by saturation of the potentiated state (Figure 6B). Therefore, the impact of memory storage on plastic synaptic function was not to enhance plasticity in all its forms, but to conserve the dynamic range for synaptic plasticity. In addition, there was no change observed in the depolarization envelope during synaptic plasticity (potentiation or depression) induction (Figure 6C). These observations suggest that long-term memory storage actively modulates the effective range of synaptic weights to frame incoming synaptic activity and regulate the subsequent processing of information (Pavlowsky and Alarcon, 2012; Park et al., 2015).

Role of fast inhibition in the expression of memory-associated synaptic changes

Finally, we explored the potential contribution of $GABA_A$ receptor mediated fast inhibition in the expression of the place memory associated CA1 changes as our electrophysiological recordings were done in GABA receptor blocker free medium. Bath application of the $GABA_A$ receptor blocker picrotoxin during slice recordings prevented detection of the training-associated changes in CA3-CA1 synaptic transmission and synaptic potentiation (Figure 7A–C) but not in CA3-CA1 PPF (Figure 7B), which was still observable. Similarly, $GABA_A$ receptor inhibition drastically reduced the trends observed in synaptic function change at the EC-CA1 input (Figure 7D–F).

These data suggest that inhibition may contribute to the memory training-induced changes of synaptic function, a possibility that highlights the functional significance of inhibitory circuits in the maintenance of learned behaviors (Ruediger et al., 2011; McKay et al., 2013).

DISCUSSION

Detecting a memory trace in hippocampus synapses has long been a subject of intense investigation because it is a key prediction of the synaptic plasticity and memory hypothesis. The present findings demonstrate persistent functional modification of a subset of synaptic inputs in the hippocampus circuit for the expression of long-term memory of at least a month; evidence that the maintenance of the memory coincides with persistent changes of synaptic function.

Synaptic function changes associated with long-term and remote memory

We examined three estimates of synaptic function, 1) strength of synaptic transmission, 2) paired-pulse facilitation and 3) synaptic plasticity expression, to test for changes associated with long-term retention of an active place avoidance memory. Each of these estimates of synaptic function has been extensively correlated with changes in synaptic plasticity mechanisms thought to underlie memory (Malenka and Bear, 2004; Mayford, 2014; Takeuchi et al., 2014). Increased synaptic transmission of CA1 synapses is observed after spatial learning (Sacchetti et al., 2001; Whitlock et al., 2006). The change is believed to involve post-synaptic modifications ranging from incorporation of new ionotropic receptors into the learning-activated so-called “tagged” synapses (Carroll RC, 2001; Esteban JA, 2003; Matsuo et al., 2008) to persistent modifications of the synaptic machinery (Sacktor, 2011; Hsieh et al., 2016; Tsokas et al., 2016) hypothesized to be maintained via activation of translation and gene expression mechanisms (Kandel, 2001; Schuman et al., 2006; Alberini and Kandel, 2015). The observation of a persistent functional increase in synaptic transmission with place avoidance memory is in agreement with the mechanistic changes expected to be associated with learning and memory processes.

There is ample evidence that presynaptic function is key for the induction of synaptic plasticity and the expression of memory (Dumas et al., 2004; Ferguson et al., 2004; Lisman and Raghavachari, 2006; Bailey et al., 2008; Lin et al., 2010). This presynaptic function is usually measured using paired-pulse facilitation analyses that report on the efficacy of probability of presynaptic vesicle release (Schulz et al., 1995). In contrast, changes of paired-pulse facilitation after learning have been less explored. Decreased paired-pulse facilitation has been observed in neurons of the lateral nucleus of amygdala after fear conditioning (Lin et al., 2010), while no changes are reported after spatial learning in the hippocampus (Sacchetti et al., 2001). Our data show changed paired-pulse facilitation one day after acquisition of active place avoidance memory, but the change did not persist after one month; suggesting that presynaptic function is not an a modification that endures with the persistence of spatial memory. Interestingly, changed paired-pulse facilitation was seen at both CA3-CA1 and EC-CA1 inputs in mice that underwent the retention memory test, while no changes in synaptic transmission or synaptic potentiation were detected at the EC-CA1 input. Although the change was not observed after recall of the 30-day active place avoidance memory, these data suggest a potential connection between activation of presynaptic mechanisms and recall; a connection that has been described in amygdala after fear conditioning (Takeda et al., 2010). Similarly, circuits other than the CA3-CA1 component might rely on presynaptic changes to support memory (O’Reilly and McClelland, 1994; Lisman J, 2006).

Changes in the expression of artificially-induced synaptic plasticity have been observed after learning (Rioult-Pedotti et al., 2000; Whitlock et al., 2006; Clem et al., 2008). In these studies, expression of synaptic potentiation was shown to decrease compared to untrained controls, after inhibitory avoidance learning (Whitlock et al., 2006), motor learning (Rioult-Pedotti et al., 2000) or sensory stimulation (Clem et al., 2008). In the present study, we also observed decreased synaptic potentiation after active place avoidance training, which endured with the persistence of remote memory. One interpretation of the decreased synaptic

potentiation is that the learning experience recruits synaptic mechanisms that occlude the subsequent induction of synaptic potentiation. After inhibitory avoidance, new bouts of induction stimuli elicited no higher synaptic potentiation, thus confirming the saturated or occluded potentiation state (Whitlock et al., 2006). The reduced synaptic potentiation may also function to constrain excessive excitation, but in the context of the synaptic plasticity and memory hypothesis, this also implies that learning would not be further improved beyond the point of the synaptic potentiation. Interestingly, we observed that higher levels of potentiation could be induced with additional bouts of induction stimuli. This indicates that the decreased synaptic potentiation after active place avoidance was not accompanied by saturation of the potentiated state. One explanation for this finding may lie on our observation that decreased synaptic potentiation was accompanied by enhanced synaptic depression. This type of metaplastic shift was also observed in the neocortex after motor skill learning (Rioult-Pedotti et al., 2000) and sensory stimulation (Clem et al., 2008). It has been proposed that subsequent synaptic potentiation could be achieved by recruiting mechanisms other than those that were used for the initial learning experience (e.g. NMDA-R activation. Candidate mechanisms for the subsequent potentiation include activation of metabotropic glutamate receptors, suggesting that a distinct mechanism may govern how continued experience can result in increasing synaptic strength over time (Clem et al., 2008). The activation of metabotropic glutamate receptors induces synaptic depression (Etkin et al., 2006) and, notably, the type of synaptic depression examined in our study is dependent on metabotropic glutamate receptor activation (Nicholls et al., 2008). We also failed to observe the synaptic function changes associated with active place avoidance memory when $GABA_A$-receptor activation was blocked. Perhaps $GABA_A$-mediated inhibition is a mechanism that keeps the level of synaptic potentiation in check, possibly by enhancing synaptic depression. Synaptic depression that depends on NMDA receptor activation appears to be sensitive to $GABA_A$-receptor inhibition (Malleret et al., 2010), but this does not seem to be the case for metabotropic glutamate receptor dependent synaptic depression (Rohde et al., 2009).

Finally, we did not observe changes that suggest there was an experience-dependent modulation of synaptic plasticity induction mechanisms (potentiation or depression). This implies that mechanisms downstream of the initial NMDA-receptor activation phase of synaptic plasticity could be the cause of the memory-associated changes in plastic function.

The manifold of findings indicate that long-term memory storage is not passive; rather, it appears to actively condition the effective range of synaptic weights to frame subsequent incoming synaptic activity and regulate the subsequent processing of information (Pavlowsky and Alarcon, 2012; Bannerman et al., 2014; Park et al., 2015).

Widespread circuit changes with long-term and remote memory

Studies of the impact of memory formation on neural circuits indicate that a limited set of neurons (~10–30%) would be recruited to store a memory (Han et al., 2009; Liu et al., 2012; Ziv et al., 2013). Similarly, just a small fraction of synapses (~15%) in these neurons would be modified by memory (Whitlock et al., 2006; Matsuo et al., 2008). One assumption from these studies is that memory-associated changes in synaptic function would be difficult to

detect at the level of populations of synapses, using the extracellular electrode recordings such as in the present study. Nonetheless, detection may be possible if the neuronal and synaptic changes occur in a widespread fashion across the neuron population. This view is consistent with evidence of large learning-associated changes in structural plasticity (Moser, 1999), widespread changes in immediate early gene activation (Tse et al., 2011) and the findings that behavioral training is sufficient to induce large and long-lasting modifications of neuropsychological variables (Lee et al., 2012; Subramaniam et al., 2012; Anguera et al., 2013; Hattori et al., 2014). The present manifold of findings suggest that when the internal representation of an experience is embedded within a neural circuit, the functioning of the circuit is substantially modified.

Inhibition and memory

It is widely accepted that modulation of both excitatory and inhibitory circuits is needed for proper encoding of information (Klausberger et al., 2003; Buzsaki, 2010; Kelemen and Fenton, 2013). Interestingly, investigations into the functional significance of inhibitory circuits in the maintenance of learned behaviors is now increasing (Lin et al., 2010; Ruediger et al., 2011; McKay et al., 2013); perhaps the relative inattention to inhibition is in part due to the fact that our central cellular model for memory mechanisms, long-term potentiation, is largely investigated on excitatory synapses. Here, we found that block of $GABA_A$ receptor-mediated fast inhibitory transmission impacts the expression of the active place avoidance memory-associated changes in synaptic transmission and synaptic potentiation but not paired-pulse facilitation. The absence of an effect at the level of paired-pulse facilitation indicates that $GABA_A$ receptor-mediated inhibition may act on postsynaptic elements and function more so than presynaptic mechanisms. One explanation of the present findings may be that inhibition tone is reduced after memory training, thus enhancing synaptic transmission, but perhaps having a lesser impact on artificially-induced synaptic potentiation and depression. $GABA_A$ receptor block would allow similar levels of excitatory synaptic drive in both trained and untrained groups, which would mask the memory-associated changes that were observed in synaptic transmission and synaptic potentiation. Alternatively, $GABA_A$ receptor mediated inhibition may be heightened after memory training (McKay et al., 2013) but it only becomes functionally effective when it is required to counterbalance exacerbated excitatory drive, as in the case of artificially induced synaptic potentiation. We cannot rule out, however, that $GABA_A$ receptor-mediated inhibition may also not play any role, and that $GABA_A$ receptor block would just carry excitatory synaptic drive to a ceiling level were outcomes of postsynaptic function in trained and untrained groups cannot be distinguished.

The present observations suggest that inhibition might play a role in shaping the active place avoidance memory training-induced changes of synaptic function, which has also been observed in vivo with persistence of active place avoidance memory (Park et al., 2015). Structural reorganization of excitatory and inhibitory synapses has been shown during expression of synaptic plasticity (Bourne and Harris, 2011). Inhibitory circuits may contribute to the reorganization of synaptic circuits with memory, however, our experiments cannot inform on the extent of the contribution or define in which manner, functionally and mechanistically, this reorganization may occur. Further research is required on how the

excitatory and inhibitory neural components are modulated in response to memory formation.

Distinct circuit requirements for different memory demands

In this study we have found changes in synaptic function that are associated with the acquisition of active place avoidance memory. These changes are not observed in untrained animals that were just exposed to the rotating arena with no shock, or yoked-shock mice that received shocks that were unavoidable and unrelated to a particular location within the arena. This pattern of findings suggest a specific connection between the observed synaptic changes and the active place avoidance memory, itself. These findings also invite speculation on how encoding of distinct kinds of experiences may demand different involvement of circuit function. Untrained, yoked-shock and active place avoidance conditioning are distinct types of experiences for the animals. In the untrained condition, mice habituate to the environment as it becomes more familiar. In the yoked-shock condition, one can speculate that the animals make a basic association between the behavioral and/or environmental context and shocks (Vafaei et al., 2007), although we have no behavioral evidence of this in the present study (but see (Jezek et al., 2002)). In the case of active place avoidance memory training, the mice form an association between shocks and a particular location. Do all three of these experiences shape circuit function? One answer would be ‘no,’ as the only persistent modulation of circuit function we could detect, followed active place avoidance training. Alternatively, these experiences may all change circuit function but the particular recruitment of the specific circuits would depend on the experience. A recent study in rat describes restricted changes in protein expression in exclusive hippocampal areas after familiarization (habituation) to a context (Cox et al., 2014). Context habituation may recruit a small subset of synaptic circuits to store information of the familiar environment, which may have escaped detection by our methods. This notion aligns well with the suggestion that acquisition of a more complex and demanding active place avoidance memory produces widespread changes in synaptic function. As others have suggested, the recruitment, extent and contribution of distinct neural circuits may depend on the need for management of resources, utilization of different learning strategies and memory demands (Gottlieb et al., 2012; Eichenbaum, 2014; Eichenbaum and Cohen, 2014; McKenzie et al., 2014; Tanaka et al., 2014; Jenkins and Ranganath, 2016; Keene et al., 2016).

Long-term memory engrams in the hippocampus

There is strong consensus in the field that memory traces undergo systems consolidation. Memory traces localized to hippocampus can also be represented in other brain areas; mainly neocortical (Czajkowski et al., 2014), and this may happen as early as during learning (Cowansage et al., 2014). However, this does not mean that upon systems consolidation hippocampal traces cease to exist. Hippocampal memory traces are generated by different experiences (Ramirez et al., 2013; Denny et al., 2014), which are embedded within distinct functional organizations of neural activity (McKenzie and Eichenbaum, 2012; McKenzie et al., 2013). Our present findings suggest that a memory trace may be maintained at least for a month within the hippocampus network. There is support for this notion in reports that describe the role of a specific protein kinase, PKMζ, which is hypothesized to be a central factor of the molecular mechanism that maintains long-term memory

(Pastalkova et al., 2006; Sacktor, 2011; Tsokas et al., 2016). PKMζ is persistently upregulated for at least a month after active place avoidance training (Hsieh et al., 2016), and 1-month old memory is erased after infusion of the selective kinase inhibitor ZIP into the hippocampus one month after active place avoidance training (Pastalkova et al., 2006). Together, these studies support the view that hippocampal function continues to be necessary for memory maintenance for at least a month, and hence is evidence for a hippocampal engram that is necessary for remote memory recall. While hippocampus independent recall can occur, reactivation of hippocampal memory traces may be necessary for proper or accurate memory retrieval (Tanaka et al., 2014).

Memory associated modulation of circuit function and cognitive theory

We speculate that widespread synaptic circuit changes at the level of hippocampal microcircuits include the embedding of explicit memory information at a particular set of synapses within a broader synaptic network that contains related information to which the newly acquired memory is associated. This is not a novel notion, cognitive psychologists have taught for over 50 years that memories form within the framework of the subject's knowledge base or schema (Piaget, 1926; Bartlett, 1932). Memories are rapidly acquired when the explicit information from experience is embedded within the subject's schema (Morris, 2006; Tse et al., 2007; McKenzie and Eichenbaum, 2011; Tse et al., 2011; Dragoi and Tonegawa, 2013; McKenzie et al., 2013; McKenzie et al., 2014). A neurobiological basis of such schema may include experience-associated widespread changes in immediate early gene activation within a neuron population (Tse et al., 2007; Tse et al., 2011) and the modification of neural firing patterns of hippocampal networks (McKenzie et al., 2013; McKenzie et al., 2014).

A widespread impact of memory on synaptic circuit function may reflect not only the storage of the explicit information, but also the establishing of a neural infrastructure for managing, regulating and coordinating knowledge of experiences that is necessary for the memory expression. Memories have been asserted to rely on the concepts of a schema, neural attractor, and a Bayesian prior (Bartlett, 1932; Huttenlocher et al., 2000; Wills et al., 2005; McNaughton et al., 2006; Buzsaki, 2010; Dragoi and Tonegawa, 2013; McKenzie et al., 2013). While the precise neural and synaptic organization of memory remains to be elucidated, the present findings suggest that these concepts may all share the same mechanistic basis, the widespread and persistent adjustments of the network of synapse functions that are engaged by the learning experience.

Concluding Remarks

This study is part of a research effort aimed at identifying changes within different hippocampus synaptic circuits with memory and how these memory circuits change with different memory experiences. We highlight the importance of identifying these memory-associated synaptic circuits to better define how memory associated neural ensemble activity originate from particular synaptic activity.

Our findings demonstrate that long-term traces of a spatial experience can be detected as persistent modifications in the function of the CA1 hippocampal circuitry lasting at least a

month. These changes in synaptic function coincided with the persistence of long-term place memories (the changes were only observed in animals that expressed robust memory and not in animals with poor memory recall). We hope to expand this research to the whole hippocampus network. Advances towards this goal have been achieved by identifying spatial memory associated synaptic changes in the Dentate Gyrus area (Park et al., 2015).

We suggest that widespread synaptic circuit changes at the level of hippocampal microcircuits include the embedding of explicit memory information at a particular set of synapses within a broader synaptic network that contains the framework of the subject's knowledge. This is consistent with the idea that memory formation changes neural ensembles to properly encode and coordinate information (Kelemen and Fenton, 2010; Singer et al., 2010; Kelemen and Fenton, 2013; Korte and Schmitz, 2016).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

Abraham WC. Metaplasticity: tuning synapses and networks for plasticity. Nature reviews Neuroscience. 2008; 9:387. [PubMed: 18401345]

Alberini CM, Kandel ER. The regulation of transcription in memory consolidation. Cold Spring Harbor perspectives in biology. 2015; 7:a021741.

Anguera JA, Boccanfuso J, Rintoul JL, Al-Hashimi O, Faraji F, Janowich J, Kong E, Larraburo Y, Rolle C, Johnston E, Gazzaley A. Video game training enhances cognitive control in older adults. Nature. 2013; 501:97–101. [PubMed: 24005416]

Bailey CP, Nicholls RE, Zhang XL, Zhou ZY, Muller W, Kandel ER, Stanton PK. Galpha(i2) inhibition of adenylate cyclase regulates presynaptic activity and unmasks cGMP-dependent long-term depression at Schaffer collateral-CA1 hippocampal synapses. Learn Mem. 2008; 15:261–270. [PubMed: 18391187]

Bannerman DM, Sprengel R, Sanderson DJ, McHugh SB, Rawlins JN, Monyer H, Seeburg PH. Hippocampal synaptic plasticity, spatial memory and anxiety. Nature reviews Neuroscience. 2014; 15:181–192. [PubMed: 24552786]

Barth AL, Poulet JF. Experimental evidence for sparse firing in the neocortex. Trends in neurosciences. 2012; 35:345–355. [PubMed: 22579264]

Bartlett, FC. Remembering: A Study in Experimental and Social Psychology. Cambridge: Cambridge University Press; 1932.

Bourne JN, Harris KM. Coordination of size and number of excitatory and inhibitory synapses results in a balanced structural plasticity along mature hippocampal CA1 dendrites during LTP. Hippocampus. 2011; 21:354–373. [PubMed: 20101601]

Buzsaki G. Neural syntax: cell assemblies, synapsembles, and readers. Neuron. 2010; 68:362–385. [PubMed: 21040841]

Buzsaki G, Csicsvari J, Dragoi G, Harris K, Henze D, Hirase H. Homeostatic maintenance of neuronal excitability by burst discharges in vivo. Cerebral cortex. 2002; 12:893–899. [PubMed: 12183388]

Carroll RCECB, von Zastrow Mark, Malenka Robert C. Role of AMPA Receptor Endocytosis in Synaptic Plasticity. Nature Reviews Neuroscience. 2001

Cimadevilla JM, Fenton AA, Bures J. New spatial cognition tests for mice: passive place avoidance on stable and active place avoidance on rotating arenas. Brain research bulletin. 2001; 54:559–563. [PubMed: 11397548]

Clem RL, Celikel T, Barth AL. Ongoing in vivo experience triggers synaptic metaplasticity in the neocortex. Science. 2008; 319:101–104. [PubMed: 18174444]

Cowansage KK, Shuman T, Dillingham BC, Chang A, Golshani P, Mayford M. Direct Reactivation of a Coherent Neocortical Memory of Context. Neuron. 2014

Czajkowski R, Jayaprakash B, Wiltgen B, Rogerson T, Guzman-Karlsson MC, Barth AL, Trachtenberg JT, Silva AJ. Encoding and storage of spatial information in the retrosplenial cortex. Proc Natl Acad Sci U S A. 2014; 111:8661–8666. [PubMed: 24912150]

Denny CA, Kheirbek MA, Alba EL, Tanaka KF, Brachman RA, Laughman KB, Tomm NK, Turi GF, Losonczy A, Hen R. Hippocampal memory traces are differentially modulated by experience, time, and adult neurogenesis. Neuron. 2014; 83:189–201. [PubMed: 24991962]

Dragoi G, Tonegawa S. Preplay of future place cell sequences by hippocampal cellular assemblies. Nature. 2011; 469:397–401. [PubMed: 21179088]

Dragoi G, Tonegawa S. Development of schemas revealed by prior experience and NMDA receptor knock-out. Elife. 2013; 2:e01326. [PubMed: 24327561]

Dumas TC, Powers EC, Tarapore PE, Sapolsky RM. Overexpression of calbindin D(28k) in dentate gyrus granule cells alters mossy fiber presynaptic function and impairs hippocampal-dependent memory. Hippocampus. 2004; 14:701–709. [PubMed: 15318329]

Eichenbaum H. Time cells in the hippocampus: a new dimension for mapping memories. Nature reviews Neuroscience. 2014; 15:732–744. [PubMed: 25269553]

Eichenbaum H, Cohen NJ. Can we reconcile the declarative memory and spatial navigation views on hippocampal function? Neuron. 2014; 83:764–770. [PubMed: 25144874]

Esteban, JAea, Roberto, Malinow. PKA phosphorykation of AMPA receptor subunits controls synaptic trafficking underlying plasticity Nature. 2003

Etkin A, Alarcon JM, Weisberg SP, Touzani K, Huang YY, Nordheim A, Kandel ER. A role in learning for SRF: deletion in the adult forebrain disrupts LTD and the formation of an immediate memory of a novel context. Neuron. 2006; 50:127–143. [PubMed: 16600861]

Ferguson GD, Wang H, Herschman HR, Storm DR. Altered hippocampal short-term plasticity and associative memory in synaptotagmin IV (−/−) mice. Hippocampus. 2004; 14:964–974. [PubMed: 15390175]

Frick A, Magee J, Johnston D. LTP is accompanied by an enhanced local excitability of pyramidal neuron dendrites. Nat Neurosci. 2004; 7:126–135. [PubMed: 14730307]

Garner AR, Rowland DC, Hwang SY, Baumgaertel K, Roth BL, Kentros C, Mayford M. Generation of a Synthetic Memory Trace. Science. 2012; 335:1513–1516. [PubMed: 22442487]

Gottlieb LJ, Wong J, de Chastelaine M, Rugg MD. Neural correlates of the encoding of multimodal contextual features. Learn Mem. 2012; 19:605–614. [PubMed: 23166292]

Green EJ, McNaughton BL, Barnes CA. Exploration-dependent modulation of evoked responses in fascia dentata: dissociation of motor, EEG, and sensory factors and evidence for a synaptic efficacy change. The Journal of neuroscience : the official journal of the Society for Neuroscience. 1990; 10:1455–1471. [PubMed: 1970602]

Gruart A, Munoz MD, Delgado-Garcia JM. Involvement of the CA3-CA1 synapse in the acquisition of associative learning in behaving mice. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2006; 26:1077–1087. [PubMed: 16436593]

Han JH, Kushner SA, Yiu AP, Hsiang HL, Buch T, Waisman A, Bontempi B, Neve RL, Frankland PW, Josselyn SA. Selective erasure of a fear memory. Science. 2009; 323:1492–1496. [PubMed: 19286560]

Hattori S, Yoon T, Disterhoft JF, Weiss C. Functional reorganization of a prefrontal cortical network mediating consolidation of trace eyeblink conditioning. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2014; 34:1432–1445. [PubMed: 24453332]

Hsieh C, Tsokas P, Serrano P, Hernandez I, Tian D, Cottrell JE, Shouval HZ, Fenton AA, Sacktor TC. Persistent increased PKMzeta in long-term and remote spatial memory. Neurobiology of learning and memory. 2016

Huttenlocher J, Hedges LV, Vevea JL. Why do categories affect stimulus judgment? J Exp Psychol Gen. 2000; 129:220–241. [PubMed: 10868335]

Jenkins LJ, Ranganath C. Distinct neural mechanisms for remembering when an event occurred. Hippocampus. 2016

Jezek K, Wesierska M, Fenton AA. Hippocampus-dependent retrieval and hippocampus-independent extinction of place avoidance navigation, and stress-induced out-of-context activation of a memory revealed by reversible lesion experiments in rats. Physiol Res. 2002; 51(Suppl 1):S35–S47. [PubMed: 12479785]

Kandel ER. The molecular biology of memory storage: a dialog between genes and synapses. Biosci Rep. 2001; 21:565–611. [PubMed: 12168768]

Keene CS, Bladon J, McKenzie S, Liu CD, O'Keefe J, Eichenbaum H. Complementary Functional Organization of Neuronal Activity Patterns in the Perirhinal, Lateral Entorhinal, and Medial Entorhinal Cortices. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2016; 36:3660–3675. [PubMed: 27030753]

Kelemen E, Fenton A. Dynamic grouping of hippocampal neural activity during cognitive control of two spatial frames. PLoS Biology. 2010; 8:e1000403. [PubMed: 20585373]

Kelemen E, Fenton AA. Key features of human episodic recollection in the cross-episode retrieval of rat hippocampus representations of space. PLoS Biol. 2013; 11:e1001607. [PubMed: 23874154]

Kirkwood A, Rioult MC, Bear MF. Experience-dependent modification of synaptic plasticity in visual cortex. Nature. 1996; 381:526–528. [PubMed: 8632826]

Klausberger T, Magill PJ, Marton LF, Roberts JD, Cobden PM, Buzsaki G, Somogyi P. Brain-state- and cell-type-specific firing of hippocampal interneurons in vivo. Nature. 2003; 421:844–848. [PubMed: 12594513]

Korte M, Schmitz D. Cellular and System Biology of Memory: Timing, Molecules, and Beyond. Physiol Rev. 2016; 96:647–693. [PubMed: 26960344]

Kozorovitskiy Y, Gross CG, Kopil C, Battaglia L, McBreen M, Stranahan AM, Gould E. Experience induces structural and biochemical changes in the adult primate brain. Proc Natl Acad Sci U S A. 2005; 102:17478–17482. [PubMed: 16299105]

Lee D, Lin BJ, Lee AK. Hippocampal place fields emerge upon single-cell manipulation of excitability during behavior. Science. 2012; 337:849–853. [PubMed: 22904011]

Lin HC, Mao SC, Su CL, Gean PW. Alterations of excitatory transmission in the lateral amygdala during expression and extinction of fear memory. Int J Neuropsychopharmacol. 2010; 13:335–345. [PubMed: 19775504]

Lisman J, Raghavachari S. A unified model of the presynaptic and postsynaptic changes during LTP at CA1 synapses. Sci STKE. 2006; 2006:re11. [PubMed: 17033044]

Lisman, JaSR. A Unified Model of the Presynaptic and Post-synaptic Changes During LTP at CA1 Synapses. Science STKE. 2006

Liu X, Ramirez S, Pang PT, Puryear CB, Govindarajan A, Deisseroth K, Tonegawa S. Optogenetic stimulation of a hippocampal engram activates fear memory recall. Nature. 2012; 484:381–385. [PubMed: 22441246]

Makara JK, Losonczy A, Wen Q, Magee JC. Experience-dependent compartmentalized dendritic plasticity in rat hippocampal CA1 pyramidal neurons. Nat Neurosci. 2009; 12:1485–1487. [PubMed: 19898470]

Malenka RC, Bear MF. LTP and LTD: an embarrassment of riches. Neuron. 2004; 44:5–21. [PubMed: 15450156]

Malleret G, Alarcon JM, Martel G, Takizawa S, Vronskaya S, Yin D, Chen IZ, Kandel ER, Shumyatsky GP. Bidirectional regulation of hippocampal long-term synaptic plasticity and its

influence on opposing forms of memory. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2010; 30:3813–3825. [PubMed: 20220016]

Martin SJ, Morris RG. New life in an old idea: the synaptic plasticity and memory hypothesis revisited. Hippocampus. 2002; 12:609–636. [PubMed: 12440577]

Matsuo N, Reijmers L, Mayford M. Spine-Type-Specific Recruitment of Newly Synthesized AMPA Receptors with Learning. Science. 2008; 319:1104–1107. [PubMed: 18292343]

Mayford M. The search for a hippocampal engram. Philosophical transactions of the Royal Society of London Series B, Biological sciences. 2014; 369:20130161. [PubMed: 24298162]

McKay BM, Oh MM, Disterhoft JF. Learning increases intrinsic excitability of hippocampal interneurons. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2013; 33:5499–5506. [PubMed: 23536065]

McKenzie S, Eichenbaum H. Consolidation and reconsolidation: two lives of memories? Neuron. 2011; 71:224–233. [PubMed: 21791282]

McKenzie S, Eichenbaum H. New approach illuminates how memory systems switch. Trends Cogn Sci. 2012; 16:102–103. [PubMed: 22138232]

McKenzie S, Robinson NT, Herrera L, Churchill JC, Eichenbaum H. Learning causes reorganization of neuronal firing patterns to represent related experiences within a hippocampal schema. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2013; 33:10243–10256. [PubMed: 23785140]

McKenzie S, Frank AJ, Kinsky NR, Porter B, Riviere PD, Eichenbaum H. Hippocampal Representation of Related and Opposing Memories Develop within Distinct, Hierarchically Organized Neural Schemas. Neuron. 2014

McNaughton BL, Battaglia FP, Jensen O, Moser EI, Moser MB. Path integration and the neural basis of the 'cognitive map'. Nature reviews Neuroscience. 2006; 7:663–678. [PubMed: 16858394]

Micheau J, Riedel G, Roloff E, Inglis J, Morris RG. Reversible hippocampal inactivation partially dissociates how and where to search in the water maze. Behavioral neuroscience. 2004; 118:1022–1032. [PubMed: 15506884]

Morris RG. Elements of a neurobiological theory of hippocampal function: the role of synaptic plasticity, synaptic tagging and schemas. The European journal of neuroscience. 2006; 23:2829–2846. [PubMed: 16819972]

Moser E, Mathiesen I, Andersen P. Association between brain temperature and dentate field potentials in exploring and swimming rats. Science. 1993; 259:1324–1326. [PubMed: 8446900]

Moser MB. Making more synapses: a way to store information? Cellular and molecular life sciences : CMLS. 1999; 55:593–600. [PubMed: 10357229]

Neves G, Cooke SF, Bliss TV. Synaptic plasticity, memory and the hippocampus: a neural network approach to causality. Nature reviews Neuroscience. 2008; 9:65–75. [PubMed: 18094707]

Nicholls RE, Alarcon JM, Malleret G, Carroll RC, Grody M, Vronskaya S, Kandel ER. Transgenic mice lacking NMDAR-dependent LTD exhibit deficits in behavioral flexibility. Neuron. 2008; 58:104–117. [PubMed: 18400167]

O'Reilly RC, McClelland JL. Hippocampal conjunctive encoding, storage, and recall: avoiding a trade-off. Hippocampus. 1994; 4:661–682. [PubMed: 7704110]

Oh MM, Kuo AG, Wu WW, Sametsky EA, Disterhoft JF. Watermaze learning enhances excitability of CA1 pyramidal neurons. J Neurophysiol. 2003; 90:2171–2179. [PubMed: 12815013]

Park EH, Burghardt NS, Dvorak D, Hen R, Fenton AA. Experience-Dependent Regulation of Dentate Gyrus Excitability by Adult-Born Granule Cells. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2015; 35:11656–11666. [PubMed: 26290242]

Pastalkova E, Serrano P, Pinkhasova D, Wallace E, Fenton AA, Sacktor TC. Storage of Spatial Information by the Maintenance Mechanism of LTP. Science. 2006; 313:1141–1144. [PubMed: 16931766]

Pavlowsky A, Alarcon J. Interaction between long-term potentiation and depression in CA1 synapses: temporal constrains, functional compartmentalization and protein synthesis. PLoS ONE. 2012; 7:e29865. [PubMed: 22272255]

Piaget, J. The language and thought of the child. New York: Harcourt Brace; 1926.

Ramirez S, Tonegawa S, Liu X. Identification and optogenetic manipulation of memory engrams in the hippocampus. Frontiers in behavioral neuroscience. 2013; 7:226. [PubMed: 24478647]

Rioult-Pedotti MS, Friedman D, Donoghue JP. Learning-induced LTP in neocortex. Science (New York, NY). 2000; 290:533–536.

Rohde M, Tokay T, Kohling R, Kirschstein T. GABA(A) receptor inhibition does not affect mGluR-dependent LTD at hippocampal Schaffer collateral-CA1 synapses. Neurosci Lett. 2009; 467:20–25. [PubMed: 19800390]

Ruediger S, Vittori C, Bednarek E, Genoud C, Strata P, Sacchetti B, Caroni P. Learning-related feedforward inhibitory connectivity growth required for memory precision. Nature. 2011; 473:514–518. [PubMed: 21532590]

Sacchetti B, Lorenzini CA, Baldi E, Bucherelli C, Roberto M, Tassoni G, Brunelli M. Long-lasting hippocampal potentiation and contextual memory consolidation. The European journal of neuroscience. 2001; 13:2291–2298. [PubMed: 11454033]

Sacktor TC. How does PKMzeta maintain long-term memory? Nature reviews Neuroscience. 2011; 12:9–15. [PubMed: 21119699]

Schulz PE, Cook EP, Johnston D. Using paired-pulse facilitation to probe the mechanisms for long-term potentiation (LTP). Journal of physiology, Paris. 1995; 89:3–9.

Schuman EM, Dynes JL, Steward O. Synaptic regulation of translation of dendritic mRNAs. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2006; 26:7143–7146. [PubMed: 16822969]

Serrano P, Friedman EL, Kenney J, Taubenfeld SM, Zimmerman JM, Hanna J, Alberini C, Kelley AE, Maren S, Rudy JW, Yin JC, Sacktor TC, Fenton AA. PKMzeta maintains spatial, instrumental, and classically conditioned long-term memories. PLoS Biol. 2008; 6:2698–2706. [PubMed: 19108606]

Singer AC, Karlsson MP, Nathe AR, Carr MF, Frank LM. Experience-dependent development of coordinated hippocampal spatial activity representing the similarity of related locations. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2010; 30:11586–11604. [PubMed: 20810880]

Subramaniam K, Luks TL, Fisher M, Simpson GV, Nagarajan S, Vinogradov S. Computerized cognitive training restores neural activity within the reality monitoring network in schizophrenia. Neuron. 2012; 73:842–853. [PubMed: 22365555]

Takeda A, Tamano H, Imano S, Oku N. Increases in extracellular zinc in the amygdala in acquisition and recall of fear experience and their roles in response to fear. Neuroscience. 2010; 168:715–722. [PubMed: 20403415]

Takeuchi T, Duszkiewicz AJ, Morris RG. The synaptic plasticity and memory hypothesis: encoding, storage and persistence. Philosophical transactions of the Royal Society of London Series B, Biological sciences. 2014; 369:20130288. [PubMed: 24298167]

Tanaka KZ, Pevzner A, Hamidi AB, Nakazawa Y, Graham J, Wiltgen BJ. Cortical Representations Are Reinstated by the Hippocampus during Memory Retrieval. Neuron. 2014

Tse D, Langston RF, Kakeyama M, Bethus I, Spooner PA, Wood ER, Witter MP, Morris RG. Schemas and memory consolidation. Science. 2007; 316:76–82. [PubMed: 17412951]

Tse D, Takeuchi T, Kakeyama M, Kajii Y, Okuno H, Tohyama C, Bito H, Morris RG. Schema-dependent gene activation and memory encoding in neocortex. Science. 2011; 333:891–895. [PubMed: 21737703]

Tsokas P, Hsieh C, Yao Y, Lesburgueres E, Wallace EJ, Tcherepanov A, Jothianandan D, Hartley BR, Pan L, Rivard B, Farese RV, Sajan MP, Bergold PJ, Hernandez AI, Cottrell JE, Shouval HZ, Fenton AA, Sacktor TC. Compensation for PKMzeta in long-term potentiation and spatial long-term memory in mutant mice. Elife. 2016; 5

Turrigiano GG, Nelson SB. Hebb and homeostasis in neuronal plasticity. Current opinion in neurobiology. 2000; 10:358–364. [PubMed: 10851171]

Vafaei AA, Jezek K, Bures J, Fenton AA, Rashidy-Pour A. Post-training reversible inactivation of the rat's basolateral amygdala interferes with hippocampus-dependent place avoidance memory in a time-dependent manner. Neurobiology of learning and memory. 2007; 88:87–93. [PubMed: 17408988]

Wen JA, DeBlois MC, Barth AL. Initiation, labile, and stabilization phases of experience-dependent plasticity at neocortical synapses. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2013; 33:8483–8493. [PubMed: 23658185]

Wesierska M, Adamska I, Malinowska M. Retrosplenial cortex lesion affected segregation of spatial information in place avoidance task in the rat. Neurobiology of learning and memory. 2009; 91:41–49. [PubMed: 18845265]

Whitlock JR, Heynen AJ, Shuler MG, Bear MF. Learning induces long-term potentiation in the hippocampus. Science (New York, NY). 2006; 313:1093–1097.

Wills TJ, Lever C, Cacucci F, Burgess N, O'Keefe J. Attractor dynamics in the hippocampal representation of the local environment. Science. 2005; 308:873–876. [PubMed: 15879220]

Ziv Y, Burns LD, Cocker ED, Hamel EO, Ghosh KK, Kitch LJ, El Gamal A, Schnitzer MJ. Long-term dynamics of CA1 hippocampal place codes. Nat Neurosci. 2013; 16:264–266. [PubMed: 23396101]

Highlights

- Memory is thought to be supported by reorganization of synaptic circuits.
- Demonstration of persistent synaptic changes accompanying memory has been elusive.
- We report changes in synaptic function coincided with the persistence of memory.
- Widespread synaptic microcircuit changes may support memory.

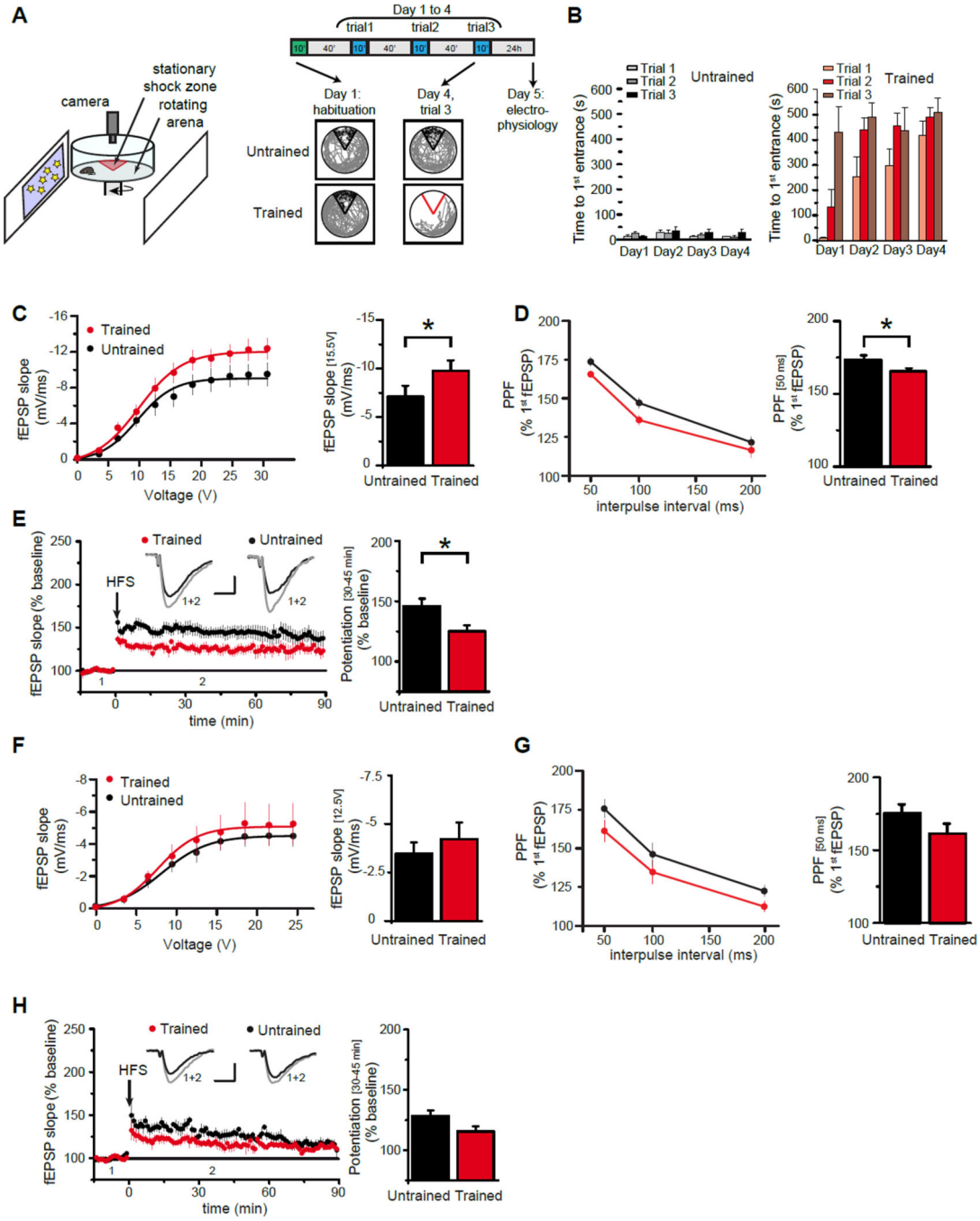

Figure 1.

Active place avoidance training modifies CA1 synapses. (**A**) The behavioral apparatus (left). Training paradigm (top right). A mouse on a continuously rotating disk-shaped arena was trained to avoid an unmarked, stationary 60° shock zone. Overhead view of an example mouse's tracked positions during pretraining and training trial 3 on day 4 (bottom right). Black lines indicate the shock zone when shock was off, red when it was on. Black dots indicate the mouse's location when shock would have been delivered within the inactive shock zone and red dots indicate the location when shock was delivered. (**B**) Formation of

place avoidance memory assessed by the time to first enter the shock zone (time to 1st entrance). Untrained mice (left, n=7) do not form an avoidance memory, whereas the trained mice (right, n=9) form a strong memory (two-way ANOVA, group: untrained vs. trained, $F_{1,23}$=125.6, P<0.001; training session: $F_{11,23}$=5.2, P<0.001; interaction: $F_{11,24}$=3.2, P <0.001). (**C–E**) Memory formation changes synaptic function at the CA3-CA1 input. (**C**) Amplitude of field excitatory postsynaptic potential (fEPSP) slope responses were larger in the trained (red, here and henceforth) vs. the untrained (black, here and henceforth) mice (left, n=7 per group), indicating increased synaptic transmission after memory formation (right, t_{13}=4.48; p<0.05 for values elicited at 15.5 V stimulation). (**D**) Paired pulse facilitation (PPF) relationships (left; untrained, n=6; trained, n=8) measured at different inter-pulse intervals (50, 100 and 200 ms). The significantly lower PPF values in the trained group indicate enhanced presynaptic function (right, t_{13}=2.13; p<0.05 for values elicited at 50 ms inter-pulse interval). (**E**) Synaptic potentiation elicited by 1 s 100 Hz stimulation (HFS) in trained and untrained mice (left, n=7 per group). Average potentiation amplitude for the 30–45 min period is decreased in trained compared to untrained mice (right, t_{13}=2.64; p<0.05). (**F–H**) Memory formation did not significantly change synaptic responses at the EC-CA1 input. (**F**) Synaptic transmission was similar in trained and untrained groups (left, n=5 per group). The average values elicited at 12.5 V stimulation were indistinguishable (right, t_9=0.98; p>0.05). (**G**) PPF is slightly reduced in trained (n=6) compared to untrained (n=4) groups (left). Comparison of the average values elicited at 50 ms inter-pulse interval reveal that the trend is not significant (right, t_9=1.05; p>0.05). (**H**) Synaptic potentiation shows a trend towards decrease in trained compared to untrained mice (left, n=5 per group). Comparison of the average potentiation amplitude values for the 30–45 min period reveal that the difference was not significant (right, , t_9=2.05; p>0.05). Representative fEPSP traces are shown, 1: baseline (black), 2: after potentiation (gray), scale bar: 2 mV/5ms. Graphs show averages ± SEM; *p<0.05. In these and all the following experiments simultaneous CA3-CA1 and EC-CA1 recordings were made in in the same recording chamber, from two separate dorsal hippocampal slices from the same animal.

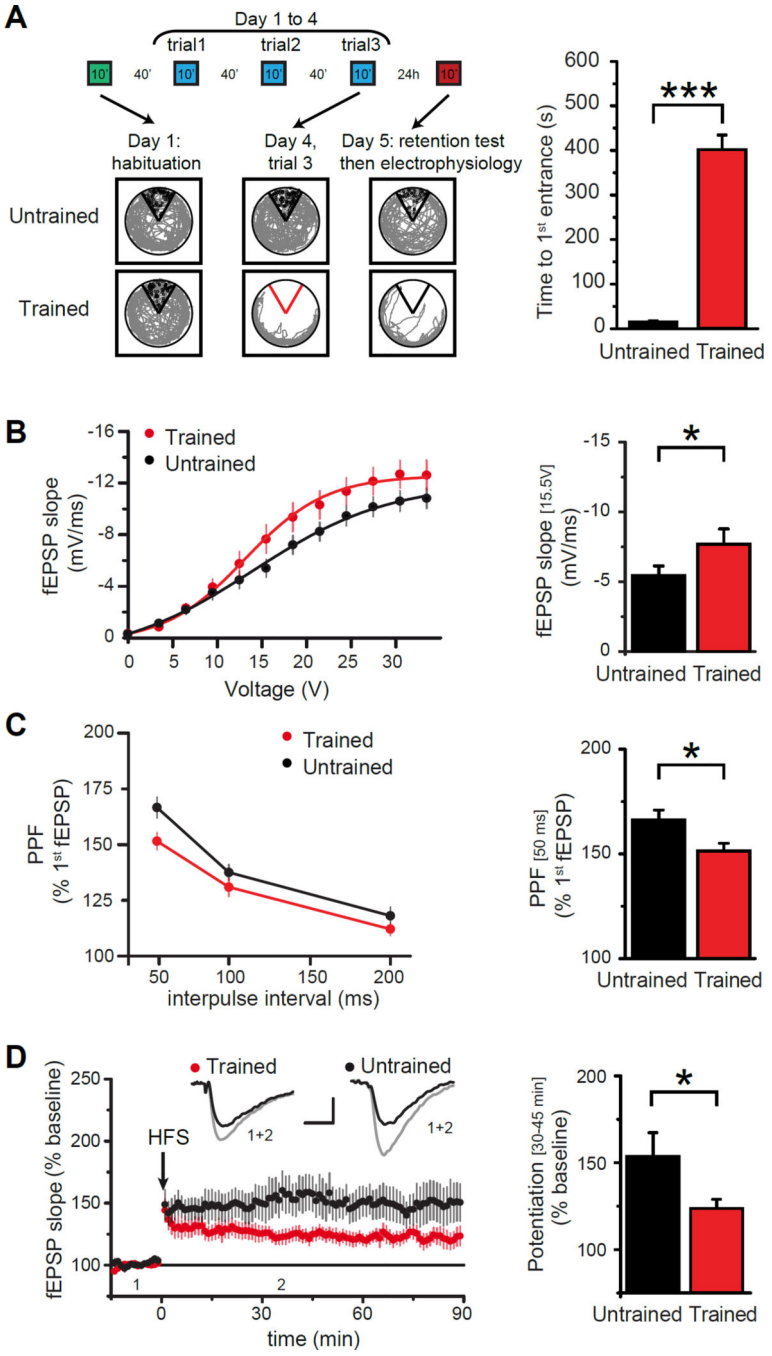

Figure 2.

Synaptic changes in the CA3-CA1 input parallel memory retention. (**A**) Training protocol (top). Trained mice avoided the shock zone (with shock off) one day after the last training session (left, position tracking examples), indicating robust memory retention (right, trained, n=30; trained, n=35; t_{63}=10.67; p<0.001). (**B**) Stimulus-response curves for trained (red) and untrained (black) mice (n=20 per group). Synaptic transmission is increased in trained (red) compared to untrained (black) mice (right, average responses to 15.5 V stimulation; t_{48}=2.18; p<0.05). (**C**) PPF curves for trained (red, n=12) and untrained (black, n=7) mice

(left). Enhanced presynaptic function in trained compared to untrained groups (right, average PPF values at 50 ms inter-stimulus-interval, t_{18}=2.46; p<0.05). (**D**) Synaptic potentiation in trained (red, n=13) and untrained (black, n=11) groups (left). Decreased synaptic potentiation in trained compared to untrained groups (right, average potentiation for the 30–45 min period, t_{23}=2.18; p<0.05). Representative fEPSP traces are shown, 1: baseline (black), 2: after potentiation (gray), scale bar: 2 mV/5ms. Graphs show averages ± SEM; *p<0.05.

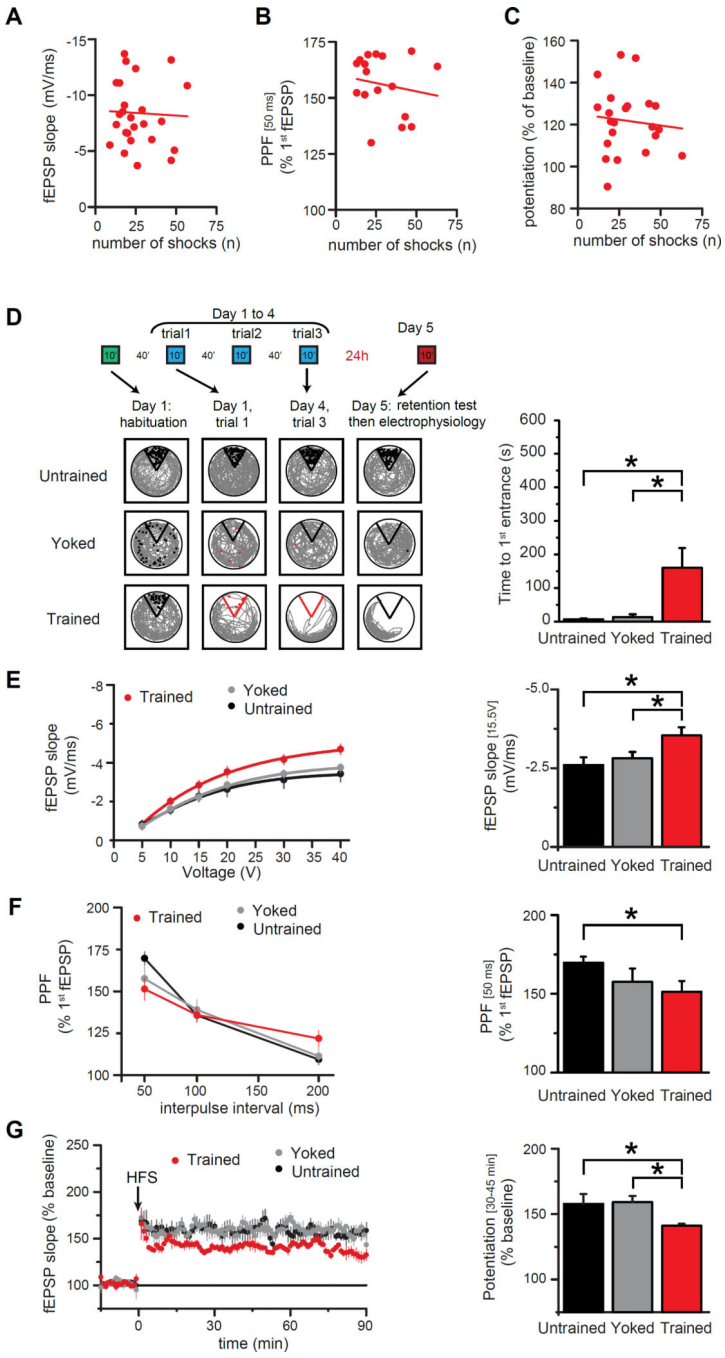

Figure 3.

During memory training, foot shock itself does not correlate with or induce changes in synaptic function. (**A**) No correlation between changes in synaptic transmission (measured at 15.5 V) and number of shocks received in the trained group (n=25, Pearson's r=0.05). (**B**) Neither are changes in PPF (measured at 50 ms inter-pulse interval) correlated with number of shocks (n=18, Pearson's r=−0.17). (**C**) Decreased synaptic potentiation (measured 30–45 min after induction) is also not associated with number of training shocks (n=22, Pearson's r=−0.1). (**D**) Unavoidable foot shock was given to a yoked-shock control group. The yoked

shocks had the same time series of shocks that a mouse received during memory training. Training protocol and example position tracking (left). Average time to 1st entrance on the retention test of untrained (black), yoked (gray) and trained (red) groups (right; $F_{2,10} = 7.6$; $p<0.01$; Tukey tests: $p<0.05$ between untrained or yoked and trained groups; n=5 per group). (**E–G**) The yoked-shock group of mice did not exhibit the changes in synaptic function associated with active place avoidance memory. (**E**) Stimulus-response curves for yoked (n=8 slices, 4 mice, gray), untrained (n=10 slices, 4 mice, black) and trained (n=10 slices, 4 mice, red) groups (left). Synaptic transmission was not different between the yoked and untrained groups but transmission was enhanced in the trained group (Average responses to 15.5 V stimulation, $F_{2,6} =4.31$; $p<0.05$ Tukey test: trained > untrained = yoked). (**F**) PPF curves for yoked (n=8 slices, 4 mice, gray), untrained (n=10 slices, 4 mice, black) and trained (n=10 slices, 4 mice, red) groups (left). Presynaptic function in trained, untrained, and yoked groups (average PPF values for the 50 ms inter-stimulus interval, $F_{2,3}=3.86$; $p>0.05$; Tukey tests: trained < untrained; trained = yoked; untrained = yoked). (**G**) Synaptic potentiation for yoked (n=8 slices, 4 mice, gray), untrained (n=10 slices, 4 mice, black) and trained (n=10 slices, 4 mice, red) groups (left). Synaptic potentiation was not different between yoked and untrained groups but was reduced in the trained group (right, average potentiation for the 30–45 min period ($F_{2,16}=24.1$; $p<0.005$; Tukey tests: trained < untrained < yoked). Graphs show averages ± SEM; *$p<0.05$.

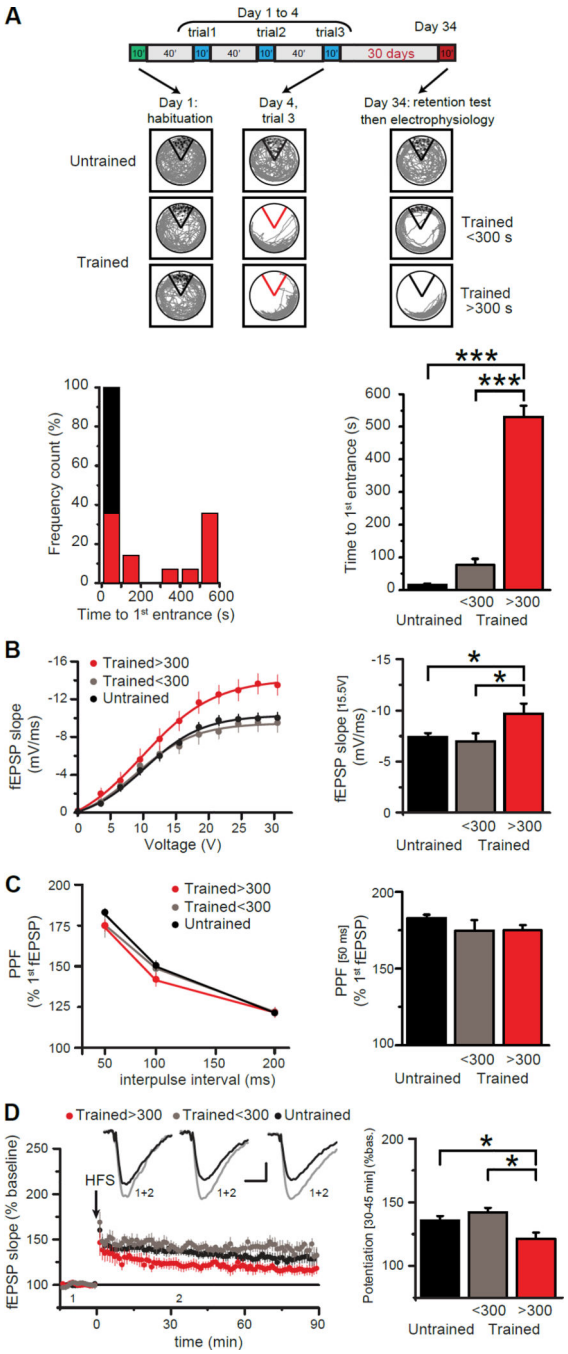

Figure 4.

Memory-associated synaptic changes in the CA3-CA1 input persist for at least one month and correlate with remote memory retention. (**A**) Training protocol and examples of position tracking (top). Distributions of 30-day memory retention scores of untrained (black) and trained (red) mice reveals bimodal memory retention in the trained group (bottom left). Average time to 1st entrance of untrained (black, n=14), and the poor (time to 1st entrance <300s, gray, n=7) and robust (time to 1st entrance >300s, red, n=7) retention subgroups (bottom right, $F_{2,25}$ = 226.5; p<0.001; Tukey tests: robust retention > poor retention =

untrained). (**B**) Stimulus-response curves for each group of mice (left, colors and numbers of subjects are described in A). Synaptic transmission was increased with remote memory. The changes in synaptic transmission were only observed in the robust retention group (right, group averages for responses to 15.5 V stimulation, $F_{2,25}$=3.9; p<0.05; Tukey tests: robust retention > poor retention = untrained). (**C**) PPF curves for each group of mice (left, colors and numbers of subjects are described in A). Unchanged presynaptic function during remote memory (right, PPF measures at 50 ms inter-stimulus interval $F_{2,25}$=1.66; p>0.05). (**D**) Synaptic potentiation for each group of mice (left, color and numbers of subjects are described in A). Synaptic potentiation was only decreased in the robust retention group (right, average potentiation for the 30–45 min period $F_{2,25}$=5.2; p<0.05; Tukey tests: robust retention < poor retention = untrained). Representative fEPSP traces are shown 1: baseline (black), 2: after potentiation (gray), scale bar: 2mV/5ms. Graphs show averages ± SEM; *p<0.05, ***p<0.001.

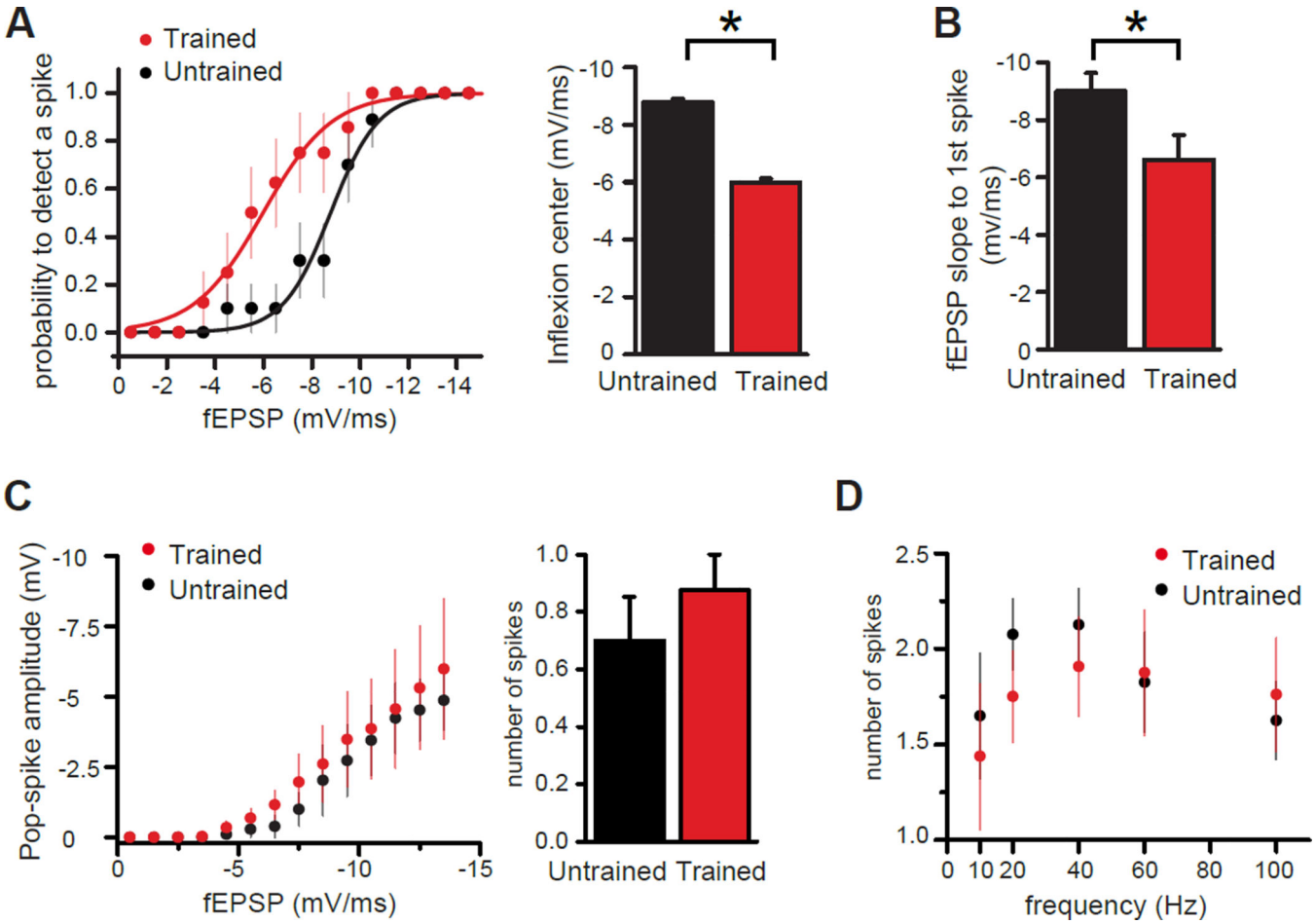

Figure 5.

Changes in CA1 spiking probability in the CA3-CA1 synaptic input after memory formation. (**A**) Recording in the *stratum pyramidale* (SP) of CA1 neurons in response to Schaffer collateral stimulation reveals increased likelihood of detecting population spike activity in the trained (n=10, red) group compared to the untrained (n=8, black) group (left), quantified by the trained group curve's lower inflexion point (right). (**B**) Lowered threshold to generate a population spike in the trained group (n=10, red) compared to the untrained group (n=8, black) (t_{17}=2.28; p<0.05). Graphs show averages ± SEM; *p<0.05. (**C**) The amplitude of fEPSP-induced CA1 population spikes was not different between the trained (n=10, red) and untrained (n=8, black) groups (left, population spike amplitude to fEPSP amplitude relationship; two-way ANOVA, Training: $F_{1,27}$=1.33, p>0.05; fEPSP: $F_{13,27}$=7.44, p<0.001; Interaction $F_{13,27}$=0.07, p>0.05). The average number of elicited CA1 population spikes was similar in trained (n=10, red) and untrained (n=8, black) groups. Average response to fEPSP slopes of 9.5mV/ms (right, t_{17}=0.86, p >0.05). (**D**) The frequency-induced number of CA1 population spikes was similar in trained (n=10, red) and untrained (n=8, black) groups (a train of four pulses was delivered at 10, 20, 40, 60 or 100 Hz and the total number of spikes per train was measured; two-way ANOVA, Training: $F_{1,9}$=0.45, p>0.05; frequency: $F_{4,9}$=0.96, p>0.05, Interaction $F_{4,9}$=0.27, p>0.05).

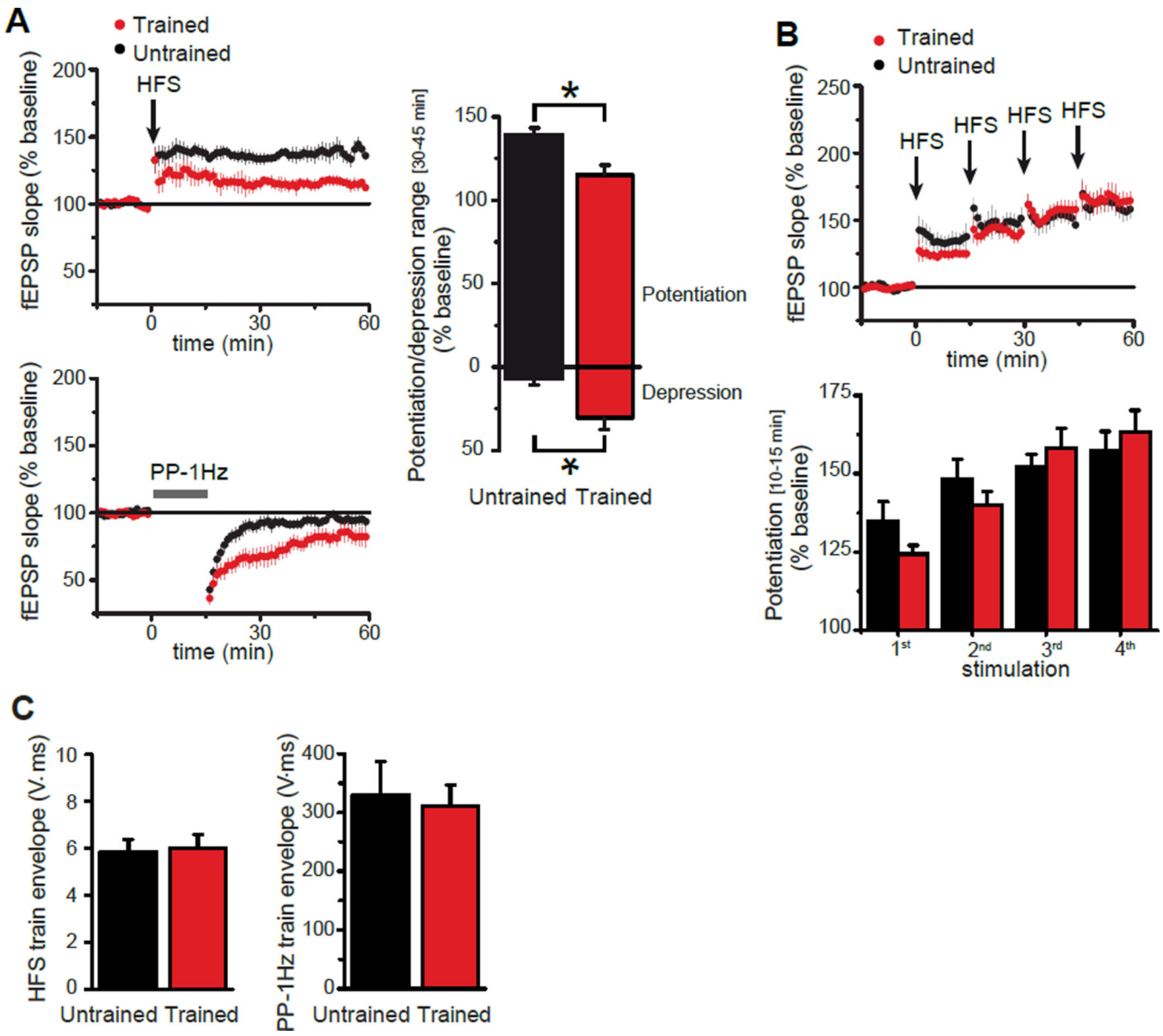

Figure 6.

: Memory training produces bidirectional rescaling of synaptic plasticity. (**A**) Bidirectional rescaling of synaptic function after memory formation. Trained mice (n=7, red) showed decreased synaptic potentiation (top left) and increased synaptic depression (bottom left) in the CA3-CA1 input compared to untrained (n=5, black) mice. Synaptic depression was induced by one 15-min train of paired-pulse (50 ms) low frequency stimulation (PP-1Hz). The plasticity range, measured as the potentiation-to-depression range for the 30–45 min time period, reveals a shift of synaptic weights towards depression in the trained group (right; potentiation: t_{13}=3.01, $p<0.05$; depression: t_{13}=4.38, $p<0.05$). In these experiments simultaneous synaptic potentiation and depression recordings were done in two slices from the same animal in the same recording chamber. (**B**) The memory-associated changes at CA3-CA1 synapses do not saturate the synaptic potentiation amplitude. Four consecutive

trains of 1-s 100 Hz stimulations applied at 15-min intervals elicited the same level of cumulative potentiation at CA3-CA1 synapses in trained (red) and untrained (black) groups (left, n=7 per group). Group averages for each 10–15 min period: t_{14}=1.64; $p_{1st\ stimulation}$=0.13; t_{14}=1.11 $p_{2nd\ stimulation}$=0.29; t_{14}=0.77 $p_{3rd\ stimulation}$=0.45; t_{14}=0.62 $p_{4th\ stimulation}$=0.55). Graphs show averages ± SEM; *p<0.05. (**C**) The 100-Hz tetanus envelope was similar in the trained (n=10, red) and untrained (n=8, black) groups (left; t_{17}=0.19, p>0.05). Similarly, the PP-1Hz train envelope was similar in the trained and untrained groups (right, n=7 per group, t_{13}=−0.29, p>0.05). Graphs show averages ± SEM.

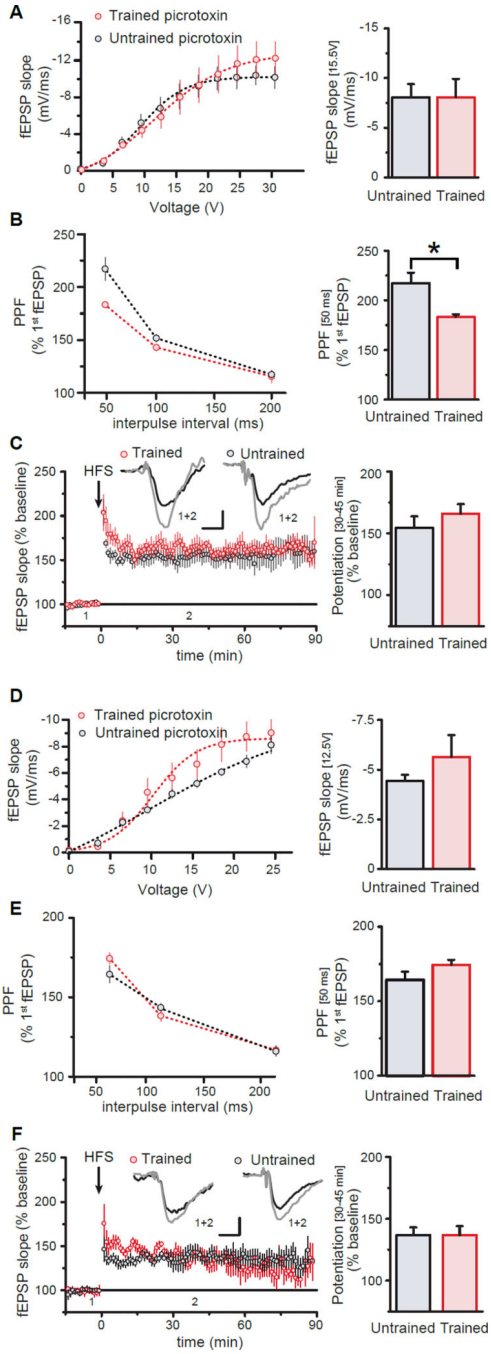

Figure 7.

Blockade of fast GABAergic inhibition by picrotoxin abolishes detection of the memory-associated changes in synaptic transmission and plasticity. **(A–C)** CA3-CA1 input: (**A**) Stimulus-response curves for trained (n=7, red) and untrained (n=6, black) groups in the presence of picrotoxin (left). Synaptic transmission is not different between the trained and untrained groups in the presence of picrotoxin (right, average responses to 15.5 V stimulation; t_{11}=0.001; p>0.05). (**B**) PPF curves for trained (red) and untrained (black) groups (left, n=6 per group) in the presence of picrotoxin. The change in presynaptic

function in the trained group is still detected under the picrotoxin block (right, average PPF values for the 50 ms inter-stimulus interval; t_{11}=3.42; p<0.05). (**C**) Synaptic potentiation for trained (red) and untrained (black) groups in the presence of picrotoxin (left, n=5 per group). Synaptic potentiation was not different between the trained and untrained groups in the presence of picrotoxin (right, average potentiation for the 30–45min period; t_9=0.95; p>0.05). (**D–F**) EC-CA1 input: (**D**) Stimulus-response curves for trained (red) and untrained (black) groups in the presence of picrotoxin (left, n=6 per group). No difference in synaptic transmission between groups (right, average responses to 12.5 V stimulation; t_{11}=2.12; p>0.05). (**E**) PPF curves for trained (red, n=6) and untrained (black, n=4) groups in the presence of picrotoxin (left). Presynaptic function was indistinguishable between trained and untrained groups (right, average PPF values at 50 ms inter-stimulus interval; t_9=0.71, p>0.05). **F**, Synaptic potentiation for trained and untrained groups in the presence of picrotoxin (left, n=6 per group). Synaptic potentiation was not different between the trained and untrained groups (right, average potentiation in the 30–45 min period; t_{11}=0.28; p>0.05). Graphs show averages ± SEM; *p<0.05.