J Neurophysiol 107: 3397–3408, 2012.
First published March 21, 2012; doi:10.1152/jn.00692.2011.

Trace fear conditioning enhances synaptic and intrinsic plasticity in rat hippocampus

Chenghui Song,[1] Julia A. Detert,[1] Megha Sehgal,[1] and James R. Moyer, Jr.[1,2]
[1]*Department of Psychology, University of Wisconsin-Milwaukee, Milwaukee, Wisconsin; and* [2]*Department of Biological Sciences, University of Wisconsin-Milwaukee, Milwaukee, Wisconsin*

Submitted 25 July 2011; accepted in final form 21 March 2012

Song C, Detert JA, Sehgal M, Moyer JR Jr. Trace fear conditioning enhances synaptic and intrinsic plasticity in rat hippocampus. *J Neurophysiol* 107: 3397–3408, 2012. First published March 21, 2012; doi:10.1152/jn.00692.2011.—Experience-dependent synaptic and intrinsic plasticity are thought to be important substrates for learning-related changes in behavior. The present study combined trace fear conditioning with both extracellular and intracellular hippocampal recordings to study learning-related synaptic and intrinsic plasticity. Rats received one session of trace fear conditioning, followed by a brief conditioned stimulus (CS) test the next day. To relate behavioral performance with measures of hippocampal CA1 physiology, brain slices were prepared within 1 h of the CS test. In trace-conditioned rats, both synaptic plasticity and intrinsic excitability were significantly correlated with behavior such that better learning corresponded with enhanced long-term potentiation (LTP; $r = 0.64$, $P < 0.05$) and a smaller postburst afterhyperpolarization (AHP; $r = -0.62$, $P < 0.05$). Such correlations were not observed in pseudo-conditioned rats, whose physiological data were comparable to those of poor learners and naive and chamber-exposed control rats. In addition, acquisition of trace fear conditioning did not enhance basal synaptic responses. Thus these data suggest that within the hippocampus both synaptic and intrinsic mechanisms are involved in the acquisition of trace fear conditioning.

learning and memory; long-term potentiation; excitability; afterhyperpolarization; spike-frequency adaptation; brain slice

PAVLOVIAN FEAR CONDITIONING paradigms are used extensively to study the neurobiology of learning and memory (see Doyere et al. 2007; Fanselow and Poulos 2005; LeDoux 2000; Moyer and Brown 2006). In these paradigms, a neutral conditioned stimulus (CS) is paired with an aversive unconditioned stimulus (US), which evokes an unconditioned response (UR). Paired CS-US presentations result in the generation of a learned or conditioned response (CR). Subtle variations in the temporal relationship between the CS and US can dramatically impact which brain structures are necessary for learning the CS-US relationship. For example, in the delay fear paradigm, the CS precedes and temporally overlaps with the US, and acquisition of delay fear conditioning requires intact amygdala and brain stem structures (for review see LeDoux 2000). However, in the trace paradigm, a stimulus-free trace interval is interposed between CS offset and US onset, which significantly changes the brain circuitry required for learning—in addition to the amygdala, medial temporal lobe and higher cortical structures are now required for learning (Gilmartin and Helmstetter 2010; Kholodar-Smith et al. 2008; McEchron et al. 1998; Quinn et al. 2002). Thus trace fear conditioning is an excellent paradigm for studying learning- and memory-related changes in higher-order brain regions, including hippocampus.

Bidirectional modification of synaptic efficiency through long-term potentiation (LTP) and long-term depression (LTD) is thought to underlie the cellular basis of certain types of learning and memory (Bear and Abraham 1996; Dudek and Bear 1993; Lynch 2004). For example, field potential recordings performed in brain slices from rabbits that learned trace eyeblink conditioning revealed a learning-specific, time-dependent enhancement of baseline synaptic responses in CA1 evoked by Schaffer collateral stimulation (e.g., Power et al. 1997). Interestingly, this enhancement of synaptic transmission was only observed in slices prepared 1 h (but not 24 h) after learning (Power et al. 1997). Likewise, field recordings from the hippocampus of freely moving animals suggest that acquisition of trace eyeblink (e.g., Gruart et al. 2006) or fear conditioning (e.g., Doyere et al. 1995) is accompanied by a facilitation of basal synaptic transmission. In each case these LTP-like changes could be observed within 1 h after learning, and suggest that an LTP-like change can occur during certain learning tasks but that this change is transient and not observed 24 h later. Interestingly, studies have also demonstrated that, after learning, LTP induction is facilitated (Barnes 1979; Boric et al. 2008; Gruart et al. 2006). One possible explanation for enhanced synaptic plasticity during learning involves alterations to the intrinsic excitability of hippocampal neurons. Numerous studies have demonstrated that learning enhances intrinsic neuronal excitability, as evidenced by a reduced postburst afterhyperpolarization (AHP) and a decrease in spike-frequency adaptation (Kaczorowski and Disterhoft 2009; McKay et al. 2009; Moyer et al. 1996; Oh et al. 2003; Saar et al. 1998). This enhancement of intrinsic neuronal excitability may contribute to memory consolidation (Moyer et al. 1996; Thompson et al. 1996), promote subsequent learning (Zelcer et al. 2006), and facilitate LTP induction (Kramar et al. 2004; Sah and Bekkers 1996). Conversely, failure to modulate intrinsic neuronal excitability is associated with slower acquisition of hippocampus-dependent learning tasks, such as trace eyeblink and contextual fear conditioning in adult animals, as well as learning deficits both in normal aging (Kaczorowski and Disterhoft 2009; Moyer et al. 2000) and in a mouse model of Alzheimer disease (Kaczorowski et al. 2011b).

To date, no studies have evaluated synaptic and intrinsic changes during or after trace fear conditioning. The present study combined trace fear conditioning with intracellular and extracellular recordings in hippocampus to investigate both intrinsic excitability and synaptic plasticity in rats as a function

Address for reprint requests and other correspondence: J. R. Moyer, Jr., Dept. of Psychology, PO Box 413, Univ. of Wisconsin-Milwaukee, Milwaukee, WI 53201 (e-mail: jrmoyer@uwm.edu).

of associative learning. The data suggest that acquisition of trace fear conditioning increases intrinsic excitability and facilitates LTP in hippocampus.

MATERIALS AND METHODS

Subjects

Subjects were 51 adult male F344 rats (4.1 ± 0.1 mo). Rats were maintained in an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)-accredited facility on a 14:10-h light-dark cycle and housed individually with free access to food and water. All rats were handled at least 1 wk prior to experiments. Procedures were conducted in accordance with protocols reviewed and approved by the University of Wisconsin-Milwaukee animal care and use committee (ACUC) and National Institutes of Health guidelines.

Apparatus

Fear conditioning chambers. Trace fear conditioning was conducted in a Plexiglas and stainless steel chamber (30.5 × 25.4 × 30.5 cm; Coulbourn Instruments, Whitehall, PA) located in a sound-attenuating box. The chamber had a standard grid floor consisting of 26 parallel steel rods (5-mm diameter and 6-mm spacing). The floor was connected to a precision adjustable shock generator (Coulbourn Instruments) for delivery of a scrambled foot shock US. Within the sound-attenuating box, a ventilation fan produced a constant background noise of ~58 dB (measured by a sound level meter, A scale; model no. 33-2050, Realistic, Fort Worth, TX). The chamber was illuminated by a miniature incandescent white lamp (28 V, type 1819) and was wiped with a 5% ammonium hydroxide solution prior to each training session. During training, the room lights were left on (illumination 20.9 lux) for the entire session.

CS testing chambers. An additional Plexiglas and stainless steel chamber served as a novel context for the auditory cue test. This chamber was located within a separate sound-attenuating box located in the same room. The test chamber was physically different from the training chamber in that it had a curved wall, the floor was black-painted Plexiglas (instead of grid bars), and it was illuminated with an infrared light. In addition, the tray below the test chamber floor contained clean bedding and the test chamber was wiped with 2% acetic acid prior to each test session to provide an olfactory stimulus different from that used during training. The room lights were turned off (illumination 0.2 lux) for the entire testing session.

Behavioral Training

Rats received one 10-trial session of auditory trace fear conditioning ($n = 15$) using a 15-s CS (80-dB white noise) followed by a 30-s trace interval (stimulus-free period) and a 1-s foot shock US (1 mA). A long (5.2 min ± 20%) intertrial interval was used to maximize CS and minimize context (i.e., training chamber) conditioning (Detert et al. 2008). Control rats were pseudoconditioned (explicitly unpaired CS and US presentations with no stimulus presented >2 consecutive times; $n = 11$), chamber exposed (placed in the training chamber for the same amount of time as conditioned rats without receiving any stimuli; $n = 4$), or experimentally naive (never exposed to the training or testing chambers; $n = 21$). To assess learning, the amount of time spent freezing during the baseline, the CS, and the 30-s trace interval following CS offset in conditioned and pseudoconditioned rats was measured (see analysis below). A PC running FreezeFrame 2.04 (Actimetrics Software, Coulbourn Instruments) controlled the delivery of all stimuli during training and testing.

Behavioral Testing

Twenty-four hours after training, trace-conditioned and pseudoconditioned rats received a brief CS test session in a novel context. After a 2-min baseline, trace-conditioned and pseudoconditioned rats received two 15-s CS presentations with a 2.9-min intertrial interval. Rats were removed 2 min after the second CS presentation. To assess memory, the amount of time spent freezing during the baseline, the first CS, and the first trace interval (defined as the first 30 s after CS offset) was measured (see analysis below). The chamber-exposed rats were placed in the same novel context for the same amount of time but without any CS presentations.

Analysis of Behavioral Data

A remote CCTV video camera (model no. WV-BP334; Panasonic, Suzhou, China), mounted to the top of each behavioral chamber, was used to record the activity of each rat during training and testing. The video data were fed to a PC running FreezeFrame 2.04. Data were analyzed with FreezeView 2.04 (Actimetrics Software, Coulbourn Instruments), and a 1-s bout of immobility was scored as freezing. Freezing was defined as the absence of all movement except that required for respiration (Blanchard and Blanchard 1969). Analyses of freezing during the probe test revealed that both conditioned and pseudoconditioned rats froze little during baseline [10.7 ± 3.8% and 6.9 ± 2.6%, respectively; $F(1,24) = 0.58$, $P = 0.46$] and during the first CS presentation [4.2 ± 2.0% and 1.1 ± 0.5%, respectively; $F(1,24) = 1.57$, $P = 0.22$] but froze much more during the trace interval [53.3 ± 7.2% and 36.0 ± 8.7%, respectively; $F(1,24) = 2.13$, $P = 0.16$]. To correlate learning ability with synaptic plasticity and intrinsic excitability, trace-conditioned rats were divided into good learners and poor learners according to behavioral performance during the CS test. Rats that froze more than 2 SD above the mean of chamber-exposed rats (9.7 + 33.3%) were defined as good learners ($n = 8$), and those below were defined as poor learners ($n = 7$). Throughout the study, freezing during the first CS-alone trial was presented because it best reflects the trace-conditioned fear memory as a result of conditioning trials on the previous day and as a function of a low level of baseline freezing without being confounded by prior CS presentation (Phillips and LeDoux 1992, 1994; Smith et al. 2007).

Slice Preparation

Brain slices were prepared within 1 h of the test session by an individual blind to training condition. Rats were deeply anesthetized with isoflurane and decapitated. The brain was quickly removed and placed in ice-cold oxygenated (95% O_2-5% CO_2) artificial cerebrospinal fluid (aCSF; composition in mM: 124 NaCl, 2.8 KCl, 1.25 NaH_2PO_4, 2 $MgSO_4$, 2 $CaCl_2$, 26 $NaHCO_3$, and 20 dextrose). The brain was then blocked, and horizontal brain slices (400 μm) were cut in aCSF at ~0°C with a temperature-controlled vibratome (model 3000, Vibratome, St. Louis, MO). The horizontal slices were located between −6.1 and −4.6 mm ventral to bregma, which corresponds to plates 104 through 110 of the rat stereotaxic atlas of Paxinos and Watson (1998). Anatomically, these slices are well within the dorsal hippocampus—defined as the septal two-thirds of the hippocampus (see Czerniawski et al. 2009; Moser and Moser 1998). Slices were then transferred to a holding chamber (Moyer and Brown 1998) containing oxygenated aCSF at room temperature (21–23°C). For experiments, slices were individually transferred as needed to an interface-type recording chamber (Warner Instrument, Hamden, CT), where they were perfused with oxygenated aCSF at 32°C for 1 h prior to starting an experiment.

Electrophysiological Recordings

All recordings were obtained with a MultiClamp 700B amplifier system (Molecular Devices, Union City, CA). Experiments were con-

trolled by pCLAMP 10 software (Molecular Devices) running on a PC, and the data were acquired with the Digidata 1440A acquisition system (Molecular Devices). All electrodes were pulled from thin-walled capillary glass (A-M Systems, Carlsborg, WA) with a Sutter Instruments P97 puller. For field potential recordings, voltage signals were filtered at 2 kHz and digitized at 50 kHz. For intracellular recordings, voltage signals were filtered at 0.5–2 kHz and digitized at 20 kHz. Figure 1*A* shows a photograph of a hippocampal slice with typical extracellular recording, intracellular recording, and stimulation sites noted.

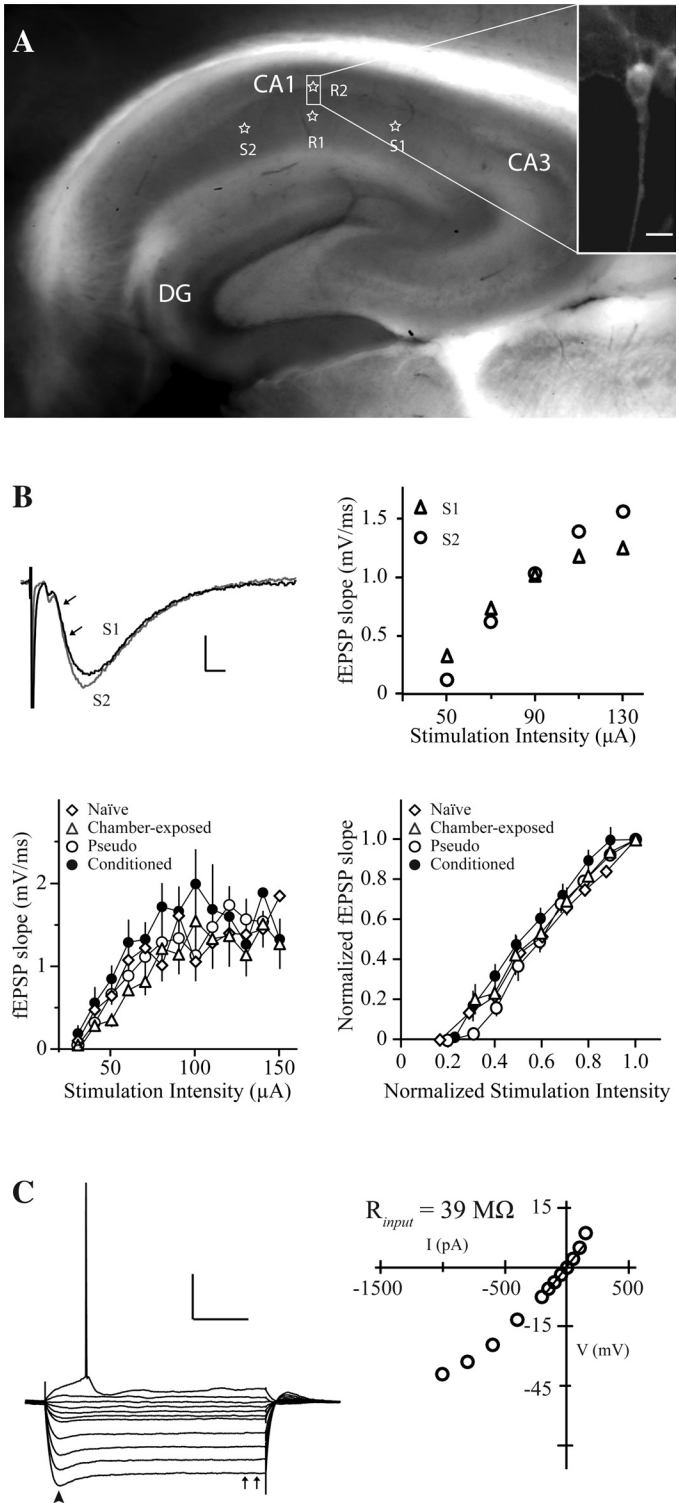

Fig. 1. Hippocampal slice preparation used to study learning-related changes in synaptic and intrinsic plasticity. *A*: photograph of hippocampal slice illustrating the location of stimulating electrodes for the test (S1) and control (S2) pathways and the location of recording electrodes for field (R1) and sharp intracellular (R2) recordings. *Inset*: representative photograph of a biocytin-filled pyramidal neuron. Scale bar, 5 μm. *B*: representative and averaged input-output (I-O) curves for extracellular field recordings. *Top left*: field excitatory postsynaptic potentials (fEPSPs) of test pathway (S1, black) and control pathway (S2, gray) were obtained by stimulating the Schaffer collaterals on either side of the recording electrode. The strength of synaptic transmission was measured as the initial slope indicated between the 2 arrows. Scale bar 0.5 mV, 2 ms. *Top right*: example of an I-O curve used to calculate the stimulation intensity required to generate a 50% maximal fEPSP slope. *Bottom left*: averaged I-O curves of the test pathway for all groups. *Bottom right*: normalized I-O curves of the test pathway for each group. *C*: voltage-current (*V-I*) relation used to calculate neuronal input resistance (R_{input}). Representative voltage responses to a series of current injections (scale bar 20 mV, 100 ms) and the accompanying *V-I* plot used to measure R_{input}. Arrowhead shows peak voltage deflection (used in measuring the depolarizing sag), and double arrows show steady-state voltage near the end of the current injection.

Synaptic Plasticity Studies

Dendritic field excitatory postsynaptic potentials (fEPSPs) were obtained from stratum radiatum of CA1 with aCSF-filled pipettes (R1; see Fig. 1*A*) with resistances of 2–6 MΩ. Two concentric bipolar stimulating electrodes (FHC, Brunswick, ME) were positioned in the stratum radiatum, one on each side of the field electrode at a distance of ~500 μm. The stimulating electrode closest to CA3 (S1) was used as the test pathway (for inducing LTP) whereas the other stimulating electrode (S2) was used as the control pathway to test for input specificity (Fig. 1*A*). Input-output (I-O) curves were used to find the stimulation intensity necessary to elicit an initial fEPSP slope that was 50% of the maximal fEPSP slope obtained in the absence of a population spike (Fig. 1*B*). This stimulation intensity was then used throughout the experiment. Although initial fEPSP slope is presented throughout this report, fEPSP amplitude yielded similar results. After a stable baseline was established for 10 min, LTP was induced in the test pathway by delivering a single tetanic stimulation (100 Hz for 1 s) to S1. Both pathways were then monitored for at least 30 min by delivering a single stimulus every 30 s (alternating every 15 s between S1 and S2). For all analyses, fEPSP slope was expressed as percent change from that observed during baseline, and LTP was reported as the mean change in fEPSP slope during the last 2 min of the 30-min posttetanic recording period. For all experiments, measurements from multiple slices were averaged for each animal. The 30-min time frame was selected because pilot data indicated that stable LTP was observed within ~15 min following high-frequency stimulation, and the amount of LTP did not differ when compared at 30, 45, or 60 min [$F(2,6) = 0.22$, $P = 0.7$]. In addition, our pilot data demonstrated that LTP was completely blocked by the *N*-methyl-D-aspartate (NMDA) receptor antagonist 2-amino-5-phosphonopentanoic acid (AP-5, 50 μM, Sigma; data not shown).

Intrinsic Excitability Studies

Somatic intracellular recordings were obtained from CA1 pyramidal neurons with sharp microelectrodes filled with 3 M potassium acetate and 20 mM KCl (60–100 MΩ). Only cells with a stable resting membrane potential (V_{rest}) more negative than −60 mV, overshooting action potentials (APs), and an input resistance (R_{input}) > 20 MΩ were used (Moyer et al. 1996). To minimize the influence of voltage-dependent changes on membrane conductances, all cells were studied at rest and at a membrane potential near −65 mV (≤0.3-nA constant-

current injection, if necessary). Neurons were recorded under current clamp according to the following protocol: *1*) Voltage-current (*V-I*) relations were obtained with 400-ms current steps (range −1.0 to +0.2 nA) and plotting of the plateau voltage deflection against current amplitude. Neuronal R_{input} was determined from the slope of the linear fit of that portion of the *V-I* plot where the voltage sweeps did not exhibit sags or active conductance. *2*) The postburst AHP (3×, at 20-s intervals) was evoked with a 100-ms depolarizing current injection sufficient to elicit a burst of four APs. AHP amplitude, duration, and integrated area were measured. The AHP amplitude was measured at various times after current offset (see Fig. 5*B*). For group comparisons and correlations, the AHP amplitude at 0.3 s was used. AP characteristics were analyzed from the first AP evoked during the AHP measurements. AP amplitude (AP_{amp}) was measured relative to baseline, and AP width (AP_{width}) was measured at one-half the AP_{amp}. *3*) Spike-frequency adaptation (accommodation; 3×, at 30-s intervals) was studied with a 1-s depolarizing current injection of the same stimulus intensity used to study the AHP. For each sweep, the number of APs elicited was counted. *4*) V_{rest} was calculated as the difference in membrane potential before and after withdrawal of the microelectrode from the cell. For statistical analyses, data were analyzed from individual cells, except for the correlation data, where neurons were averaged per rat.

Biocytin Staining

A subset of neurons was filled with biocytin to confirm the position and identity of pyramidal cells in the CA1 area. For these recordings, sharp electrodes were filled with 2% (wt/vol) biocytin dissolved in 1 M potassium acetate (80–120 MΩ). After stable V_{rest} was obtained, biocytin was injected iontophoretically by using 300-ms, 800-pA depolarizing current pulses delivered every 600 ms for 10–20 min. Slices were permitted to recover in the recording chamber for 30 min after biocytin injection (adapted from Yankova et al. 2001) and were then fixed in 10% neutral-buffered formalin at 4°C for 1–3 days before further processing. To visualize hippocampal neurons labeled by biocytin, the slices were incubated with 1% $NaBH_4$ for 30 min and washed with 0.1 M PBS for 10 min (3 times). Slices were then incubated in 3% H_2O_2-10% methanol for 45 min and washed with PBS for 10 min (3 times), followed by 0.25% Triton X-100–2% BSA for 60 min and 2% BSA for 10 min. The slices were then incubated with 1:500 streptavidin Alexa Fluor 488 (Invitrogen) for 135 min in the dark and washed with PBS for 10 min (3 times), rinsed in dH_2O, incubated for 90 min in 10 mM $CuSO_4$, rinsed with dH_2O, and rinsed in PBS (15 min, 3 times). They were mounted onto slides, coverslipped with Ultra Cruz Mounting Medium (Santa Cruz Biotechnology, Santa Cruz, CA), and sealed with nail polish. The neurons were viewed under a fluorescence microscope (BX51WI, Olympus) at ×20 and photographed. A representative biocytin-filled hippocampal CA1 pyramidal neuron and the *V-I* relation are shown in Fig. 1.

Statistical Analyses

The overall treatment effects were examined by one-way ANOVA or paired *t*-tests with SPSS 13.0 (SPSS, Chicago, IL). A repeated-measures ANOVA was used to compare freezing levels across training trials and AHP across time for each group of rats. For significant main effects (α 0.05), unless otherwise noted, a Fisher's protected least significant difference (PLSD) test was used for post hoc comparisons. All data are expressed as means ± SE.

RESULTS

Effects of Trace Fear Conditioning on Hippocampal Synaptic Plasticity

To examine how acquisition of trace fear conditioning affects synaptic plasticity in hippocampus, rats received a probe test 24 h after conditioning, and then LTP experiments were conducted. Analysis of percent freezing during training indicated that both good and poor learners exhibited comparable levels of freezing throughout the training session (see Fig. 2*A*). The pseudoconditioned rats also expressed a similar pattern of freezing, presumably due to contextual fear acquisition (Amano et al. 2010).

When memory was tested 24 h later, freezing levels were comparably low during the 2-min baseline and the 15-s CS (see Fig. 2*B*). In contrast, a statistically significant group effect was observed during the 30-s trace interval following the CS offset [$F(2,23) = 12.11$, $P < 0.01$]. As illustrated in Fig. 2*B*, post hoc analyses indicated that good learners froze significantly more than both poor learners ($P < 0.001$) and pseudoconditioned rats ($P < 0.001$).

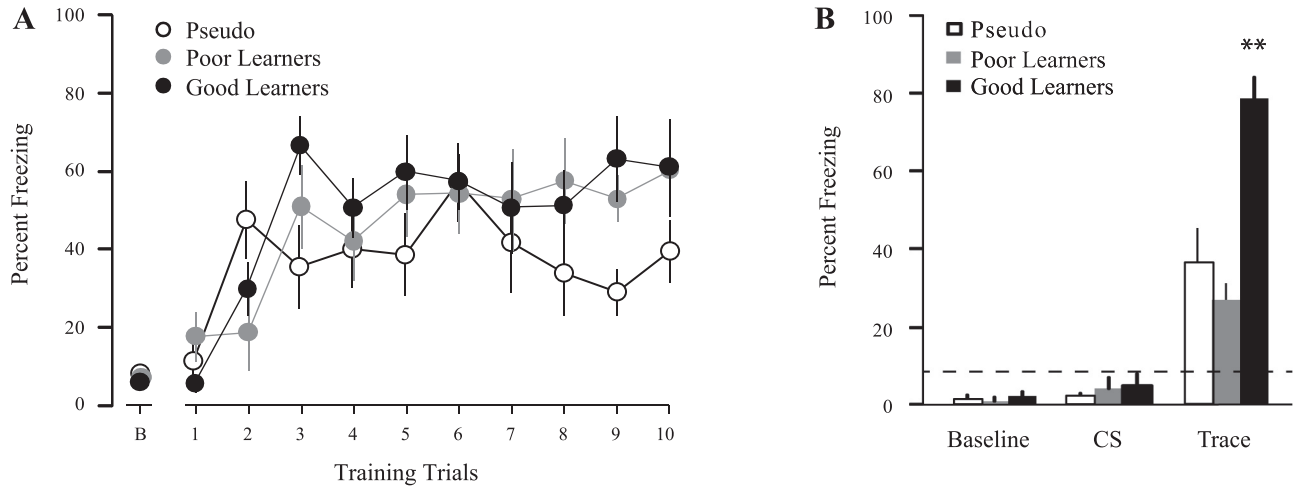

Fig. 2. Behavioral responses of trace fear-conditioned and pseudoconditioned rats during training and testing. *A*: throughout training, good learners ($n = 8$), poor learners ($n = 7$), and pseudoconditioned rats ($n = 11$) froze at comparable levels during the trace interval. A repeated-measures ANOVA of % freezing revealed a significant main effect of training trials [$F(6.18, 142.1) = 16.15$, $P < 0.01$; Greenhouse-Geisser corrected], group by training trial interaction [$F(12.4,142.1) = 1.95$, $P < 0.05$; Greenhouse-Geisser corrected], but no significant effect of group [$F(2,23) = 1.64$, $P = 0.22$]. *B*: during the probe test, good learners froze significantly more during the trace interval following offset of the conditioned stimulus (CS) than both poor learners and pseudoconditioned rats (**$P < 0.01$). Neither baseline nor CS freezing was significantly different between the 3 groups. Dashed line indicates the mean freezing of chamber-exposed control group.

To evaluate the learning-specific effects of trace fear conditioning on synaptic plasticity, LTP was compared between brain slices prepared from naive rats, rats exposed to the training and testing chambers only (chamber exposed), pseudoconditioned rats, poor learners, and good learners (Fig. 3). Two pathways were studied, but LTP was induced only in the test pathway. While all groups exhibited LTP in the test pathway, the good learners showed significantly enhanced LTP compared with the other groups [$F(4,46) = 5.13$, $P < 0.01$]. In all groups, LTP was input specific because no changes were observed in the control pathway (see Fig. 3, *inset*). Interestingly, Fig. 4*A* shows that the amount of LTP observed in the hippocampus of the trace fear-conditioned rats was positively correlated ($r = 0.64$, $P < 0.05$) with their behavioral performance. This correlation was learning specific because it was not observed in the pseudoconditioned rats ($r = -0.05$, $P = 0.88$; see Fig. 4*B*). Furthermore, acquisition of trace fear conditioning did not appear to alter basal synaptic transmission in the Schaffer collateral CA1 pathway—the average I-O curves did not differ between groups (for all individual intensities, $P > 0.25$ in raw I-O curves; see Fig. 1*B*). In addition, the baseline fEPSP (measured prior to LTP induction, see MATERIALS AND METHODS) was not significantly different in slices taken from naive, chamber-exposed, or pseudoconditioned rats, poor learners, or good learners [see Table 1; $F(4,46) = 0.40$, $P = 0.81$]. These data demonstrate that the enhanced synaptic plasticity following trace fear conditioning is learning specific and does not involve enhanced basal transmission.

Effects of Trace Fear Conditioning on Hippocampal CA1 Intrinsic Neuronal Excitability

Intracellular recordings revealed a significant reduction of the postburst AHP in good learners compared with poor learners and pseudoconditioned, chamber-exposed, and naive rats (see representative traces in Fig. 5*A* and average values in Fig. 5*B*). The AHP amplitude was measured at different time points following offset of the somatic current injection (from 50 ms to 3 s). A repeated-measures ANOVA showed that good learners had significantly smaller AHPs than the other groups [main effect of group, $F(4,52) = 4.77$, $P < 0.01$]. There was also a significant effect of time point [within-subject effect, $F(2.0,103.8) = 210.9$, $P < 0.001$; Greenhouse-Geisser corrected] and a group by time point interaction [$F(8.0,103.8) = 3.36$, $P < 0.01$; Greenhouse-Geisser corrected]. Follow-up analyses using a one-way ANOVA revealed that from 0.1 s to 2 s after current offset, a statistically significant group effect on AHP amplitude was observed (all values, $P < 0.01$). Post hoc comparisons confirmed that the AHP was significantly smaller in CA1 neurons from good learners compared with those from poor learners and pseudoconditioned, chamber-exposed, and naive rats ($P < 0.05$; see Fig. 5*B*). Our findings indicate that successful acquisition of trace fear conditioning results in a significant decrease in the amplitude of the postburst AHP of hippocampal CA1 neurons. Similar findings were also observed during analysis of the area and duration of the postburst AHP (Table 2).

Percent freezing during the CS test was negatively correlated with the amplitude, area, and duration of the AHP in trace fear-conditioned rats, such that better behavioral performance was associated with a smaller AHP (Fig. 6*A*). In contrast, percent freezing during the CS test was not significantly correlated with the amplitude, area, or duration of the AHP in CA1 neurons from pseudoconditioned rats (Fig. 6*B*). The significant correlation between behavioral performance and the AHP in trace fear-conditioned but not pseudoconditioned rats further demonstrates that the enhancement of intrinsic excitability after trace fear conditioning is learning specific.

In addition to altering the size and duration of the postburst AHP, trace fear conditioning also altered spike-frequency adaptation (or accommodation), another index of intrinsic neuronal excitability. To quantify spike-frequency adaptation, the somatic current injection used to study the AHP was extended to deliver a 1-s depolarizing current injection and the number of APs was counted. Accommodation was significantly reduced after acquisition of trace fear conditioning [$F(3,52) = 5.62$, $P < 0.01$; see Fig. 5*C* and Table 2]. Post hoc analysis revealed that CA1 neurons from good learners fired significantly more APs in response to the prolonged current injection than those from poor learners ($P < 0.05$), pseudoconditioned rats ($P < 0.01$), chamber-exposed rats ($P < 0.05$), or naive rats ($P < 0.01$; see Table 2). Similar reductions in spike-frequency adaptation have been reported in hippocampal neurons after acquisition of other hippocampus-dependent tasks (e.g., see McKay et al. 2009; Moyer et al. 1996, 2000; Thompson et al.

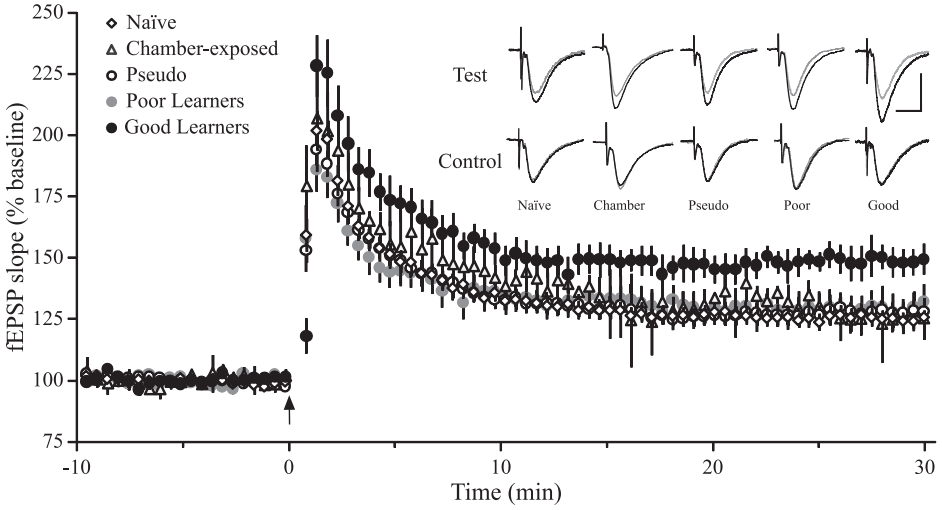

Fig. 3. Acquisition of trace fear conditioning enhanced Schaffer collateral to CA1 synaptic plasticity. One train of high-frequency stimulation (100 Hz, 1 s), delivered at *time 0* (upward arrow) was used to induce long-term potentiation (LTP) in slices from all groups. Slices from good learners showed significantly enhanced LTP compared with poor learners or pseudoconditioned, chamber-exposed, or naive rats ($P < 0.01$). Poor learners showed LTP that was comparable to the other 3 control groups. *Inset*: representative fEPSP waveforms before (gray) and 30 min after (black) LTP induction. Scale bar, 1 mV, 10 ms.

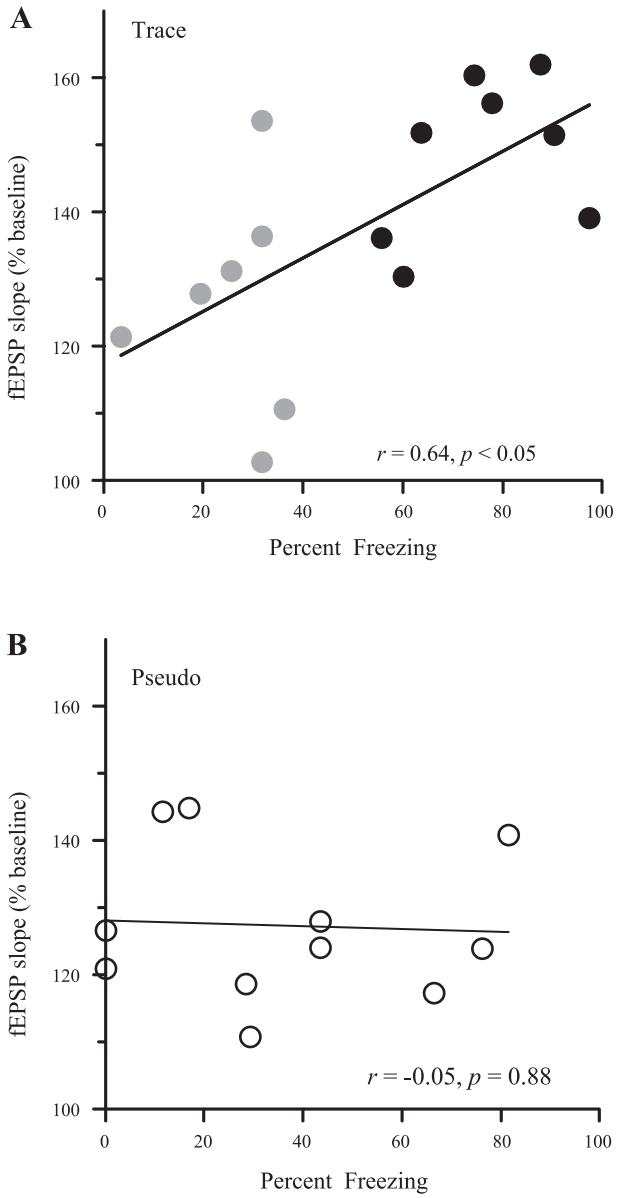

Fig. 4. Acquisition of trace fear conditioning is correlated with hippocampal synaptic plasticity. The percentage of time spent freezing during the CS test session was significantly correlated with amount of LTP in slices taken from trace fear-conditioned (*A*; gray, poor learners; black, good learners) but not pseudoconditioned (*B*) rats.

1996; Zelcer et al. 2006), suggesting that in the present study acquisition of trace fear conditioning results in a more efficient neuronal I-O function in CA1 neurons. Furthermore, the learning-specific increase in CA1 pyramidal cell excitability was observed in the absence of any changes in V_{rest}, R_{input}, depolarizing sag, or AP properties (see Table 3). These changes were also unlikely to result from any bias in cell selection for several reasons. First, all recordings were conducted by an individual who was blind to the training condition. Second, the cell selection criteria were established a priori (see MATERIALS AND METHODS). Finally, the percentage of cells lost during a recording was comparable between groups [$F(4,31) = 1.35$, $P = 0.27$]. Taken together, these data suggest that acquisition of trace fear conditioning enhances intrinsic excitability in a learning-specific manner.

DISCUSSION

The present study demonstrates that acquisition of trace fear conditioning results in learning-specific changes in hippocampal synaptic plasticity and intrinsic excitability. The use of a single, brief CS test revealed that trace fear memory was correlated with hippocampal intrinsic excitability as well as synaptic plasticity in the same animals. Recordings in brain slices from rats that were classified as good learners revealed not only a learning-specific facilitation of LTP but also a learning-specific reduction in both the postburst AHP and spike-frequency adaptation. These data suggest that both intrinsic excitability and synaptic plasticity are integrally involved in shaping the efficiency of hippocampal processing during acquisition of trace fear conditioning.

Acquisition of Trace Fear Conditioning Is Correlated with Synaptic Plasticity

Percentage of time spent freezing in trace fear-conditioned rats was positively correlated with synaptic plasticity (Fig. 4). Although this is the first report of a learning-related enhancement in synaptic plasticity following trace fear conditioning, these data are consistent with other in vivo or in vitro synaptic plasticity studies using other hippocampus-dependent learning paradigms. For example, in adult rats the magnitude of LTP in hippocampus was strongly correlated with behavioral performance in the Morris water maze (Boric et al. 2008). Similarly, in vivo recordings from hippocampal CA1 neurons in behaving mice demonstrated that LTP induced during (but not before) trace eyeblink conditioning lasted longer and was more resistant to extinction-induced depotentiation (Gruart et al. 2006). Other manipulations that either impair or enhance learning have also resulted in a corresponding impairment or enhancement of LTP. For example, not only did mice exposed to environmental enrichment perform better in hippocampus-dependent contextual fear conditioning but also hippocampal slices from these animals exhibited greater LTP (e.g., Duffy et al. 2001). Conversely, exposure to stress resulted in both impaired hippocampal LTP and impaired retention of spatial learning in the Morris water maze (e.g., Kim et al. 2001). The present finding of a positive correlation between acquisition of trace fear conditioning and amount of hippocampal LTP suggests that the enhanced synaptic plasticity is learning specific. This is supported by the fact that hippocampal LTP was highest in the good learners whereas LTP in the poor learners was comparable with the other control groups (Fig. 3). Furthermore, although some of our pseudoconditioned rats exhibited high levels of freezing following CS offset, LTP was not

Table 1. *Facilitation of LTP after trace fear conditioning is learning specific*

Group (no. of rats)	Baseline fEPSP Slope, mV/ms	% LTP, % of baseline
Naive (21)	−0.63 ± 0.07	125.0 ± 3.0
Chamber exposed (4)	−0.59 ± 0.07	126.1 ± 2.5
Pseudo (11)	−0.67 ± 0.05	127.3 ± 3.5
Poor learners (7)	−0.76 ± 0.14	126.2 ± 6.4
Good learners (8)	−0.72 ± 0.12	148.4 ± 4.2*

Data are means ± SE. LTP, long-term potentiation; fEPSP, field excitatory postsynaptic potential; pseudo, pseudoconditioned. *Statistically different from all other groups ($P < 0.01$).

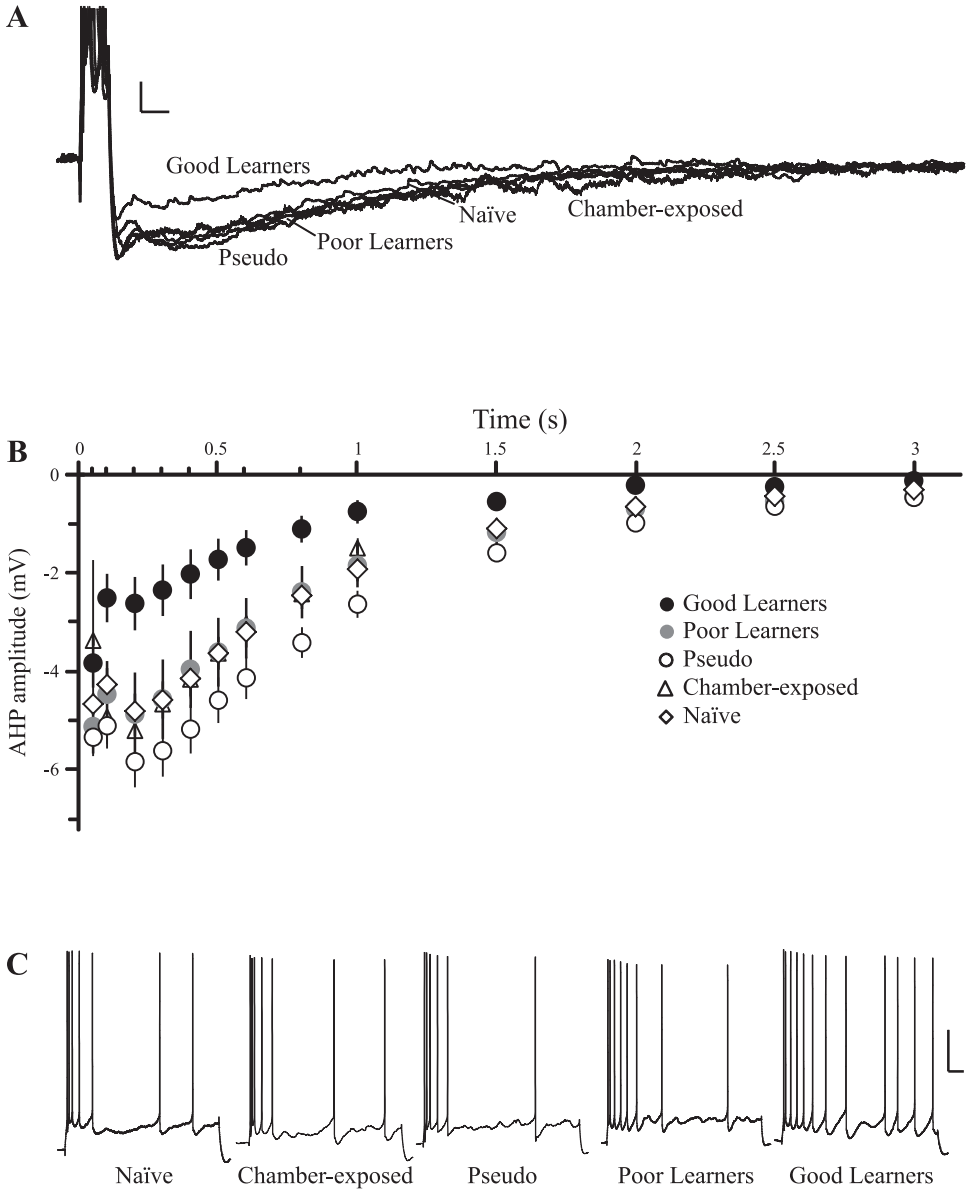

Fig. 5. Acquisition of trace fear conditioning increased the intrinsic excitability of hippocampal CA1 pyramidal neurons. *A*: representative traces of the postburst afterhyperpolarization (AHP) illustrating that CA1 neurons from good learners had smaller AHPs compared with those from poor learners and pseudoconditioned, chamber-exposed, and naive rats. Scale bar, 2 mV, 100 ms. *B*: time course of the postburst AHP amplitude as a function of training condition. Neurons from good learners had a significantly smaller AHP compared with all other groups when measured at 0.1–0.8 s after current offset ($P < 0.05$). *C*: action potential output of CA1 neurons in response to a prolonged 1-s current injection. Note that CA1 pyramidal neurons from good learners fired more action potentials than CA1 neurons from poor learners or pseudoconditioned, chamber-exposed, or naive rats. Scale bar, 20 mV, 100 ms.

significantly correlated with freezing levels in pseudoconditioned rats (Fig. 4*B*). Other studies have observed high freezing levels in pseudoconditioned rats following CS offset (e.g., Gilmartin and Helmstetter 2010; Majchrzak et al. 2006). Since trace interval freezing (i.e., freezing following CS offset) is of particular interest in trace fear conditioning studies, care should be taken to minimize post-CS freezing in pseudoconditioned animals (for further discussion of this topic see Smith et al. 2007). That LTP was not correlated with freezing in our pseudoconditioned rats suggests that the relatively high freezing levels observed in some of these animals did not result from hippocampal plasticity. Taken together, these data sug-

Table 2. *Summary of learning-related changes in CA1 neurons after trace fear conditioning*

Group (no. of rats)	Postburst AHP			Accommodation, no. of action potentials
	Amplitude, mV	Duration, s	Area, mV·s	
Naive (12)	−4.82 ± 1.50 (18)	3.13 ± 0.17 (18)	−5.56 ± 1.55 (18)	6.8 ± 0.5 (18)
Chamber exposed (3)	−5.22 ± 0.46 (6)	3.29 ± 0.33 (6)	−6.25 ± 0.80 (6)	7.8 ± 0.4 (6)
Pseudo (10)	−5.85 ± 0.52 (16)	3.57 ± 0.21 (16)	−7.43 ± 0.74 (16)	6.3 ± 0.4 (16)
Poor learners (5)	−4.89 ± 0.85 (8)	3.05 ± 0.26 (8)	−5.88 ± 1.00 (8)	7.8 ± 0.8 (8)
Good learners (6)	−2.64 ± 0.54 (9)*†	2.10 ± 0.22 (9)*†	−2.85 ± 0.81 (9)*†	10.0 ± 0.8 (9)*†

Data are means ± SE for no. of cells in parentheses. AHP, afterhyperpolarization. For AHP amplitude: significantly different from all controls (*poor learners, $P < 0.05$; †naive, chamber exposed, and pseudo, $P < 0.01$). For AHP duration: significantly different from all controls (*poor learners, $P < 0.05$; †naive, chamber exposed, and pseudo, $P < 0.01$). For AHP area: significantly different from all controls (*naive, chamber exposed, and poor learners, $P < 0.05$; †pseudo, $P < 0.01$). For accommodation: significantly different from all controls (*poor learners and chamber exposed, $P < 0.05$; †naive and pseudo, $P < 0.01$).

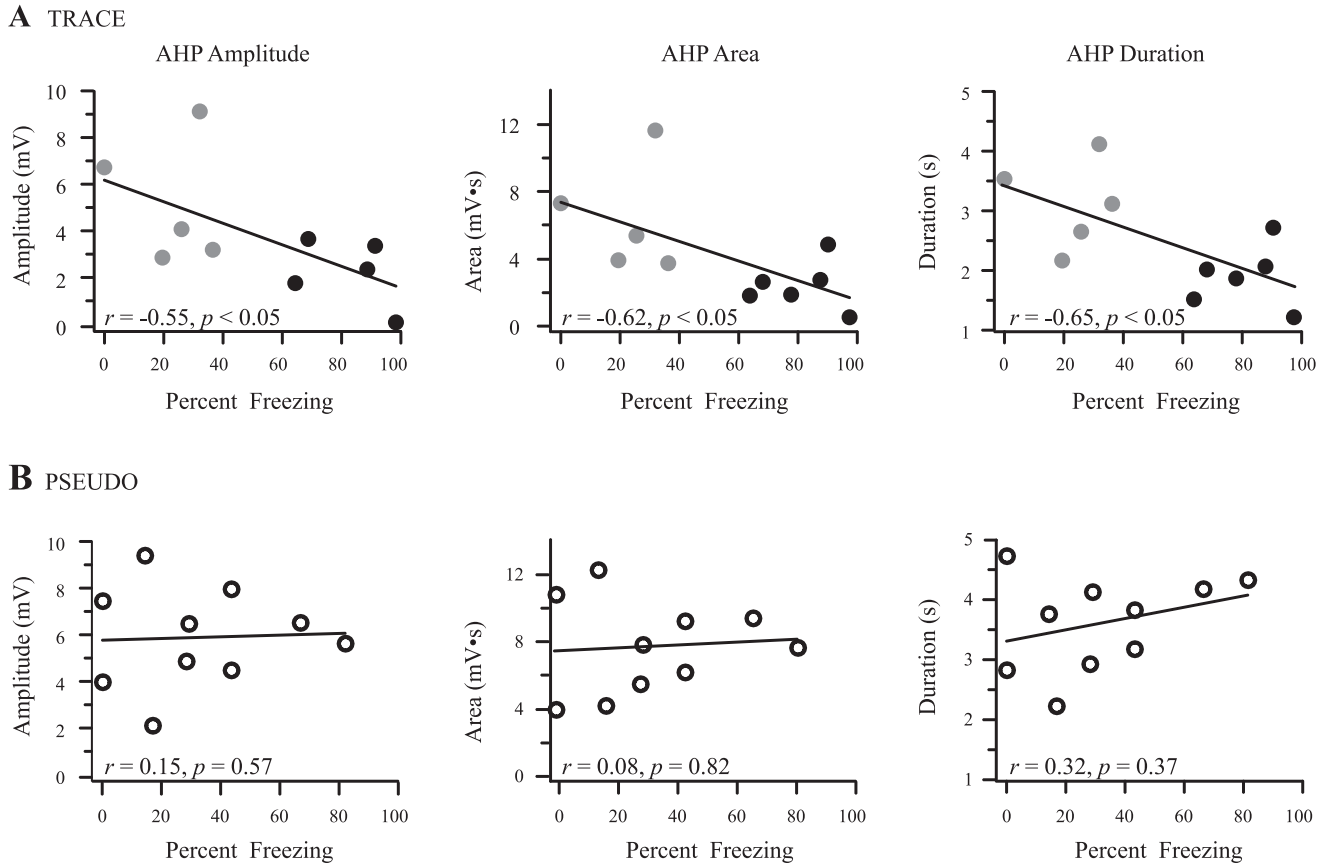

Fig. 6. Acquisition of trace fear conditioning is correlated with intrinsic excitability. The % freezing during the CS test session was significantly correlated with the amplitude, area, and duration of the postburst AHP in trace fear-conditioned (*A*; gray, poor learners; black, good learners) but not pseudoconditioned (*B*) rats. The postburst AHP amplitude was measured at 300 ms after current offset, and the neurophysiological data from each neuron were averaged to obtain the mean value of the AHP for that animal.

gest that acquisition of trace fear conditioning facilitates synaptic plasticity in hippocampal neurons in a learning-specific manner.

Acquisition of Trace Fear Conditioning Is Correlated with Intrinsic Excitability

Trace fear conditioning induced a learning-specific increase in the intrinsic excitability of CA1 pyramidal neurons, which was due to reductions in the postburst AHP and spike-frequency adaptation (Fig. 5). Furthermore, the percentage of time spent freezing in the trace fear-conditioned rats was negatively correlated with the amplitude, area, and duration of the postburst AHP (see Fig. 6*A*). The fact that these correlations were not observed in neurons recorded from pseudoconditioned rats (see Fig. 6*B*) further supports the idea that these intrinsic changes were learning specific and not a general result of the training or testing procedures.

Numerous studies have investigated intrinsic plasticity following learning with both invertebrate and vertebrate preparations (for reviews, see Disterhoft and Oh 2006; Zhang and Linden 2003). Our observed AHP reductions following trace fear conditioning are reminiscent of intrinsic plasticity in CA1 observed after acquisition of trace eyeblink conditioning (e.g., de Jonge et al. 1990; Moyer et al. 1996; Oh et al. 2009). Furthermore, a recent study by McKay et al. (2009) reported reduced AHPs in CA1 neurons from rats that received three trials of trace fear conditioning. Although the present data also showed reduced AHPs after trace fear conditioning, one major

Table 3. *Properties of CA1 neurons that do not change after trace fear conditioning*

Group (no. of rats)	V_{rest}, mV	R_{input}, MΩ	AP Characteristics		Sag, mV
			AP_{amp}, mV	AP_{width}, ms	
Naive (12)	−68.6 ± 0.8 (18)	38.9 ± 2.1 (18)	90.4 ± 0.8 (18)	1.03 ± 0.01 (18)	5.9 ± 0.5 (18)
Chamber exposed (3)	−66.3 ± 2.1 (6)	38.4 ± 4.1 (6)	88.2 ± 2.7 (6)	1.00 ± 0.02 (6)	6.1 ± 0.7 (6)
Pseudo (10)	−68.9 ± 1.0 (16)	42.5 ± 2.1 (16)	90.4 ± 0.8 (16)	1.04 ± 0.03 (16)	5.8 ± 0.4 (16)
Poor learners (5)	−69.7 ± 2.2 (8)	46.2 ± 3.5 (8)	89.0 ± 1.6 (8)	1.08 ± 0.05 (8)	7.4 ± 0.9 (8)
Good learners (6)	−70.5 ± 2.0 (9)	41.8 ± 3.5 (9)	88.4 ± 0.8 (9)	1.00 ± 0.04 (9)	5.6 ± 0.5 (9)

Data are means ± SE for no. of cells in parentheses. V_{rest}, resting membrane potential; R_{input}, input resistance; AP, action potential; AP_{amp}, AP amplitude; AP_{width}, AP half-width.

difference between the two studies is the number of training trials. The present study used 10 training trials and found that freezing during the test session was significantly correlated with the size of the AHP, and that this correlation was observed in trace fear-conditioned but not pseudoconditioned rats (see Fig. 6). In contrast, McKay and colleagues used very few training trials and demonstrated the labile nature of these learning-related AHP reductions through the use of three extinction trials. Although rats did not receive an extinction session in the present study, our prior work suggests that extinction does not begin to emerge until at least three or more CS presentations (see Fig. S1 of Kaczorowski et al. 2011a). Thus our use of two test trials did not obscure our ability to observe learning-related changes in CA1 excitability—we observed reduced AHPs in CA1 neurons from trace fear-conditioned but not pseudoconditioned rats.

Our studies employed a brief CS test session in order to relate freezing behavior to measures of hippocampal physiology. This test, although essential for getting a read-out of fear memory, is also likely to engage brain mechanisms associated with the well-described phenomenon of reconsolidation (for review, see Nader et al. 2000). Much has been learned about the cellular and molecular mechanisms of reconsolidation (e.g., Clem and Huganir 2010; Miller and Marshall 2005; Nader and Einarsson 2010), but exactly how this process may influence our observed electrophysiological changes is unclear and beyond the scope of the present study. Although we cannot rule out an important influence of this CS test, we believe it is unlikely that our use of a brief test session substantially influenced our electrophysiological measurements for several reasons. First, using rats that received a test session 1 h prior to slice preparation, Quirk and colleagues (Santini et al. 2008) demonstrated electrophysiological changes in prefrontal neurons—changes that could be reversed by an interposed extinction session. Thus the only variable was the presence of an extinction session, as all rats received the same test session prior to slice preparation. Similarly, Restivo and colleagues (2009) demonstrated changes in spine density in hippocampal neurons after context fear conditioning—changes that were independent of whether the rat received a test session or not. Perhaps the most compelling data come from a study by McKay and colleagues (2009), who demonstrated that changes in the intrinsic excitability of CA1 neurons after trace fear conditioning were similar between rats that did and those that did not receive a test trial prior to slice preparation. Taken together, these data suggest that acquisition of trace fear conditioning enhances intrinsic plasticity in a learning-specific manner.

Putative Interaction Between Synaptic and Intrinsic Plasticity During Learning

The present study found that acquisition of trace fear conditioning induced both an increase in the intrinsic excitability of CA1 neurons and a facilitation of LTP. The observed enhancement of LTP following acquisition of trace fear conditioning can be accomplished in a variety of ways. First, drugs or other treatments that reduce the AHP have been found to facilitate the induction of LTP. For example, the adrenergic agonist isoprenaline reduces the slow AHP and converts short-term potentiation (STP) into LTP (Sah and Bekkers 1996). Similarly, pharmacological stimulation of metabotropic glutamate receptors reduces the AHP of hippocampal CA1 neurons (Cohen and Abraham 1996) and also facilitates LTP induction, without affecting basal synaptic transmission (Cohen et al. 1999). Thus the AHP may act as an adjustable gain control where larger AHPs (or even the presence of the AHP) can shunt synaptic inputs (Sah and Bekkers 1996). Indeed, a recent study demonstrated that the larger postsynaptic AHP observed in aged CA1 neurons significantly impairs synaptic throughput in a frequency-dependent manner (Gant and Thibault 2009). Second, enhancement of synaptic plasticity can also be achieved by downregulation of transient A-type K^+ channels. These transient K^+ channels are highly expressed in distal dendrites and shape AP backpropagation through the dendrites. Furthermore, pharmacological downregulation or deletion of A-type K^+ channels has been associated with an enhancement of both dendritic excitability and LTP (Chen et al. 2006; Hoffman and Johnston 1998). Third, LTP can be enhanced by application of brain-derived neurotrophic factor (BDNF). BDNF facilitates LTP induction (e.g., Figurov et al. 1996) by enhancing intrinsic excitability (reduced AHP), which involves the activation of small-conductance Ca^{2+}-activated K^+ (SK2) channels in hippocampal neurons (Kramar et al. 2004).

Another line of evidence illustrating an interaction between synaptic stimulation and intrinsic plasticity comes from a recent report demonstrating that intrinsic plasticity can be induced independent of synaptic plasticity. For example, Barkai and colleagues (Cohen-Matsliah et al. 2010) recorded from CA1 pyramidal neurons and demonstrated that high-frequency synaptic stimulation (e.g., 20 stimuli at 50 Hz), which alone was incapable of inducing LTP, was able to cause a significant reduction of the postburst AHP. That we saw reduced AHPs in the absence of a significant alteration in baseline synaptic responses suggests the possibility that acquisition of trace fear conditioning may alter intrinsic neuronal excitability, which might then facilitate synaptic plasticity. Although this is highly speculative, support for this possibility comes from our within-animal analyses of the relationship between the size of the AHP and the amount of LTP. Figure 7 shows that there was a negative correlation between the size of the AHP and the amount of LTP such that hippocampal LTP was greater in animals whose CA1 neurons had smaller AHPs. This correlation was significant not only for the amplitude ($r = -0.38$, $P < 0.05$; Fig. 7*A*) but also for the area ($r = -0.41$, $P < 0.05$; Fig. 7*B*) and the duration ($r = -0.37$, $P < 0.05$; Fig. 7*C*) of the postburst AHP. Interestingly, if good learners are removed from the plots, the correlation is no longer significant (Fig. 7, dashed lines). Thus the data suggest that under baseline conditions (e.g., in the absence of learning-related changes), the AHP and LTP are not correlated. This correlation only emerges when there is a perturbation, such as a learning-related AHP reduction. Additional experiments, beyond the scope of this study, involving multiple time points (e.g., monitoring intrinsic and synaptic plasticity at different times throughout training) would be required to further address this complex relationship.

Implications of Learning-Induced Synaptic and Intrinsic Plasticity

The present study demonstrated an increase in both intrinsic excitability and synaptic plasticity in hippocampal CA1 neurons following trace fear conditioning. Our data (see also

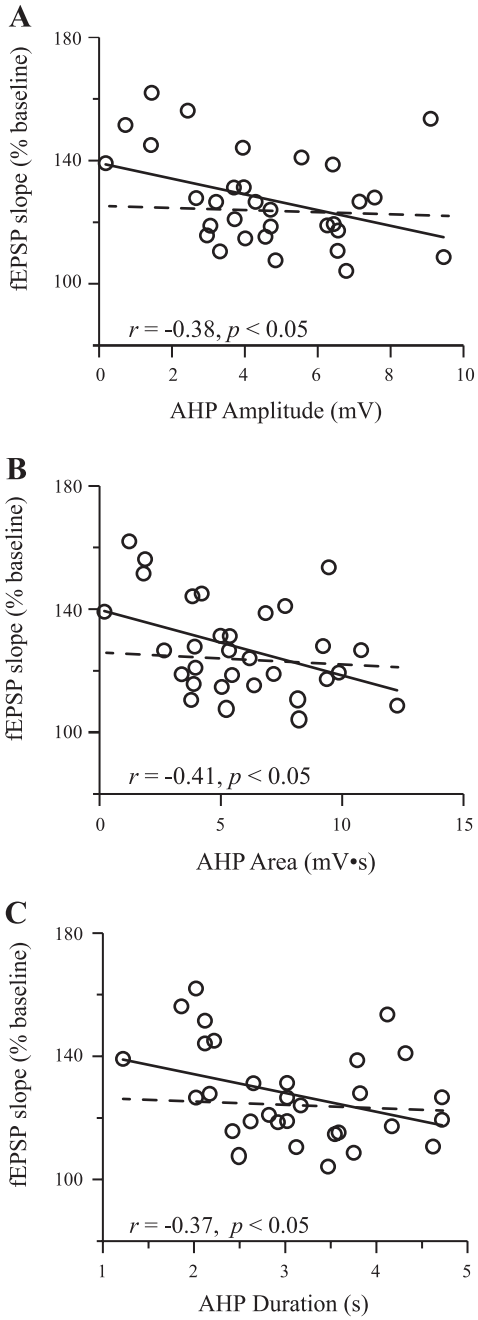

Fig. 7. Synaptic plasticity is correlated with intrinsic excitability. The magnitude of LTP was significantly correlated with the amplitude (*A*), area (*B*), and duration (*C*) of the postburst AHP (solid lines). Data are mean values for each animal where both intrinsic excitability and synaptic plasticity were studied in the same slice. Interestingly, when good learners are removed from the plot, the correlation is no longer significant (dashed line indicates slope of the line in the absence of good learners).

review by Zhang and Linden 2003) suggest that both intrinsic and synaptic plasticity play important roles in trace fear learning. These are dynamic and time-dependent processes. For example, it is known from prior studies that learning can rapidly induce, within an hour, an LTP-like enhancement of synaptic transmission (Power et al. 1997; Rumpel et al. 2005; Whitlock et al. 2006), which has been shown to occlude subsequent induction of LTP. In hippocampus, this LTP-like enhancement of basal synaptic transmission has been shown to result from a rapid and transient delivery of AMPA receptors at activated synapses (see Whitlock et al. 2006). However, in contrast, this enhancement of basal synaptic transmission has not been consistently observed in hippocampus 24 h after learning (LoTurco et al. 1988; Power et al. 1997; Zelcer et al. 2006), suggesting that, at least in hippocampus, this learning-related LTP-like phenomenon is short lived. Furthermore, it has also been shown that intrinsic excitability is altered in hippocampus as early as 1 h after learning (Moyer et al. 1996; Thompson et al. 1996) and that this change persists for several days (Moyer et al. 1996; Thompson et al. 1996; Zelcer et al. 2006). Interestingly, it has recently been shown that synaptic activity, even activity that does not induce LTP, can cause a protein synthesis-dependent increase in intrinsic excitability, as measured by AHP reductions (Cohen-Matsliah et al. 2010). Thus it is possible (albeit speculative at this point) that as the animal learns the trace fear conditioning task, pairing of the CS and US transiently increases basal transmission at hippocampal synapses that leads to increased intrinsic excitability (e.g., a smaller AHP), and that this increased excitability contributes to our observed facilitation of LTP.

How does learning-induced intrinsic and synaptic plasticity impact further learning? Few studies have directly addressed this issue. However, a learning-related increase in CA1 excitability may facilitate learning of another hippocampus-dependent task. Support for this hypothesis comes from rule-learning studies, in which rats show an increased learning capacity in discriminating between new pairs of odors once they have learned to discriminate the first pair (Saar and Barkai 2003; Saar et al. 1998). In addition, odor discrimination also facilitates acquisition of hippocampus-dependent Morris water maze, but only within a brief time window of 1–2 days after rule learning, while the AHP is reduced (Zelcer et al. 2006). Thus learning-induced intrinsic and synaptic modifications of postsynaptic neurons are capable of facilitating subsequent learning. In contrast, prior training in trace eyeblink conditioning did not enhance learning of hippocampus-dependent Morris water maze; however, simultaneous training in both tasks facilitated trace eyeblink but not water maze learning (Kuo et al. 2006). Additional studies will be required to determine the extent to which intrinsic and/or synaptic changes following trace fear conditioning impact learning of other hippocampus-dependent learning tasks or whether learning of other tasks affects the acquisition of trace fear conditioning.

Conclusion

The present data are the first to demonstrate that trace fear conditioning is significantly correlated with both synaptic plasticity and intrinsic excitability in the hippocampus. Acquisition of trace fear conditioning enhanced intrinsic excitability and facilitated the induction of LTP in the absence of significant changes in basal synaptic transmission. These observations were learning specific because they were not observed in pseudoconditioned, chamber-exposed, or naive rats. In addition, there was a negative correlation between the size of AHP and the amount of LTP such that animals whose CA1 neurons had smaller AHPs tended to display greater LTP. Thus the data suggest a model whereby as acquisition occurs hippocampal intrinsic excitability increases, which then leads to a facilita-

tion of synaptic plasticity, which occurs during memory consolidation.

ACKNOWLEDGMENTS

The authors thank Devon O'Hara, Scott Davis, and Trevor Van Langendon for technical assistance.

GRANTS

This study was supported by a Research Growth Initiative from University of Wisconsin-Milwaukee (J. R. Moyer, Jr) and Quincy Bioscience (J. R. Moyer, Jr.).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: C.S., J.A.D., and M.S. performed experiments; C.S. and J.R.M.J. analyzed data; C.S. and J.R.M.J. interpreted results of experiments; C.S. and J.R.M.J. prepared figures; C.S., J.A.D., M.S., and J.R.M.J. edited and revised manuscript; J.R.M.J. conception and design of research; J.R.M.J. drafted manuscript; J.R.M.J. approved final version of manuscript.

REFERENCES

Amano T, Unal CT, Pare D. Synaptic correlates of fear extinction in the amygdala. *Nat Neurosci* 13: 489–494, 2010.

Barnes CA. Memory deficits associated with senescence: a neurophysiological and behavioral study in the rat. *J Comp Physiol Psychol* 93: 74–104, 1979.

Bear MF, Abraham WC. Long-term depression in hippocampus. *Annu Rev Neurosci* 19: 437–462, 1996.

Blanchard RJ, Blanchard DC. Crouching as an index of fear. *J Comp Physiol Psychol* 67: 370–375, 1969.

Boric K, Munoz P, Gallagher M, Kirkwood A. Potential adaptive function for altered long-term potentiation mechanisms in aging hippocampus. *J Neurosci* 28: 8034–8039, 2008.

Chen X, Yuan LL, Zhao C, Birnbaum SG, Frick A, Jung WE, Schwarz TL, Sweatt JD, Johnston D. Deletion of Kv4.2 gene eliminates dendritic A-type K^+ current and enhances induction of long-term potentiation in hippocampal CA1 pyramidal neurons. *J Neurosci* 26: 12143–12151, 2006.

Clem RL, Huganir RL. Calcium-permeable AMPA receptor dynamics mediate fear memory erasure. *Science* 330: 1108–1112, 2010.

Cohen-Matsliah SI, Motanis H, Rosenblum K, Barkai E. A novel role for protein synthesis in long-term neuronal plasticity: maintaining reduced postburst afterhyperpolarization. *J Neurosci* 30: 4338–4342, 2010.

Cohen AS, Abraham WC. Facilitation of long-term potentiation by prior activation of metabotropic glutamate receptors. *J Neurophysiol* 76: 953–962, 1996.

Cohen AS, Coussens CM, Raymond CR, Abraham WC. Long-lasting increase in cellular excitability associated with the priming of LTP induction in rat hippocampus. *J Neurophysiol* 82: 3139–3148, 1999.

Czerniawski J, Yoon T, Otto T. Dissociating space and trace in dorsal and ventral hippocampus. *Hippocampus* 19: 20–32, 2009.

de Jonge MC, Black J, Deyo RA, Disterhoft JF. Learning-induced afterhyperpolarization reductions in hippocampus are specific for cell type and potassium conductance. *Exp Brain Res* 80: 456–462, 1990.

Detert JA, Kampa ND, Moyer JR Jr. Differential effects of training intertrial interval on acquisition of trace and long-delay fear conditioning in rats. *Behav Neurosci* 122: 318–327, 2008.

Disterhoft JF, Oh MM. Learning, aging and intrinsic neuronal plasticity. *Trends Neurosci* 29: 587–599, 2006.

Doyere V, Debiec J, Monfils MH, Schafe GE, LeDoux JE. Synapse-specific reconsolidation of distinct fear memories in the lateral amygdala. *Nat Neurosci* 10: 414–416, 2007.

Doyere V, Redini-Del Negro C, Dutrieux G, Le Floch G, Davis S, Laroche S. Potentiation or depression of synaptic efficacy in the dentate gyrus is determined by the relationship between the conditioned and unconditioned stimulus in a classical conditioning paradigm in rats. *Behav Brain Res* 70: 15–29, 1995.

Dudek SM, Bear MF. Bidirectional long-term modification of synaptic effectiveness in the adult and immature hippocampus. *J Neurosci* 13: 2910–2918, 1993.

Duffy SN, Craddock KJ, Abel T, Nguyen PV. Environmental enrichment modifies the PKA-dependence of hippocampal LTP and improves hippocampus-dependent memory. *Learn Mem* 8: 26–34, 2001.

Fanselow MS, Poulos AM. The neuroscience of mammalian associative learning. *Annu Rev Psychol* 56: 207–234, 2005.

Figurov A, Pozzo-Miller LD, Olafsson P, Wang T, Lu B. Regulation of synaptic responses to high-frequency stimulation and LTP by neurotrophins in the hippocampus. *Nature* 381: 706–709, 1996.

Gant JC, Thibault O. Action potential throughput in aged rat hippocampal neurons: regulation by selective forms of hyperpolarization. *Neurobiol Aging* 30: 2053–2064, 2009.

Gilmartin MR, Helmstetter FJ. Trace and contextual fear conditioning require neural activity and NMDA receptor-dependent transmission in the medial prefrontal cortex. *Learn Mem* 17: 289–296, 2010.

Gruart A, Munoz MD, Delgado-Garcia JM. Involvement of the CA3-CA1 synapse in the acquisition of associative learning in behaving mice. *J Neurosci* 26: 1077–1087, 2006.

Hoffman DA, Johnston D. Downregulation of transient K^+ channels in dendrites of hippocampal CA1 pyramidal neurons by activation of PKA and PKC. *J Neurosci* 18: 3521–3528, 1998.

Kaczorowski CC, Davis SJ, Moyer JR Jr. Aging redistributes medial prefrontal neuronal excitability and impedes extinction of trace fear conditioning. *Neurobiol Aging* (Apr 30, 2011a). doi:10.1016/j.neurobiolaging.2011.03.020.

Kaczorowski CC, Disterhoft JF. Memory deficits are associated with impaired ability to modulate neuronal excitability in middle-aged mice. *Learn Mem* 16: 362–366, 2009.

Kaczorowski CC, Sametsky E, Shah S, Vassar R, Disterhoft JF. Mechanisms underlying basal and learning-related intrinsic excitability in a mouse model of Alzheimer's disease. *Neurobiol Aging* 32: 1452–1465, 2011b.

Kholodar-Smith DB, Boguszewski P, Brown TH. Auditory trace fear conditioning requires perirhinal cortex. *Neurobiol Learn Mem* 90: 537–543, 2008.

Kim JJ, Lee HJ, Han JS, Packard MG. Amygdala is critical for stress-induced modulation of hippocampal long-term potentiation and learning. *J Neurosci* 21: 5222–5228, 2001.

Kramar EA, Lin B, Lin CY, Arai AC, Gall CM, Lynch G. A novel mechanism for the facilitation of theta-induced long-term potentiation by brain-derived neurotrophic factor. *J Neurosci* 24: 5151–5161, 2004.

Kuo AG, Lee G, Disterhoft JF. Simultaneous training on two hippocampus-dependent tasks facilitates acquisition of trace eyeblink conditioning. *Learn Mem* 13: 201–207, 2006.

LeDoux JE. Emotion circuits in the brain. *Annu Rev Neurosci* 23: 155–184, 2000.

LoTurco JL, Coulter DA, Alkon DL. Enhancement of synaptic potentials in rabbit CA1 pyramidal neurons following classical conditioning. *Proc Natl Acad Sci USA* 85: 1672–1676, 1988.

Lynch MA. Long-term potentiation and memory. *Physiol Rev* 84: 87–136, 2004.

Majchrzak M, Ferry B, Marchand AR, Herbeaux K, Seillier A, Barbelivien A. Entorhinal cortex lesions disrupt fear conditioning to background context but spare fear conditioning to a tone in the rat. *Hippocampus* 16: 114–124, 2006.

McEchron MD, Bouwmeester H, Tseng W, Weiss C, Disterhoft JF. Hippocampectomy disrupts auditory trace fear conditioning and contextual fear conditioning in the rat. *Hippocampus* 8: 638–646, 1998.

McKay BM, Matthews EA, Oliveira FA, Disterhoft JF. Intrinsic neuronal excitability is reversibly altered by a single experience in fear conditioning. *J Neurophysiol* 102: 2763–2770, 2009.

Miller CA, Marshall JF. Molecular substrates for retrieval and reconsolidation of cocaine-associated contextual memory. *Neuron* 47: 873–884, 2005.

Moser MB, Moser EI. Functional differentiation in the hippocampus. *Hippocampus* 8: 608–619, 1998.

Moyer JR Jr, Brown TH. Impaired trace and contextual fear conditioning in aged rats. *Behav Neurosci* 120: 612–624, 2006.

Moyer JR Jr, Brown TH. Methods for whole-cell recording from visually preselected neurons of perirhinal cortex in brain slices from young and aging rats. *J Neurosci Methods* 86: 35–54, 1998.

Moyer JR Jr, Power JM, Thompson LT, Disterhoft JF. Increased excitability of aged rabbit CA1 neurons after trace eyeblink conditioning. *J Neurosci* 20: 5476–5482, 2000.

Moyer JR Jr, Thompson LT, Disterhoft JF. Trace eyeblink conditioning increases CA1 excitability in a transient and learning-specific manner. *J Neurosci* 16: 5536–5546, 1996.

Nader K, Einarsson EO. Memory reconsolidation: an update. *Ann NY Acad Sci* 1191: 27–41, 2010.

Nader K, Schafe GE, Le Doux JE. Fear memories require protein synthesis in the amygdala for reconsolidation after retrieval. *Nature* 406: 722–726, 2000.

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

Oh MM, McKay BM, Power JM, Disterhoft JF. Learning-related postburst afterhyperpolarization reduction in CA1 pyramidal neurons is mediated by protein kinase A. *Proc Natl Acad Sci USA* 106: 1620–1625, 2009.

Paxinos G, Watson C. *The Rat Brain in Stereotaxic Coordinates*. San Diego, CA: Academic, 1998.

Phillips RG, LeDoux JE. Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. *Behav Neurosci* 106: 274–285, 1992.

Phillips RG, LeDoux JE. Lesions of the dorsal hippocampal formation interfere with background but not foreground contextual fear conditioning. *Learn Mem* 1: 34–44, 1994.

Power JM, Thompson LT, Moyer JR Jr, Disterhoft JF. Enhanced synaptic transmission in CA1 hippocampus after eyeblink conditioning. *J Neurophysiol* 78: 1184–1187, 1997.

Quinn JJ, Oommen SS, Morrison GE, Fanselow MS. Post-training excitotoxic lesions of the dorsal hippocampus attenuate forward trace, backward trace, and delay fear conditioning in a temporally specific manner. *Hippocampus* 12: 495–504, 2002.

Restivo L, Vetere G, Bontempi B, Ammassari-Teule M. The formation of recent and remote memory is associated with time-dependent formation of dendritic spines in the hippocampus and anterior cingulate cortex. *J Neurosci* 29: 8206–8214, 2009.

Rumpel S, LeDoux J, Zador A, Malinow R. Postsynaptic receptor trafficking underlying a form of associative learning. *Science* 308: 83–88, 2005.

Saar D, Barkai E. Long-term modifications in intrinsic neuronal properties and rule learning in rats. *Eur J Neurosci* 17: 2727–2734, 2003.

Saar D, Grossman Y, Barkai E. Reduced after-hyperpolarization in rat piriform cortex pyramidal neurons is associated with increased learning capability during operant conditioning. *Eur J Neurosci* 10: 1518–1523, 1998.

Sah P, Bekkers JM. Apical dendritic location of slow afterhyperpolarization current in hippocampal pyramidal neurons: implications for the integration of long-term potentiation. *J Neurosci* 16: 4537–4542, 1996.

Santini E, Quirk GJ, Porter JT. Fear conditioning and extinction differentially modify the intrinsic excitability of infralimbic neurons. *J Neurosci* 28: 4028–4036, 2008.

Smith DR, Gallagher M, Stanton ME. Genetic background differences and nonassociative effects in mouse trace fear conditioning. *Learn Mem* 14: 597–605, 2007.

Thompson LT, Moyer JR Jr, Disterhoft JF. Transient changes in excitability of rabbit CA3 neurons with a time course appropriate to support memory consolidation. *J Neurophysiol* 76: 1836–1849, 1996.

Whitlock JR, Heynen AJ, Shuler MG, Bear MF. Learning induces long-term potentiation in the hippocampus. *Science* 313: 1093–1097, 2006.

Yankova M, Hart SA, Woolley CS. Estrogen increases synaptic connectivity between single presynaptic inputs and multiple postsynaptic CA1 pyramidal cells: a serial electron-microscopic study. *Proc Natl Acad Sci USA* 98: 3525–3530, 2001.

Zelcer I, Cohen H, Richter-Levin G, Lebiosn T, Grossberger T, Barkai E. A cellular correlate of learning-induced metaplasticity in the hippocampus. *Cereb Cortex* 16: 460–468, 2006.

Zhang W, Linden DJ. The other side of the engram: experience-driven changes in neuronal intrinsic excitability. *Nat Rev Neurosci* 4: 885–900, 2003.