

SYNAPSE-SPECIFIC COMPARTMENTALIZATION OF SIGNALING CASCADES FOR LTP INDUCTION IN CA3 INTERNEURONS

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Abstract—Inhibitory interneurons with somata in *strata radiatum* and *lacunosum-moleculare* (SR/L-M) of hippocampal area CA3 receive excitatory input from pyramidal cells via the recurrent collaterals (RCs), and the dentate gyrus granule cells via the mossy fibers (MFs). Here we demonstrate that Hebbian long-term potentiation (LTP) at RC synapses on SR/L-M interneurons requires the concomitant activation of calcium-impermeable AMPARs (CI-AMPA) and N-methyl-D-aspartate receptors (NMDARs). RC LTP was prevented by voltage clamping the postsynaptic cell during high-frequency stimulation (HFS; 3 trains of 100 pulses delivered at 100 Hz every 10 s), with intracellular injections of the Ca²⁺ chelator BAPTA (20 mM), and with the NMDAR antagonist D-AP5. In separate experiments, RC and MF inputs converging onto the same interneuron were sequentially activated. We found that RC LTP induction was blocked by inhibitors of the calcium/calmodulin-dependent protein kinase II (CaMKII; KN-62, 10 μM or KN-93, 10 μM) but MF LTP was CaMKII independent. Conversely, the application of the protein kinase A (PKA) activators forskolin/IBMX (50 μM/25 μM) potentiated MF EPSPs but not RC EPSPs. Together these data indicate that the apical dendrites of SR/L-M interneurons compartmentalize synapse-specific Ca²⁺ signaling required for LTP induction at RC and MF synapses. We also show that the two signal transduction cascades converge to activate a common effector,

protein kinase C (PKC). Specifically, LTP at RC and MF synapses on the same SR/LM interneuron was blocked by postsynaptic injections of chelerythrine (10 μM). These data indicate that both forms of LTP share a common mechanism involving PKC-dependent signaling modulation.

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Key words: recurrent commissural LTP and LTD, calcium impermeable AMPARs, CaMKII; PKA; PKC, CA3 interneurons, feed-forward inhibition.

INTRODUCTION

The majority of excitatory synapses in hippocampal area CA3 originate from the extensive recurrent collateral (RC) axons of pyramidal cells (Amaral and Witter, 1989; Ishizuka et al., 1990). The RC circuitry underlies many autoassociative network models that simulate pattern completion, i.e. the recall of activity patterns stored in area CA3 via mossy fibers (MFs), the axons of dentate gyrus granule cells (Marr, 1971; McNaughton and Morris, 1987; O'Reilly and McClelland, 1994; Rolls, 1996). One key assumption in these computational models is that RC synapses exhibit N-methyl-D-aspartate receptor (NMDAR)-mediated long-term potentiation (LTP) to recreate the original episode (O'Reilly and McClelland, 1994). Indeed, NMDAR activation in area CA3 is required for memory recall (Nakazawa et al., 2002; Fellini et al., 2009), and the induction of RC LTP in CA3 pyramidal cells (Harris and Cotman, 1986; Zalutsky and Nicoll, 1990; Magee and Johnston, 1997; Debanne et al., 1998; Bains et al., 1999). The retrieval of previously stored activity patterns also relies on the inhibitory input from local interneurons to constrain the activation of non-assembly pyramidal cells (Sahay et al., 2011). Therefore, preservation of the excitatory-to-inhibitory balance for optimal pattern separation requires that RC synapses undergo near simultaneous LTP on pyramidal cells and feed-forward interneurons (Lamsa et al., 2005). However, previous investigations of synaptic plasticity at RC synapses on CA3 interneurons have yielded varying results. Early studies reported NMDAR-independent LTD at RC synapses on *stratum radiatum* (SR) interneurons (Laezza et al., 1999). In contrast, NMDAR-dependent RC LTD in SR interneurons was detected during persistent bursting activity in the disinhibited slice (Stoop et al., 2003). A more recent study on the same interneuron synapse uncovered the bidirectional induction of NMDAR-dependent plasticity

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Abbreviations: AC, adenylyl cyclase; ACSF, artificial cerebrospinal fluid; ANOVA, analysis of variance; CaMKII, calcium/calmodulin-dependent protein kinase II; CI-AMPA, calcium-impermeable AMPAR; CP-AMPA, calcium permeable AMPAR; DCG-IV, (2S,2'R,3'R)-2-(2',3'-dicarboxycyclopropyl)glycine; DIC, differential intensity contrast; EDTA, ethylenediaminetetraacetic acid; FSK, forskolin; HEPPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HFS, high-frequency stimulation; ISI, inter-stimulus interval; LTP, long-term potentiation; MF, mossy fiber; NMDAR, N-methyl-D-aspartate receptor; PFA, paraformaldehyde; PKA, protein kinase A; PKC, protein kinase C; PPF, paired pulse facilitation; PPR, paired pulse ratio; RC, recurrent collateral; RI, rectification index; SR/L-M, *strata radiatum* and *lacunosum-moleculare*; VGCC, voltage-gated calcium channel.

(LTP/LTD), contingent on the level of postsynaptic depolarization (Laezza and Dingledine, 2004).

Hippocampal interneurons with somata in *stratum radiatum* and *lacunosum-moleculare* (SR/L-M) of area CA3 belong to a larger population of dendritic targeting GABAergic cells providing feed-forward inhibition to pyramidal cells (Lacaille and Schwartzkroin, 1988; Williams et al., 1994; Vida et al., 1998). MF synapses on SR/L-M interneurons exhibit NMDAR-independent LTP induced by cytosolic Ca^{2+} increase from the coactivation of L-type voltage-gated calcium channels (VGCCs) and mGluR₁. This form of MF LTP requires postsynaptic activation of protein kinases A (PKA) and C (PKC) (Galván et al., 2011). Here we show that RC synapses on SR/L-M interneurons exhibit a form of Hebbian LTP that requires calcium entry via NMDARs. High-frequency stimulation (HFS) of RC and MF inputs synapsing on the same interneuron revealed that blockade of calcium/calmodulin-dependent protein kinase II (CaMKII) prevented LTP induction at RC but not at MF synapses. Conversely, PKA stimulation resulted in a potentiation of MF synapses but did not affect RC synapses. We conclude that the aspiny dendrites of SR/L-M interneurons are able to compartmentalize the initial Ca^{2+} signaling cascades that trigger LTP at two different synaptic inputs. Nevertheless, PKC inhibition prevented the induction of both forms of LTP suggesting that PKC activation provides a point of convergence of signaling cascades originating from RC and MF synaptic activity.

EXPERIMENTAL PROCEDURES

Ethical approval

For the electrophysiological recordings, animal use was in accordance with the University Institutional Animal Care and Use Committee. Male Sprague–Dawley rats (35 ± 5 days old; Zivic Miller Company, Portersville, PA) were deeply anaesthetized (Nembutal, IP, 5 mg/100 g body weight) and perfused intracardially with a modified artificial cerebrospinal fluid (ACSF) in which sucrose was substituted for NaCl (in mM): 210 sucrose, 2.8 KCl, 1.25 NaH₂PO₄, 26 NaHCO₃, 10 D-Glucose, 1 CaCl₂, 4 MgCl₂, at 4 °C. Following 1–2 min of perfusion, animals were decapitated and the brains removed. Blocks of hippocampal tissue were glued to the stage of a Leica VT1000S and cut in 380- μ m-thick sections. Slices were maintained for at least 120 min in an incubation solution of the following composition (in mM): 125 NaCl, 2.5 KCl, 1.2 NaH₂PO₄, 25 NaHCO₃, 10 D-Glucose, 0.4 ascorbic acid, 1 CaCl₂ and 6 MgCl₂. The solution was maintained at pH 7.3 and bubbled with O₂ (95%)/CO₂ (5%) mixture at room temperature. Slices were then transferred to a submersion recording chamber and superfused at constant flow (3.5 ml/min) with the following solution (in mM): 125 NaCl, 3 KCl, 1.25 Na₂HPO₄, 25 NaHCO₃, 2 CaCl₂, 1 MgCl₂, 10 Glucose). The temperature of the solution in the recording chamber was set at 33 ± 1 °C. Interneurons were identified visually with infrared video (IR) microscopy and differential intensity contrast (DIC) optics. Patch pipettes were pulled from borosilicate

glass and had resistances of 5–8 M Ω when filled with a solution containing (in mM), 120 K-methylsulfate, 10 NaCl, 10 KCl, 10 HEPES, 0.5 EGTA, 4 Mg-ATP, 0.3 Na₂-GTP, 14 phosphocreatine. In some experiments, biocytin, (0.1%) was added to the pipette solution to allow subsequent morphological identification and reconstruction of the interneurons (Fig. 1A). For the AMPAR rectification experiments (Fig. 1B–F), the intracellular solution was modified to contain (in mM), 120 Cesium Methanesulfonate, 10 KCl, 10 HEPES, 0.5 EGTA, 8 phosphocreatine, 4 Mg-ATP, 0.3 Na₂-GTP and 0.5 QX-314. Osmolarity was adjusted to 295–300 mOsm with pH 7.2–7.3. Access resistance was monitored throughout the length of experiments using a 5-ms, 5-mV voltage steps. Interneurons were accepted for analysis only if the seal resistance was > 1 G Ω and the series resistance did not change > 15% of its initial value during the course of the experiment.

RC and MF EPSPs were evoked by extracellular stimulation via concentric bipolar electrodes (12.5- μ m inner pole diameter, 125- μ m outer pole diameter; FHC Inc., ME, USA). To activate the RC input, the electrode was positioned in the *stratum radiatum* near the border between CA3b and CA3a. To activate the MF input, the electrode was placed in the suprapyramidal blade of the dentate gyrus (SDG; Fig. 1A). RC EPSPs exhibited shorter latency and time-to-peak than MF EPSPs (latency = 1.74 ± 0.12 ms and 3.32 ± 0.13; $p < 0.001$; time to peak = 3.7 ± 0.22 ms and 5.61 ± 0.41; $p < 0.001$ for RC and MF EPSPs, respectively) as previously reported (Laezza and Dingledine, 2004; Calixto et al., 2008). Pairs of stimuli were delivered at 60-ms inter-stimulus interval (ISI) to each input separately. Each pair consisted of monophasic pulses (100–400 μ A; 85–100- μ s duration) applied at 0.25–0.16 Hz. We applied stimulation current intensities that evoked monosynaptic RC and MF EPSP amplitudes $\leq 30\%$ of the threshold amplitude required to elicit action potentials in the recorded interneurons. Cells with composite postsynaptic responses were discarded from the study. For each input paired pulse facilitation (PPF) was calculated as the ratio (PPR at 60-ms ISI) of the amplitude of the second EPSP over the first EPSP in the pair. The rectification index (RI) of the synaptic responses was obtained from the ratio of RC EPSCs at +40 and –80 mV, as previously reported (see Laezza et al., 1999). Synapses exhibiting RI > 0.6 were considered to be composed of a majority of calcium impermeable (CI) AMPARs whereas a RI < 0.3 was indicative of rectifying synapses mainly containing calcium permeable (CP) AMPARs (see Fig. 1B, C). Synapses exhibiting rectification values ranging from 0.31 to 0.59 were considered to contain a mixed population of CP- and CI-AMPA receptors and were discarded from this study. Sequential activation of RC and MF inputs converging onto the same interneuron was delivered at 1000-ms ISI to minimize synaptic temporal summation. Control experiments were performed to confirm the long-lasting duration of RC and MF LTP in the absence of the drugs used in this study. Both RC LTP ($n = 3$) and MF LTP ($n = 4$) exhibited duration and time-course similar to those reported in the results section. Specifically, LTP was stable for at least

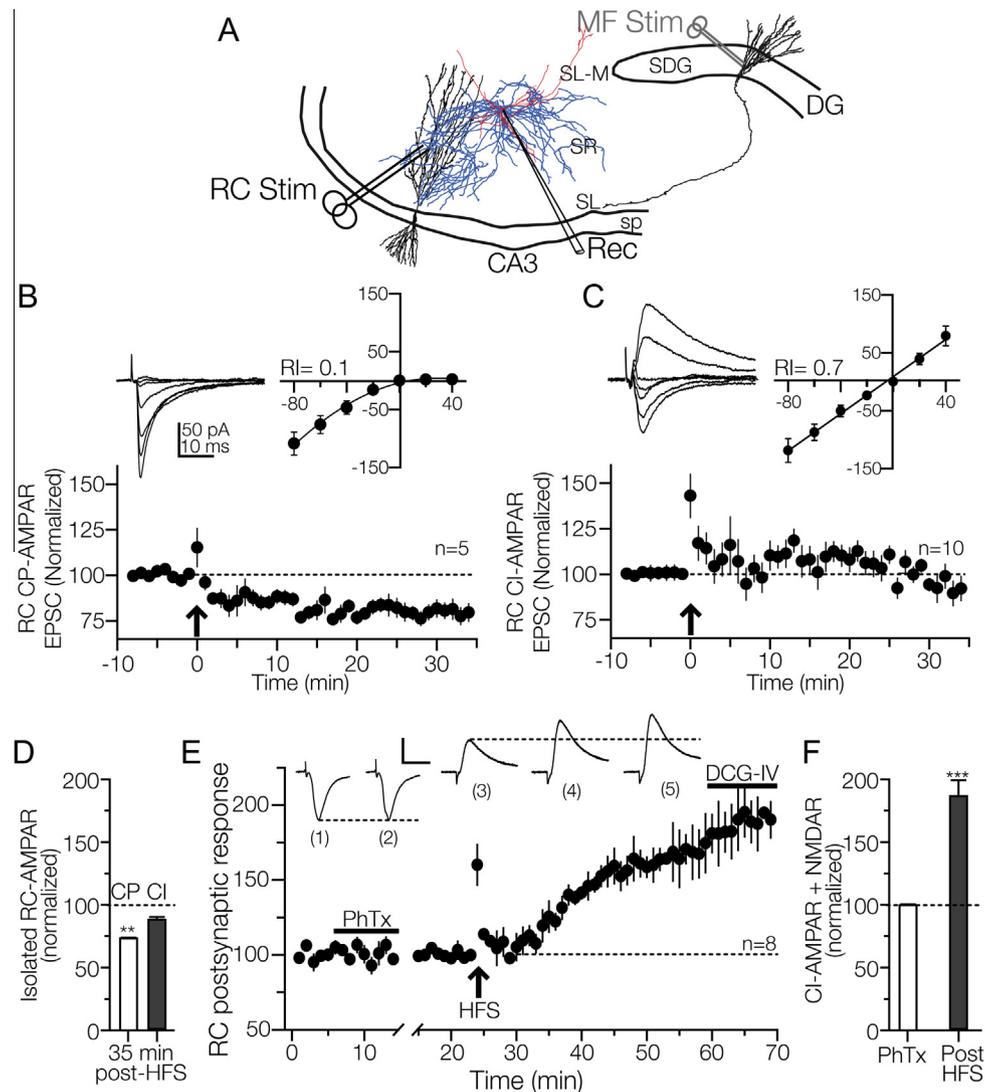


Fig. 1. Composition of AMPARs at recurrent commissural (RC) synapses on CA3 interneurons. (A) Placement of stimulation electrodes and the whole-cell recording pipette. Recordings were obtained from interneurons with soma in the *stratum radiatum* (SR) and *lacunosum-moleculare* (SL-M). RC responses were evoked by stimulating the *stratum radiatum*; MF responses were evoked stimulating on the suprapyramidal blade of the dentate gyrus (SDG). (B, C) (Top panels) The voltage–current relationships from RC EPSCs revealed that RC synapses are comprised of CP- (19%) and Cl-AMPA receptors (73%). (Lower panels) HFS delivered to RC synapses containing CP-AMPA receptors induced LTD whereas HFS to RC synapses formed by Cl-AMPA receptors results in a transient potentiation. (D) Bar graph summarizing the effect of HFS on isolated RC CP-AMPA receptors and Cl-AMPA receptors. (E) Time-course graph averaged from eight independent experiments in the presence of bicuculline (10 μ M). The RC EPSC insensitivity to philanthotoxin (PhTx, 10 μ M) was used to identify Cl-AMPA receptors. HFS applied to this glutamatergic synapse composed of Cl-AMPA receptors and NMDARs induces stable LTP, as summarized in the bar chart (F). ** $p < 0.01$; *** $p < 0.001$ or higher statistical significance. Error bars indicate SEM for all the figures. Calibration bar for panel E, 50 pA/5 ms for the voltage clamp and 2 mV/5 ms for current clamp traces.

100-min post-HFS (RC LTP = $204 \pm 14\%$; MF LTP = $164 \pm 7.4\%$ of baseline; $p < 0.0001$ for both inputs). Current and voltage clamp recording were obtained with an Axopatch 200B (Axon Instruments) in the presence of (–)-bicuculline methiodide (10 μ M) to block GABA_A-mediated responses. Signals were low-pass filtered at 5 kHz, digitized at 10 kHz, and stored for off-line analysis. Data acquisition and analysis were performed using PClamp 10 (Molecular Devices, Sunnyvale, CA). Lack of sensitivity (< 5%) of RC EPSPs to the application of the group II metabotropic glutamate receptor agonist (2S,2'R,3'R)-2-(2',3'-Dicarboxycyclopropyl)glycine (DCG-IV; 5 μ M) was confirmed at the end of the experiments. Although DCG-IV inhibition of MF transmission in pyramidal cells is $\geq 90\%$ (Kamiya et al., 1996), it is variable in

interneurons (Alle et al., 2001; Lawrence and McBain, 2003; Galván et al., 2008). Therefore, synaptic responses were considered of MF origin if the DCG-IV application resulted in $\geq 70\%$ inhibition (Lawrence and McBain, 2003; Galván et al., 2008). LTP was induced in each input by HFS consisting of three trains of 100 pulses each at 100 Hz, repeated every 10 s paired with a postsynaptic depolarizing current step (30 ± 0.6 pA).

Drugs

1(S),9(R)-(–)-Bicuculline methbromide; D(–)-2-Amino-5-phosphonopentanoic acid, D-AP5; (2S,2'R,3'R)-2-(2',3'-Dicarboxycyclopropyl)glycine, DCG-IV; 2-Methyl-6-(phenylethynyl)pyridine hydrochloride, MPEP; (S)-(+)- α -Amino-4-carboxy-2-methylbenzeneacetic acid, LY 367385;

KN-62, KN-93 were purchased from TOCRIS (Ellisville, MO) or Sigma Chemical (St. Louis, MO). Forskolin, IBMX and PDA were dissolved in DMSO at concentrations of 100, 10, 2, respectively, and then added to the bath solution. The concentration of DMSO in the final bath solution was 0.1%. Otherwise, drugs were dissolved in double-distilled H₂O.

Immunofluorescence determinations

In the first set of experiments, six rats were anesthetized and perfused intracardially with phosphate buffer (PB) 0.1 M, pH 7.4 followed by 4% paraformaldehyde (PFA). Brains were post-fixed overnight in 4% PFA, and then transferred into 30% sucrose solution. Serial coronal sections of the brain were cut at 30 μ m using a cryostat at -19°C (Leica CM1510). For immunostaining, we selected 1 every 10 slices. Sections were pretreated with an antigen retrieval citrate buffer at 80°C for 15 min and rinsed several times in 50 mM Tris-buffered saline (TBS). Sections were incubated with BSA 5% in PBS 0.3% Triton X-100 for 1 h at room temperature to prevent nonspecific staining. Sections were then incubated with primary antibodies at 4°C for 24 h in BSA 5% in PBS 0.3% Triton X-100.

A second set of experiments were conducted directly on vibratome-cut slices. The first group consisted of naïve slices incubated for 20 min with ACSF. The second group comprised slices in which the HFS protocol was applied on the *stratum radiatum* of CA3c, as described above. Slices were next fixed in PFA (4%) 5 and 30 min after delivering the tetanic stimulation. Both groups were post-fixed during 24 h and next they were transferred to 30% sucrose solution. Slices were then resectioned into 30- μ m sections for the immunohistochemical experiments. Following antibodies were used: mouse anti-CAMKII (1:500, Abcam, Cambridge, MA), rabbit anti-CAMKII (1:1000, Abcam, Cambridge, MA), mouse anti-Cam α -phospho (1:500 Abcam, Cambridge, MA), rabbit anti-GAD67 (1:100, Santa Cruz, Dallas, Tx), mouse anti-GAD67 (1:1000, Millipore, Billerica, MA) and rabbit anti-Calbindin D-28 K (1:300, Millipore, Billerica, MA). As negative controls, some slices of the same tissue cut into 30- μ m thickness were simultaneously processed in the absence the primary antibody. After rinsing three times, slices were incubated with secondary antibodies for 2 h at room temperature as follows: goat anti-rabbit Alexa 488 (1:500, Molecular probes, Eugene, OR), goat anti-mouse Cy5 (1:500, Molecular probes, Eugene, OR), goat anti-mouse TRITC (1:100, Jackson ImmunoResearch, West Grove, PA) and goat anti-rabbit TRITC (1:100, Jackson ImmunoResearch, West Grove, PA). Finally, slices were rinsed three times in PBS and mounted in slides using Vectashield with DAPI mounting medium (Vector Laboratories, Burlingame, CA). All slices were examined with an epifluorescent microscope (Axio Scope, Carl Zeiss).

Morphological reconstructions

Following recordings, slices were fixed in cold 4% paraformaldehyde for 72 h, transferred into an anti-freeze

solution (a one-to-one mixture of glycerol and ethylene glycol in 0.1 M phosphate buffer), and stored at -80°C . Slices were then cut into 60- μ m sections on a vibratome, reacted with 1% H₂O₂, and placed in blocking serum with 0.5% Triton X-100 for 2 h at room temperature. Biocytin-labeled neurons were incubated with ABC-peroxidase and developed using the Ni-enhanced DAB chromogen. Interneurons were reconstructed using the NeuroLucida tracing system (MicroBrightField, Inc., Williston, VT, USA) on a AxioPlan 2 Zeiss microscope equipped with DIC, a 100 \times (NA = 1.4) planapochromatic lens and additional Optovar magnification of 1.6 \times (final optical magnification, 1600 \times ; screen magnification, 7200 \times). For the reconstructions, all sections containing the cell were used.

Statistics

Group measures are expressed as mean \pm S.E.M. Normality of the populations were tested with Kolmogorov–Smirnov test ($p < 0.05$), followed by a one-way analysis of variance (ANOVA) and a Student–Newman–Keuls all pairwise comparisons ($p < 0.05$). In all cases differences were considered significant if p was less than alpha = 0.05. In the figures, statistical significance is denoted as follows: * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ (or higher).

RESULTS

Anatomical and electrophysiological properties of SR/L-M CA3 interneurons

Whole-cell recordings were obtained from 90 SR interneurons and 37 L-M localized in area CA3b. Interneuron somata were typically positioned $120 \pm 10 \mu\text{m}$ to $300 \pm 10 \mu\text{m}$ from the boundary between *stratum pyramidale* and *stratum lucidum* and 150–250 μm from the medial extend of the superior blade of the dentate gyrus and 50–150 μm below the slice surface (Fig. 1A). The predominant morphology of the recorded cells was bipolar with dendritic arborizations extended horizontally, and had similar passive properties (Calixto et al., 2008; Ascoli et al., 2009). Both types of interneurons exhibited adapting (accommodating) firing patterns with spike adaptation ratio of the first to last inter-spike interval > 3.0 . These interneurons provide feedforward and lateral inhibition to pyramidal cells (Chitwood et al., 1999; Ascoli et al., 2009).

Characterization of AMPARs targeted by the RC input to SR/L-M CA3 interneurons

It is well known that glutamatergic synaptic transmission in hippocampal area CA3 is mediated by GluR2-lacking calcium permeable CP-AMPA, and GluA2-containing CI AMPARs. In addition, different forms of synaptic plasticity of these responses have been characterized for MF synapses on CA3 interneurons (Toth and McBain, 1998; Toth et al., 2000) and RC synapses (Laezza et al., 1999).

In the presence of bicuculline and D-AP5, RC-evoked voltage–current relationships obtained from -80 mV to

+40 mV in steps of 20 mV revealed that the RC input to SR/L-M CA3 interneurons forms at least three types of AMPAR synapses. The first group was characterized by a strong inward rectification curve (five out of 26 recorded cells; RI: 0.1 ± 0.17 ; Fig. 1B; upper panel). HFS of these synapses induced a stable RC-LTD (RC EPSC, $74.2 \pm 0.8\%$ of baseline at 35 min post HFS; $p < 0.001$ RM-ANOVA; Fig. 1B, lower panel; $N = 5$). A second group (19 out of 26) expressed a linear V–I relationship (RI: 0.7 ± 0.13 ; Fig. 1C, upper panel). In 10 of these interneurons, HFS induced a transitory potentiation that lasted up to 20 min before returning to baseline values followed by a mild synaptic depression (RC EPSC, $94.6 \pm 2.2\%$ of baseline at 35 min post HFS; $p < 0.001$ RM-ANOVA; Fig. 1C, lower panel; $N = 10$). The remaining nine cells of this group showed a small PTP but no potentiation (RC EPSC, $104.6 \pm 4\%$ of baseline at 35 min post HFS). Two additional cells displayed an irregular V–I response. Similar responses were previously described for mixed AMPAR-containing synapses (Toth and McBain, 1998; Toth et al., 2000); these cells were discarded from the present study. These results support the notion that in CA3 interneurons the isolated RC CP AMPARs express LTD whereas MF CI AMPARs express NMDAR-independent LTP (Galván et al., 2008). In contrast, RC synapses composed of isolated CI AMPARs are unable to undergo LTP.

It has been previously shown that early on postnatal development (P12–P20) HFS stimulation applied to RC synapses on CA3 interneurons containing CI-AMPA/NMDARs do not exhibit LTP (Laezza and Dingledine, 2004). To test if this is the case for mature hippocampi, experiments were performed in slices from P30 to P40 animals. Following a stable 8-min baseline of RC EPSCs (recorded at -70 mV) in the presence of bicuculline, pihanthotoxin ($10 \mu\text{M}$) was added to the perfusion medium. In eight interneurons, RC EPSCs were minimally sensitive to PhTx ($3.1 \pm 2\%$ of sensitivity; $p > 0.1$, one-way ANOVA; Fig. 1E). After the washout of PhTx, the recordings were switched to current clamp mode, and a 5-min baseline was recorded followed by HFS of the RC input. The RC EPSPs exhibited PTP ($149.05 \pm 8.28\%$ of baseline; $p < 0.001$) followed by LTP that lasted up to 1 h. RC EPSPs were insensitive to DCG-IV ($5 \mu\text{M}$; RC LTP = $183.9 \pm 10\%$ of baseline at 40 min post HFS; $p < 0.001$; RM ANOVA; RC LTP in the presence of DCG-IV = $191.2 \pm 7\%$ of baseline at 60 min post HFS; $p < 0.001$; RM-ANOVA). No statistical difference in RC LTP magnitude was found in the presence of DCG-IV ($p > 0.15$; one-way ANOVA). In two additional cells, RC EPSCs were highly sensitive to PhTx ($78.13 \pm 9\%$ of sensitivity) indicating that these synapses were mainly composed of CP-AMPA. These cells were discarded. In summary, the majority of RC synapses on SR/L-M interneurons were mainly comprised of CI-AMPA (75%). The remaining synapses contained either CP-AMPA (19.4%) or a mixed population of CP- and CI-AMPA (5.5%). These data suggest that RC LTP may require the co-activation of CI-AMPA and NMDARs.

Requirements for the induction of RC LTP in CA3 interneurons

One distinct anatomical feature of area CA3 is the dense local connectivity via the RC axons of CA3 pyramidal cells making synapses on neighboring CA3 pyramidal cells (Ishizuka et al., 1990; Sik et al., 1993; Li et al., 1994). In contrast to MF LTP, RC LTP in CA3 pyramidal cells requires NMDAR activation for the postsynaptic Ca^{2+} influx (Harris and Cotman, 1986; Zalutsky and Nicoll, 1990). In a separate group of interneurons, HFS was applied while the membrane potential was voltage clamped at -100 mV to prevent action potential firing. In these conditions, no significant changes in the RC EPSP slope was observed ($102.3 \pm 1\%$ at 5 min pre-HFS; $102.5 \pm 2\%$ at 15 min post-HFS; $p > 0.45$, RM-ANOVA; $N = 6$; Fig. 2A–D). A second HFS train paired with a depolarizing pulse (50 pA) to evoke burst of action potentials, was delivered to the RC input while the cell was held in current clamp mode at -60 mV. This paired stimulus protocol resulted in a robust increase in RC EPSPs slope that lasted up to 45 min and was insensitive to DCG-IV (PTP = $152 \pm 7\%$ of baseline; RC LTP = $195 \pm 8\%$ of baseline at 30 min post-HFS; $p < 0.001$; RM ANOVA; RC LTP in the presence of DCG-IV = $218.9 \pm 16\%$ of baseline at 50 min post HFS; $p < 0.001$; RM ANOVA; $N = 6$; Fig. 2A–D). The requirement for concomitant pre- and postsynaptic activation for RC LTP induction in SR/L-M interneurons indicates that this form of synaptic plasticity is Hebbian. To further investigate the locus of expression of RC LTP, the paired pulse facilitation (PPF, at ISI 60 ms) was monitored in all the experiments. Forty minutes after the induction of RC LTP, RC PPF exhibited a systematic reduction compared to control (RC PPR control = 1.46 ± 0.02 ; RC PPR at 40 min post HFS = 1.02 ± 0.06 ; $p < 0.0001$, one-way ANOVA; Fig. 2D). We also plotted the coefficient of variation (CV^{-2}) against the mean of the RC EPSP values at 40 min following the application of HFS. The distribution of the data values (Fig. 2E) were closely located to the identity line (dashed line). The decrease in the PPF and the changes in the CV^{-2} following the induction of RC LTP, strongly suggest that RC LTP has a presynaptic component of expression (Malinow and Tsien, 1990; Alle et al., 2001).

It has been reported that RC LTP induction in CA3 pyramidal cells is prevented with postsynaptic Ca^{2+} chelation (Zalutsky and Nicoll, 1990). Thus, we investigated whether RC synapses on CA3 interneurons also require postsynaptic Ca^{2+} influx to induce LTP. Cells were loaded with BAPTA (20 mM) for at least 15 min before the experiments. BAPTA did not affect PTP ($142 \pm 9\%$ of baseline; $p < 0.001$) but prevented changes in RC EPSPs slopes at 15 min ($100 \pm 4.1\%$ of baseline; $p > 0.05$; one-way ANOVA) and 35 min post-HFS ($94.8 \pm 5\%$ of baseline; $p > 0.05$; one-way ANOVA, $N = 4$; Fig. 2C).

CA3 interneurons also express group I mGluRs (Baude et al., 1993; Lujan et al., 1996), which contribute to several forms of synaptic plasticity in hippocampal interneurons. For example, at MF synapses on SR/L-M interneurons,

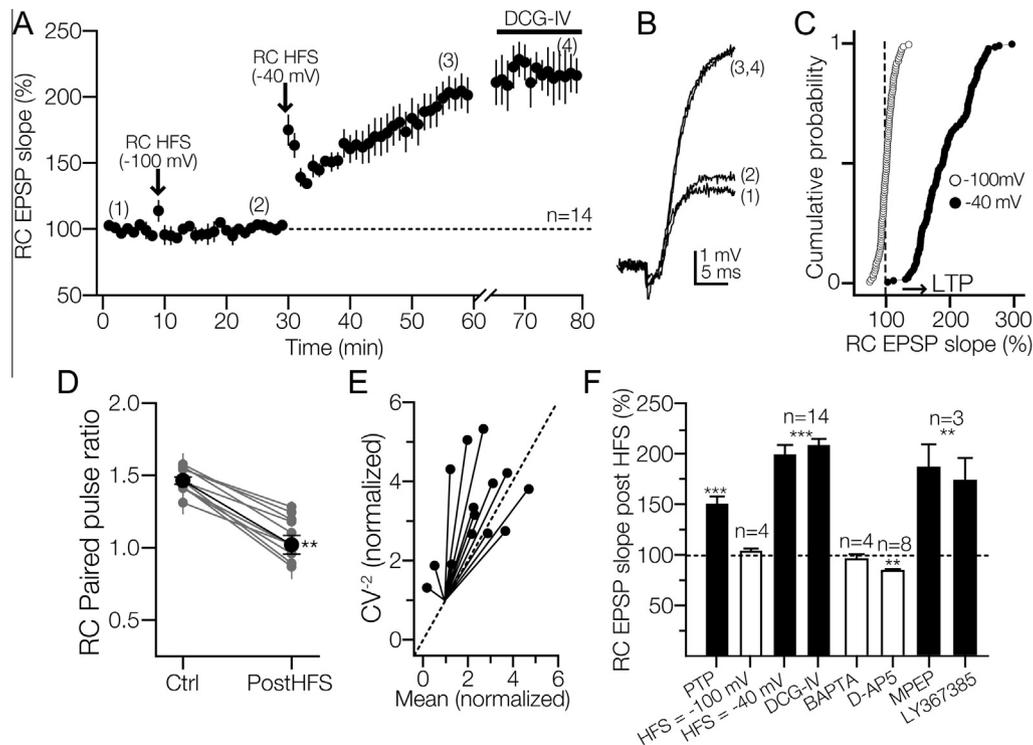


Fig. 2. The requirements of LTP induction at RC synapses on CA3 SR/SL-M interneurons. All experiments were performed in the presence of bicuculline (10 μ M). (A) Average time-course of RC EPSPs from six separate experiments. HFS applied while cells were held in voltage clamp at -100 mV did not express RC LTP (2) but subsequent HFS paired with a postsynaptic depolarization step (-40 mV) elicited RC LTP that lasted up to 1 h (3), and was insensitive to DCG-IV (4). (B) Average of RC EPSP slopes from 10 consecutive sweeps at the times indicated in A. (C) Cumulative probability of responses when HFS was applied at -100 mV (empty circles) or HFS at -40 mV (filled circles). Dashed line represents the RC EPSP baseline. Arrowhead indicates an increase in the EPSP slope above 25% of baseline value. (D) The paired pulse ratio of RC EPSPs at 60-ms ISI showed a persistent decrease at 40 min after the application of the HFS. (E) Coefficient of variation (CV^{-2}) plotted against mean of the synaptic responses at 40-min post HFS; the values were normalized to their respective controls. The distribution of the data points with respect to the identity line (dashed) is indicative of a presynaptic locus of expression for RC LTP. (F) Bar graph summarizing the magnitude of RC PTP and LTP with respect to baseline and lack of effect of DCG-IV (eight additional experiments were performed without PhTx; the LTP magnitude was similar to that shown in A). In cells loaded with BAPTA (20 mM) HFS failed to induce LTP. In the presence of D-AP5 (50 μ M), HFS triggered a significant depression of RC EPSPs. RC LTP was insensitive to the pharmacological blockade of group I mGluRs with MPEP (50 μ M) or LY367385 (100 μ M). ** $p < 0.01$; *** $p < 0.001$ or higher statistical significance.

mGluR₁ is a metaplastic switch controlling the polarity of long-term synaptic plasticity (Galván et al., 2008). At CA1 *stratum oriens* interneuron synapses, mGluR₁ is required for the induction of Hebbian LTP (Perez et al., 2001; Lapointe et al., 2004; Topolnik et al., 2006). In the next series of experiments, we investigated whether the group I mGluRs is involved in RC LTP induction in SR/L-M interneurons. The mGluR₅ antagonist MPEP (50 μ M) did not block the induction of RC LTP (PTP = $162.7 \pm 29\%$; LTP at 30 min post HFS = $185 \pm 23\%$ of baseline; $p < 0.001$; one-way ANOVA; $N = 3$; Fig. 2C). Similar results were found from experiments in which the mGluR₁ was blocked with bath perfused LY 367385 (100 μ M) for at least 10 min before the experiment. RC HFS was delivered after EPSP baseline was collected for 8 min. In three cells, HFS applied to the RC input induced PTP followed by LTP with a magnitude similar to those obtained in the experiments described in Fig. 2A (PTP = $142 \pm 11\%$ of baseline; LTP at 30 min post HFS = $172.2 \pm 22.4\%$ of baseline; $p < 0.001$; RM-ANOVA; $N = 3$; Fig. 2C). Collectively these data show that the induction of RC LTP in SR/L-M CA3 does not require activation of the group I mGluRs.

Induction of RC LTP in CA3 interneurons requires CaMKII activity

CaMKII plays a key role in the induction of NMDAR-dependent LTP of CA1 pyramidal cells of the hippocampus (Malinow et al., 1989; Hvalby et al., 1994; Lledo et al., 1995; Wang and Kelly, 1995, 1996). In addition, CaMKII up-regulates the glutamatergic transmission of CA1 fast-spiking non-pyramidal cells (Wang and Kelly, 2001), and is required for the induction of NMDAR-dependent LTP in interneurons located in CA1 *stratum radiatum* (Lamsa et al., 2007). Furthermore, the dependence on CaMKII activation for the induction of CA3-CA3 LTP has been documented in organotypic slices (Pavlidis et al., 2000; Lu and Hawkins, 2006). Given the dependency of NMDAR-mediated LTP on CaMKII in CA1 interneurons (Lamsa et al., 2007), we postulated that RC LTP in CA3 SR/L-M interneurons also requires CaMKII autophosphorylation. To test this hypothesis, we sought to determine whether CaMKII inhibition prevented induction of RC LTP. Hippocampal slices were incubated in the presence of the cell-permeable inhibitor of CaMKII, KN-62 (10 μ M) or the more selective and potent CaMKII blocker KN-93

(10 μ M) for 50–60 min prior to the experiment. In these experiments, RC and MF inputs converging onto the same interneuron were consecutively stimulated (see Fig. 1A for stimulation electrodes position) at 1000-ms ISI (see Experimental procedures). Following the incubation with KN-62 or KN-93, stable EPSP slopes were recorded for 8 min prior to the delivery of HFS to the RC input. As predicted, the slope of the RC EPSP was unchanged following the incubation with KN-62 ($91.7 \pm 3.76\%$ at 5 min post-HFS; and $89.9 \pm 3.3\%$ at 15 min of baseline post-HFS; $p > 0.5$ RM-ANOVA; $N = 5$) or KN-93 ($91 \pm 5\%$ at 5 min post-HFS; and $85 \pm 12\%$ at 15 min post-HFS; $p > 0.5$ RM-ANOVA; $N = 6$; Fig. 3A, top panel). In the same experiment, D-AP5 (50 μ M) was subsequently added to the perfusion bath to isolate the AMPAR component of the MF-mediated transmission. A second HFS applied to the MF input induced a robust PTP followed by a sustained increase in MF EPSP slope that lasted 30 min and was sensitive to DCG-IV (5 μ M) (PTP = 228.6 ± 13.6 of baseline; $p < 0.001$; LTP = $176.7 \pm 5\%$ at 30 min post HFS; $p < 0.001$; DCG-IV depression of the MF response = $32.9 \pm 4\%$ of baseline; $p < 0.001$; RM-ANOVA; $N = 6$; Fig. 3A, bottom panel). In contrast, RC EPSPs were insensitive to DCG-IV ($94.8 \pm 2.75\%$ of baseline 1 h post-FS; $p > 0.15$; one-way ANOVA; Fig. 3A, top panel; Fig. 3A–C).

The results described above indicate that CaMKII activity is required for LTP in CA3 SR/L-M interneurons. However, CaMKII has not been directly observed in CA1 interneurons (Liu and Jones, 1996; Sik et al., 1998) but see Lamsa et al. (2007). Therefore, to determine whether CaMKII is detected in these interneurons, we performed double-immunofluorescence staining on hippocampal sections for the CaMKII isoforms (see the Experimental procedures for details) and glutamate decarboxylase enzyme (GAD-67), the limiting enzyme for GABA synthesis present in interneurons. In slices prepared from rats that were transcardially perfused with PFA, the coexpression of GAD and CaMKII in interneurons of the *stratum lucidum* was virtually inexistent (three interneurons in 150 slices analyzed). We therefore conducted immunohistochemical experiments in slices prepared for *in vitro* recordings before and 5 min after HFS. We found that 32 out of 89 (36%) interneurons co-expressed the phosphorylated α subunit of CaMKII and GAD⁺ whereas in non-stimulated slices, only four out of 90 were immunopositive. As shown in Fig. 4, the merging of the confocal images revealed that GAD-67 immunopositive populations of interneurons located in SR/L-M of area CA3 also were immunopositive for CaMKII. Together, these results suggest that CaMKII is postsynaptically

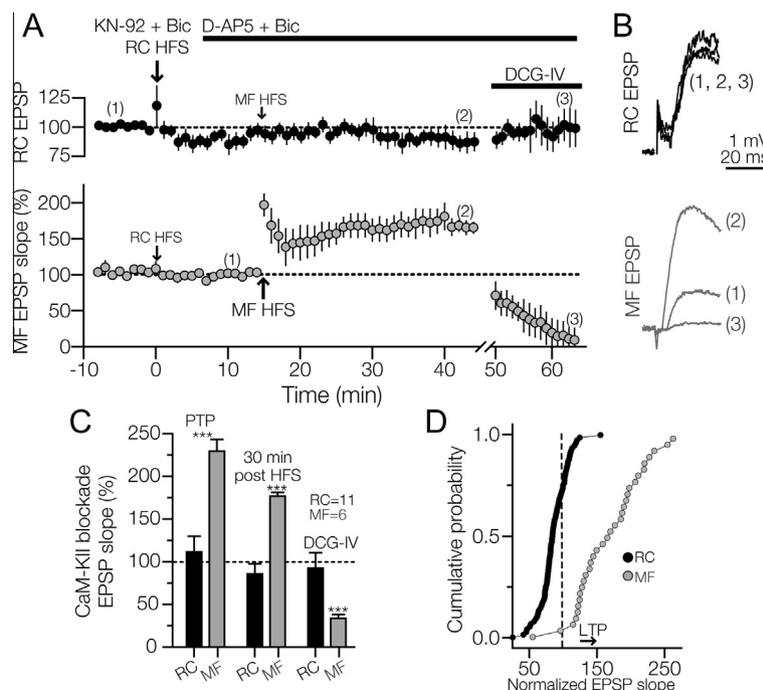


Fig. 3. RC LTP but not MF LTP requires CaMKII activity. The slices were incubated with the CaMKII inhibitor KN-62 (10 μ M, $n = 5$) or KN-93 (10 μ M, $n = 6$) for 50–60 min before experiments and simultaneous recordings of RC and MF EPSPs in the same interneuron were performed in the presence of bicuculline. (A) Averaged time-course graph of RC (upper panel) and MF EPSP slopes (lower panel). RC HFS failed to induce LTP. Ten minutes after HFS, D-AP5 was added to the bath and a second HFS train was applied to the MF input. MF HFS induced a PTP followed by a robust MF LTP sensitive to DCG-IV. HFS on RC did not affect MF EPSPs nor did the HFS on MF affected RC EPSPs indicating that the two inputs converging onto the same cell were functionally independent. (B) RC and MF EPSPs slopes averaged from 10 continuous sweeps obtained at the times indicated in the experiment of A. (C) Summary of the changes for RC and MF EPSPs in the presence of CaMKII blockade. Because similar effects were obtained with KN-62 and KN-93, the data were pooled. Error bars indicate SEM. (D) Cumulative probability chart shows induction of MF LTP and blockade of RC LTP in the presence of KN-93. Dashed line represents the EPSP baseline, for both MF and RC EPSP slopes. Arrowhead indicates a change in the slope above (right arrowhead) or below (left arrowhead) 25% of baseline value. In some experiments, RC HFS induced a depression of the synaptic response in the presence of CaMKII antagonists. *** $p < 0.001$ or higher statistical significance.

expressed in CA3 interneurons in an activity-dependent manner.

Application of forskolin/IBMX does not potentiate RC EPSPs in CA3 interneurons

Among the multiple kinases required for LTP induction, the cAMP-dependent PKA plays an essential role at the Schaffer to CA1 pyramidal cell synapse (Frey et al., 1993; Huang et al., 1994; Blitzer et al., 1995; Duffy and Nguyen, 2003) and at the MF to CA3 pyramidal cell synapse (Weisskopf et al., 1994; Villacres et al., 1998; Calixto et al., 2003). PKA activity is also required for the induction of MF LTP in dentate gyrus basket cells (Alle et al., 2001), and CA3 interneurons in SL-M (Galvan et al., 2010). However, adenylyl cyclase (AC) stimulation has been reported to have mild effects on RC EPSPs in CA3 pyramidal cells and interneurons (Weisskopf et al., 1994; Galvan et al., 2010). We tested whether the signal transduction through the cAMP-PKA cascade plays a role in RC LTP induction in CA3 interneurons. In the presence of bicuculline, a stable baseline of RC and MF EPSPs were concurrently evoked in the same interneuron for 8 min. The co-application of the AC stimulator forskolin (FSK, 50 μ M) with the non-specific inhibitor of cAMP phosphodiesterase IBMX (25 μ M) had contrasting effects on the EPSPs evoked from RC and MF. RC EPSPs were insensitive to AC stimulation during or after washout of the drugs (105.3 \pm 8% of baseline at 10 min after the

onset of FSK + IBMX; $p > 0.05$, RM-ANOVA. 97 \pm 3% of baseline at 30 min after washout; $p > 0.15$; $N = 7$; Fig. 5A, top panel; Fig. 5B, C). In contrast, the FSK + IBMX treatment induced a fast and sustained potentiation of MF EPSPs for at least 30 min after the washout of drugs (440 \pm 29.6% of baseline 10 min after the onset of FSK + IBMX; $p < 0.001$; 265 \pm 42% of baseline after 30 min washout; $p < 0.0001$, RM-ANOVA, $N = 7$; Fig. 5A, bottom panel; Fig. 5B, C). DCG-IV (5 μ M) depressed the MF EPSPs but had no effect on RC EPSPs (RC EPSP in the presence of DCG-IV, 105 \pm 2% of baseline; $p > 0.05$; MF EPSP sensitivity to DCG-IV = 58.7 \pm 8% of baseline; $p < 0.001$, RM-ANOVA). In addition, the PPF ratio of the EPSPs was monitored during these experiments, as illustrated in Fig. 5D. The RC EPSPs remained unchanged in the presence or after a 30-min washout of FSK + IBMX (RC-PPF control = 1.18 \pm 0.02; during FSK + IBMX = 1.1 \pm 0.8; 30 min after washout = 1.15 \pm 0.08, $p > 0.6$; one-way ANOVA). In agreement with our previous results (Galvan et al., 2010), the FSK/IBMX-induced potentiation of the MF EPSP was associated with a decrease in the PPF ratio during the drug application but exhibited a slight recovery after a 30-min washout (MF-PPF control = 1.57 \pm 0.02; during FSK + IBMX = 1.1 \pm 0.3; $p < 0.001$; 30 min after washout = 1.46 \pm 0.03; $p < 0.05$, one-way ANOVA). Although presynaptic PKA activation is sufficient to produce a robust but transient potentiation of transmission at MF synapses on CA3

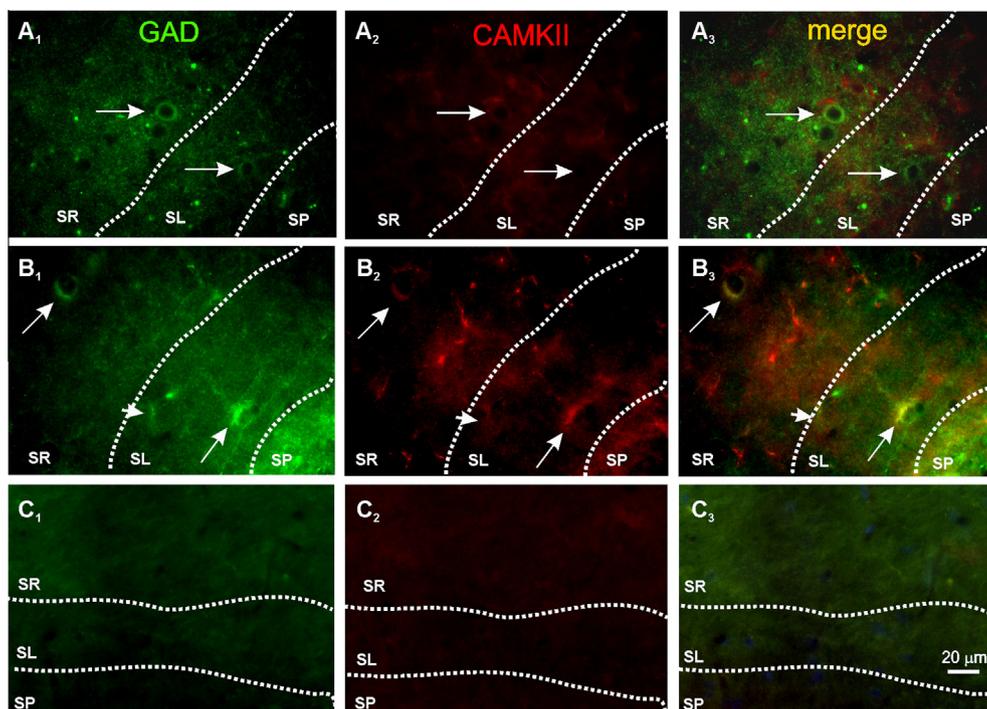


Fig. 4. Co-localization of phospho-CaMKII and GAD₆₇ in CA3 interneurons. Photomicrographs depicting the co-expression of phospho-CaMKII and GAD₆₇. (A1–3) Immunofluorescent images of CA3 hippocampal area of a non-stimulated slice. (B) Co-expression of GAD₆₇ (B1; green) and phospho-CaMKII (B2; red) was observed in the somatic region of two interneurons, one located in stratum radiatum (SR; arrow), and a second one in stratum lucidum (SL; arrow) of CA3b (merge in yellow; B3) of a stimulated slice. The arrowhead signals a GAD₆₇-positive interneuron devoid of CaMKII immunoreactivity. These results were obtained from a slice that was fixated 5 min after HFS. (C) Control experiment in which the IHC was conducted in the absence of the first antibody. The calibration bar at the bottom of the right hand panel corresponds to 20 μ m and applies to all photomicrographs. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

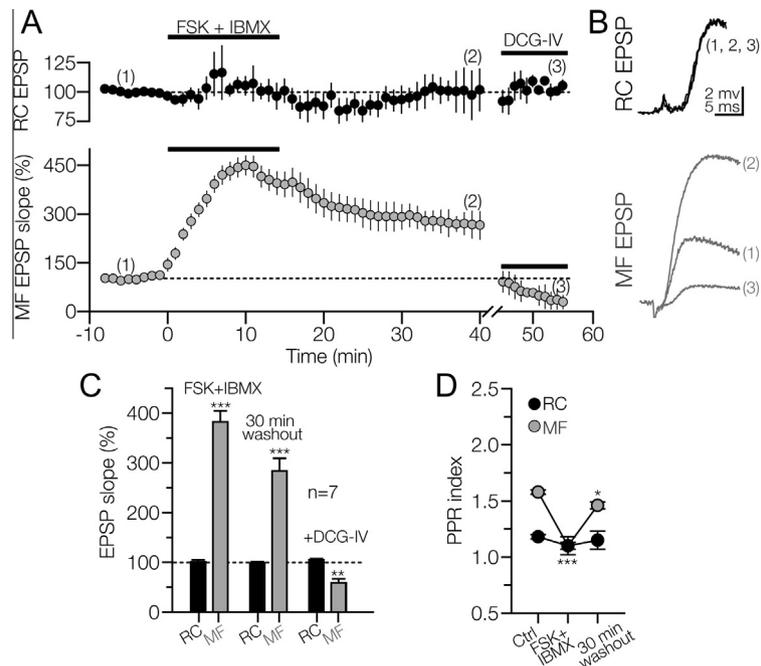


Fig. 5. Forskolin and IBMX potentiate MF EPSPs but not RC EPSPs in CA3 interneurons. Simultaneous recordings of RC and MF EPSPs in the same interneuron in the presence of bicuculline. (A) Graph averaged from six independent experiments showing the time course of RC and MF EPSP and the effect of the adenylyl cyclase stimulator forskolin (FSK; 50 μ M) and the nonspecific phosphodiesterase inhibitor IBMX (25 μ M). RC EPSPs were unaffected by the FSK/IBMX or by DCG-IV treatment. In contrast, MF EPSPs dramatically increased during the FSK/IBMX treatment (2, filled circles) and 30 min after washout the MF EPSP remained potentiated. MF EPSPs were reduced by DCG-IV (3, filled circles). (B) Representative RC and MF EPSP slopes averaged from 10 continuous sweeps at the times indicated by the corresponding numbers in A. (C) Summary of the effects of FSK/IBMX application on MF and RC EPSP slopes. (D) The paired pulse ratio (PPR) for the MF EPSP was decreased during the FSK/IBMX stimulation, and partially recovered 30 min after the washout of the drugs. RC PPR did not change during the experiment. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ or higher statistical significance.

interneurons, the increased PKA activation in the postsynaptic cell is required for the maintenance of FSK/IBMX-induced MF potentiation (Galván et al., 2010). Lack of effects of PKA on RC synapses suggests that in CA3 interneurons PKA is exposed to compartmentalized pools of cAMP locally generated by adenylate cyclases and phosphodiesterases (Michel and Scott, 2002).

Induction of RC and MF LTP in CA3 interneurons rely on postsynaptic PKC activation

Previous studies have shown that PKC is essential for LTP induction at the Schaffer/collateral to CA1 pyramidal cell synapse (Malinow et al., 1989; Hvalby et al., 1994; Wang and Kelly, 1995; Hussain and Carpenter, 2005) and at the MF to CA3 pyramidal cell synapse (Son et al., 1996; Hussain and Carpenter, 2005; Kwon and Castillo, 2008). To assess whether postsynaptic PKC is required for the induction of RC LTP we loaded interneurons with PKC blocker chelerythrine (10 μ M); (Kwon and Castillo, 2008; Galván et al., 2010). In these experiments, a baseline for RC and MF EPSPs was recorded in the same interneuron in the presence of bicuculline. Chelerythrine had little effect on PTP of RC and MF EPSPs but prevented LTP induction at both inputs (RC PTP = $133.2 \pm 5.7\%$ of baseline; $p < 0.001$; RC at 30 min post-HFS = $91.5 \pm 4\%$ of baseline; $p > 0.05$, one-way ANOVA; MF PTP = $188.2 \pm 10\%$ of baseline; $p < 0.001$; MF at 30 min

post-HFS, 85.5 ± 4.4 of baseline; $p < 0.01$; one-way ANOVA; $N = 9$, for both inputs; Fig. 6A–D). DCG-IV decreased the MF responses without affecting the RC EPSP slopes of CA3 interneurons (RC EPSP in the presence of DCG-IV = $105.4 \pm 5\%$ of baseline; $p > 0.05$, one-way ANOVA; MF EPSP in the presence of DCG-IV = $62.6 \pm 5\%$ of baseline; $p < 0.001$, one-way ANOVA). The blockade of PKC with chelerythrine demonstrates that postsynaptic PKC signaling is required for the induction of RC and MF LTP in SR/L-M CA3 interneurons (see model in Fig. 7).

DISCUSSION

The contribution of NMDARs to the induction of long-term plasticity in hippocampal interneurons may be different at synapses expressing CI- and CP-AMPA receptors (Lei and McBain, 2002; Laezza and Dingledine, 2011). For example, previous investigations on CA3 stratum radiatum interneurons reported a form of RC NMDAR-independent LTD that required the coactivation of postsynaptic CP-AMPA receptors and presynaptic mGluR₇ (Laezza et al., 1999). A subsequent study of the same interneuron synapse revealed a form of LTP mediated by CP-AMPA receptors and NMDARs (Laezza and Dingledine, 2004). In the same study, RC LTD was induced by calcium influx either through CP-AMPA receptors or NMDARs, depending on the postsynaptic membrane potential. However, a comparison between those data and our present results may be

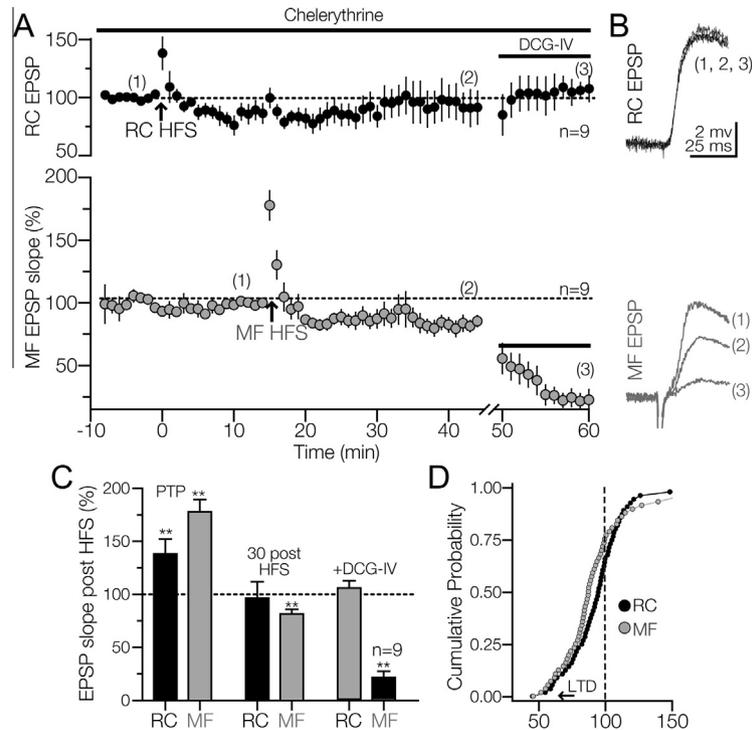


Fig. 6. Postsynaptic PKC activity is required for LTP at RC and MF synapses on CA3 interneurons. Cells were loaded with the PKC inhibitor, chelerythrine (10 μ M) for at least 15 min before the experiment. (A) HFS failed to induce RC LTP and triggered a significant MF LTD. (B) Representative RC and MF EPSP slopes obtained from 10 consecutive traces at the indicated time of A. (C) Summary of the effects of HFS; RC and MF PTP were unaffected but induction of LTP was blocked. (D) Cumulative probability of responses for RC and MF following the HFS. Dashed line represents the EPSPs baseline. Arrowhead indicates a decrease in the slope below 25% of the baseline value. $**p < 0.01$ or higher statistical significance.

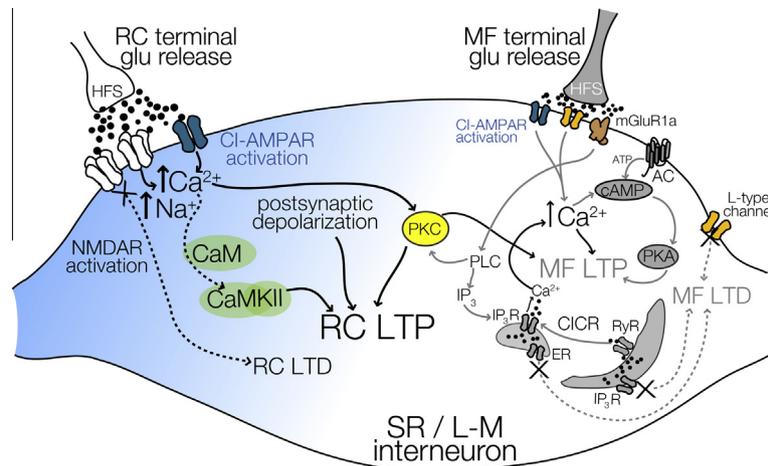


Fig. 7. Schematic representation of the compartmentalized signaling cascades for LTP induction at RC and MF synapses on the spiny dendrite of CA3 SR/L-M interneurons. LTP at both synapses mostly containing Ci-AMPA receptors has a strong dependence on presynaptic activation and rise in postsynaptic calcium levels. MF LTP induction requires two sources of calcium: (1) from the extracellular space via L-type VGCCs and (2) via mGluR₁ dependent phospholipase C (PLC) pathway to promote IP_3 -mediated Ca^{2+} mobilization and the AC-cAMP-PKA signaling cascade (see Galván et al. (2008, 2010) for details). In contrast, RC LTP only requires calcium from the activation of NMDARs, and is dependent on CaMKII activation. PKC-dependent signaling modulation is the point of convergence for signaling molecules originating from RC and MF synaptic inputs.

problematic because of age differences in the rats used in the two studies (P9–P12 vs. P30–P40, respectively). Here we show that in the absence of functional NMDARs, RC synapse mostly containing Ci-AMPA receptors exhibit a comparatively small but significant LTD that relies on calcium entry, possibly via L-type VGCCs (Galván et al., 2008).

We also demonstrate that RC LTP exclusively depends on CaMKII activity, in agreement with the findings that GAD-67-positive SR/L-M interneurons are immunoreactive to CaMKII isoforms. However, by conducting immunohistochemistry experiments to detect CAMKII and phospho-CAMKII, we found

phospho-CaMKII in 36% of interneurons of SL and SR only if the recorded slices were fixed 5 min after the HFS. If the slices were fixed after more than 30 min post-HFS, the labeling of CaMKII and phospho-CaMKII was not detected. This may suggest that HFS transiently elicits phosphorylation of CaMKII or *de novo* expression of phospho-CaMKII. Earlier work on CA1 interneurons with somata in *stratum pyramidale* revealed that CaMKII activity up-regulates AMPAR-mediated transmission by inducing the conversion of inactive-to-active synapses (Wang and Kelly, 2001). While all four CaMKII isoforms (α , β , γ , and δ) are present in the brain (Takaishi et al., 1992), CaMKII α and CaMKII β are predominantly found in neurons. CaMKII α expression is localized to excitatory neuronal populations (Jones et al., 1994) but it has not been found in GABAergic neurons (Benson et al., 1992; Ochiishi et al., 1994; Sik et al., 1998). Autophosphorylation of CaMKII α is essential for NMDAR-dependent LTP in the hippocampus (Lisman et al., 2002) and in the neocortex (Hardingham et al., 2003). In the CaMKII α T286A-mutant mice, NMDAR-dependent LTP expression at the Schaffer commissural-CA1 pyramidal cell synapse is absent (Giese et al., 1998; Cooke et al., 2006). However, in the same strain of mutant mice, LTP is inducible at the medial perforant path input to dentate gyrus granule cells (Cooke et al., 2006), and in CA1 inhibitory interneurons (Lamsa et al., 2007). Therefore, the induction of some forms of NMDAR-dependent LTP do not rely on the auto phosphorylation of threonine 286 in the CaMKII α isoform (Lamsa et al., 2007). Because there are no isoform-selective inhibitors of CaMKII, we were unable to determine whether the specific activation of CaMKII α plays a key role in RC LTP. In agreement with previous reports that CaMKII auto phosphorylation is not involved in MF LTP in CA3 pyramidal cells (Salin Paul et al., 1996; Kakegawa et al., 2004). CaMKII inhibition did not prevent the subsequent induction of MF LTP in the same interneuron. Taken together, our data suggest that the initial steps required for the induction of RC LTP in SR/L-M interneurons are similar to those reported to underlie NMDAR-dependent LTP at synapses containing CI-AMPA located on the spiny dendrites of pyramidal cells.

The sustained activation of the AC-cAMP-PKA effector system by forskolin elicited robust MF potentiation but did not affect RC synapses in the same interneuron. The contrasting effects of forskolin on RC and MF synapses have been previously documented in CA3 pyramidal cells (Weisskopf et al., 1994). Interestingly, the signaling cascades for LTP induction differ across different interneuron subtypes, likely reflecting a diversity in dendritic Ca²⁺ signaling in these cells (Goldberg and Yuste, 2005; Camire and Topolnik, 2012). For example, MF synapses on dentate gyrus basket cells and SR/L-M interneurons also undergo long-lasting synaptic enhancement during AC stimulation with forskolin (Alle et al., 2001; Galván et al., 2010). In contrast, naïve MF synapses in *stratum lucidum* interneurons are insensitive to forskolin stimulation (Maccafferri et al., 1998; Lawrence and McBain, 2003) indicating lack of PKA-mediated signaling.

Irrespective of the main source of postsynaptic Ca²⁺ influx that triggers RC and MF LTP, both forms of

Hebbian plasticity involve PKC activation. Furthermore, postsynaptic application of chelerythrine prevented the induction of both forms of LTP, thus confirming the participation of PKC activation in NMDAR-dependent LTP (Ling et al., 2002) and NMDAR-independent LTP at MF synapses (Kwon and Castillo, 2008; Galván et al., 2010).

SR/L-M interneurons lack dendritic spines, which provide the necessary biochemical compartment for input-specific plasticity in pyramidal cells (Yuste and Denk, 1995; Goldberg et al., 2003; Bourne and Harris, 2008). However, the dendritic shafts of CA1 interneurons possess specialized asymmetric synaptic junctions that use glutamate as neurotransmitter (Harris and Landis, 1986), and experience dendritic remodeling driven by synaptic activity (Chen et al., 2011; Guirado et al., 2013). Another example of complex signaling in aspiny dendrites is present in fast-spiking interneurons of the neocortex. These interneurons possess highly localized Ca²⁺ signaling due to the presence of microdomains associated with CP-AMPA, potentially allowing synapse-specific biochemical compartmentalization in the absence of dendritic spines (Goldberg et al., 2003; Goldberg and Yuste, 2005). In part, dendritic compartmentalization in the aspiny dendrite may be due to specific barriers to calcium diffusion, and the movement of second-messenger molecule (Soler-Llavina and Sabatini, 2006). We hypothesize that at RC and MF synapses, CI-AMPA also have spatially restricted Ca²⁺ micro domains associated with NMDARs and L-type VGCCs/mGluR₁, respectively. The contrasting induction requirements for RC and MF LTP also suggest that scaffolding and anchoring proteins adjacent to RC and MF synapses are different. While little information is available regarding the anchoring proteins expressed on hippocampal interneurons (Sik et al., 2000), our data suggest that different groups of scaffolding proteins may be coupled to excitatory synapses on interneurons (Wong and Scott, 2004; Sanderson and Dell'Acqua, 2011). It is possible that compartmentalization of signaling cascades also could be due to the spatial segregation of MF and RC synapses onto different dendritic branches (Cosgrove et al., 2010).

At the Schaffer-CA1 pyramidal cell synapse, LTP expression requires incorporation of new AMPARs following HFS. The delivery of GluR1-containing AMPAR requires CaMKII activity in a PDZ protein-dependent fashion (Hayashi et al., 2000; Poncer et al., 2002; Malinow, 2003) but see (Adesnik and Nicoll, 2007). Similarly, in CA3 pyramidal cells RC LTP but not MF LTP is expressed by the replacement of AMPARs with newly incorporated CP AMPARs. Although we have no direct evidence for the incorporation of newly synthesized CP-AMPA in SR/L-M interneurons, RC LTP occurs at synapses mainly comprised of CI-AMPA and requires NMDAR and CaMKII activation. A parsimonious hypothesis is that RC LTP expression in these interneurons results from the incorporation of newly synthesized CP-AMPA. The trafficking of CP-AMPA is triggered by postsynaptic CaMKII activity, a mechanism that is absent at the MF synapse (Kakegawa et al., 2004). This is in

agreement with our findings showing that MF LTP in SR/L-M interneurons is unaffected by CaMKII blockade.

Computational and behavioral studies (McNaughton and Morris, 1987; Treves and Rolls, 1992; O'Reilly and McClelland, 1994; Lisman, 1999; Leutgeb et al., 2007) have proposed that during pattern separation, the dentate gyrus has the ability to generate sparse memory representations conveyed to the CA3 network via the MF pathway. These studies also suggest that the RC connectivity between CA3 pyramidal cells operates as an autoassociative network capable of reestablishing previously stored representations based on noisy or degraded cues via pattern completion. Pattern separation and pattern completion involve the obligatory contribution of the parallel activation of feed-forward inhibitory interneurons to maintain the temporal window for synaptic integration and restrict the spurious activation of non-assembly pyramidal cells (Pouille and Scanziani, 2001; Perez-Orive et al., 2002; Sahay et al., 2011). The preservation of the balance between monosynaptic excitation and disinaptic inhibition requires near simultaneous LTP induction at excitatory synapses on pyramidal cells and interneurons (Lamsa et al., 2005; Carvalho and Buonomano, 2009; Rolls, 2013). Our results indicate that SR/L-M feed-forward inhibitory interneurons in area CA3 have the ability to express two mechanistically distinct forms of Hebbian LTP at CI-AMPA synapses. Functionally, synapse-specific compartmentalization of MF and RC LTP signaling in the aspiny dendrite enables SR/L-M interneurons to participate in the dual mnemonic processes of pattern separation and pattern completion.

CONCLUSION

The aspiny dendrites of CA3 SR/L-M interneurons compartmentalize the initial steps in the signaling transduction cascades implicated in the induction of Hebbian LTP at RC and MF synapses predominantly containing CI-AMPA receptors. Both forms of synaptic plasticity were prevented by postsynaptic injections of the calcium chelator BAPTA. However, RC LTP depends on Ca^{2+} influx via the NMDARs whereas MF LTP requires cytosolic Ca^{2+} increase from the coactivation of L-type VGCCs and mGluR₁ (Galvan et al., 2008). Despite the absence of dendritic spines, SR/L-M interneurons have the capability to spatially restrict the signaling calcium cascades that lead to two mechanistically distinct forms of Hebbian LTP.

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