Brief Communications

Activation Conditions for the Induction of Metabotropic Glutamate Receptor-Dependent Long-Term Depression in Hippocampal CA1 Pyramidal Cells

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Two forms of homosynaptic long-term depression (LTD) are distinguished in hippocampal CA1 pyramidal cells, one which is NMDA receptor dependent and the other metabotropic glutamate receptor (mGluR) dependent. Although the molecular processes involved in mGluR-LTD are well characterized, the conditions of circuit activation required for its induction remain unclear. We show that mGluR-LTD cannot be induced in synaptically coupled CA3–CA1 pyramidal cell pairs. Experiments to address the underlying mechanisms indicate that, even when glutamate transporters are blocked, one presynaptic cell releases insufficient glutamate to evoke an mGluR-mediated current in a connected CA1 cell. These findings imply that extrasynaptic diffusion is not a limiting factor and are consistent with a sparse distribution of functional mGluRs in the dendritic tree of pyramidal cells. Thus, the discharge of multiple Schaffer collaterals to a targeted cell is necessary for mGluR-LTD. Our experiments indicate that approximately eight CA3 inputs to a CA1 pyramidal cell must be activated to induce mGluR-LTD.

Introduction

Homosynaptic long-term depression (LTD) in the hippocampus is well characterized as an NMDA receptor-dependent form of synaptic plasticity (NMDAR-LTD), as first described at the Schaffer collateral input to CA1 pyramidal cells (Dudek and Bear, 1992; Mulkey and Malenka, 1992). An additional form of LTD at Schaffer collateral synapses onto CA1 pyramidal cells that requires activation of metabotropic glutamate receptors (mGluRs) (mGluR-LTD) was also reported (Stanton et al., 1991; Bolshakov and Siegelbaum, 1994; Oliet et al., 1997). Significant progress has been made in understanding the induction mechanisms and intracellular transduction pathways involved in each form (Kemp and Bashir, 2001; Anwyl, 2006; Bellone et al., 2008). Both appear to be induced postsynaptically (Oliet et al., 1997), but whether their expression relies exclusively on postsynaptic processes (Snyder et al., 2001; Xiao et al., 2001; Zhou et al., 2004; Zhang et al., 2006; Brager and Johnston, 2007), or rather involves presynaptic reduction in neurotransmitter release probability (Bolshakov and Siegelbaum, 1994; Fitzjohn et al., 2001; Watabe et al., 2002; Enoki et al., 2009), remains under debate (Lisman, 2009). For the induction of NMDAR-LTD, it has been shown that cooperative interactions of multiple presynaptic inputs are not essential, such that appropriately timed discharge of a single CA3 pyramidal cell can lead to homosynaptic LTD in a targeted CA1 pyramidal cell (Debanne et al., 1996). A corresponding investigation of the circuit requirements for induction of mGluR-LTD is at present lacking. Here, we recorded from synaptically connected pairs of CA3 and CA1 hippocampal pyramidal cells in organotypic slice cultures and find that, in contrast to NMDA-LTD, multiple Schaffer collateral input is required for the induction of mGluR-LTD in a targeted CA1 pyramidal cell.

Materials and Methods

Slice cultures and electrophysiology. Hippocampal organotypic slice cultures were prepared from 6-d-old Wistar rats, as described previously (Gähwiler et al., 1998). After 3–4 weeks, *in vitro* slice cultures were transferred to a recording chamber mounted on an upright microscope (Axioskop FS; Zeiss). Slices were superfused at 1.5 ml/min with artificial CSF (ACSF) containing the following (in mM): 137 NaCl, 2.7 KCl, 11.6 $NaHCO_3$, 0.4 NaH_2PO_4, 2.0 $MgCl_2$, 2.8 $CaCl_2$, 5.6 D-glucose, and 10 mg/L phenol red, pH 7.4 (~305 mOsm; 33°C; saturated with 95% O_2, 5% CO_2). Both low-resistance (3–5 MΩ) and high-resistance (10–15 MΩ) recording pipettes were filled with the following (in mM): 120 K-gluconate, 10 L-glutamic acid, 5 KCl, 10 HEPES, 1 EGTA, 2 MgATP, 5 creatine phosphate, 0.4 NaGTP, 0.07 $CaCl_2$, pH 7.2 (~290 mOsm). For paired recordings, postsynaptic pipettes were filled with the following (in mM): 126.6 CsF, 8.4 CsCl, 10 HEPES, 10 EGTA, 2 MgATP, 5 creatine phosphate, 0.4 NaGTP, pH 7.2 (~290 mOsm). Neuronal responses were recorded with an Axopatch 200B amplifier (pClamp 9; Molecular Devices) and corrected for liquid junction potentials. Signals were filtered at 5 kHz and analyzed off-line. All numerical data are means ± SEM. Significance was tested using the two-tailed *t* test.

For perforated patch-clamp recording, a stock solution of amphotericin B (60 mM) in DMSO was added to the pipette solution to a final concentration of 0.3 mM. Fresh solution was prepared every 1.5 h, sonicated 5–10 min, and kept in the dark.

Extracellular stimulation. For extracellular stimulation of Schaffer collaterals, glass pipettes filled with ACSF were placed in CA3 stratum ra-

Received Nov. 13, 2009; accepted Dec. 15, 2009.

This work was supported by the Swiss National Science Foundation. W.F. was supported by a Neuroscience Center Zurich PhD fellowship. We thank D. Göckeritz-Dujmovic, H. Kasper, S. Giger, and R. Schöb for excellent technical assistance. We are grateful to B. Gähwiler and G. Coiret for suggestions and for critically reading this manuscript and to the members of the Helmchen Laboratory for valuable discussions.

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DOI:10.1523/JNEUROSCI.5619-09.2010

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diatum, 100–200 μm from the recorded cell. In supplemental Figure 3 (available at www.jneurosci.org as supplemental material), the monopolar stimulation pipette was positioned in CA3 stratum oriens. Stimulation intensity was 10–20 μA, which induced a response of 50–70% of maximum. To evoke mGluR-mediated current, high-frequency stimulation (100 Hz; one to four pulses; 100 μs duration) was applied. AMPA, NMDA, $GABA_A$, and $GABA_B$ receptors were blocked by 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) (50 μM), D-(−)-2-amino-5-phosphonopentanoic acid (D-AP5) (50 μM), picrotoxin (100 μM), and ((3–1-(*R*)-((4-methoxyphenyl-methyl)hydroxyphosphinyl)-2-(*S*)-hydroxypropyl)aminoethyl)benzoic acid (CGP 62349) (5 μM), respectively.

Drugs. CNQX, D-AP5, and (*S*)-α-methyl-4-carboxyphenylglycine [(*S*)-MCPG] were purchased from Ascent Scientific. DL-Threo-b-benzyloxyaspartic acid (DL-TBOA) was from Tocris. CGP 62349 was kindly provided by Novartis. All other chemicals were from Sigma-Aldrich/Fluka.

Results

A single CA3 pyramidal cell does not induce mGluR-LTD in a connected CA1 pyramidal cell

In the presence of D-AP5 (50 μM), a standard LTD protocol evoking a low-frequency train of action potentials (APs) in a CA3 pyramidal cell (1 Hz; 900 APs) failed to induce mGluR-LTD in a synaptically connected CA1 pyramidal cell in hippocampal slice cultures (98 ± 4% of baseline, $n = 6$, $p = 0.5$; recording pipette resistance, 3–5 MΩ) (Fig. 1*A*; supplemental Fig. 1, available at www.jneurosci.org as supplemental material). Because certain forms of synaptic plasticity are disrupted by cytoplasmic washout (Kullmann and Lamsa, 2007), this experiment was repeated using high-resistance recording pipettes (10–15 MΩ). Again, LTD was not observed (98 ± 3% of baseline; $n = 3$; $p = 0.6$) (Fig. 1*A*). Paired-pulse low-frequency stimulation (PP-LFS) represents a more efficient protocol for the induction of mGluR-LTD (Kemp and Bashir, 1997). However, even PP-LFS (1 Hz; 900 paired APs; 50 ms interstimulus interval; 10–15 MΩ for the CA1 electrode) did not induce mGluR-LTD in CA3–CA1 connected cell pairs (99 ± 4% of baseline; $n = 4$; $p = 0.6$) (Fig. 1*B*). As postsynaptic mGluRs in hippocampal pyramidal cells are localized in perisynaptic and extrasynaptic zones (Lujan et al., 1996), membrane transporters may prevent glutamate from accessing these receptors. But even in the presence of TBOA (10 μM), a glutamate transporter blocker (Jabaudon et al., 1999), LTD was not induced (102 ± 6% of baseline; $n = 3$; $p = 0.8$) (Fig. 1*B*). Together, these results suggest that activity in a single CA3 pyramidal cell is insufficient to induce mGluR-LTD in a targeted CA1 pyramidal cell. In contrast, when Schaffer collaterals were stimulated with an extracellular electrode (LFS, stimulation intensity, 10–20 μA), thereby activating several CA3 pyramidal cell axons, mGluR-LTD was consistently observed (70 ± 3% of baseline; $n = 8$; $p < 0.001$) (Fig. 1*C*). Interestingly, mGluR-LTD induced by extracellular stimulation was significantly enhanced when recording with high-resistance pipettes (42 ± 3% of baseline; $n = 5$; $p < 0.001$; high- vs low-resistance pipettes, $p < 0.001$) (Fig. 1*D*), indicating that cytoplasmic dialysis significantly disrupts metabotropic signaling. Application of (*S*)-MCPG (500 μM) prevented mGluR-LTD (low-resistance pipettes: 100 ± 4% of baseline, $n = 5$, $p = 0.95$; high-resistance pipettes: 103 ± 6%, $n = 5$, $p = 0.6$) (Fig. 1*C*). In the relatively young slice cultures used here (3–4 weeks), the PP-LFS protocol did not further enhance mGluR-LTD ($n = 5$; $p = 0.98$), and blocking glutamate transporters with TBOA had no significant effect ($n = 5$; $p = 0.4$) (supplemental Fig. 2, available at www.jneurosci.org as supplemental material).

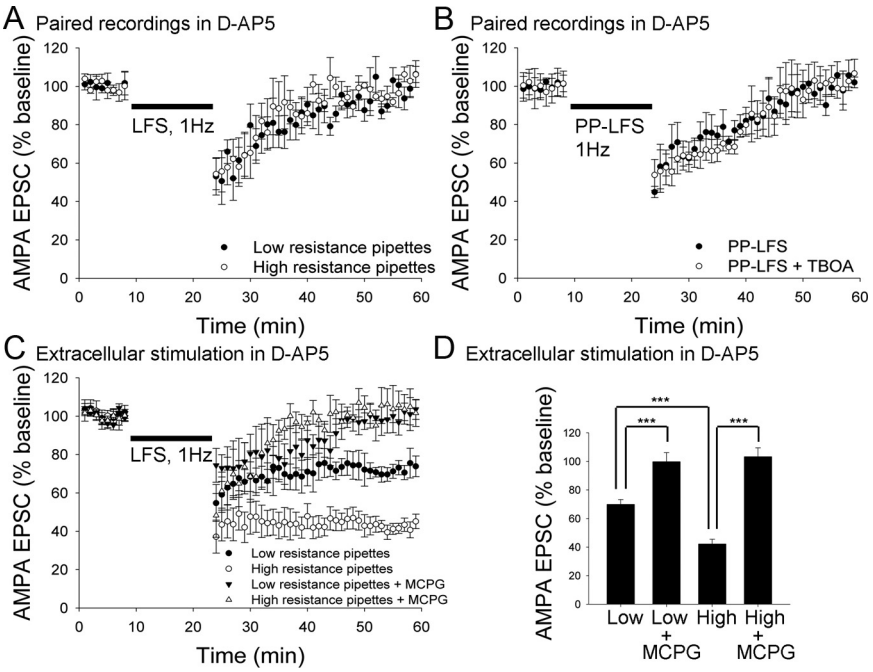

Figure 1. Activation of a single CA3 pyramidal cell is insufficient to induce mGluR-LTD in a synaptically connected CA1 pyramidal cell. ***A***, In the presence of D-AP5, a low-frequency train of action potentials (1 Hz; 900 APs) in a CA3 cell did not induce mGluR-LTD in a CA1 cell ($n = 6$), even when high-resistance recording pipettes were used to minimize dialysis ($n = 3$). ***B***, A low-frequency train of paired action potentials (PP-LFS) also failed to induce mGluR-LTD ($n = 4$) even when glutamate transporters were blocked with TBOA ($n = 3$). ***C***, mGluR-LTD can be induced in a CA1 pyramidal cell with extracellular stimulation of Schaffer collaterals (1 Hz; 900 pulses; 10–20 μA; in D-AP5). The amplitude of mGluR-LTD was significantly greater when high-resistance pipettes were used ($n = 8$ and 5 for low- and high-resistance pipettes, respectively; $p < 0.001$). (*S*)-MCPG (500 μM) prevented mGluR-LTD ($n = 5$). ***D***, Summary of results from extracellular stimulation experiments. Error bars indicate SEM. ***$p < 0.001$.

When Schaffer collaterals are stimulated extracellularly, it can be difficult to rule out that the perforant pathway, other CA1 pyramidal cells, or subicular cells are inadvertently activated. In our slice cultures, however, the subiculum is removed, and perforant path fibers have completely disappeared after 2 weeks in culture (Zimmer and Gähwiler, 1984). Furthermore, monosynaptic connectivity between CA1 pyramidal cells is 0.8% in acute slices (Thomson and Radpour, 1991) and marginally higher in slice cultures. Nevertheless, we performed experiments in which Schaffer collaterals were activated with the stimulating electrode positioned in the stratum oriens instead of the stratum radiatum of CA3 ($n = 4$), making it unlikely that CA1 pyramidal cell axons are activated. The data show that mGluR-LTD was not significantly different ($p = 0.9$; stratum oriens: 43 ± 4% of baseline, $n = 4$; stratum radiatum: 42 ± 3% of baseline, $n = 5$) (supplemental Fig. 3, available at www.jneurosci.org as supplemental material).

A single CA3 pyramidal cell does not evoke a somatic current mediated by mGluRs in a connected CA1 pyramidal cell

To examine why mGluR-LTD could not be induced between pyramidal cell pairs, we checked whether an mGluR-mediated current could be evoked in a CA1 pyramidal cell in response to firing of a single connected CA3 pyramidal cell. After blockade of

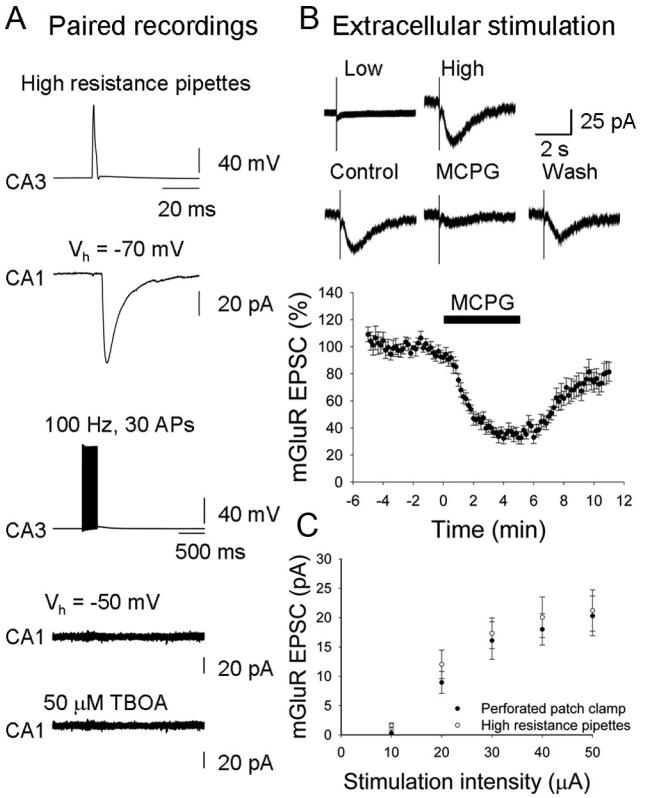

Figure 2. Activation of a single CA3 pyramidal cell does not evoke a discernible somatic current mediated by mGluRs in a synaptically connected CA1 pyramidal cell. ***A***, To enhance mGluR-mediated currents, postsynaptic CA1 cells were voltage clamped at −50 mV using recording pipettes with a resistance of 10–15 MΩ. Top, A single AP in a CA3 pyramidal cell elicited a fast EPSC in a CA1 pyramidal cell ($V_h = -70$ mV). Bottom, Blocking NMDA (50 μM D-AP5), AMPA/kainate (50 μM CNQX), $GABA_A$ (100 μM picrotoxin), and $GABA_B$ receptors (5 μM CGP 62349) failed to reveal a slow metabotropic current in response to a 100 Hz train of 30 APs. Inhibition of glutamate transporters (50 μM TBOA) under these conditions also did not reveal an mGluR-mediated current. Traces represent averages of 10 sweeps. ***B***, Top, Extracellular stimulation of Schaffer collaterals (100 Hz; 4 pulses; 30 μA; $V_h = -50$ mV) did not evoke an mGluR-mediated response in CA1 pyramidal cells when recording pipettes of 5 MΩ were used. The same stimulation evoked a slow EPSC when using high-resistance recording pipettes (12 MΩ). Bottom, The slow EPSC was inhibited by (*S*)-MCPG (500 μM; $n = 13$). Responses were elicited at 0.1 Hz. Traces represent averages of five sweeps. ***C***, Average amplitudes of mGluR EPSCs evoked with extracellular stimulation ($n = 8$ and 5 for high-resistance pipettes and perforated patch-clamp, respectively). Error bars indicate SEM.

NMDA, AMPA/kainate, $GABA_A$, and $GABA_B$ receptors, even a high-frequency train of 30 APs at 100 Hz failed to evoke a metabotropic response in a CA1 pyramidal cell recorded with a high-resistance pipette at −50 mV, a membrane potential at which mGluR-mediated currents are maximal (Gee et al., 2003) ($n = 3$) (Fig. 2*A*, bottom). Blocking glutamate transporters (TBOA, 50 μM) also did not reveal a metabotropic response ($n = 3$) (Fig. 2*A*, bottom). A similar negative result was obtained with the perforated patch-clamp method ($n = 3$) (supplemental Fig. 4*A*, available at www.jneurosci.org as supplemental material).

With extracellular stimulation of Schaffer collaterals (100 Hz; four pulses; 20–50 μA; 100 μs duration), mGluR-mediated EPSCs were consistently evoked in CA1 pyramidal cells, as previously reported (Congar et al., 1997; Nakamura et al., 1999). A metabotropic response was, however, only observed with high-resistance recording pipettes ($n = 10$ and 28 for low- and high-resistance pipettes, respectively) (Fig. 2*B*, top). The time to peak current (933.3 ± 67.6 ms; $n = 28$) and the 90–10% decay time (2927.2 ± 187.0 ms; $n = 28$) of these EPSCs were two orders of magnitude greater than those of ionotropic AMPA EPSCs. Consistent with responses mediated by group I mGluRs, the EPSCs were blocked by (*S*)-MCPG (500 μM; $n = 13$) (Fig. 2*B*, bottom). Furthermore, TBOA (10 μM) significantly potentiated mGluR EPSCs evoked by extracellular stimulation (192 ± 24% of baseline; $n = 6$; $p < 0.05$) (supplemental Fig. 5, available at www.jneurosci.org as supplemental material). Similar results were obtained with the perforated patch-clamp method, with no significant difference in response amplitude obtained with high-resistance pipettes ($n = 8$) versus perforated patch-clamp ($n = 5$; $p > 0.3$) (Fig. 2*C*; supplemental Fig. 4*B*, available at www.jneurosci.org as supplemental material).

Estimating the number of Schaffer collaterals required to induce mGluR-mediated responses

As stimulation of a single CA3 pyramidal cell did not induce a metabotropic current in a CA1 pyramidal cell, we performed experiments to estimate the minimum number of synaptic inputs to a CA1 pyramidal cell that must be activated to evoke an mGluR-mediated response. We determined that a threshold stimulation intensity of 20 μA applied to the Schaffer collaterals (100 Hz; two pulses;100 μs duration) is necessary to evoke a discernible mGluR-mediated current of ~10 pA in CA1 pyramidal cells voltage clamped at −50 mV ($n = 5$) (Fig. 3*A*; supplemental Fig. 6*A*, available at www.jneurosci.org as supplemental material). The identical two pulse stimulation protocol evoked two AMPA EPSCs, whose mean amplitude was ~470 pA at 20 μA, derived from the exponential fit of the curve ($f = y_0 + a^*(1 - \exp(-b^*x))$) (Fig. 3*B*; supplemental Fig. 6*B*, available at www.jneurosci.org as supplemental material). As the median amplitude of the two unitary AMPA responses was 23.1 pA ($n = 22$ pairs) (Fig. 3*C*; supplemental Fig. 6*C*, available at www.jneurosci.org as supplemental material), we can estimate that ~20 CA3 synaptic inputs released glutamate to yield a response of 470 pA. Therefore, ~20 CA3 pyramidal cell axons targeting a given CA1 pyramidal cell must be activated concurrently to induce a somatic current mediated by group I mGluRs. We then checked whether the threshold stimulation intensity for mGluR-LTD is similar to that required to induce an mGluR-mediated current. We found that a much lower stimulation intensity of 10 μA (at 1 Hz) was sufficient to induce mGluR-LTD. At a still lower stimulation intensity of 5 μA, an AMPA receptor-mediated current was no longer observed. As glutamate binds with a ~30-fold higher affinity to mGluRs (Conn and Pin, 1997) than to AMPA receptors (Jonas and Sakmann, 1992), it is nonetheless possible that this lower stimulation intensity might induce mGluR-LTD. However, when stimulation intensity was reduced to 5 μA, LTD was no longer observed (5 μA: 101 ± 4% of baseline, $p = 0.9$; 10 μA: 46 ± 7% of baseline, $p < 0.05$; $n = 3$) (Fig. 3*D*). Thus, an intensity of 10 μA is close to threshold for inducing mGluR-LTD. A stimulus of 10 μA evokes an extracellular AMPA receptor-mediated response of ~190 pA (Fig. 3*B*), which when divided by the median value of a single unitary AMPA response (23.3 pA; $n = 22$ pairs) (Fig. 3*C*) provides an estimate of eight active CA3 inputs required to induce mGluR-LTD in a targeted CA1 pyramidal cell.

Discussion

The main finding of this study is that, in contrast to NMDAR-LTD (Debanne et al., 1996), mGluR-LTD assessed by somatic recording in CA1 pyramidal cells could not be induced by stimulating a single synaptically connected CA3 pyramidal cell. A number of possibilities may account for this difference. First,

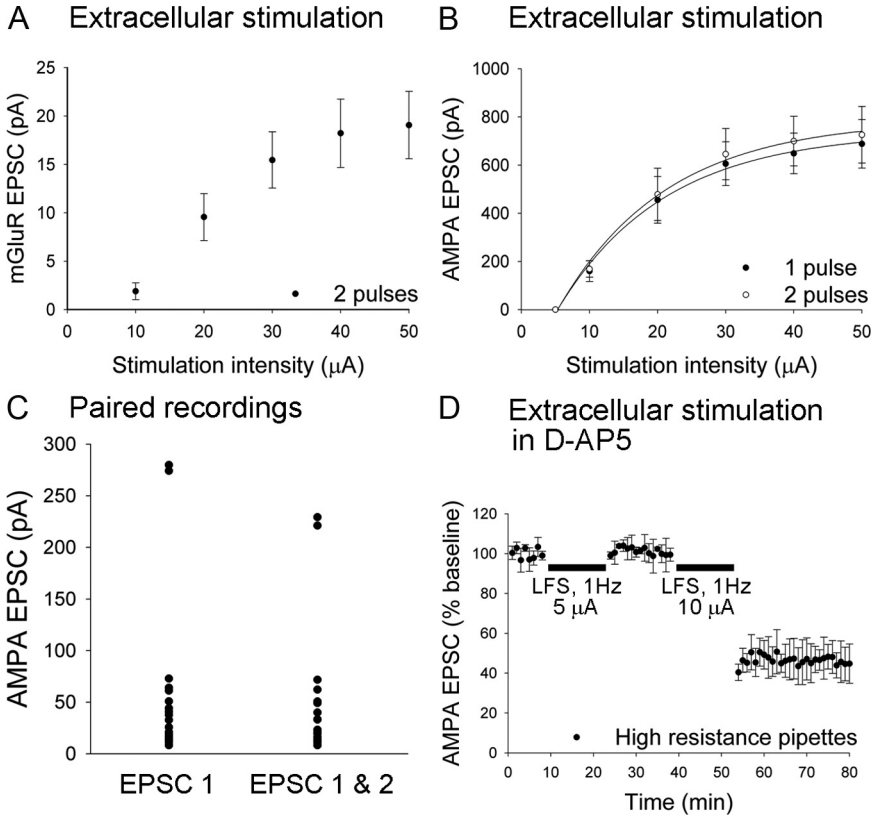

Figure 3. Estimating the number of CA3 axons targeting one CA1 pyramidal cell that must be activated to induce an mGluR-mediated current or mGluR-LTD. ***A***, Average amplitudes of mGluR EPSCs evoked with two pulses at 100 Hz were plotted against stimulation intensity (10–50 μA; $V_h = -50$ mV; $n = 5$; high-resistance pipettes). ***B***, AMPA EPSCs in CA1 pyramidal cells elicited with extracellular stimulation of Schaffer collaterals with one or two pulses at 100 Hz ($V_h = -70$ mV). Average amplitudes of AMPA EPSCs were plotted against stimulation intensity ($n = 7$). ***C***, Paired recordings between CA3–CA1 pyramidal cells. Four APs (100 Hz) triggered in a CA3 pyramidal cell elicited AMPA EPSCs in a connected CA1 pyramidal cell voltage-clamped at -70 mV. Amplitudes of the first EPSCs and the average amplitudes of the first and second EPSCs are shown ($n = 22$). Outliers may reflect multiquantal release (Tong and Jahr, 1994). ***D***, mGluR-LTD can be induced in a CA1 pyramidal cell with extracellular stimulation of Schaffer collaterals (1 Hz; 900 pulses; in 50 μM D-AP5) with a stimulation intensity of 10 μA, but not 5 μA ($n = 3$). The amplitude of the AMPA response, evoked with a stimulation intensity corresponding to the threshold to induce mGluR-LTD or an mGluR-mediated EPSC, was divided by the amplitude of a unitary AMPA response to estimate the number of inputs to a CA1 pyramidal cell required for an mGluR-mediated response. Error bars indicate SEM.

NMDA receptors in CA1 pyramidal cells are usually located in the middle of the synaptic disk (Racca et al., 2000), whereas metabotropic receptors display a perisynaptic or extrasynaptic localization (Lujan et al., 1996). Thus, cooperative presynaptic activity could be necessary to allow pooling and diffusion of neurotransmitter to the more distantly positioned metabotropic receptors, as demonstrated for activation of $GABA_B$ receptors (Scanziani, 2000). This mechanism, however, does not appear to be involved in inducing mGluR current, as inhibition of glutamate transporters with TBOA to promote synaptic spillover of neurotransmitter did not reveal a response. A second possibility is that, as opposed to NMDA receptors, which are expressed in virtually all spines in CA1 pyramidal cells (Takumi et al., 1999; Racca et al., 2000), the expression of dendritic mGluRs may be relatively low, such that diffusion of transmitter from a single release site will rarely reach an mGluR-positive spine. Evidence for a sparse distribution of functional dendritic mGluRs is provided in a recent study showing that metabotropic responses in CA1 pyramidal cells could only be induced in a subset of endoplasmic reticulum-containing mushroom spines (Holbro et al., 2009), which represent <10% of all spines. Furthermore, the failure rate with glutamate uncaging for mGluR-mediated responses in mGluR-positive spines was ~80%. Even though a single CA3 pyramidal cell can make multiple functional contacts with a synaptically coupled CA1 pyramidal cell (Hsia et al., 1998)—estimated at between two and four (Sorra and Harris, 1993)—the probability that one of these few contacts will produce a metabotropic response is therefore very low. Indeed, our experiments indicate that ~20 CA3 pyramidal cell inputs to a CA1 pyramidal cell must be activated for reliable induction of somatic mGluR-mediated current. This is, nevertheless, a relatively small number considering that a CA1 pyramidal cell *in vivo* receives, on average, input from 5500 CA3 pyramidal cells (Amaral et al., 1990). Moreover, we estimate that even fewer CA3 inputs (<10) must be activated to induce mGluR-LTD. When an mGluR-positive spine is first identified and then specifically stimulated by two-photon glutamate uncaging, it is, however, possible to obtain mGluR-LTD (Holbro et al., 2009). This difference to our experiments is likely to reflect the fact that previous identification of mGluR-positive synapses is not possible at present with paired recording, and, as there are multiple functional contacts between CA3–CA1 pyramidal cell pairs, LTD of at best one of these synapses may not be discernible within the compound synaptic response.

A lower threshold for mGluR-LTD compared with mGluR-mediated current in CA1 pyramidal cells could reflect differences in the respective intracellular transduction mechanisms. Thus, an mGluR-dependent calcium signal to initiate LTD induction may have a lower stimulation threshold than an mGluR-mediated current, as demonstrated in cerebellar Purkinje cells (Finch and Augustine, 1998; Takechi et al., 1998). It has not been possible to repeat this type of experiment in CA3–CA1 paired recordings because of the difficulty in localizing the very few activated spines in the large dendritic tree of a pyramidal cell.

A second finding emerging from our study is the pronounced sensitivity of mGluR-mediated responses to cytoplasmic dialysis. This phenomenon may explain why in most studies using whole-cell patch recording, concentrations of agonists to activate postsynaptic mGluRs exceed EC_{50} values for group I mGluRs (Conn and Pin, 1997) by one to two orders of magnitude. The rundown of metabotropic responses can be avoided with the perforated patch-clamp method (Horn and Marty, 1988), but this approach is technically demanding. Here, we show that a simpler method using patch pipettes with a higher resistance (10–15 MΩ) allows mGluR-mediated currents to be recorded with similar amplitudes to those obtained with the perforated patch-clamp technique.

In conclusion, we have shown that, even under conditions in which cytoplasmic dialysis is minimized, mGluR-LTD is not observed in CA3–CA1 pyramidal cell pairs. Temporally contiguous input from multiple Schaffer collaterals must target a CA1 pyra-

midal cell to induce mGluR-mediated currents and mGluR-LTD. Interestingly, the requirement for multiple inputs does not reflect a cooperative mechanism involving synaptic spillover of glutamate (Arnth-Jensen et al., 2002) but rather is consistent with a sparse expression of functional mGluRs in the dendritic tree of pyramidal cells.

References

Amaral DG, Ishizuka N, Claiborne B (1990) Neurons, numbers and the hippocampal network. Prog Brain Res 83:1–11.

Anwyl R (2006) Induction and expression mechanisms of postsynaptic NMDA receptor-independent homosynaptic long-term depression. Prog Neurobiol 78:17–37.

Arnth-Jensen N, Jabaudon D, Scanziani M (2002) Cooperation between independent hippocampal synapses is controlled by glutamate uptake. Nat Neurosci 5:325–331.

Bellone C, Lüscher C, Mameli M (2008) Mechanisms of synaptic depression triggered by metabotropic glutamate receptors. Cell Mol Life Sci 65:2913–2923.

Bolshakov VY, Siegelbaum SA (1994) Postsynaptic induction and presynaptic expression of hippocampal long-term depression. Science 264: 1148–1152.

Brager DH, Johnston D (2007) Plasticity of intrinsic excitability during long-term depression is mediated through mGluR-dependent changes in I_h in hippocampal CA1 pyramidal neurons. J Neurosci 27:13926–13937.

Congar P, Leinekugel X, Ben-Ari Y, Crépel V (1997) A long-lasting calcium-activated nonselective cationic current is generated by synaptic stimulation or exogenous activation of group I metabotropic glutamate receptors in CA1 pyramidal neurons. J Neurosci 17:5366–5379.

Conn PJ, Pin JP (1997) Pharmacology and functions of metabotropic glutamate receptors. Annu Rev Pharmacol Toxicol 37:205–237.

Debanne D, Gähwiler BH, Thompson SM (1996) Cooperative interactions in the induction of long-term potentiation and depression of synaptic excitation between hippocampal CA3-CA1 cell pairs in vitro. Proc Natl Acad Sci U S A 93:11225–11230.

Dudek SM, Bear MF (1992) Homosynaptic long-term depression in area CA1 of hippocampus and effects of *N*-methyl-D-aspartate receptor blockade. Proc Natl Acad Sci U S A 89:4363–4367.

Enoki R, Hu YL, Hamilton D, Fine A (2009) Expression of long-term plasticity at individual synapses in hippocampus is graded, bidirectional, and mainly presynaptic: optical quantal analysis. Neuron 62:242–253.

Finch EA, Augustine GJ (1998) Local calcium signalling by inositol-1,4,5-trisphosphate in Purkinje cell dendrites. Nature 396:753–756.

Fitzjohn SM, Palmer MJ, May JE, Neeson A, Morris SA, Collingridge GL (2001) A characterisation of long-term depression induced by metabotropic glutamate receptor activation in the rat hippocampus in vitro. J Physiol 537:421–430.

Gähwiler BH, Thompson SM, McKinney RA, Debanne D, Robertson RT (1998) Culturing nerve cells. In: Organotypic slice cultures of neural tissue (Banker G, Goslin K, eds), pp 461–498. Cambridge, MA: MIT.

Gee CE, Benquet P, Gerber U (2003) Group I metabotropic glutamate receptors activate a calcium-sensitive transient receptor potential-like conductance in rat hippocampus. J Physiol 546:655–664.

Holbro N, Grunditz A, Oertner TG (2009) Differential distribution of endoplasmic reticulum controls metabotropic signaling and plasticity at hippocampal synapses. Proc Natl Acad Sci USA 106:15055–15060.

Horn R, Marty A (1988) Muscarinic activation of ionic currents measured by a new whole-cell recording method. J Gen Physiol 92:145–159.

Hsia AY, Malenka RC, Nicoll RA (1998) Development of excitatory circuitry in the hippocampus. J Neurophysiol 79:2013–2024.

Jabaudon D, Shimamoto K, Yasuda-Kamatani Y, Scanziani M, Gähwiler BH, Gerber U (1999) Inhibition of uptake unmasks rapid extracellular turnover of glutamate of nonvesicular origin. Proc Natl Acad Sci U S A 96:8733–8738.

Jonas P, Sakmann B (1992) Glutamate receptor channels in isolated patches from CA1 and CA3 pyramidal cells of rat hippocampal slices. J Physiol 455:143–171.

Kemp N, Bashir ZI (1997) NMDA receptor-dependent and independent long-term depression in the CA1 region of the adult rat hippocampus *in vitro*. Neuropharmacology 36:397–399.

Kemp N, Bashir ZI (2001) Long-term depression: a cascade of induction and expression mechanisms. Prog Neurobiol 65:339–365.

Kullmann DM, Lamsa KP (2007) Long-term synaptic plasticity in hippocampal interneurons. Nat Rev Neurosci 8:687–699.

Lisman JE (2009) The pre/post LTP debate. Neuron 63:281–284.

Lujan R, Nusser Z, Roberts JD, Shigemoto R, Somogyi P (1996) Perisynaptic location of metabotropic glutamate receptors mGluR1 and mGluR5 on dendrites and dendritic spines in the rat hippocampus. Eur J Neurosci 8:1488–1500.

Mulkey RM, Malenka RC (1992) Mechanisms underlying induction of homosynaptic long-term depression in area CA1 of the hippocampus. Neuron 9:967–975.

Nakamura T, Barbara J-G, Nakamura K, Ross WN (1999) Synergistic release of Ca^{2+} from IP3-sensitive stores evoked by synaptic activation of mGluRs paired with backpropagating action potentials. Neuron 24:727–737.

Oliet SH, Malenka RC, Nicoll RA (1997) Two distinct forms of long-term depression coexist in CA1 hippocampal pyramidal cells. Neuron 18:969–982.

Racca C, Stephenson FA, Streit P, Roberts JD, Somogyi P (2000) NMDA receptor content of synapses in stratum radiatum of the hippocampal CA1 area. J Neurosci 20:2512–2522.

Scanziani M (2000) GABA spillover activates postsynaptic $GABA_B$ receptors to control rhythmic hippocampal activity. Neuron 25:673–681.

Snyder EM, Philpot BD, Huber KM, Dong X, Fallon JR, Bear MF (2001) Internalization of ionotropic glutamate receptors in response to mGluR activation. Nat Neurosci 4:1079–1085.

Sorra KE, Harris KM (1993) Occurrence and three-dimensional structure of multiple synapses between individual radiatum axons and their target pyramidal cells in hippocampal area CA1. J Neurosci 13:3736–3748.

Stanton PK, Chattarji S, Sejnowski TJ (1991) 2-Amino-3-phosphonopropionic acid, an inhibitor of glutamate-stimulated phosphoinositide turnover, blocks induction of homosynaptic long-term depression, but not potentiation, in rat hippocampus. Neurosci Lett 127:61–66.

Takechi H, Eilers J, Konnerth A (1998) A new class of synaptic response involving calcium release in dendritic spines. Nature 396:757–760.

Takumi Y, Ramírez-León V, Laake P, Rinvik E, Ottersen OP (1999) Different modes of expression of AMPA and NMDA receptors in hippocampal synapses. Nat Neurosci 2:618–624.

Thomson AM, Radpour S (1991) Excitatory connections between CA1 pyramidal cells revealed by spike triggered averaging in slices of rat hippocampus are partially NMDA receptor mediated. Eur J Neurosci 3:587–601.

Tong G, Jahr CE (1994) Multivesicular release from excitatory synapses of cultured hippocampal neurons. Neuron 12:51–59.

Watabe AM, Carlisle HJ, O'Dell TJ (2002) Postsynaptic induction and presynaptic expression of group 1 mGluR-dependent LTD in the hippocampal CA1 region. J Neurophysiol 87:1395–1403.

Xiao MY, Zhou Q, Nicoll RA (2001) Metabotropic glutamate receptor activation causes a rapid redistribution of AMPA receptors. Neuropharmacology 41:664–671.

Zhang XL, Zhou ZY, Winterer J, Müller W, Stanton PK (2006) NMDA-dependent, but not group I metabotropic glutamate receptor-dependent, long-term depression at Schaffer collateral–CA1 synapses is associated with long-term reduction of release from the rapidly recycling presynaptic vesicle pool. J Neurosci 26:10270–10280.

Zhou Q, Homma KJ, Poo MM (2004) Shrinkage of dendritic spines associated with long-term depression of hippocampal synapses. Neuron 44:749–757.

Zimmer J, Gähwiler BH (1984) Cellular and connective organization of slice cultures of the rat hippocampus and fascia dentata. J Comp Neurol 228: 432–446.