Published in final edited form as:

Hippocampus. 2010 September ; 20(9): 1003–1009. doi:10.1002/hipo.20713.

Area CA3 interneurons receive two spatially segregated mossy fiber inputs

Kathleen E. Cosgrove, Emilio J. Galvan, Stephen D. Meriney, and German Barrionuevo
Department of Neuroscience, University of Pittsburgh, Pittsburgh, PA, USA

Abstract

Area CA3 receives two extrinsic excitatory inputs, the mossy fibers (MF) and the perforant path (PP). Interneurons with somata in str. lacunosum moleculare (L-M) of CA3 modulate the influence of the MF and PP on pyramidal cell activity by providing strong feed-forward inhibitory influence to pyramidal cells. Here we report that L-M interneurons receive two separate MF inputs, one to the dorsal dendrites from the suprapyramidal blade of the dentate gyrus (MF_{SDG}), and a second to ventral dendrites from the str. lucidum (MF_{SL}). Responses elicited from MF_{SDG} and MF_{SL} stimulation sites have strong paired-pulse facilitation, similar DCG-IV sensitivity, amplitude, and decay kinetics but target spatially segregated domains on the interneuron dendrites. These data demonstrate that certain interneuron subtypes are entrained by two convergent MF inputs to spatially separated regions of the dendritic tree. This anatomical arrangement could make these interneurons considerably more responsive to the excitatory drive from dentate granule cells. Furthermore, temporal summation is linear or slightly sublinear between PP and MF_{SL} but supralinear between PP and MF_{SDG}. This specific boosting of the excitatory drive to interneurons from the SDG location may indicate that L-M interneurons could be specifically involved in the processing of the associational component of the recognition memory.

Keywords

hippocampus; str. lacunosum moleculare; str. lucidum; perforant path; coincidence detection

A distinctive connectivity feature of hippocampal area CA3 is that it receives two extrinsic excitatory afferent inputs. One input is conveyed via the perforant path (PP), the axons of the stellate cells in entorhinal cortex (EC) layer II. The second extrinsic input is provided by the mossy fibers (MF), the axons of dentate gyrus granule cells, which in turn receive input from the same layer II cells in EC (Tamamaki and Nojyo, 1993). Each projection pathway gains access to area CA3 through compact axonal bundles. The PP enters dorsally near the suprapyramidal blade of the dentate gyrus and the MF enters ventrally at str. lucidum. Area CA3 also contains a heterogeneous pool of feed-forward GABAergic interneurons (Freund and Buzsaki, 1996; Ascoli et al., 2009) receiving excitatory drive both from the PP and MF inputs (Buhl et al., 1994; Penttonen et al., 1998). Interneurons with soma residing in str. lacunosum moleculare (L-M interneurons) are known to mediate the influence of the EC on pyramidal cell activity by providing strong feed-forward inhibition (Lacaille and Schwartzkroin, 1988; Williams et al., 1994; Khazipov et al., 1995; Freund and Buzsaki, 1996; Chitwood and Jaffe, 1998; Savic et al., 2001; Cope et al., 2002). The somata of the majority of L-M interneurons are bipolar, with primary dendrites arising from the polar extremes of the cells. When viewed in a coronal section, dendrites extend dorsally into the

str. lacunosum moleculare of CA3 where they overlap with the trajectory of PP fibers (Ascoli et al., 2009). Ventrally, the dendritic branches of L-M interneurons arborize within the str. lucidum where they receive MF input (MF_{SL}) via *en passant* synapses arising from the main axonal trunk and filipodial extensions from the giant MF boutons on CA3 pyramidal cells (Acsady et al., 1998). However, MFs exiting the dentate gyrus *en route* to the str. lucidum form extensive collateral plexuses within the polymorphic layer and hilus of the dentate gyrus (Acsady et al., 1998; Claiborne et al., 1986). Some of the dorsally extending dendrites of L-M interneurons coexist with MF collateral plexus axons near the suprapyramidal blade of the dentate gyrus (SDG). Because of these anatomical relations, we hypothesize that the dentate gyrus granule cells furnish separate excitatory input to L-M interneurons at two spatially distinct locations on the dendritic tree. Dorsally projecting axon collaterals provide one of these inputs in the vicinity of the SDG and the other is derived from *en passant* boutons and filopodial extensions arising from the giant MF thorny excrescences in str. lucidum.

The present study analyzed the electrophysiological properties of MF inputs impinging on the dorsal and ventral dendritic branches of the same L-M interneuron. Whole-cell recordings were obtained from visually identified L-M interneurons (200–300 μ m from the boundary between s. pyramidale and s. lucidum, 50–180 μ m from tip of dentate gyrus; Ascoli et al., 2009) in the presence of bicuculine, and D-2-amino-5-phosphonopentanoic acid (D,L-AP5) to block $GABA_A$ - and NMDAR-mediated transmission (Calixto et al., 2008; Galvan et al., 2008). Extracellular stimulation of the str. lucidum in CA3b (MF_{SL} ; Fig 1A, green) and the medial extent of SDG (MF_{SDG} ; Fig 1A, blue) evoked isolated AMPAR-mediated MF EPSPs and EPSCs with similar kinetics (Table 1). However, differences in EPSP/EPSC kinetics between the two inputs due to synaptic location could be obscured if MF responses evoked from the stimulation sites share common presynaptic fibers. To determine if this was the case, we applied a paired-pulse stimulation protocol in current-clamp conditions (-70 mV). Pairs of stimuli were first delivered at 60 ms interstimulus interval (ISI) to each pathway individually (“homosynaptic paired pulse stimulation”), and the paired pulse ratio (PPR) was calculated as the amplitude of the second EPSP divided by the amplitude of the first. The primary assumption underlying the paired-pulse test is that a $PPR > 1$ (facilitation; PPF) in homosynaptic permutations is due to changes in presynaptic function produced by the first pulse in the pair. A previous study has shown that MF synapses on str. lucidum have a limited expression for short-term plasticity (Toth et al., 2000). However, our current data revealed that both MF inputs to L-M interneurons displayed similarly robust PPF ($PPR = 1.5 \pm 0.06$ for MF_{SL} EPSP; $P < 0.001$; $N = 13$; and 1.5 ± 0.06 for MF_{SDG} EPSP; $P < 0.001$; $N = 13$; Fig. 1C, black bars). Significance was determined as $p < .05$ using Student's t-test and ANOVA with multiple comparisons. (2S,2'R,3'R)-2-(2'3'-dicarboxycyclopropyl) glycine (DCG-IV; 1 μ M), the group II metabotropic glutamate receptor agonist, was bath applied to confirm the MF origin of the inputs (MF_{SL} EPSP, $68.9 \pm 3.23\%$ decrease, $P < 0.001$, $n=7$; MF_{SDG} EPSP, $67.63 \pm 5.36\%$ decrease, $P < 0.001$, $n=7$). DCG-IV inhibition is variable in interneurons (Alle et al., 2001; Lawrence and McBain, 2003; Galvan et al., 2008). Therefore, synaptic responses were classified to be of MF origin if their DCG-IV sensitivity was $>50\%$ inhibition (Lawrence and McBain, 2003). There were no differences in DCG-IV sensitivity between MF_{SL} and MF_{SDG} EPSPs ($P > 0.12$). To test whether the two stimulation locations shared common presynaptic fibers, a pair of stimuli (60 ms ISI) was given to first one MF stimulation site and then the other (“heterosynaptic paired pulse stimulation”; Bradler and Barrionuevo, 1990). Facilitation was not observed during the heterosynaptic paired-pulse stimulation when MF_{SDG} EPSP was followed by MF_{SL} EPSP (0.88 ± 0.05 ; $N = 13$; $P < 0.001$, Fig. 1C, grey bars and 1D, grey waveforms). Reversing the stimulation order produced similar results (1.0 ± 0.02 ; $P > 0.05$; $N = 13$ Fig. 1C, grey bars and 1D, grey waveforms). The absence of heterosynaptic paired-pulse facilitation indicates that activation of MF_{SDG} and MF_{SL} gave rise to EPSPs from a

population of non-overlapping presynaptic fibers. From these experiments we conclude that the two MF inputs are functionally independent. The functional separation between the two MF inputs may be due to the truncation of the MF axons in the hilus as a result of the slicing procedure (Fig. 1A). However, MF synapses onto CA3 interneurons have only one or two MF contacts each with a single active zone (Acsady et al., 1998). This sparse connectivity would make it improbable that a single MF axon would have synaptic contacts on the same interneuron at both the hilar region and the str. lucidum. Therefore, it is possible that in the intact hippocampus each MF synapse on L-M interneurons is furnished by a single dentate granule cell.

The findings that the two stimulation locations evoke MF inputs to L-M interneurons that are functionally independent does not rule out the possibility that the activated MF synapses are positioned at the same site on the dendritic tree. However, the spatial orientation of the dorsal dendritic arbor suggests that MF axons from SDG make synaptic contacts on dendrites within the str. lacunosum moleculare of CA3b whereas the ventral dendritic arbor receives MF input within the str. lucidum. On the basis of this anatomical evidence, we predicted that focal blockade of AMPAR-mediated synaptic transmission in the str. lacunosum moleculare would suppress the MF_{SDG} input while sparing the MF_{SL} input. To test this hypothesis, EPSCs were recorded from the soma of L-M interneurons at a holding potential of -70 mV in the presence of bicuculline and AP5. We also activated the PP input from s. lacunosum moleculare of area CA1 as a control to determine if the focal application of CNQX in a constantly perfusing bath was sufficient to suppress AMPAR-mediated responses within the s. lacunosum moleculare of area CA3 (Calixto et al., 2008; Fig. 1A). PP EPSCs (40.02 ± 9.48 pA; N = 6; Fig. 2C) were evoked from s. lacunosum moleculare in hippocampal area CA1. MF EPSCs (Fig. 2C) were evoked using stimulation locations described above (MF_{SDG}; 26.28 ± 2.75 pA; N = 7; and MF_{SL}; 28.43 ± 7.88 pA; N = 6; Fig. 1A). The site of MF_{SL} stimulation was ~ 200 μ m from the s. lacunosum moleculare, the site of the presumed location of PP and MF_{SDG} synapses. There was no significant difference in EPSC amplitude or kinetics among the pathways (Table 1). DCG-IV reduced PP EPSC amplitude by $21.84 \pm 3.15\%$ ($p < 0.05$; N = 6; Fig. 2C) but produced an inhibition greater than 50% on MF_{SDG} and MF_{SL} EPSC amplitudes ($61.89 \pm 3.65\%$; $P < 0.001$; N = 7; and $67.82 \pm 6.87\%$; $P < 0.001$; N = 6, Fig. 2C), respectively. The slight decrease in PP EPSC amplitude with the application of DCG-IV is consistent with previous reports in CA1 demonstrating inhibition of glutamate release from the PP following application of group II mGluR agonists (Macek, 1996; Price, 2005). Following DCG-IV application, a pressure ejection pipette containing CNQX (50 μ M) was positioned within 20 μ m of the interneuron soma. CNQX was then focally applied with a pulse of pressure (2-4 mmHg) for ~ 2 to 4 minutes, creating a plume that encompassed the s. lacunosum moleculare (Fig. 2A). The pressure pipette solution contained Fast Green (3% or in some cases fluorescein; Fig. 2A) to allow visual confirmation that the application of the CNQX solution was restricted to the s. lacunosum moleculare. CNQX further reduced the EPSC amplitudes from PP by $77.46 \pm 4.26\%$ ($P < 0.001$; N = 6, Fig. 2) and MF_{SDG} by $61.72 \pm 4.78\%$ ($P < 0.05$; N = 7, Fig. 2) such that the remaining response was only $17.69 \pm 3.64\%$ ($p < 0.001$; N = 6) and $14.44 \pm 2.23\%$ ($P < 0.001$; N = 7) of control values, respectively. In contrast, CNQX did not significantly decrease the MF_{SL} EPSC amplitude ($P > 0.05$; N = 6; Fig. 2). These data demonstrate the spatial segregation of the two MF inputs to L-M interneurons – one within the str. lacunosum moleculare, and the other more ventral, likely near the str. lucidum.

Together these data indicate that while responses elicited from MF_{SDG} and MF_{SL} stimulation sites both have similar DCG-IV sensitivity, homosynaptic PPF, and similar amplitude and decay kinetics (indicating that the corresponding synapses were positioned at similar distances from the interneuron soma), they target spatially segregated domains on the interneuron dendrites. By stark contrast, the location of the synapses made by the giant MF

boutons on CA3 pyramidal cells is restricted to the proximal apical dendrite. Although the MF projection is sparse, the interneuron drive from the dentate gyrus is very effective (Henze et al., 2002), due to the combination of large mean quantal amplitude and high probability of release by MF terminals (Lawrence & McBain, 2003), increased number and faster kinetics of postsynaptic AMPA/kainate receptors (Geiger et al., 1997; Nusser et al., 1998; Walker et al., 2002; Jonas et al., 2004), and the presence of dendritic voltage dependent conductances (Martina et al., 2000) in the proximity of MF synaptic sites (Calixto et al., 2008). The current data demonstrate that certain interneuron subtypes are entrained by two convergent MF inputs to spatially separated regions of the dendritic tree. This anatomical arrangement could also make these interneurons considerably more responsive to the excitatory drive from dentate granule cells.

Lastly, we compared temporal summation between PP and MF_{SDG} vs. MF_{SL} EPSPs. We recently reported that coactivation of PP and MF_{SDG} at the 10-ms ISI elicits supralinear summation of subthreshold AMPAR-mediated EPSPs in L-M interneurons (Calixto et al., 2008). In this earlier work, we showed that the boosting to the second EPSP in the pair relies on the recruitment of dendritic T-type Ca²⁺ currents (I_{CaT}) by the first EPSP in the pair (Calixto et al., 2008). Based on theoretical and experimental studies (Polsky et al., 2004; Losonczy and Magee, 2006; Carter et al., 2007), we hypothesized that the two coincidental inputs are required to synapse in close proximity with each other within the str. lacunosum moleculare to elicit the EPSP boosting provided by the I_{CaT}. If the supralinear boosting between PP and MF_{SDG} requires neighboring synapses, then PP and MF_{SL} inputs are likely to interact linearly or sublinearly (Cash and Yuste, 1999; Tamas et al., 2002; Polsky et al., 2004; Gasparini and Magee, 2006; Losonczy and Magee, 2006; Carter et al., 2007). To test this prediction, we first assessed summation at the 10-ms ISI between PP EPSP and MF_{SDG} EPSP, and then between PP EPSP and MF_{SL} EPSP. The first EPSP waveform of the pair was digitally subtracted from the combined EPSP waveform. This resultant 'subtracted EPSP' waveform represents the second EPSP evoked by the paired stimulation. We calculated temporal summation as the ratio of the peak amplitude of the subtracted EPSP to the peak amplitude of the EPSP elicited alone from the second 'actual EPSP'. A summation ratio (SR) = 1 indicates linear summation (the amplitude of the second EPSP in the pair is identical to the amplitude of the same EPSP evoked singly, i.e. subtracted EPSP = actual EPSP). When the amplitude of the subtracted EPSP is larger (SR>1) or smaller (SR<1) than that of the actual EPSP, summation is supra or sublinear, respectively (Urban & Barrionuevo, 1998; Calixto et al., 2008). That is, the combined effect of the subthreshold responses either exceeds or is below the arithmetic sum of the individual responses, respectively. In each cell, stimulation of MF_{SDG} or MF_{SL} preceded PP stimulation ('MF_{SDG} or MF_{SL} EPSP first'), and then the order was reversed ('PP EPSP first'). At this ISI, PP EPSP and MF_{SDG} EPSP yielded supralinear SR values (1.29 ± 0.07 for MF_{SDG} EPSP first; $P < 0.05$; $N = 10$; Fig 3B, top traces, and 1.33 ± 0.20 for PP EPSP first; $P < 0.05$; $N = 6$; Fig. 3B bottom traces) in 10/15 cells examined. In agreement with our previous findings, the supralinear interaction between MF_{SDG} and PP input was symmetrical in time, i.e. prestimulation with either input amplified the second EPSP in the pair. We hypothesize that in the cells in which concurrent MF_{SDG} and PP inputs did not show supralinear summation ($SR = 0.95 \pm 0.05$ for MF_{SDG} EPSP first; $P > 0.01$; $N = 5$; and 1.04 ± 0.04 for PP EPSP first; $P < 0.01$; $N = 5$) the activated synapses were located on different dendrites within the str. lacunosum moleculare and thus, their respective postsynaptic domains were separated by greater distances. Consistent with this interpretation, summation at the same ISI was sublinear for MF EPSP_{SL} first ($SR = 0.80 \pm 0.08$; $P < 0.05$; $N = 8$; Fig. 3D, top traces), and linear for PP EPSP first ($SR = 0.93 \pm 0.04$; $P > 0.05$; $N = 8$; Fig. 3D, bottom traces) in all cells. Together these results support the view that the arithmetic of summation (supra vs. linear/sublinear) between PP and MF is determined by the dendritic position of the synaptic inputs and active properties of the dendritic membrane regardless of the origin of the input.

In addition, it is possible that the lack of supralinear summation between some pairs of coincident inputs may be due to spatial heterogeneity in the distribution of I_{CaT} channels along the different branches of the spineless dendrites of L-M interneurons. Regardless of the mechanism responsible for the selective EPSP boosting, the supralinear summation between MF and PP inputs would likely increase the feed-forward inhibitory input to CA3 pyramidal cells. In turn, the resulting feedforward inhibition would constrain the timing of action potential discharge in CA3 pyramidal cells elicited by input from the recurrent collaterals during the CA3-generated gamma oscillations (Csicsvari et al., 2003).

L-M interneurons are adapting interneurons with an accommodation ratio > 1 in response to depolarizing current steps (Ascoli, 2009; Calixto, 2008). Physiologically, this means that these interneurons do not fire in prolonged bursts in response to suprathreshold synaptic input. However, data from in vivo experiments suggest that input from a single granule cell is rarely able to elicit postsynaptic firing in CA3 interneurons (Henze, 2002). Consequently, multiple inputs must be integrated to provide effective inhibitory drive to CA3 pyramidal cells. Although the likelihood of the supralinear summation may be low because it requires that inputs be close spatially and temporally, it could exert a powerful influence over L-M interneuron firing. What might be the functional significance of the afferent-specific supralinear summation between PP and MF_{SDG} in feed-forward interneurons? Labeling studies have shown that the SDG receives a stronger projection from the lateral than from the medial EC (Tamamaki, 1997). Although the functional significance of this segregated input pattern is not clear, the lateral EC mainly conveys non spatial object-specific information from the perirhinal cortex (Hargreaves et al., 2005). Perhaps, the specific boosting of the excitatory drive to CA3 feed-forward interneurons resulting from coincidental MF_{SDG} and PP inputs is specifically involved in the processing of the associational component of the recognition memory representations conveyed by the perirhinal cortex (Brown and Aggleton, 2001).

Acknowledgments

Grant sponsor: NINDS NS 24288 and Central Research Development Fund of the University of Pittsburgh.

References

- Acsady L, Kamondi A, Sik A, Freund T, Buzsaki G. GABAergic cells are the major postsynaptic targets of mossy fibers in the rat hippocampus. *J Neurosci*. 1998; 18(9):3386–403. [PubMed: 9547246]
- Alle H, Jonas P, Geiger JR. PTP and LTP at a hippocampal mossy fiber-interneuron synapse. *Proc Natl Acad Sci U S A*. 2001; 98(25):14708–13. [PubMed: 11734656]
- Ascoli GA, Brown KM, Calixto E, Card JP, Galván EJ, Perez-Rosello T, Barrionuevo G. Quantitative morphometry of electrophysiologically identified CA3b interneurons reveals robust local geometry and distinct cell classes. *The Journal of Comparative Neurology*. 2009; 515(6):677–695. [PubMed: 19496174]
- Bradler JE, Barrionuevo G. Heterosynaptic correlates of long-term potentiation induction in hippocampal CA3 neurons. *Neuroscience*. 1990; 35(2):265–271. [PubMed: 1974327]
- Brown MW, Aggleton JP. Recognition memory: what are the roles of the perirhinal cortex and hippocampus? *Nat Rev Neurosci*. 2001; 2(1):51–61. [PubMed: 11253359]
- Buhl EH, Halasy K, Somogyi P. Diverse sources of hippocampal unitary inhibitory postsynaptic potentials and the number of synaptic release sites. *Nature*. 1994; 368(6474):823–8. [PubMed: 8159242]
- Calixto E, Galvan EJ, Card JP, Barrionuevo G. Coincidence detection of convergent perforant path and mossy fibre inputs by CA3 interneurons. *J Physiol*. 2008; 586(Pt 11):2695–712. [PubMed: 18388134]

- Carter AG, Soler-Llavina GJ, Sabatini BL. Timing and location of synaptic inputs determine modes of subthreshold integration in striatal medium spiny neurons. *J Neurosci.* 2007; 27(33):8967–77. [PubMed: 17699678]
- Cash S, Yuste R. Linear summation of excitatory inputs by CA1 pyramidal neurons. *Neuron.* 1999; 22(2):383–94. [PubMed: 10069343]
- Chitwood RA, Jaffe DB. Calcium-dependent spike-frequency accommodation in hippocampal CA3 nonpyramidal neurons. *J Neurophysiol.* 1998; 80(2):983–8. [PubMed: 9705484]
- Claiborne BJ, Amaral DG, Cowan WM. A light and electron microscopic analysis of the mossy fibers of the rat dentate gyrus. *J Comp Neurol.* 1986; 246(4):435–58. [PubMed: 3700723]
- Cope DW, Maccaferri G, Marton LF, Roberts JD, Cobden PM, Somogyi P. Cholecystokinin-immunopositive basket and Schaffer collateral-associated interneurons target different domains of pyramidal cells in the CA1 area of the rat hippocampus. *Neuroscience.* 2002; 109(1):63–80. [PubMed: 11784700]
- Csicsvari J, Jamieson B, Wise KD, Buzsaki G. Mechanisms of gamma oscillations in the hippocampus of the behaving rat. *Neuron.* 2003; 37(2):311–22. [PubMed: 12546825]
- Freund TF, Buzsaki G. Interneurons of the hippocampus. *Hippocampus.* 1996; 6(4):347–470. [PubMed: 8915675]
- Galvan EJ, Calixto E, Barrionuevo G. Bidirectional Hebbian Plasticity at Hippocampal Mossy Fiber Synapses on CA3 Interneurons. *J Neurosci.* 2008; 28(52):14042–14055. [PubMed: 19109487]
- Gasparini S, Magee JC. State-dependent dendritic computation in hippocampal CA1 pyramidal neurons. *J Neurosci.* 2006; 26(7):2088–100. [PubMed: 16481442]
- Geiger JRP, Lübke J, Roth A, Frotscher M, Jonas P. Submillisecond AMPA Receptor-Mediated Signaling at a Principal Neuron-Interneuron Synapse. *Neuron.* 1997; 18(6):1009–1023. [PubMed: 9208867]
- Gulyas AI, Miles R, Sik A, Toth K, Tamamaki N, Freund TF. Hippocampal pyramidal cells excite inhibitory neurons through a single release site. *Nature.* 1993; 366(6456):683–7. [PubMed: 8259211]
- Hargreaves EL, Rao G, Lee I, Knierim JJ. Major dissociation between medial and lateral entorhinal input to dorsal hippocampus. *Science.* 2005; 308(5729):1792–4. [PubMed: 15961670]
- Henze DA, Wittner L, Buzsaki G. Single granule cells reliably discharge targets in the hippocampal CA3 network in vivo. *Nat Neurosci.* 2002; 5(8):790–795. [PubMed: 12118256]
- Jonas P, Bischofberger J, Fricker D, Miles R. Interneuron Diversity series: Fast in, fast out--temporal and spatial signal processing in hippocampal interneurons. *Trends Neurosci.* 2004; 27(1):30–40. [PubMed: 14698608]
- Khazipov R, Congar P, Ben-Ari Y. Hippocampal CA1 lacunosum-moleculare interneurons: modulation of monosynaptic GABAergic IPSCs by presynaptic GABAB receptors. *J Neurophysiol.* 1995; 74(5):2126–37. [PubMed: 8592201]
- Lacaille JC, Schwartzkroin PA. Stratum lacunosum-moleculare interneurons of hippocampal CA1 region. II. Intracellular and intradendritic recordings of local circuit synaptic interactions. *J Neurosci.* 1988; 8(4):1411–24. [PubMed: 3357024]
- Lawrence JJ, McBain CJ. Interneuron diversity series: containing the detonation--feedforward inhibition in the CA3 hippocampus. *Trends Neurosci.* 2003; 26(11):631–40. [PubMed: 14585604]
- Losonczy A, Magee JC. Integrative properties of radial oblique dendrites in hippocampal CA1 pyramidal neurons. *Neuron.* 2006; 50(2):291–307. [PubMed: 16630839]
- Macek TA, Winder DG, Gereau RW, Ladd CO, Conn PJ. Differential involvement of group II and group III mGluRs as autoreceptors at lateral and medial perforant path synapses. *J Neurophysiol.* 1996; 76(6):3798–3806. [PubMed: 8985877]
- Martina M, Vida I, Jonas P. Distal Initiation and Active Propagation of Action Potentials in Interneuron Dendrites. *Science.* 2000; 287(5451):295–300. [PubMed: 10634782]
- Nusser Z, Lujan R, Laube G, Roberts JDB, Molnar E, Somogyi P. Cell Type and Pathway Dependence of Synaptic AMPA Receptor Number and Variability in the Hippocampus. *Neuron.* 1998; 21(3):545–559. [PubMed: 9768841]
- Penttonen M, Kamondi A, Acsády L, Buzsaki G. Gamma frequency oscillation in the hippocampus of the rat: intracellular analysis in vivo. *Eur J Neurosci.* 1998; 10(2):718–28. [PubMed: 9749733]

- Polsky A, Mel BW, Schiller J. Computational subunits in thin dendrites of pyramidal cells. *Nat Neurosci.* 2004; 7(6):621–7. [PubMed: 15156147]
- Price CJ, Karayannis T, Pál BZ, Capogna M. Group II and III mGluRs-mediated presynaptic inhibition of EPSCs recorded from hippocampal interneurons of CA1 stratum lacunosum moleculare. *Neuropharmacology.* 2005; 49(Supplement 1):45–56. [PubMed: 15998525]
- Savic N, Pedarzani P, Sciancalepore M. Medium afterhyperpolarization and firing pattern modulation in interneurons of stratum radiatum in the CA3 hippocampal region. *J Neurophysiol.* 2001; 85(5): 1986–97. [PubMed: 11353015]
- Tamamaki N. Organization of the entorhinal projection to the rat dentate gyrus revealed by Dil anterograde labeling. *Exp Brain Res.* 1997; 116(2):250–8. [PubMed: 9348124]
- Tamamaki N, Nojyo Y. Projection of the entorhinal layer II neurons in the rat as revealed by intracellular pressure-injection of neurobiotin. *Hippocampus.* 1993; 3(4):471–80. [PubMed: 8269038]
- Tamas G, Szabadics J, Somogyi P. Cell type- and subcellular position-dependent summation of unitary postsynaptic potentials in neocortical neurons. *J Neurosci.* 2002; 22(3):740–7. [PubMed: 11826103]
- Urban NN, Barrionuevo G. Active summation of excitatory postsynaptic potentials in hippocampal CA3 pyramidal neurons. *Proc Natl Acad Sci U S A.* 1998; 95(19):11450–5. [PubMed: 9736757]
- Walker HC, Lawrence JJ, McBain CJ. Activation of Kinetically Distinct Synaptic Conductances on Inhibitory Interneurons by Electrotonically Overlapping Afferents. *Neuron.* 2002; 35(1):161–171. [PubMed: 12123616]
- Williams S, Samulack DD, Beaulieu C, LaCaille JC. Membrane properties and synaptic responses of interneurons located near the stratum lacunosum-moleculare/radiatum border of area CA1 in whole-cell recordings from rat hippocampal slices. *J Neurophysiol.* 1994; 71(6):2217–35. [PubMed: 7931512]

Figure 1. MF_{SDG} and MF_{SL} inputs are functionally independent

(A) Schematic diagram of area CA3 of hippocampus showing typical soma location of L-M interneurons relative to the suprapyramidal blade of the dentate gyrus (SDG) and the arrangement of stimulating and recording electrodes. Perforant path fibres (red, PP) were activated from the str. lacunosum moleculare in area CA1. Mossy fibers were activated from two locations: 1) the suprapyramidal blade of the dentate gyrus (blue, MF_{SDG}); and 2) from the str. lucidum dorsal to the ventral tip of the DG (green, MF_{SL}). Dotted line indicates truncation of the MF axons by the slicing procedure. Abbreviations: SO, str. oriens; SP, str. pyramidale; SL, str. lucidum; SR, str. radiatum; SLM, str. lacunosum moleculare; DG, dentate gyrus; PP, perforant path; MF_{SDG}, mossy fiber originating from the suprapyramidal blade of the dentate gyrus; MF_{SL}, mossy fiber bundle in str. lucidum. (B) Computer reconstruction of one biocytin-filled L-M interneuron. Dendritic (red) and axonal (blue) arbors were reconstructed from serial coronal slices using the NeuroLucida software. Same abbreviations as in A. Scale bar, 100 μ m. (C) Bar graph showing the population data (N = 13) from the paired pulse experiments. Paired pulse facilitation (PPF) of the MF EPSP is expressed as the paired pulse ratio (PPR) between the peak amplitude of the second EPSP to the amplitude of the first EPSP at 60 ms ISI. Each pulse in the pair was delivered to the same site (homosynaptic SDG or SL, black bars), and to a different site (heterosynaptic SDG - SL or SL - SDG, grey bars). PPF was robust for homosynaptic stimulations but was not significant in heterosynaptic permutations. (D) Average traces (N = 10) from a representative experiment in one L-M interneuron. Superimposed traces reveal the lack of heterosynaptic PPF between MF_{SL} and MF_{SDG} (grey traces, second EPSP in the pair).

*Significant, $p < .05$. Error bars indicate S.E.M. Scale bars, 1.0 mV, 20 ms.

Figure 2. PP and MF_{SDG} synapses on L-M interneurons coexist within the s. lacunosum molecular far from MF_{SL} synapses

(A) Bright field image of hippocampal slice and typical placement of pressure ejection pipette (dashed lines). (Inset) Fluorescence image demonstrating the spread of drug application using fluorescein as a marker. Dashed outline marks region estimated to be affected by drug application. Scale bar, 200 μ m. (B) Summary graph illustrating EPSC amplitude changes after bath application of DCG-IV (dark grey bars), and subsequent focal application of CNQX to the s. lacunosum molecular (light grey bars). EPSC amplitudes were normalized to control. DCG-IV (1 μ M) significantly decreased the amplitude of MF_{SDG} and MF_{SL} relative to control. Additionally, CNQX (50 μ M) significantly decreased the amplitudes of MF_{SDG} and PP EPSCs, but did not change the amplitude of MF_{SL} EPSCs. * $p < .01$; ** $p < .001$. Error bars indicate S.E.M. (C) Average EPSCs (N = 10 to 30, black traces) from three L-M interneurons illustrating the reduction of EPSCs by DCG-IV (1 μ M; dark grey traces) and the selective suppression of PP and MF_{SDG} EPSC amplitudes by focal application of CNQX (50 μ M; light grey traces) to s. lacunosum molecular. Scale bars, 10 pA, 25 ms. (D) Scatter plots displaying individual normalized values (grey symbols and lines) following bath application of 1 μ M DCG-IV and focal application of 50 μ M CNQX to the str. lacunosum molecular. Mean values overlaid in black (PP: left; MF_{SDG}: middle; MF_{SL}: right). Error bars indicate S.E.M.

Figure 3. Supralinear summation between PP and MF_{SDG}

(A) The population data ($N = 10$) for PP and MF_{SDG} summation at the 10-ms ISI shows supralinear summation ratios for MF EPSP_{SDG} first and for PP EPSP first (amplitude of ‘Subtracted EPSP’ was larger than that of same EPSP evoked alone). (B) Traces are average EPSPs ($N = 10$) evoked from the same cell in A. Dotted line indicates the amplitude of the second EPSP in the pair evoked alone. $*p < 0.05$. Error bars indicate S.E.M. Scale bars, 2.0 mV, 25 ms. (C) The population data ($N = 8$) for PP and MF_{SL} summation at the 10-ms ISI shows sublinear summation ratios for MF EPSP_{SL} first and linear for PP EPSP first (amplitude of ‘Subtracted EPSP’ was small or similar compared to the same EPSP evoked alone, respectively). (D) Traces are average EPSPs ($N = 10$) evoked from a typical experiment in one L-M interneuron. Dotted line indicates the amplitude of the second EPSP in the pair evoked alone.

Table 1

Properties of synaptic responses in L-M interneurons

	N	Latency (ms)	20-80% Rise Time (ms)	Tau Decay (ms)	Peak Amplitude	Paired-pulse ratio	DCG-IV (1 μ M) Sensitivity
EPSP _{SDG}	7	2.3 $\pm$ 0.16	2.1 $\pm$ 0.19	25.3 $\pm$ 1.95	4.2 $\pm$ 0.53 mV	1.5 $\pm$.06*	-67.6 $\pm$ 5.36%
EPSP _{SL}	7	2.1 $\pm$ 0.13	2.1 $\pm$ 0.19	22.6 $\pm$ 2.67	3.1 $\pm$ 0.32 mV	1.5 $\pm$.06*	-68.9 $\pm$ 3.23%
EPSP _{pp}	7	2.0 $\pm$ 0.20	1.9 $\pm$ 0.25	28.2 $\pm$ 4.66	2.8 $\pm$ 0.59 mV	-	-
EPSC _{SDG}	7	3.1 $\pm$ 0.14	1.6 $\pm$ 0.19	10.4 $\pm$ 1.7	26.3 $\pm$ 2.7 pA	-	-61.9 $\pm$ 3.65%
EPSC _{SL}	6	2.8 $\pm$ 0.32	1.9 $\pm$ 0.28	10.6 $\pm$ 2.5	28.4 $\pm$ 7.9 pA	-	-67.8 $\pm$ 6.87%
EPSC _{pp}	6	2.6 $\pm$ 0.21	1.7 $\pm$ 0.31	8.5 $\pm$ 1.8	40 $\pm$ 9.5 pA	-	-21.8 $\pm$ 3.15%

Values are means $\pm$ SE.

* N=15

- Indicates values not determined.