

Origin of the Apparent Asynchronous Activity of Hippocampal Mossy Fibers

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Henze, Darrell A., Nathaniel N. Urban, and German Barrionuevo. Origin of the apparent asynchronous activity of hippocampal mossy fibers. *J. Neurophysiol.* 78: 24–30, 1997. Fiber volleys (FVs) from the stratum lucidum of rat hippocampal area CA3 were recorded extracellularly from in vitro slices in the presence of 10 mM kynurenic acid. In agreement with previous work, bulk stimulation of the dentate gyrus (DG) near the hilar border leads to an asynchronous FV. Transection of the stratum lucidum between the DG stimulation site and the CA3 recording site reduced or eliminated the early components of the asynchronous FV, indicating that they are of mossy fiber (MF) origin. In contrast, moving the stimulating electrode away from the hilus toward the hippocampal fissure reduced or eliminated the late components of the FV. Subsequently, we found that bulk stimulation on the DG/hilar border induces an antidromic population spike in CA3 pyramidal cells. Finally, the MFs and associational collaterals have different conduction velocities (0.51 and 0.37 m/s, respectively; temperature = 33°C). From these data, we conclude that the late components of the asynchronous FV are due to antidromic activation of CA3 collaterals that have been shown to be present in the DG and hilus. A corollary of these findings is that bulk stimulation on the DG/hilar border can lead to at least two different monosynaptic inputs to CA3 pyramidal cells: the MFs and the antidromically activated associational collaterals. We suggest that when MF synaptic responses are being evoked with the use of bulk stimulation, stimulating electrodes should be placed in the outer molecular layer of the DG to prevent the activation of hilar-projecting associational collaterals. This procedure should be added to the previously proposed criteria for preventing polysynaptic contamination of the intracellularly recorded evoked MF synaptic response.

INTRODUCTION

The entorhinal cortex provides synaptic input to hippocampal area CA3 via a mono- and a disynaptic pathway. The mossy fibers (MFs)—the axons of the dentate gyrus (DG) granule cells—are a critical component of this system in that they provide the second synapse of the disynaptic pathway from the entorhinal cortex to CA3 pyramidal cells. The MF-to-CA3 pyramidal cell synapse has several distinctive features that distinguish it from other cortical synapses. The MF synapse location is primarily restricted to the proximal apical dendrite in the stratum lucidum (Blackstad et al. 1970). The MF presynaptic bouton is both large (3–10 μm diam) and structurally complex. The presynaptic MF bouton completely envelopes a large, complex, postsynaptic “thorn” and gives off long fingerlike processes (Amaral 1979; Blackstad and Kjaerheim 1961; Hamlyn 1962). In addition to its complex ultrastructure, the MF synapse displays multiple forms of synaptic plasticity: a prolonged post-

tetanic potentiation (Langdon et al. 1995) and both Hebbian and non-Hebbian forms of *N*-methyl-D-aspartate-receptor-independent long-term potentiation (Castillo et al. 1994; Johnston et al. 1992; Katsuki et al. 1991; Langdon et al. 1995; Urban and Barrionuevo 1996; Urban et al. 1996; Xiang et al. 1994; Zalutsky and Nicoll 1990).

Interpretation of much of the previous work studying synaptic plasticity at this synapse relies on the “purity” of the evoked MF response. The MF synapse originally was presumed to be a particularly tractable model system for the study of excitatory synaptic function in the CNS (Brown and Johnston 1983; Johnston and Brown 1983). This assumption was based on the close proximity of the MF synapse to the soma of CA3 pyramidal cells, which facilitates voltage-clamp analysis and interpretation of synaptic currents (Spruston et al. 1993). It also was believed that selective activation of the MF synapse could be accomplished by bulk stimulation of the cell body layer of the DG. However, subsequent studies revealed that polysynaptic activation of the CA3 associational collateral network due to the firing of CA3 pyramidal cells could contaminate the DG-evoked MF synaptic response (Claiborne et al. 1993; Langdon et al. 1993). As a result, a series of criteria has been proposed to minimize polysynaptic contamination of the evoked MF excitatory postsynaptic potential/current (EPSP/C) that includes a requirement for a smooth, short (<3-ms) rising phase (Claiborne et al. 1993).

However, other work has demonstrated that the smooth, short rising phase criterion may lead to the rejection of a population of monosynaptic DG-evoked excitatory postsynaptic currents (EPSCs). In particular, despite blockade of polysynaptic activation, EPSCs in CA3 pyramidal cells evoked by bulk stimulation of the DG exhibited complex rising phases (Langdon et al. 1993). In addition, this work demonstrated that the fiber volley (FV) evoked by stimulation of the DG and recorded in the stratum lucidum is asynchronous. Because the asynchronous FV observed under these conditions was believed to reflect sole activation of MFs, it was suggested that MFs are intrinsically asynchronous. In another study, researchers using minimal stimulation of the DG also reported that evoked EPSCs in CA3 pyramidal cells may have complex rising phases (Jonas et al. 1993).

There are three possible origins of the observed asynchrony in the DG-evoked FV recorded in the stratum lucidum. 1) Anti-/orthodromic MF collateral activation (Fig. 1A) could lead to apparent asynchrony because of the in-

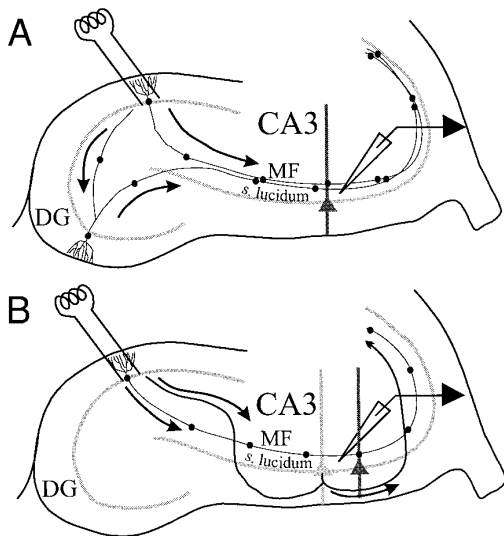


FIG. 1. Schematic illustrations of hippocampal circuitry in CA3 that could lead to asynchrony of the dentate gyrus (DG)-evoked stratum lucidum fiber volley (FV). Both *A* and *B* show DG and area CA3 of a transverse hippocampal slice. Arrows: possibly active pathways. Schematic recording electrode is placed in area CA3. *A*: direct, orthodromic activation is shown alongside possible indirect anti-/orthodromic activation of mossy fiber (MF) hilar collaterals. *B*: direct, orthodromic activation is shown alongside possible antidromic activation of CA3 associational collaterals that leads to antidromic spiking of CA3 cells.

creased conduction distance of an action potential. 2) Antidromic activation of CA3 associational collaterals in the hilus (Fig. 1*B*) could lead to apparent asynchrony if either the conduction velocities and/or conduction distances of these fibers are different from those of MFs. Finally, 3) intrinsic delayed spiking of the large MF boutons could lead to apparent asynchrony if different boutons exhibit different delays to spike.

In this paper we further examine the origins of the apparent asynchrony of the DG-evoked FV recorded in the stratum lucidum of area CA3. With the use of a combination of carefully placed stimulating and recording electrodes, as well as a series of cuts in the slice, we conclude that the MF FV is not intrinsically asynchronous. All the observed asynchrony in the FV recorded from the stratum lucidum arises from direct stimulation of MF and CA3 collaterals in the hilus.

METHODS

In vitro slice preparation

Slices were prepared from 27- to 40-day-old male Sprague-Dawley rats (Zivic-Miller). Animals were anesthetized deeply with equithesin and their chest cavities were rapidly opened. The rats were then perfused transcardially with a cold oxygenated sucrose solution (Aghajanian and Rasmussen 1989) with the following composition (in mM): 229 sucrose, 1.9 KCl, 1.2 Na₂PO₄, 33 NaHCO₃, 10 glucose, and 6.0 MgCl₂. Before the transcardial perfusion, the sucrose solution was bubbled with 95% O₂-5% CO₂ to adjust the pH to 7.4 at 4°C. After 1 min of transcardial perfusion, the rats were decapitated and the hippocampi were dissected from the brain. Each hippocampus was then cut into 400- μ m-thick slices perpendicular to its long axis with the use of a vibratome.

After sectioning, slices were maintained at room temperature

(22°C) in an artificial cerebrospinal fluid (ACSF) consisting of (in mM) 126 NaCl, 3.5 KCl, 1.2 Na₂PO₄, 11 glucose, 25 NaHCO₃, 3.0 MgCl₂, and 3.0 CaCl₂. The ACSF was bubbled with 95% O₂-5% CO₂ (pH = 7.4). Individual slices were transferred as needed (between 2 and 12 h after slice preparation) to a submerged chamber where they were constantly superfused with oxygenated ACSF at 33°C or room temperature. All figures show data recorded at 33°C; however, most experiments were also repeated at room temperature (~22°C), as indicated in the text.

Electrophysiological recording

FVs were recorded extracellularly with an Axoclamp 2A amplifier (Axon Instruments) via 5-M Ω electrodes filled with 0.5 M NaCl. Intracellular recordings also were made with the Axoclamp 2A amplifier via 100-M Ω electrodes filled with 1 M KCl. All FV recordings were obtained in the presence of 10 mM kynurenic acid (Sigma) to block excitatory neurotransmission. We have found that at this concentration both α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid and *N*-methyl-D-aspartate receptors are completely blocked (See Urban and Barrionuevo 1996) and no further changes are observed when 10 μ M 6-cyano-7-nitroquinoxaline-2,3-dione and 25 μ M D-2-amino-5-phosphonovaleric acid are added to the bath. We also observed no change in the waveform following addition of 10 μ M bicuculline methiodide, indicating that γ -aminobutyric acid-A-receptor-mediated currents do not contribute to our waveforms. The lack of effect of bicuculline also indicates that axons of interneurons do not contribute to the FV. Data were digitized (10 kHz) and then stored on computer disk. Data were collected and analyzed with the use of LabView (National Instruments) and Origin (Microcal). All average values are given as means \pm SE. All distances reported were measured with the use of a calibrated reticule eyepiece on a dissecting stereo microscope.

Placement of cuts in slices

All cuts in slices were accomplished by the use of a jet of ACSF ejected from the tip of a broken pipette (tip diameter ~25–50 μ m). By mounting the “cutting” pipette on a standard micromanipulator, precise cuts were made with little, if any, damage to surrounding tissue. Cuts were considered effective if we could see completely through the slice at the location of the cut. In some experiments, the efficacy of the cuts was confirmed by completely transecting the slice between the stimulating and recording electrodes and verifying that this caused a complete abolition of the evoked FV.

Analysis and quantification of the FVs

The FVs were quantified by measuring the amplitude of troughs for both the early and the late components. Quantification of early versus late components was accomplished by dividing the FV waveforms into two separate regions. With the use of the most asynchronous waveform for each group, the beginning and end of the FV were determined. The beginning of the FV was defined as the point before the first positive or negative deflection from baseline. The end of the FV was defined as the point at which the FV returned to or crossed baseline. The separation into early and late components was made at the point at which the FV showed the first peak following the first trough (see arrows in Figs. 2*B* and 4*B*). We found that these criteria provided a consistent means to quantify visually apparent changes in the evoked FV.

RESULTS

Degree of asynchrony in the DG-evoked FV is correlated with proximity of the stimulating electrode to the hilus

Previously, Langdon et al. (1993) demonstrated that cuts placed in the hilus reduce the asynchrony of the DG-evoked

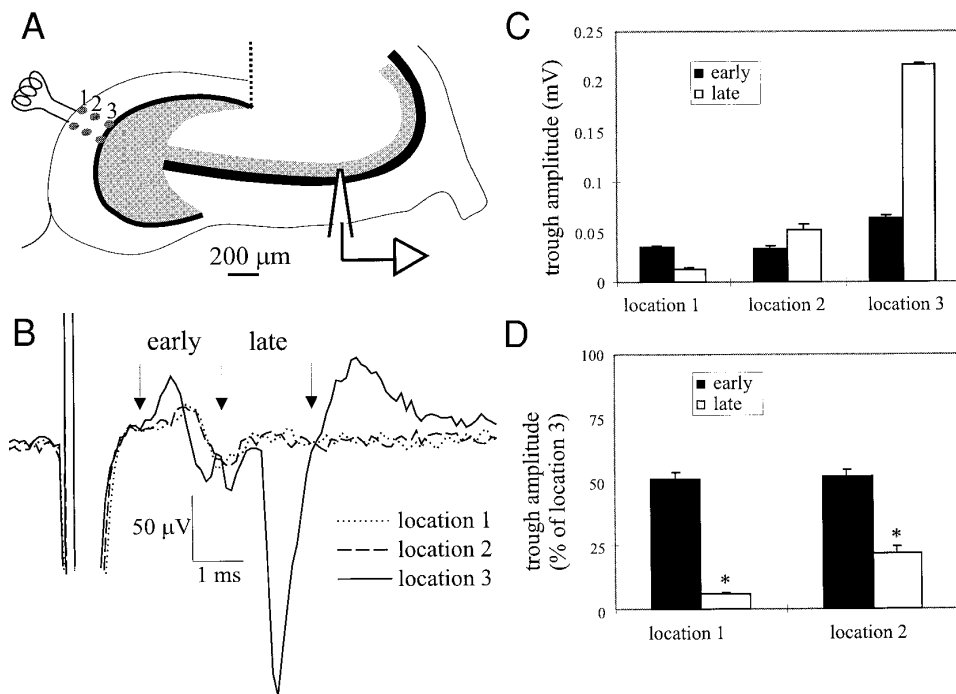


FIG. 2. Degree of asynchrony in FV is correlated with proximity of stimulating electrode to the hilus. *A*: tested stimulation sites. Vertical dotted line at tip of DG: cut placed to prevent direct perforant path activation from occurring in CA3. *B*: effect of moving stimulating electrode closer to the hilus on the DG-evoked FV waveforms (each trace is the average of 20 responses). Arrows: points of separation of waveform into early and late components. *C*: average early and late trough amplitude for the group of experiments (mean \pm SE) at each of the 3 stimulation locations ($n = 8$ for location 1 and $n = 12$ for locations 2 and 3). *D*: % difference in average early and late trough amplitude for stimulation in the DG molecular layer (1, 2) compared with stimulation on the hilar border (3). For *B–D*, location 1 corresponds to distal molecular layer, location 2 to middle molecular layer, and location 3 to granule cell layer of DG. Asterisk: $P < 0.001$, 1-tailed Student's *t*-test.

FV. It was concluded that direct activation of MF hilar collaterals by the stimulating electrode placed in the DG could lead to an axon reflex where there is antidromic followed by orthodromic conduction of an action potential in some MFs. The additional distance incurred by the antidromic conduction in some MFs delays the arrival of the FV relative to those MFs that exhibit only orthodromic conduction. However, the cuts did not completely abolish the asynchrony of the FV, suggesting the existence of an additional mechanism of asynchrony intrinsic to MFs. In the present work, we used two different approaches to eliminate/reduce activation of hilar MF collaterals so that the intrinsic asynchrony of MFs could be studied in isolation. The first approach was to place the stimulating electrode in the outer molecular layer near the hippocampal fissure, in the region of the dendrites of granule cells. Stimulating in this location should be generally as effective in activating granule cells (and subsequently MFs) while reducing the probability of activating hilar collaterals. This stimulation site resulted in a synchronous FV (Fig. 2*B*). When the stimulating electrode was moved closer to the hilus, there was an increase in the asynchrony of the FV. In particular, late components appeared when the stimulating electrode was placed closer to the hilus. Figure 2*C* shows that the amplitudes of the largest trough for both the early and late components of the FV increase as the stimulation site is moved toward the hilus; however, the late components are much more affected. The amplitude of the early components of the FV evoked by stimulating near the hippocampal fissure (location 1) was $50.8 \pm 3.0\%$ (mean \pm SE) of the amplitude of the early component amplitude when the stimulation was at the hilar border (location 3). In contrast, the amplitude of the late component of the FV when the stimulation was at the fissure was only $6.0 \pm 0.8\%$ of the late component when stimulation was at the hilar border (Fig. 2*D*). The relative changes of the early and late components are significantly different ($P < 0.001$, 1-tailed

paired Student's *t*-test; $n = 8$), suggesting that moving the stimulating electrode closer to the hilus differentially affects the late component of the FV. In all cases, the FV evoked from stimulation near the hippocampal fissure was apparently synchronous. Similar results were obtained at room temperature ($n = 6$).

The second approach to reduce hilar collateral activation was to create a "minislice" that contained only the stratum lucidum and stratum pyramidale of CA3. This preparation is useful for two reasons. First, it eliminates the influence of the hilar collaterals from both MFs and CA3 pyramidal cells. Second, it transects most, if not all, of the associational collaterals of CA3 pyramidal cells that could be activated by stimulating CA3 directly as shown in Fig. 3*A*. As a result, the conduction of the FV from one end of the minislice to the other is predominantly carried by MFs. We found that stimulating at one end and recording at the other end of these minislices yields a synchronous FV (Fig. 3; $n = 4$ at 33°C , $n = 7$ at room temperature). Reversing the stimulating and recording electrode positions so that activation was antidromic for the MFs also yielded a synchronous FV (data not shown).

Antidromic activation of CA3 pyramidal cells contributes to asynchrony of the DG-evoked FV

In addition to the anti-/orthodromic activation of MF hilar collaterals, a second possible origin of apparent asynchrony of the FV is the direct activation of CA3 pyramidal cell axon collaterals located in the hilus (see Fig. 1*B*) (Ishizuka et al. 1990; Li et al. 1994). We tested this possibility by transecting the MF axons between the stimulation and recording sites (see Fig. 4*A*). We found that the transection of the stratum lucidum and stratum pyramidale, which should cut all MFs, removes the early component(s) of the FV and leaves the late component intact (Fig. 4*B*). Figure 4*C* shows

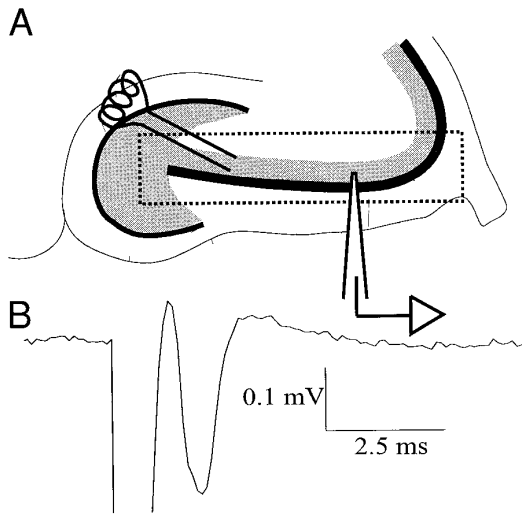


FIG. 3. Direct stimulation of the isolated stratum lucidum and stratum pyramidale of CA3 results in a synchronous FV. A minislice consisting of only stratum lucidum and stratum pyramidale was prepared and the MFs were directly stimulated. Minislice used is the area of a typical transverse slice enclosed within the dotted box (A). B: average of 10 responses recorded 500 μm from stimulating electrode. Similar results were found in 4 slices at 33°C and in 7 slices at room temperature.

the effect of transecting the stratum lucidum on the amplitude of the largest trough on the early and late components. Transection of the stratum lucidum attenuated the late component of the FV to $74.4 \pm 8.9\%$ of control, whereas the early component was reduced to $17.7 \pm 1.83\%$ of control (Fig. 4D). Because the only fibers that remain intact after the stratum lucidum transection are the CA3 collaterals, the late components of the FV are due to the direct activation of CA3 collaterals in the hilus and DG ($n = 8$ at 33°C; $n = 6$ at RT). Similar results were obtained when cuts extended from the stratum lucidum all the way through the stratum oriens, suggesting that the observed asynchrony is not due to infrapyramidal MFs (data not shown).

The relatively large amplitude of the late CA3-collateral-mediated component suggests that the FV is augmented by an antidromically activated population spike of the CA3 pyramidal cells. A laminar analysis of the extracellular signal that remains after the transection (Fig. 5A) provides additional support for this hypothesis. As indicated in Fig. 5A, the recording electrode was placed at several locations in area CA3 between the middle of the stratum radiatum and the middle stratum oriens. It was observed that the extracellular currents are largest in the stratum pyramidale and reverse polarity in the stratum radiatum, which is consistent with a population spike of the CA3 pyramidal cells. This was pattern was observed in six of six slices tested. As a final test of whether CA3 pyramidal cells are activated antidromically by stimulation on the DG/hilar border, we looked for antidromic action potentials in CA3 pyramidal cells. Figure 5C shows an example of the outcome of a collision test performed for a stimulation site on the DG/hilar border. The records show that an antidromic action potential is blocked by a preceding action potential elicited by a depolarizing current step injected into the CA3 pyramidal cell. Similar antidromic responses were obtained in three additional cells (all at 33°C).

Conduction velocity of the MFs is faster than that of the CA3 collaterals

Our results (see Fig. 4) suggest that the MFs have a faster conduction velocity than the associational/Schaffer collaterals. We verified these observations by comparing the conduction velocities for the MFs and CA3 collaterals recorded from the same slices (Fig. 6). MF conduction velocity was measured with the use of either a stimulation location in the molecular layer of the DG and recording in the stratum lucidum (see above), antidromically activating the MFs and recording in the DG, or with the use of stratum lucidum minislices. All of the preparations yield nominally pure FVs and no differences in conduction velocity were observed

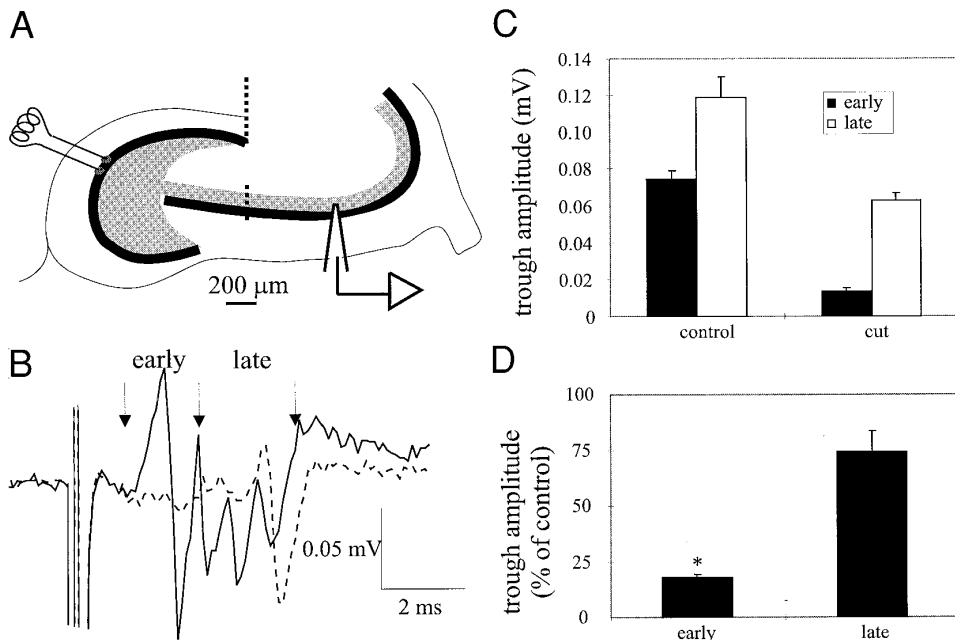


FIG. 4. Late component of FV is not due to activation of MFs. A: location of electrodes and placement of cuts (vertical dotted lines). Cut through stratum lucidum was made after control traces were recorded. B: DG-evoked FVs before (—) and after (---) transection of stratum lucidum. Note that transection of stratum lucidum abolishes the early components while leaving the late component relatively unchanged. C: amplitude (mean \pm SE) of early and late troughs (as defined by \downarrow in B) for control and stratum-lucidum-transected cases ($n = 8$). D: relative amplitude of early and late components after transection of stratum lucidum expressed as % of control (mean \pm SE). These relative changes are significantly different ($P < 0.05$, 1-tailed paired Student's *t*-test).

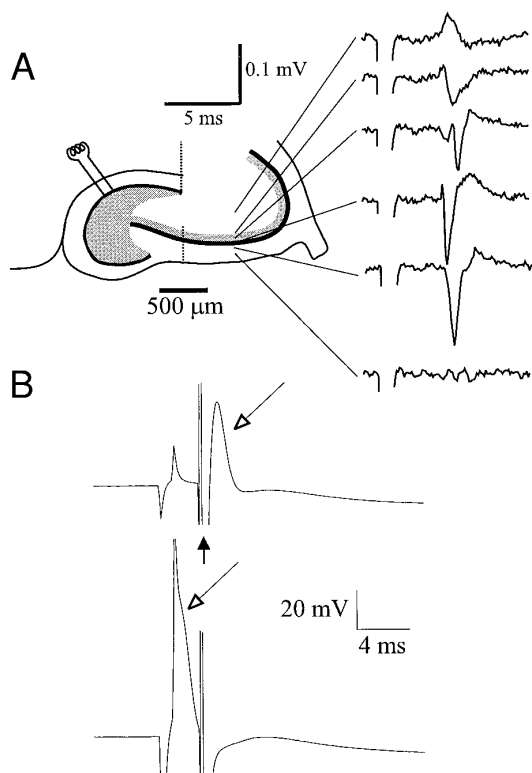


FIG. 5. Stimulation in the DG near the hilus results in an antidromic activation of CA3 pyramidal cells. Stimulating electrode was placed in the suprapyramidal blade as illustrated in A. A cut (vertical dotted lines) was placed to transect stratum lucidum, stratum pyramidale, and stratum oriens, as well as a cut to transect the direct perforant path projections to CA3. This arrangement of cuts left a small window of intact fibers in stratum radiatum. DG-evoked FVs were then recorded at various locations in CA3, as indicated by lines from the FV traces. Laminar profile of the DG-evoked FV shows a reversal at stratum lucidum/stratum radiatum border, which suggests that the DG-evoked FV recorded under these conditions is actually an antidromically evoked population spike of CA3 pyramidal cells. B: outcome of a collision test. An intracellular recording was obtained from a CA3 pyramidal cell. An antidromic action potential was first evoked by stimulation on the DG/hilus border (*top trace*). Prior activation of an action potential by a brief depolarization of the cell blocked the antidromically activated action potential (*bottom trace*).

across methods. The associational collateral conduction velocity was measured by placing the stimulating electrode in the stratum oriens near the fimbria and recording FVs in the stratum oriens between the DG and the stimulating electrode. At 33°C, we find that the mean conduction velocity is 0.51 m/s for the MFs and 0.37 m/s for the associational collaterals ($n = 7$, $P < 0.001$, Student's t -test).

DISCUSSION

There are three main findings from the present study. 1) The MF FV is intrinsically synchronous. 2) Electrical stimulation of the DG near the hilus leads to antidromic activation of CA3 pyramidal cells. 3) MFs have a faster conduction velocity than the associational collaterals of CA3 pyramidal cells.

Synchrony of the MF FV in CA3

Langdon et al. (1993) observed that the asynchrony of EPSCs evoked by stimulation of the DG granule cell layer is correlated with an apparent asynchrony of the FV. This result suggested that the asynchrony of the EPSC is due to the asynchronous depolarization of MF boutons. Two possible mechanisms were proposed to underlie the observed asynchrony. The first mechanism was termed the anti-/orthodromic mechanism; this involved antidromic activation of MF hilar collaterals followed by orthodromic conduction in the main MF axon (see Fig. 1 B). The second mechanism proposed was a delayed sodium spiking of the large MF boutons. It was shown that although the anti- or orthodromic mechanism could account for some of the asynchrony, it could not account for all that is observed. Although the remaining proposed mechanism of delayed spiking would be physiologically interesting, our data suggest that this mechanism does not contribute to the asynchrony of the FV recorded in the stratum lucidum. If MFs are intrinsically asynchronous, then stimulation of the dendritic layer of the DG as well as direct stimulation of stratum lucidum in CA3 minislices will result in an asynchronous FV. In the present study, however, no asynchrony was observed with either of these two experimental paradigms. These results indicate

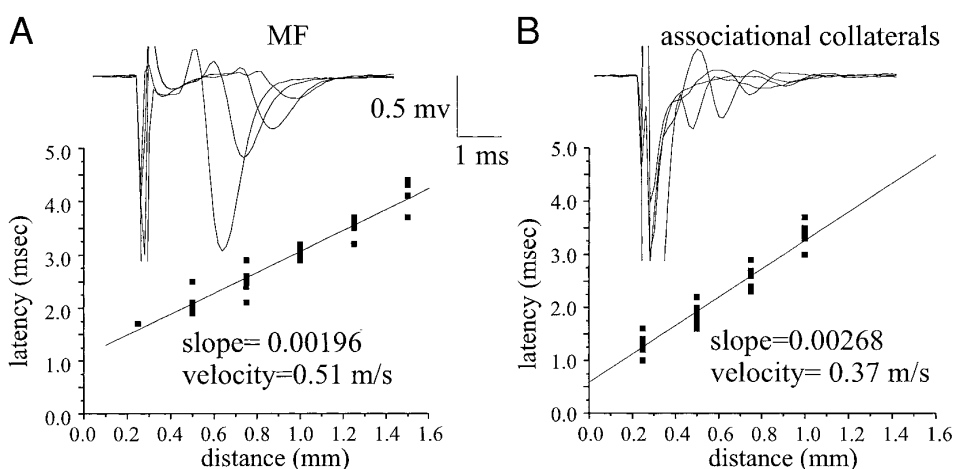


FIG. 6. MFs have a faster conduction velocity than CA3 associational collaterals. For the conduction velocity of MFs as shown in A, an antidromic population spike was recorded in the DG while the stimulating electrode was placed at various distances in stratum lucidum. For the CA3 collaterals as shown in B, stimulating electrode was placed in the fimbria and recording electrode was placed at various distances in stratum oriens. Latencies of peak amplitudes for FVs/population spikes vs. conduction distance (measured by a reticule) are shown with the best-fit linear regression line. Conduction velocities were calculated from the inverse slope from the fit. MF conduction velocity = 0.51 m/s, CA3 collateral conduction velocity = 0.37 m/s ($n = 7$, $P < 0.001$, 1-tailed Student's t -test). *Insets*: representative traces from each of the 2 fiber systems.

that the MFs are a homogeneous population of axons with a uniform conduction velocity and have no asynchronous spiking of the presynaptic boutons. Therefore, if there is asynchrony in the unitary MF EPSC, as suggested by data collected with the use of minimal stimulation (Jonas et al. 1993), it probably arises from nonelectrogenic mechanisms across the multiple release sites within a single MF bouton.

Antidromic activation of CA3 pyramidal cells following bulk stimulation of the DG

Over time, hippocampal synaptic circuitry has been shown to be much more complex than the originally described hippocampal trisynaptic circuit (For review see Amaral 1993). In addition to the functional implications of the newly appreciated complexity of hippocampal circuitry, there are several technical implications for studies in which bulk stimulation is used to evoke synaptic activity. Bulk stimulation of the DG to evoke MF synaptic activity in CA3 pyramidal cells has two possible monosynaptic non-MF contaminants. The first monosynaptic non-MF input is the direct perforant path connection from entorhinal cortex to the distal dendrites of CA3 pyramidal cells. At least some of these fibers course through the molecular layer of the DG on their way to CA3 (Tamamaki and Nojyo 1993; and personal observations). As a result, bulk stimulation of the DG molecular layer could result in activation of both the perforant path and MF synaptic inputs to CA3 pyramidal cells. The second monosynaptic non-MF input is by the hilar-projecting collaterals of CA3 pyramidal cells (Ishizuka et al. 1990), some of which have been shown to project to the inner third of the DG (Li et al. 1994). These fibers also can be activated by bulk stimulation in the granule cell body layer. The direct activation of these fibers causes antidromic spiking of CA3 associational collaterals, which would, in turn, evoke monosynaptic responses in CA3 pyramidal cells. In most cases, the monosynaptic responses arising from the antidromic activation of associational collaterals would be indistinguishable from monosynaptic responses arising from the MFs.

Our results, therefore, suggest that the EPSP/C time course criteria designed to eliminate polysynaptic contamination (see Claiborne et al. 1993) are insufficient to ensure a pure monosynaptic MF EPSP/C. Stimulation of the DG can result in activation of at least three possible monosynaptic pathways to CA3 pyramidal cells: 1) the MFs, 2) the perforant path, and 3) the antidromic activation of hilar-projecting CA3 associational collaterals. None of the criteria proposed by Claiborne et al. (1993) will rule out contamination by pathway 2 or 3. EPSCs from synapses of the perforant path have been observed to have rise times <2.5 ms (personal observations) and synapses from the associational collaterals can have EPSC rise times as fast those observed at the MF synapse (Henze et al. 1996; Spruston et al. 1993). As a result, we suggest that when evoking MF synaptic responses with the use of bulk stimulation, stimulating electrodes should be placed in the outer molecular layer of the DG to prevent the activation of hilar-projecting associational collaterals. This stimulation site should be accompanied by a cut at the tip of the suprapyramidal granule cell layer to prevent monosynaptic perforant path activation of CA3 pyramidal cells. This procedure should be added to the pre-

viously proposed criteria for preventing polysynaptic contamination of the evoked MF synaptic response. It should be noted that stimulation in the molecular layer of the DG may lead to a very strong activation of the granule cells. This would happen because additional granule cells would be activated synaptically by perforant path fibers activated by the stimulating electrode.

Initially, we were concerned about the apparent discrepancy between the high reliability of the antidromic activation of CA3 pyramidal cells as observed in the FV (present in all slices) and the low incidence of observed antidromic activation at the single-cell level when recording intracellularly (personal observations). We feel, however, that this apparent discrepancy can be explained by the anatomic connectivity, membrane biophysical properties, and experimental selection of stimulation site and intensity. First, in a slice, there is a low probability of finding a monosynaptic connection between any DG stimulation site and any recording site in CA3 (Xiang et al. 1994). This low probability applies to both the orthodromic activation of MFs as well as the antidromic activation of associational collaterals. However, when recording from a single cell, the probability of observing a monosynaptic MF EPSC is higher than that of observing an antidromic action potential. This is because activation of a single MF axon produces synaptic input in multiple CA3 pyramidal cells (~ 14) (Amaral et al. 1990), whereas activation of a single associational collateral leads to an antidromic somatic spike in only one cell. A second reason for the apparent discrepancy is that the current density from an action potential in an MF axon and/or bouton is certain to be smaller than that produced by an action potential in an adjacent CA3 pyramidal cell. This is because there is much more cell membrane (and therefore ionic channels) associated with a single CA3 pyramidal cell soma than a single MF axon. The two mechanisms above, when taken together, explain why the FV almost always has an antidromic spike component from CA3 pyramidal cells whereas single-cell recordings rarely, if ever, do. A final reason for the apparent discrepancy is that in cases in which an action potential is observed in a single cell recording, we reduce the stimulation intensity and/or move the stimulation electrode to another site. This point was made even clearer to us when we attempted to perform the collision test experiments (see Fig. 5) and easily found CA3 pyramidal cells that demonstrated antidromic spiking following DG stimulation.

MFs conduct faster than CA3 associational collaterals

It has been widely believed that CA3 associational collaterals have a faster conduction velocity than the MFs. This belief has been based primarily on the reports that the MFs lack myelin (Hamlyn 1962), whereas the associational/Schaffer collaterals are partially myelinated (Andersen 1975). A review of the literature reveals a range of values for the conduction velocity of the associational/Schaffer collaterals of 0.47 m/s (guinea pig in vitro slices at 37°C) (Miles et al. 1988) to 0.51 m/s (rat in vivo) (Finnerty and Jefferys 1993) to 0.3 m/s (rat in vitro slices at unknown temperature) (Andersen et al. 1978). With respect to the MFs, a single previous work reports a conduction velocity of 0.67 m/s (rat in vitro slices at 33°C) (Langdon et al.

1993). Because of the variety of conditions under which the associational/Schaffer collateral conduction velocity has been measured, it is difficult to compare the values with the single reported conduction velocity for MFs. Several possible reasons may account for the variations in the conduction velocity values in the literature including temperature, species, animal age, and perhaps external divalent concentrations. The data presented here demonstrate that when measured in the same slice prepared from a young adult rat, the MFs do, in fact, conduct action potentials at a higher velocity than do the associational/Schaffer collaterals of CA3 pyramidal neurons.

These conduction velocity data also address a previous concern regarding polysynaptic contamination of DG-evoked MF synaptic activity in that the partially myelinated (Andersen 1975) associational collaterals activated polysynaptically might conduct action potentials fast enough to overtake the direct MF activity (Langdon et al. 1993). Our data concerning the relative conduction velocities of MFs and CA3 associational collaterals suggest that this scenario can never occur because of the faster conduction velocity of the MFs. The differential conduction velocities of the different fiber systems within the hippocampal formation also may have important implications for the function of the hippocampus, particularly when considering the timing of arrival for synaptic input from the perforant path, the MF, and associational collateral synapses onto CA3 pyramidal cells.

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