

Timing and efficacy of transmitter release at mossy fiber synapses in the hippocampal network

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Abstract It is widely accepted that the hippocampus plays a major role in learning and memory. The mossy fiber synapse between granule cells in the dentate gyrus and pyramidal neurons in the CA3 region is a key component of the hippocampal trisynaptic circuit. Recent work, partially based on direct presynaptic patch-clamp recordings from hippocampal mossy fiber boutons, sheds light on the mechanisms of synaptic transmission and plasticity at mossy fiber synapses. A high Na⁺ channel density in mossy fiber boutons leads to a large amplitude of the presynaptic action potential. Together with the fast gating of presynaptic Ca²⁺ channels, this generates a large and brief presynaptic Ca²⁺ influx, which can trigger transmitter release with high efficiency and temporal precision. The large number of release sites, the large size of the releasable pool of vesicles, and the huge extent of presynaptic plasticity confer unique strength to this synapse, suggesting a large impact onto the CA3 pyramidal cell network under specific behavioral conditions. The characteristic properties of the hippocampal mossy fiber synapse may be important for pattern separation and information storage in the dentate gyrus-CA3 cell network.

Keywords Presynaptic recording · Mossy fiber boutons · Mossy fiber synapses · Hippocampus · Autoassociative networks · Episodic memory · Synaptic efficacy

The mossy fiber synapse: a key connection in the hippocampal network

The hippocampal formation consists of three types of principal neurons: granule cells in the dentate gyrus, CA3 pyramidal cells, and CA1 pyramidal neurons. These cells are interconnected by glutamatergic synapses, forming the classical trisynaptic circuit (Fig. 1a,b). Dentate gyrus granule cells receive excitatory glutamatergic input from layer 2 pyramidal cells of the entorhinal cortex and project to CA3 pyramidal cells. CA3 pyramidal cells project to CA1 cells, which in turn project to the subiculum and back to the entorhinal cortex [4]. In addition to this trisynaptic circuit, there is also a direct input from the entorhinal cortex to both CA3 and CA1 pyramidal cells. Furthermore, CA3 pyramidal neurons are extensively connected to each other via recurrent collateral synapses.

The nonmyelinated axons of the granule cells, the so-called mossy fibers, show several structural properties that distinguish them from the other synaptic pathways [39]. They project to the CA3 region, mainly traveling within a narrow band termed “stratum lucidum.” Several (~15) large “giant” boutons (~3–5- μ m diameter) emerge from a single mossy fiber axon, either arranged in an en passant manner or attached to the main axon via a short perpendicular axonal branch [1, 8, 29, 31] (Fig. 1c,d). A large mossy fiber bouton typically contacts only a single CA3 pyramidal neuron [21], and a mossy fiber axon contacts a given CA3 pyramidal neuron only once, as boutons are ~150 μ m apart. As the rat hippocampus contains ~1 million granule cells

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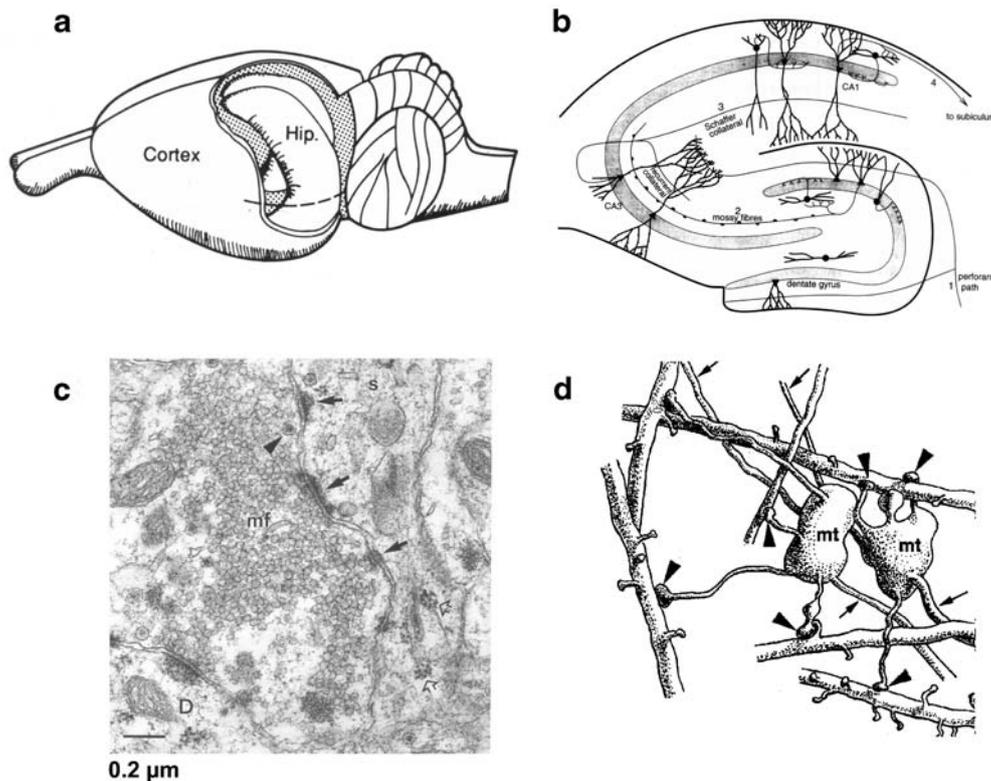


Fig. 1 Anatomical position of mossy fiber synapses in the hippocampal formation. **a** Macroscopic anatomy of the hippocampus in rat brain. The neocortex was fenestrated to show the location of the underlying hippocampal formation. **b** Schematic diagram of the trisynaptic circuit of the hippocampus. The major components are: perforant path–dentate gyrus granule cells–mossy fibers–CA3 pyramidal neurons–Schaffer collaterals–CA1 pyramidal neurons–subiculum. **c** Electron micrograph of a large hippocampal mossy fiber bouton in stratum lucidum of the

CA3 region. *mf* Mossy fiber bouton, *s* spine, *D* dendrite (of CA3 pyramidal neuron). *Arrows* indicate synaptic membrane specializations, *arrowhead* points to large dense-core vesicle, and *open arrows* indicate ribosomes at the spine base. Electron micrograph taken from Frotscher [30]. **d** Schematic illustration of two mossy fiber boutons. *Arrows* indicate mossy fiber axons, *arrowheads* show filopodial extensions, which contact interneuron dendrites. Illustration from Acsády et al. [1], with permission of the Society for Neuroscience, copyright (1998)

and ~300,000 CA3 pyramidal neurons (per hippocampus; [4]), and as a single granule cell contacts ~15 CA3 pyramidal neurons, a single CA3 pyramidal neuron on average receives input from $\sim 15 \times 1,000,000 / 300,000 = 50$ granule cells. Thus, the mossy fiber–CA3 pyramidal neuron synapse forms a sparse connection.

In addition to the large boutons on CA3 pyramidal neurons, the mossy fiber forms three other types of synaptic terminals, contacting distinct postsynaptic target cells. (1) Large boutons emerging from hilar collaterals (~15 per mossy fiber axon) form synapses on glutamatergic hilar mossy cells [1]. Mossy cells, in turn, establish an excitatory feedback circuit by forming synapses on granule cells of the ipsilateral dentate gyrus and on granule cells of the contralateral side. (2) Filopodial extensions emerging from large mossy fiber boutons (~2.5 filopodia per large bouton, [1]) form synapses on GABAergic interneurons in the stratum lucidum [1, 81] (Fig. 1d). In addition, large mossy fiber boutons can contact the dendritic shafts of GABAergic interneurons in the stratum lucidum directly [29]. (3) Small boutons emerging from the main axon (~15) or

from hilar collaterals (~150 per mossy fiber axon) form synapses on GABAergic interneurons in the stratum lucidum and the hilus [1]. Thus, the total number of synapses formed on GABAergic interneurons (in CA3 and hilus) via filopodial extensions and small boutons greatly exceeds the total number of synapses formed on glutamatergic neurons (CA3 pyramidal neurons and mossy cells) via large boutons [1].

Presynaptic action potential and voltage-gated Na^+ and K^+ channels in hippocampal mossy fiber boutons

After the development of methods for patch-clamp recording in brain slices [26, 38], techniques were gradually refined to a point when it became possible to obtain recordings from presynaptic elements, including mossy fiber boutons [12, 28, 34]. The key steps of improvement were as follows: (1) the enhanced visibility provided by high-numerical aperture objectives and infrared illumination [75]; (2) the development of vibratomes with minimal blade vibration in the

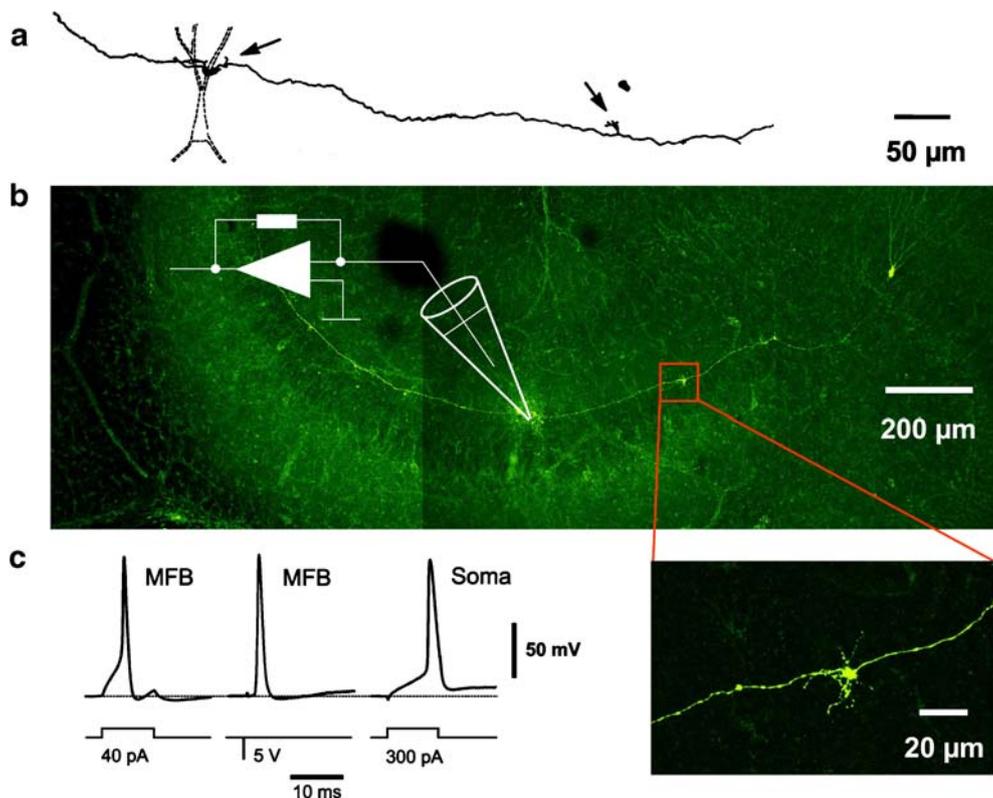


Fig. 2 Direct patch-clamp recording from hippocampal mossy fiber terminals. **a** Hippocampal mossy fiber bouton, filled with biocytin during whole-cell recording, stained with avidin-horseradish peroxidase complex, and reconstructed using a camera lucida. *Arrows* show the recorded bouton (*left*) and an adjacent bouton emerging from the same axon (*right*). Image from N. Spruston and P. Jonas (1993), unpublished. **b** Hippocampal mossy fiber bouton, filled with biocytin during whole-cell recording, stained using Alexa-conjugated avidin, and examined with a confocal laser scanning microscope. The

recorded bouton is indicated by the attached schematic pipette. The *square box* indicates the adjacent bouton, shown at higher magnification in the *inset*. The granule cell soma is visible on the *right*. Image from G. Stocca and J. Bischofberger, unpublished. **c** Presynaptic action potentials in mossy fiber boutons evoked by direct current injection (*left*) and extracellular stimulation of the axon (*middle*). The action potential recorded in a granule cell soma is shown for comparison (*right*). Note that the presynaptic action potential is ~two times briefer than the somatic action potential. Data from Bischofberger et al. [6]

vertical direction, resulting in an improved preservation of superficial tissue layers [34], although mossy fiber terminal recordings with commercially available tissue slicers were also reported [72]; (3) the use of sucrose-based cutting solutions with reduced Na^+ and Ca^{2+} concentrations, which further improves tissue preservation during cutting and maintenance of slices [34]. Collectively, these refinements made it possible to routinely obtain patch-clamp recordings from large hippocampal mossy fiber boutons in the stratum lucidum [6, 27, 33, 37].

Direct patch-clamp recording allowed us, for the first time, to examine the presynaptic action potential that triggers glutamate release at a cortical glutamatergic synapse (Fig. 2). Kinetic analysis revealed that the presynaptic action potential was very brief, with a half-duration of 915 μs at 22°C and 380 μs at 34°C [6, 33]. A comparison of action potentials in mossy fiber boutons with those in granule cell somata revealed that the time course of the presynaptic action potential was ~two times faster than that of the somatic action potential in the same cell type [6, 33] (Fig. 2c). Thus, the properties of the presynaptic action potential are highly

specialized. Brief presynaptic spikes may ensure the synchrony of transmitter release at mossy fiber synapses.

The characteristic properties of the presynaptic action potential in hippocampal mossy fiber boutons are conferred by the expression of a distinct set of voltage-gated ion channels. Voltage-clamp analysis revealed that several types of voltage-gated channels are expressed in hippocampal mossy fiber boutons. First, these boutons contain a high density of voltage-gated Na^+ channels [27]. Estimates based on outside-out patch recordings suggested that the Na^+ conductance density was $\sim 50 \text{ mS cm}^{-2}$, corresponding to $\sim 40 \text{ channels } \mu\text{m}^{-2}$ and $\sim 2,000 \text{ channels per typically sized bouton}$. The Na^+ channel density in hippocampal mossy fiber boutons is comparable to previously reported Na^+ channel densities in peripheral axons [41, 42]. Thus, mossy fiber boutons have axon-like properties. Immunocytochemical studies showed a high level of Nav1.2 immunoreactivity in the mossy fiber tract [83]. Furthermore, Nav1.2 and Nav1.6 immunoreactivity are often colocalized in nonmyelinated axons throughout the entire brain [18]. This suggests that native presynaptic channels in mossy fiber

boutons are assembled from either Nav1.2 or Nav1.2/Nav1.6 alpha subunits.

Mossy fiber boutons also express functionally specialized voltage-gated K^+ channels [33]. These channels show low activation threshold, relatively fast inactivation, and are blocked by external tetraethylammonium ions (TEA) and dendrotoxin (a toxin from the green mamba, which blocks K^+ channels assembled from Kv1.1, 1.2, and 1.6 subunits). These functional and pharmacological properties of the presynaptic Kv channels greatly constrain their subunit composition. The low activation threshold and the sensitivity to TEA and dendrotoxin suggest that the presynaptic channel is assembled from Kv1 subunits. Furthermore, the fast inactivation suggests that the presynaptic channel contains either Kv1.4 subunits or Kvbeta subunits. Consistent with this hypothesis, Kv1.1, Kv1.4, and Kvbeta immunoreactivity is found in the mossy fiber tract [22, 69]. Thus, the results suggest that the native K^+ channel in mossy fiber boutons is a Kv1.1/Kv1.4 heteromer or a Kv1.1 alpha/beta subunit heteromer [33].

Are hippocampal mossy fibers involved in information processing [23]? Or do they follow the rule, “The axon does not think. It only ax.” [7]? Experimental evidence suggests that the propagation of action potentials in mossy fiber axons is reliable, in both the orthodromic [6] and the antidromic direction [70, 72]. Furthermore, simulation of action potential propagation in mossy fiber boutons showed that the experimentally determined Na^+ conductance density of 50 mS cm^{-2} , if homogeneously implemented in boutons and axons, provides a high safety factor of action potential propagation [27]. These findings suggest that mossy fiber axons are reliable transmission devices.

However, two lines of evidence suggest that mossy fiber axons are also capable of information processing. First, the duration of the presynaptic action potential is not constant, but shows broadening during repetitive stimulation [33]. Action potential broadening is much more pronounced in mossy fiber boutons than in the calyx of Held in the auditory brainstem [11], implying that broadening is a specific property of cortical presynaptic terminals. This may suggest that information in the mossy fiber system is not only coded by action potential frequency and timing but also as action potential duration [25]. Second, recent experiments revealed that subthreshold excitatory postsynaptic potentials (EPSPs) can be passively propagated from the somatodendritic domain of granule cells to mossy fiber boutons [2]. This may suggest that information in the mossy fiber system is coded by both action potential (“digital”) and subthreshold (“analogue”) signals. Both spike duration coding and analogue coding may enrich the computational repertoire of mossy fiber axons.

Action potential–exocytosis coupling in hippocampal mossy fiber boutons

As in many other synapses, exocytosis at hippocampal mossy fiber boutons requires the opening of voltage-gated presynaptic Ca^{2+} channels. Voltage-clamp analysis of Ca^{2+} currents in hippocampal mossy fiber boutons revealed that the presynaptic Ca^{2+} channels show a high activation threshold [6]. Ca^{2+} currents recorded in mossy fiber boutons are largely blocked by the selective Ca^{2+} channel blockers ω -agatoxin IVa and ω -conotoxin GVIA, suggesting that they are of the P/Q-type (Cav2.1) and N-type (Cav2.2; [5]) Evoked mossy fiber–CA3 pyramidal neuron excitatory postsynaptic currents (EPSCs) are also reduced by ω -agatoxin and ω -conotoxin, demonstrating that both P/Q- and N-type channels couple to glutamate release [19]. Thus, converging evidence suggests that synaptic transmission at the mossy fiber–CA3 synapse is mediated by P/Q- and N-type Ca^{2+} channels. Ca^{2+} -imaging experiments recently suggested the presence of R-type Ca^{2+} channels in a subset of hippocampal mossy fiber boutons [59]. It remains to be shown whether these findings can be confirmed in direct recordings and what role R-type Ca^{2+} channels play in mossy fiber synaptic transmission [16, 32].

Analysis of the gating kinetics of the presynaptic Ca^{2+} channels revealed that both activation and deactivation of the presynaptic Ca^{2+} channels are fast, with time constants in the submillisecond range [6] (Fig. 3). As a consequence, the presynaptic Ca^{2+} channels are activated very efficiently during the rising phase of the presynaptic action potential. Maximal activation occurs shortly after the peak of the action potential. However, little Ca^{2+} current is flowing at that time because the driving force is small (Fig. 3a,b). During action potential repolarization, the driving force for Ca^{2+} current flow increases rapidly. However, concurrently the Ca^{2+} channels begin to deactivate. The temporally overlapping processes of changes in driving force and channel deactivation result in an approximately symmetrical Ca^{2+} current with short half-duration ($570 \mu\text{s}$ at 22°C and $170 \mu\text{s}$ at 34°C) and large amplitude. The peak of the current occurs in the center of the repolarization phase [6, 33]. Detailed computational analysis of Ca^{2+} channel gating in hippocampal mossy fiber boutons further suggested that the gating kinetics of activation and deactivation are optimized to generate a maximal Ca^{2+} inflow during a minimal period of time (Fig. 3c,d). Optimized Ca^{2+} channel gating will be important for efficacy and synchrony of transmitter release. Remarkably, the sequence of rapid activation and deactivation of presynaptic Ca^{2+} channels in mossy fiber boutons shows many similarities to the calyx of Held, which plays a key role in temporal signal processing in the auditory brainstem [10–12].

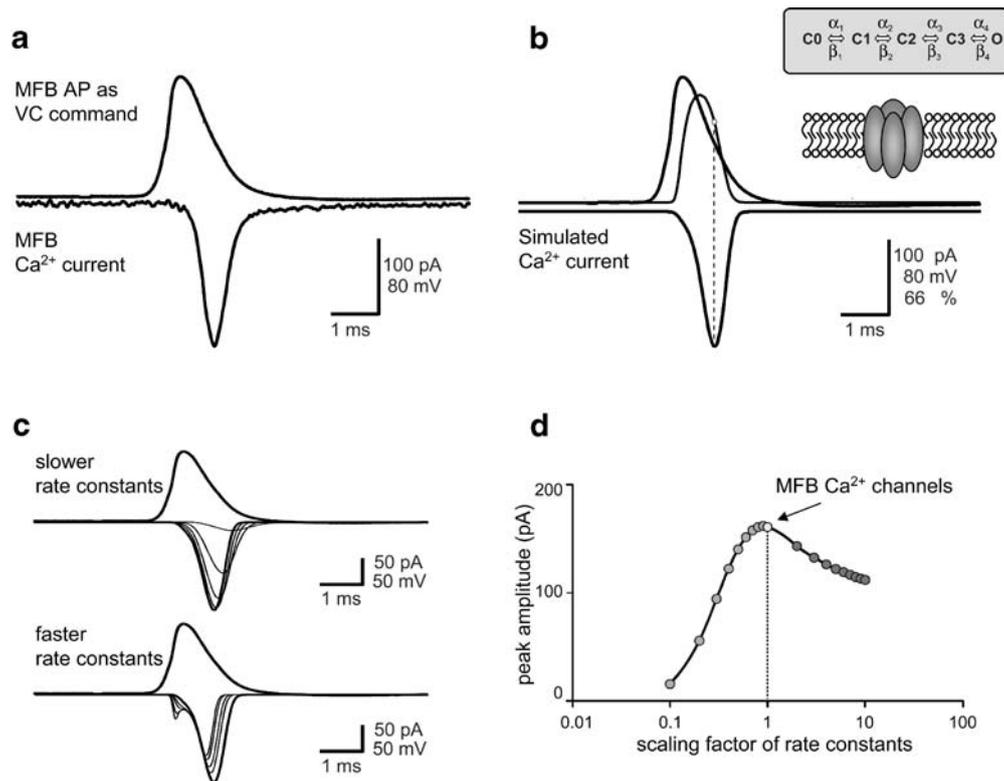


Fig. 3 Gating of presynaptic Ca^{2+} channels in mossy fiber boutons is optimized for high efficacy and temporal precision. **a** Presynaptic action potential waveform (*upper trace*) and presynaptic Ca^{2+} current (*lower trace*) in a whole-cell recorded hippocampal mossy fiber bouton. **b** Model of Ca^{2+} channel gating with four closed states and an open state linked by voltage-dependent forward and backward rates accurately reproduces the experimental observations. *Grey curve*, relative open probability of the Ca^{2+} channels. The relative open probability at the peak of the Ca^{2+} current (*dashed line*) is ~ 0.68 . **c** Activation of presynaptic Ca^{2+} channels

The coupling between Ca^{2+} channels and Ca^{2+} sensor of exocytosis at the mossy fiber synapse has not been analyzed at a quantitative level. However, the presynaptic Ca^{2+} current evoked by an action potential has a large amplitude, with an average value of ~ 170 pA [6]. Assuming a single channel current of ~ 0.2 pA at physiological divalent concentrations [15, 35], this would correspond to ~ 850 Ca^{2+} channels open at the peak in a typical terminal. Further assuming that P/Q- and N-type Ca^{2+} channels are restricted to active zones and that the number of release sites is 37 in a large bouton [21], we estimate that ~ 23 Ca^{2+} channels per release site are open at the peak. Thus, although the estimate is rough, the number of Ca^{2+} channels per release site appears to be large. Early studies suggested that the slow Ca^{2+} chelator ethylene glycol bis-2-aminoethyl ether-*N,N,N,N'*-tetraacetic acid (EGTA) applied extracellularly in a membrane-permeant form (200- μM EGTA-AM) reduces the peak amplitude of mossy fiber-CA3 pyramidal neuron EPSCs by $\sim 40\%$ [71]. Likewise, the fast Ca^{2+} chelator 1,2-bis(*o*-aminophenoxy)ethane-*N,N,N,N'*-tetraacetic acid (BAPTA)

with altered gating properties by presynaptic action potential waveforms. Slowing of gating (*upper traces*; factors 2–10) reduces the peak amplitude of the Ca^{2+} current. Acceleration of gating (*lower traces*; factors 0.9–0.1) reduces the Ca^{2+} current and leads to a biphasic Ca^{2+} current for large changes. **d** Plot of action potential-evoked Ca^{2+} current peak amplitude against scaling factor of the rates. The physiological value of the rates corresponds to a scaling factor of 1 (*vertical dashed line*). Data from Bischofberger et al. [6]

applied intracellularly (10 mM) completely blocks transmission at granule cell autapses [79]. Thus, the distance between Ca^{2+} source and Ca^{2+} sensor might be relatively large. In conclusion, the available data are consistent with the view that transmitter release at hippocampal mossy fiber synapses is triggered by a large number of presynaptic Ca^{2+} channels, which might result in a precisely timed presynaptic Ca^{2+} signal even in the presence of stochastic single channel gating. However, the distance between Ca^{2+} source and Ca^{2+} sensor may allow modulation of the amplitude of the presynaptic Ca^{2+} transient by endogenous Ca^{2+} buffers, such as calbindin- $\text{D}_{28\text{k}}$ [62].

The large size of the mossy fiber boutons and their proximal location on the apical dendrites of CA3 pyramidal neurons [17] suggested that the mossy fiber-CA3 pyramidal neuron synapse is a uniquely strong synapse, at least if compared to other synapses in the hippocampus. Indeed, unitary mossy fiber-CA3 pyramidal neuron EPSPs have amplitudes of 2–10 mV, and unitary EPSCs show amplitudes of up to 1 nA [2, 17, 33, 40, 43] (Figs. 4 and 5).

Three distinct but interrelated factors contribute to the high efficacy of this synapse. First, large boutons have a large number of release sites (37 sites, [21]; 31 sites, [1]). Second, although release probability is low under conditions of low-frequency stimulation [48], it increases substantially during trains of stimuli, due to paired-pulse facilitation, frequency facilitation, posttetanic potentiation (PTP), and long-term potentiation (LTP; [71]). The mechanisms for the induction and expression of LTP have remained controversial, as reviewed elsewhere [64]. Finally, mossy fiber boutons contain a very large pool of releasable vesicles (“maxi-pool”). Capacitance measurements from hippocampal mossy fiber boutons revealed that voltage pulses that produce sustained Ca^{2+} inflow (30 ms to 0 mV) evoke capacitance increases of ~ 100 fF, corresponding to the fusion of $\sim 1,400$ vesicles [37]. Thus, the “readily releasable pool” at this synapse is large. In contrast, in the calyx of Held, a threefold larger number of vesicles ($\sim 4,000$) is released from an approximately 20-fold larger number of release sites (~ 600 ; [76, 87]). Thus, the number of releasable vesicles per release site in the mossy fiber terminal is substantially higher than in the calyx of Held. Serial electron microscopic analysis suggested that the total number of synaptic vesicles in large mossy fiber terminals is $>10,000$ ($\sim 11,000$ small clear vesicles and ~ 340 large dense core vesicles in a fully reconstructed large mossy fiber bouton from a 28-day-old rat with 19 active zones; J. Lübke et al. unpublished), suggesting a large reserve pool. Both the large readily releasable pool and the huge reserve pool could be important factors to develop and maintain a high efficacy of synaptic transmission during repetitive stimulation.

Differential synaptic transmission at mossy fiber–pyramidal neuron and mossy fiber–interneuron synapses

Recent work revealed substantial differences between mossy fiber synapses on CA3 pyramidal neurons via large boutons and on stratum lucidum interneurons via filopodial extensions and small boutons [55, 80]. First, the release probability at mossy fiber synapses on stratum lucidum interneurons is larger, while the number of release sites is smaller than at mossy fiber–CA3 pyramidal neuron synapses [48]. Second, significant differences in frequency facilitation are observed. In the mossy fiber–CA3 pyramidal neuron synapse, an increase in stimulation frequency from 0.05 to 1 Hz results in an $\sim 600\%$ increase in EPSC amplitude ([71, 80]—although facilitation is less pronounced in mature synapses, [61]). In contrast, in stratum lucidum interneurons the extent of frequency facilitation is much smaller ($\sim 200\%$, [80]). Finally, marked differences in plasticity were observed. In CA3 pyramidal neurons, high-

frequency stimulation (HFS) leads to a large posttetanic potentiation ($\sim 800\%$) and long-term potentiation ($\sim 200\%$; [36, 68, 71, 88, 89]). In contrast, in stratum lucidum interneurons HFS induces synaptic depression rather than potentiation [50, 67]. Likewise, activation of the cAMP pathway by forskolin leads to a large chemical potentiation at mossy fiber–CA3 pyramidal neuron synapses [45, 82], but has only minimal effects on monosynaptic transmission at mossy fiber–stratum lucidum interneurons [55]. As synaptic plasticity at mossy fiber synapses is believed to be expressed presynaptically [64], these results suggest that the release properties of filopodial extensions and small boutons in the CA3 region differ substantially from those of large mossy fiber boutons.

However, the properties of small boutons emerging from axonal collaterals in the hilus are more similar to those of the large boutons. At the mossy fiber synapses on fast-spiking basket cells in the dentate gyrus, HFS induces both PTP and LTP [3], but the extent of plasticity at these synapses appears to be smaller than at mossy fiber–CA3 pyramidal neuron synapses [71]. For example, the extent of LTP (300 stimuli) at the mossy fiber–basket cell synapse is 133% [3], whereas it is 236% in mossy fiber–CA3 pyramidal neuron synapses [89]. Furthermore, both forskolin and phorbol esters induce a chemical potentiation of mossy fiber–dentate gyrus basket cell EPSCs [3], but again the extent of potentiation is less [239% by 50- μM forskolin; 217% by 1- μM phorbol-12,13-diacetate (PDA)] than that at the mossy fiber–CA3 pyramidal neuron synapse (418 and 670%; [45]).

What is the function of differential synaptic transmission and plasticity? If granule cells fire at low frequency, the net output onto the CA3 region will be inhibitory [1, 13, 14, 60]. In contrast, if granule cells fire high-frequency bursts, differential synaptic dynamics will shift the balance between monosynaptic excitation and disinaptic inhibition towards excitation. This may implement a highly nonlinear gating mechanism, by which only the granule cells with the highest activity levels relay excitation to the CA3 network.

The mossy fiber synapse—a teacher synapse for completion, separation, and linkage of patterns in the dentate gyrus–CA3 cell network

Several lines of evidence suggest that the hippocampus is involved in the processing, storage, and recall of spatial information [74]. A large proportion of granule cells in the dentate gyrus and pyramidal neurons in the CA3 and CA1 region are “place cells,” showing a firing rate that is modulated by the animal’s position in space [65]. It is thought that the hippocampus processes spatial information by both pattern completion (restoration of original patterns from degraded or incomplete inputs; [49]) and pattern

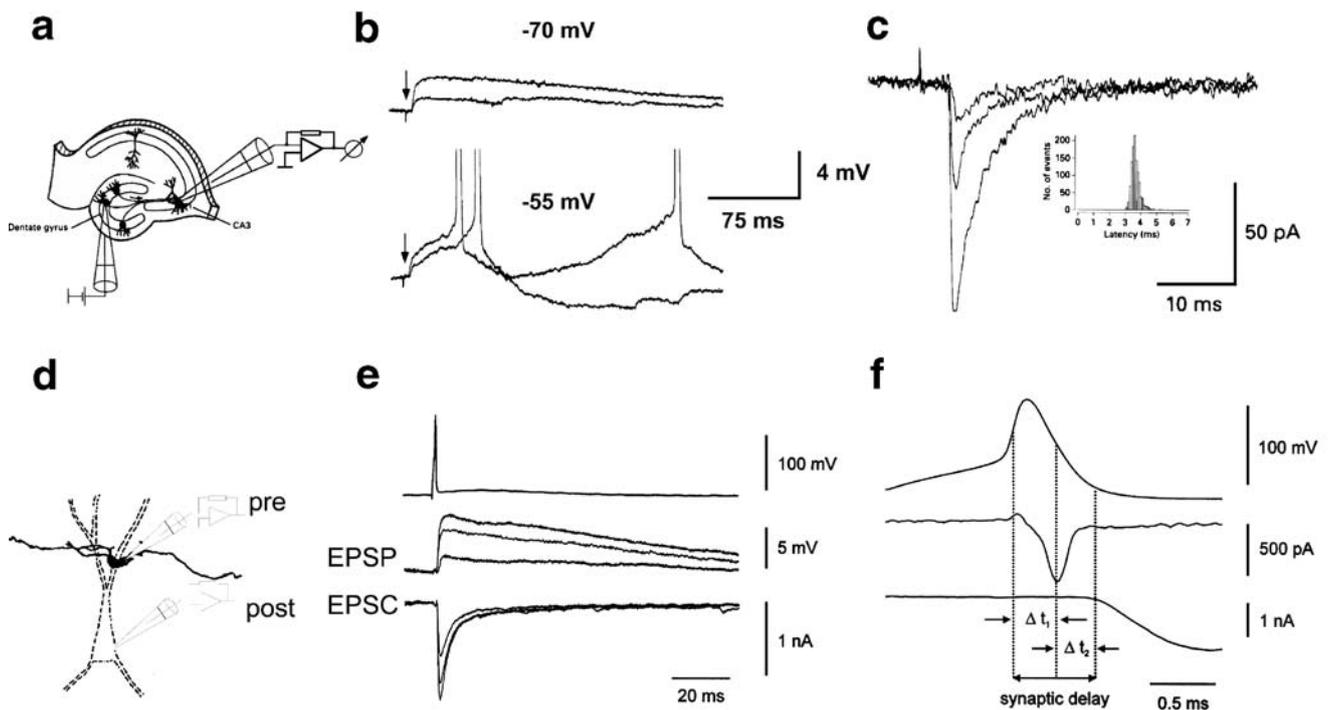


Fig. 4 Efficacy and timing of synaptic transmission at the hippocampal mossy fiber–CA3 pyramidal neuron synapse. **a–c** Putative unitary mossy fiber–CA3 pyramidal neuron EPSPs (**b**) and EPSCs (**c**) evoked by electrical stimulation of single granule cell somata. **a** Schematic illustration of the stimulation and recording method. **b** Unitary EPSPs evoked at -70 mV (close to the resting potential) or -55 mV (during sustained current injection). Note that unitary EPSPs trigger action potentials when the postsynaptic cell is depolarized by tonic current, suggesting that the mossy fiber–CA3 synapse acts as a conditional detonator (stimulation frequency: 1 Hz, $10 \mu\text{M}$ bicuculline). **c** Unitary EPSCs at -70 mV. Note that evoked transmitter release is synchronized, as expected for a single bouton with a large number of active zones. *Inset*, synaptic latency histogram. Data from Jonas et al. [43].

d–f Unitary mossy fiber–CA3 pyramidal neuron EPSPs and EPSCs evoked by paired recording from a presynaptic mossy fiber bouton and a postsynaptic CA3 cell. **d** Schematic illustration of the pre- and postsynaptic recording configuration. **e**, Presynaptic action potential (*top*), unitary EPSPs (*center*), and unitary EPSCs (*bottom*). **f** Simultaneous recording of presynaptic action potential waveform (*top*), resulting Ca^{2+} current (*center*), and evoked EPSC (*bottom*). Δt_1 , Δt_2 indicate components of the synaptic delay. Unitary EPSCs in the MFB–CA3 pyramidal neuron paired recordings (**d–f**) are larger than those in the granule cell–CA3 pyramidal neuron recordings (**a–c**), presumably because of bias towards large boutons in the presynaptic recordings. Data from Geiger and Jonas [33]

separation (amplification of differences between input patterns; [51, 52]). Experimental evidence for pattern completion is that CA3 pyramidal neurons generate consistent activity patterns after small changes in the environment [49]. Experimental evidence for pattern separation is that CA3 cells show abrupt and coordinated place field remapping when the environment is changed to a larger extent [51, 77, 85]. Finally, it is believed that the hippocampus is able to store sequences of places. Experimental support for this hypothesis is the replay of firing sequences during sleep [86] and the phenomenon of phase precession [73]. As an animal moves along a linear track, action potentials shift from late to early time points within a theta cycle, which has been interpreted as a cued and time-compressed recall of previously stored spatial information [53, 54].

How could mossy fiber synapses assist in these complex network functions? If a granule cell fires at low frequency (<0.5 Hz, [44]), e.g., if the animal is located outside the corresponding place field, the cell is likely to have minimal

impact on the CA3 network because the mossy fiber–CA3 synapse is unfacilitated and inhibition will dominate over excitation [1, 13, 14, 60]. In contrast, when the animal is located in a place field center (Fig. 5a; [44, 73]) or during delayed nonmatch-to-sample tasks [84], granule cells will fire at higher frequencies (~ 50 and ~ 10 Hz, respectively), and the differential synaptic dynamics will lead to conditions in which unitary mossy fiber synaptic events trigger spikes in postsynaptic CA3 pyramidal neurons, as suggested by both in vitro and in vivo experiments [39, 40, 47, 48] (Figs. 4 and 5). Likewise, activation of the direct perforant path input onto CA3 cells (presumably signaling information about context, [53]) may lead to a scenario in which unitary mossy fiber synaptic events discharge postsynaptic CA3 pyramidal neurons, as suggested by in vitro recordings [43] (Fig. 4b). Thus, mossy fiber synapses can be viewed as “conditional detonators,” discharging their postsynaptic CA3 pyramidal targets under certain conditions. Remarkably, action potentials in CA3 cells are

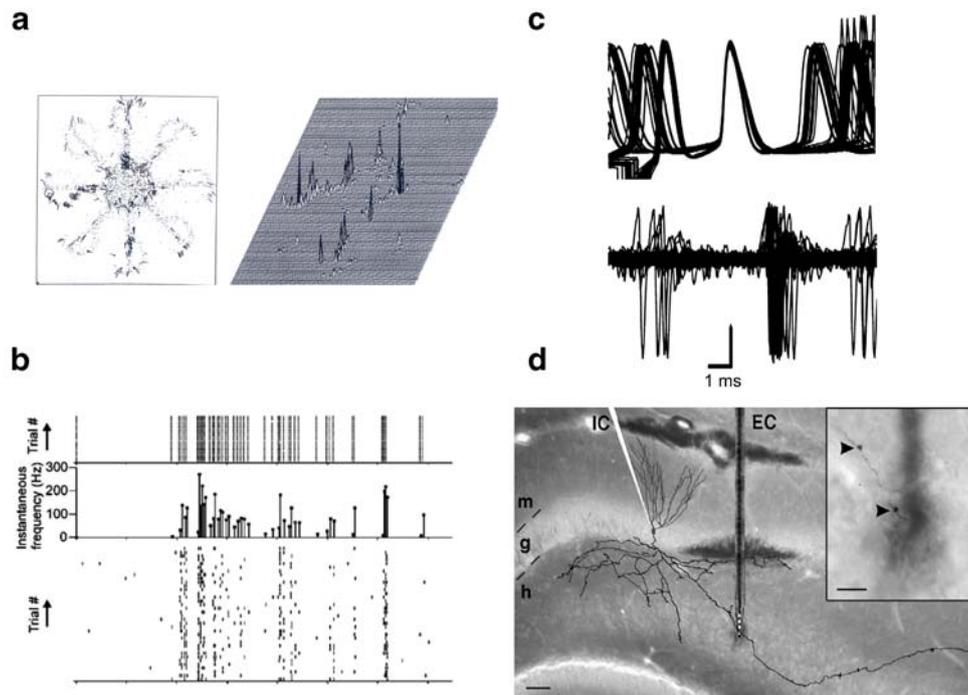


Fig. 5 The mossy fiber–CA3 pyramidal neuron synapse is a conditional detonator in vivo. **a** Spatial firing pattern of a granule cell in vivo at different locations in a spatial environment (eight-arm maze). Mean firing rate is plotted in a grid of 100×100 position bins. Data from Jung and McNaughton [44], with permission of Wiley, copyright (1993). **b** (*top*) Action potential pattern of an extracellularly recorded granule cell during traversal of its place field. **b** (*center*) Corresponding instantaneous action potential frequency (inverse of interspike interval). **b** (*bottom*) Raster plot of action potential activity extracellularly recorded in a CA3 pyramidal neuron while a presynaptic granule cell was stimulated intracellularly with the pattern shown in **a**. **c** Presynaptic action potentials in an intracellularly recorded granule cell (*top*) and postsynaptic action potentials in the synaptically

connected, extracellularly recorded CA3 pyramidal neuron (*bottom*). Note that bursts of three to five spikes in intracellularly recorded granule cells trigger action potentials in extracellularly recorded CA3 pyramidal neurons with high probability (~ 0.8) within a very narrow time window (standard deviation: 0.8 ms; Henze et al. [40]). **d** Recording configuration for the experiments shown in **b** and **c**. In vivo paired (intracellular–extracellular) recording from a granule cell and a synaptically connected CA3 pyramidal neuron. Image shows a camera lucida drawing of the presynaptic granule cell (biocytin labeled), superimposed with the hippocampal tissue. *IC* Intracellular electrode, *EC* extracellular electrode, *m* molecular layer, *g* granule cell layer, *h* hilus. Data from Henze et al. [40], with permission of Macmillan Publishers Ltd: Nat Neurosci, copyright (2002)

generated within a narrow time window under these conditions [40] (Fig. 5b,c), showing that detonation is conditional, but temporally precise.

The detonator function of the mossy fiber synapses has various implications for pattern completion, pattern separation, and storage of sequences of events in the hippocampal network. Mossy fiber synapses may contribute to pattern formation by acting as an unsupervised “teacher” synapse for the synaptic matrix in CA3 [54, 56, 57, 78] (Fig. 6). During storage and recall of spatial information, the role of the mossy fiber system may be envisaged as follows. (1) If an animal is located at a particular place in the environment, a subset of granule cells fires, and a subset of downstream CA3 pyramidal neurons is activated reliably via mossy fiber synapses. Additionally, information about context may be signaled via the direct perforant path input onto CA3 cells [53]. (2) If the animal visits the same place several times, the same CA3 pyramidal neuron ensemble is activated repeatedly, and the CA3–CA3 cell synapses between the neurons of the activated ensemble will be

potentiated [20, 24, 58]. (3) If the animal visits the same place with a modified environment, i.e. with a partial set of cues, the potentiated CA3–CA3 synapses will enable the completion of the original pattern [54, 57, 78]. Consistent with these ideas, burst stimulation of mossy fiber inputs induces heterosynaptic LTP in CA3–CA3 cell synapses in vitro [46]. Furthermore, transgenic mice in which the NR1 N-methyl-D-aspartate (NMDA) receptor subunit gene is specifically deleted in CA3 pyramidal neurons show decreased memory performance in a partial-cue, but not in a full-cue environment [63].

Additionally or alternatively, the mossy fiber pathway could contribute to pattern separation [66, 78]. The sparse connectivity between dentate gyrus granule cells and CA3 pyramidal neurons [4] and the differential dynamics of mossy fiber synapses on pyramidal neurons, which facilitate markedly, and those on interneurons, which facilitate less or even depress [80], may implement a “winner takes all” mechanism in which only the most active granule cells relay excitation to the CA3 network. Computational studies

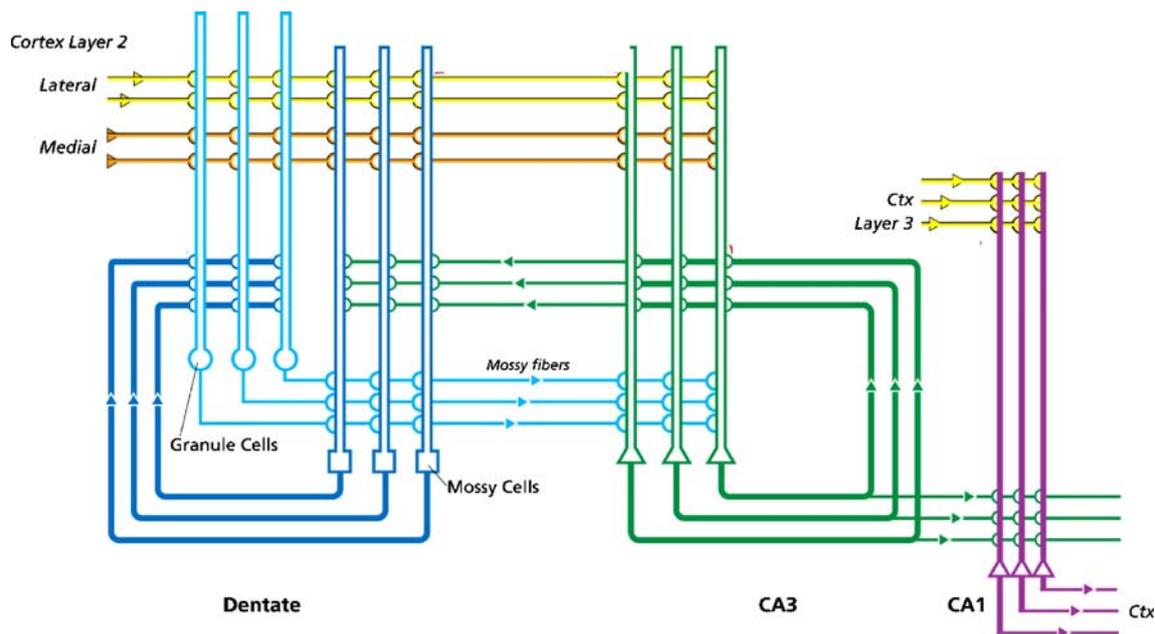


Fig. 6 Role of the mossy fiber pathway in storage and recall of episodic memory in autoassociative and heteroassociative networks. Granule cells and mossy fiber axons forming mossy fiber–CA3 cell synapses are drawn in *cyan*. CA3 pyramidal cells and their collaterals forming CA3–CA3 cell synapse are drawn in *green*. Hilar mossy cells are drawn in *blue*. The input from the perforant path to granule cells and CA3 cells is shown on *top*. The output to the CA1 region is shown at the *bottom*. Scheme kindly provided by John Lisman [53]. Storage of information may be envisaged as follows: when the animal is exposed to an episode (i.e., a series of items), subpopulations of granule cells encoding items 1, 2, 3, ... are activated sequentially. Activation of granule cells is thought to occur via a multi-item buffer (presumably in associative cortical areas), which may activate granule cells repeatedly in a time-compressed manner via the perforant path [53, 54], rather than directly by sensory input. The mossy fiber pathway relays the activation of the subpopulations of granule cells encoding for item 1, 2, 3, ... to corresponding subpopulations of CA3 cells. Because of the Hebbian properties of CA3–CA3 pyramidal cell synapses, information about individual items could be stored as an increase in synaptic strength in the autoassociative CA3 network. The

excitatory polysynaptic excitatory loop granule cells–CA3 pyramidal cells–mossy cells–granule cells “plays back” the original granule cell activity to the granule cell layer with a time delay (e.g., due to axonal conduction). Thus, the activation of granule cells corresponding to item 2 will coincide with the played-back signal originally generated by item 1. Because of the Hebbian synaptic properties, the link between the two items could be stored as an increase in synaptic strength in the heteroassociative granule cell network, e.g., in mossy cell–granule cell synapses [53, 54]. Recall of information may be envisaged as follows. If an animal is reexposed to item 1 or a partial version of it, this activates the subpopulation of granule cells encoding item 1. Additionally, the excitatory polysynaptic loop granule cells–CA3 pyramidal cells–mossy cells–granule cells is activated. Within this loop, the autoassociative CA3 cell network helps to complete the original pattern. The completed pattern is then played back to the granule cell layer, where it may activate the subpopulation of granule cells encoding item 2 via potentiated synapses, e.g., mossy cell–granule cell synapses. Thus, the network may be able to ‘predict’ future events

suggested that pattern separation can be implemented by this mechanism [66]. In such a model, regulation of the strength of the mossy fiber–CA3 pyramidal neuron synapse, e.g., by non-Hebbian frequency facilitation, PTP, or LTP, could set the balance between pattern completion and pattern separation [66].

Finally, mossy fiber synapses may also help to establish heteroassociative links between individual items in a sequence, such as series of places [9] (Fig. 6). It was proposed that heteroassociative links are established in the dentate gyrus [54]. To link two consecutive items by spike timing-dependent plasticity, the action potentials encoding the first item need to be “played back” to the granule cells at a time point matching the activity period corresponding to the second item. This would require a connection

among granule cells, e.g., by the polysynaptic excitatory pathway granule cell → CA3 pyramidal neuron → mossy cell → granule cell [54] (Fig. 6). If action potentials corresponding to subsequent items in a sequence were separated by a time interval corresponding to a gamma oscillation cycle (~20 ms; [54]), the polysynaptic granule cell → CA3 pyramidal neuron → mossy cell → granule cell pathway would need to relay activity back precisely within 20 ms. The unique anatomical and functional properties of the mossy fiber axon, including reliable action potential propagation, precisely tuned presynaptic Ca^{2+} signaling and transmitter release, and large impact on target cells under certain conditions, appears to be an ideal design to implement such a reliable and temporally precise delay line [27].

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