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COMMENTARY

THE MULTIFARIOUS HIPPOCAMPAL MOSSY FIBER PATHWAY: A REVIEW

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Abstract—The hippocampal mossy fiber pathway between the granule cells of the dentate gyrus and the pyramidal cells of area CA3 has been the target of numerous scientific studies. Initially, attention was focused on the mossy fiber to CA3 pyramidal cell synapse because it was suggested to be a model synapse for studying the basic properties of synaptic transmission in the CNS. However, the accumulated body of research suggests that the mossy fiber synapse is rather unique in that it has many distinct features not usually observed in cortical synapses. In this review, we have attempted to summarize the many unique features of this hippocampal pathway. We also have attempted to reconcile some discrepancies that exist in the literature concerning the pharmacology, physiology and plasticity of this pathway. In addition we also point out some of the experimental challenges that make electrophysiological study of this pathway so difficult.

Finally, we suggest that understanding the functional role of the hippocampal mossy fiber pathway may lie in an appreciation of its variety of unique properties that make it a strong yet broadly modulated synaptic input to postsynaptic targets in the hilus of the dentate gyrus and area CA3 of the hippocampal formation.

Key words: dentate gyrus, hippocampus, area CA3, pharmacology, anatomy, physiology.

CONTENTS

"The more one finds out about properties of different synapses, the less grows one's inclination to make general statements about their mode of action!"—Bernard Katz, 1966.

The hippocampal mossy fiber (MF) axons arise from the granule cells (GCs) of the dentate gyrus (DG) and provide synaptic input to neurons in the hilus and area CA3. The MF input to CA3 comprises the second synapse of the classical trisynaptic hippocampal circuit providing input to the proximal apical dendrites of CA3 pyramidal cells. In this classical

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Abbreviations: ACPD, (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; AP-4 or APB, 2-amino-4-phosphonobutyric acid; cAMP, cyclic adenosine monophosphate; CCK, cholecystokinin; DCG-IV, (2S,1′R,2′R,3′R)-2-(2,3-dicarboxycyclopropyl) glycine; DG, dentate gyrus; EPSC, excitatory postsynaptic current; GC, granule cell; HFS, high-frequency stimulation; ISI, inter-stimulus interval; LE, Long–Evans rat; LTD, long-term depression; LTP, long-term potentiation; MF, mossy fiber; mGluR; metabotropic glutamate receptor; NMDA, *N*-methyl-D-aspartate; NPY, neuropeptide Y; PTP, post-tetanic potentiation; SD, Sprague–Dawley rat.

trisynaptic model of the hippocampus, the MF pathway provides the only extrinsic input to CA3, and thus the basic functional role of the MF pathway is clear.[10,11] However, many studies have highlighted the importance of the non-trisynaptic components of the hippocampal circuitry, and have shown that these pathways may be critical to our understanding of how this brain structure functions. For example, *in vivo* and *in vitro* studies[18,26,206,248] have shown that area CA3 also receives substantial monosynaptic input from the entorhinal cortex via the perforant path. One functional consequence of this circuit arrangement is that area CA3 receives extrahippocampal input other than via the MF pathway.

Thus, as has been previously suggested,[6,61] we must change the view of the MF pathway from one where it provides the sole extrahippocampal input to area CA3 to one in which it, at least, shares that functional role with the perforant path. This raises the question of what is the specific role of the MF input in the activation of area CA3. An important step towards specifying the role of the MF pathway in the hippocampal network is to determine its anatomical, pharmacological, and physiological properties. Knowledge of these basic properties can then be used to guide and refine a complete understanding of the MF contribution to hippocampal function. Many of the basic properties that have been described indicate that, in comparison to other cortical projections, the MF pathway has many unusual morphological, pharmacological, and physiological properties. Therefore, one could speculate that these atypical features have evolved to satisfy unique requirements of the information processing carried out by this synapse within the hippocampal network. Unfortunately, the literature also contains discrepancies and conflicts concerning the pharmacology, physiology and plasticity of this pathway that prevent the emergence of a clear picture of the details of the unique properties of the MF pathway.

Therefore, our goals for this review are threefold. First, we attempt to summarize the published data about the basic properties of the MF pathway. Second, we point out areas of ongoing debate and, where possible, offer possible explanations for contradictory data. Finally, we propose that understanding the functional role of the MF pathway may lie in an appreciation of its variety of unique properties that allow it to provide highly reliable synaptic input to its postsynaptic targets in the hilus of the dentate gyrus and hippocampal area CA3.

1. ANATOMY OF THE MOSSY FIBER PATHWAY

1.1. *Projections of the mossy fiber pathway*

The hippocampal MF pathway was first noted and named for the distinctive anatomical appearance of the MF axons. The MF axons were first described by Golgi[72] and Sala[176] and eventually named by Cajal[34] for their anatomical similarity to the fibers found in the cerebellum that Cajal had previously termed “mossy fibers” (see Fig. 1). The large varicosities and filamentous extensions of the cerebellar and hippocampal MFs are reminiscent of moss, and thus suggested the name of these structures. Although Cajal named the MFs based upon their own appearance, the specialized postsynaptic structures on hilar mossy cells and CA3 pyramidal cells (see below) is also reminiscent of moss and is often confused as the reason for the MFs name. In rats, each of the approximately 1 million granule cells of the dentate gyrus[23] (approximately 15 million in humans[192,234]) gives rise to a single unmyelenated MF axon that has a diameter of approximately 1 μm.[45] Immediately after exiting the granule cell layer of the DG, the main MF axon gives rise to an extensive set of fine collaterals (generally less than 0.2 μm thick) which provide input to the polymorphic neurons of the hilus.[2,31,45,60,172] In the rodent, the main MF axons leave the hilus and travel through area CA3 in a narrow band called the stratum lucidum which approximately corresponds to the proximal 100 μm of the apical dendrites of CA3 pyramidal cells. For most of its course through area CA3, the MF pathway can be considered the only true lamellar fiber system of the hippocampal formation because it shows only a limited degree of septo-temporal divergence.[9] However, the MF pathway does makes a significant longitudinal projection in the temporal direction (1–2 mm) once it reaches the border of CA1[2,9,34,233] The degree of the longitudinal projection varies as a function of the septo-temporal location of the parent granule cell such that septally located GCs give rise to MFs with the longest longitudinal projection.[209] The average total length of the main MF axon for GCs in the dorsal (septal) DG in the rat is about 3250 ± 72 μm.[2]

Pyramidal cells in rodent area CA3c also receive a limited MF projection to the proximal basilar dendrites, which is referred to as the infrapyramidal projection.[20] The GCs in the infrapyramidal blade of the DG are the primary source of the infrapyramidal MF axons which eventually cross through the pyramidal cell layer and enter the stratum lucidum at the border of CA3 c and b.[128] The extent of the infrapyramidal MF projection varies across species, and even across strains within species. The infrapyramidal MF projection is also observed to increase following epileptiform activity induced by kindling via the amygdala.[169] Finally, there has been some data suggesting that animal performance in spatial tasks correlates with the magnitude of this basilar MF input but this finding is still a matter of debate (see Ref. 187 for review). Interestingly, the MF projection to CA3 in the human has a strong intra- and infra-pyramidal component throughout the majority of the CA3 region.[121]

1.2. *Cellular targets of the mossy fiber pathway*

As suggested by the axonal projections of the MF pathway, MF axons form synapses with excitatory and inhibitory cells of the hilus and area CA3. Each MF axon makes approximately 140–150 synapses with cells of the hilus. The hilar cells are primarily inhibitory interneurons but the MF collaterals of a single GC make approximately 10 synapses with excitatory hilar mossy cells. In area CA3, each MF provides 11–18 synapses with CA3 pyramidal cells.[2,45] The MF projection to CA3 also provides a robust innervation of all types of interneurons that have dendrites in the stratum lucidum (40–50 synapses per MF axon).[2,58,198] In particular, there is a population of interneurons that have their somata located within the stratum lucidum.[61,74,90,198,200,224] In many cases, the dendrites of these interneurons also are contained mainly within the borders of the stratum lucidum.[74,90,198,200] A subset of these stratum lucidum interneurons are spiny, are calretinin positive, and also stain positive for glutamate.[1,74,150,198] The possibility that these cells contain glutamate raises the possibility that unlike most hippocampal non-pyramidal cells, they may not be inhibitory interneurons but instead maybe local circuit excitatory cells. Most recently, another sub-type of the

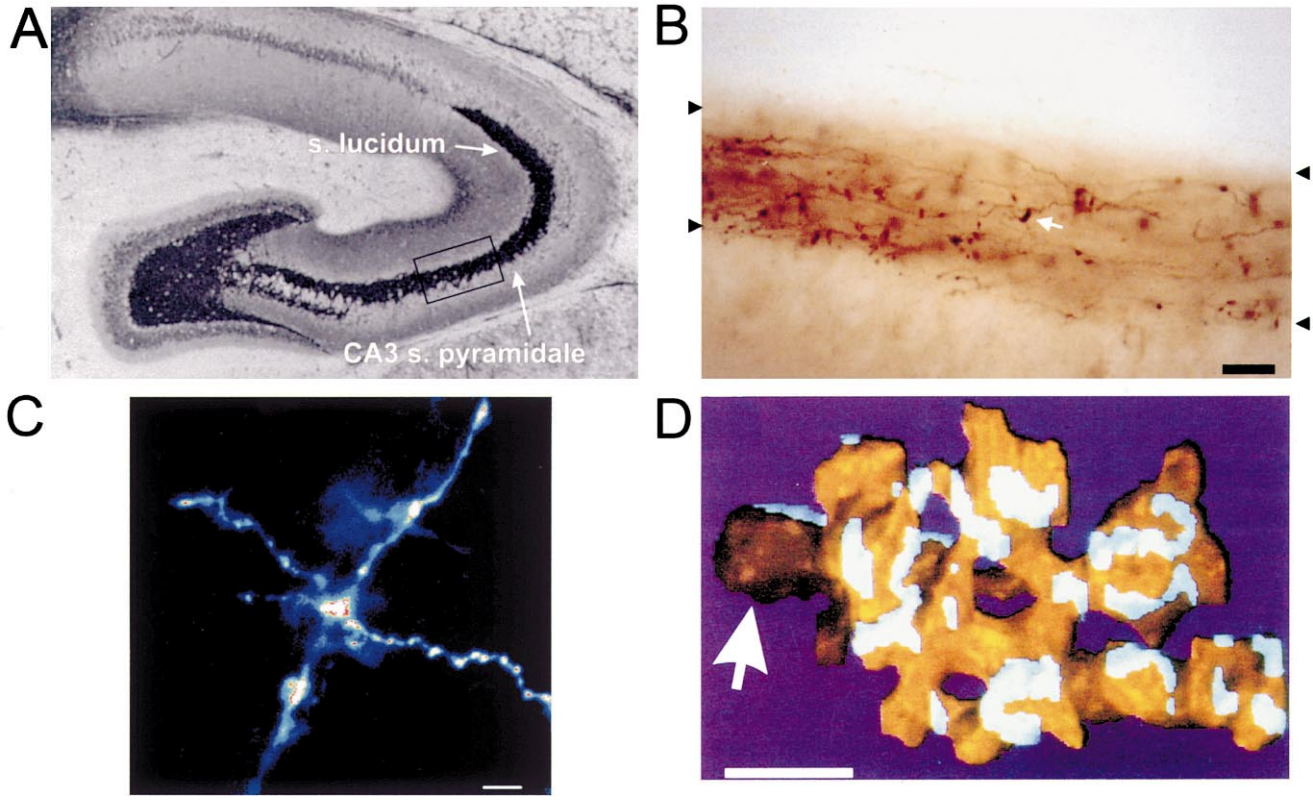


Fig. 1. Gross anatomy of the hippocampal mossy fibers in the rat. (A) A transverse hippocampal section stained with a Timm's stain for zinc. The dark band of labeled mossy fibers is labeled as stratum lucidum (s. lucidum) and the CA3 pyramidal cell layer is labeled as stratum pyramidale (s. pyramidale). Figure adapted from Ref. 80. (B) Example of biocytin labeled mossy fibers in the s. lucidum of area CA3b. The box in part A indicates the equivalent location of the view presented in this panel. Biocytin was iontophoresed into multiple sites in the dentate gyrus resulting in partial labeling of mossy fibers in the s. lucidum of area CA3. Individual giant MF boutons are apparent with one example indicated by the white arrow. The vertical boundaries of the s. lucidum are also indicated by black arrow heads. (C) Example of a single giant MF bouton that was fluorescently labeled with DiI placed into the granule cell layer of the dentate gyrus. After several weeks of dye diffusion, a single bouton was selected and scanned with a confocal microscope. The main MF axon runs from lower left to upper right. The fibers extending towards the upper left and lower right are filopodial extensions from the main MF bouton. (D) A serial electronmicrographic reconstruction of a single thorny excrescence from a CA3 pyramidal cell. The excrescences are complex postsynaptic spine-like structures that are specific to the mossy fiber synaptic complexes. The surface of the thorn is shown in orange and the locations of postsynaptic densities are shown in light blue. The large white arrow indicates the place where the thorn was attached to the proximal apical dendrite of the CA3 pyramidal cell. A total of 12 heads are present on this thorn. Figure adapted from Ref. 44. Scale bars = 20 μm (B), 5 μm (c), 1 μm (D).

stratum lucidum interneuron was described as being aspiny and having dendrites in the stratum radiatum and stratum oriens and an axonal arbor localized to the stratum lucidum. These interneurons were shown to provide GABAergic input to CA3 pyramidal cells and are thus positioned to provide feedforward inhibition following mossy fiber activity.[224]

The numbers of synapses described above indicate that activity in the MF pathway is likely to result in the activation of greater numbers of inhibitory interneurons than excitatory hilar mossy cells and CA3 pyramidal cells. Therefore, contrary to the prediction from the trisynaptic circuit model of the hippocampus, activation of the MF pathway may cause a net inhibition of the hippocampal CA3 network.[2,25,163] More specifically, activity of the GCs may lead to activation of a very specific subset of CA3 pyramidal cells (10–18) at the same time as the majority of CA3 pyramidal cells are suppressed. Further work will be necessary to determine the extent to which these competing excitatory and inhibitory influences elicited by the granule cells via the MF pathway affect the activity of CA3 pyramidal cells. To resolve this issue it would be critical to know whether or not interneurons activated by a given MF axon make synaptic contacts on pyramidal cells that also receive input from the same MF axon.

1.3. *Mossy fiber pathway synaptic morphology*

The MF axons form three different types of synaptic contacts with their targets in the hilus and area CA3. First we consider the characteristic large boutons for which the MF are named. These boutons form synapses with the hilar mossy cells and proximal dendrites of CA3 pyramidal cells. A single presynaptic MF bouton is large (4–10 μm diameter see Figs 1C, 2A), and is found arranged either in an *en passant* fashion or attached via a short process to the main axon.[2,7,21,76,252] Each MF bouton contains thousands of small (~40 nm), clear vesicles and many large, dense-core vesicles (Fig. 2A; Refs 7, 44). There are also reports of clear vesicles ranging up to 200 nm in diameter (Refs 7, 44; personal observations). The number and size range of MF vesicles can be compared to the tens to hundreds of small clear vesicles that do not exceed 60 nm in diameter reported for other cortical synapses.[78] In addition to the vesicles, each MF bouton contains smooth endoplasmic reticulum and approximately eight mitochondria (see Fig. 2A)[7,21,45,59] and often a lamellar structure of unknown function is observed.[7] The main body of the presynaptic MF bouton envelopes a large multi-headed postsynaptic spine, called a thorny excrescence, that protrudes from the proximal dendrites of hilar mossy cells

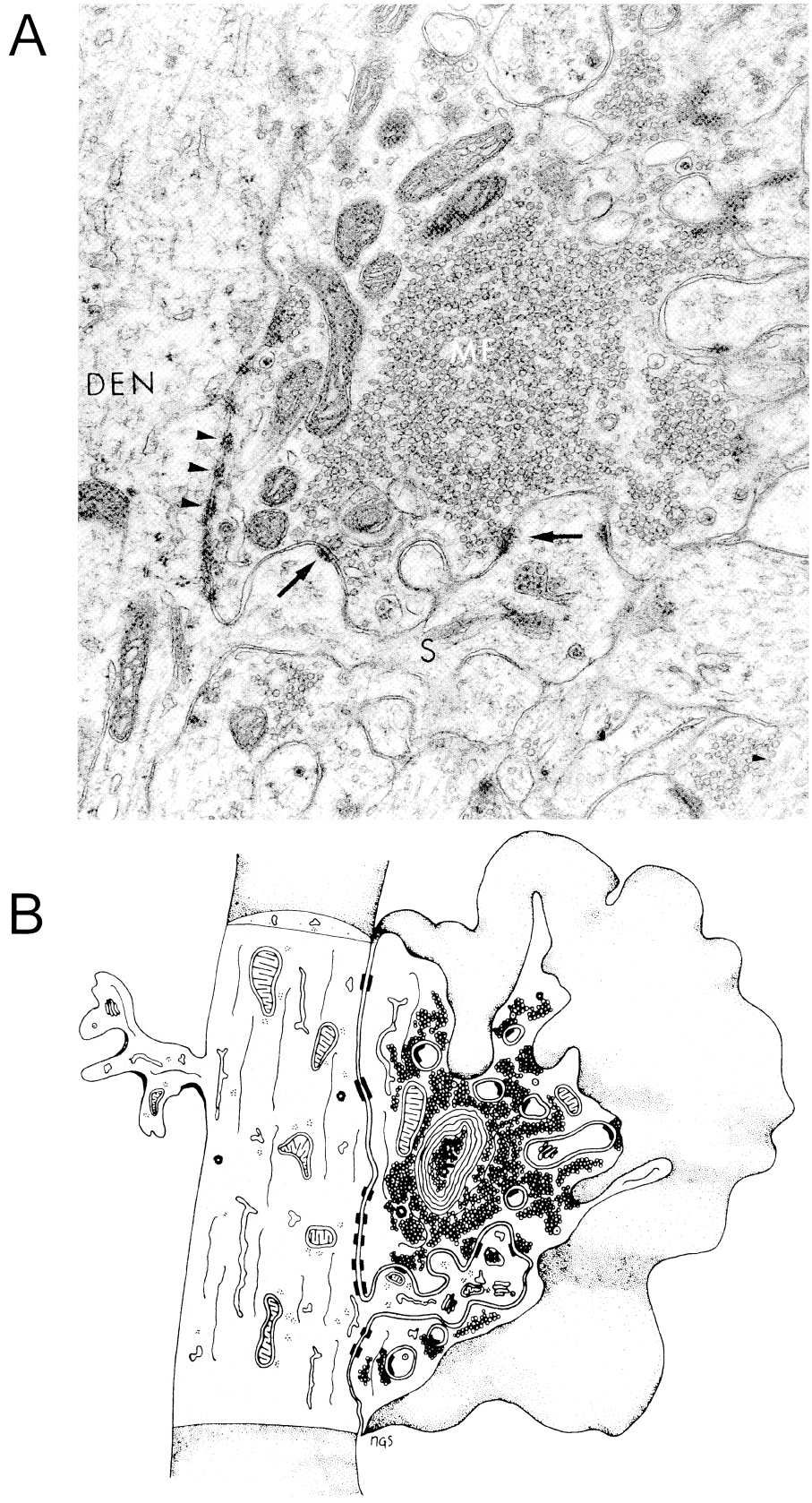


Fig. 2. Ultrastructure of the mossy fiber synaptic complex. (A) Electron micrograph through a MF synaptic complex. The postsynaptic CA3 pyramidal cell dendrite is indicated (DEN) to the left of the presynaptic MF terminal (MF). The presynaptic terminal contains numerous small vesicles and mitochondria. The postsynaptic thorny excrescence is indicated (S) where it penetrates the terminal. Several symmetrical junctions between the dendritic shaft and the terminal are observed (arrowheads) and are representative of puncta adhaerentia. Notice that these symmetric junctions do not have any vesicles located nearby. In contrast, asymmetrical junctions (arrows) on to the thorn shown a significant clustering of vesicles. (B) Schematic representation of a MF synaptic complex depicting the various features observed at the electronmicroscopic level. Both A and B are adapted from Ref. 7.

and CA3 pyramidal cells (Figs 1D and 2).[7,21,34,44] MF boutons appear to be anchored around the excrescence to the dendritic shaft of CA3 pyramidal cells via a series of symmetric thickenings called puncta adhaerentia.[7,76]Taken together, we will refer to a giant MF bouton and its associated thorny excrescence as the MF synaptic complex. These anatomical features are descriptive of adult MF boutons (>21 days post-natal in the rat).[7] However, it is important to note that, in the rat, these features develop gradually during the first 21 post-natal days.[7] There is also evidence to suggest that the post-synaptic thorny excrescences on CA3 pyramidal cells and hilar mossy cells are induced by the arrival of MF axons during development.[173]

One of the most interesting morphological aspects of the MF synaptic complex is the presence of multiple active zones and their associated post-synaptic densities (Fig. 1D). The largest reported number of active zones from a single MF synaptic complex with a CA3 pyramidal cell is thirty-five, which was observed for an incompletely reconstructed complex.[44] Another study reported 31 active zones from a single completely reconstructed terminal.[2] Due to the challenges of serial EM reconstruction, the average number of active zones per MF synaptic complex is not known. However, if we assume a value of 14 active zones per complex, which is in the middle of the reported range, and 50 MF boutons per CA3 cell,[8] then each CA3 pyramidal cell is associated with an average of 700 active zones associated with the MF pathway. Recall also that the MF synapses are localized to the proximal 100 μm of the apical dendrite. Therefore, despite the relatively small number of MF synaptic complexes per CA3 pyramidal cell (compared to >10,000 for non-MF synapses), the MF pathway has the potential to provide a strong excitatory drive close to the somatic region of action potential generation in CA3 pyramidal cells.[208] It is not known how many MF axons contact and form complexes with a single hilar mossy cell. Mossy cells receive the MF input onto their proximal dendrites, similar to what is observed for CA3 pyramidal cells. However, since mossy cells have numerous primary dendrites to receive the MF input, they appear to receive a greater number of MF inputs than a CA3 pyramidal cell which usually has only one primary apical dendrite.

The remaining two types of MF synaptic contacts are associated with interneurons of the hilus and area CA3. The interneuron-associated boutons are smaller than the giant pyramidal cell associated boutons, do not form multiple release sites, and the synapses are either *en passant* or at the ends of synaptic filopodia which extend from the giant MF boutons.[2,5,7,34,58,74,198] Giant MF boutons often give rise to several such filopodia (up to nine) which, in the adult, can extend for up to 30 μm from the main body of the giant bouton.[2,5] The average length of the filopodia varies during development, achieving a maximum average length (30 μm) in the rat at two weeks of age followed by a pruning back to adult levels (12 μm) by four weeks of age.[5] Although the MF boutons associated with interneurons are smaller than the giant MF boutons associated with pyramidal cells, it should be noted that the average size of the active zones at these synapses is larger than the actives zones observed at other excitatory synapses in CA3, CA1, and cortex.[2] If it is assumed that synaptic active zone is correlated with synaptic strength (e.g. Ref. 158), then the above findings suggest that all synapses made by the MF pathway (on to both excitatory and inhibitory targets) are relatively strong when compared to other excitatory cortical synapses.

1.4. *Anatomical plasticity*

Yet another unusual feature of this pathway that has been revealed through anatomical techniques is that the GCs are continuously undergoing turnover throughout the life of the animal.[4] Granule cells are being continuously generated from stem cells located in the hilus. The new born GCs then migrate outwards into the granule cell layer.[107] It is important to note that the number of GCs apparently does not increase as a function of the age of the animal. Other evidence suggests that the number of GCs is regulated dynamically by environmental factors. Exposure to novel environments increases GC proliferation[111] and stress decreases their proliferation (reviewed in Ref. 145). If we assume that the newly formed GCs also generate MF axons that form synapses on CA3 pyramidal cells, then the hardwiring of the MF pathway also must be undergoing continuous remodeling.

The MF pathway also demonstrates a strong propensity for sprouting following periods of strong activity, such as epilepsy induced by kainate injection or kindling.[16,167,168] However, not all the MF sprouting is due to the generation of new granule cells[162] suggesting that existing granule cells can each innervate more CA3 pyramidal cells.

Finally, recent evidence indicates that chronic stress decreases GC proliferation, and also causes dramatic changes in the appearance of the MF synaptic complexes on CA3 pyramidal cells.[134] Following repeated restraint stress the clear vesicles of the MF boutons were more clustered near release sites, and the number of mitochondria per terminal increased compared to controls. These findings suggest that synaptic transmission at the MF synaptic complexes is strongly influenced by chronic stress.

2. PHARMACOLOGY OF THE MOSSY FIBER PATHWAY

2.1. *Amino acid transmitters*

Aside from the distinct morphology of the presynaptic bouton, another unique feature of the MF synapse is the presence of a distinct complement of neurotransmitters and their associated receptors. Like most excitatory CNS synapses, the transmitter for ionotropic transmission at the MF synapse is glutamate[49,179,207,212] which acts on post-synaptic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid sensitive receptors (AMPA-Rs) and kainate receptors.[15,37,104,136,157,171,225,226] However, the MF synapse differs from most CNS synapses in that it has few *N*-methyl-D-aspartate (NMDA) sensitive receptors.[104,154,190,228,230] Some of these studies have demonstrated that NMDA receptors are expressed at very low levels in the stratum lucidum and can be activated by activity in the MFs. However, it still not known whether the MF-activated NMDA receptors are localized to the postsynaptic density or are instead extrasynaptic and possibly activated by glutamate spillover.[151]

Based upon anatomical colocalization of high-affinity kainate receptors with the MF pathway[17,171,220] and data from synaptosome preparations[136–138] it was proposed that high-affinity kainate receptors were located on MF presynaptic terminals.[55,62,171] However, electrophysiological studies have suggested that the high-affinity kainate receptors are not

presynaptic and are instead located on the proximal apical dendrite of the postsynaptic CA3 pyramidal cells.[37,180,181,225]

Two recent reports have demonstrated that the kainate receptors that are localized to the stratum lucidum of CA3 pyramidal cells (see above) can be activated by short bursts of high-frequency stimulation of the MF pathway.[37,226] The response evoked by activation of these MF-pathway-associated kainate receptors has slow kinetics and thus provides a prolonged excitatory drive in CA3 pyramidal cells.

In addition to the ionotropic glutamate receptors, metabotropic glutamate receptors (mGluRs) are localized on both the pre- and postsynaptic membranes of the MF synaptic complex.[22,42,71,105,106,139,151,164,183,189,227,251] Activation of the presynaptic mGluRs inhibits glutamate release. The metabotropic GluR agonist 2-amino-4-phosphonobutyric acid (AP-4 or APB) was found to inhibit glutamate release in the guinea pig [50,69,119,139,215,245,247,251] but not in the rat.[119] However, other work in the rat using mGluR agonists such as (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid (ACPD) or (2S,1′R,2′R,3′R)-2-(2,3-dicarboxycyclopropyl) glycine (DCG-IV) has shown that MF synaptic transmission in the rat is also is inhibited by activation of mGluRs.[106] These findings suggest that there are specific, albeit subtle, differences in the mGluRs expressed at MF synapses in different species. Regardless of interspecies differences in the pharmacology, exposure of mGluRs on MF boutons to the endogenous agonist, glutamate, will result in a subsequent inhibition of glutamate release.[227] On the postsynaptic side of the MF synapse, activation of mGluRs increases cytosolic calcium in the CA3 pyramidal cells[249] and may be necessary for the induction of at least one form of LTP observed at this synapse (see below).[13,99,249]

2.2. *Neuropeptide transmitters*

Yet another unusual feature of the MF pathway is that it is one of the few excitatory pathways in the telencephalon to contain and release neuropeptides from large dense-core vesicles. The giant MF boutons have been shown to contain several peptides including dynorphin,[146] enkephalin[47,48,66,68,146,202] cholecystokinin (CCK),[41,63,65,66,68,188,204] neuropeptide Y(NPY),[40,66,141,186,199] and neurokinin-B.[186]

Many of the details of the opioids of the MF synapse have been reviewed by Simmons and Chavkin.[193] One complicating factor in the study of MF neuropeptides is that the presence of each of the peptides and their receptors appears to vary across species. Dynorphin has been found in the stratum lucidum of all species examined but κ-opioid receptors are present in the stratum lucidum at higher levels in the guinea pig than the rat. In contrast, the rat has high levels of κ-opioid receptors in the CA3 pyramidal cell layer.[147] Enkephalin is also expressed in the MFs of all species but seems to be expressed at varying levels; e.g. guinea-pig has high basal levels while rats and mice have lower basal expression.[64,147,202] Similar to κ-opioid receptors, compared to the guinea pig, μ-opioid receptors are almost absent in the stratum lucidum of the rat but are highly expressed in CA3 pyramidal layer.[147] Finally, CCK appears to be present in guinea pig but not in rats[63,65,66,68,188,204] (but see Ref. 41).

Enkephalin and dynorphin are believed to act via G-protein coupled μ- and κ-opioid receptors, respectively. Activation of opioid receptors has been shown to inhibit adenylyl cyclase (reviewed in Ref. 194). This is of note since synaptic plasticity at the MF synapse is critically dependent upon activation of adenylyl cyclase[93,94,219,229] and dynorphin has been reported to modulate MF synaptic potentiation.[231,238] Of particular interest for understanding MF pathway function is the finding that the MF synapses may inhibit one another heterosynaptically via release of dynorphin.[231] This phenomenon seems to be species and even strain specific with Sprague–Dawley rats showing less dynorphin induced depression than Long–Evans rats which in turn show less depression than guinea pigs.[178]

CCK receptors are also coupled to G-proteins. In addition, CCK receptor activation has been shown to increase phospholipase C activity [185] and also activate tyrosine kinases.[129]

Unfortunately, the functional importance of these peptides is unclear due to contradictory reports in the literature. Although some of the variability in reports of effects of CCK might be explained by species and strain differences, this cannot explain all the discrepancies observed (see Table 1). Interestingly, the expression levels of the neuropeptides contained in the MFs is affected by epileptogenic treatments, such as kindling or kainate injections.[186] For example, CCK and enkephalin[64] and NPY[144] are up regulated in the MFs following seizures. The up regulation or *de novo* expression of these neuromodulators may be a compensatory reaction to the epileptiform activity.

2.3. *Miscellaneous contents*

In addition to glutamate and peptides, the MF boutons contain and release several other neuromodulators. Like most other CNS excitatory synapses, the MF boutons release ATP/adenosine.[213] The adenosine probably plays an automodulator role since it has been shown that activation of presynaptic A1 receptors inhibits MF glutamate release.[123,161,246]

The MF boutons also contain very high levels of chelatable zinc. In fact, histochemical staining for zinc (Timm's sulfide silver stain) is often used to assay for the presence of the MF boutons (e.g. see Fig. 1).[7,156] The zinc contained in the MF bouton is able to be released in a stimulus-dependent manner[32,88] and a specific transporter for zinc is found in glutamate-containing vesicles.[232] Although there has been no conclusive study which has indicated the physiological role of the high levels of zinc in the MF pathway, several possibilities exist. In general, exogenous zinc tends to lead to excitatory bursting. The mechanism of this excitatory effect is likely to be related to an inhibition of potassium channels[12,79,191] as well as an inhibition of GABA[235] and a potentiation of AMPA binding to its receptors.[27] In contrast to its excitatory effects, zinc also blocks NMDA receptors[235] and has been shown to interfere with NMDA dependent LTP induction in area CA3.[242] Zinc also has been shown to inhibit the binding of opioids to their receptors[203,211] which may be particularly relevant given the opioid content of the MF synapse.

Finally, there are several reports that the MF pathway contains and releases GABA.[179,186,195,210] These anatomical and biochemical findings await electrophysiological confirmation. If GABA is released from MF synapses under physiological circumstances, it could act as an automodulator by activating presynaptic $GABA_B$ receptors on the giant bouton. $GABA_B$ receptor activation has been shown to inhibit MF

Table 1. Published data on peptide modulation of the mossy fiber pathway in area CA3

Modulator	Preparation	Effect on MF transmission	Reference
Enkephalin/ mu receptor	SD rat *in vivo*	Required for LTP	53
	SD rat *in vivo*	Facilitates LTP	51
	Guinea-pig slice	Inhibits transmission	178
	SD rat slice	Inhibits transmission	
	SD rat slice	Enhance LTP	101
Enkephalin/ delta receptor	SD rat slice	Increase CA3 cell excitability	155
	SD rat *in vivo*	Delta not required for LTP	53
Dynorphin/ kappa receptor	SD rat *in vivo*	Kappa not required for LTP	53
	SD rat slice	No effect	
	LE rat slice	Inhibits transmission	
	Guinea-pig slice	Inhibits transmission	178
	Mouse slice	Inhibits transmission	
	Hamster slice	Inhibits transmission	
	Guinea-pig slice	Inhibits transmission	38
	Guinea-pig slice	heterosynaptic inhibit trans. Inhibits LTP	231
	Guinea-pig slice	Inhibits transmission	193
	SD rat slice	No effect	238
	Guinea-pig slice	Inhibits transmission	
	Rat *in vivo*	Increases CA3 cell excitability	81
	SD rat slice	Reduces CA3 cell excitability	155
Opioid antagonist Naloxone	SD rat slice	No effect on LTP	178
	SD rat *in vivo*	Blocks LTP	54
	Guinea-pig slice	Facilitates LTP	231
	SD rat slice	Inhibits LTP	238
	Guinea-pig slice	No effect on LTP	
	Primate slice	No effect on LTP	223
CCK	SD rat slice	Reduces CA3 cell excitability	3
	SD rat slice	Increases CA3 cell excitability	24
NPY	SD rat slice	Reduces CA3 cell excitability	112
	SD rat slice	Reduces CA3 spont. EPSCs	149

Animal species and strain are indicated where available

synaptic transmission in both acute slices (Refs 85, 118, 152 but see Ref. 95) and organotypic culture.[214]

2.4. *Extrinsic neuromodulation of the mossy fiber synapse*

In addition to the endogenous neurochemicals of the MF pathway discussed above, the MF synapse onto CA3 pyramidal cells also appears to be modulated by several exogenous neuromodulators and chemical agents. Table 2 details the many agents both endogenous and exogenous which have been reported to influence neurotransmission at the MF to CA3 pyramidal cell synapse.

2.5. *Mossy fiber synapses onto interneurons*

Not much is known about the pharmacology of the MF to interneuron synapse. It is known that, at least for one population of interneurons whose somata are located in the stratum lucidum, MF synaptic input is mediated by AMPA receptors which lack the GluR2 subunit.[216] This is in contrast to other non-MF synapses onto these same interneurons which are mediated by AMPA receptors containing the GluR2 subunit. Interestingly, similar to MF input to pyramidal cells, the MF input to these stratum lucidum interneurons is inhibited by application of the MGluR agonists DCG-IV and ACPD.[130,216]

3. MOSSY FIBER PATHWAY SYNAPTIC PHYSIOLOGY

3.1. *Complicating factors in the electrophysiological study of the mossy fibers*

The MF synapse has received considerable attention because originally it was proposed as a model synapse to study neurotransmission in the CNS.[29] This proposal primarily was based upon the proximal dendritic location of the MF synapse on CA3 pyramidal cells and the apparent ease for accurate and selective activation of the MF pathway via bulk stimulation of the DG, hilus, or stratum lucidum. The location of the MF synapse near the soma suggested that MF synaptic currents could be measured more precisely using somatic voltage clamp than could most other excitatory synapses, which generally are located far from the soma.[29,35,82,102] Unfortunately, there are several complicating factors associated with the electrophysiological study of this pathway.[46] First, the sparse connectivity between GCs and the CA3 pyramidal cells makes it very difficult to study synaptic transmission at individual MF synaptic complexes between

Table 2. Published data on the pharmacological modulation of the mossy fiber pathway in area CA3

Modulator	Preparation	Effect on MF transmission	Reference
Norepinephrine	SD rat *in vitro*	Facilitates and augments LTP	86,87
	Rat *in vivo*	Required for LTP	175
	Rat organotypic slice	Depresses transmission	182
	SD rat *in vitro*	Facilitates MF LTP induction	92
Serotonin	Guinea-pig slice	5-HT3-mediated inhibition of LTP induction	133
$GABA_B$/baclofen	Guinea-pig slice	Depresses transmission	85
	Rat slices	Depresses transmission	118
	Guinea-pig slice	Depresses transmission	152
Acetylcholine	Rat slice	Muscarine depresses transmission	236
	Rat slice	Muscarine inhibits LTP induction Carbachol depresses transmission	237
	Guinea-pig slice	Low-dose carbachol inhibits LTP induction High-dose carbachol facilitates LTP induction	132
	SD rat slice	Nicotine facilitates transmission	73
Somatostatin	Guinea-pig slice	Facilitates MF LTP	143
Bifemelane	Guinea-pig slice	Augments MF LTP	97
Interleukin 1-beta	Mouse slice	Inhibits MF LTP	110
Arachidonic acid	Rat synaptosomes	Facilitates release	57
TRH	Guinea-pig slice	Augments LTP	96

Animal species and strain are indicated where available

MF axons and single CA3 pyramidal cells. Due to the sparseness of the projection to CA3, it is often difficult to find a bulk stimulation site in the DG or stratum lucidum that results in a monosynaptic MF response in the subject pyramidal cell. This problem is even more severe for experiments in which the goal is to carry out paired recordings between single GCs and CA3 pyramidal cells (see below).

In addition to the sparse connectivity, another challenge to the study of the properties of MF synapses is that bulk electrical stimulation of the DG, the hilus, or the stratum lucidum, (the usual methods of MF stimulation) can lead to activation of at least three different synaptic inputs to CA3 pyramidal cells besides the intended orthodromic activation of the MF-CA3 synapse (reviewed in Ref. 46). MF synaptic responses evoked by DG stimulation can be contaminated by synaptic responses evoked by activation of non-MF inputs in two ways. One contamination can result from strong stimulation of the DG, hilus or stratum lucidum that leads to firing of CA3 pyramidal cells synaptically via the MF pathway. In turn, firing of these CA3 pyramidal cells evokes synaptic responses on the CA3 pyramidal cell being recorded (Fig. 3C). The second source of contamination of MF synaptic responses evoked by DG, hilus or stratum lucidum stimulation stems from the antidromic activation of the hilar projecting associational collaterals of CA3 pyramidal cells.[98,120,153,184,239,243] Propagation of action potentials via these CA3 axon collaterals may then evoke a monosynaptic non-MF synaptic response in CA3 pyramidal cells (Fig. 3D). This possible contamination of DG-evoked MF responses has been suggested several times[46,116,240] and also has been functionally demonstrated.[84,113,239] The third source of contamination of MF synaptic responses is bulk stimulation of the DG or hilus often results in activation of the hilar collaterals of MF axons. The activation of a hilar MF axon collateral evokes an action potential that travels antidromically to the main MF axon, and then is conducted orthodromically into CA3 in a so-called anti-orthodromic sequence (Fig. 3B). Whereas activation of this anti-orthodromic mechanism still yields a "pure", i.e. uncontaminated MF synaptic responses in area CA3, the additional conduction distance introduced by the antidromic travel causes differential synaptic delays, and thus leads to complexities in the evoked MF waveform (Fig. 4).[84,116]

The existence of these contaminating factors has led to a set of criteria to optimize the probability of studying a "pure" MF synaptic response in CA3 pyramidal cells.[46,116,241] In general, it has been suggested that a "pure" MF excitatory postsynaptic current (EPSC) should have relatively fast rising phase kinetics ($<$3 ms rise-time measured at 31°C) due to their close electronic location to the soma. The response should not have inflected rising and decay phases that may otherwise occur due to polysynaptic recruitment or anti-orthodromic activation of MF hilar collaterals. Finally, the response should not have a variable latency that can indicate polysynaptic recruitment. In addition, several pharmacological protocols can be employed to validate a response as being a pure MF response. Polysynaptic recruitment contamination can be minimized in "suppressing" conditions (such as low concentrations of AMPA receptor antagonists or high divalent ion concentrations) that reduce the probability of polysynaptic transmission via the interconnected CA3 pyramidal cells (Fig. 3C).[116,240] The selective inhibition of the MF synaptic response by mGluR agonists has been used successfully because it can help to discriminate between monosynaptic responses evoked by the MFs and responses due to antidromic activity in CA3 axons.[50,69,106,119,130,139,215,245,247,249,251]

An important point to bear in mind is that amplitude is not a useful criterion for distinguishing MF from non-MF synaptic responses. Although it is predicted from the close electrotonic location of the MF synapses that on average the MF EPSP/Cs are larger than non-MF EPSP/Cs, synchronous polysynaptic contamination also yields large amplitude synaptic responses. For example, it has been our experience that by increasing the stimulation intensity, an apparently contaminated small MF response paradoxically can be made to meet criteria for a pure MF response as described above. This may happen because increasing the stimulation intensity recruits more cells and fibers (both mossy and non-mossy in nature) into participating in the evoked response. The net result is that the activation of any individual contaminating fiber contributes less to the overall response and thus the contamination is much more difficult to detect. However, the initial contaminating

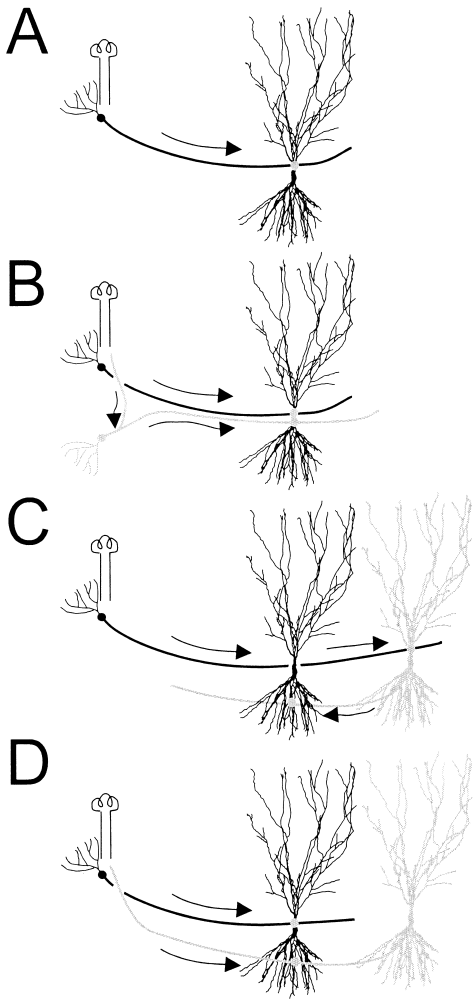


Fig. 3. Bulk stimulation of the DG can lead to excitatory postsynaptic activation of individual CA3 pyramidal cells via four different "pathways". (A) Bulk stimulation of the DG may lead to direct monosynaptic MF activation of a single CA3 pyramidal cell. (B) DG stimulation can also activate MF collaterals (indicated in grey) in the hilus which causes antidromic action potentials until the main MF axon is reached, followed by orthodromic AP propagation into area CA3 (so-called anti-orthodromic activation). This "pathway" can result in "pure" MF responses that have complex kinetics due to the differential conduction distances of various MF axons involved. (C) Alternatively, DG stimulation can synaptically activate an interposed pyramidal cell (indicated in grey) which then provides a synaptic input to the subject CA3 cell (so-called polysynaptic contamination). (D) DG stimulation can also directly activate hilar projecting collaterals of CA3 pyramidal cells. Activation of the CA3 pyramidal cell collaterals (indicated in grey) can cause monosynaptic non-MF activity in the subject pyramidal cell.

responses still remain despite that they may contribute less to the overall waveform kinetics.

As a result of the afore-mentioned complicating factors, particular care must be taken both in designing new experiments as well as interpreting prior experiments which were carried out without concern for these possible sources of contamination. Unfortunately, many of the early pharmacological studies listed in Tables 1 and 2 have not fully considered these issues and so the conclusions from these studies must be evaluated with this caveat in mind. On the other hand, the exclusive use of conservative kinetic criteria to obtain "pure" MF synaptic events also is likely to exclude responses that are "purely" MF in origin but possibly are different in their kinetics due to other properties of the MF-CA3 pyramidal cell synapse (see below).[83]

3.2. *Basic synaptic properties*

Despite the fact that the MF-CA3 synapse was one of the first synapses studied in *in vitro* slices,[243] we still lack a complete and accurate description of the basic aspects of neurotransmission at this synapse (i.e. the probability of release, the average quantal content, the average kinetics). The primary reason for this lack of information is that, as mentioned above, it has proved very difficult to achieve a selective monosynaptic activation of MF synapses onto CA3 pyramidal cells. The best method for studying the basic properties of transmission at a synapse is to record simultaneously from the pre and postsynaptic cells. This approach has proved intractable at the MF to CA3 synapse because of the extremely low probability that a given GC and CA3 pyramidal cell pair are connected. However, two alternative methods which approximate this ideal have been used. Yamamoto[245] used focal application of glutamate to the DG while simultaneously measuring extracellular GC spike activity. Using this approach, it was possible to find locations where EPSPs were evoked in CA3 pyramidal cells recorded with sharp intracellular electrodes and where only one granule cell was observed to spike in response to the glutamate application. In these experiments, the average unitary EPSP had a latency of 3.3 ms, a peak conductance of 1.5 nS and a time-to-peak of 5.5 ms. The average MF quantal conductance was calculated to be approximately 150 pS.[245] Unfortunately, this study was conducted before the advent of whole-cell recording and thus was carried out in current clamp with sharp intracellular electrodes. Therefore, the reported values of peak conductance and quantal content are calculated based upon voltage measurements. In addition, the time-to-peak measured from the EPSP does not reflect the actual time course of the synaptic current. Finally, interpretation of these results is made difficult by the possibilities that the focal application of glutamate evoked spikes in cells that were not detectable by the extracellular electrode or that GC spikes from multiple cells were not isolated properly. Thus, the events recorded may not have been truly unitary.

A more recent report was presented by Jonas *et al.*[104] a different approach whereby a small stimulating pipette was placed in the granule cell layer to stimulate single granule cells. Using this technique, it was possible to find a site that evoked unitary MF EPSCs in CA3 pyramidal cells. The evoked EPSCs were determined to be the result of stimulating a single GC because movement of the stimulating electrode by more than 25 μm abolished the evoked synaptic response. This approach yielded results very similar to those of Yamamoto.[245] It was found that the average unitary EPSC had a latency of 4.2 ms, a 20–80% rise-time of 0.6 ms, a decay time constant of 6.2 ms, and a maximal peak conductance of 1 nS. The average quantal conductance was determined to be 133 pS.[104] However, since the main purpose of this study was to perform a quantal analysis of the MF synapse and not to describe the synaptic properties of the MFs *per se*, the authors imposed kinetic criteria on the EPSC waveform (mean 20–80% rise-time less than 1 ms) to further ensure analysis of pure unitary MF EPSCs As a result, the values reported are for a subset of the MF synapses which have the fastest rising kinetics and, therefore, we still

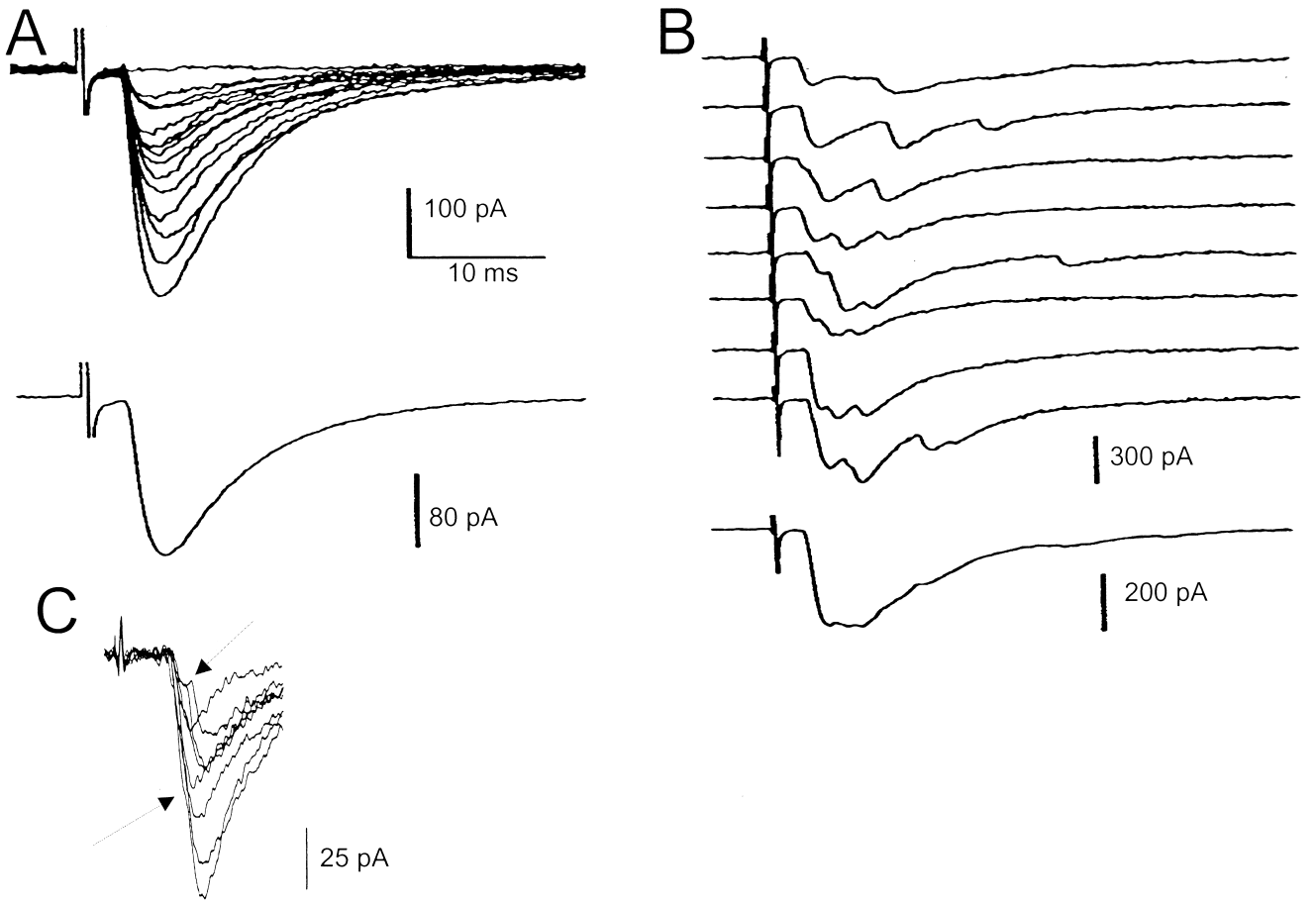


Fig. 4. Examples of DG evoked EPSCs recorded in CA3 pyramidal cells. (A) examples of MF EPSCs evoked using minimal stimulation of the DG. The upper panel shows overlaid individual responses that are believed to be simple, uncontaminated, monosynaptic MF responses in a CA3 pyramidal cell. The lower panel shows the average response. (B) The upper traces are individual DG-evoked traces that show distinct complexities. The lower trace shows the average which does not resemble the individual responses. Such waveform complexities are subsequently reduced or abolished by pharmacological manipulations that minimize polysynaptic activity[240] (see Fig. 3C). (C) Example of what are believed to be monosynaptic MF EPSCs recorded by another laboratory using a minimal stimulation protocol where polysynaptic activity was not likely to play a role. Note that, despite the use of the minimal stimulation paradigm, some subtle complexities are observed in the individual waveforms (arrows). Although it is possible that contamination from some non-MF source (see Fig. 3B–D) occurred even with the minimal stimulation, it is also possible that these complexities reflect real properties of transmission at the MF synapse. Parts A and B are adapted from Ref. 240, while part C is adapted from Ref. 104.

do not know the full distributions of unitary MF EPSC amplitudes and their associated kinetic parameters. Specifically, these kinetic criteria may exclude a subset of MF EPSCs with slower kinetics.

Recent work from our laboratory suggests that while the quantal parameters of EPSCs at the MF-CA3 synapse are similar to the values given above, there exists at least a small population of MF EPSCs that are larger, and have slower kinetics than has been reported previously. We have found that, in the presence of TTX, the largest MF spontaneous miniature EPSC averages 350 pA corresponding to a conductance change of 4 nS and individual mEPSCs can be as large as 20 nS. We have also found that the linear portion of the rising phase (10–85% risetime) can be as long as 7 ms.[83] Assuming a single channel conductance of 8.5 pS, the amplitude of the largest spontaneous mEPSCs requires that the number of AMPA receptors activated be up to two orders of magnitude larger than that activated for the reported quantal MF EPSC.[104] A recent report has indicated that although the MF-CA3 synapse contains a higher mean number of AMPA receptors than non-MF-synapses (~fourfold), the maximum number of receptors observed in a single synapse was only slightly greater (37%) for the MFs compared to Schaffer collateral synapses onto CA1 PCs.[159] Given the anatomy and physiology of the MF synapse, we feel that there are two possible mechanisms that can account for the presence of the "maxi" mEPSCs. First, "spontaneous" release from multiple active zones in a MF synaptic bouton may be synchronized by some intraterminal signal. Unfortunately, we have found no reliable evidence to support this mechanism, and instead have found that treatments, such as the application of hyperosmotic saline that increase the frequency of miniature EPSCs through the the asynchronous release of vesicles increase the frequency of mEPSCs at all amplitudes equally (unpublished observations). Alternatively, if the large clear vesicles observed in the MF boutons (e.g. Ref. 7) contain and release glutamate, then the release of glutamate from these vesicles could account for the large amplitude and slow kinetics of the "maxi" EPSCs. When glutamate is spontaneously released from one of these large vesicles at one of the MF bouton's multiple release sites, the "spillover" of glutamate from the single release site could result in the activation of AMPARs throughout the MF synaptic complex. The glutamate spillover to adjacent postsynaptic densities would result in the activation of the large numbers of AMPA receptors necessary to generate the "maxi" mEPSCs. This proposed

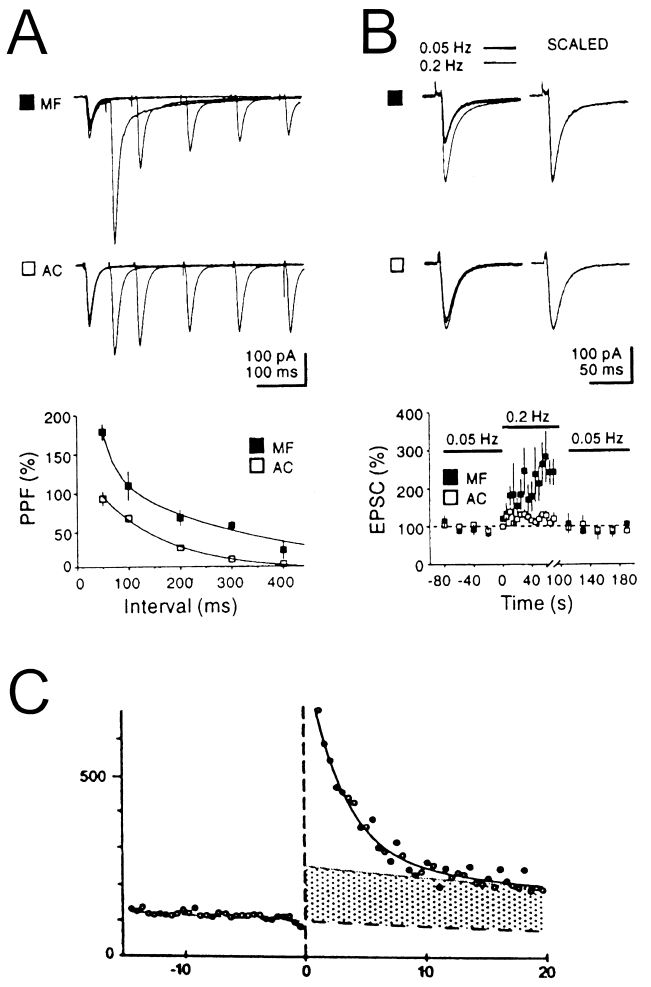


Fig. 5. The MF synapse onto CA3 pyramidal cell exhibits robust short-term facilitation and plasticity. (A) The magnitude of paired-pulse facilitation of the MF (filled squares) and associational/commissural (open squares) inputs to CA3 pyramidal cells. The MFs show almost twice as much facilitation as the associational/commissural synapses at the same inter-event intervals. (B) The MFs also selectively show frequency facilitation with stimulation frequencies as low as 0.2 Hz. Symbols as in A. (C) Following the induction of MF LTP using L-HFS (3×100 Hz for 1 s) MFs exhibit a large short-term plasticity that decays over a period of 10 min to a more stable long-lasting potentiated level than control. This period is indicated by the unshaded region immediately following the tetanus (vertical dashed line). Panels A and B are adapted from Ref. 177 and panel C is adapted from Ref. 117.

mechanism can account for the large observed ranges in the both the amplitude and rise-times of mEPSCs arising from the MF synapse. Recent work has provided some additional support for this hypothesis by demonstrating that evoked MF synaptic responses can be reduced by minimizing glutamate diffusion[151] and that glutamatergic MF heterosynaptic inhibition is mediated by spillover.[227]

4. PLASTICITY OF THE MOSSY FIBER PATHWAY

4.1. *Short-term plasticity*

Short-term plasticity at the MF to CA3 pyramidal cell synapse differs significantly from that observed at most CNS synapses in that the MF synapse exhibits very high levels of paired-pulse and frequency facilitation. Salin *et al.*[177] have demonstrated that, at room temperature, MF paired pulse facilitation is approximately two times greater in amplitude but has a similar time-course to commissural/associational synapses. However, while frequency facilitation for the C/A synapses was observed for inter-stimulus intervals (ISIs) less than 10 s, MF synapses showed frequency facilitation at ISIs as long as 40 s. Moreover, the maximal frequency facilitation for C/A synapses was only 125% of control where as for MF synapses it was 600%. The ability to induce frequency facilitation at MF synapses with low stimulation frequencies (>0.1 Hz) was also shown to be dependent on calcium/calmodulin dependent kinase II and was also partially occluded by induction of LTP (Fig. 5A, B).[177] In addition, there is also a notable difference in the time-course of the short-lasting form of plasticity known as post-tetanic potentiation (PTP) between the MF synapse and the Schaffer collateral to CA1 synapse. PTP at the Schaffer collateral synapses decays with a time constant of less than one minute (e.g. Ref. 253). In contrast, PTP at MF synapses decays with a time constant of about 3 min (Fig. 5C).[117,165,253] Calcium imaging data suggest that at least part of the PTP is mediated by residual calcium in the MF bouton. However, the same work concludes that some other presynaptic mechanism must play a role in the prolonged expression of MF PTP since calcium levels return to baseline before the PTP has fully decayed.[165,166] Although it remains a question as to whether MF LTP is ever induced under physiological conditions (due to the reports that GCs normally do not exhibit the sustained high firing rates needed to induce MF LTP, see below), short-term forms of potentiation may be more relevant in understanding the normal functioning of the MF pathway in the hippocampal network.

4.2. *Long-term plasticity—potentiation*

One of the most investigated aspects of the MF to CA3 pyramidal cell synapse has been the nature of and mechanisms underlying long-lasting changes in synaptic strength. Early on, the MF synapse was recognized to have a form of LTP that was distinct from the NMDA-receptor dependent form regularly observed at the Schaffer collateral synapse in CA1 because MF LTP was not blocked by NMDA-R antagonists such as D-APV[77,253] (reviewed in Ref. 103). The NMDA-receptor independence of MF LTP is noteworthy because for most forms of LTP, the unique properties of the NMDA receptor serve as a mechanism for requiring coincident pre- and postsynaptic activity for subsequent changes in synaptic strength. That is, the dependence on the NMDA receptor activation accounts for the Hebbian nature of LTP. Hebbian forms of plasticity allow alterations in synaptic strength to depend on the association of activity at different synaptic inputs onto a given cell, thus forming a biological basis of associative memory formation. In contrast, non-Hebbian forms of LTP would not lead to the associations of simultaneously active synaptic inputs, but may correspond to some kind of sensitization of a repeatedly activated input. In many studies, the induction of MF LTP has been shown to be non-associative, non-cooperative, and non-Hebbian (e.g. Refs 43, 117, 254 but see below). The physiological or functional relevance of non-Hebbian forms of MF LTP has not been well studied; however these forms of LTP may prove to be important in modulating the induction of Hebbian LTP at other synapses onto CA3 pyramidal cells.[43,221]

Until recently, the conclusions from numerous studies that

have examined the properties of MF LTP fell into two opposing categories. According to one group of studies, the induction of MF LTP depends only on presynaptic activity and therefore is non-Hebbian.[39,99,109,117,201,253] In contrast, another group of studies concluded that, although MF LTP is NMDA receptor independent, its induction is Hebbian because it requires both pre- and postsynaptic activity.[51,100,103] A solution to this apparent contradiction was provided by findings that, depending on the specific pattern of high-frequency stimulation, LTP at the MF synapse onto CA3 pyramidal cells is either non-Hebbian or Hebbian.[221] Specifically, long-lasting high-frequency stimulation (HFS) (three 1-s-duration 100-Hz trains presented at 0.1 Hz, L-HFS) induced MF-LTP regardless of the level of postsynaptic hyperpolarization.[109,117] In contrast, a brief tetanus (eight 0.1-s 100-Hz trains presented at 0.2 Hz, B-HFS) induces a form of MF LTP that requires depolarization of the postsynaptic CA3 cell as well as the activity of the MFs. Further studies have indicated that the B-HFS induced Hebbian form of MF LTP requires postsynaptic calcium influx through voltage-dependent L-type calcium channels.[100,108]

Yeckel *et al.*[249] presented data indicating that, despite the differences in the induction protocols, both the L-HFS and B-HFS induced forms of MF LTP require postsynaptic calcium elevation. The difference in the Hebbian vs non-Hebbian induction protocols arises from differences in the source of the calcium elevation in the postsynaptic cell. For B-HFS, postsynaptic calcium is elevated by influx via L-type calcium channels thus requiring postsynaptic depolarization to activate the voltage-gated calcium channels.[108,249] In contrast, for L-HFS, efflux of calcium from postsynaptic intracellular stores is sufficient to induce MF LTP.[249] Specifically, they have reported that the activation of mGluRs during L-HFS is sufficient to cause release of calcium from intracellular stores and thus, calcium elevation may take place in the absence of postsynaptic depolarization. In summary, the inducing signal for LTP can be provided either by the calcium influx via L-type calcium channels *or* by the calcium released from intracellular stores. Because the postsynaptic calcium elevation from either source is *sufficient* to induce MF LTP, this may explain why several studies have failed to demonstrate that mGluR antagonism blocks MF LTP induction[89,140,250] (but also see Refs 13 and 99).

MF LTP induced in either manner (L-HFS or B-HFS) appears to rely on cyclic adenosine monophosphate (cAMP)-dependent signaling cascades, as pharmacological and genetic manipulations that interfere with these cascades seem to block completely the induction of MF LTP.[87,92,93,94,127,229,249] It has been proposed that the L-HFS induced form of MF-LTP is caused by presynaptic calcium influx which triggers a cAMP cascade, ultimately leading to long-term changes in neurotransmitter release.[94,229] The mechanisms for B-HFS induced MF-LTP are believed to be due to increased postsynaptic calcium that then activates a postsynaptic cAMP cascade.[87,108,249] The postsynaptic cAMP cascade is believed to then lead to the generation of a retrograde messenger which, in turn, activates a presynaptic cAMP cascade. There is also some evidence that presynaptic PKC activation may play role.[196,197,244]

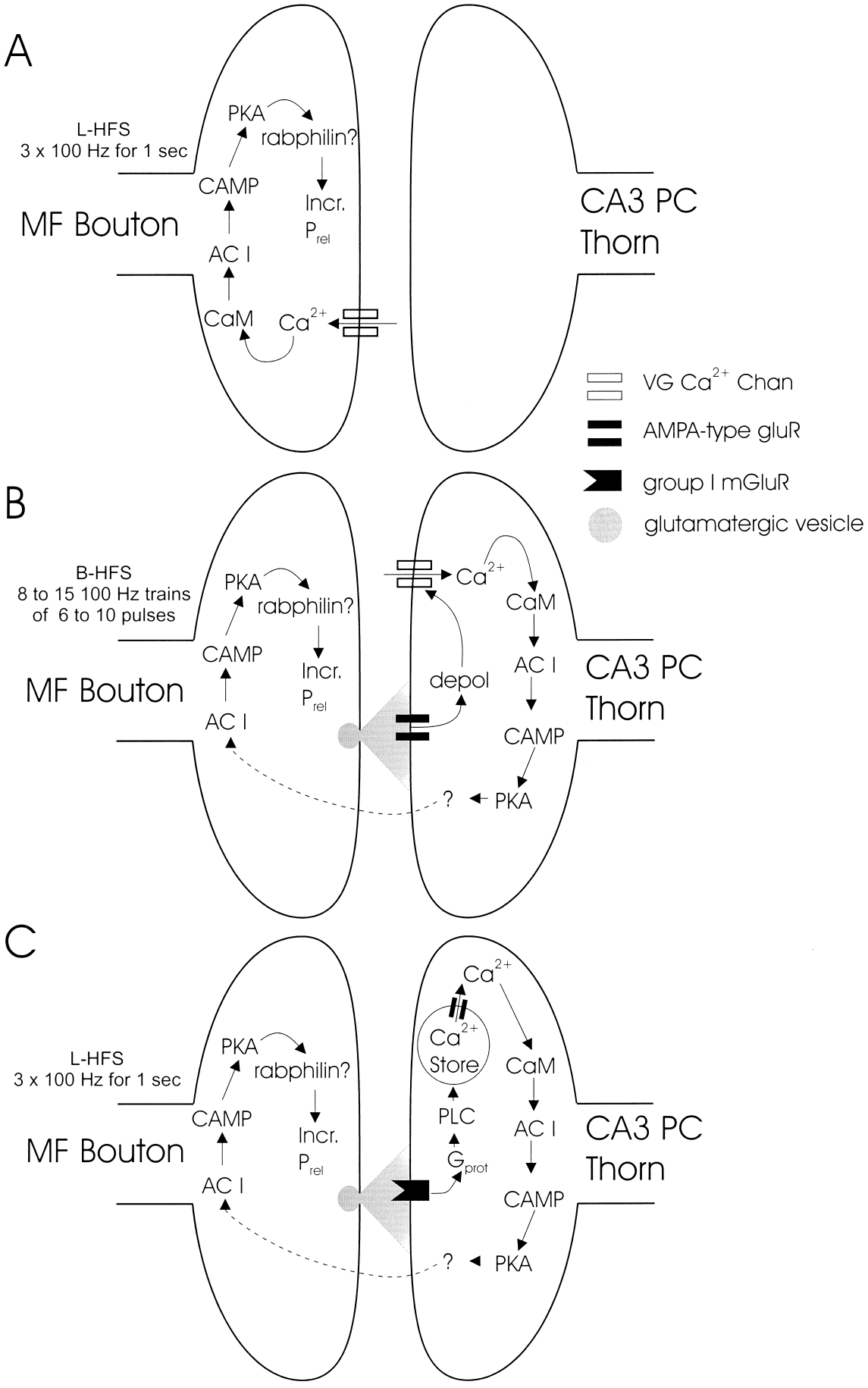
Importantly, regardless of the method used to induce MF LTP, it is widely believed that the expression of MF LTP is presynaptic and due to increase in glutamate release.[126,131,230,241,244] Further work has investigated the downstream targets of PKC and PKA that might be important effectors for MF LTP expression. With respect to downstream effects of PKC, following MF LTP induction, the PKC substrate GAP-43 shows increased phosphorylation.[196,197] This is interesting because GAP-43 (also called B-50) has been implicated in the release process (reviewed in Ref. 160). Similarly, MF LTP induced PKA activation appears to directly modulate proteins involved in the release process. The synaptic protein rabphilin is a known substrate for PKA and has been demonstrated to have increased phosphorylation following MF LTP induction.[125] It is also known that another synaptic protein, rab3A, is required for MF LTP expression and that rab3a binds to rabphilin.[36,124] The full duration of MF LTP is not known. L-HFS induced MF LTP has been reported to last at least for several hours *in vitro*[87,92] and the B-HFS induced form has been observed to last at least 30 minutes *in vitro*.[108,221,249] However, the real durations may be considerably longer since the durations reported above are limited by the duration of experimental observation.

As mentioned above (see Table 1), dynorphin-activated κ-opioid receptors have been reported to modulate the induction of the L-HFS form of MF LTP. Unfortunately, it is controversial whether activation of κ-opioid receptors is required for L-HFS induced MF LTP,[238] or whether they inhibit its induction,[231] or whether they have no effect on its induction at all.[178,223] This issue is unresolved and in part may involve species differences between rats, guinea pigs, and primates.[178,223,238] *In vivo* work also has reported that enkephalin-activated μ-opioid receptors are required for MF LTP induction,[53] however this is at odds with *in vitro* work showing that opioid receptor antagonism with naloxone has no effect on MF LTP.[178] As with most of the pharmacological modulation of the MF synapse, further studies will be required to clarify these issues.

Although the data from Yeckel *et al.*[249] go a long way towards explaining much of the confusion in the literature, there are still a few unreconciled issues. These issues revolve around experiments when MF LTP was induced despite manipulations that should have prevented postsynaptic calcium elevations (because of high postsynaptic calcium chelators or dialysis with CsFl).[117,221,253] These data leave open the possibility that yet another form of MF LTP still exists that is induced independent of postsynaptic calcium elevation.

It is interesting to note that although the mGluR-mediated form of MF-LTP has a postsynaptic locus of induction, it is still a non-Hebbian form of LTP.[249] LTP that does not require postsynaptic depolarization is unlikely to depend on or be facilitated by the coincident activation of many cells and thus cannot produce the association of weak and strong

Fig. 6. Three possible mechanisms of MF LTP induction. (A) An entirely presynaptically induced form that may be induced by L-HFS stimulation protocols. (B) A postsynaptically induced form that is dependent on calcium entry through postsynaptic voltage gated calcium channels. This form is induced by B-HFS and L-HFS stimulation protocols. (C) A postsynaptically induced form that is dependent on mGluR triggered calcium release from intracellular calcium stores. This form is induced by L-HFS stimulation protocols. Note that all three forms share a common mode of expression in the presynaptic terminal. Also note that these mechanisms are not mutually exclusive and could occur in various combinations.


A
L-HFS
3 x 100 Hz for 1 sec
PKA
rabphilin?
CAMP
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AC I
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CA3 PC
Thorn
VG Ca 2+ Chan
AMPA-type gluR
group I mGluR
glutamatergic vesicle
B
B-HFS
8 to 15 100 Hz trains
of 6 to 10 pulses
PKA
rabphilin?
CAMP
Incr.
P rel
MF Bouton
AC I
Ca 2+
CaM
AC I
depol
CA3 PC
Thorn
CAMP
? PKA
C
L-HFS
3 x 100 Hz for 1 sec
PKA
rabphilin?
CAMP
Incr.
P rel
MF Bouton
AC I
Ca 2+
Ca 2+
Store
CaM
PLC
AC I
G prot
CA3 PC
Thorn
CAMP
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Fig. 6.

inputs[43] However, if this mGluR-mediated form of LTP is facilitated by the simultaneous activation of other synaptic inputs (perhaps due to calcium influx through voltage-dependent calcium channels), then this form of LTP should be considered to be Hebbian because it would cause an increase in synaptic strength that reflected the association of inputs from multiple sources.

In summary, there appear to be at least two forms of NMDA-receptor independent LTP present at the MF synapse that are distinguishable by their induction protocols. One form is induced by repeated brief tetanic trains and is Hebbian in nature i.e. it requires simultaneous pre and postsynaptic activity. The other form is induced by long tetanic trains and is non-Hebbian in nature in that it does not require postsynaptic depolarization. Figure 6 summarizes in schematic form the hypothesized mechanisms of MF LTP induction along with the hypothesized common presynaptic expression mechanism. Unfortunately, there have been no parametric studies investigating the minimum duration of a tetanus that must be used to induce the L-HFS form of MF-LTP *in vitro*. *In vivo* data suggest that at least 30 pulses during a single train are necessary to induce L-HFS MF LTP.[52] A knowledge of the threshold number of pulses for L-HFS induced MF-LTP is important when determining the functional relevance of this form of synaptic plasticity. Although there is some debate over the precise firing patterns of GCs *in vivo*, it is accepted that under normal physiological conditions they do not fire prolonged high-frequency bursts. Therefore, depending on the minimal duration of the tetanus required to induce the non-Hebbian form of MF-LTP, it is possible that the non-Hebbian form is most relevant when considering pathological states with prolonged activation of GCs such as occurs during epileptic bursting. However, the few (8–15) repeated short 100-Hz trains of spikes (6–10) necessary to induce Hebbian LTP could be in the range of normal physiological GC activity.

4.3. *Long-term plasticity—depression*

Besides the various types of MF potentiation, there is also evidence for a long-lasting depression of MF synaptic transmission. A recent report shows that the MF to CA3 pyramidal cell synapse undergoes a homosynaptic NMDA-receptor independent long-term depression (LTD) following a long, low-frequency train of stimuli (1 Hz for 15 min; Ref. 114). This LTD lasts for at least 1 h which is the longest time point examined. Similar to the non-Hebbian form(s) of MF LTP, MF LTD does not require postsynaptic activity of CA3 pyramidal cells.[114] Further work reported that slices prepared from knockout mice lacking the mGluR2 subunit also fail to show MF LTD. This finding suggests that the MF LTD induction requires the presence of presynaptic mGluR2 subunits.[250] Other work has demonstrated that the effects of a single 1-s 100-Hz train varies as a function of development. In rats less than 11 days old a single 1-s 100-Hz train consistently induces LTD whereas in rats greater than 14 days old this same tetanus induces LTP.[14,56] This early developmental form of MF LTD is also NMDA receptor independent but it does require elevation of postsynaptic calcium.[75] In addition, the postsynaptically induced form of MF LTD does not occlude the induction of the non-Hebbian form of MF LTD.[56] Taken together with the data concerning MF LTP, there is now good evidence that the MF synapse can be bi-directionally modulated in a use-dependent manner that is dependent solely on GC activity.

5. WHAT IS THE FUNCTIONAL ROLE OF THE MOSSY FIBERS?

From the studies described above, it is clear that the hippocampal MF synapse has many properties that make it unique among cortical synapses. Thus, the question is raised, do these unique properties indicate a unique functional role for the MF synapse? The large synaptic terminals, proximal dendritic location, large frequency facilitation, and large mean (or median) EPSP/C size all suggest that MF synapses are designed to have a higher net probability of release than most other cortical synapses. That is, whenever a GC fires, a limited group of hilar cells, CA3 pyramidal cells and CA3 interneurons that receive input from that GC all experience some amount of depolarization. However, the role of this depolarization in modulating the output of the target cells remains a matter of debate.

Perhaps the most widely proposed functional role of the MF synapse onto CA3 pyramidal cells is that it is a detonator or "teacher" synapse.[30,122,142,148,174,217,218] The use of terms such as "detonator" or "teacher" are meant to indicate that whenever a GC initiates an action potential in a MF axon, all 14 CA3 pyramidal cells innervated by that MF axon will fire action potentials. This concept is attractive from a computational stand point because it allows the CA3 network to be seen as associating representations entering CA3 from the strong MF input, with those coming in via the weaker synapses made by the perforant path.[174,217,218] The detonator hypothesis assumes that a CA3 pyramidal cell has a fixed threshold for action potential initiation and that MF synaptic input is capable of reliably exceeding that threshold. However, action potential initiation in pyramidal cells is a complex event that results from the interaction of many factors such as the recent firing history of the cell, the recent history of subthreshold membrane potential changes, and the ratio of excitatory to inhibitory input.[115,135,205] Due to these complexities, and because MF EPSPs are quite variable and thus are not always large, it seems unlikely that activation of a single MF synapse onto a CA3 pyramidal cell will *always* fire a CA3 pyramidal cell. However, it is important to point out that under certain conditions, the MF EPSP can be quite large and would be expected to initiate an action potential in a CA3 pyramidal cell (e.g. during short bursts of firing by GCs or when large MF quanta are released). It remains an important goal to understand the rules that determine the conditions when such high synaptic strength conditions are achieved.

An alternative role for a MF synapse with a high probability of release is the subthreshold membrane potential of CA3 pyramidal cells. One obvious role would be to provide sufficient depolarization for the induction of NMDA-receptor-mediated Hebbian forms of LTP at non-MF synapses on CA3 pyramidal cells.[33,43] Another possibility is that the MF input serves to regulate the subthreshold activation of voltage-gated ion channels. Recent work has suggested that MF synaptic activity can gate the influence of synaptic activity from the perforant path input to CA3 pyramidal cells via indirect effects on dendritic voltage gated channels.[222]

The broader functional role of the complex pharmacology of the MF synapse has yet to be investigated experimentally or computationally. The sheer number and diversity of the substances that can modulate MF activation of CA3 pyramidal

cells suggests that multiple points of modulation are important for the normal function of this pathway. Hopefully future work will clarify the current confusion concerning the various effects (or lack thereof) of the modulators listed in Tables 1 and 2.

6. CONCLUSIONS

We hope that the issues discussed above will serve as a starting point for debate on the larger question of what the MF do in the hippocampal network as a whole. We would like to conclude by explicitly pointing out several experimental considerations and areas that we feel need to be addressed.

There are at least two important technical considerations that should be kept in mind by investigators. First, the properties of the MF pathway are not the same across species and even across development for a single species. In fact, the ongoing development of the GCs and MFs is likely to be an important aspect of understanding the functional role of this pathway. Anatomically, the MF synaptic complexes in the rat are still undergoing changes even after 21 days post birth.[7] Furthermore, the induction of LTD versus LTP following L-HFS is also a function of age.[14] Additional complications arise from the fact that different species and even different strains of the same species develop at different rates. These developmental issues may account for some of the confusion in the current literature (see Tables 1 and 2). Developmental studies are likely to help clarify some issues concerning the pharmacology and plasticity of the MF synapse.

Second, as has been warned by others,[46,84,116] the use of bulk extracellular stimulation to study the MF synapses must be carefully considered. The use of large stimulating electrodes placed in the DG leads to polysynaptic and antidromic activation of CA3 pyramidal cells which can subsequently contaminate alleged MF synaptic responses. We would like to expand on the previous warnings of Claiborne *et al.*[46] by suggesting that the use of overly conservative criteria may lead to the exclusion of "pure" MF responses with unexpected properties. The application of type II mGluR agonists (such as DCG-IV) to inhibit MF EPSP/Cs selectively is one particularly useful approach for validating MF responses that does not require the use of essentially arbitrary criteria. As such, the application of type II MGluR agonists should be a routine component of experimental paradigms involving evoked MF responses.

Assuming that necessary technical challenges can be overcome, there are many outstanding scientific issues that need to be resolved. For example, the basic properties of quantal synaptic transmission of the MFs onto all cellular targets (both in the hilus and CA3) have yet to be completely described without any data being excluded based upon kinetic criteria. The use of the mGluR agonists or the recently developed direct patching of the MF boutons[19,70] may prove useful in this regard. Other general areas include understanding the effects of the continuous development of this pathway throughout the life of the animal; understanding the importance of the broad pharmacological modulation of this pathway; and finally understanding how GC firing patterns influence the activity of both pyramidal cells and interneurons in the hilus and area CA3 *in vivo*. These all are important steps towards a complete understanding of the functional role of the MFs in the hippocampal network.

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