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Revisiting the Role of the Hippocampal Mossy Fiber Synapse

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ABSTRACT: The mossy fiber pathway has long been considered to provide the major source of excitatory input to pyramidal cells of hippocampal area CA3. In this review we describe anatomical and physiological properties of this pathway that challenge this view. We argue that the mossy fiber pathway does not provide the main input to CA3 pyramidal cells, and that the short-term plasticity and amplitude variance of mossy fiber synapses may be more important features than their long-term plasticity or absolute input strength. *Hippocampus 2001;11:408–417.*

KEY WORDS: hippocampus; CA3; mossy fiber; synapse; plasticity; LTP; synaptic integration

CLASSICAL VIEW: THE "DETONATOR" SYNAPSE OF THE TRISYNAPTIC CIRCUIT

Early observers of the hippocampus noted the large size of mossy fiber presynaptic boutons and the location of mossy fiber synapses close to the soma of CA3 pyramidal cells and suggested that mossy fibers provide a strong excitatory input to CA3 pyramidal cells (Blackstad and Kjaerheim, 1961). Some authors even have referred to the mossy fiber synapse as a "detonator" in reference to the idea that the input from a single mossy fiber synapse may be sufficient to fire a CA3 pyramidal cell (Brown and Zador, 1986; McNaughton and Morris, 1987; Andersen and Loyning, 1962). This simplified view of mossy fiber synaptic physiology lead to an equally idealized picture of the function of the mossy fiber input to CA3, as featured in the trisynaptic circuit picture of the hippocampus. In classical "trisynaptic circuit" models of the hippocampus, the mossy fiber input is considered to be the only source of excitatory input to CA3 pyramidal cells; no other sources of input are included in the model (Andersen et al., 1969). More sophisticated models propose that the role of the mossy fiber input to CA3 might be understood by viewing area CA3 as storing an association between (strong) mossy fiber and (weak) perforant path inputs (McNaughton and Morris, 1987; Treves and Rolls, 1992; Lisman, 1999), but still these models require that individual mossy fibers are able to fire CA3 pyramidal cells reliably. In short, they require that a mossy fiber synapse be a detonator.

The growing body of work on the synaptic physiology and plasticity of the synapses providing input to CA3 pyramidal cells suggests that the picture of hippocampal function outlined by the trisynaptic circuit and related models is overly simplistic. For example, the strength of the various inputs to CA3 pyramidal cells and interneurons is differentially modulated by recent activity of the projecting neurons (Maccaferri et al., 1998; Salin et al., 1996), and mossy fiber excitatory postsynaptic potentials (EPSPs) summate sublinearly with other sources of input to CA3 pyramidal cells (Urban and Barrionuevo, 1998). Moreover, the average amplitude of single mossy fiber synaptic responses, while large (Jonas et al., 1993; Henze et al., 1997), is unlikely to be sufficient to allow single mossy fiber synapses to fire CA3 pyramidal cells, unless the cell is substantially depolarized. These data highlight the fact that the relationship between activity of mossy fiber synapses and CA3 pyramidal cell firing is not as simple as suggested by the picture of the mossy fiber synapse as detonator. Collectively, these results suggest that the relative strength of mossy fiber and nonmossy fiber input pathways varies dynamically as a function of the activity of granule cells, CA3 pyramidal cells, and cells in the entorhinal cortex. Moreover, mossy fibers directly activate several populations of hippocampal interneurons (Frotscher et al., 1994; Acsady et al., 1998; Spruston et al., 1997), providing a further brake on the excitatory drive of CA3 pyramidal cells by their mossy fiber synapses. Here we argue that while individual mossy fiber synapses are stronger than the other synapses received by CA3 pyramidal cells, the mossy fiber pathway is unlikely to be the primary source of excitation to the CA3 network. This conclusion requires that many ideas about the function of area CA3 be rethought.

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In this review we highlight some of the recent work on mossy fiber synaptic physiology and synaptic plasticity, and place this work in the context of recent proposals (Markram and Tsodyks, 1996; Tsodyks and Markram, 1997; Abbott et al., 1997) that revise the way in which synaptic strength and plasticity are described. Initially, we consider the two related questions of whether individual mossy fiber synapses function as detonators for CA3 pyramidal neurons, and whether the mossy fiber pathway provides the main source of excitation to CA3 pyramidal cells. In this context, we propose a general method for calculating an estimate of the relative strength of the different sources of input received by a cell. Finally, we will argue that the mossy fiber input to CA3 should be compared not to a detonator that can reliably fire CA3 pyramidal cells, but rather to a discriminator, in several senses of the term. Generally, a discriminator is a device whose output is a function of a particular feature of its input, such as its amplitude, frequency, or latency. For example, in electronics, a window discriminator produces an output only when its input is between two values of voltage, whereas the amplitude of the output of a frequency discriminator varies with the frequency of the input. We also discuss the implications that this shift in thinking may have on ideas of hippocampal function.

HOW STRONG IS THE MOSSY FIBER PATHWAY?

The mossy fiber synapse onto CA3 pyramidal cells is unusual in many respects (see Henze et al., 2000), and in particular, activation of even a single mossy fiber synapse is able to produce quite a strong depolarization at the soma of a CA3 pyramidal cell. In light of the strength of individual mossy fiber synapses, the mossy fiber *pathway* has long been considered the primary source of afferent input to CA3 pyramidal cells, a distinction that has earned it a key place in the "trisynaptic circuit" model of the hippocampus (Andersen et al., 1969). However, in addition to receiving mossy fiber synapses onto their proximal apical dendrite, CA3 pyramidal neurons receive excitatory synapses from stellate cells of layer II of the entorhinal cortex (EC) onto their distal apical dendrite (Steward, 1976; Yeckel and Berger, 1990; Berzhanskaya et al., 1998), and from other CA3 axon collaterals onto the remainder of the apical and the entire basal dendrite (Li et al., 1994) (Fig. 1A). Theoretical work has considered the perforant path and collateral inputs to be an important source of excitation for CA3 pyramidal cells (Treves and Rolls, 1992; O'Reilly and McClelland, 1994; Hasselmo et al., 1995; Hasselmo and McClelland, 1999; Kali and Dayan, 2000; Levy et al., 1998), and recent experimental work (Yeckel and Berger, 1990; Berzhanskaya et al., 1998; Urban et al., 1998; Breindl et al., 1994) has begun to explore the properties of the perforant path projection to CA3 pyramidal neurons. The collateral synapses onto CA3 pyramidal cells are thought to be similar to the Schaffer collateral synapses on CA1 pyramidal cells (Pavlidis and Madison, 1999; Miles and Wong, 1986; Malinow, 1991; Debanne et al., 1995, 1998), a synapse which is exceedingly well studied.

Given that CA3 pyramidal cells receive excitatory inputs from three distinct sources, the question of the relative strength of these inputs is of critical importance to our understanding of hippocampal function. In many areas of the brain, neurons receive synaptic inputs from multiple sources; thus, the general question of which source of input received by a cell is most important for determining how it fires is a complex and important one. For example, does the activity of spiny stellate cells in layer 4 of the primary visual cortex mostly reflect the activity of thalamic relay cells, or is it more a function of intracortical inputs? To what extent is the activity of Purkinje cells determined by the activity of climbing fibers vs. parallel fibers? In these and other cases, one would like to know by how much the firing rate of a given postsynaptic cell (such as a CA3 pyramidal cell) would change for a given change in the average firing rate of a population of presynaptic cells (such as dentate granule cells). Thus, if the average rate of granule cell firing is reduced by 10%, what change in the firing rate of CA3 pyramidal cells would result (assuming that the firing rates of other cells projecting to CA3 pyramidal cells are unchanged)? Expressed mathematically, one would like to determine the partial derivative of the firing rate of the target cell with respect to the firing rate of the cells in the various areas presynaptic to the target. Such a measurement is virtually impossible to make experimentally. However, if we make the simplifying assumption that over a range of values, firing rate varies approximately linearly with current injection from a given source, then the relative contribution of a given source of input to a cell's firing rate can be estimated by simply computing the relative amount of current injected by all the synapses that that cell receives from the given source.

Although such a calculation of the relative strength of inputs from various sources is an estimate, it serves as a useful first approximation for determining how much the activity of one population of cells should depend on the activity of another. Note that we are only interested in the contribution of an entire population of inputs (e.g., all of the mossy fiber synapses) to the average firing rate of another entire population of cells (e.g., all the CA3 pyramidal cells). We are not interested (for now) in knowing which input caused any particular CA3 cell to fire. Thus, in what follows, we will calculate the average proportions of current injected by the population of inputs with the understanding that individual cells will differ in the amounts of input that they receive from different sources. So first, we will make an estimate for the relative strengths of the three main sources of excitatory input to area CA3, and then we will discuss cases in which this estimate is likely to break down.

The strength of the input provided by a given source region will be determined by the total amount of synaptic current injected into the target cell by the synapses formed on the target cell by cells in the source region. Three main factors will determine the amount of synaptic current injected into a target cell by the cells in a given region that serves as a source of input: 1) the average amplitude of a unitary synaptic current onto the target cell from the synapses made by the specific set of input cells, 2) the average number of connections made by cells in the input region to the target cell, and 3) the average firing rate of the cells in the input region. Once we

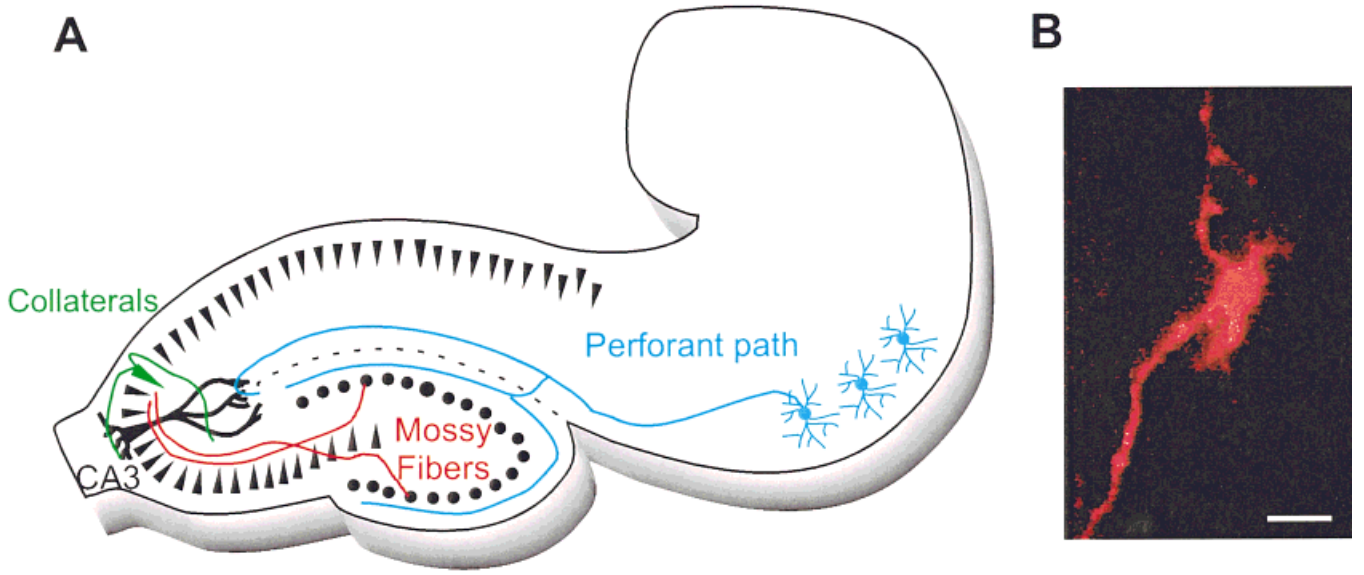

FIGURE 1. Pyramidal cells in hippocampal area CA3 receive excitatory synaptic inputs from three sources. A: Mossy fiber axons of dentate granule cells form synapses on the proximal apical dendrite of CA3 pyramidal cells. Collateral axons from other CA3 pyramidal cells synapse on the medial apical and basal dendrites of CA3 pyramidal cells. Axons of stellate cells in entorhinal cortex synapse on the distal apical dendrite of CA3 pyramidal cells. B: A single mossy fiber bouton filled with DiI. Scale bar, 4 μm.

know these three factors, the strengths of various sources of input to a given input to a cell can be calculated simply by comparing the products of these three numbers for the input sources. All three of these factors may change due to development, changes in the behavioral and neuromodulatory state of the animal, and changes in the sensory environment of the animal, with the modulation of firing rate as a function of the behavior or environment of the animal perhaps producing the most dramatic changes. The question of which input is most important can be refined by considering any of these additional variables. For example, one can ask what the relative strength is of these input pathways in a sleeping juvenile mouse, or, as we will below, one can ask about the relative strength of inputs in an adult rat during spatial exploration.

Given this method for estimating the relative strength of an input pathway, we can compare the strength of the mossy fiber input to CA3 to that of the perforant path and collateral inputs. Each pyramidal cell of area CA3 is known to receive roughly 50 mossy fiber synapses, 4,000 perforant path synapses, and 12,000 synapses from collateral axons (Amaral et al., 1990). As a result, mossy fibers are at a distinct numerical disadvantage which must be overcome if they are to dominate the activation of CA3 pyramidal cells. The mean amplitude of unitary mossy fiber excitatory postsynaptic currents (EPSCs) recorded in CA3 pyramidal cell somata is approximately 70 pA (Jonas et al., 1993; Henze et al., 1997). For comparison, the mean unitary EPSCs at Schaffer collateral synapses onto CA1 (and presumably also onto other CA3) pyramidal cells have an amplitude of ~7 pA (Bolshakov and Siegelbaum, 1994; Pavlidis and Madison, 1999), and the mean amplitude of unitary perforant path EPSCs in CA3 pyramidal cells is less than 2 pA (unpublished observations, and Henze et al., 1996). Although mossy fiber EPSCs are on average almost 10-fold larger than collateral EPSCs, it is unlikely that the difference in EPSC amplitude could offset the 200-fold larger number of collateral synapses. Moreover, the firing rate of dentate granule cells during behavioral tasks has been estimated at less than 0.2 Hz or <3% that of EC neurons (6 Hz), while CA3 pyramidal cells fire at an average rate of about 2.5 Hz, or 40% of EC cells (Jung and McNaughton, 1993; Barnes et al., 1990).

When one computes the average synaptic current provided to a CA3 pyramidal cell by the inputs from these three cell populations (Table 1), then the collateral synapses seem to be providing by far the most input. Individual mossy fiber synapses, despite having a unitary EPSC amplitude almost 10-fold larger than that of collateral synapses, provide the least total input to the CA3 pyramids. Thus, this simple calculation of input strength suggests that the mossy fiber pathway as a whole is expected to play a very small role in the overall activity of CA3 pyramidal cells.

The most likely source of error in the parameters that go into this estimate originates from the data on the average firing rate of the various cell populations. There is not a great deal of data on the average firing rates of cells in EC, dentate gyrus (DG), or even CA3 cells during behavioral tasks. Specifically with respect to DG granule cells, there is some controversy as to what their average firing rate is, because of the difficulty of unambiguously identifying granule cells from extracellularly recorded action potentials (Jung and McNaughton, 1993; Rose et al., 1983; Buzsaki et al., 1983; Mizumori et al., 1989). Moreover, average firing rates can change dramatically with changes in the behavioral state of the animal, or with environmental conditions (Wilson and McNaughton, 1994). Thus, although our estimate of the relative strength of these three inputs may be modified, the hypothesis that the mossy fibers serve as the main input to CA3 pyramidal cells clearly needs to be reconsidered.

TABLE 1.

*Relative Strength of Three Excitatory Inputs to CA3 Pyramidal Cells**

Input	Quantal size	Active zones/syn.	No. CA3 cell	Mean unitary amplitude	Activity (% of EC)	Relative strength[a]
MF	9 pA[b]	20	50[c]	70 pA[b]	3%[d]	105
Collateral	5 pA	1	12,000[c]	7 pA[e]	40%[d]	24,000
PP	?	?	4,000[c]	1 pA	100%[d]	4,000

*MF, mossy fiber; PP, perforant path.

[a]Relative strengthis obtained simply by multiplying the number of synapses by their mean unitary amplitude by the relative activity of the cells of origin for the inputs.

[b]Jonas et al., 1993; Henze et al., 1997.

[c]Amaral et al., 1990.

[d]Barnes et al., 1990.

[e]Bolshakov and Siegelbaum, 1994; Stevens and Wang, 1994.

This estimate is a simplification in that we have ignored many aspects of hippocampal physiology, including synaptic plasticity and the possibility that the inputs to CA3 cells may be activated synchronously rather than randomly in time. However, given this estimate, we see that if factors such as plasticity or synchrony are to play an important role in determining the relative strengths of various pathways providing input to CA3 pyramidal cells, then the effects of these phenomena must be quite large. Moreover, if factors such as synchrony of inputs play a large role in modifying the relative strength of these input pathways to CA3, then they must occur selectively in some input areas and not others. Much of the synchronous population activity that has been observed in the hippocampal complex is notable in that it seems to occur in several of the hippocampal subfields at once (Bragin et al., 1995; Chrobak and Buzsaki, 1996, 1998a,b).

In looking for ways in which the accuracy of our estimate might be improved, we next consider the following four different sources of error. First we examine the importance of the large variability of individual mossy fiber synaptic events. Second, we consider how the differential integration of inputs from various sources might be important in determining the conditions under which mossy fiber and nonmossy fiber inputs might be most effective. Third, we consider how short- and long-term modification of the strength of mossy fiber synapses might alter the balance of excitation received by CA3 pyramidal cells. Finally we discuss briefly how our estimates may be altered by the fact that each input pathway to CA3 activates a population of inhibitory interneurons in addition to activating pyramidal cells.

QUANTAL VARIABILITY AT MOSSY FIBER SYNAPSES

The estimate of the relative strength of the various inputs to CA3 pyramidal cells makes use of data about the average strength of the various excitatory synapses made onto CA3 pyramidal cells. In this case, synaptic strength was quantified as the average amplitude of a unitary synaptic event. An alternative view of the strength of an input pathway can be gained by considering the probability that activation of a single synapse will result in a postsynaptic action potential, and then scaling this probability by the number of synapses that make up the pathway. On this view, the strength of a connection is not simply equal to the average amplitude of the EPSP that it produces, but rather depends on the probability that activation of the synapse results in an EPSP of sufficient amplitude to exceed the threshold for action potential initiation. The probability that a single synapse will drive a given cell to threshold is equal to the probability that the EPSP from that synapse will be greater than the difference between the cell's voltage and its threshold. For two synapses producing the same average amplitude response, if that average response is insufficient to drive the cell to threshold, then the synapse with the higher variance and/or skewness has a greater chance of firing the cell. In this case, the strength of a synapse can be related to its variability or, more specifically, to the area under the tail of the histogram of EPSP amplitudes (O'Reilly and McClelland, 1994).

Despite providing a relatively small component of the total synaptic current to CA3 cells, individual mossy fiber synaptic responses are on average almost 10-fold larger than collateral responses. The average amplitude of mossy fiber EPSCs (and EPSPs) suggests that a single mossy fiber synapse cannot reliably fire a CA3 cell sitting near its resting membrane potential (−75 to −70 mV). However, the variance and skewness, and thus the range of mossy fiber EPSC amplitudes, are larger than would be expected if mossy fiber responses were just scaled collateral synapse responses. Unitary evoked mossy fiber EPSCs can be more than 500 pA or about 50-fold larger than their quantal size, and miniature EPSCs larger than 1 nA have been reported in CA3 cells (Jonas et al., 1993; Henze et al., 1997) bathed in tetrodotoxin (TTX). In contrast, collateral EPSCs do not exceed an amplitude of 10 times their mean quantal size (Bolshakov and Siegelbaum, 1994; Stevens and Wang, 1994; Malinow, 1991) (Fig. 2). If we take unitary connection strength to be a measure of the probability that activation of a given synapse will result in the generation of an action potential, then the large variability of mossy fiber EPSC amplitudes can be seen as a component of mossy

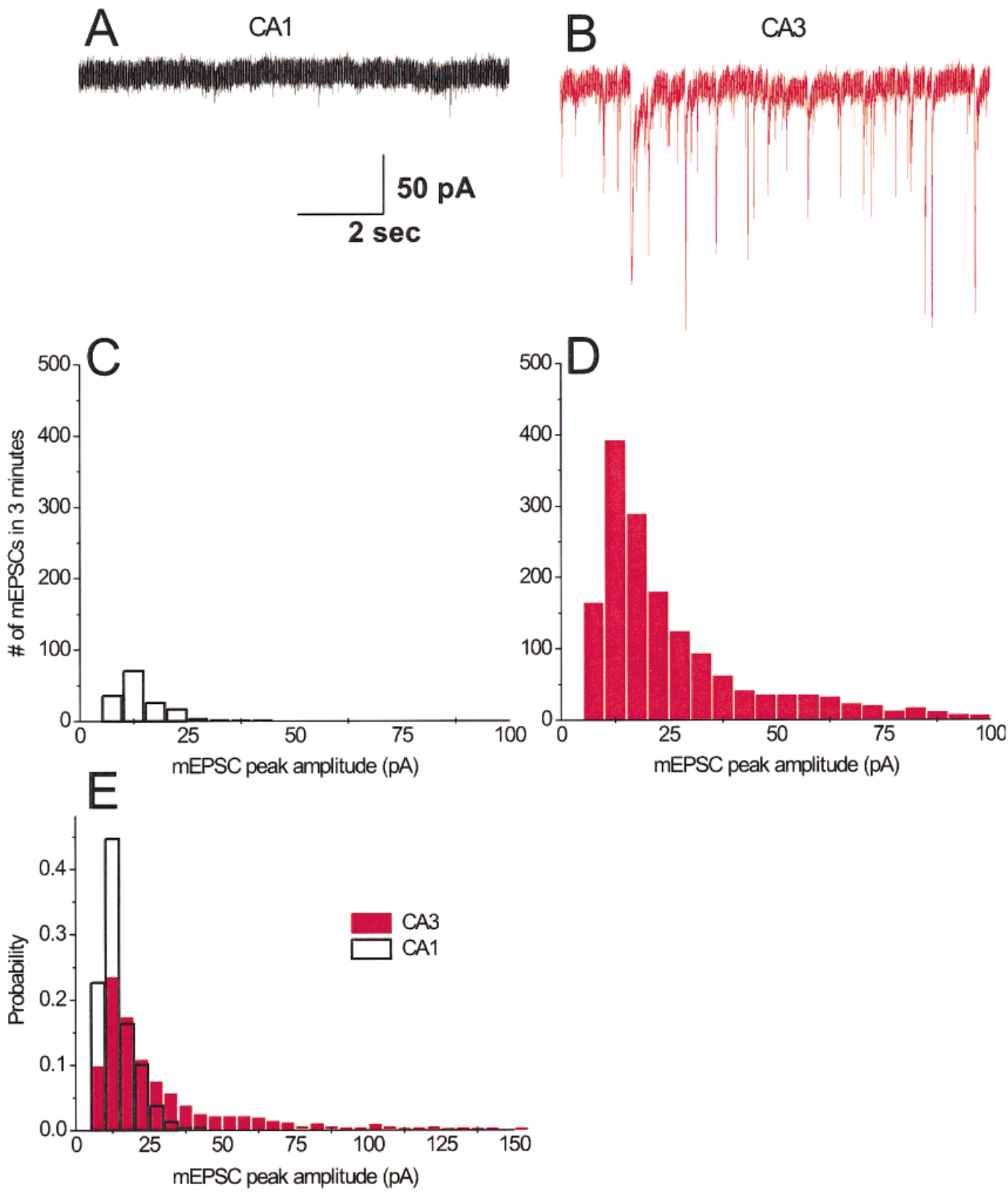

FIGURE 2. EPSCs in CA3 pyramidal cells show much larger range than EPSCs in CA1 pyramids. A, B: Sample traces of spontaneous EPSCs recorded from CA1 (A) and CA3 (B) pyramidal cells in the presence of 1 μM TTX and 10 μM bicuculline, with the cell held at −80 mV. C, D: Histograms showing amplitudes of all spontaneous EPSCs recorded from the same cells shown in A and B above, during a 3-min period. E: Histograms of same data shown in C and D, but showing probability rather than number of events. Note that the range of the events recorded in CA3 pyramidal cells is much larger than the range recorded in CA1.

fiber strength. Interestingly, this is an example in which the usual measure of synaptic strength (average EPSC or EPSP amplitude) is not a good indicator of how potent a synapse is in its ability to alter the firing of the postsynaptic cell. The strength of mossy fiber synapses may be derived in large part from the particular details of the statistical distribution of their amplitudes. This analysis points to the importance of studying synapses individually, e.g., by doing paired recordings from pre- and postsynaptic cells. These techniques allow the details of the amplitude distributions of synaptic responses to be determined for individual synapses. In contrast, experiments in which bulk stimulation is used to activate large populations of synapses will not allow the details of the statistics of individual synapses to be studied.

In the hippocampus, the high variability of the input from the mossy fibers may allow novel stimuli to activate a randomly selected population of CA3 pyramidal cells. On those occasions when mossy fiber EPSPs are above threshold, Hebbian synaptic plasticity may then strengthen collateral and perforant path synapses to this population of CA3 pyramidal cells (Magee and Johnston, 1997; Debanne et al., 1998; Chattarji et al., 1989). Such plasticity would allow future presentations of similar patterns to drive the same group of CA3 cells, even in the absence of strong mossy fiber input. This mechanism could be further facilitated if a single stimulus (e.g., in the case of the hippocampus, a single location in space) was sampled repeatedly, resulting in frequency facilitation of the mossy fiber synapses (see below).

INTEGRATION OF MOSSY FIBER AND NONMOSSY FIBER EPSPS

A second issue complicating the straightforward assessment of input strength is that synaptic potentials must be combined or integrated to produce the changes in membrane potential that lead to action potential initiation. Activation of mossy fiber synapses within a short (0–15 ms) interval before activation of perforant path synapses results in the reduction of the amplitude of perforant path EPSPs (Urban and Barrionuevo, 1998) (Fig. 3). This reduction is caused by the activation of transient voltage-dependent potassium channels ($I_{K(A)}$) by the rapidly rising mossy fiber EPSP, and thus we have termed this process "active summation." We have found that active summation makes the integration of mossy fiber EPSPs with perforant path EPSPs sensitive to the temporal order in which these two inputs arrive (Fig. 3).

When considering the strength of the mossy fiber input, one must know whether it is acting alone, or in conjunction with other inputs to depolarize the CA3 cell. If it is acting in conjunction with other inputs, then the time between the arrival of mossy fiber and nonmossy fiber inputs also must be considered: granule cells firing at the beginning of a wave of synaptic excitation to CA3 pyramidal cells will reduce the effectiveness of those nonmossy fiber inputs to CA3 pyramidal neurons that arrive during a 15-ms time window following the MF input. By contrast, if the mossy fiber input arrives at the end of a barrage of synaptic inputs, when transient

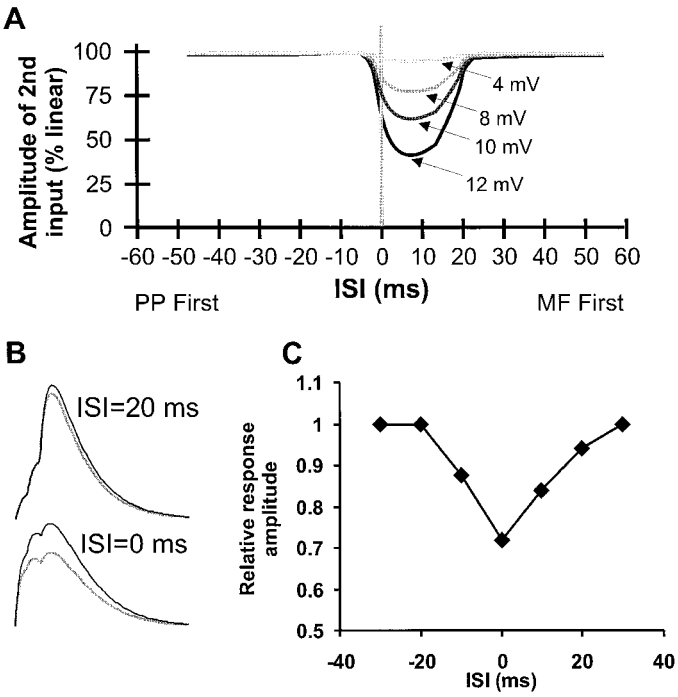

FIGURE 3. Sublinear summation of EPSPs in CA3 pyramidal cells reduces benefit of cooperative mossy fiber inputs. A: Summary of data showing the effect of prestimulation of mossy fiber or perforant path axons on the opposite input. Prior stimulation of mossy fiber EPSPs results in a reduction of perforant path EPSPs. Amount of reduction depends on amplitude of the mossy fiber EPSP and the interval between stimulation of the two pathways. B: Simulated data showing the effect of sublinear summation when mossy fiber EPSP arrives before (B top) or after (B bottom) a train of three perforant path EPSPs. C: The effect of sublinear summation is negligible when mossy fiber EPSPs follow EPSPs from the perforant path, and is largest when mossy fiber EPSPs precede the arrival of perforant path EPSPs.

potassium channels are inactivated, then it is more likely to drive an already depolarized cell over its firing threshold. Activation of the entorhinal cortex has been shown to result in both monosynaptically and disynaptically driven population spike activity in the CA3 pyramidal cell layer, with monosynaptically evoked activity preceding the disynaptically evoked activity by 0.5–2 ms (Yeckel and Berger, 1990). If we assume that the difference in population spike latency reflects the difference in the arrival of synaptic input via these two pathways, then this predicts that a synchronous increase in activity in EC will result in two successive "waves" of input to CA3, separated by a few milliseconds. The first wave of input from the EC is the monosynaptic input from the perforant path, and the second wave of input is the disynaptic input from the mossy fibers. Thus, the postsynaptic response to these two events will sum linearly. In contrast, if activity in the EC is not synchronous, or if there are multiple peaks in EC activity separated by less than 15 ms, then direct perforant path inputs that arrive "late" relative to the mossy fiber inputs will be selectively suppressed, resulting in improved synchrony in the activity of CA3 pyramidal cells, relative to the inputs that these cells receive. Thus, active summation allows CA3 pyramidal cells to discriminate between various temporal patterns of incoming excitatory inputs.

SYNAPTIC DYNAMICS AND FREQUENCY DISCRIMINATION

Our estimate of the relative strengths of the various input pathways to CA3 pyramidal cells above was based in part on data about the average amplitude of unitary synaptic responses of the various input pathways. Such data are generally derived from experiments in which synapses are stimulated at low frequencies (<1 Hz). Recent work on short-term plasticity at cortical synapses has highlighted the importance of synaptic dynamics as a component of synaptic strength (Abbott et al., 1997; Tsodyks and Markram, 1997). If the amplitude of a synaptic response depresses or facilitates with repeated stimulation at certain frequencies, then the strength of this synapse will vary with the frequency with which it is activated.

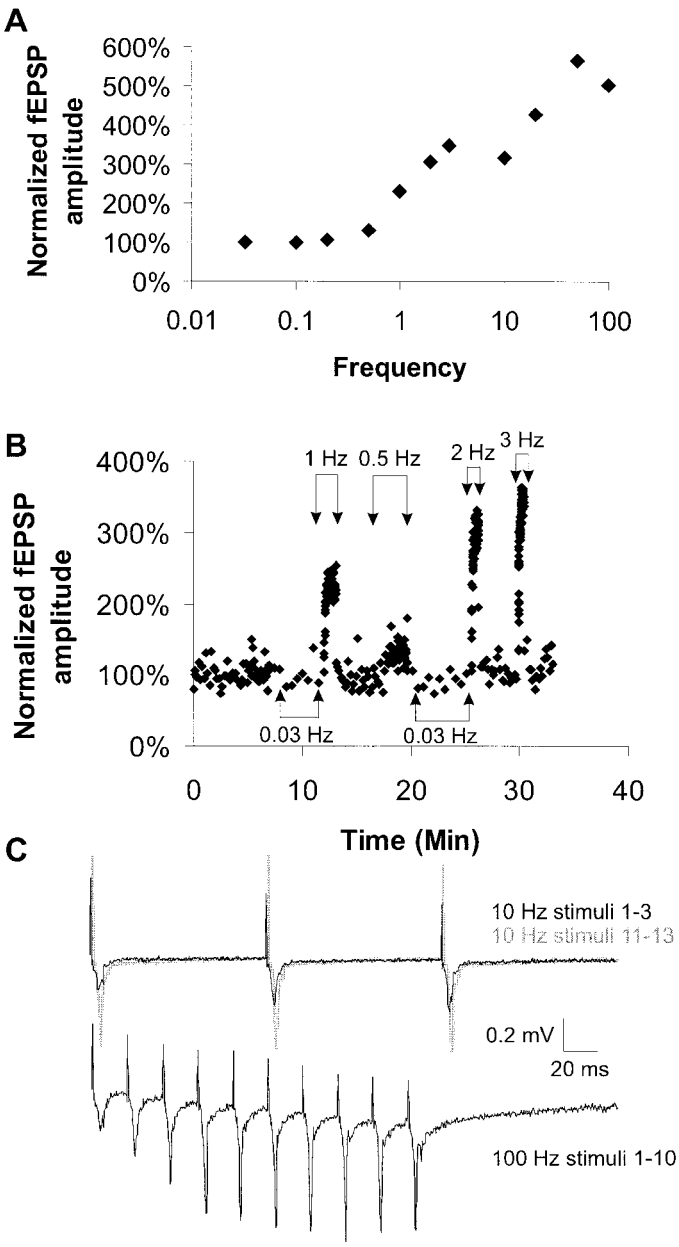

FIGURE 4. Mossy fiber EPSPs show profound frequency facilitation. A: Plot of relative amplitude of mossy fiber field EPSPs, evoked at a variety of frequencies. All values are expressed as percentage of amplitude at 0.1 Hz, which is approximately the average firing rate of dentate granule cells. B: Example of experiment in which frequency of stimulation was varied from 0.3–3.0 Hz while recording mossy fiber field EPSPs. Stimulation rate was 0.1 Hz, unless indicated otherwise. C: Example of EPSCs from stimulation at 10 Hz and 50 Hz. For the 10-Hz traces, responses to the first through third and eleventh through thirteenth pulses at this frequency are shown.

Mossy fiber synapses on pyramidal cells differ from most excitatory synapses made onto pyramidal cells (Thomson and Deuchars, 1997; Markram et al., 1998; Reyes et al., 1998) in that mossy fiber synapses show pronounced facilitation rather than depression when stimulated at high frequencies (Salin et al., 1996; Langdon et al., 1995). The magnitude and time course of this facilitation vary with the frequency of stimulation, but they are larger and occur with lower frequencies than facilitation and depression observed at neocortical synapses or at Schaffer collateral synapses onto CA1 pyramidal cells. Reports of mossy fiber facilitation range from a 2-fold potentiation of field EPSPs for frequencies as low as 0.2 Hz (Salin et al., 1996), to more than a 40-fold potentiation of EPSCs for 100-Hz stimulation (Langdon et al., 1995). Frequency facilitation at mossy fiber synapses on pyramidal cells persists during repeated stimulation: 15 min of 1-Hz stimulation results in a maximal facilitation of 5-fold within the first minute and a persistent 3-fold potentiation at the end of the 15 min of stimulation (Yokoi et al., 1996). The magnitude and duration of this frequency facilitation suggest that this process may serve to amplify the influence of dentate granule cells when they fire in a repeated fashion, even at low rates (e.g., <1 Hz). In this case, increases in the firing frequency of granule cells will result in higher amplitude unitary inputs. Thus, the mossy fiber synapse will function as a frequency discriminator. Because of this strong frequency facilitation of mossy fiber synapses, the effect of a 50% reduction in granule cell firing will depend on how this reduction is achieved. For example, if half of the granule cells are silenced, this will lead to a 50% reduction in the total current injected into CA3 cells by their mossy fiber synapses. However, if the firing rates of all granule cells are reduced by 50%, this may reduce the total mossy fiber synaptic current by more than 50%, because the active mossy fiber synapses will show less frequency facilitation. In this case, knowing the average firing rate of granule cells may not be sufficient to know the contribution of activity in this pathway to CA3 pyramidal cell firing. One needs to know the distribution of firing rates across individual granule cells.

MOSSY FIBER LONG-TERM PLASTICITY

Long-term synaptic potentiation and depression result in modifications in the average amplitude of synaptic responses, and as such could alter the relative strengths of the various input pathways that activate CA3 pyramidal cells. Mossy fiber synapses have been

shown to display a variety of forms of long-term synaptic plasticity, including both Hebbian and non-Hebbian forms of long-term potentiation (LTP) and depression (LTD) (Jaffe and Johnston, 1990; Zalutsky and Nicoll, 1990; Yokoi et al., 1996; Urban and Barrionuevo, 1996; Urban et al., 1996; Derrick and Martinez, 1996; Domenici et al., 1998). Despite the proliferation of forms of mossy fiber long-term plasticity, there has yet to be demonstrated a correlation between impairment of any form of mossy fiber long-term plasticity and performance on a learning task. Knockout mice in which mossy fiber LTP or LTD are eliminated have been shown to be unimpaired in learning tasks (Huang et al., 1995; Yokoi et al., 1996). Interestingly, none of the genetic or pharmacological manipulations that have resulted in impaired mossy fiber LTP or LTD have been reported to affect paired-pulse facilitation or other measures of short-term plasticity of the kind we characterized above as synaptic dynamics. This raises the intriguing possibility that short-term mossy fiber plasticity may be of greater behavioral relevance than mossy fiber LTP. Short-term mossy fiber plasticity is reduced by inhibitors of Ca^{2+}/calmodulin-dependent protein kinase (CaM kinase) (Salin et al., 1996), genetic and pharmacological manipulations of which are known to affect performance on spatial learning tasks (Silva et al., 1992a,b; Chapman et al., 1995; Mayford et al., 1995). However, the effectiveness of these manipulations of CaM kinase at impairing learning has been attributed to their blockade of Schaffer collateral-CA1 LTP. Thus, more specific experiments must be performed to isolate a possible role for the effect of CaM kinase blockade on mossy fiber short-term plasticity and its relationship to spatial learning.

DISYNAPTIC INHIBITORY INPUTS

In the calculation of the relative strength of the various pathways given above, we have not taken into account the fact that the excitatory input pathways to area CA3 activate interneurons as well as the pyramidal cells. The number of interneurons activated in a feedforward fashion by an input pathway will of course play an important role in determining the amount to which a given input activates the pyramidal cells of area CA3. The relative degrees of activation of interneurons in area CA3 by the mossy fiber, collateral/associational, and perforant pathways could, in theory, be estimated by the same calculation used for the pyramidal cells given above. Arriving at such an estimate is, in practice, quite difficult. Very little is known about the anatomy and physiology of synapses made by mossy fibers, collateral axons, and perforant path axons onto the interneurons of area CA3. In part this is because CA3 is the home of a large number of types of interneurons, each of which may receive different kinds of input from these three main sources of synaptic input to area CA3. The consideration of inhibitory influence on pyramidal cells is further complicated in that it involves estimating the degree to which interneuron firing suppresses firing of principal cells. Because of these unresolved issues, at present any extension of our analysis to include interneurons is premature.

CONCLUSIONS

Recent data suggest that, collectively, the mossy fibers are a relatively a minor input pathway for CA3 pyramidal cells, inconsistent with the idea that this pathway functions as the main source of input to CA3 pyramidal neurons. The unique anatomical and physiological properties of mossy fiber synapses allow them to provide strong input, but only under specific conditions. We propose that in most cases, the mossy fiber synapse functions not as a simple detonator, but also as a discriminator, in at least three different ways. The synapse can function as a frequency discriminator, i.e., as a device for converting a frequency-modulated signal into an amplitude-modulated signal (higher granule cell firing rate yields larger synaptic current injected into the CA3 pyramidal cell). Second, the mossy fiber synapse can function as an amplitude discriminator: the largest mossy fiber EPSPs can produce a kind of detonator effect that others have proposed as the mossy fiber's normal mode of operation (McNaughton and Morris, 1987; Treves and Rolls, 1992). Third, mossy fibers can function as a temporal window discriminator, by selectively suppressing those nonmossy inputs that arrive during a brief interval following the arrival of mossy fiber inputs.

These unique physiological properties suggest that the mossy fiber synapse onto CA3 pyramidal cells plays a special role in the function of the hippocampus. When granule cells fire at rates >1 Hz, CA3 pyramidal cells will be driven strongly by their mossy fiber input. Under these conditions, the strength of mossy fiber input to CA3 pyramidal cells may be sufficiently strong to allow it to function in a manner similar to that required by various computational and conceptual models of hippocampal function operation (McNaughton and Morris, 1987; Treves and Rolls, 1992, 1994; O'Reilly and McClelland, 1994; Lisman, 1999; Kali and Dayan, 2000; Levy et al., 1998).

We would, however, argue that given the reported low rate of granule cell firing and the modest mean amplitude of mossy fiber EPSCs, conditions allowing for robust, consistent activation of CA3 pyramidal cells by mossy fibers in isolation are likely to be somewhat rare. During periods of low activity in the dentate, CA3 pyramidal neurons may be thought of as functioning in the absence of a significant mossy fiber input, which may allow the hippocampus to shift from performing pattern separation operations to performing pattern completion (O'Reilly and McClelland, 1994). Thus, an additional consequence of the properties of the mossy fiber synapse may be to shift the function of the CA3 network, depending on the frequency of granule cell activity.

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