H.E. Scharfman (Ed.)
Progress in Brain Research, Vol. 163
ISSN 0079-6123

CHAPTER 12

Hilar mossy cells: functional identification and activity in vivo

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Abstract: Network oscillations are proposed to provide the framework for the ongoing neural computations of the brain. Thus, an important aspect of understanding the functional roles of various cell classes in the brain is to understand the relationship of cellular activity to the ongoing oscillations. While many studies have characterized the firing properties of cells in the hippocampal network including granule cells, pyramidal cells and interneurons, information about the activity of dentate mossy cells in the intact brain is scant. Here we review the currently available information and describe biophysical properties and network-related firing patterns of mossy cells in vivo. These new observations will assist in the extracellular identification of this unique cell type and help elucidate their functional role in behaving animals.

Keywords: mossy cell; hilus; network patterns; sharp wave; gamma; slow oscillation

Introduction

The granule cell is the most numerous cell type of the dentate gyrus (1 million in one hemisphere in the rat, (Amaral et al., 1990), serves to integrate entorhinal input and sends its messages to three major target cell populations. These three cell groups include mossy cells (MCs) of the hilus, CA3 pyramidal cells and interneurons of both dentate and CA3 regions. Although the MCs are the least numerous cell type in the hilar region, their unique properties suggest that they are likely to be a very important cell type of the dentate region. This chapter examines the properties of the MCs and their possible role in overall hippocampal function.

Mossy cells are perhaps the most underinvestigated neurons in the hippocampal formation. Part of the scarcity of information can be explained by the low number of MCs (approximately 10,000 in the rat) (Amaral et al., 1990). Unlike other principal (excitatory) cell types of the hippocampus, MCs do not form recognizable layers with densely packed somata. Instead, they are scattered in the hilar region under the granule cell layer, making their in vivo accessibility for physiological studies difficult. Most of what we know about the functions of MCs comes from the pioneering in vitro studies of Scharfman (Scharfman and Schwartzkroin, 1988; Scharfman et al., 1990, 2001; Scharfman, 1991, 1992a, b, 1994a–c, 1995), in vivo studies of Schwartzkroin et al. (Buckmaster et al., 1992, 1993, 1996; Buckmaster and Schwartzkroin 1995; Wenzel et al., 1997) and pathoanatomical studies of epilepsy and ischemia by Sloviter et al. (Sloviter, 1989, 1991a, 1994; Sloviter et al., 1991,

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DOI: 10.1016/S0079-6123(07)63012-X

2003; Goodman et al., 1993; Zappone and Sloviter, 2004). Behavioral correlates of MCs are unknown due to the lack of criteria for the reliable identification of MCs with extracellular methods. A major goal of this chapter is to provide an overview of the available knowledge about the firing patterns and biophysical properties of anatomically identified MCs and their network-related behavior, and discuss how this information can facilitate studies on MCs in the intact, behaving animal.

Mossy cell anatomy

Mossy cells of the hilus were first recognized by Cajal (1911) and Lorente de No (1934) for their dendrites covered with large spines. These cells were later given the name "mossy cells" by Amaral (1978) due to the "mossy" appearance of their large spines (see Fig. 1A). It has also been demonstrated that all MCs selectively stain for GluR2/3 receptors as opposed to other cells of the hilus (Petralia and Wenthold, 1992; Leranth et al., 1996; Fujise and Kosaka, 1999). Interestingly, there appears to be differences in the peptide content of the mouse and hamster MC population, with ventral MCs containing calretinin (Murakawa and Kosaka, 2001) which is largely absent in MCs of the dorsal hilus. This is in contrast to the rat where no MCs contain calretinin. In addition, calcitonin gene related peptide has been reported to selectively label MCs in the rat (Freund et al., 1997). Because these peptide markers are conspicuously absent in the pyramidal cells of the hippocampus proper, their differential staining can be taken as clear justification of separating MCs of the dentate gyrus and pyramidal neurons of the Ammon's horn.

The MCs are usually multipolar and have tapering dendrites that largely remain restricted to the hilus proper, although occasional dendrites are observed in the molecular layer of the dentate gyrus in both rats (Scharfman, 1991), and more often in primates (Frotscher et al., 1991; Buckmaster and Amaral, 2001). These dendrites in the molecular layer can receive direct input from the entorhinal cortex bypassing the granule cells. This variability in direct EC input is likely to be important for physiological function. All CA3 pyramidal cells, including those with mostly horizontal dendrites residing in zone 3 of Amaral (1978), send at least one dendritic branch to the stratum lacunosum-moleculare and therefore

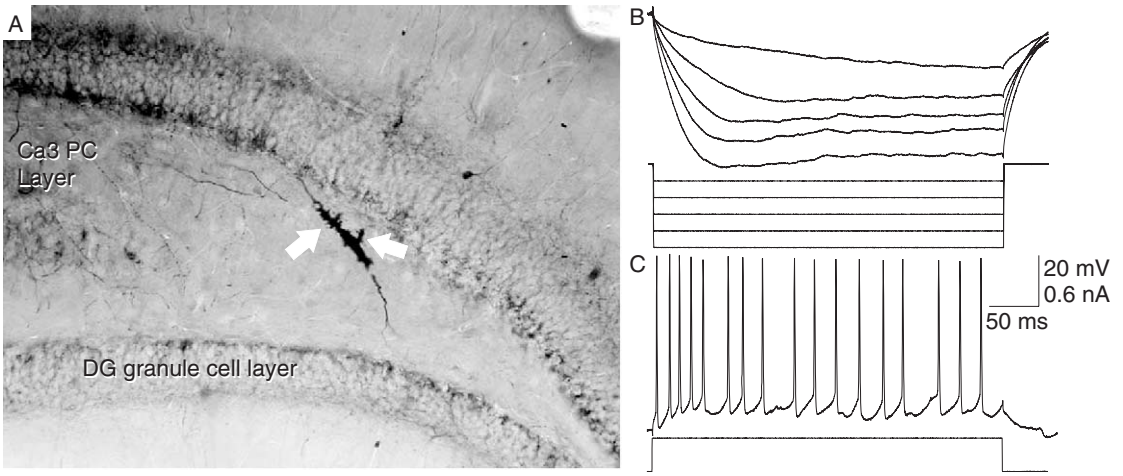

Fig. 1. Hilar mossy cell and associated basic properties. (A) Biocytin labeled mossy cell. Note the large spines covering the soma and proximal dendrites (arrows). Example current step evoked traces from the mossy cell shown in A. (B) Responses to a series of hyperpolarizing steps from a resting potential of ~57 mV. Note the robust "sag" observed for the larger hyperpolarizing steps and the long charging curve for the smallest hyperpolarizing step. For this cell, the in vivo input resistance was 52 MΩ. (C) The response of this cell to a strong depolarizing step (1.0 nA). Note that there is only very weak accommodation observed.

receive inputs from layer 2 neurons of the entorhinal cortex. In contrast, MCs without dendrites in the molecular layer generally receive only indirect information from the entorhinal input relayed by the granule cells (but see Kohler, 1985; Deller, 1998).

The proximal dendrites and somata of MCs are covered by large "thorny excrescences" (Fig. 1). While the thorny excrescences at first seem similar to those of the proximal apical dendrite of CA3 pyramidal cells, they are qualitatively different in that they resemble "clusters of spheres" (Amaral, 1978) where as CA3 excrescences appear more irregular and thorny in their shape (Chicurel and Harris, 1992). As in CA3 pyramidal cells, the thorny excrescences of MCs receive synaptic input from the mossy fibers of the granule cells (Amaral, 1978; Murakawa and Kosaka, 2001). Recent findings suggest that the mossy fiber input to CA3 is a mixed glutamatergic/GABAergic input for the first three weeks of development in the rat or the young guinea pig (Walker et al., 2001). Under normal conditions in the adult, there is no detectable GABAergic transmission. However, mossy fiber GABAergic transmission is restored in the adult after periods of strong repetitive stimulation or hyperexcitability such as epilepsy (see Gutierrez, 2003, for review). There are also significant GAD-positive, inhibitory inputs to the somatic region of MCs suggesting a strong perisomatic inhibitory input to these cells (Acsady et al., 2000; Murakawa and Kosaka, 2001). In fact, the perisomatic inhibition to MCs is very strong (15–40 times more synapses per soma) compared to the inhibitory cells of the hilus. The perisomatic inhibitory innervation of MCs is primarily from terminals that contain parvalbumin or cholecystokinin (CCK) (Acsady et al., 2000), similar to the pyramidal cells (Freund and Buzsáki, 1996). There is also a perisomatic and proximal dendritic input from cholinergic fibers (Deller et al., 1999) which may serve to provide excitatory tone to the MCs during oscillations such as theta. The more distal, or so-called peripheral dendrites, are innervated by a variety of inputs including more mossy fiber inputs onto regular small spines as well as other anatomically uncharacterized inputs making asymmetric and symmetric synapses (Frotscher et al., 1991). It is possible that at least some of the input to the more peripheral dendrites is from CA3 pyramidal cells which extend axons back into the hilus and granule cell layer (Li et al., 1994). However, direct anatomical evidence for a CA3-mossy cell communication is still lacking (but see below for discussion of functional data). The dendrites of MCs are extensive, extending several hundred micrometers in both medio-lateral and dorso-caudal directions, suggesting a largely cylindrical dendritic tree arborizing largely in the subgranular zone. The large span of the dendritic arbor suggests that MCs are innervated by spatially distributed granule cells. The 100:1 ratio of granule cells to MCs predicts that a typical MC receives inputs from as many as a hundred granule cells. Given the relative sparse distribution of mossy fiber terminals in the hilus, it is also likely that each granule cell innervates only one or two MCs with large mossy fiber boutons. This low divergence and convergence should be contrasted to the widespread reciprocal innervation of granule cells by the MCs (see below).

The MCs make glutamatergic (Soriano and Frotscher, 1994; Wenzel et al., 1997) asymmetric synapses with both excitatory and inhibitory postsynaptic targets (Frotscher et al., 1991; Buckmaster et al., 1996). In general, a single MC's synaptic targets can be divided into three classes: local hilar targets; longitudinal targets (located >1 mm either septally or temporally); and contralateral targets. These three target classes have been observed in both rodents and primates. Excitatory innervation of CA3 pyramidal cells by the MCs has been repeatedly suggested (Buckmaster et al., 1996; Buckmaster and Amaral, 2001) but conclusive anatomical evidence for this alleged connection is missing.

The main local hilar targets are mostly aspiny dendrites of interneurons and potentially dendrites of other MCs, although anatomical proof for mutual MC communication is lacking. CA3 recurrent collaterals also innervate aspiny interneurons in the hilus (Buckmaster et al., 1996) but conspicuously avoid spiny interneurons of which there are many in the hilus (Wittner et al., 2006). The major bulk of the axon cloud of MCs ($>90\%$ of ipsilateral synaptic contacts) target dentate

granule cell dendrites in the inner third of the molecular layer of the dentate gyrus rostral and caudal to the soma and dendrites of the parent MC (Buckmaster et al., 1992, 1996; Wenzel et al., 1997). This longitudinal arrangement is of major physiological consequence because it eliminates the possibility of a local granule cell–mossy cell–granule cell recurrent excitatory loop. Granule cells, which drive discharge of the MCs, do not receive excitatory information about the firing status of their targets. Instead, the output of the activated MC is distributed over the septal-temporal extent of the dentate. In these target areas, MCs innervate both granule cells and interneurons, giving rise to potentially interesting physiological scenarios. First, a granule cell discharging a target MC can silence competing granule cells over large territories via the feed-forward excitation of interneurons by the MC. However, if this were the sole function of such widespread axon arborization, there would be no need for MC excitatory innervation of granule cells. Through the latter numerically dominant excitatory pathway, the possibility exists that MCs can synchronize spatially distinct granule cells in the septotemporal axis, although the functional efficacy of the mossy cell–granule cell synapse is not well characterized (but see Scharfman, 1995). From the latter perspective, the main function of MCs would be to integrate functions of large numbers of active granules cells in the septotemporal axis of the hippocampus.

The contralateral cellular targets of MCs are largely undefined. However, in addition to innervating granule cells, at least hilar neuropeptide Y (NPY) containing cells may receive input from contralateral MCs (Deller and Leranth, 1990). It will be interesting to learn the extent and identity of the contralateral targets and how they compare to the predominant granule cell targets ipsilaterally.

Functional cellular connectivity of MCs

The anatomical connectivity described above provides a prospective framework for understanding the functional role of the MCs in normal hippocampal function. This can be summarized most simply as primarily providing distributed excitatory feedback to dentate granule cells and secondarily providing excitatory drive to local inhibitory interneurons of the hilus. The functional importance of the contralateral projection is harder to predict since the anatomical targets are not yet characterized. A body of work by Scharfman and colleagues (Scharfman and Schwartzkroin, 1988; Scharfman et al., 1990, 2001; Scharfman, 1991, 1992a, b, 1994a–c, 1995) has provided functional data to support the predictions of the anatomical connectivity to and from MCs. For example, MCs that have dendrites that extend into the DG molecular layer have a lower threshold to fire in response to perforant path stimulation (Scharfman, 1991). A challenging series of studies in ventral slices used paired recordings from anatomically confirmed hilar, dentate and CA3 pyramidal cells to investigate the functional connectivity in this region. Paired recording of granule cells or CA3 pyramidal cells with MCs showed that single action potentials in either GCs or PCs can evoke EPSPs in MCs (Scharfman, 1994b). Another paired recording study demonstrated that MCs monosynaptically excite both granule cells and inhibitory interneurons. In addition, evidence was observed for polysynaptic inhibition of GCs in response to MC activity (Scharfman, 1995).

The hypothesized physiological function of the low convergence and divergence of granule cells onto CA3 pyramidal cells is to disperse (“orthogonalize”) the entorhinal information onto the large recurrent system of CA3 neurons during the encoding of memories and provide multiple but sparse representation (Treves and Rolls, 1992, 1994). In the retrieval process, the auto-associative CA3 recurrent system, in turn, can recover the whole memory representation from partial or fragmental information (“pattern completion”) (McNaughton and Morris, 1987; Kanerva, 1988). Given this model of memory encoding and retrieval, we can ask what role might MCs play in this system? Although the anatomical connectivity between granule cells and MCs is similar to the granule cell–CA3 connections, given the small number of MCs and the lack of their reciprocal

excitation with one another makes it unlikely that MCs are part of the pattern completion mechanism. Instead, they may assist in increasing the sparseness of the memory representation in the recurrent CA3 system by the hypothesized feed forward suppression of granule cells in the septotemporal axis or by allowing a sparse but coordinated transfer of information from different segments of the entorhinal cortex onto the CA3 system. This may be an important combinatorial mechanism because the metric of spatial representation increases in the dorso-caudal axis of the entorhinal cortex with corresponding projections to different segments of the septotemporal axis of the hippocampus (Hafting et al., 2005). The synchronizing mechanism of granule cells by the MCs in the longitudinal axis would secure the simultaneous but distributed representation of the different sampling metric of the entorhinal cortex in the associative CA3 network. This hypothesis differs from an alternative proposed by Buckmaster and Schwartzkroin (1994) dubbed the "granule cell association" hypothesis. In that hypothesis, the MCs provide the necessary links to form associative connections within the dentate network analogous to the associational collaterals of pyramidal cells in the CA3 region.

Cellular properties of MCs

The basic cellular properties of MCs are quite distinctive from other cell types in the hilar region. The MCs tend to have higher input resistance and strong inward rectification in response to hyperpolarization. Figure 1B shows a typical MC from a set of 10 cells we have recorded in vivo in response to a series of hyperpolarizing steps. Each anatomically verified MC recorded in urethane anesthetized rats had action potentials that crossed 0 mV, a mean resting membrane potential of -58 ± 1.8 mV, and a mean input resistance of 61 ± 8.6 MΩ (means ± SEM). The resting potential is similar to that of CA3 (−63 mV; $n = 84$) and CA1 (−65 mV; $n = 280$) pyramidal cells but different from the more hyperpolarized granule cells (−74 mV; $n = 41$). Of the principal cell types, MCs had the largest input resistance (CA3 = 53.8 mΩ; $n = 8$; CA1 = 48.4 MΩ (Henze and Buzsaki, 2001)). The smallest hyperpolarizing step applied (−0.2 nA) resulted in a hyperpolarization of ~22 mV and the time constant is best fit with a double exponential with $tau_1 = 7.5$ ms and $tau_2 = 250$ ms. A strong inward rectification can be seen with a step injection of −0.4 nA that results ultimately in a maximum hyperpolarization of 35 mV. The hyperpolarizations of the membrane potential by small step currents were best fit by a double exponential in seven of the nine cells where it could be measured; the mean time constant values were 15.9 and 188.2 ms. The remaining two MCs were best fit with single exponentials with time-constants of 32 and 26 ms. Figure 1C shows that the MCs typically do not show burst firing in response to depolarizing steps (1.0 nA) and only show weak accommodation for the duration of the step depolarization. This behavior can be contrasted to the typical burst pattern and strong spike accommodation in response to current steps in CA3 pyramidal cells (personal observations, Bilkey and Schwartzkroin, 1990; Buckmaster et al., 1993; Scharfman, 1993b).

The background synaptic activity in MCs has been reported to be quite high, both in vivo and in vitro (Strowbridge et al., 1992; Scharfman, 1993a).We also have observed high background activity that included some very large events (>10 mV; Fig. 2). It is likely that these giant PSPs with fast rise times arise from the mossy fiber synaptic inputs to the MCs reflecting either synchronous multivesicular release from a complex MF bouton or perhaps the release of large individual quanta as has been reported in CA3 pyramidal cells (e.g. see Henze et al., 2002). Although the magnitude of the giant PSPs is quite variable, it is unlikely that it reflects varying convergence of activity from multiple granule cells. First, the convergence of granule cells onto MCs is low (~100). Second, the density of mossy boutons in the hilus is quite low. Third, intracellularly labeled neighboring granule cells never showed spatially clustered boutons that would otherwise suggest common targets (Acsady et al., 2000).

One feature of MC activity that we have observed that has not been previously described

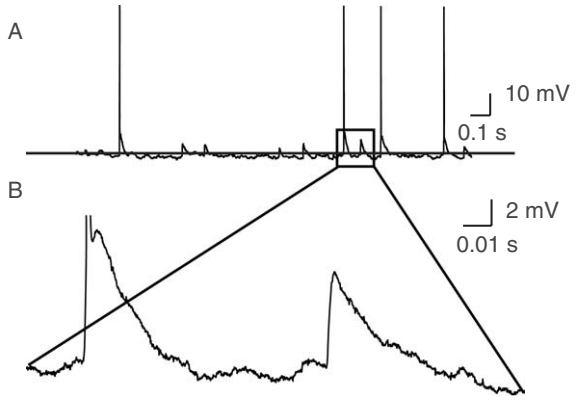

Fig. 2. (A) Intracellular recording of a hilar mossy cell resting at −55 mV (solid line). Notice the large spontaneous postsynaptic potentials that occur from the baseline that sometimes lead to action potentials. (B) Higher resolution depiction of two large EPSPs from the box in (A).

is their relatively high background firing rate (e.g. see Figs. 3, 5, and 9C). Our findings under urethane anesthesia are partly consistent with Scharfman (1993a), who showed that there is often a high rate of firing but it waxes and wanes in vitro. However, it is not consistent with other studies in vivo (Soltesz et al., 1993) under ketamine/xylazine where rates of less than 1 Hz were reported. The source of this high firing rate is not well understood because granule cells under urethane anesthesia are typically hyperpolarized and fire at a low rate. One hypothesis is that spontaneous release from MF boutons can lead to MC action potentials (Henze et al., 2002). If the high firing pattern is confirmed by investigations in the freely behaving animal, it can be contrasted with the more clustered firing patterns of CA3 pyramidal cells. Figure 3 illustrates the autocorrelograms calculated from our MC recordings. These autocorrelograms should facilitate the comparison of the spike dynamics of MCs under anesthesia and in the drug-free animal in future studies.

Another essential piece of information in the process of physiological identification of MCs is the extracellular features and shape of the extracellular action potential. To this end, we have recorded from a single MC using simultaneous intracellular and extracellular electrodes (Fig. 4). The waveform we have observed is somewhat unusual in that the extracellular unit waveform has a rounded trough that has not been observed in other hippocampal unit waveforms. It is possible that the rounded (wide) pattern derives from the summed extracellular currents from the soma and the thick dendrites of MCs. In support of this interpretation, the extracellular spikes were recorded by three shanks of the silicon probe, a total distance of 300 μm. We would suggest that the horizontally wide current distribution of MC spikes may be used as a distinguishing feature in extracellular recordings from the granule cells and other nearby smaller size interneurons.

MC cellular activity in a network

Although there is a relative lack of information about the physiological role of MCs under normal conditions, their role has been a frequently discussed topic of debate in the epilepsy literature. This is because MCs are often observed to be reduced in post-mortem tissue taken from people who have had temporal lobe epilepsy (TLE) (Sloviter et al., 1991). Sloviter and colleagues proposed the so-called ''dormant basket cell'' hypothesis of epilepsy (Sloviter, 1991b). The dormant basket cell hypothesis holds that the importance of the excitatory tone provided by the MCs is to provide excitation of hilar inhibitory interneurons which in turn then provide strong inhibition of granule cells. When MCs are lost due to neurodegeneration associated with TLE, the inhibitory basket cells lose their tonic excitatory drive resulting in a net disinhibition of dentate gyrus granule cells (Sloviter, 1994; Sloviter et al., 2003). A contrasting hypothesis has been called the ''irritable mossy cell'' hypothesis as proposed by Soltesz and colleagues. In this view, it is not the loss of MCs that leads to a net excitation of granule cells, instead it is the remaining MCs that have higher firing rates and provide uncontrolled excitatory feedback to the dentate gyrus granule cells thus exacerbating the epileptic process (Santhakumar et al., 2000; Ratzliff et al., 2002).

Both of the ''dormant basket cell'' and ''irritable mossy cell'' hypotheses have their attractions. Recent studies by both camps have provided

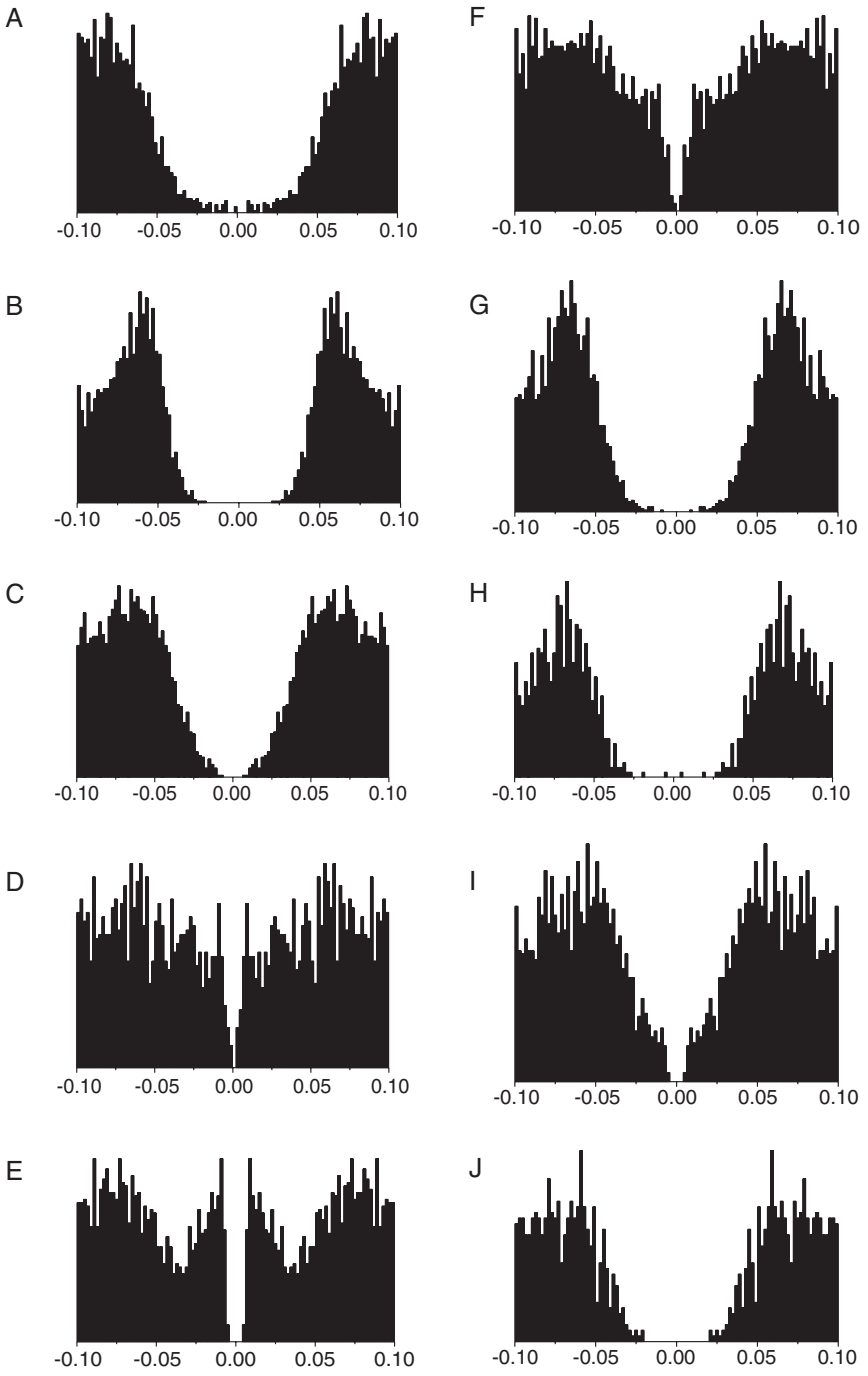

Fig. 3. Hilar mossy cells show a variety of firing patterns as assessed by autocorrellograms (A–J). The autocorrelogram for each of the 10 MCs in our dataset was calculated from spontaneous spiking from the resting potential (no injected current). The majority of the cells show a pattern that is more reminiscent of that seen for repetitively firing interneurons than the more bursty firing of CA1 or CA3 pyramidal cells (e.g. Csicsvari et al., 1998).

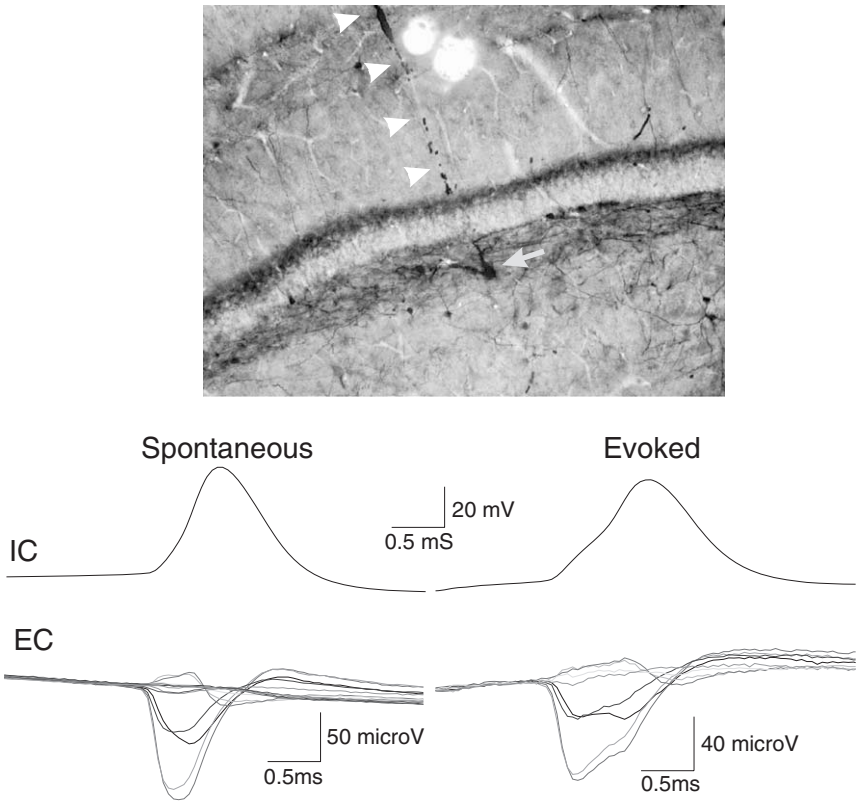

Fig. 4. Extracellular waveforms of mossy cells during spontaneously and evoked spiking. A recording was obtained where the intracellularly recorded mossy cell was also observed on the extracellular electrode. The extended shape of the mossy cell dendrites allowed the extracellular signal to be observed on three shanks of the extracellular silicon probe (150 μm between shanks). The extracellular electrode track is indicated by the white arrows. The soma of the mossy cell is indicated by the yellow arrow. There was a difference in the shape of the action potentials, both intracellular and extracellular, suggesting differences in the site of spike initiation for spontaneous and evoked spikes. (See Color Plate 12.4 in Color Plate Section.)

evidence to support the respective theories. Three days after kainic acid induced status epilepticus, there is reduction in inhibition and thus increase in excitability of the dentate gyrus that correlates with the degree of MC loss (Zappone and Sloviter, 2004). However, Ratzliff et al. (2004) showed that in the hippocampal slice, if they acutely removed MCs via manual destruction, there was a net reduction in the excitability of the dentate gyrus presumably due to the loss of direct excitatory input from MCs. In all likelihood, both hypotheses are at least partially correct and strict interpretation of these studies is confounded by the technical challenges of studying the MCs in vivo under non-pathological conditions. The intrinsic firing rates of MCs in the intact unanesthetized animal is not known, and both low (Soltesz et al., 1993) and high firing rates (Figs. 3, 5, and 9C) have been observed under anesthesia. Bulk stimulation of fiber pathways such as the perforant path input to the dentate gyrus is inherently not physiological in that the precise synchrony of synaptic input that results very rarely, if ever, happens in natural processing. As such, the ratio of synchronously activated excitatory to inhibitory inputs may be very different than that observed during normal ongoing hippocampal function.

Although the hilus also contains a large variety of interneurons (Amaral, 1978; Mizumori et al., 1990; Halasy and Somogyi, 1993; Buhl et al., 1994; Sik et al., 1997), the contribution of these interneurons to the rich variety of dentate area network

patterns is not known. Our overriding hypothesis is that the main goal of both MCs and hilar interneurons is to control network patterns during various behaviors. This rationale has been successfully applied to characterize CA1 interneurons (Csicsvari et al., 1999; Klausberger et al., 2003, 2004, 2005), where the initial network pattern-based classification (theta phase and sharp waves) in freely behaving rats was followed by juxtacellular labeling of similarly characterized interneurons under anesthesia. We suggest that a similar approach in the dentate gyrus can be equally fruitful. Here we have preliminary yet more advanced knowledge of the network contribution of MCs under anesthesia than in the behaving animals. In the dentate area, several distinct oscillatory patterns are present.

Network oscillations are known to involve a rhythmic pattern of periods of active inhibition that are counterbalanced by periods of permissive excitability (e.g. theta in CA1) (Buzsaki, 2002). Perhaps the unique role of the MCs is to provide active excitation to granule cells in the periods between the rhythmic inhibitory inputs driven by interneurons. Although all the various patterns are mediated by a limited set of excitatory pathways, it is expected that MCs and the various interneuron groups may differentially participate in these network patterns because the dynamics of activation can differentially affect neurons with different properties. The following patterns can be used to characterize the network contribution of MCs and contrast them to granule cells, CA3 pyramidal cells and hilar area interneuron types.

1. In the exploring rat and during REM sleep, large amplitude *theta oscillations* are present in the dentate gyrus. Theta waves in the dentate region are coherent with but phase-shifted by approximately 270° relative to the theta oscillation in the CA1 pyramidal layer (Buzsaki et al., 1983).
2. Concurrent with theta, another prominent pattern in the dentate area is the *gamma frequency oscillation* (40–100 Hz). The power of gamma oscillations is strongly phase modulated by the slower theta rhythm and both theta and gamma oscillations in the dentate gyrus depend mainly on inputs from the entorhinal cortex (Bragin et al., 1995a; Penttonen et al., 1998). In addition to the classical gamma frequency, slower (12–40 Hz; beta) oscillations are also observed, often with a larger amplitude in the hilus. It is not clear whether the slow oscillation is simply a slower version of gamma or comprises a physiologically distinct rhythm.
3. The largest amplitude dentate gyrus event is the "*dentate spike*". This is a short duration (<60 ms), large amplitude (>0.5–2.5 mV) field potential characterized by synchronous discharge of granule cells and interneurons and suppression of CA3 pyramidal cells (Bragin et al., 1995b; Penttonen et al., 1997). Two types of dentate spikes have been distinguished. The first type is a short burst of gamma oscillation consisting of 2–5 waves, one of which of excessively high amplitude with large sinks in the outer third of the dentate molecular layer. The second type, observed in a subset of animals, has a somewhat different voltage vs. depth profile with a large sink located in the middle third of the dentate molecular layer. Our unpublished observations suggest that type 2 dentate spikes occur when thalamocortical high voltage spindles (Buzsaki et al., 1988) invade the dentate area.
4. Neocortical *slow oscillations* (Steriade et al., 1990) also exert an impact on the firing patterns of the hippocampus, likely by way of the entorhinal cortex (Isomura et al., 2006; Wolansky et al., 2006). During the UP state of slow oscillations, gamma power in the dentate gyrus and spiking of neurons increases dramatically. In contrast, dentate gamma activity decreases during the DOWN state (corresponding to delta waves of deep sleep in the neocortex) but CA3 pyramidal cells may increase their firing rates and generate gamma oscillations (Isomura et al., 2006)
5. *Sharp waves-ripple complexes* (SPW) are truly self-organized endogenous hippocampal events that occur during slow-wave sleep, immobility and consummatory behaviors

(Buzsaki et al., 1983). They arise in the CA3 recurrent system and can spread to the CA1 region and the dentate region. The transient excitation of CA1 neurons gives rise to a short-lived fast oscillation ("ripple") (O'Keefe and Nadel, 1978; Buzsáki et al., 1992; Ylinen et al., 1995). No ripples are associated with SPW in the dentate area but putative interneurons and, occasionally, granule cells are depolarized and discharge in synchrony with CA1 ripples, although the typical response is hyperpolarization (Penttonen et al., 1997).

We submit that these five unique population patterns can be used in future studies to functionally classify dentate region neurons in the freely behaving animal. In turn, these same patterns can be used in anesthetized rats where they can be labeled by intracellular or juxtacellular methods. The network-based classification should be combined with the spike dynamics and wave-shape features of extracellular spikes. Fortunately, the repertoire of rat hippocampal network activity patterns under urethane anesthesia largely reflects what is observed in the drug-free rat. Using this approach we have been able to observe how MCs behave in relation to some of these naturally occurring network patterns.

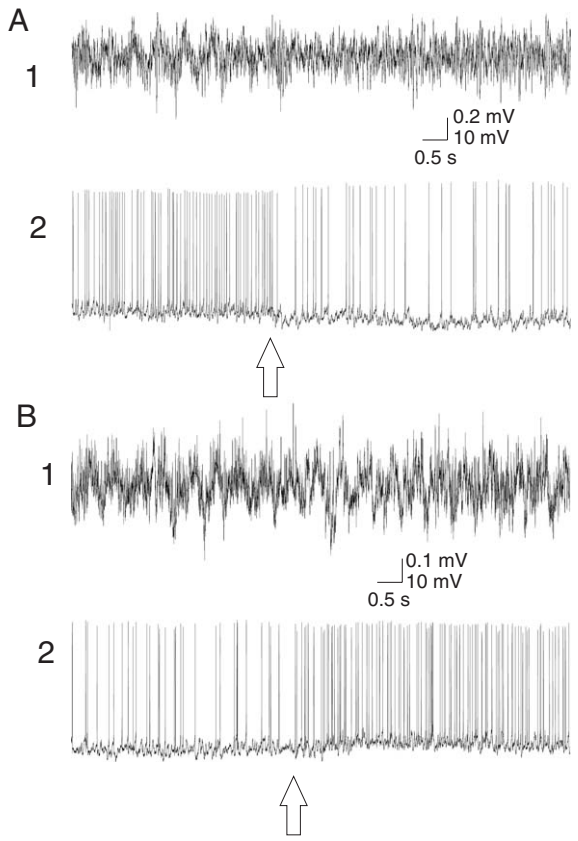

Fig. 5. (A) Example of mossy cell inhibition by transition to theta rhythm evoked by tail pinch in urethane anesthetized rat. Extracellular recording (A1) from area CA1 recorded simultaneously with a hilar mossy cell (A2). A tail pinch was applied at the arrow and the mossy cell responded by slowing its firing rate. (B) Example of mossy cell excitation by transition to theta rhythm evoked by tail pinch in urethane anesthetized rat. Extracellular recording (B1) from area CA3 recorded simultaneously with (B2) a hilar mossy cell that was excited by tail pinch (arrow) evoked theta.

Theta

It has been previously reported that MC membrane potential shows rhythmic oscillations in the theta band that are phase-locked to the extracellular theta oscillation in the contralateral hippocampus (Soltesz et al., 1993). However, as noted above, this study reported a very low (<1 Hz) basal firing rate for the MCs. Nevertheless, our observations support the involvement of MCs in theta oscillations. Individual MCs can either be depolarized (8 of 10) or hyperpolarized (2 of 10) by a transition from slow wave sleep to theta evoked by a tail pinch (Fig. 5). This behavior is similar to pyramidal cells (Kamondi et al., 1998). In addition, the membrane potential of MCs shows a co-variation with the extracellular field, with peak depolarization and discharge slightly after the peak of the locally derived theta oscillation (Fig. 6) and coherent with the discharge of some interneuron types. We predict that MCs in the behaving rat will keep a similar relationship to local hilar/CA3c theta oscillations.

Beta/gamma oscillations

The power of gamma frequency oscillation in the hilus is phase modulated by the slower theta (Bragin et al., 1995a). This gamma frequency

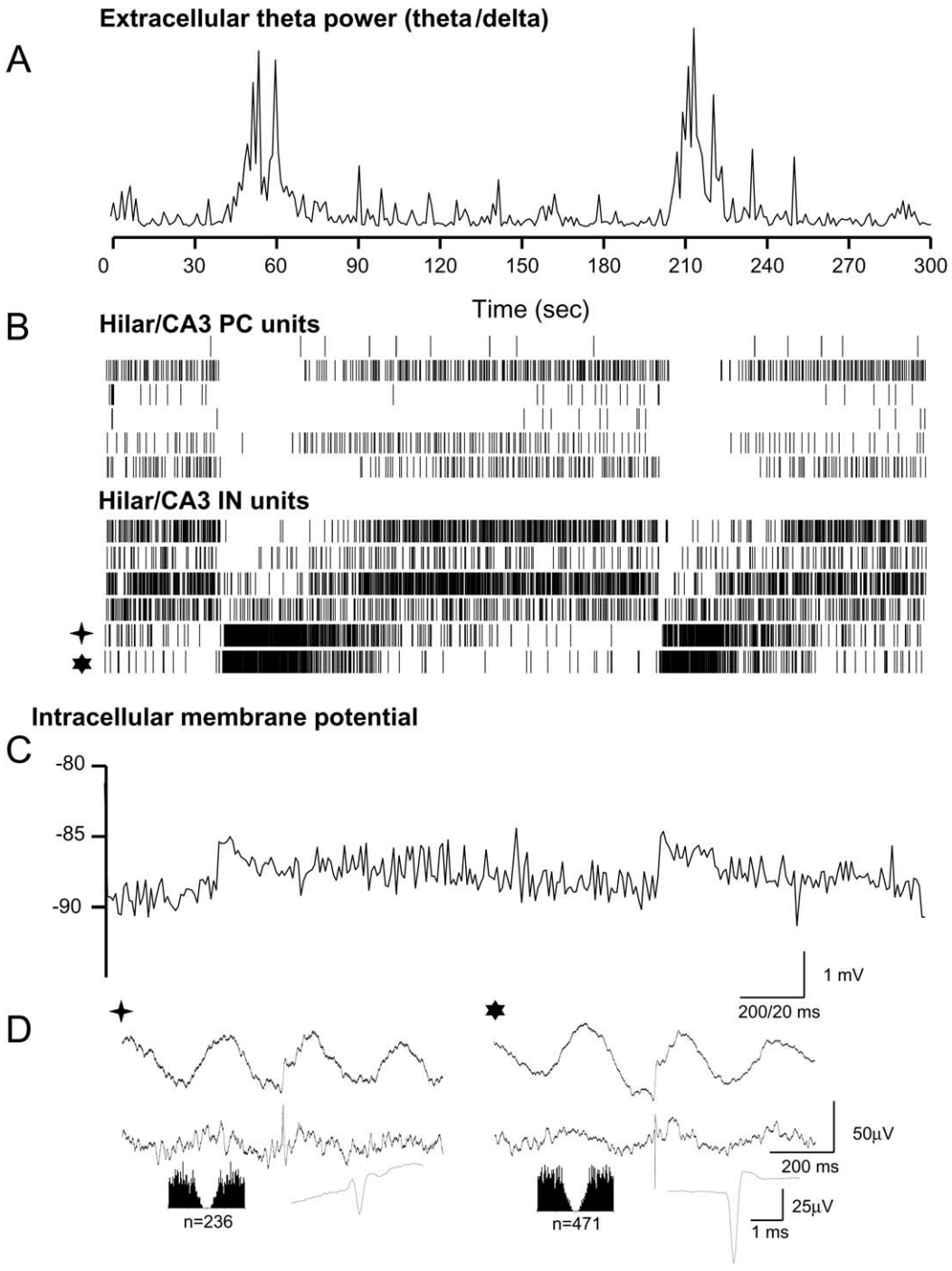

Fig. 6. (A) Continuous theta/delta power ratio from an extracellular electrode located in the hilus. The increases in theta power at ~45 and 200 s was induced via tail pinch. (B) rastergrams of isolated units recorded from three extracellular tetrodes in the hilus/area CA3c. (C) Intracellular membrane potential recorded from a mossy cell during this same recording epoch while passing hyperpolarizing current to maintain the resting potential near −80 mV. Notice that during the periods of theta power increase, the mossy cell experiences a depolarization from the −80 mV holding potential. (D) Two examples of average mossy cell membrane potential time aligned to the two ''theta on'' putative interneuronal units indicated by the start symbols in (C). The upper trace is the average membrane potential of the MC. The lower trace is the average wide-band extracellular trace. The autocorrelogram and waveform for the isolated IN units are also shown as insets.

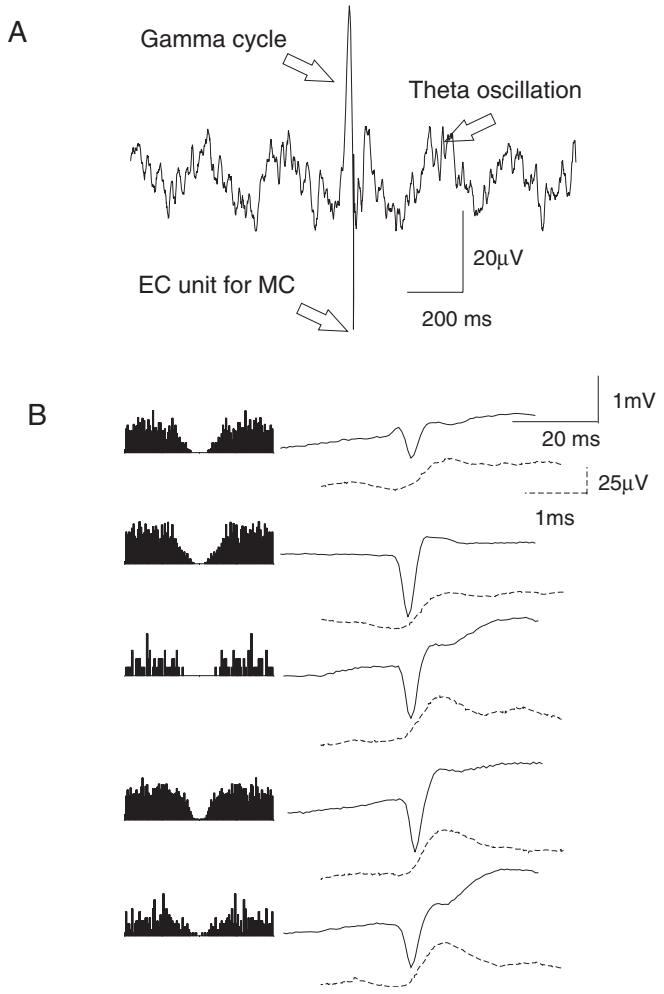

Fig. 7. (A) Average of extracellular hilar field potential aligned to the peaks of the intracellular MC spontaneous action potentials recorded over a 200 sec recording period. This is the same case as in Fig. 4 where the extracellular electrode picked up the same MC as was recorded intracellularly. The extracellular unit correlate of the intracellular AP is indicated. Notice that the MC fires on the end of the gamma cycle waveform and there is an overall theta oscillation present that is time aligned to the intracellular spike. (B) Red traces: MC membrane potential averaged and time aligned on the spike times of isolated interneurons recorded in the hilus/CA3c. Left column: IN unit autocorrelogram, black traces: average IN unit waveforms. Note similar phase relationship of the interneuron-timed intracellular gamma frequency oscillation.

modulation is also evident in the relationship between the local field and fast spiking interneurons and their effect on the MC (Fig. 7). When the action potentials of MCs are used as reference for averaging local field potentials, phaselocking of MC spikes to the gamma oscillation, superimposed on the peak of theta waves becomes evident (Fig. 7A). Furthermore, when nearby fast-spiking putative interneurons are used as the reference, membrane oscillation of MCs in the gamma frequency band becomes evident (Fig. 7B). Similar phase-locked behavior has been also observed in the beta oscillation frequency range as well.

Slow oscillations

Slow oscillations arise in neocortical networks (Steriade et al., 1993a–c) and spread to the

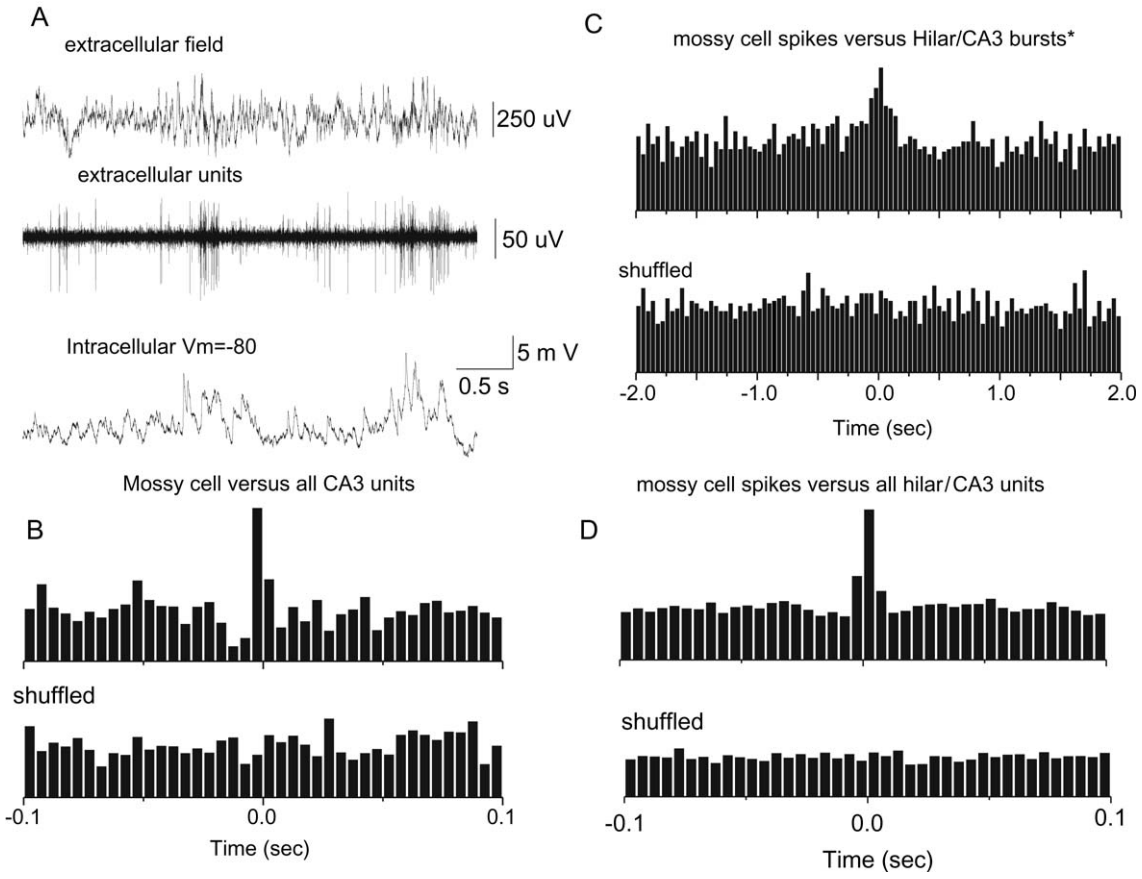

Fig. 8. (A) CA3 unit activity and MC membrane potential fluctuations. Mossy cells show synaptic activity correlated with unit activity in CA3 (2/2 cells recorded with extracellular electrode placed in CA3c. Upper traces show extracellular wideband recording middle trace bandpass filtered between 800 Hz and 3 kHz. Lower traces show intracellular membrane potential. (B) Cross-correlation between spontaneous MC spikes recorded intracellularly and all extracellular units combined. Lower panels are shuffled controls. (C) Hilar/CA3c population bursts correlate with spontaneous mossy cell spikes. Hilar/CA3c bursts were defined as five unit spikes with less than 20 ms inter-spike interval. The spikes could originate from any unit. Shuffled correlograms are shown for controls. (D) Cross-correlation between spontaneous mossy cell spikes and all hilar/CA3 units combined for a second extracellular/intracellular recording pair.

entorhinal cortex and subiculum from where they can invade the dentate gyrus as well (Isomura et al., 2006; Wolansky et al., 2006). At the single cell level, most cortical neurons show a bimodal distribution of the membrane potential and these UP and DOWN states alternate relatively rhythmically at 0.5–2 Hz. Slow oscillations are most prominent under anesthesia but are also present in deep stages of slow wave sleep (Achermann and Borbely, 1997), where the transient delta waves correspond to the DOWN (or silent) state. At the transition of the entorhinal DOWN–UP state, the surge of excitation induces a strong discharge of granule cells and also gamma frequency oscillations (Isomura et al., 2006). Often one of these gamma waves becomes excessively large and has been referred to as the dentate spike (Bragin et al., 1995b). In this case, the MC firing is likely timed by feed forward excitation from dentate gyrus granule cells that are driven by the entorhinal input. The surge of activity in the input from entorhinal cortex is also reflected by the sudden

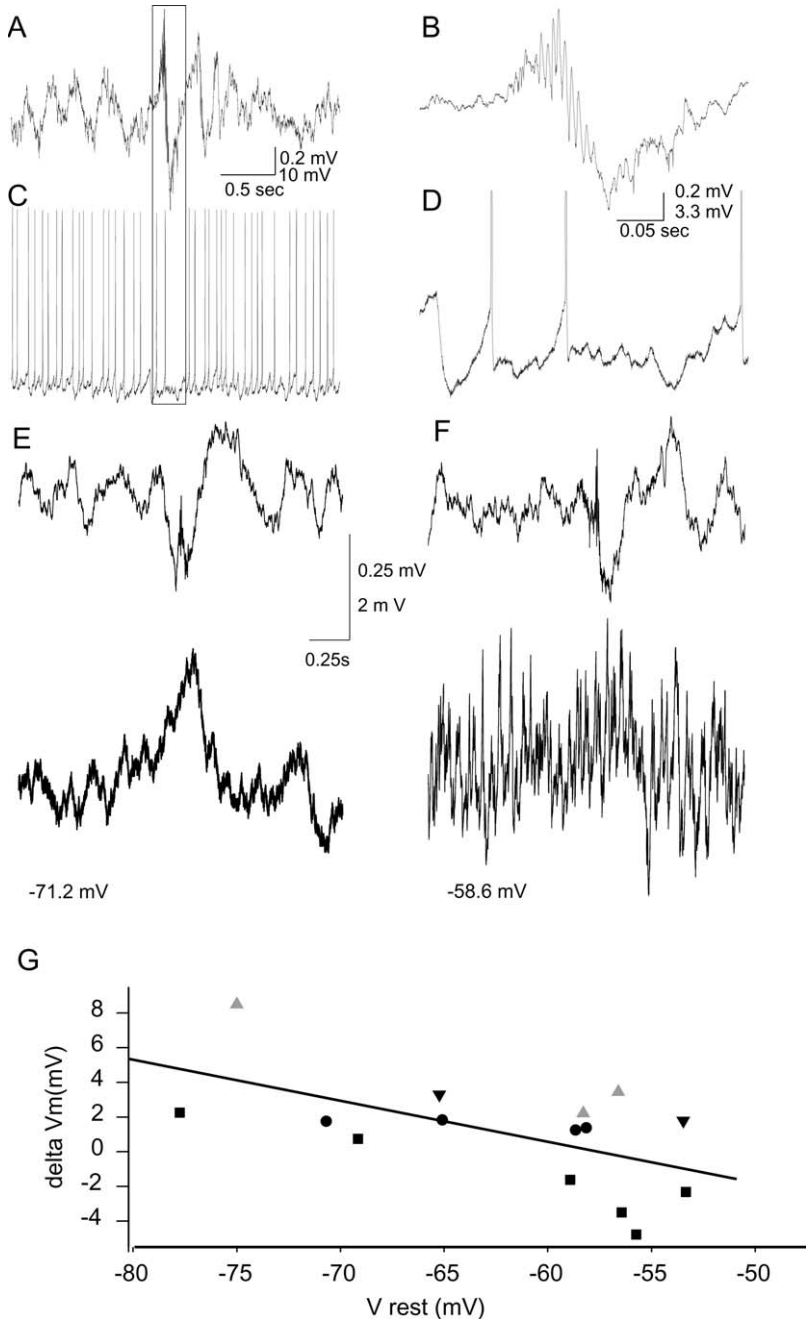

Fig. 9. CA1 sharpwave/ripples are associated with inhibition of mossy cells. (A) Example of a CA1 sharpwave/ripple complex recorded extracellularly in the pyramidal layer of CA1. (B) Intracellular spiking activity of mossy cell is suppressed during SPW. (C, D) Temporal expansion of region in box in A and B. Mossy cell membrane response during SPW/ripples has a reversal near -60 mV. (E, F) Average extracellular SPW and mossy cell membrane potential from a hyperpolarized baseline (E; $n = 12$ SPWs) and a more depolarized baseline (F; $n = 11$ SPWs). (G) Plot of the peak change in mossy cell membrane potential during CA1 SPWs from 15 recording epochs at different membrane potentials from four mossy cells (different symbols). The best fit linear regression line is shown ($R = -0.57621$, $P<0.025$).

increase of population firing in the dentate. We exploited the high levels of CA3 activity that occur during the slow oscillatory pattern for the examination of the behavior of MCs. The transition from DOWN to UP state was defined when a group of dentate/hilar/CA3 neurons fired a burst of activity. This analysis revealed that MCs become periodically active and silent according to the level of activity in the entorhinal–hippocampal network (Fig. 8).

CA1 sharp waves

Although SPW arises in the CA3–CA1 regions, the recurrent axon collaterals of CA3 can directly excite hilar interneurons and even granule cells (Li et al., 1994; Penttonen et al., 1997). Typically, feed-forward inhibition prevails by this mechanism as reflected by the SPW-locked hyperpolarization of granule cells. However, this occasional failure of inhibition and/or a concerted activation of a single granule cell by the recurrent CA3 axons can robustly discharge granule cells during SPWs (Penttonen et al., 1997). Our observations, to date, suggest that SPWs are associated with net inhibition of MCs. Figure 9A–D show the temporal relationship between a spontaneous SPW recorded in area CA1 (Fig. 9A, B) while recording from a MC in the hilus (Fig. 9C, D). As can be appreciated from this example, the ongoing spontaneous firing of the MC is inhibited in the period overlapping with and following the SPW/ripple complex. This inhibitory effect is probably mediated via activation of chloride flux through $GABA_A$ receptors since the change in MC membrane potential associated with a CA1 SPW has a reversal potential near −60 mV similar to $GABA_A$ reversal potentials in vivo (Fig. 9E–G). The potential source of this inhibition is the increased SPW-related firing of hilar interneurons driven either directly by the recurrent CA3 collaterals or the rarely discharging granule cells (Penttonen et al., 1997). It appears that, at least under anesthesia, the strong inhibition can prevent MCs from discharging in response to their granule cell inputs during SPWs. However, the situation might be quite different in the drug-free animal and it is expected that future studies will clarify whether MCs can become active participants in SPW events. It is worth noting here that in slices treated with a $GABA_A$ receptor antagonist, or slices from an epileptic rat, CA3 and MCs are engaged in population bursts while granule cells remain hyperpolarized, suggesting that CA3 pyramidal cells may directly excite MCs (Scharfman, 1994a; Scharfman et al., 2001). The failure of MCs to discharge during SPWs in the intact brain would imply that the hypothesized SPW-mediated consolidation of synaptic circuits in the CA3–CA1 networks (Buzsáki, 1989) can proceed independent of the modification of the synapses established by the MCs.

Conclusion

Although MCs are well-known and critical components of the dentate circuitry, their physiological function and exact involvement in various hyperexcitable phenomena has remained elusive. A major technical problem is the lack of reliable physiological criteria that may be used in extracellular recordings in freely behaving animal for the positive identification of MCs. Our intracellular characterization of some of their biophysical features and network-related behavior are the first steps in this direction.

Abbreviations

CCK	cholecystokinin
EPSP	excitatory postsynaptic potential
GABA	gamma-aminobutyric acid
GAD	glutamic acid decarboxylase
MC	mossy cell
NPY	neuropeptide Y

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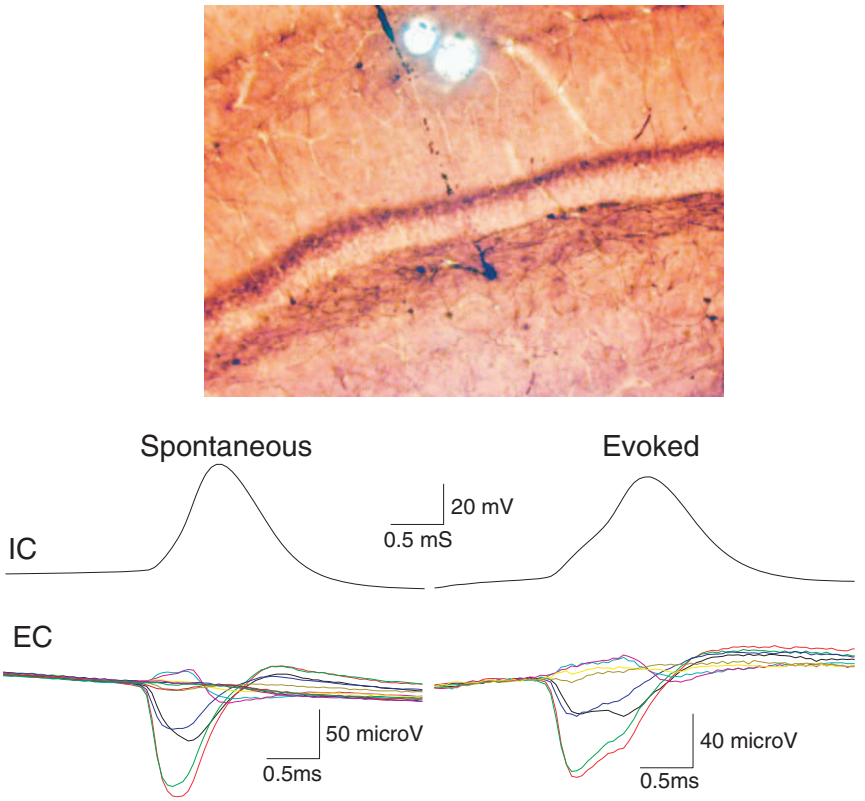

Plate 12.4. Extracellular waveforms of mossy cells during spontaneously and evoked spiking. A recording was obtained where the intracellularly recorded mossy cell was also observed on the extracellular electrode. The extended shape of the mossy cell dendrites allowed the extracellular signal to be observed on three shanks of the extracellular silicon probe (150 μm between shanks). The extracellular electrode track is indicated by the white arrows. The soma of the mossy cell is indicated by the yellow arrow. There was a difference in the shape of the action potentials, both intracellular and extracellular, suggesting differences in the site of spike initiation for spontaneous and evoked spikes. (For B/W version, see page 206 in the volume.)