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Advances in understanding hilar mossy cells of the dentate gyrus

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Abstract

Hilar mossy cells (MCs) of the dentate gyrus (DG) distinguish the DG from other hippocampal subfields (CA1–3) because there are two glutamatergic cell types in the DG rather than one. Thus, in the DG, the main cell types include glutamatergic granule cells (GCs) and MCs, whereas in CA1–3, the only glutamatergic cell type is the pyramidal cell. In contrast to GCs, MCs are different in morphology, intrinsic electrophysiological properties, afferent input and axonal projections, so their function is likely to be very different from GCs. Why are MCs necessary to the DG? In past studies, the answer has been unclear because MCs not only excite GCs directly but also inhibit them disynaptically, by exciting GABAergic neurons that project to GCs. Results of new studies are discussed that shed light on this issue. These studies take advantage of recently available transgenic mice with Cre recombinase expression mostly in MCs and techniques such as optogenetics and DREADDs (designer receptors exclusively activated by designer drugs). The recent studies also address in vivo behavioral functions of MCs. Some of the results support past hypotheses whereas others suggest new conceptualizations of how the MCs contribute to DG circuitry and function. While substantial progress has been made, additional research is still needed to clarify the characteristics and functions of these unique cells.

Keywords

Hippocampus; Granule cell; Interneuron; CA3; Entorhinal cortex; Memory; Pattern separation

Introduction

The dentate gyrus (DG) plays a critical role in the hippocampus, as demonstrated by deficits in behavior following experimental lesions or other impairments to the DG (Kesner 2007, 2017). Although hilar cells are not studied specifically in these experiments, there is a large body of literature about the hilus that provides compelling evidence that hilar neurons play a

critical role in the normal DG and in disease (Henze and Buzsaki 2007; Sloviter et al. 2012; Jinde et al. 2013; Scharfman and Myers 2013; Scharfman 2016).

Within the hilus is a glutamatergic cell called the mossy cell (MC) that is fairly common, comprising more than 30% of hilar cells in rodents (Buckmaster and Jongen-Relo 1999; Jiao and Nadler 2007; Morgan et al. 2007). Here, we discuss the characteristics and fundamental properties of MCs and what is known about their function in the local circuitry of the DG. In addition, the contribution of MCs to behavior is addressed.

The original descriptions of MCs

MCs were first discussed in the 1970–1980s as ‘commissurally projecting’ hilar cells, based on the evidence that the primary cell type projecting to the contralateral DG in the rodent was a hilar cell (Zimmer 1971; Gottlieb and Cowan 1973; Hjorth-Simonsen and Laurberg 1977; Fricke and Cowan 1978; Berger et al. 1981; Laurberg and Sorensen 1981). Moreover, this hilar projection seemed very important in these early studies because the projecting axon terminals comprised the vast majority of fibers in the inner molecular layer (IML), covering the most proximal third of the GC dendritic tree in the molecular layer of the DG. The high specificity of the terminals of these commissurally-projecting cells complement the entorhinal cortex (EC) perforant path (PP) fibers, which innervate the outer 2/3 of the molecular layer. Most of these hilar cells had large somata, suggesting that the commissurally-projecting cells were unlike GCs and interneurons. Therefore, the GC dendrites were thought to receive commissural input proximally and EC input distally.

The commissural projection to the IML became the “associational/commissural pathway” when the hilar cells of origin were found to not only project contralaterally but also ipsilaterally. Thus, hilar commissurally-projecting neurons gave rise to axons that seemed to cover the ipsilateral and contralateral septotemporal axis of the DG. In 1978, the results of an investigation of the hilus of the rat using Golgi staining was published, identifying a cell type with a large soma that had dense and complex spines (Amaral 1978). Because of the large somata, this cell type became a candidate for the associational/commissurally-projecting cells. At low power, the cells appeared to be covered in moss, leading to the term ‘mossy cell’. At high power, the ‘moss’ corresponded to large thorny excrescences, like those in area CA3.

At about this time, physiological studies of electrical stimulation of the commissure were providing insight into the function of commissurally-projecting cells. These experiments typically used a stimulating electrode in the commissure and perforant path, using the perforant path electrode to evoke a GC population spike and the electrode in the commissure to trigger a stimulus just before the PP stimulus (Buzsaki and Czeh 1981; Buzsaki and Eidelberg 1981; Douglas et al. 1983; Bilkey and Goddard 1987). Commissural stimulation inhibited the population spike in response to the electrical stimulation of the PP (Buzsaki and Czeh 1981; Buzsaki and Eidelberg 1981; Douglas et al. 1983; Bilkey and Goddard 1987), although there were some reports of excitatory effects where the PP response was potentiated by a prior stimulus to the commissure (Assaf and Miller 1981). Another study found that hilar stimulation induced a field EPSP in the molecular layer ipsilaterally or

contralaterally (Deadwyler et al. 1975; Steward et al. 1977). From these data, it was inferred that the commissurally-projecting cells were powerful in their inhibition of the GCs. However, the exact nature of the circuit was unclear.

There were two possibilities that were considered: commissurally-projecting hilar neurons (MCs) sent projections to the distal ipsilateral and contralateral IML where they activated local circuit GABAergic neurons (interneurons), which in turn inhibited GCs. Alternatively, the commissurally-projecting neurons were GABAergic themselves and directly inhibited the distal GCs. Two critical questions needed to be answered: (1) did MCs use glutamate or GABA as their neurotransmitter and (2) was the net effect of MCs primarily excitatory or inhibitory to GCs?

The diversity of >20 hilar cell types based on Golgi studies (Amaral 1978) complicated these hypotheses about circuitry. When the hippocampal slice preparation became accepted as a valid approach, intracellular recordings from hilar cells were made (Scharfman and Schwartzkroin 1988). These recordings and those in subsequent studies (Scharfman 1992, 1993, 1999) showed that the morphology and physiology of hilar cells could be used to divide them into two groups, so-called regular-spiking cells with characteristics of pyramidal cells and fast-spiking or moderately fast-spiking neurons that correspond to GABAergic neurons (Scharfman 1992, 1999). The regular-spiking cells were spiny and usually had thorny excrescences, with dendrites mostly in the hilus (Scharfman 1992). These cells were called spiny hilar cells and, later, MCs (Scharfman and Schwartzkroin 1988; Scharfman and Myers 2013). The faster-spiking cells had no thorny excrescences, usually few spines and dendrites in all DG cell layers. They were and still are discussed as GABAergic neurons and have been characterized into subtypes (Halasy and Somogyi 1993; Han et al. 1993; Freund and Buzsaki 1996; Houser 2007; Morgan et al. 2007; Hosp et al. 2013).

MCs are extremely active cells electrophysiologically, reflected by the high frequency of spontaneous EPSPs compared to other DG and hippocampal cell types (Scharfman 1992, 1993, 1995a, 1999) and these EPSPs can trigger action potentials (Scharfman and Schwartzkroin 1988; Scharfman 1993). Consistent with these characteristics, it has recently been shown that MCs express the immediate early gene *c-fos* even without behavioral testing, whereas most other hippocampal neurons require behavioral activation (Duffy et al. 2013). Some regular spiking cells had dendrites in the molecular layer, which was also shown by morphological studies (Scharfman 1991; Blackstad et al. 2016). These studies raised the possibility that there were subtypes of MCs, some with and some without thorny excrescences. Other work also suggest subtypes, such as MCs in the ventral versus dorsal hippocampus (Fujise et al. 1998; Fujise and Kosaka 1999; Jinno et al. 2003; Duffy et al. 2013).

The similarity of MCs to regular-spiking pyramidal cells led to the presumption that MCs were glutamatergic. However, investigators struggled to prove this because the specificity of antibodies to glutamate was questioned at that time and the standard for proving a cell was glutamatergic physiologically was difficult, requiring recordings from a presynaptic MC and postsynaptic cell at the same time. Two papers significantly advanced the field: one was by Soriano and Frotcher (1994) showing that a validated antibody to glutamate combined with

Golgi staining clearly marked MCs. Later, the glutamatergic nature of MCs was further supported by studies illustrating that an antibody to the GluR2/3 receptor subunit stained MCs but not other hilar cells that are GABAergic (Leranth et al. 1996), an antibody that has become a standard method to define glutamatergic neurons. These studies showed that MCs were glutamatergic cells.

The second valuable study addressed physiological definitions of a glutamatergic neuron using hippocampal slices and two microelectrodes to show that presynaptic action potentials in MCs depolarized GCs with minimal latency between the MC action potential and postsynaptic depolarization, consistent with a monosynaptic connection (Scharfman 1995b). Disynaptic IPSPs were also recorded from GCs in response to a single presynaptic MC action potential, providing evidence that MCs activated interneurons that hyperpolarized GCs. Evidence that MCs innervated interneurons was shown later by paired whole cell recordings of MCs and hilar inter-neurons, where again the short latency between MC action potential and interneuron response was extremely brief, consistent with a monosynaptic connection from MC to interneuron (Larimer and Stowbridge 2008).

Interestingly, the MC effects on GCs were only weakly excitatory, with a high failure rate and a requirement that GABA_A receptors were blocked (Scharfman 1995b). Because of the difficulty with the technique, few connected pairs could be examined (Scharfman 1995b). Therefore, it remained controversial whether MCs primarily excite the GC network or primarily inhibit the network by activating interneurons. These studies also identified a significant complication to the circuitry: MCs projected locally in addition to their well-known distant projections (Scharfman and Schwartzkroin 1988; Buckmaster et al. 1992). Other studies at the time showed that, within a hippocampal slice, effects of MCs can be detected (Jackson and Scharfman 1996). Whether these actions were the same or different from distal projections was unclear.

Additional studies of the anatomical characteristics of MCs provided important information to help clarify the DG circuitry involving MCs. An elegant study employed MCs that were filled with biocytin in vivo and their axons quantified (Buckmaster et al. 1996). Electron microscopy, still the gold standard for this type of work, defined synapses. Distal to the MC soma in the ipsilateral hippocampus, the vast majority of terminals of the biocytin-labeled MCs innervated GCs dendrites. There were only a minority of synaptic contacts on putative interneurons, defined by their relatively smooth dendrites in the IML. While critical, these studies and other functional studies described above did not definitively address the net effects of MCs on GCs.

Inferences about MCs based on their experimentally-induced death

In studies that started in 1983 and continue to the present day, Robert Sloviter and colleagues provided evidence that the primary effect of MCs is not excitation of GCs but inhibition of GCs, because MCs activate interneurons that in turn silence GCs (Sloviter 1983, 1987, 1989, 1991, 1994). Initial studies supporting this idea first came from rats where perforant path (PP) stimulation of GCs was prolonged and strong. The results showed that MCs were highly vulnerable to excitotoxic stimulation of this kind, i.e., produced by the

strong continuous stimulation of GCs in vivo. There was also damage to somatostatin-expressing interneurons of the DG after prolonged repetitive PP stimulation. Other cells such as the GCs and parvalbumin (PV)-expressing interneurons (also called basket cells) were relatively spared (Sloviter 1983, 1987, 1989, 1991, 1994). Following the stimulation, rats were urethane-anesthetized and examined physiologically. The GC population spike showed hyperexcitability, meaning more than one population spike of GCs was recorded per PP stimulus (Sloviter 1991). Together, the data suggested that GCs show less inhibition when MCs are killed, although PP stimulation also had other effects.

In an experimental model of traumatic brain injury (TBI), a similar effect was found, where MCs were highly vulnerable to the TBI and, shortly after their injury, the GC population spike evoked by a PP stimulus reflected hyperexcitability (Lowenstein et al. 1992). It was hypothesized that MC input to PV-expressing basket cells normally activates them, so without MCs there was disinhibition of GCs because PV cells lacked activation. This ‘dormant basket cell hypothesis’—originally proposed on the basis of the experiments using prolonged, repetitive PP stimulation—was debated for many years (Sloviter 1991; Bekenstein and Lothman 1993; Bernard et al. 1998).

Several caveats about these studies were proposed. One was the fact that the vast majority of terminals in the IML from MCs were on GCs, at least in the ipsilateral hippocampus far away from the MC soma (Buckmaster et al. 1996). On the basis of numbers of terminals, the data suggested that the primary effect of MCs was to excite GCs. A counterargument was that there is significant divergence of PV-expressing basket cells, where one basket cell, for example a basket cell in area CA1, can innervate up to 500 CA1 pyramidal cells (Freund and Buzsaki 1996). Also, the activation of GCs by MCs is weak at the normal GC resting potential and has low probability (Scharfman 1995b), being potentially weak compared to the MC to basket cell synapse. However, the weak MC-to-GC synapse may be stronger far away from the MC soma. This is important because paired recordings of the MC-to-GC synapse with hippocampal slices has relied on synapses relatively close to the MC soma (Scharfman 1995b).

Additional studies supported the idea that the net effect of MCs was primarily an excitatory effect on GCs. For example, Soltesz and colleagues used a laser to reduce the number of hilar MCs in slices and found the GC response to a PP stimulus did not become disinhibited (Ratzliff et al. 2004). Using voltage-sensitive dyes and microelectrodes, the response to PP stimulation in the GC layer in slices was significantly reduced after lesions of the molecular layer, which would impair transmission by the PP and leave a dependence on circuitry in the hilus (Jackson and Scharfman 1996). The slice data supporting a proexcitatory role of MCs on GCs have recently been replicated with other techniques. For example, ChR2 has been expressed in the IML fibers and a large EPSC recorded in GCs with patch clamp methods (Chancey et al. 2014). These experiments require cautious interpretation because they were conducted in slices where the MC axon is severely truncated. Therefore, the work of Soltesz and colleagues in vivo has been especially important (Santhakumar et al. 2000). They found that, after TBI, there is considerable preservation of MCs, suggesting that MCs are not always vulnerable to injury. Also, the results of electrophysiological experiments after TBI (albeit in slices) suggested that the GCs are disinhibited in this condition despite MC

preservation. MCs showed hyperexcitability in slices of injured rats, suggesting that MCs potentially cause GC hyperexcitability by exciting them too much and not by disinhibition.. This 'irritable MC hypothesis' was an alternative to the 'dormant basket cell hypothesis' (Santhakumar et al. 2000).

Additional experiments suggested that, both near and distant to the MC soma, the net effects of MCs were to inhibit GCs. The study supporting this idea suggested that MC projections are likely to excite interneurons near as well as distal to their soma (Zappone and Sloviter 2004). However, Buckmaster and Jongen-Relo showed that long-distance projections of somatostatin-expressing hilar neurons could have contributed (Buckmaster and Jongen-Relo 1999). In other words, either the loss of MCs or somatostatin-expressing neurons in one part of the DG could lead to distal disinhibition.

Resolving the role of MCs in the DG circuit has been facilitated by the development of additional methods explained below.

Advances in our understanding of MCs

One of the advances in our understanding of DG circuitry arose from studies of a cell type in the DG that few had considered very important, the semilunar GC. This cell type is located in the IML of the rodent and in elegant studies by Larimer and Strowbridge, it was shown that the semilunar GCs activate hilar neurons (both MCs and interneurons), which in turn lead to long-lasting inhibition of GCs (Williams et al. 2007; Larimer and Strowbridge 2010).

One of the implications of SLGCs and their activity was that they could mediate the effects of EC activation of the DG. This circuit allows the majority of the GC population to be quiet, which is considered optimal for DG functions related to pattern separation. SLGC activation of hilar neurons also helps explain why MCs had a relatively low threshold to the activation of PP fibers in the hippocampal slice compared to GCs (Scharfman 1991). Although MCs have dendrites in the molecular layer (Scharfman 1991; Blackstad et al. 2015) and there is a correlation between the presence of these dendrites and a low threshold to PP stimuli (Scharfman 1991), proof of a direct connection has not been available. The EC does have fibers that penetrate the hilus but those appear to mostly be on inter-neurons (Deller et al. 1996). One of the reasons for the low threshold could be an EC-to-SLGC- to-MC pathway (Fig. 1).

After 2010, a series of technical advances allowed greater insight into whether MCs were primarily inhibitory or excitatory to GCs. One study made use of a transgenic mouse that expressed Cre recombinase primarily in MCs (Jinde et al. 2012). It turns out that this mouse has expression of Cre recombinase in some of CA3 in the ventral hippocampus but the studies were very important nonetheless because it was the first time MCs could be manipulated with some specificity. The first paper published about these mice showed that the deletion of MCs using diphtheria toxin led to hyperexcitability of DG GCs in anesthetized mice using a PP stimulus. Hyperexcitability was demonstrated by multiple population spikes evoked by a stimulus rather than the normal response, which is one population spike per stimulus. These data supported the idea that MCs primarily inhibited GCs. However, the

study did not look distal to the stimulated fibers to test the hypothesis that local to the activated MCs there is inhibition of GCs but distal to the MC there is excitation (Scharfman and Myers 2013; Scharfman 2016). Another caveat was that hyperexcitability was transient. In other words, hyperexcitability could be recorded after MC loss but only within a certain temporal window following deletion of MCs.

This transgenic mouse was also tested to determine what behaviors might be affected by MC deletion. Importantly, behaviors were impaired that are considered to be dependent on the DG, such as contextual discrimination. The idea that MCs are important to the discrimination of context has been further supported by others in recent years (see below).

A subsequent paper that shed significant light on MC function that used more ‘modern’ approaches took advantage of the method developed by Soltesz and colleagues. A virus is injected in one dorsal hippocampus that is transported retrogradely to the contralateral hippocampus. Any cell projecting contralaterally will express Cre recombinase. If the virus is limited to the DG, only contralaterally-projecting DG cells will express Cre, which are primarily MCs. However, a limitation is that a number of GABAergic hilar neurons have contralateral projections (Seress and Ribak 1983; Ribak et al. 1986; Goodman and Sloviter 1992; Deller et al. 1995).

After the first viral injection, a second virus was used in the DG contralateral to the first injection to express the excitatory opsin channelrhodopsin (ChR2) in those cells expressing Cre. Then, slices were prepared from that hippocampus to examine effects of ChR2 on the network. GCs and interneurons were patch clamped to determine the effects of ChR2 on all of the cell types; MCs were shown to have excitatory effects on all cell types. To then determine what effects the excitation had on the network of GCs, extracellular recordings were made. Stimulation of the PP terminals in the slice normally evoked a population spike in the GC layer and a prepulse of light to activate ChR2 reduced the population spike amplitude. The conclusion was that the effects of MCs were primarily inhibitory. Furthermore, the excitation of GABAergic neurons persisted during a train of stimuli but the excitation of GCs did not, suggesting a reason for the greater inhibition of GCs compared to excitation produced by MCs (Fig. 2).

Role of MCs in the awake behaving animal

After the defects in contextual discrimination were identified by Jinde et al. (2012), other investigators have advanced our understanding of the DG behavior that is influenced by MCs.

Using c-fos as a marker of MC activity, Duffy et al. (2013) showed that MCs in the ventral DG are normally active even when most other ventral DG cells are not. The consistent finding that ventral but not dorsal MCs show c-fos under this condition supports earlier anatomical and physiological evidence of distinctions between ventral and dorsal MCs (Fujise et al. 1998; Fujise and Kosaka 1999; Jinno et al. 2003). In the mice that showed c-fos in ventral MCs, the olfactory areas and lateral EC superficial layers were also c-fos immunoreactive, suggesting that MCs are normally activated by the LEC in response to

normal activity in the home cage. These data suggested that ventral MCs might be especially active in normal conditions by the ongoing stimuli in the environment, such as olfactory cues and this was supported by showing that acclimation to the laboratory reduced c-fos expression (Duffy et al. 2013). This study complements the work of Weeden et al. who showed that ventral DG is important to olfactory pattern separation (Weeden et al. 2014) and Myers and Scharfman (2009, 2011) who showed that hilar neurons are important in pattern separation by computational modeling.

A second study using c-fos (Moretto et al. 2017) showed a rapid sensitivity of MCs to behavioral stress. Restraint in a nose cone or transparent plexiglas holder led to a reduced pattern of c-fos expression (Moretto et al. 2017). Interestingly, the MC response was fast, appearing 20 min after restraint that lasted 10 min. However, it then was absent at later times. This type of rapid “on” and “off” suggests that MCs could act as novelty detectors or sentinels for environmental change, because they would rapidly signal but then stop, potentially being ready to signal again (Scharfman and Myers 2013; Scharfman 2016; Moretto et al. 2017).

New insights into MCs from recent publications

Important new information about mossy cells in vivo has been provided in four new studies published in 2017.

- A. Danielson et al. (2017) injected a virus in one DG to express Cre recombinase in the hilar cells of origin in the contralateral DG, similar to others who had used this approach before, described above. Danielson et al. used GCamp6f to study activity in vivo using calcium imaging and a chronic imaging window to fix the DG in place so anesthesia could be avoided. Using the head-fixed preparation, they examined calcium dynamics as the mice ran on a treadmill positioned under the mouse. Animals were trained to run to obtain water and different linear tracks were placed in front of the mouse so it visualized different spatial cues.

They found MCs were very active using GCamp6f, supporting the findings of slice electrophysiology before where the spontaneous synaptic activity of MCs was extremely high (Scharfman and Schwartzkroin 1988; Livsey and Vicini 1992; Scharfman 1993) and c-fos (Duffy et al. 2013). In some ways, the work probably underestimated MC activity, if restraint stress transiently suppresses MC activity. Importantly, GCs downregulated c-fos for longer periods of time than MCs after restraint stress (Moretto et al. 2017), so the GC activity could also have been underestimated.

They also found that GCs had relatively ‘diffuse spatial tuning profiles’ compared to MCs and that MCs discriminated context more than GCs. In this regard, their conclusions were consistent with Goodsmith et al. (2017) and Senzai and Buszaki (2017) (see below), who also suggested that MCs contributed to spatial coding and they had more plasticity in remapping than GCs. Danielson et al. (2017) also conducted computational modeling, which suggested that MCs contributed to sparse GC coding. These findings supported

prior computational modeling (Myers and Scharfman 2009). This work placed MCs in a central role in the processing of spatial information by the DG.

- B.** Goodsmith et al. (2017) used tetrode and juxtacellular recordings in the awake rat to compare attributes of GCs and MCs. Using an innovative classification system to differentiate cell types and sampling of MCs with arduous juxtacellular recordings without anesthesia, they made several findings. First, they found that GCs had sparse firing and, when they did fire, there were single firing fields, which one might expect from cells that are relatively quiescent. Here, a single firing field corresponds to a cell that fires mostly in one location in their environment. On the other hand, MCs fired a lot more, which is consistent with prior conceptions of MCs as very active (as described above). MCs also had multiple firing fields, meaning they fired in more than one location in a given environment. These data were the first to identify that MCs had a firing field of any kind, because they had never been recorded before in vivo without anesthesia as the rat moved freely in its environment.

These results suggested that previous studies of GCs in vivo in the rat, where multiple firing fields and plasticity of the fields were described, had actually been recordings of MCs (Leutgeb et al. 2007). This is plausible, because in vivo it is hard to know if the tip of the recording electrode is in the GC layer or the hilus, just beside the GC layer. These results were supported by the other two papers published in the same journal issue (Danielson et al. 2017; Senzai and Buzsaki 2017).

- C.** Senzai and Buzsaki (2017) developed a strategy to discriminate between GCs and MCs using extracellular unit recordings. Results were similar to those of Goodsmith et al. discussed above. Thus, Senzai and Buzsaki found that GCs had single place fields primarily and MCs had more than one place field primarily. Furthermore, MCs underwent place field remapping differently than GCs. Although the procedure was different, GoodSmith et al. reported differences between MCs and GCs also. Here, the term “remapping” reflects a change in the preference of a cell to fire in a specific location as the local environment is changed.

Interestingly, Senzai and Buzsaki found that GCs could remap if different rooms were used during recordings, rather than the same room but with different contextual cues. They suggested that this could be explained by a greater influence on GCs of head direction cells relative to MCs. Thus, different afferent inputs to the two cell types, GCs and MCs, may allow them to encode different aspects of the environment. This idea is similar to the one proposed by Goodsmith et al., that GCs and MCs encode the environment differently.

Senzai and Buzsaki also used optogenetics in a mouse where Cre recombinase is expressed in neurons that have the dopamine type 2 receptor. In the DG, these cells are either MCs or are a small subset of interneurons (Gangarossa et al. 2012). Senzai and Buzsaki found that there was more GC firing in response to silencing MCs with Archaelhodopsin, a light-sensitive opsin that causes a cell to

be hyperpolarized during a pulse of light. These data supported the view that MCs inhibit GCs primarily. It is hard to discount these data because they were conducted in vivo without anesthesia, in contrast to prior studies either in the slice preparation or with anesthesia. Prior studies also killed MCs to understand their role better but loss of MCs can lead to complex compensatory changes that make the effects of MCs hard to distinguish. The results of Senzai and Buzsaki are also consistent with the idea that MCs excite GCs but only under specific conditions, such as a time when the GCs are concurrently depolarized by the brainstem afferents to the DG (Scharfman and Myers 2013; Scharfman 2016).

By analyzing relative firing of GCs and MCs, the investigators also found that GCs excited MCs with high reliability. This was then discussed in relation to the DG network. It was suggested that the powerful mossy fiber synapses of GCs ‘detonated’ MCs, which agrees with the known anatomy with MCs having numerous thorny excrescences close to their soma (although there are exceptions; Scharfman and Myers 2013; Scharfman 2016). Furthermore, it agrees with simultaneous intracellular recordings in rat hippocampal slices, where it has been shown that GC-to-MC transmission is robust and highly reliable (Scharfman et al. 1990). Senzai and Buzsaki also showed that there was strong frequency facilitation of the GC-to-MC input, which supported previous demonstrations either with unit recordings or paired intracellular recordings in hippocampal slices that the GC-to-MC synapse has robust frequency facilitation (Scharfman et al. 1990; Henze and Buzsáki 2007).

In light of the strong activation of MCs by GCs, Senzai and Buzsaki suggested that it seemed counterintuitive that MCs and GCs would have different place fields because GCs would almost always activate MCs (Senzai and Buzsaki 2017). The authors suggested that this might be explained by the very different inputs to GCs and MCs, which are likely to encode different aspects of the environment (Senzai and Buzsaki 2017). Another possibility is that an active GC will indeed be likely to cause its downstream MC to fire but then the original GC would be likely to stop firing because of its characteristics (spike frequency adaptation) and feedback inhibition. In contrast to the GC that stops firing, MCs fire repetitively even after one stimulus. Therefore, the patterns of activity of GCs and MCs are likely to diverge after the first action potential. The divergence in encoding could arise because of divergence in the response of the GC and MC to a single input.

- D.** Another significant body of work was recently published using a modified rabies virus to define presynaptic inputs to MCs (Sun et al. 2017). Rabies pseudovirus was injected in the DG of one hemisphere and used to label commissurally-projecting hilar neurons as other studies had done (described above). Studies were made of the inputs to the Cre recombinase-expressing neurons. The results showed that the major inputs to MCs in the DG are from local interneurons and GCs and the CA3 input—the “backprojection” (Scharfman 2007)—is also significant, potentially more significant than has been realized. Furthermore, there were projections from the cholinergic nuclei of the septum and diagonal

band. These projections had been identified (Deller et al. 1999) but the rabies study made them seem more important than ever because few other extrinsic afferents were labeled.

These ideas based on data from the rabies approach supported prior work using other techniques (Li et al. 1994; Scharfman 1994a, b, 2007; Hyde and Strowbridge 2012). Surprisingly, extrinsic input from extra hippocampal areas were rarely found, because it has been suggested that numerous brainstem inputs influence the hilus (Scharfman and Myers 2013). The conception of MCs based on this paper is that they are primarily neurons that receive hippocampal input and send axons to the DG.

However, the rabies approach could have underestimated projections to MCs from extrahippocampal sites, because it only allows a short time for retrograde transport before animals need to be examined. That makes long projections less easily labeled. It should also be considered that extrahippocampal areas influence MCs in an atypical way. For example, dopaminergic input might not synapse directly on MCs (Etter and Krezel 2014). Instead, dopamine might travel by diffusion from fibers in the hilus to MCs. Alternatively, noradrenergic input to the DG might release NE that is metabolized to DA and that influences MCs by diffusion or uptake.

Conclusions

New techniques are allowing greater insight than ever into understanding MCs. However, no technique is perfect and there are still unresolved questions. Here are some aspects of MCs where there seems to be agreement:

1. They are glutamatergic and project to the IML of the same hemisphere and contralaterally. They are highly active under normal conditions and vulnerable to excitotoxicity.
2. They innervate both GCs and interneurons of the DG and have cholinergic input from the septum/diagonal band.
3. They have robust input from GCs. They are also targeted by DG interneurons and CA3 pyramidal cells.
4. They produce inhibition of GCs as well as excitation but the excitation seems limited by characteristics such as frequency depression (Sun et al. 2017). Conditional excitation of GCs is one way to think about the circuitry (Scharfman and Myers 2013).
5. MCs are active during behavior such as spatial navigation and behavioral stress can reduce the activity. MCs have spatial tuning, and plasticity to change it. Furthermore, their spatial tuning differs from GCs (Scharfman and Myers 2013). This could allow the DG to perform pattern separation better, because input from the EC would potentially be “separated” (orthogonalized) better, allowing more storage capacity, as has been proposed (GoodSmith et al. 2017; Nakazawa 2017).

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Fig. 1.

Potential circuitry underlying EC activation of MCs. **a** A summary of some of the cell types and layers used in **B–D** is shown (for a complete view, see Scharfman 2016). *MOL* molecular layer, *GCL* granule cell layer; *EC* entorhinal cortex; *GC* granule cell; *PP* performant path. **b** The classic view is that the EC activates GC dendrites and GCs innervate MCs. The *red arrows* indicate the excitatory pathway from EC to MCs. **c** An alternate way for the EC to activate MCs is by directly contacting their processes in the hilus or molecular layer. **d** A new way to conceptualize the EC activation of hilar cells is by semilunar GCs (*SLGCs*) located in the inner third of the molecular layer

Fig. 2.

Changes in the effects of MCs with afferent fiber frequency. **a** A summary of some of the cell types and layers used in B–D is shown (for a complete view, see Scharfman (2016)). *OML* outer molecular layer; *MML* middle molecular layer, *IML* inner molecular layer, *GCL* granule cell layer; *SGZ* subgranular zone; *GC* granule cell; *GABA* GABAergic interneuron, *PP* perforant path. **b** A schematic illustrating one view of how the EC activates the DG. The common view is that PP fibers in the molecular layer activate the GCs, which in turn activate MCs. MCs then excite GCs by their direct input and also activate interneurons that inhibit GCs. Only one subtype of the GCs and interneurons is shown. A second way that the DG is activated is by EC exciting MCs in a feed-forward manner and then MCs excite GCs and interneurons. **c** After a single afferent stimulus, MCs have the ability to directly excite GCs and indirectly inhibit them by activation of interneurons. The outcome is probably a mixture of excitation and inhibition because there are numerous synapses of MCs on GCs (Buckmaster et al. 1996) but MCs also produce GABAergic inhibition of GCs (Scharfman 1995b; Larimer and Strowbridge 2008; Hsu et al. 2016) and excite DG interneurons (Larimer and Strowbridge 2008; Hsu et al. 2016) in the slice preparation. **d** During a train of stimuli, at frequencies which are physiological, the EPSCs of GCs produced by MCs appear to decline but IPSCs often persist (Hsu et al. 2016). Therefore, during patterns of firing of the MCs in vivo, GCs are likely to be inhibited more than excited. Stars indicate altered synapses.