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EPILEPSY-RELATED ABNORMALITIES/CHANGES IN BRAIN STRUCTURE

New insights into the role of hilar ectopic granule cells in the dentate gyrus based on quantitative anatomic analysis and three-dimensional reconstruction

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Summary

The dentate gyrus is one of two main areas of the mammalian brain where neurons are born throughout adulthood, a phenomenon called postnatal neurogenesis. Most of the neurons that are generated are granule cells (GCs), the major principal cell type in the dentate gyrus. Some adult-born granule cells develop in ectopic locations, such as the dentate hilus. The generation of hilar ectopic granule cells (HEGCs) is greatly increased in several animal models of epilepsy and has also been demonstrated in surgical specimens from patients with intractable temporal lobe epilepsy (TLE). Herein we review the results of our quantitative neuroanatomic analysis of HEGCs that were filled with Neurobiotin following electrophysiologic characterization in hippocampal slices. The data suggest that two types of HEGCs exist, based on a proximal or distal location of the cell body relative to the granule cell layer, and based on the location of most of the dendrites, in the molecular layer or hilus. Three-dimensional reconstruction revealed that the dendrites of distal HEGCs can extend along the transverse and longitudinal axis of the hippocampus. Analysis of axons demonstrated that HEGCs have projections that contribute to the normal mossy fiber innervation of CA3 as well as the abnormal sprouted fibers in the inner molecular layer of epileptic rodents (mossy fiber sprouting). These data support the idea that HEGCs could function as a "hub" cell in the dentate gyrus and play a critical role in network excitability.

KEY WORDS: Postnatal neurogenesis, Migration, Granule cell, Mossy fiber, Sprouting, Epileptogenesis, Hub cell.

There are two locations in the adult mammalian brain where new neurons are generated throughout life, a process called postnatal neurogenesis (Kempermann, 2006; Gage et al., 2008). One area where neurogenesis occurs in adulthood is the subventricular zone, and the other is the dentate gyrus (Kempermann, 2006; Gage et al., 2008).

In the dentate gyrus, the majority of the adult-born neurons become granule cells (GCs), which are the primary cell type in the region, and use glutamate as a neurotransmitter (Amaral et al., 2007). Adult-born GCs appear to be generated primarily from progenitors in the subgranular zone (SGZ), a 50- to 100-μm–thick layer located at the border of the GC layer (GCL) and the hilus (Fig. 1). In normal development, GCs are primarily born in the hilus; as the dentate gyrus matures, the primary site of proliferation shifts from the hilus to the SGZ (Li & Pleasure, 2007).

Remarkably, the generation of adult-born neurons and their migration into the GCL seems to follow a normal pattern, that is, similar to that of the majority of GCs, which are born in early life. Adult-born GCs develop cell bodies that are similar in diameter, shape, and other characteristics, compared to GCs born much earlier in life. In addition, the orientation of dendrites and dendritic spines appear similar, and mossy fiber axons have comparable trajectories. Afferent input from the major cortical pathway to the dentate gyrus, the perforant path, appears to make similar

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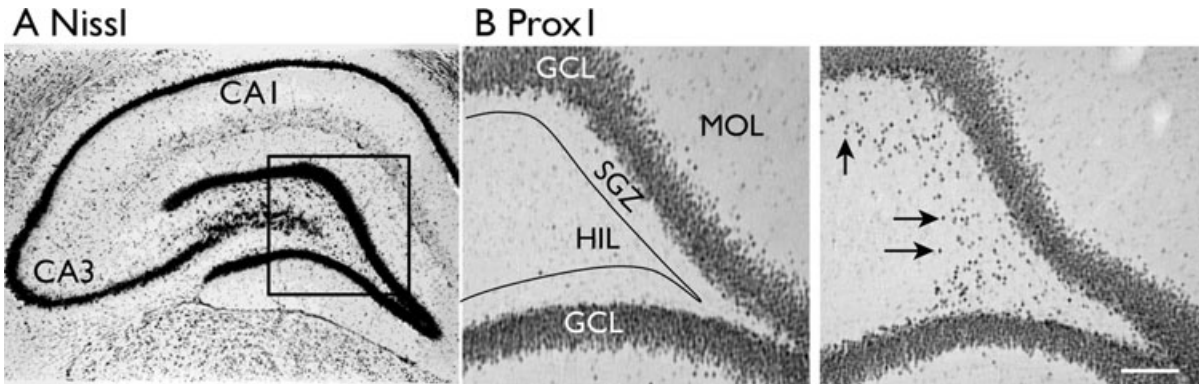

Figure 1.
Hilar ectopic granule cells in a rat model of temporal lobe epilepsy. (**A**) A Nissl-stained section showing the hippocampus in the transverse axis. The boxed area is shown in part **B**. (**B**) A section from a pilocarpine-treated rat that had SE and then spontaneous recurrent seizures. The section is stained with an antibody to Prox1, a specific marker of GC nuclei in the dentate gyrus. In the saline-treated control, Prox1-labeled cells in the subgranular zone (SGZ) and hilus (HIL) are rare. In the pilocarpine-treated epileptic rat, there are numerous hilar and SGZ cells (arrows). MOL, molecular layer; GCL, granule cell layer; HIL, hilus. Calibration = 250 μm (**A**) and 150 μm (**B**).

synapses on the dendrites of adult-born GCs and other GCs. Therefore, in the normal brain, adult-born progenitors appear to have a sufficient intrinsic program and adequate extrinsic cues to develop and migrate normally.

There is evidence, however, that not all adult-born GCs follow such a normal pattern of development. Notably, some of them are located in the hilar region. These neurons presumably migrated incorrectly from the SGZ, although it is also possible that they developed in the hilus from the vestiges of the tertiary matrix where GCs emerge in early life. In a normal adult Sprague-Dawley rat, these hilar ectopic granule cells (HEGCs) are rare (McCloskey et al., 2006; Fig. 1), but in animal models of temporal lobe epilepsy (TLE) their numbers are increased greatly (Parent et al. 1997; Scharfman et al., 2000; Jessberger et al., 2005; McCloskey et al., 2006; Jessberger et al., 2007; Jiao & Nadler, 2007; Fournier et al., 2009; Sugaya et al., 2010) (Fig. 1). HEGCs also appear to occur in humans, based on the GC marker calbindin D28K (calbindin) (Sloviter et al., 1991) and more selective marker, prospero homeobox 1 (Prox1) (Parent et al., 2006). In addition, early life status epilepticus produces (SE) HEGCs (Muramatsu et al., 2008), which is important because early life insults are known to be a risk factor for TLE, but all postnatal ages are not necessarily vulnerable (Porter, 2008). It is important to note how robust the finding of HEGCs are after adult SE in rodents, developing even when the methods to induce status epilepticus or species differ (Parent et al., 1997; Scharfman et al., 2000; Jung et al., 2004; Jessberger et al., 2005; Jung et al., 2006; Jessberger et al., 2007; Jiao & Nadler, 2007; Chu et al., 2008; Sugaya et al., 2010).

The first study to demonstrate HEGCs in an epileptic condition used an animal model of TLE where pilocarpine-induced status epilepticus is induced in adult life in the rat, and in the weeks and months that follow, spontaneous recurrent seizures emerge. That study (Parent et al., 1997) and a report of another study published in the same year using kindling stimulations (Bengzon et al., 1997), were very important because they identified—for the first time—a pronounced increase in proliferation in the GCL following status epilepticus or afterdischarges. HEGCs were not a focus of these studies, but evidence was presented that they were produced in the study of pilocarpine-induced SE (Parent et al., 1997). Based on that study, it did not appear that the proliferation after SE led to long-term neuronal survival, but, subsequent reports that focused on the hilus showed that HEGCs induced by either pilocarpine or kainic acid–induced status epilepticus in adult rat or mouse led to HEGCs that survive long-term, at least 18 months, the longest time that has been studied to our knowledge (Scharfman et al., 2000; Jessberger et al., 2005; McCloskey et al., 2006; Jessberger et al., 2007).

The idea that HEGCs developed in large numbers after status epilepticus, and could survive, led us to study HEGC characteristics in greater detail. Herein we review the quantitative anatomic data and correlated electrophysiology that emerged from our studies, published between 2000 and the present. We also include additional findings that have emerged from other laboratories where studies of HEGCs have also been conducted. After comparing the characteristics of HEGCs and GCL GCs, we discuss the hypothesis that has emerged as a result, that HEGCs contribute to seizure generation in the status epilepticus model of TLE, and potentially contribute to cognitive impairment in TLE.

Anatomic Characterization of HEGCs

HEGCs in the normal and epileptic brain

Before discussing the epileptic animal, it is important to mention what is now known about granule-like cells that are located outside the GCL in normal rodents, although they appeared to represent a relatively small population. The initial descriptions of granule-like neurons in the hilus were based primarily on Golgi material (Amaral, 1978; Marti-Subirana et al., 1986; Scharfman et al., 2007) and used purely morphologic criteria to categorize this subgroup of hilar cells. Factors such as the size and shape of the soma and the density and morphology of spines suggested that the granule-like hilar cells were similar to GCs located in the GCL. However, the hilar granule-like cells were sometimes considered to be a rare type of interneuron and not a GC, probably because the concepts of adult neurogenesis and mismigration had not yet been well developed. At a later time, calbindin-immunoreactive neurons that were similar to GCs in shape and size, but located in the hilus, were shown in human tissue, and they were also considered to be interneurons (Sloviter et al., 1991). Ramón y Cajal also noted that GCs exist in the inner molecular layer and named them ''semilunar'' GCs; these cells have been studied recently in depth in the normal rodent (Williams et al., 2007). Another elegant study showed recently that GCs exist in area CA3 in the normal rodent (Szabadics et al., 2011).

It is important to note that the studies of HEGCs in normal and epileptic rodents did not attempt to quantify the size of the populations and that they generally have been considered rare. Therefore, one of our first efforts was to measure the number of HEGCs in a normal rat using a GC-specific marker, Prox1, and unbiased stereologic techniques (McCloskey et al., 2006). Although relatively few HEGCs were observed in a given 50-μm–thick section in a normal rat, the HEGC population greatly increased after status epilepticus, reaching a maximum of approximately 22,000 cells per hippocampus (McCloskey et al., 2006) (Fig. 1). Compared to one of the most common cell types in the hilus, the hilar mossy cell (MC), which normally comprises about one third of all hilar cells (see Jiao & Nadler, 2007 for an excellent discussion of the values for MCs in normal and epileptic rodents), HEGCs were a relatively small population normally. However, after status epilepticus the numbers of HEGCs could approach the number of MCs in the hilus. In addition, the number of MCs is greatly reduced, and because MCs and HEGCs are the only glutamatergic hilar cells, one might conclude that HEGCs replaced MCs after status epilepticus and were restorative. However, there was a significant correlation between the size of the HEGC population and the numbers of spontaneous convulsive seizures in the same animals (McCloskey et al., 2006). Therefore, instead of restoring excitability, the data suggested that HEGCs contributed to seizure generation, a hypothesis that has been explored by many laboratories (Scharfman, 2004; Shapiro & Ribak, 2005; Scharfman & Gray, 2007; Scharfman & Hen, 2007; Scharfman et al., 2007; Danzer, 2008; Parent & Murphy, 2008; Scharfman & McCloskey, 2009) and is discussed in the context of recent findings below (see part III).

It is notable that other laboratories have also addressed the numbers of HEGCs using a different procedure and marker. Jiao & Nadler (2007) evaluated HEGCs by immunolabeling with a marker of glutamatergic neurons, an antibody to GluR2/3. They focused on cells with the shape of granule-like neurons to ensure that MCs (which also express GluR2/3) were not counted, although this is difficult because some MCs have a relatively granule-like somatic shape (Scharfman & Schwartzkroin, 1988; Scharfman, 1991, 1992, 1993, 1999). Jiao & Nadler (2007) showed that in their pilocarpine-treated rats with status epilepticus that 10–25 weeks after status epilepticus, there was a population of putative HEGCs that was similar quantitatively to the studies of HEGCs by our group using Prox1, although we used a slightly different stereologic approach with a slightly different method for inducing pilocarpine-induced SE. The main difference in the Jiao & Nadler (2007) study and our own (McCloskey et al., 2006) was the lack of HEGCs in normal rats in their hands, but a significant number of HEGCs in the normal rat in our analyses. In subsequent studies we have continued to find HEGCs in normal rats and mice, albeit in small numbers (Winawer et al., 2007). Because endogenous molecules like brain-derived neurotrophic factor (BDNF) appear to influence the generation of HEGCs (Scharfman et al., 2005), it is possible that age, strain, housing, or environment (which all modulate BDNF potentially, or other chemicals that could influence neurogenesis and migration) were responsible for the differences in normal HEGC numbers.

To clarify the structure and physiologic characteristics of HEGCs, hippocampal slices were prepared in our laboratory from animals that had pilocarpine-induced status epilepticus and chronic seizures or saline-treated, age-matched controls. In slices, hilar neurons were recorded randomly using ''blind'' techniques, and those cells that had GC-like electrophysiologic properties (Scharfman, 1992; Scharfman et al., 2000) were filled with Neurobiotin (Vector Laboratories, Burlingame, CA, U.S.A.). Slices were fixed by immersion in acrolein and subsequently resectioned in agar, and they were processed in preparation for light and electron microscopic examination (Scharfman et al., 2000, 2003; Pierce et al., 2011). The cells were analyzed quantitatively to identify their dendritic and axonal characteristics (Pierce et al., 2011). Comparisons were made to GCL GCs and HEGCs in the saline-treated

controls, as well as GCL GCs from an online database (http://NeuroMorpho.Org; Ascoli et al., 2007), where reconstructions of the HEGCs are now also available. In addition, separate studies employed electron microscopic dual immunolabeling techniques to examine HEGC synaptic input, using immunogold labeling for calbindin to label HEGC dendrites, in combination with immunoperoxidase labeling for either the zinc transporter 3 (Pierce et al., 2005) to label mossy fiber terminals, or calcitonin gene-related peptide (Pierce et al., 2007) to label the terminals of MCs.

Similarities among GCs in the GCL and hilus of epileptic rodents

In most HEGCs that have been sampled to date in our studies, the cell body has been very similar to GCL GCs; the shapes were spherical or ovoid, and the diameters were indistinguishable from those of GCL GCs. In addition, dendrites of HEGCs were covered by small spines, like GCL GCs (Scharfman et al., 2000, 2003; Pierce et al., 2005, 2007; Fig. 2). There have been some exceptional HEGC cell bodies that do not resemble GCs (Scharfman et al., 2002; Fig. 2B), but these are relatively rare. Similar conclusions have been made by others (Dashtipour et al., 2001). At the ultrastructural level, HEGCs also appear to be similar to GCs: the nucleus of HEGCs fills most of the cell body, leaving only a small perimeter for cytoplasm; a characteristic of GC somata (Ribak & Shapiro, 2007; Seress, 2007). However, there are exceptions where the ultrastructure of the cell body of HEGCs is atypical in that they have an infolded nucleus (Dashtipour et al., 2001), which is unusual in the rodent but present in primates (Seress & Ribak, 1992).

Quantitative studies revealed another characteristic of HEGCs that was similar to GCL GCs: the total length of dendrites of HEGCs and GCL GCs that we have studied was statistically indistinguishable (Pierce et al., 2011). Indeed, all of the GC types that we have studied, whether from saline controls or epileptic rats, had similar total dendritic lengths, although some of the groups were too small to compare statistically (Pierce et al., 2011). This surprising observation suggests that some influences on granule cell dendritic development are conserved, regardless of location. This may allow plasticity in the location of dendrites and dendritic branching, but once dendrites reach a specific length, further growth is inhibited. It has been suggested that constraints on this type of growth may limit competition between cells for space (Luczak, 2006).

It is notable that a recent study from another laboratory (Cameron et al., 2011) did not find as much homogeneity among dendritic length of HEGCs. Some HEGCs had smaller dendritic arbors than others. It was suggested that these HEGCs might be immature (Cameron et al., 2011). The discrepancy in our analyses and those of Cameron et al. (2011) could be related to the way the two studies sampled HEGCs. In our experiments, HEGCs that were

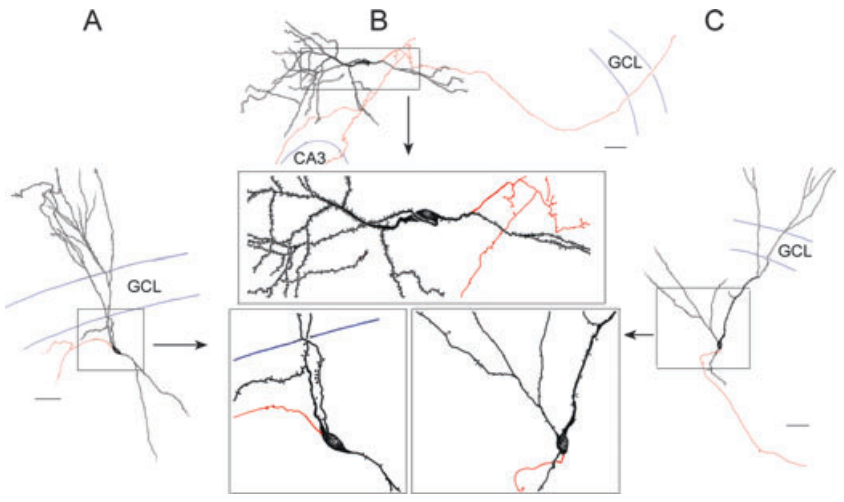

Figure 2.
Subtypes of HEGCs in normal and epileptic rats. (**A**) A proximal HEGC is similar in orientation to normal GCs in the GCL. Epileptic rat. (**B**) A distal HEGC, with dendrites exclusively in the hilus. Epileptic rat. To the right of the cell is an axon collateral (red) entering the inner molecular layer. (**C**) A distal HEGC with dendrites extending into the molecular layer. Saline-treated control rat. From (Scharfman et al., 2003). For **A**–**C**, the areas outlined by the box are shown at higher magnification as indicated by the arrows. Axons are red. Borders of the cell layers are blue. Calibrations in **A**–**C** are 20 μm for low power drawings and 5 μm for high power images.

immature were unlikely to have been sampled because sharp electrodes were used. This technique is difficult to use for immature cells and cells that have small cell bodies. In the study of Cameron et al. (2011), HEGCs were patched after visualizing a small granule-like cell in the hilus. This method is easier to use for immature neurons, and visualization enhances the opportunities to sample small cells. Taken together, the two studies suggest that when HEGCs do mature, they are likely to be similar to GCL GCs is their somata and total dendritic length. However, there is also likely to be an immature subset of HEGCs that are unlike GCL GCs in that their dendrites are not as complete. Another possibility mentioned by Cameron et al. (2011) is that some HEGCs are abnormal in that they exhibit stunted growth.

Unique characteristics of HEGCs

Although HEGCs in our sample were similar in many respects to GCL GCs, as indicated in preceding text, there appeared to be other characteristics that were distinct, but only for those HEGCs that were distal to the GCL (outside the subgranular zone, typically >100 μm from the border of the GCL and hilus). Therefore, we suggest that there are two major subtypes of HEGCs, proximal HEGCs with more similarity to GCL GCs and distal HEGCs with less similarity to GCL GCs.

Proximal HEGCs

As shown in Fig. 2A, proximal HEGCs displayed an elaborate apical dendritic arbor, like GCL GCs, which develops from either one or two primary dendrites that branch repeatedly after entering the molecular layer and sometimes before it (Scharfman et al., 2003; Pierce et al., 2011). Apical dendritic arbors of proximal HEGCs were confined primarily within the transverse axis, like GCL GCs (Carnevale et al., 1997). Basal dendrites were very similar to basal dendrites of GCL GCs in the site of origin (they originated from the opposite pole of the cell body as the apical dendrites) and trajectory (extended into the hilus; Fig. 2A). Proximal HEGC basal dendrites were much less complex than their apical dendrites, with fewer and shorter branches, similar to basal dendrites of GCL GCs (Fig. 2A). Although rare, differences between proximal HEGCs and GCL GC dendrites did exist, however. For example, dendritic branches of proximal HEGCs in the molecular layer were sometimes unusual, for example, extending at right angles to the parent dendrite (Fig. 2A).

Other laboratories have studied proximal HEGCs after retrograde labeling from stratum lucidum, and the analysis identified important differences in synaptic coverage of proximal HEGCs compared to GCL GCs (Dashtipour et al., 2001). Axosomatic synapses on the soma and proximal dendrites were more numerous compared to GCL GCs (Dashtipour et al., 2001). The results are important because they provide structural arguments in support of the idea that HEGCs are more excitable than GCL GCs.

Distal HEGCs

As shown in Fig. 2B, distal HEGCs were different from GCL GCs, and many of these differences have been quantified (Pierce et al., 2011). For example, HEGC dendritic trees rarely entered the molecular layer (Fig. 2B; Pierce et al., 2011). Within the hilus, distal HEGCs developed significantly more primary dendrites than GCL GCs, and these dendrites displayed no consistent pattern in terms of their orientation. Dendrites extended toward the molecular layer, CA3c, or both. Of interest, despite the diversity displayed in orientation, distal HEGC dendrites appeared to maintain a polarized dendritic tree, with larger, more elaborate dendrites similar to apical dendrites exiting from one pole and a smaller dendritic tree, more basal-like, emerging from the other pole. When viewed as a merged image, this was not always apparent. However, three-dimensional reconstruction allowed one to appreciate that primary dendrites often made sharp turns to arborize in a different plane (Fig. 3). Three-dimensional reconstruction and rotation was thus required to thoroughly appreciate the dendritic patterns of distal HEGCs. Another difference of distal HEGCs and GCL GCs was that the dendrites of distal HEGCs were topologically more complex than those of GCL GCs, displaying a more torturous pattern of branching and significantly more branch points (Pierce et al., 2011).

HEGC axons

In a normal GCL GC, the mossy fiber axon has a main projection that targets the proximal apical dendrites of area CA3 pyramidal cells, traveling parallel to the pyramidal cell layer in stratum lucidum and forming periodic massive boutons that are opposed to the thorny excrescences of CA3 pyramidal cells (Blaabjerg & Zimmer, 2007). The mossy fibers also collateralize densely in the hilus (Claiborne et al., 1986; Acsady et al., 1998). Many of the boutons formed by mossy fibers are notable because of their size (called ''giant'' or ''massive'' boutons; Laatsch & Cowan, 1966; Jaffe & Gutierrez, 2007), and are packed with both glutamatergic vesicles and dense core vesicles, which are associated with zinc or neuropeptides (McCarthy et al., 1998; Pierce et al., 1999). These large boutons also have more than one postsynaptic density (Laatsch & Cowan, 1966; Chicurel & Harris, 1992) and filamentous extensions that mainly contact γ-aminobutyric acid (GABA)ergic interneurons (Amaral, 1979; Acsady et al., 1998). Mossy fiber axons in the hilus have massive boutons on thorny excrescences of MCs, and also form smaller boutons that contact many cell types.

In animal models of TLE, mossy fiber axons collateralize, and additional boutons are found throughout the GCL and inner molecular layer, a phenomenon called mossy

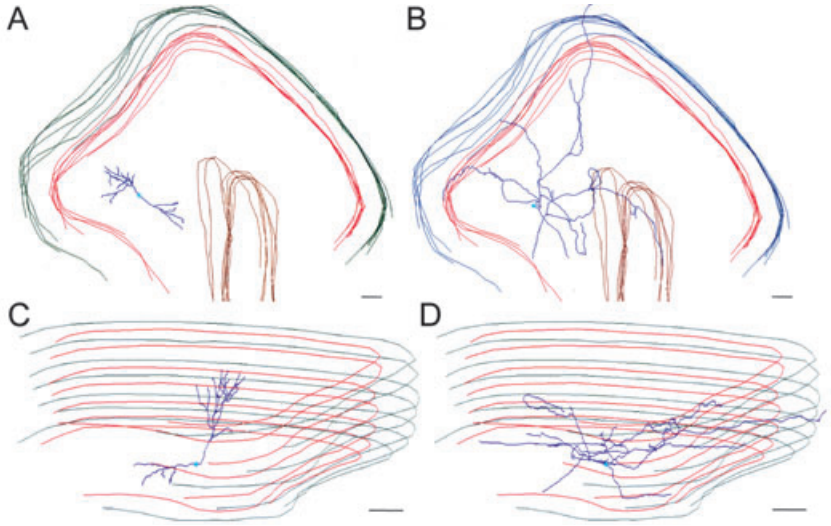

Figure 3.
HEGCs have dendrites and axons that are oriented in the transverse and longitudinal axis. (**A**) Reconstruction of the dendrites of an HEGC from the resectioned hippocampal slice where it was filled with Neurobiotin. (**B**) Reconstruction of the axon of the cell in **A**. (**C**, **D**) Projection of the dendrites and axon in three dimensions. Red: Border of the GCL and SGZ. Green: Hippocampal fissure (edge of the outer molecular layer). Brown: Borders of CA3c. Turquoise: Cell body. Navy blue: Dendrites or axons. Calibration = 50 μm.

fiber sprouting (Dudek & Sutula, 2007; Sutula & Dudek, 2007). Although the sprouted fibers innervate interneurons in the inner molecular layer, suggesting that sprouting may exert an inhibitory influence (Sloviter, 1992; Sloviter et al., 2006), the number of mossy fiber boutons that innervate dendrites of GCL GCs exceeds the synapses on dendrites of interneurons, suggesting that the net effect of mossy fiber sprouting is to increase recurrent excitatory circuitry in the dentate gyrus (Buckmaster et al., 2002).

Remarkably, HEGCs appear to have mossy fiber axons that are extremely similar to those of GCL GCs in the epileptic rat. All of the HEGCs that have been examined had collaterals in the hilus and projected into stratum lucidum of area CA3. When the stratum lucidum projection was lengthy, it traveled parallel to the pyramidal cell layer and gave rise to evenly spaced, periodic giant boutons, like mossy fibers of GCL GCs (Scharfman et al., 2000; Pierce et al., 2011). In addition, mossy fiber sprouting into the molecular layer was observed in all instances where the HEGC axon was reconstructed. Furthermore, HEGC sprouting could be more extensive and more divergent than sprouting of GCL GCs, because one to three collaterals of the HEGC axon entered the GCL and inner molecular layer compared with zero to one collateral per GCL GCs, sampled from the same animals. HEGC axon collaterals that entered the GCL were far apart, and therefore could have influenced more of the GCL GC population (Fig. 3) than the GCL GC axon collaterals that entered the molecular layer, which were close to the parent cell body when present (H.E. Scharfman, unpublished data). However, other laboratories have documented that the GCL GCs can have sprouted collaterals that enter parts of the GCL that are far apart (Sutula & Dudek, 2007). Even if HEGCs have an equivalent contribution to mossy fiber sprouting as GCL GCs, and it is no greater than GCL GCs, it is still an important characteristic that they contribute to mossy fiber sprouting, because most individuals consider mossy fiber sprouting to originate only from GCL GCs.

As indicated earlier, three-dimensional reconstruction of HEGCs revealed that HEGC dendritic trees were often oriented at right angles to each other, in contrast to GCL GC dendrites, which are restricted primarily within the transverse plane (Fig. 3). Mossy fiber axons of HEGCs also exhibited this characteristic, because mossy fibers of distal HEGCs were oriented longitudinally as they projected to CA3 (Fig. 3), but mossy fibers of GCL GCs are limited primarily to the transverse plane. Although a small number of HEGCs were reconstructed in three dimensions, which limits the degree the data can be generalized, the reconstructed HEGCs all showed the characteristic, that is, axons with projections in diverse planes of section. Therefore, it is likely to be a robust characteristic, although a larger sample size would be valuable.

Afferents to HEGCs

A remarkable set of electrophysiologic observations suggested that the perforant path made similar excitatory projections to proximal HEGCs as GCL GCs, and robust

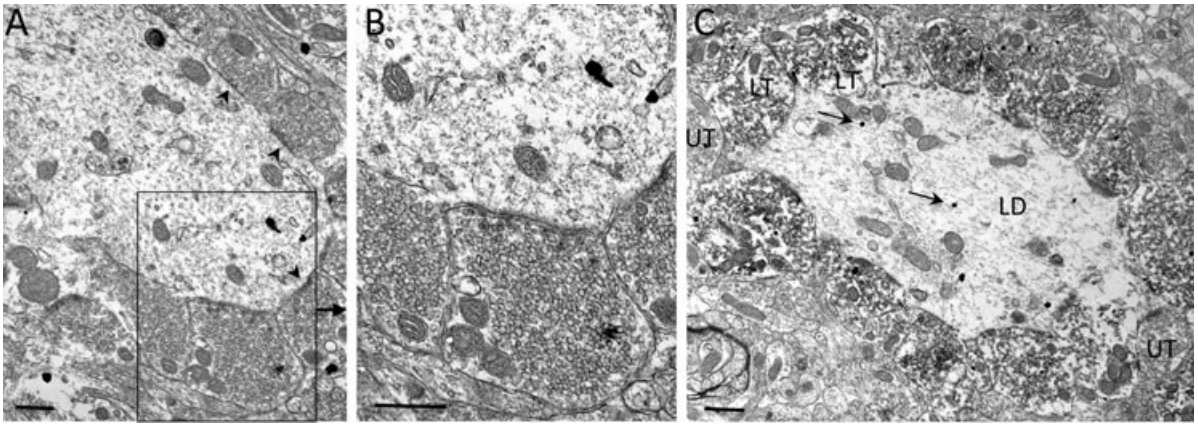

Figure 4.
Dense mossy fiber input to hilar dendrites of HEGCs. (**A**, **B**) Ultrastructural analysis of hilar dendrites of calbindin-labeled HEGCs show numerous juxtaposed mossy fiber boutons labeled (arrowheads) innervating a single dendritic shaft of a HEGC. The area outlined by a box is shown at higher magnification in part **B**. Dynorphin was used to label mossy fiber terminals. Calibration = 0.5 μm for parts **A** and **C**, 0.3 μm for part **B**. (**C**) Calbindin immunogold-labeled HEGC dendrite (LD, labeled dendrite) surrounded by zinc transporter-3 immunoperoxidase-labeled mossy fiber terminals (LT, labeled terminal) and unlabeled terminals (UT). Part **C** is from (Pierce et al., 2005).

disynaptic or polysynaptic activation of distal HEGCs (Scharfman et al., 2003). Ultrastructural observations helped identify additional sources of input to HEGCs. These studies suggested that mossy fiber axons represent the predominant source of afferent input to hilar dendrites of HEGCs (Pierce et al., 2005) (Fig. 4). Mossy fiber input to hilar dendrites of HEGCs was quantitatively greater than mossy fiber input to the spiny dendrites in the inner molecular layer of the same tissue section, which were presumably the proximal dendrites of GCL GCs. Mossy fiber input to hilar dendrites of HEGCs also was distinctive because mossy fiber boutons often were present in an array on dendritic shafts (Fig. 4) rather than intermittent input to spines. Based on these characteristics, it would be expected that HEGCs would have a greater excitatory input from mossy fibers than GCL GCs.

Ultrastructural analyses also showed that cells other than GCs innervate HEGC dendrites in the hilus. MCs are one source of this input (Pierce et al., 2007). This is important because in the epileptic tissue used for these studies, most of the MCs that survived status epilepticus exhibited periodic burst discharges (Scharfman et al., 2001), like CA3 pyramidal cells (Scharfman et al., 2000; McCloskey & Scharfman, 2011). Therefore, both CA3 pyramidal cells and MCs would provide strong excitatory input to HEGCs and be likely to initiate the periodic burst discharges that were recorded from many HEGCs (Scharfman et al., 2000, 2003, 2007; Pierce et al., 2011).

Relative to excitatory input, there were fewer inhibitory synaptic contacts on hilar dendrites of HEGCs compared to dendrites of presumed GCL GCs in the inner molecular layer (Pierce et al., 2005). This finding is similar to observations of greater excitatory versus inhibitory input to basal dendrites of GCL GCs (Thind et al., 2008) and somatic input of HEGCs (Dashtipour et al., 2001). Taken together, the results support the hypothesis that GCs with hilar dendrites are more excitable than GCL GCs, and are part of a recurrent excitatory circuit between GCL GCs, HEGCs, and possibly other cell types (Scharfman, 2004; Scharfman & Hen, 2007; Scharfman et al., 2007; Scharfman & McCloskey, 2009).

Implications of anatomic data

What emerges from anatomic analyses is an image of an aberrant cell type, HEGCs, and in particular the distal HEGCs, whose unique position and structure relative to GCL GCs are striking. The orientation of some dendrites along the transverse axis and other dendrites along the longitudinal axis suggests diverse input would converge onto HEGCs. In addition, the fact that hilar dendrites of EGCs develop extensive mossy fiber input suggests that recurrent excitatory circuits exist between GCL GCs and HEGCs. The fact that HECGs have dendrites that are similar to normal GCL GCs in term of total length and surface area suggests that it is the orientation of their dendrites that is important, not the amount of available surface.

In addition, HEGCs have axons that could have the potential to be highly divergent because they project to many locations. Divergence is also likely because the

axon targets many cell types: CA3 pyramidal cells, GCL GCs, as well as other hilar neurons. The potential for convergent input coupled with divergent output suggests that distal HEGCs could act as ''hub'' cells, or nodes in a network, a cell type that has been proposed to be a possible key player in the initiation of seizures (Morgan & Soltesz, 2008), and a hypothesis we have discussed previously (Pierce et al., 2011) as have others (Cameron et al., 2011) Therefore, HEGCs could support an amplification of excitatory activity, and possibly exert a proconvulsant influence in the epileptic brain. Similarly, HEGCs could potentially interrupt normal information processing in the hippocampal formation, by disrupting the complex relay of information from entorhinal cortex, to GCL GCs, and to area CA3. In summary, the structural characteristics of HEGCs suggest that they support hyperexcitability in the epileptic brain, and would be likely to impair cognitive function that depends on the dentate gyrus.

Electrophysiologic Characterization of HEGCs

Recordings of reconstructed HEGCs have shown that HEGCs have intrinsic properties that are similar to GCL GCs (Scharfman et al., 2000). These studies identified a set of core characteristics of GCL GCs that were also present in HEGCs, whether they were proximal or distal. These included (1) a relatively hyperpolarized membrane potential compared to other hilar neurons and pyramidal cells; (2) strong spike frequency adaptation, that is, decremental spiking in response to a maintained depolarizing current pulse; quantitatively distinct from other neurons in the hilus or CA3; (3) a regular spike in duration and slope, compared to fast spiking hilar cells; and (4) an afterhyperpolarization similar to GCL GCs, not hilar cells or pyramidal cells (Scharfman, 1992, 1999; Scharfman et al., 2000, 2007).

Patch clamp recordings do not necessarily discriminate hilar cells with different electrophysiologic characteristics (Lubke et al., 1998), although sharp recordings do, in our experience (Scharfman, 1992; Scharfman et al., 2007). However, recent patch-clamp recordings of HEGCs from animals that experienced status epilepticus have shown a number of electrophysiologic distinctions of HEGCs and GCL GCs. For example, the resting potential of HEGCs is relatively depolarized—approximately 5 mV more depolarized—compared to GCL GCs (Zhan & Nadler, 2009). In addition, there is more tonic inhibition in HEGCs than GCL GCs (Zhan & Nadler, 2009). Miniature excitatory postsynaptic currents (EPSCs) are more frequent, whereas miniature inhibitory postsynaptic currents (IPSCs) are less frequent (Zhan et al., 2011). Taken together, it was suggested that HEGCs are generally more excitable than GCL GCs, with potential compensatory changes such as increased tonic inhibition (Zhan & Nadler, 2009; Zhan et al., 2011).

Evidence that HEGCs Contribute to the Pathophysiology of TLE

One of the first pieces of evidence that HEGCs might have an adverse effect on the dentate gyrus, rather than a restorative effect, was based on recordings in slices of epileptic rodents. Most of the HEGCs exhibited rhythmic burst discharges that were driven by periodic epileptiform discharges of the same rhythm, originating in the area CA3 population (Scharfman et al., 2000, 2003, 2007). Based on the excitatory input to HEGCs from CA3, presumably due to the CA3 backprojection (Scharfman, 2007) or MCs, and divergent mossy fiber projections of HEGCs to both CA3 and GCL GCs, it seemed logical to suggest that HEGCs would increase excitability of the dentate-CA3 network by facilitating recurrent excitation (Scharfman, 2004; Scharfman & Gray, 2007; Scharfman & Hen, 2007; Scharfman et al., 2007; Scharfman & McCloskey, 2009).

To address the activity of HEGCs in vivo, epileptic rats were perfused 2 h after a spontaneous stage 4–5 seizure in their home cage, to identify whether HEGCs were active during the spontaneous seizure (Scharfman et al., 2002). This approach was taken because all previous work had been conducted in vitro, which is not necessarily predictive of in vivo behavior. We found that numerous hilar cells expressed c-fos, and many of them were double-labeled by calbindin (Scharfman et al., 2002) and Prox1 (Scharfman et al., 2007), suggesting that they were HEGCs. We also found that hilar parvalbumin- and neuropeptide Y-immunoreactive hilar interneurons exhibited a c-fos-immunoreactive nucleus (Scharfman et al., 2002). Together the results suggested that hilar cells were active during spontaneous convulsive seizures and HEGCs behaved like other hilar cells. This experiment did not prove that the HEGCs caused the seizure, but did show that the HEGCs were part of the circuitry activated by spontaneous convulsive seizures, and therefore they were functionally integrated into circuitry involved in these types of limbic seizures.

Experiments by others suggest a role of HEGCs in seizure generation. In the first of these studies, a mitotic inhibitor was used in the month after lithium-pilocarpine–induced status epilepticus, and seizures were evaluated approximately 4 weeks after status epilepticus (Jung et al., 2004). The results showed that there was a dramatic loss of HEGCs in the treated group, although the treatment was not selective because glia were also affected. Nevertheless, there was a reduction in the number of spontaneous seizures in treated animals compared to controls,

supporting the hypothesis that HEGCs contribute to seizure generation after SE. Of interest, when seizures occurred in the treated group, they were as severe as controls, based on the Racine scale. These data are consistent with inhibition of a focus of seizure generation, possibly involving HEGCs, but not the expression of seizures in motor structures, where HEGCs do not exist. More recently the same laboratory has used the cyclooxygenase-2 inhibitor celecoxib (Jung et al., 2006), and erythropoietin (Chu et al., 2008) to reduce HEGCs, and these treatments also reduced seizures, but they did not reduce HEGCs selectively either.

Several other studies have provided support for the view that HEGCs contribute to seizure generation in TLE. One was the identification of HEGCs using Prox1 in human tissue resected from patients with intractable TLE (Parent et al., 2006). Several transgenic animals with seizures have also been shown to exhibit HEGCs (Scharfman et al., 2007). However, these studies are correlational; direct evidence that HEGCs cause seizures is not yet available.

There are some arguments against the idea that HEGCs influence the dentate gyrus in a robust manner, or influence it adversely. Pekcec et al. (2008) tested the idea that interference with polysialylated-neural cell adhesion molecule would inhibit seizures. In the process, HEGCs were reduced, but seizures were not. However, their treatment increased basal dendrites of GCL GCs, and there were other effects of treatment that could have been epileptogenic. In a study of newborn neurons in the GCL after status epilepticus, it was found that these neurons were not more excitable; indeed they appeared to be less excitable. Although these neurons were not HEGCs, the study suggested that GCs born after status epilepticus, and possibly HEGCs by extension, had limited excitability (Jakubs et al., 2006). The fact that HEGCs exhibit more tonic inhibition than GCL GCs also is an argument against the idea that HEGCs are highly excitable, and contribute to network excitability in the epileptic brain (Zhan & Nadler, 2009). However, the authors noted that despite the increase in tonic inhibition, the HEGCs had a more depolarized resting potential, and appeared to be more excitable because miniature EPSCs were greater and miniature IPSCs less frequent than GCL GCs. In addition, they reported that the HEGCs exhibited spontaneous burst discharges, replicating previous findings (Zhan & Nadler, 2009; Zhan et al., 2011).

Conclusion

In summary, HEGCs could represent one of the epileptogenic developments after status epilepticus in rodents that contributes to the pathophysiology of epilepsy, and could also play a role in intractable TLE. It is tempting to suggest that one of the reason drugs fail and seizures stop after surgery in intractable cases is that HEGC removal is therapeutic. However, direct evidence in support of a causative role of HEGCs in TLE is still not available. Nevertheless, even if the seizures are not affected greatly by HEGCs, it is highly likely that HEGCs would impair dentate gyrus network function, and contribute to comorbidity such as cognitive impairment and depression.

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Disclosure

None of the authors has any conflict of interest to disclose. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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