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NEWLY GENERATED DENTATE GRANULE CELLS FROM EPILEPTIC RATS EXHIBIT ELONGATED HILAR BASAL DENDRITES THAT ALIGN ALONG GFAP-IMMUNOLABELED PROCESSES

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Abstract—Previous studies showed that neurogenesis occurs in the dentate gyrus of the adult rodent. Recent evidence suggests that the resulting newly born neurons integrate into pre-existing hippocampal circuitry. Newly born neurons in the developing and adult dentate gyrus exhibit a transient basal dendrite. In adult pilocarpine-induced epileptic rats, basal dendrites persist and are ectopically located in the hilus where they receive synaptic input from mossy fiber axons. We hypothesize that these hilar basal dendrites are derived from newly born neurons that are born after the pilocarpine-induced seizures. To test this hypothesis, the length of basal dendrites from epileptic rats was compared with that from control rats using doublecortin immunocytochemistry, which labels newly born neurons and their processes for up to 3 weeks after their genesis. The data on hilar basal dendrites in pilocarpine animals indicate that those from newly born neurons are significantly longer than those found in the control rats. We also demonstrate that 20% of newly born neurons in the epileptic rat have a basal dendrite that enters the hilus at an angle greater than 30° from its cell body as compared with <2% in the control rats. Lastly, we provide evidence that the hilar basal dendrites in the epileptic rats are adjacent to glial fibrillary acidic protein-labeled astrocytic processes in the hilus and suggest that an ectopic glial scaffold in the hilus is involved with the formation of hilar basal dendrites. In conclusion, the data show that newly born neurons from epileptic rats have longer hilar basal dendrites and their formation might relate to gliosis which occurs as a result of hilar neuronal cell loss after status epilepticus.

Key words: adult neurogenesis, hippocampus, dentate gyrus, pilocarpine, doublecortin.

Granule cell neurogenesis occurs in adults and can be observed along the hilar border of the granule cell layer (GL) in the hippocampal dentate gyrus (Altman and Das, 1965; Kempermann et al.,1998; Cameron and McKay, 2001). Recent evidence suggests that in the adult, newly born neurons (NNs) are born from astrocytes (Seri et al., 2001). These NNs are born in the subgranular zone (SGZ) and migrate to the GL where they are integrated into existing granule cell circuitry (Van Praag et al., 2002). Several studies used nuclear markers such as bromodeoxyuridine (BrdU) and [^{3}H]thymidine to label the NNs, while others have incorporated more structurally revealing immunohistochemical markers such as TUC-4, β-III-tubulin, Prox-1, CRMP-4, and doublecortin (DCX) to analyze these newly generated granule cells.

DCX is a protein found in NNs associated with the growth cones, processes, and surrounding perikaryal cytoplasm (Francis et al., 1999). DCX antibody labels newly generated granule cells for up to 3 weeks after their division (Kempermann et al., 2003; Rao and Shetty, 2004), and recent evidence suggests that DCX immunocytochemistry can detect neuronal precursor cells in the adult rodent dentate gyrus (Steiner et al., 2004). Unlike BrdU which only labels the nucleus, DCX preparations allow for high resolution light microscopic observations of dendrites and immature processes in the dentate gyrus of the adult hippocampus. For example, we have previously shown that DCX-labeled dendrites display growth cones, including lamellipodia and filopodia (Ribak et al., 2004).

Several reports suggested that NNs in the dentate gyrus undergo a programmed progression of dendritic process outgrowth as they migrate from the SGZ into the GL (Jones et al., 2003; Shapiro et al., 2005). One of these processes appears to arise from the apical portion of the cell and typically projects toward the molecular layer as the apical dendrite. In addition to an apical dendrite, 31%–55% of newly born granule cells exhibit a basal dendrite (Rao and Shetty, 2004; Ribak et al., 2004), which appears to be transient for granule cells during neonatal development (Seress and Pokorny, 1981; Jones et al., 2003). Many of these basal dendrites curve into the GL as a recurrent basal dendrite (Dashtipour et al., 2002; Yan et al., 2001).

In pilocarpine-induced epileptic rats, granule cells with hilar basal dendrites (HBDs) were reported (Spigelman et al., 1998; Ribak et al., 2000). Ribak et al. (2000) demonstrated that these basal dendrites have spines and contribute to additional recurrent excitatory circuitry. Austin and Buckmaster (2004) later showed that the circuitry involving HBDs contributes to increased bursting activity of granule cells. Most granule cells with HBDs are located at the hilar border, a site where neurogenesis occurs. Therefore, it is possible that the appearance of granule cells with HBDs in epileptic rats is related to the overall increase in neurogenesis following pilocarpine-induced seizures (Parent et al., 1997). Unlike normal development, these latter basal dendrites are a persistent feature of granule cells in epileptic rats and represent a seizure-induced neuro-

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Abbreviations: BrdU, bromodeoxyuridine; DAB, diaminobenzidine; DCX, doublecortin; GFAP, glial fibrillary acidic protein; GL, granule cell layer; HBDs, hilar basal dendrites; NNs, newborn neurons; PBS, phosphate-buffered saline; SGZ, subgranular zone.

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plastic change that could be specific for NNs. Thus, one of the goals of the present study is to determine whether HBDs arise from NNs that are born after the induction of status epilepticus by pilocarpine-treatment.

The present study was performed using light microscopic preparations of DCX-immunolabeled granule cells to determine whether basal dendrites from NNs are longer in epileptic rats and if these basal dendrites course into the hilus. We also examined the relationship of the basal dendrites with glial fibrillary acidic protein (GFAP)-immunolabeling for astrocytic processes.

EXPERIMENTAL PROCEDURES

Induction of status epilepticus

For this study, adult male, Sprague–Dawley rats (n=9; 450–600 g; Simonsen, Gilroy, CA, USA) were used. All protocols were approved in advance by the Institutional Animal Care and Use Committee at the University of California at Irvine. All experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. In addition, the number of rats in this study was kept to a minimum, and when necessary, all animals were anesthetized to minimize their suffering. Experimental rats (n=5) were first injected with methylscopolamine in solution with saline (1 mg/kg, i.p.), followed by an injection of pilocarpine hydrochloride (320–340 mg/kg, i.p.) to induce status epilepticus (Turski et al., 1983). S.E. was terminated after 3 h with two separate injections of Nembutal (pentobarbital sodium; 25 mg/kg, i.p.) 10 min apart.

Control rats (n=4) were first injected with methylscopolamine in saline solution (1 mg/kg, i.p.), followed by a saline injection substituted for pilocarpine. The control rats were also subjected to two separate injections of Nembutal (pentobarbital sodium; 25 mg/kg, i.p.) 10 min apart at the 3 h time point. All rats were monitored for 30 days after injections. Using a video monitoring system the presence of spontaneous seizures was verified in the rats prior to kill.

DCX immunocytochemistry

At 30 days after the injections, both groups of rats were anesthetized with an overdose of Nembutal (50 mg/kg; i.p.) and then perfused intracardially with 150 ml of 0.9% saline followed by 200–300 ml of 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS). Brains remained intact within the cranium for 48 h at 4 °C prior to removal. Blocks containing the hippocampus were extracted and sectioned at 50 μm with a vibratome. All immunohistochemical reactions were carried out simultaneously using the same reagents for all animals.

Sections containing the hippocampus separated by 250 μm in the coronal plane were rinsed in PBS for 30 min. Sections were then incubated in 0.5%, 1.0%, and 0.5% PBS buffered H_2O_2 for 30, 60, and 30 min, respectively. Sections were then rinsed in PBS for 30 min and incubated in a combination of N- and C-termini targeted DCX antibodies (1:250, goat polyclonal antibody in 5% normal horse serum, Santa Cruz Biotechnology, Santa Cruz, CA, USA) overnight at 4 °C. These sections were then washed in 0.05% Tween 20-PBS for 15 min and incubated for 60 min in biotinylated anti-goat IgG that was raised in rabbit and dissolved in 5% normal horse serum (1:500; Vector Laboratories, Burlingame, CA, USA). Sections were rinsed again in Tween 20-PBS for 15 min and then incubated 60 min in avidin-biotin solution (Vectastain Elite ABC Kit, Vector Laboratories). After a 15 min PBS rinse, the antibody–antigen conjugate was visualized by incubating for 5 min in 0.025% diaminobenzidine (DAB), 0.002% H_2O_2, and nickel ammonium sulfate. The reaction was halted in PBS followed by 30 min PBS rinse. The sections were mounted onto gel-coated glass slides, dried, counterstained with thionin, dehydrated and then coverslips were applied.

GFAP-immunohistochemistry

GFAP-immunohistochemistry was performed on coronal sections containing the hippocampus. These sections were first labeled

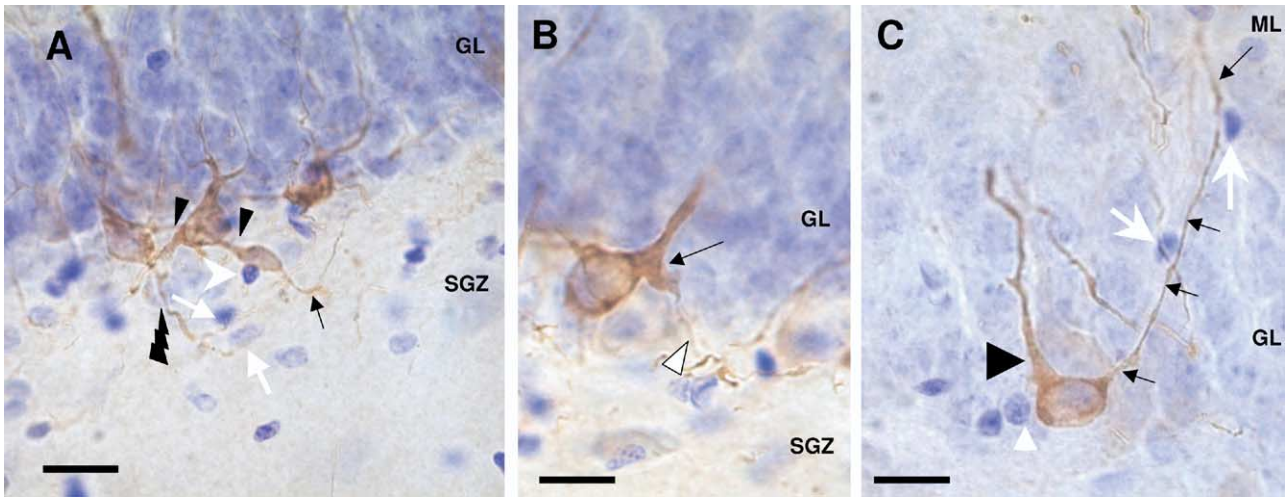

Fig. 1. Light photomicrographs of DCX-labeled NNs from adult control rats in the dentate gyrus. In A, NNs are shown in both the SGZ and at the base of the GL. Note the NN in the SGZ with its apical dendrite directed toward the GL (black arrowheads), its recurrent basal dendrite curving from the hilus toward the GL (black arrow), and a thionin-stained glial cell beneath its cell body (white arrowhead). Note that the NN to the left of this cell is located at the base of the GL and exhibits a basal dendrite (black lightning bolt) that loops back toward the GL, via three dendritic collaterals, two of which are adjacent to two glial cells (white arrows). In B, a NN is shown at the base of the GL that exhibits a bifurcating apical dendrite (black arrow). It is difficult to determine if the branch directed toward the SGZ is a dendrite or an axon (white arrowhead). In C, a NN is shown in the GL that has two apical dendrites, one that is thick (black arrowhead) and one that is thinner (black arrows). It should be noted that the thinner process lies adjacent to two glial cells (white arrows). Note that this NN in the GL lacks an apparent basal dendrite. However, it has a glial cell apposed to its base (white arrowhead). Scale bars=10 μm for A and 8 μm for B and C.

with DCX as described above. After the DAB reaction was halted, the sections containing NNs labeled with brown DAB reaction product were rinsed in PBS for 30 min. The sections were then incubated in GFAP antibody (1:1000, Sigma) rotating for 24 h at room temperature. These sections were then washed in PBS for 15 min and incubated for 60 min in biotinylated anti-rabbit IgG (1:500; Vector Laboratories) with 5% normal horse serum. Sections were again rinsed in PBS for 15 min and then incubated for 60 min in avidin–biotin solution (Vectastain Elite ABC Kit, Vector Laboratories). After a 15 min PBS rinse, the antibody–antigen conjugate was visualized by incubating for 2 min in Vector V.I.P. solution in order to obtain a purple reaction product for the GFAP.

Analysis of DCX-labeled basal dendrites

DCX-immunolabeled hippocampal sections were viewed with a Zeiss Axioplan light microscope. Images were captured with an Axiocam digital camera and prepared with Jasc Paint Shop Pro 8. Basal dendrites were examined for all DCX-labeled cells. As previously shown for control rats (Ribak et al., 2004), DCX-labeled basal dendrites arise from the base of the granule cell body and course along the hilar border. Some were shown to turn into the GL where they join the field of DCX-labeled apical dendrites.

The length of the basal dendrites was quantified by measuring only those dendrites that were observed arising from the basal portion of DCX-labeled cell bodies. The cells chosen for measurement had a well-defined cell body located either in the GL, the SGZ, or at the border between the GL and the SGZ. Images of the dentate gyrus were captured with a Zeiss Axioplan camera using either a 40× objective lens (NA=0.75) or a 100× oil immersion lens (NA=0.7). Both the supra- and infra-pyramidal blades were divided into four respective areas along each blade, and sequential images were used to catalogue results. For the quantitative analysis, a standard micrometer scale was photographed using the same 40× or 100× objective lens as used to capture the images. The image of the micrometer scale was then superimposed onto images of DCX-labeled basal dendrites to obtain linear measurements of their length. The length of the dendrite was determined by measuring the process from the site of origin at the soma, to the point where it was no longer visible or had terminated as a growth cone. Measurements were confirmed by two independent individuals and the final numbers from each were comparable.

In order to confirm these measurements and account for *x*, *y* and *z* axis measurements, 3-D reconstructions of 30 and 32 DCX-labeled NNs were analyzed from the epileptic (n=6/rat) and control (n=8/rat) rats, respectively. Image stacks were taken at 100× through 50 μm thick sections at 1 μm intervals using a microscope (Olympus BH-2) with an automated Z stage controlled by the Neurolucida system (MBF V.6.1). The main segment of basal dendrites was traced and the minor branches were excluded from analysis. The length and angle that the DCX-labeled NNs traveled through the hilus were calculated using Neuroexplorer (MBF V.6.1). The results from these analyses confirmed the significant differences reported from the two-dimensional analysis.

RESULTS

DCX-positive cells were typically found in the SGZ, at the border between the SGZ and the GL, or in the GL. Consistent with previous studies (Parent et al., 1997), epileptic rats had a greater number of NNs. Basal dendrites were observed on DCX immunolabeled NNs for both control and epileptic rats. Morphological analysis of the DCX immunolabeled cells with basal dendrites revealed that the NNs with basal dendrites

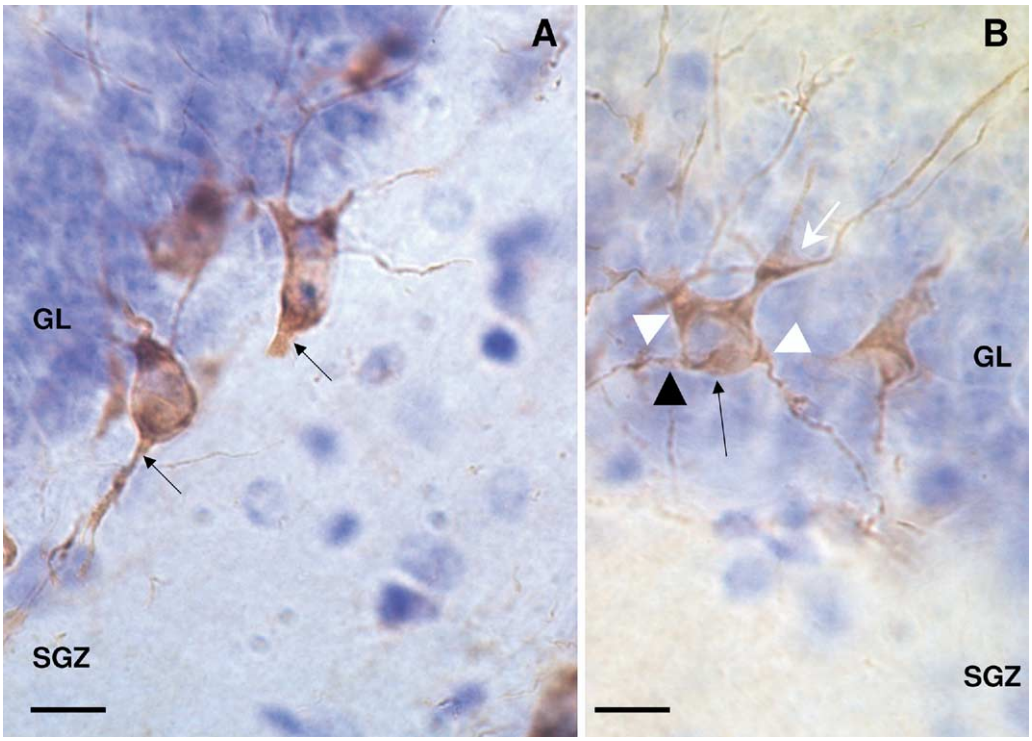

Fig. 2. Light micrographs of DCX-labeled NNs from adult epileptic rats. A shows two DCX-labeled NNs at the border between the SGZ and the GL. Both of these NNs have basal dendrites (black arrows) that extend from their cell bodies and one of these extends horizontally along the base of the GL. In B, a DCX-labeled NN (black arrow) located in the GL extends an apical dendrite that bifurcates (white arrow) into two thinner branches. Note that this same NN exhibits a basal process (black arrowhead) and two lateral processes (white arrowheads) extending from the cell body. Such morphology is rarely encountered in control animals. Scale bars=10 μm.

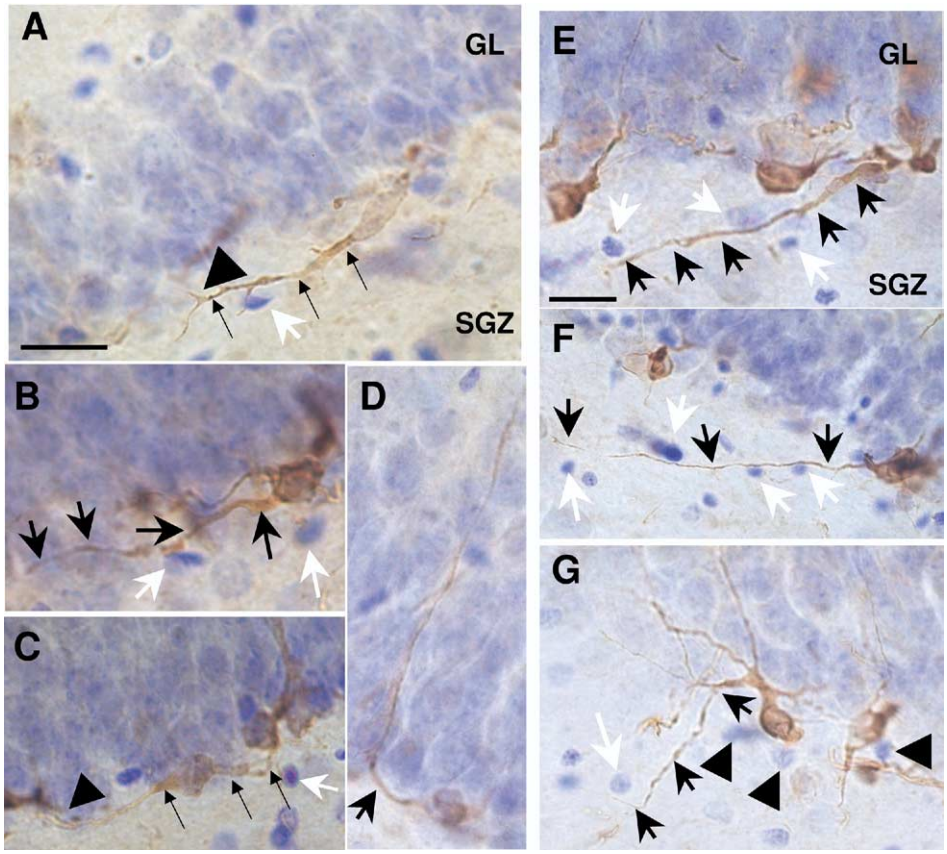

Fig. 3. A comparison of the morphology of DCX-labeled NNs and their dendrites from control (A–D) and epileptic (E–G) rats. A shows a bipolar DCX-labeled NN at the border between the SGZ and the GL. This NN has a basal process extending horizontally along the border of the GL (black arrows). Note that a glial cell (white arrow) is adjacent to a part of this process that also branches at this site. Another branch curves into the GL (arrowhead). In B, a NN is shown at the base of the GL that also exhibits a basal dendrite extending horizontally along the GL border (black arrows). Note the proximity of two glial cells (white arrows). C shows another bipolar NN with two basal dendrites that curve into the GL (black arrows) as described for recurrent basal dendrites. The one on the left curves into the GL further away from its cell body (black arrowhead) than the one on the right. Note the glial cell body at the elbow of the recurrent basal dendrite (white arrow). In D, a typical recurrent basal dendrite is shown (black arrow) that extends through the entire GL. In E, a DCX-labeled HBD is shown from an epileptic rat (black arrows). Note that glial cells (white arrows) are adjacent to both sides of this HBD along its length. F is another example of a DCX-labeled HBD (black arrows) from an epileptic rat that travels deep into the hilus. Note that once again, glial cells are adjacent to both sides of this HBD along its length. G is another example of a DCX-labeled NN with a HBD (black arrows) from an epileptic rat. The cell body of this NN is adjacent to glial cells (black arrowheads). In this case, the HBD arises from an apical dendrite with at least six branches that are directed in all directions. Note that this HBD (black arrows) displays a glial cell at its elbow. This relationship of an HBD arising from an apical dendrite was previously demonstrated by Spigelman et al. (1998), Fig. 4F. Other glial cells (black arrowheads) are adjacent to the NN cell bodies. Scale bars=8 μm A–D and 6 μm E–G.

have a distinctly different appearance in the epileptic animals compared with the controls (Figs. 1–3). Therefore, the morphology of the NNs from control rats will first be presented for reference, followed by the morphological appearance of the NNs with basal dendrites from epileptic rats.

NNs in the dentate gyrus of controls have basal dendrites adjacent to glial cells

Most of the DCX-labeled granule cells in the SGZ were located within 30 μm of the GL (Fig. 1). They often showed a fusiform cell body with its long axis oriented parallel to the GL (Fig. 1). Typically these bipolar cells had two processes, one extending from each end. Many dendrites remained in the SGZ, whereas others curved into the GL as described for recurrent basal dendrites (Figs. 1 and 3). In addition, both the cell bodies of NNs and their processes were frequently observed adjacent to glial cells (Fig. 1A). Double-labeling with DCX and GFAP revealed that DCX-labeled basal dendrites are frequently observed to be aligned along GFAP-expressing astrocytic processes.

The NNs found at the border between the SGZ and GL typically resemble the NNs found in the GL of the control rats. They have apical dendrites which extend toward the molecular layer and sometimes exhibit a basal dendrite that extends horizontally before turning into the GL (Figs. 1 and 3). The basal dendrites of the NNs in this location were also typically observed to be adjacent to GFAP-labeled astrocytic processes.

DCX-labeled cells in the GL displayed a different morphology from those in the SGZ. These cells showed either fusiform or round cell bodies. Most of these immunolabeled cells had apical dendrites extending through the GL. Some of the labeled DCX cells in the GL had basal dendrites that extended horizontally along the border of the GL

for up to 30 μm and then entered the GL and the molecular layer (Fig. 3). The basal dendrites could typically be observed adjacent to glial cells, whether they ran horizontal to, or curved into the GL (Fig. 3). It is pertinent to note that apical dendrites were also frequently observed adjacent to glial cells in the GL (Figs. 1 and 3).

NNs in epileptic rats have basal dendrites extending deep into the hilus

In pilocarpine-treated animals DCX-positive granule cells were found in the same three locations as described for the control rats, the SGZ, the hilar border and the GL (Figs. 2 and 3). These NNs had basal dendrites that projected from the cell body, some directed along the GL border and others that projected deep into the hilus (Fig. 3). DCX-labeled basal dendrites that projected deep into the SGZ were longer than those found parallel to the GL border. Basal dendrites could typically be observed adjacent to glial cells, regardless if they ran parallel to the GL, curved into the GL, or projected into the hilus (Fig. 3).

NNs found along the border of the SGZ and GL were also observed but had different dendritic morphology than those cells in the SGZ (Fig. 2). These NNs were distinct because of their location, relatively larger cell size and the long length of their dendrites. Similar to the control rats, some basal dendrites turned into the GL and were identified as recurrent basal dendrites. Others projected deep into the hilus and often entered the hilus at an angle greater than 30°. The DCX-labeled NNs and their basal dendrites were frequently observed adjacent to glial cells and processes (Fig. 4).

DCX-labeled granule cells in the GL were relatively larger with their cell soma diameter ranging between 6 and 12 μm. Most cells were oriented perpendicular with the GL and typically had one or more thick apical dendrites that projected toward the molecular layer (Fig. 2B). Those that did not have one or more thick processes had two or more smaller dendrites projecting from their cell body. These DCX-labeled NNs in the GL were also observed to have a basal dendrite that extended deep into the SGZ (Fig. 5B). However, these basal dendrites typically extended along

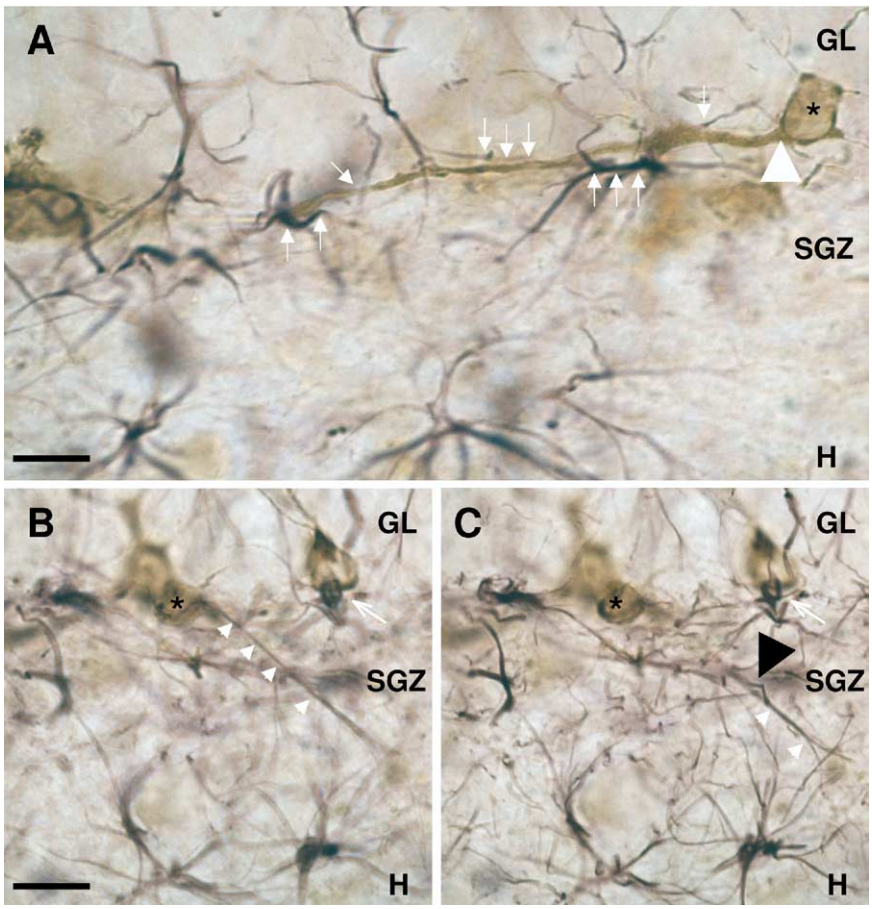

Fig. 4. Light micrographs of DCX-labeled NNs with basal dendrites adjacent to GFAP-labeled processes from epileptic rats. A shows a DCX-labeled NN (asterisk) at the border between the SGZ and the GL. Note the basal dendrite (white arrowhead) emanating from the basal portion of the cell body and extending horizontally along the base of the GL. GFAP-positive astrocytic processes can be seen along the extent of the basal dendrite with their processes (white arrows) adjacent to the basal dendrite. B and C represent two different planes of focus of a DCX-labeled NN (asterisk) located in the SGZ. Note the basal dendrite (white arrowheads) extending into the hilus. The close association of this dendrite with a GFAP-labeled process can best be visualized in C, where a kink occurs in this process (black arrowhead). Scale bars=8 μm.

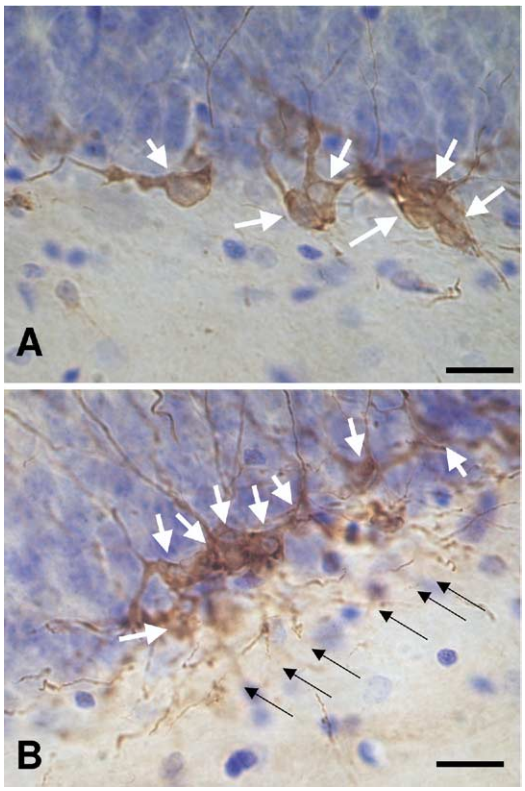

Fig. 5. Examples of clusters of cells from both control (A) and epileptic rats (B). In A, DCX-labeled NNs (white arrows) from a control rat are sometimes found in clusters of two or three NNs. Note that the majority of dendritic processes from these cells are orientated along the SGZ–GL border or enter the GL. B shows a cluster of NNs from an epileptic rat that is distinctly different from those in A. Note the extensive amount of processes extending from the basal sides of these NNs (black arrows). Note that the NNs in B are more densely packed into the clusters and display many more dendritic processes as compared with the control rats. Scale bar=8 μm.

the border between the GL and SGZ (Fig. 2B). These HBDs did not typically branch in the GL and were often found adjacent to glial cells (Fig. 3E and F).

NNs from epileptic rats have significantly longer HBDs

At low magnification where groups of cells are observed, the controls show only a few processes extending into the SGZ (Fig. 5A), while the epileptic rats show many processes extending into the hilus forming a plexus (Fig. 5B). At high magnification we identified and measured the length of the basal dendrites from isolated DCX-labeled cells (Fig. 3). The mean length of the basal dendrites from NNs in the control rats was 7.36 μm (±1.5 S.D.) and 17.28 μm (±3.2 S.D.) in the epileptic rats. Quantitative analysis using a Student's *t*-test revealed that the length of the basal dendrites in the epileptic rats was significantly longer compared with control rats ($t=6.7$, $P<0.05$). A graph of the means and standard error bars is shown in Fig. 6A. Analysis of the percentage of basal dendrites entering the hilus at an angle greater than 30°, revealed that <2% of the NNs in the control rats exhibit this morphology, whereas 20% of the NNs in the epileptic rat do. It is pertinent that of the <2% of basal dendrites that did enter the hilus in the control animals, all but two of these were observed to turn back toward the GL as described by Ribak et al. (2000) for recurrent basal dendrites (See Fig. 1A). This is in contrast to epileptic animals where the basal dendrites project deep into the hilus (Fig. 3E–G).

Three-dimensional reconstruction of 36 (four/animal) DCX-labeled NNs with basal dendrites using an automated Z-stage controlled by the Neurolucida system and analyzed using Neuroexplorer confirmed the differences in the length of the basal dendrites. In addition, Neuroexplorer was used to calculate the angle of the basal dendrites and these analyses confirmed that at least 20% of the NNs with basal dendrites from the pilocarpine-treated rats entered the hilus at an angle greater than 30°, compared with the less than 2% of NNs from control rats. In addition, the three-dimensional analysis revealed that the DCX-labeled HBDs from the pilocarpine-treated rats typically zigzagged deeper into the hilus, and glial cell bodies were frequently observed adjacent to the HBD at the point where the dendrite curves (Fig. 7).

The mean length of the basal dendrites from NNs in the control rats was 18.39 μm (±2.6 S.D.) and 66.64 μm (±2.2 S.D.) in the epileptic rats. Quantitative analysis of the length of the basal dendrites from 3-D reconstructions was done using a Student's *t*-test. The results confirmed that the length of the basal dendrites in the epileptic rats was significantly longer compared with control rats ($t=23.225$, $P<0.001$). A graph of the means and standard error bars is shown in Fig. 6B.

Glial cells are adjacent to DCX-labeled dendrites of NNs

In both the control and epileptic rats, every basal dendrite has at least one or more glial cells adjacent to it. This arrangement does not appear to be random because as shown in Fig. 3, glial cells are adjacent to alternating sides of HBDs from an epileptic rat. Further examination of DCX-labeled dendrites in the control and epileptic rat often showed apposition to glial cells in the hilus (Figs. 1A and C, 3 and 4), as well as in the molecular layer (not shown). Consistent with previous studies (Seki and Arai, 1999; Shapiro et al., 2005) the cell bodies of DCX-labeled NNs were also apposed to glial cells (Fig. 3).

DISCUSSION

The results from this study demonstrate that the newly born granule cells from epileptic rats have significantly longer HBDs and that they enter the hilus at an angle greater than 30° from its cell body. In addition, glial cells are often adjacent to these HBDs in the hilus (Fig. 3E and F). Consistent with previous data (Ribak et al., 2004), most of the basal dendrites on granule cells from control rats remain at the hilar border before entering the GL as recurrent basal dendrites. When basal dendrites from control NNs enter the hilus they form a loop as described previously by Dashtipour et al. (2002) and often display a glial cell at the curve (Figs. 1A and 3C). Also consistent with previous studies is that NNs were typically

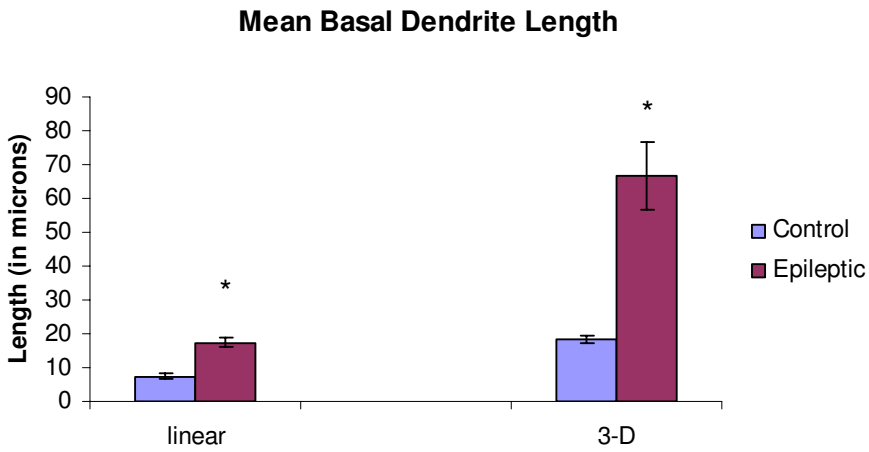

Fig. 6. Graph of the mean lengths of basal dendrites on DCX-labeled NNs in control and epileptic rats. In A, linear analysis of the basal dendrites using a Student's *t*-test revealed that the length of the basal dendrites from NNs in the epileptic rats (X=17.28 μm) was significantly longer than that in the control rats (X=7.36 μm). * P=0.022. In B, three-dimensional analysis of the basal dendrites using a Student's *t*-test confirmed that the length of the basal dendrites from NNs in the epileptic rats (X=66.64 μm) was significantly longer than that in the control rats (X=18.39 μm). * P<0.001. Error bars are S.E.M.

found in clusters. In the control rats, these clusters are relatively loosely packed and their processes typically run along the hilar border or in the GL (Fig. 5A). In contrast, the clusters in the epileptic rats are densely packed and the NNs have dendritic processes that form a dense plexus in the hilus (Fig. 5B).

Our findings of DCX-immunolabeled granule cells with HBDs in epileptic rats are consistent with the hypothesis

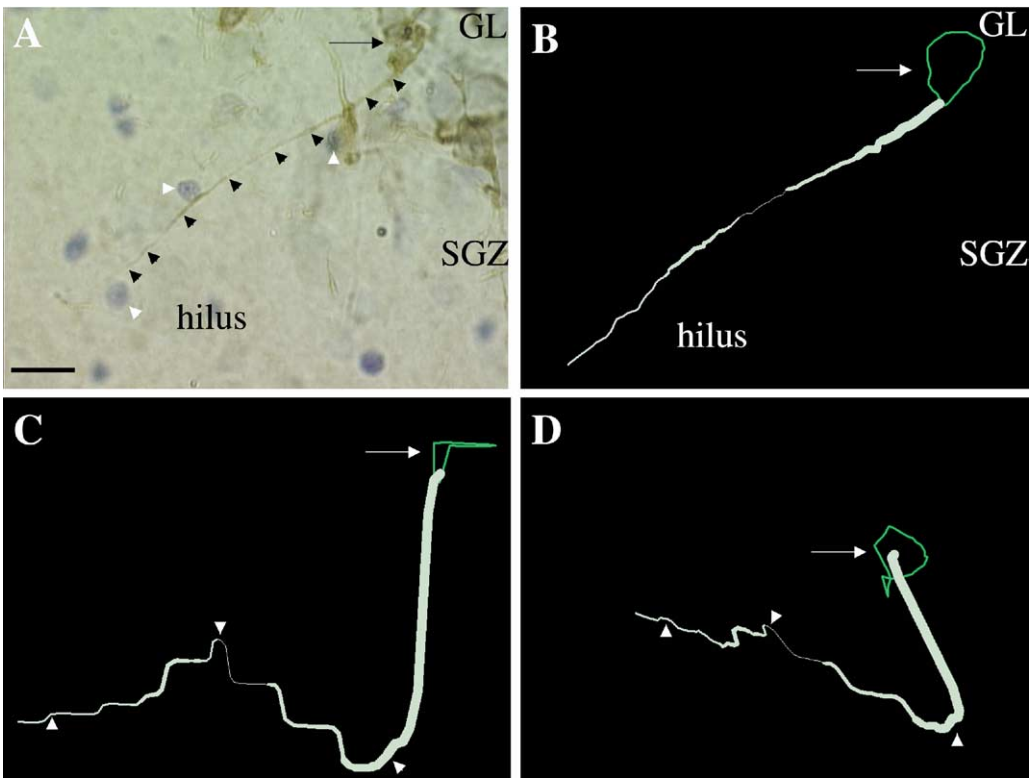

Fig. 7. 3-D reconstruction of a DCX-labeled HBD from a pilocarpine-induced epileptic rat. In A, a light photomicrograph is shown depicting a NN (arrow) with an HBD (black arrowheads). Note that there are three glial cell bodies (white arrowheads) adjacent to the HBD. In B, a two-dimensional tracing of the DCX-labeled NN and HBD from A is shown. Linear analysis of this dendrite showed that it measured 51.8 μm. Linear measurement in the two-dimensional plane underestimates the actual length of the dendrite because it does not take into account the *x*, *y* and *z* axis. In C and D, the three-dimensional tracing of this DCX-labeled NN with a HBD is shown in two different orientations in order to demonstrate how the dendrite traverses through the tissue. The measured length of this dendrite taking into account the *x*, *y* and *z* axis is 86.2 μm. Note that the three glial cell bodies (white arrowheads) seen in A are located at some of the HBD curves. Scale bar=8 μm.

that many granule cells with HBDs are NNs. Previous studies using several different labeling methods for NNs in control and epileptic rats showed that the base of the GL is the location of these NNs (Parent et al.1997; Cameron and McKay, 2001). Further data to support this hypothesis were obtained by Dashtipour et al. (2003), where biocytin labeling at several time points following epilepsy, showed the presence of HBDs on granule cells as early as 1 week following the induction of status epilepticus. It should be noted that such granule cells were located at the GL-SGZ border and had HBDs with similar morphology as that shown in the present study (cf. Dashtipour et al., 2003, Fig. 2C and present study, Fig. 3E).

These findings raise an important question: Why do HBDs course into the hilus in epileptic rats? There are several possibilities that involve changes in the hilus of epileptic rats, such as: neuronal loss, gliosis, increased neurogenesis and synaptic plasticity. The first two changes are observed in the hilus and are thought to be causally related (i.e. hilar neuronal loss is followed by hypertrophy of astrocytes and gliogenesis; Mello et al., 1993). It should be noted that DCX-labeled apical dendrites and growth cones of NNs in control rats are apposed to the processes of radial glial cells running perpendicular through the GL (Shapiro et al., 2005). Because the radial glial cell scaffold in the GL guides the apical dendrites of granule cells to grow toward the molecular layer, we suggest that HBDs in epileptic rats, grow aberrantly into the hilus using an ectopic glial scaffold that forms in the hilus subsequent to neuronal cell loss in this region. Evidence for this suggestion is derived from the appearance of glial cell bodies adjacent to curved portions of HBDs in the hilus of epileptic rats (Fig. 3). Moreover, DCX-labeled HBDs are typically observed adjacent to GFAP-labeled astrocytic processes (Fig. 4). Further ultrastructural studies are needed to confirm this relationship for HBDs and glial cells.

Another possible factor for the formation of HBDs is that they are targeted by the robust sprouting of mossy fibers that occurs in the epileptic rats (Sutula et al., 1989; Tauck and Nadler, 1985). It is likely that these mossy fibers are actively seeking synaptic targets, and subsequently, the basal dendrite of the NN is encountered in the hilus. Ribak et al. (2000) showed that mossy fibers synapse on HBDs in epileptic rats, and it is reasonable to suggest that this aberrant synaptogenesis contributes to the survival and elongation of HBDs (Shapiro and Ribak, 2005). Thus, we provide two possible mechanisms (ectopic glial scaffold and mossy fiber sprouting) that contribute to the basal dendrite not retracting and not curving into the GL as it does in the control.

CONCLUSIONS

Our data show that NNs from epileptic rats have HBDs that are longer than those in control rats. The fact that DCX-labeled HBDs from epileptic rats are longer than those for control rats, is consistent with previous findings that HBDs are not transient structures in epileptic rats. Also, these HBDs lie adjacent to glial cells in the hilus. The formation of these HBDs might relate to an altered glial scaffold, as well as to the sprouting mossy fiber phenomenon previously documented for the dentate gyrus. In the future, we plan to determine whether HBDs form in the absence of neurogenesis following seizures by blocking mitotic division in the dentate gyrus following induction of status epilepticus.

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