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Newly born dentate granule neurons after pilocarpine-induced epilepsy have hilar basal dendrites with immature synapses

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Abstract

Neurogenesis in the subgranular zone of the dentate gyrus persists throughout the lifespan of mammals, and the resulting newly born neurons are incorporated into existing hippocampal circuitry. Seizures increase the rate of neurogenesis in the adult rodent brain and result in granule cells in the dentate gyrus with basal dendrites. Using doublecortin (DCX) immunocytochemistry to label newly generated neurons the current study focuses on the electron microscopic features of DCX-labeled cell bodies and dendritic processes in the dentate gyrus of rats with pilocarpine-induced epilepsy. At the base of the granule cell layer clusters of cells that include up to six DCX-labeled cell bodies were observed. The cell bodies in these clusters lacked a one-to-one association with an astrocyte cell body and its processes, a relationship that is typical for newly born granule cells in control rats. Also, DCX-labeled basal dendrites in the hilus had immature synapses while those in control rats lacked synapses. These results indicate that increased neurogenesis after seizures alters the one-to-one relationship between astrocytes and DCX-labeled newly generated neurons at the base of the granule cell layer. The data also suggest that the synapses on DCX-labeled hilar basal dendrites contribute to the persistence of hilar basal dendrites on neurons born after pilocarpine-induced seizures.

Keywords: Newly born neurons; Hippocampal dentate gyrus; Pilocarpine-induced seizures; Adult neurogenesis

1. Introduction

Adult neurogenesis persists in the adult mammalian dentate gyrus, and these newborn neurons survive and become incorporated into the existing hippocampal circuitry (Markakis and Gage, 1999; Hastings and Gould, 1999; van Praag et al., 2002). Many experimental conditions can alter the rate of neurogenesis including seizures (Parent et al., 1997; Scott et al., 1998). Seizures also alter the proliferation rate of glial cells (Niquet et al., 1994; Huttmann et al., 2003).

Several reports suggested that newly generated neurons in the dentate gyrus undergo a programmed progression of dendritic process outgrowth as they migrate from the SGZ into the granule cell layer (Jones et al., 2003; Shapiro and Ribak, 2005; Shapiro et al., 2005a). One of these processes appears to arise from the apical portion of the cell, is aligned along a radial glial process

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(Seki and Arai, 1999; Shapiro et al., 2005a) and typically projects toward the molecular layer as the apical dendrite. In addition to an apical dendrite, 31–55% of newly born dentate granule cells in the adult dentate gyrus 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 granule cell layer as recurrent basal dendrites (Dashtipour et al., 2002; Yan et al., 2001). However, it is not known whether these basal dendrites grow along a glial scaffold in the same way for that of the apical dendrites.

Recently, Shapiro et al. (2005b) reported that newly generated neurons from epileptic rats have longer basal dendrites compared to those from control rats. In addition, the basal dendrites projected deep into the hilus, as compared to those in the control rats, where the basal dendrites typically coursed horizontally along the base of the granule cell layer. Because previous studies have not examined synapses on these basal dendrites, electron microscopy was used to assess synapses on basal dendrites from control and pilocarpine-induced epileptic rats. This ultrastructural analysis used preparations immunolabeled for doublecortin (DCX). DCX is a microtubule associated protein expressed by newly generated neurons from ~3 h to 3 weeks after they are generated (Brown et al., 2003; Kempermann et al., 2003) and has been demonstrated to be an excellent marker to elucidate the morphology of the immature neurons because it is expressed in the perikaryal cytoplasm as well as within both axonal and dendritic processes and their growth cones (Nacher et al., 2001; Friocourt et al., 2003; Rao and Shetty, 2004; Ribak et al., 2004; Shapiro et al., 2005a). Therefore, the goal of the present study was to examine the DCX-labeled cell bodies and their apical and basal dendrites in the dentate gyrus of epileptic rats to determine whether their relationship with astrocytes is similar to that for those from adult control rats.

2. Methods

2.1. Animals

Adult male, Sprague–Dawley rats ($n = 28$; 450–600 g; Charles River) were used. All protocols were approved in advance by the Institutional Animal Care and Use Committee at the University of California at Irvine. Experimental rats ($n = 16$) 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 (SE) (Turski et al., 1983). SE was terminated after 3 h with two separate injections of Nembutal (pentobarbital sodium; 25 mg/kg, i.p.) 10 min apart.

Sham ($n = 4$) and untreated control rats ($n = 8$) were used for comparison. The sham animals were first injected with methylscopolamine in saline solution (1 mg/kg, i.p.), followed by a saline injection substituted for pilocarpine. These 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. Both the sham and control rats from this experiment had similar results and will be referred to collectively as controls. All rats were monitored for 30 days after injections and the presence of spontaneous seizures in the pilocarpine-injected rats was verified using a video monitoring system.

At 30 days after the injections, all 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.

2.2. DCX-immunoelectron microscopy

Sections containing the dentate gyrus were rinsed in PBS for 30 min and then incubated in 0.5%, 1.0%, and 0.5% PBS buffered H_2O_2 for 30, 60, and 30 min, respectively. Sections were next rinsed in three PBS baths for 10 min each, then incubated rotating in anti-DCX (1:500, goat polyclonal antibody in 5% normal horse serum, Santa Cruz Biotechnology Inc., 8066 and 8067) at 4 °C for 24 h. Following this primary incubation, the sections were washed in 0.05% Tween-20 in PBS for 15 min. Sections were then incubated

for 60 min in secondary antibody (1:500, biotinylated anti-goat IgG, raised in rabbit, in 5% normal horse serum, Vector Laboratories), followed by a 15 min rinse in Tween-20 in PBS. The avidin biotin complex (ABC) (Vectastain Elite ABC Kit, Vector Laboratories) incubation was for 60 min and the ABC was visualized using 0.025% diaminobenzidine with 0.002% hydrogen peroxide in PBS. The reaction was halted using PBS after 4 min and washed for 15 min in PBS. In addition, control sections were reacted without the primary DCX antibody, to verify antibody specificity. No reaction product was observed at either the light or electron microscopic level in these control sections. Several sections from each animal were mounted onto glass slides, counterstained with thionin, dehydrated, and then coverslips were applied, in order to verify labeling in serial sections prepared in the same batch and intended for electron microscopy.

Following the DCX immunocytochemical processing, hippocampal blocks containing DCX-positive cells were obtained from the above described sections and post-fixed in 1% glutaraldehyde for 1 h, then rinsed in PBS and placed in 1% osmium tetroxide for 20–60 min, and dehydrated by ethanol and propylene oxide immersion. A flat-embedding procedure was used after which the tissue block was trimmed using a single-edged razor blade under a dissecting microscope (Nikon). A short series of ultrathin (60–80 nm) sections containing the dentate gyrus from each block was cut with an ultramicrotome (Reichert-Jung) and sequential sections were collected on mesh and formvar-coated slot grids. The sections were stained with uranyl acetate and lead citrate to enhance contrast. Sections containing granule cells and the hilus were examined with a Philips CM-10 transmission electron microscope and images of DCX-labeled somata and processes were captured with a Gatan digital camera.

2.3. Analysis of DCX-labeled dendritic processes

Any DCX-labeled dendrites in the granule cell layer were classified as apical dendrites even if they were not traced back to their cell body of origin. For the analysis of the basal dendrites, only DCX-labeled dendrites in the SGZ were included in the analysis. An example of one of these at the light microscopic level is shown in Fig. 1.

2.4. Analysis of DCX-labeled cells within clusters and their relationship with glial cells

The DCX-labeled cells within the clusters were quantified and an analysis was performed on several serial thin sections from each cluster. A total of eight clusters from pilocarpine-injected rats and eight clusters from control rats containing DCX-labeled cells were assessed. Within these clusters, the DCX-labeled cell bodies were analyzed for the percentage of glial apposition by measuring the somal circumference of the cell bodies and determining the portion apposed by astrocytes. An example of one of these clusters from an epileptic rat is shown at the light microscopic level in Fig. 1.

2.5. Stereological quantification of synapses on DCX-labeled basal dendrites

To determine if more synapses occurred on DCX-labeled basal dendrites from the pilocarpine-induced epileptic rats, a stereological method was used that employed the double dissector (Sterio, 1984). This method allowed us to estimate the numbers of axodendritic synapses on DCX-labeled basal dendrites in the hilus. Using this method, the counts are independent of the shape, size or orientation of the objects being counted (synapses). Images of basal dendrites were captured from 11 epileptic and 8 control rats. Some animals were excluded from our stereological analysis because the fixation and reaction product in thin sections appeared insufficient to allow us to resolve membranes on DCX-labeled basal dendrites. Between 15 and 20 grids from each animal were examined, and each grid contained at least three thin sections. A Gatan digital camera was used to capture the images from each of three serial sections per Formvar-coated slot grid. Initially, a low magnification electron micrograph was captured followed by a photomontage (if necessary) and a grid with equally spaced points was placed over the low magnification image of the DCX-labeled basal dendrites to be used as sampling points. A simple computer program was written to randomly select points on the grid (numbered 1–50). A grid size was selected so that at least five sampling points from each basal dendrite could be examined for synapses. For every five grid squares that fit on the DCX-labeled dendrite, three points were selected at random. At each

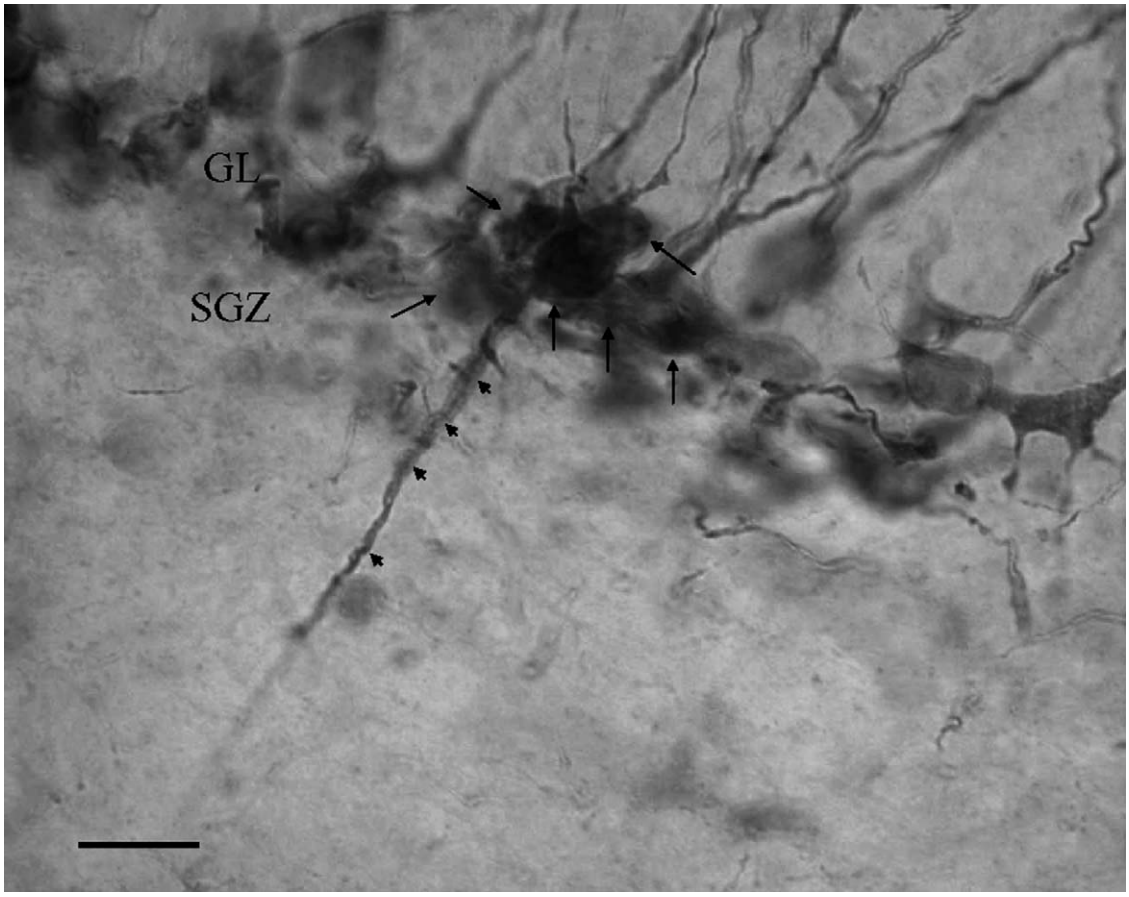

Fig. 1. Light photomicrograph of a cluster of DCX-labeled cell bodies (arrows) at the base of the granule cell layer (GL) and a hilar basal dendrite (arrowheads) that arises from one of the cells in the cluster and extends into the subgranular zone (SGZ). It is necessary to use electron microscopy to resolve the number and glial apposition of DCX-labeled cell bodies in these clusters. Electron microscopy is also necessary to identify any synapses on the hilar basal dendrite. Scale bar = 10 μm.

analysis point selected, an area of approximately 1 μm^2 was photographed on three serial sections to serve as the reference and look-up sections. The identification of axodendritic synapses on these DCX-labeled basal dendrites used the following criteria: a membrane bound profile that was apposed to the DCX-labeled basal dendrite and contained three or more synaptic vesicles in the presynaptic terminal with at least one of these vesicles located adjacent to a synaptic cleft with parallel pre- and postsynaptic membranes (Murray and Goldberger, 1986). Synapses were marked on the reference section, and then it was compared with the look-up section. Synapses that were observed in the reference but were not observed in the look-up section were counted. Then, the reference and look-up sections were switched, and the process was repeated. A Mann–Whitney *U* nonparametric analysis of variance test was used to assess the effects of seizures on the frequency of synapses. These data provided an estimate of the prevalence of axodendritic synapses on DCX-labeled basal dendrites in the dentate gyrus.

3. Results

3.1. Control rats

The description of DCX-immunolabeled cells in the adult dentate gyrus was similar to that previously reported by Shapiro et al. (2005a). DCX-labeled cells

with no processes in the SGZ were typically round or elliptical and 4–6 μm in diameter, and were adjacent to astrocyte cell bodies in a one-to-one relationship. The cell body of the astrocyte in such pairs was located within 10 μm of the basal lamina of the capillary. DCX-labeled cell bodies at the base of the granule cell layer had a rudimentary apical process, were adjacent to the cell body of an astrocyte, and were wrapped on their basal and lateral sides by astrocytic processes from the adjacent astrocyte. DCX-labeled cells within the granule cell layer were also found adjacent to an astrocyte and the growth cone of their apical dendritic process was adjacent to the radial process of the adjacent astrocyte (Fig. 2). These data are consistent with previous data (Shapiro et al., 2005a) showing that newly born granule cells in the SGZ are cradled by radial glial cells and their processes and that at the base of the granule cell layer the newly born neurons extend an apical dendrite along a radial glial process.

DCX-labeled dendrites from control rats were analyzed for synapses. No synapses were observed on the DCX-labeled dendrites in the SGZ. These dendrites are

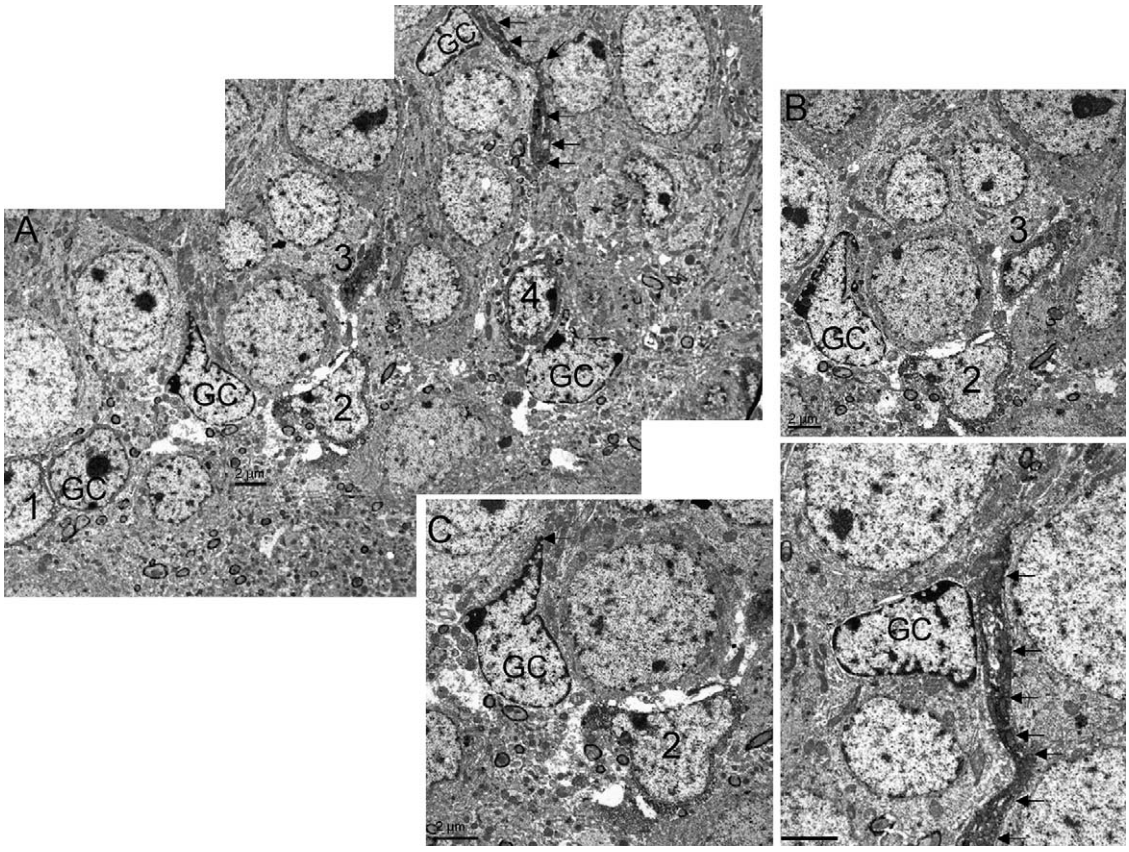

Fig. 2. Electron micrographs from a control rat showing several DCX-labeled cells within one field of view, each associated with an astrocyte. In (A), a photomontage shows four DCX-immunolabeled cells 1–4. Note that astrocytes (GC) are adjacent to cells 1, 2, and 4. It is pertinent to note that cell 3 does not have its nucleus in the plane of this section, nor is its adjacent astrocyte cell body present. Cell 4 has an apical dendrite (arrows) that branches in the granule cell layer (GL) near an astrocyte cell body (GC). (B) Cells 2 and 3 in a serial section where the nucleus of cell 3 is present. Note that cell 3 has its own astrocytic process surrounding 75% of its somal surface. The cell body of this astrocyte is not within the plane of this section. Also note the intimate relationship between cell 2 and the cell body of the astrocyte (GC) to its left. (C) Enlargement of cell 2 and its associated astrocyte (GC). Note that this astrocyte has a sawtooth nucleus with its dense chromatin-filled tip (arrow) pointing radially towards the granule cell layer. (D) The DCX-labeled apical dendrite (arrows) from cell 4 that is adjacent to an astrocyte (GC) cell body in the GL. This apical dendrite is filled with mitochondria suggesting that it is a filipodia of a growth cone. Also note that this dendrite has no synapses along its full extent, and that it is enveloped by glial processes (not visible at this magnification). Scale bars = 2 μm for all figures.

assumed to be transient basal dendrites as described by Jones et al. (2003) and Ribak et al. (2004). In addition, no synapses were found on DCX-labeled dendrites in the granule cell layer. Instead, radial glial processes were adjacent to most of the DCX-labeled processes interpreted to be apical dendrites.

Because Seri et al. (2004) described DCX-labeled D cells in clusters of two to four cells at the base of the granule cell layer, DCX-labeled cells that appeared at this location were analyzed in the present study. Occasionally, DCX-labeled cells were observed in a group containing up to four DCX-immunolabeled cells. However, each of these DCX-labeled cells was separated from the others and had its own astrocyte adjacent to it (Fig. 2). Serial section analysis revealed that the astrocytic processes from their adjacent astrocyte cell body enveloped 70% (mean value) of the cell body of DCX-labeled cells in these groups. Thus, the DCX-labeled cells within these groups maintain a one-to-one relationship with an astrocyte as described previously for individual DCX-labeled cells in this location (Shapiro et al., 2005a).

3.2. DCX-labeled cells from pilocarpine-induced epileptic rats

DCX-immunolabeled cells in the dentate gyrus from epileptic rats were different from those observed from

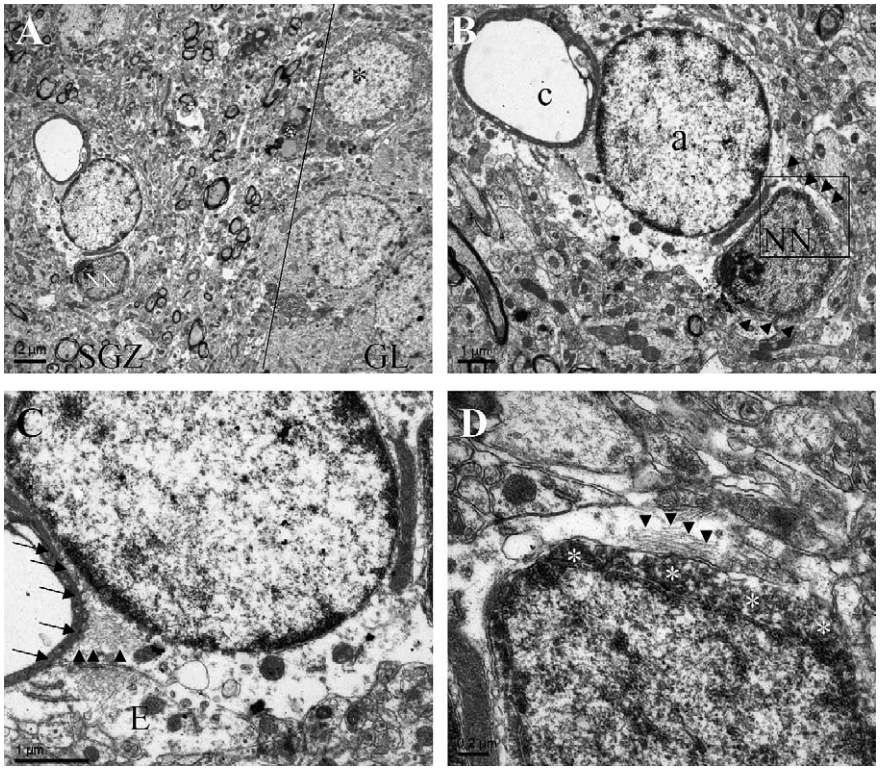

Fig. 3. Electron micrographs of a DCX-labeled cell (NN)–astrocyte (a) pair from an epileptic rat that is adjacent to a capillary in the subgranular zone (SGZ). (A) The location of this NN–astrocyte pair next to a capillary and its proximity to the granule cell layer (GL) on the right. Note the line that separates the GL from the SGZ. Also, note a second DCX-labeled cell (black asterisk) in the upper right portion of the micrograph. This latter cell lacks the one-to-one astrocyte–NN relationship that is seen in the controls. (B) Enlargement of the capillary (c) and the adjacent astrocyte cell body (a) that displays two cytoplasmic extensions (arrowheads) that surround or cradle the DCX-labeled cell (NN). (C) The direct apposition of the astrocyte cell body to the basal lamina (arrows) of the capillary wall. Both the cell body and the adjacent swollen glial endfoot (E) display bundles of glial filaments (arrowheads). (D) Enlargement of the boxed area in (B) to show part of this astrocyte's process apposed to a portion of the DCX-labeled cell. Note immunoreaction product in the perikaryal cytoplasm (white asterisks) of the DCX-labeled cell and glial filaments (arrowheads) in the astrocytic process. Scale bars = 2 μm for (A), 1 μm for (B) and (C), and 0.2 μm for (D).

control rats. First, DCX-labeled cell–astrocyte pairs in the SGZ were adjacent to the basal lamina of capillaries. Also, DCX-labeled cells in the clusters at the base of the granule cell layer from epileptic rats had significantly less ($P < 0.05$) glial apposition than that observed for those from control rats. In fact, these DCX-labeled cells lacked the one-to-one relationship with an astrocyte as described for control rats (Shapiro et al., 2005a). Moreover, DCX-labeled cells in the dentate gyrus of epileptic rats had hilar basal dendrites with synapses on them, whereas no such synapses were found for those from control rats. These differences will be described below.

In the SGZ, DCX-immunolabeled cells were typically round and 4–6 μm in diameter. They were found adjacent to astrocyte cell bodies that were apposed to the basal lamina of capillaries (Fig. 3A–C). The cell body of such astrocytes adjacent to the basal lamina was closely apposed to the DCX-labeled cell body and wrapped up to 74% of the surface of the DCX-labeled cell. Both the astrocyte cell body and processes contained the characteristic bundles of glial

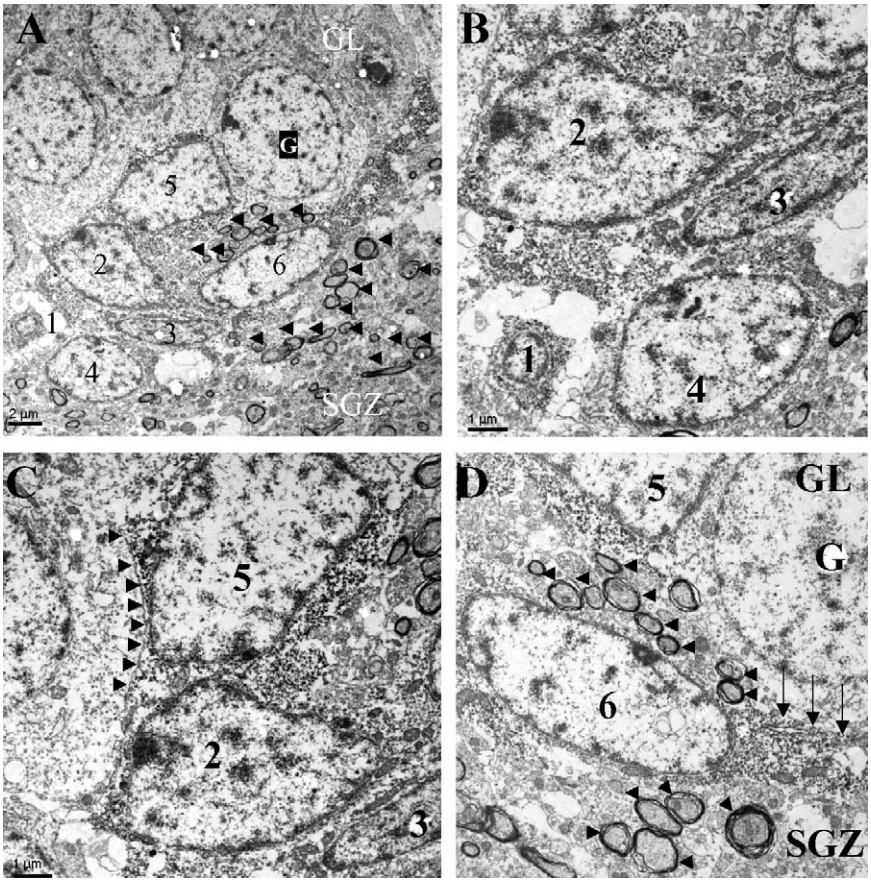

Fig. 4. Electron micrographs of a cluster of six DCX-labeled cells 1–6 at the base of the granule cell layer (GL) from an epileptic rat. In (A), all six cells appear at low magnification to show their location between the GL and the subgranular zone (SGZ). Note several myelinated axons (arrowheads) in the SGZ and the mature granule cell (G). (B) Cells 1–4, and each cell body of which has a distinctly different shape than the other cells instead of all being round. (C) Enlargement of cells 2, 3, and 5. Note the rectangular shape of the soma of cell 5. Also, note a thin astrocyte process surrounding one edge of cells 2 and 5 (arrowheads). No glial cells intervene between cells 2 and 5 in this section. (D) Cell 6 separated by a group of myelinated axons (arrowheads) from cell 5. Also note that cell 6 has an oval nucleus with a DCX-labeled dendritic process (arrows) extending horizontally along the border between the GL and the SGZ. Note the lack of an adjacent radial process from an astrocyte for this DCX-labeled dendrite (cf. Fig. 1C). Scale bars = 2 μm in (A) and 1 μm in (B–D).

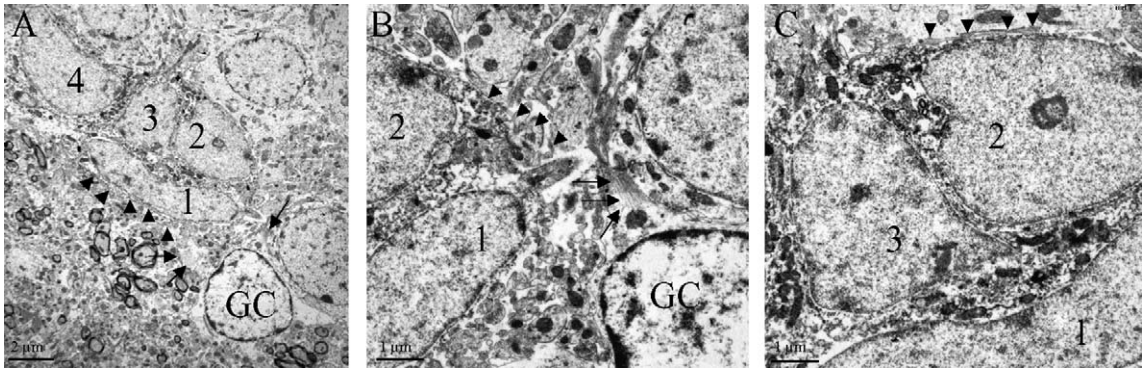

Fig. 5. Electron micrographs of another cluster of DCX-labeled cells at the base of the granule cell layer from an epileptic rat. In (A), the cell body of an astrocyte (GC) extends processes that appose the lateral sides of the four DCX-labeled cells 1–4 in this cluster. Note the myelinated axons in the subgranular zone. Also note the glial filaments (arrows) in the astrocyte cell body and the glial process (arrowheads) apposed to the base of cell 1. (B) Enlargement of a portion of the astrocyte (GC) to demonstrate the glial filaments (arrows) and shows its apposition (arrowheads) to cell 2. Note that no intervening glial process is found between cells 1 and 2. (C) Enlargement of cells 1–3 to demonstrate the direct apposition between these cell bodies and the lack of any intervening astrocytic processes. Scale bars = 2 μm in (A) and 1 μm in (B) and (C).

filaments (Fig. 3C and D). Because of the close juxtaposition of the astrocyte cell body and basal lamina there were no classical astrocytic endfeet (Peters et al., 1991) observed for the astrocyte in the pair at this location. Moreover, glial endfeet (assumed to be from other glial cells) adjacent to this astrocyte cell body appeared hypertrophied (Fig. 3C). The DCX-labeled cell in this pair had minimal perikaryal cytoplasm (Fig. 3D) and no apparent processes. The presence of the DCX-labeled cell–astrocyte pair adjacent to capillaries is different than that in controls where this pair was located 5–10 μm from the basal lamina of capillaries.

At the base of, and within the granule cell layer, clusters containing up to six DCX-labeled cells were observed (Figs. 4 and 5). These DCX-labeled cells appeared in the single sections as either round or fusiform. The round ones were 2–7 μm in diameter while the fusiform ones were 2–3 μm across their short axis and 6–8 μm across their long axis (Fig. 4A). Within these clusters, some of the DCX-labeled cells were apposed to each other with little or no intervening astrocytic processes (Fig. 5C). The individual DCX-labeled cells within the cluster lacked a one-to-one relationship with an astrocyte cell body and its processes (Fig. 5A). However, some portions of the surface of these cells were apposed by a glial process (Fig. 5B). The mean value of this apposition was only 27%. Serial section analysis showed that this process emanated from an adjacent astrocyte cell body that was apposed to a small portion of the cluster of DCX-labeled cells (Fig. 5A and B). It should be noted that the extent of astrocyte apposition to a single DCX-labeled cell was significantly less ($P < 0.05$) than the 70% observed in control rats in this location.

Additionally, single DCX-labeled cells were also found scattered throughout the SGZ and granule cell layer. These cells also lacked any apparent one-to-one relationship with an astrocyte cell body (Fig. 3A, asterisk). It is pertinent to note that with the exception of the DCX-labeled cells that are adjacent to the astrocytes that are apposed to the basal lamina or capillaries, all of the others observed in this study (within or outside of the clusters) lacked the one-to-one astrocyte–newly born neuron relationship that is observed in control rats (Shapiro et al., 2005a).

3.3. Synapses on DCX-labeled dendrites from epileptic rats

DCX-labeled apical and basal dendrites were apposed by glial membranes to varying extents and were aspiny (Figs. 6 and 7). No synapses were found on the DCX-labeled apical dendrites within the granule cell layer (Fig. 6A and B). In contrast, DCX-immunolabeled basal dendrites had synapses that were found in the hilus. These synapses often appeared immature in that there were few synaptic vesicles in

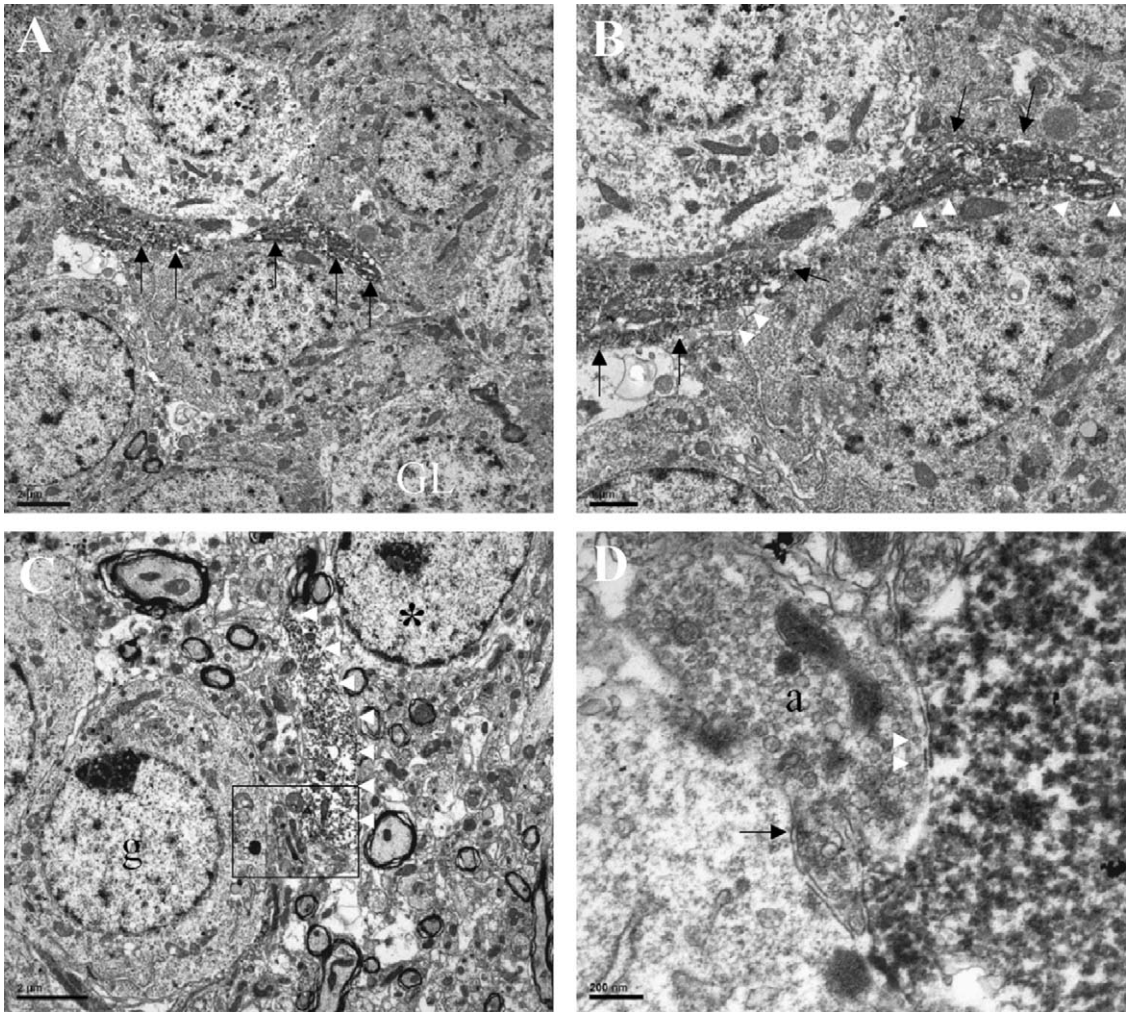

Fig. 6. Electron micrographs of DCX-labeled apical and basal dendrites from epileptic rats. (A) A DCX-labeled apical dendrite (arrows) that extends into the granule cell layer (GL). (B) Enlargement of (A), and shows that the DCX-labeled apical dendrite (arrows) is adjacent to a thin astrocytic process (white arrowheads). In (C), a DCX-labeled basal dendrite in the SGZ is adjacent to a granule cell (g). Note the astrocyte (asterisk) in the upper right hand corner of the micrograph. The boxed area is shown at higher magnification in (D) to demonstrate the synapse formed by an axon terminal. In (D), the synapse is shown at higher magnification from a serial section. Vesicles (white arrowheads) are accumulated within the axon terminal (a) at the pre-synaptic membrane of this axodendritic synapse on the DCX-labeled basal dendrite. Note that the axon also synapses on the adjacent granule cell body (arrow). Scale bars = 2 μm in (A) and (C), 1 μm in (B), and 0.2 μm in (D).

the presynaptic profile (Fig. 7) and the pre- and post-synaptic membranes were parallel for only a short length (Blue and Parnavelas, 1983). Mann–Whitney *U* nonparametric analysis of variance test of the stereological data revealed that there were significantly more synapses on basal dendrites from epileptic rats compared to control rats ($z = 1.982$, $P < 0.05$, one-tailed test). It should also be noted that there were no synapses found on the DCX-labeled cell bodies from either the epileptic or control rats.

4. Discussion

The present electron microscopic study of DCX-labeled cells demonstrates two major findings. First,

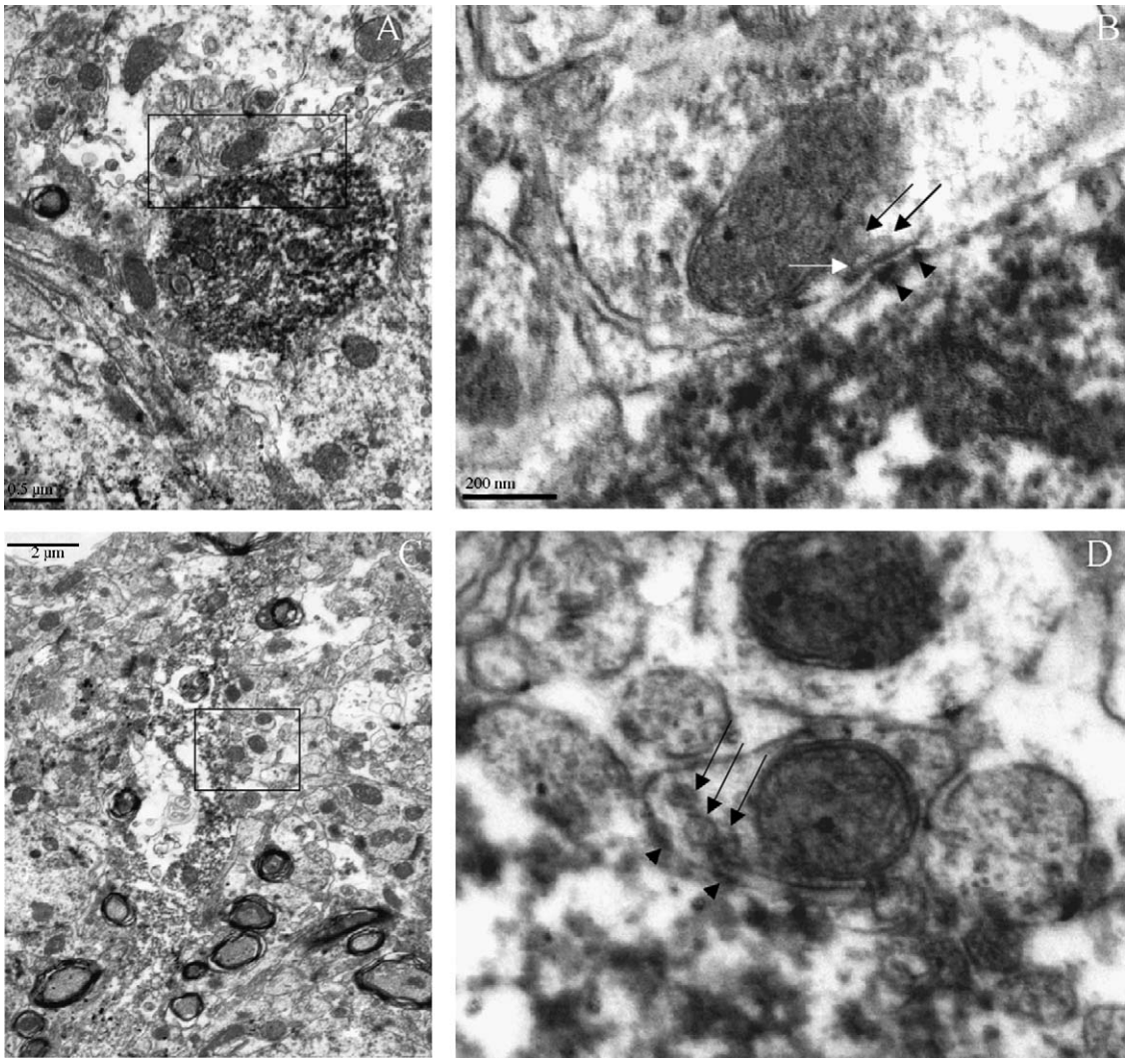

Fig. 7. Electron micrographs of two additional synapses on DCX-labeled basal dendrites in the subgranular zone from epileptic rats. (A and B) A cross-sectioned DCX-immunolabeled basal dendrite that is postsynaptic to an axon terminal with a mitochondrion in it. The box in (A) is enlarged in (B) to show three synaptic vesicles (arrows) between the presynaptic membrane and the mitochondrion. Note the enhanced electron density of the synapse (arrowheads) associated with an active zone. Scale bars = 0.5 and 0.2 μm, respectively. (C and D) Another example of an axodendritic synapse on a DCX-labeled hilar basal dendrite. This dendrite is transversely sectioned and the boxed area in (C) is enlarged in (D) and rotated 90° counterclockwise to show three synaptic vesicles (arrows) in this small axon terminal. The enhanced electron density of the presynaptic membrane is associated with the two synaptic vesicles (arrows) apposed to the presynaptic membrane. Scale bar in (C) = 2 μm and in (D) = 0.2 μm.

DCX-labeled basal dendrites in the dentate gyrus of epileptic rats had synapses on them. Second, the DCX-labeled cells at the base of the granule cell layer from epileptic rats often appeared in a cluster with no intervening processes of astrocytes. Although light microscopy has been used in the past to study newly generated neurons from rats with pilocarpine-induced seizures (Parent et al., 1997; Radley and Jacobs, 2003; Shapiro et al., 2005b), the use of electron microscopy in the present study provides the ability to resolve these fine details of DCX-labeled cells and adjacent astrocytes.

4.1. Significance of synapses on hilar basal dendrites in epileptic rats

The presence of synapses on DCX-labeled basal dendrites in the hilus, albeit immature ones, indicates an important morphological difference between epileptic and control rats. This difference may provide a basis for the formation of hilar basal dendrites that are found following epileptic seizures (Spigelman et al., 1998; Ribak et al., 2000; Buckmaster and Dudek, 1999). It is likely that the axon terminals forming these synapses on DCX-labeled basal dendrites are sprouted mossy fiber collaterals because Ribak et al. (2000) identified mossy fibers synapsing on hilar basal dendrites using biocytin labeling. These aberrant synapses were shown to provide the basis for additional recurrent excitatory circuitry following epilepsy (Ribak et al., 2000; Austin and Buckmaster, 2004). Thus, the data support a previous hypothesis that newly formed synapses on DCX-labeled basal dendrites in the hilus contribute to their persistence in epileptic rats (Shapiro and Ribak, 2005). Note that the synapses shown are exclusively onto dendritic shafts and no DCX-labeled spines were observed, while those in Ribak et al. (2000) were onto both dendritic spines and shafts. Therefore, these DCX-labeled dendrites are immature. It should also be noted that no synapses were observed in the present study on DCX-labeled basal dendrites from control rats. In addition, the portion of the DCX-labeled apical dendrites in the granule cell layer from both control and epileptic rats lacks synapses probably because this region is mainly surrounded by astrocytic processes, and these radial glial processes appear to act as a scaffold for apical dendritic outgrowth (Seki and Arai, 1999; Shapiro et al., 2005a). Furthermore, the radial glia cell that is cradling the newly born neuron and providing a scaffold for process outgrowth in the control rat might also prevent synapses from forming on the basal and lateral sides of its cell body.

The physiological significance of the formation of hilar basal dendrites and their mossy fiber synapses has been previously shown to increase excitatory responses from granule cells in pilocarpine-treated rats (Okazaki et al., 1999). It is possible that this mechanism is indirectly related to the increased neurogenesis observed following seizures (Parent et al., 1997). Weissman et al. (2005) reported that spreading calcium waves can induce ATP-mediated radial glial cell proliferation during development. Because GFAP-expressing radial glia-like cells in the adult brain are the neuronal precursor cells (Mignone et al., 2004; Garcia et al., 2005), it is possible that they are induced to proliferate as a result of the increased calcium bursts occurring in the epileptic brain. Another possibility is that an increase in transient precursor cell proliferation contributes to this phenomenon (Jessberger et al., 2005). It is also possible that some of the elevated trophic factors that are induced following seizures (Binder et al., 2001) contribute to increased neuronal differentiation and survival relative to that of astrocytes leading to the lack of the one-to-one astrocyte–newly born neuron relationship at the base of the granule cell layer in epileptic rats, as well as the formation of hilar basal dendrites on the newly born neurons (Danzer et al., 2002).

4.2. Clusters of DCX-labeled cells in epileptic rats

In the present study we describe clusters of three to six DCX-labeled cell bodies that are surrounded by a glial wrapper at the base of the granule cell layer from epileptic rats (Fig. 3A). Within these clusters, only 27% of the somal surface of each DCX-labeled cell is apposed by an astrocyte, as compared to 70% found in control rats. Moreover, a one-to-one relationship between DCX-labeled cells and an adjacent astrocyte was not observed for DCX-labeled cells in clusters from the epileptic rats. Previous studies have identified clusters containing newly born neurons, astrocytes and apoptotic cells in control rats at the light and confocal microscopic levels (Seki and Arai, 1999; Palmer et al., 2000; Seki, 2002; Kempermann et al., 2003; Filippov et al., 2003), as well as at the electron microscopic level (Seki, 2002; Seri et al., 2004; Shapiro et al., 2005a). Seki (2002) did not examine whether a one-to-one relationship with a DCX-labeled newly born neuron and a glial cell existed within their cluster. Although Palmer et al. (2000) described doublets, they did not identify the cells in this pair as a newly generated neuron and an astrocyte. Seri et al. (2004) reported that neurons within the clusters are surrounded by glial processes, but they never reported a percentage of somal surface apposed by glial, nor did they identify the one-to-one relationship between each newly born neuron and an astrocyte. In the present study and in an earlier one (Shapiro et al., 2005a), electron microscopy showed

that DCX-labeled cells in control rats each had an associated astrocyte adjacent to it. Therefore, the clusters of DCX-labeled cells at the base of the GL in epileptic rats indicate an alteration.

It is pertinent to note that a recapitulation of developmental mechanisms might occur following various types of neurodegenerative events and seizures (Emery et al., 2003; Haas et al., 2004). The seizure-induced increase in neurogenesis may alter cell–cell interactions (Mendis et al., 2000), resulting in clusters that lack the one-to-one astrocyte–newly born neuron relationship. Perhaps the DCX-labeled cells in these clusters regain a glial relationship as they mature.

Using video time-lapse microscopic preparations, Noctor et al. (2004) and Garcia et al. (2005) showed that during development and in the adult, neurons are derived from GFAP-expressing radial glial cells. Furthermore, Noctor et al. (2004) described symmetric and asymmetric radial glial cell division during development that gives rise to newborn daughter cells. At certain time points and locations, daughter cells were shown to differentiate into transit amplifying cells in the developing neocortex and they acted as progenitors for newly born neurons (Noctor et al., 2004). Based on ultrastructural features of progenitor cells (Seri et al., 2001, 2004), some of the DCX-labeled cells in the clusters from epileptic rats display the features of precursor cells. Therefore, it is suggested that the expansion of the progenitor cell population after seizures alters the one-to-one numerical relationship between astrocytes and DCX-labeled cells.

4.3. Altered radial glia–newly born neuron relationship may contribute to aberrant dendritic growth after seizures

In the control rat, the normal one-to-one relationship between a DCX-labeled newly born neuron and a radial glial cell may be essential for the normal growth profile of the newly born neuron (Shapiro et al., 2005a). For example, the apical dendrite of the granule cell grows along the radial glial process in the GL (Seki and Arai, 1999; Shapiro et al., 2005a). This relationship is not afforded by the lack of the one-to-one relationship for the DCX-labeled cells within the clusters from epileptic rats. Might this contribute to the growth of basal dendrites from newly generated granule cells? The data from the current study do not directly address this issue. However, it was hypothesized that hilar basal dendrites found after seizures result from a lack of the appropriate radial glial cell relationship where astrocytes provide guidance for the process outgrowth of the newly generated neurons (Shapiro and Ribak, 2005).

Additional data in support of this hypothesis are derived from two other studies in the adult rodent hippocampus. In the first, a subset of GFAP-expressing astrocytes was shown to express the extracellular matrix (ECM) glycoprotein SPARC, which is thought to be important in influencing the differentiation of newly generated neurons (Mendis et al., 1995). The second study showed that ECMs are altered following reactive astrogliosis (Mendis et al., 2000), and is pertinent to our data because GFAP-positive reactive astrogliosis occurs following seizures (Represa et al., 1993; Garzillo and Mello, 2002), and hypertrophied astrocytes with swollen endfeet were observed in the present study. Thus, alterations to the one-to-one newly born neuron–radial glial cell relationship in the present study may contribute to the ectopic growth of basal dendrites into the hilus where they are subject to aberrant synaptogenesis.

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