Title
GFAP-expressing radial glia-like cell bodies are involved in a one-to-one relationship with doublecortin-immunolabeled newborn neurons in the adult dentate gyrus

Journal
Brain Research, 1040(1-2)

ISSN
1385-299X

Authors
Shapiro, Lee A
Korn, Matthew J
Shan, Zhiyin
et al.

Publication Date
2005-04-01

DOI
10.1016/j.brainres.2005.01.098

Available online at www.sciencedirect.com
SCIENCE DIRECT
Brain Research 1040 (2005) 81–91
BRAIN RESEARCH
www.elsevier.com/locate/brainres

Research report

GFAP-expressing radial glia-like cell bodies are involved in a one-to-one relationship with doublecortin-immunolabeled newborn neurons in the adult dentate gyrus

Lee A. Shapiro, Matthew J. Korn, Zhiyin Shan, Charles E. Ribak*

Department of Anatomy and Neurobiology, College of Medicine, University of California, Irvine, CA 92697-1275, USA

Accepted 21 January 2005
Available online 9 March 2005

Abstract

The present study examined the relationship between radial glial cells and newborn neurons in the adult dentate gyrus using three different methods. Single labeling immunocytochemistry for newly born neurons using doublecortin, as well as double labeling using an additional antibody to glial fibrillary acidic protein (GFAP) to label astrocytes were used at the light microscopic level. Furthermore, doublecortin immunoelectron microscopy was used to examine the ultrastructural relationship between newborn neurons and astrocytes in the adult dentate gyrus. These data showed an intimate one-to-one relationship between GFAP-expressing radial glia-like cell bodies and their non-radial processes that wrap around the basal and lateral sides of newborn neurons to cradle them in the subgranular zone. A similar relationship is observed for the newborn neurons at the base of the granule cell layer, but the cell body of the GFAP-expressing radial glia-like cells is not as intimately associated with the cell body of the newborn neurons at this site. Furthermore, newborn neurons with apical dendritic processes and growth cones in the granule cell layer extend them along radial glial processes. These newborn neurons do not receive axosomatic or axodendritic synapses indicating the absence of basket cell innervation. These data show that GFAP-expressing radial glia-like cells in the dentate gyrus cradle newborn neurons in the subgranular zone and that their radial processes provide a scaffold for neuronal process outgrowth.

Theme: Development and regeneration
Topic: Genesis of neurons and glia

Keywords: Hippocampus; Dentate gyrus; Granule cell layer; Adult neurogenesis; Radial glial cell; Astrocyte

1. Introduction

Granule cell neurogenesis occurs throughout the lifespan of mammals and can be observed along the border of the dentate granule cell layer (GL) with the hilus. Previous studies have shown newly born granule cells in the subgranular zone (SGZ) of the dentate gyrus, and later, these neurons are found in the GL [7,9]. Recent studies have shown that these granule cells born in the adult rat become integrated into neural circuitry and mature into functional neurons [3,22].

Doublecortin (DCX) is a microtubule associated protein expressed in newly born neurons (NNs) for the first 3 weeks of their life, and studies show DCX antibody to be a reliable marker for newborn neurons [2,7,14]. DCX labeling is observed in the perikaryal cytoplasm of migrating neurons, as well as in the growth cones of their growing axons and dendrites [11,15]. In addition, Kempermann et al. [8] using DCX-labeling recently reported NNs with no processes in the adult dentate gyrus. Filippov et al. [5] also showed that these NNs exhibit physiological properties of immature neurons.

Abbreviations: GL, Granule cell layer; DCX, Doublecortin; NN, Newly born neuron; SGZ, Subgranular zone; GFAP, Glial fibrillary acidic protein

* Corresponding author. Fax: +1 949 824 8549.
E-mail address: ribak@uci.edu (C.E. Ribak).

0006-8993/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2005.01.098

It is pertinent to the present study to note that radial glial cells persist in the adult dentate gyrus [4] and are present in cell clusters containing NNs in the adult dentate gyrus [19]. Moreover, astrocytes have been shown to be neuronal precursor cells in the adult dentate gyrus [19]. Recently, Alvarez-Buylla and Lim [1] have reviewed the features of NNs derived from the SGZ in the adult brain. However, there have not been detailed electron microscopic studies of the relationship between newly born dentate granule cells and radial glial cells in the adult brain. The present study using light and electron microscopy attempts to examine this relationship.

2. Methods

2.1. Animals

Thirteen adult male (4–6 months) Sprague–Dawley derived rats (400–550 g; Simonsen, Gilroy, CA) were used in this study. All protocols were approved in advance by the Institutional Animal Care and Use Committee at the University of California at Irvine. Rats were injected with an overdose of Nembutal (sodium pentobarbital; 50 mg/kg, i.p.), monitored until deeply anesthetized, and then perfused intracardially with 150 ml of saline followed by 200–300 ml of 4% paraformaldehyde in 0.1 M phosphate buffered saline (PBS). Brains were post-fixed for 48 h and 50 μm vibratome sections were collected.

2.2. DCX immunocytochemistry

Sections containing the dentate gyrus were rinsed in PBS for 30 min 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 at 4 °C for 24 h in anti-DCX (1:500, goat polyclonal antibody in 5% normal horse serum, Santa Cruz 8066 and 8067). Following 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 Labs) followed by a 15 min rinse in Tween-20 in PBS. The avidin biotin complex (ABC) (Vectastain Elite ABC Kit, Vector Labs) incubation was for 60 min and the ABC was visualized using 0.025% diaminobenzidine (DAB) 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. Sections were mounted onto glass slides, counterstained with thionin, dehydrated, and then coverslips were applied. In addition, adjacent sections were used for double immunolabeling with a glial fibrillary acidic protein (GFAP) antibody to observe intermediate filaments of astrocytes at the light microscopic level.

2.3. GFAP immunocytochemistry

Sections used for double-labeling with GFAP had excellent brown DAB reaction product for DCX. Several sections from 3 rats were rinsed for 30 min in PBS. Then, the sections were incubated on a rotator for 24 h in GFAP antibody (1:1000, rabbit polyclonal antibody in 5% normal goat serum, Sigma Chemicals) at 4 °C. After which, the sections were washed in 0.05% Tween-20 in PBS for 15 min. Sections were then incubated for 1 h in a secondary antibody (1:500, biotinylated anti-rabbit IgG in 5% normal goat serum, Sigma Chemicals) followed by a 15 min rinse in Tween-20 in PBS. The ABC (Vectastain Elite ABC Kit, Vector Labs) incubation was for 1 h and the entire complex was visualized using the Vector VIP kit (Vector Labs) that results in a purple reaction product. This latter reaction was stopped after 1 min by rinsing in distilled H_2O for 1 min and then in PBS for 15 min. These double labeled sections were mounted onto gel coated slides, dehydrated in alcohol, cleared with xylene, and then coverslips were applied. Light microscopic analysis of the double labeled sections showed purple (VIP) reaction product within astrocytes and their processes and brown (DAB) reaction product in the NNs and their processes.

2.4. Electron microscopy

Following the DCX immunocytochemical processing, hippocampal blocks containing DCX-positive NNs were post-fixed in 1% glutaraldehyde for 1 h, rinsed in PBS, placed in 1% osmium tetroxide for 20–60 min, and then dehydrated by immersion in ethanol and propylene oxide. 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 and analyzed (including measuring somal circumference and glial apposition) with a Gatan digital camera.

2.5. Dentate gyrus anatomy

The hilus, granule cell, and molecular layers were identified using known morphological features [6]. Briefly, the GL was identified by the presence of granule cells forming a layer 5–6 cells deep. The molecular layer was distinguished by the thick apical dendrites arising from

granule cell bodies. In contrast, the hilus was identified by large polymorphic neurons, myelinated axons, several capillaries, and DCX-immunolabeled somata and processes.

3. Results

3.1. Light microscopy of DCX-labeled NNs in the dentate gyrus

The distribution of DCX immunolabeled granule cells in the dentate gyrus was similar to that previously described [7,11,15]. DCX-positive cells were found in the SGZ, at the border of the GL and in the GL. Most of the DCX-labeled granule cells in the SGZ were located within 30 μm of the GL (Fig. 1). They either had a round cell body with no processes (Figs. 1A–D) or a fusiform cell body with two processes and its long axis oriented parallel to the GL (not shown). The round cell bodies of DCX-labeled NNs in the SGZ were confirmed by using a through focus analysis (see Figs. 1A–D). In addition, the cell bodies of NNs were frequently observed adjacent to glial cell bodies (Figs. 1A–D).

The NNs found at the border between the SGZ and GL either have apical dendrites that extend into the GL (Fig. 1E)

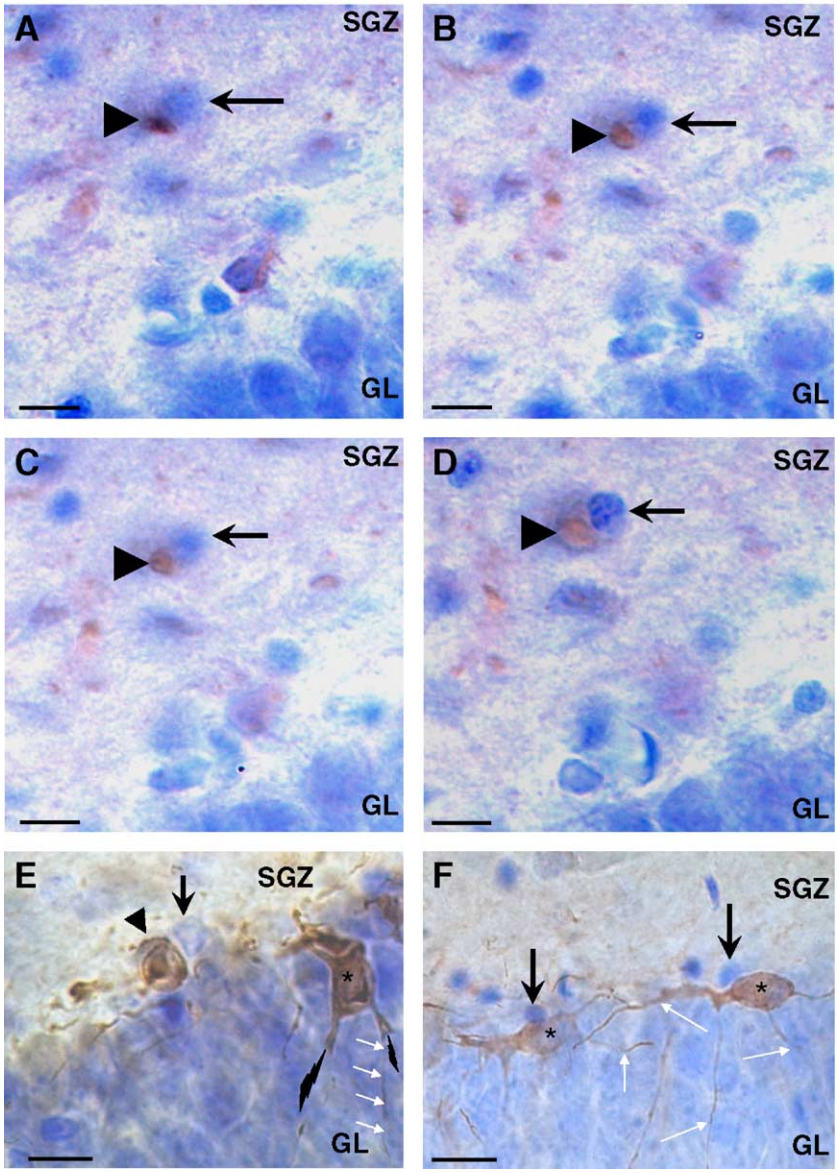

Fig. 1. Light photomicrographs of DCX-labeled NNs with adjacent thionin-stained glial cells. Panels A–D show a through focus series of a DCX-labeled NN (arrowhead) with no visible processes and an adjacent glial cell (arrow) in the subgranular zone (SGZ). Note the dense chromatin pattern of the nucleus of the glial cell in panel D where it is optimally focused. In panel E, a DCX-labeled NN (arrowhead) is adjacent to a glial cell (large black arrow) at the base of the GL. Note that the other labeled NN in this panel (asterisk) is in the GL and has two apical dendritic growth cones with lamellipodia (lightning bolts) and filopodia (white arrows). In panel F, 2 DCX-labeled NNs (asterisks) in the GL are shown with adjacent glial cells (black arrows). Note that these NNs have elaborate processes (white arrows) extending into the GL. Scale bars for panels A–D = 5 μm, panels E and F = 8 μm.

or basal dendrites that extend horizontally before turning into the GL (Fig. 1F). These NNs and their basal dendrites were typically observed to be adjacent to glial cells.

DCX-labeled cells in the GL showed either fusiform or round cell bodies. Most of these immunolabeled cells had one or two apical dendrites with growth cones extending into the GL (Fig. 1E). Some of the DCX-labeled cells in the GL also had basal dendrites that extended horizontally along the border with the SGZ for up to 30 μm and then entered the GL and the molecular layer (not shown). Once again, DCX-labeled NNs had glial cells adjacent to them (Fig. 1F). The relationship between glial cell bodies and DCX-labeled NNs was further examined in double-labeled preparations.

3.2. NNs are partially enveloped by the non-radial processes of GFAP-expressing radial glia-like cells

To determine the type of glial cells apposed to NNs in Fig. 1, double-labeled preparations were examined. The double-labeled sections showed purple reaction product to indicate GFAP-immunoreactivity within astrocytes and their processes and brown reaction product in the DCX-positive NNs and their processes, when present (Fig. 2).

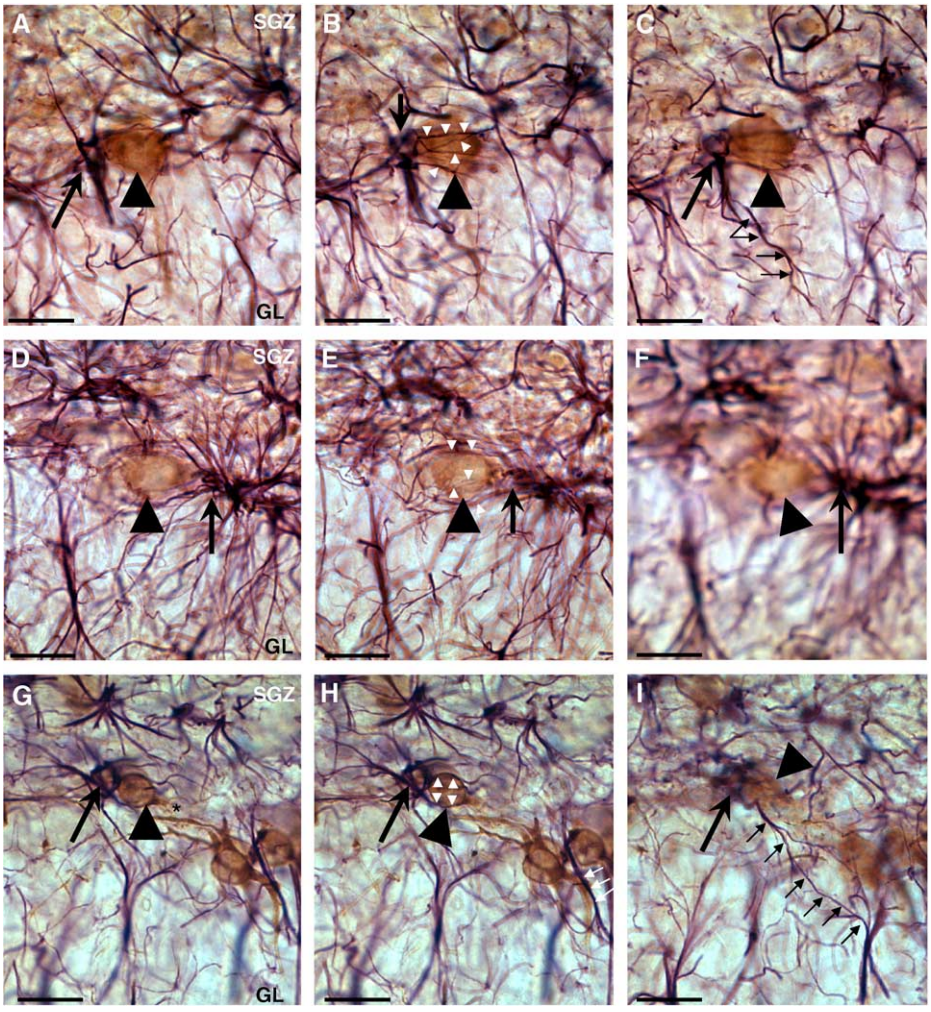

Fig. 2. Light photomicrographs of double labeled preparations showing DCX-positive NNs (brown) and GFAP-positive astrocytes (purple). In panels A–C, a DCX-labeled NN (black arrowhead) that lacks processes is shown in three planes of focus at the border between the subgranular zone (SGZ) and granule cell layer (GL). This NN is adjacent to a GFAP-immunolabeled triangular shaped cell body of an astrocyte (large arrow). In panel B, fine GFAP-immunolabeled bundles (white arrowheads) emanate from the area of the astrocyte cell body and wrap around the NN. In panel C, a GFAP-immunolabeled radial glial process (small arrows) arising from the labeled astrocyte extends through the GL (see label in panel A). It is pertinent to note that GFAP stains only the intermediate filaments of the glial cytoskeleton whereas the entire glial cytoplasm is not visualized using GFAP antibody. Electron microscopy will show details of this glial cell–NN relationship in subsequent figures. Panels D–F show a second through focus series of a DCX-labeled NN (black arrowhead) that lacks processes and is adjacent to a GFAP-immunolabeled astrocyte cell body (large arrow). In panel E, fine GFAP-immunolabeled bundles (white arrowheads) surround the NN. Panels G–I show a third through focus series of a DCX-labeled NN (black arrowhead) that has a rudimentary process that displays a lamellipodia (asterisk) with two filopodia. A GFAP-immunolabeled astrocyte (large black arrow) is adjacent to this NN. In panel H, fine GFAP-immunolabeled bundles (white arrowheads) wrap around the NN. Note that this astrocyte has a radial process (small arrows) extending through the GL in panel I. Another GFAP-positive radial process (white arrows) is adjacent to an apical dendrite of another DCX-labeled NN in panel H. Scale bars = 8 μm in panels A–F and 10 μm in panels G–I.

A consistent observation was the wrapping of NNs by the GFAP-immunolabeled bundles arising from astrocytic cell bodies (Fig. 2). Through focus microscopic analysis was performed to demonstrate that the astrocytes adjacent to NNs had round or triangular-shaped cell bodies outlined by GFAP-immunolabeling (Fig. 2). It should be noted that many of the NNs observed in these pairs lacked any apparent processes and were enveloped by the non-radial GFAP-positive processes arising from a GFAP-positive glia cell body (Figs. 2A–F). The NNs at the base of the GL had rudimentary processes emerging from the pole of the NN opposite to the pole apposed by the GFAP-positive cell body (Fig. 2G). The GFAP-positive astrocytes associated with the NNs in this location also had radial processes extending through the GL (Figs. 2C,I). DCX-positive apical dendrites of NNs in the GL were closely aligned with GFAP-positive radial processes (Fig. 2H). The labeling of the glial cells with GFAP and the fact that they

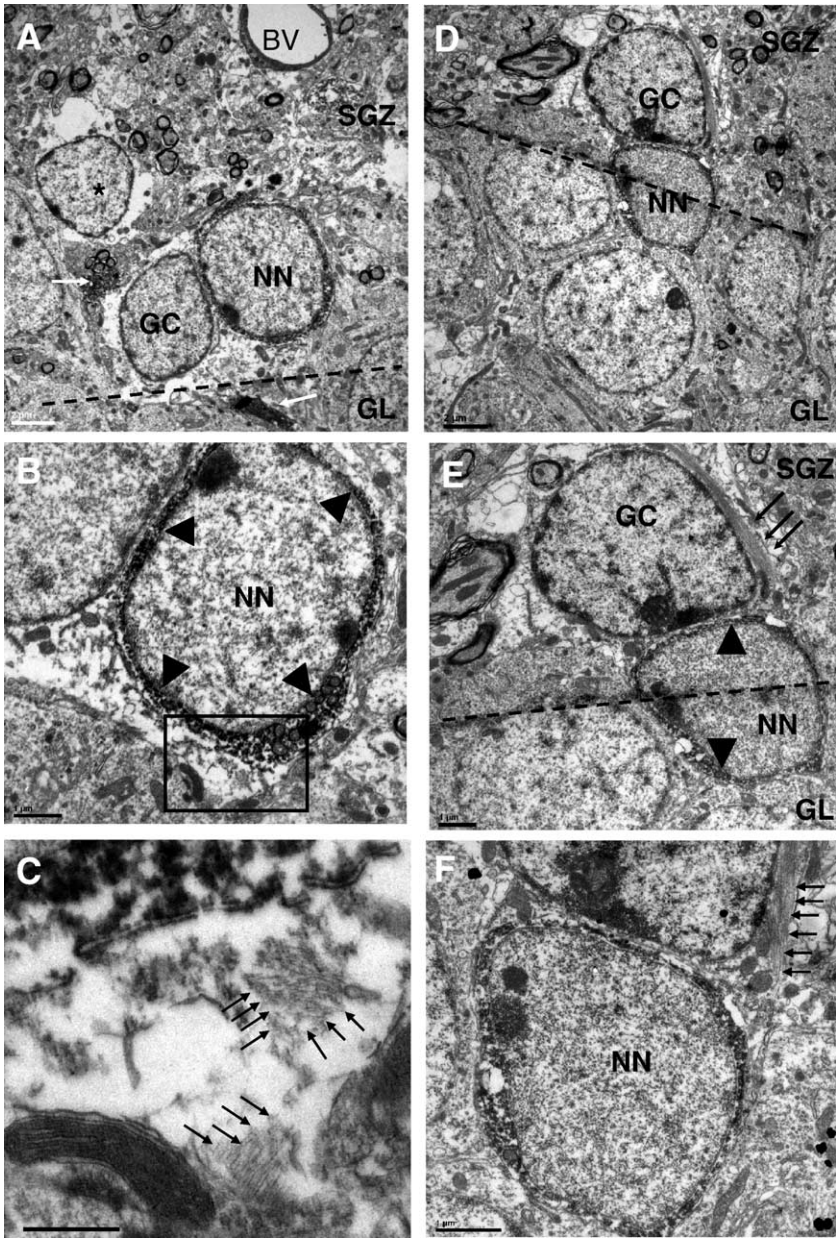

Fig. 3. Electron micrographs of DCX-immunolabeled NNs that have no apparent processes and are juxtaposed to astrocytes in the SGZ. In panel A, the cell body of an astrocyte (GC) is juxtaposed to a DCX-labeled soma of a NN that lacks processes (confirmed in serial sections in Fig. 4). Note the proximity of this cell pair to a blood vessel (BV), another astrocyte (asterisk), and two DCX-labeled dendritic processes (white arrows) from other NNs. Panel B is an enlargement of a section adjacent to the one in panel A that shows a thin shell of DCX-immunolabeling within its perikaryal cytoplasm (arrowheads). The boxed area is enlarged in panel C to illustrate two bundles of glial filaments (small arrows) within the watery cytoplasm of the astrocyte. Panel D is a second example of a NN that lacks processes and is juxtaposed to an astrocyte (GC). Panels E and F are enlargements of panel D showing the bundles of intermediate glial filaments (small arrows) within the cytoplasm of the astrocyte (GC). Note the DCX-immunolabeling within the perikaryal cytoplasm in panel E (arrowheads). Scale bars = 2 μm for panels A and D, 1 μm for panels B, E, F, and 0.5 μm for panel C.

have a radially oriented process indicate that they are GFAP-expressing radial glia-like cells. Relationships between astrocytic cell bodies and processes and DCX-labeled NNs and processes were further examined in electron microscopic preparations to determine their definitive membrane proximities and appositions.

3.3. Electron microscopic identification of NNs and astrocytes

At the electron microscopic level, the DCX-immunolabeled NNs in this study were identified by electron-dense immunoreaction product in the perikaryal cytoplasm that also extended into their processes (if present). Most of the organelles in the perikaryal cytoplasm of NNs were obscured by the DCX-immunoreaction product, except for the mitochondria.

Astrocytes were identified by the presence of bundles of glial filaments within a light cytoplasm [13]. In addition, astrocytes also displayed irregular membrane contours that entered the neuropil. In the present study, some astrocytes had a watery cytoplasm even though all of the surrounding profiles appeared well-preserved.

3.4. NNs in the SGZ are partially enveloped by astrocytes

NNs in the SGZ only had a thin shell of perikaryal cytoplasm (Figs. 3 and 4), and these DCX-immunolabeled

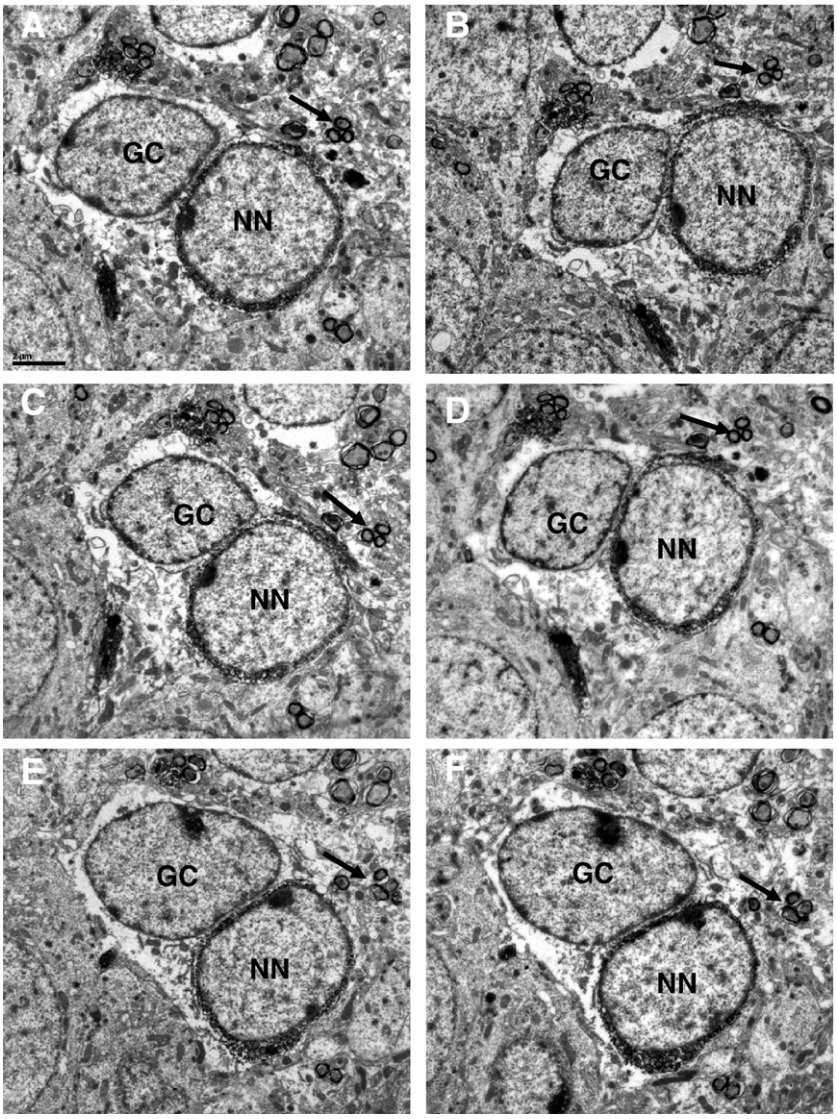

Fig. 4. Electron micrographs through a series of serial sections of the same DCX-positive NN and juxtaposed glial cell (GC) shown in Figs. 3A–C. These micrographs were captured from a series of 8 grids spanning a 3 μm thickness. These sections show the same one-to-one astrocyte–DCX-immunolabeled NN relationship as shown in random sections. The proximity between the nuclei of the astrocyte and NN remains relatively constant throughout the series. Bundles of glial filaments in both its cell body and processes identified the glial cell as an astrocyte. The astrocytic process at the top of the NN in panel A is thinner relative to the appearance of this same astrocytic process in panels E and F. The arrow in each of these panels depicts a group of three transversely-sectioned myelinated axons that appears in all of these serial sections as well as the one in Fig. 3A. Scale bar in panel A = 2 μm and is applicable to all panels.

cells lacked any apparent processes. Consistent with the light microscopic results in Figs. 2B,E, each DCX-labeled cell body was intimately apposed by the cell body and processes of an astrocyte (Fig. 3). Two DCX-labeled cell bodies were partially reconstructed from serial sections and these reconstructions confirm the observation obtained from random sections. One of these glial cell-NN pairs is shown in a series of sections in Fig. 4, and it represents 3 μm through the central portion of this 6 μm diameter NN. It is important to note that the cell body and processes of the astrocyte in such pairs envelope 60–75% (depending on the individual section in the series) of the basal and lateral surfaces of the DCX-immunolabeled NNs in the SGZ (Figs. 3 and 4). This leaves only the apical side of these neurons relatively free of astrocytic processes and in contact with the neuropil or neuronal somata of granule

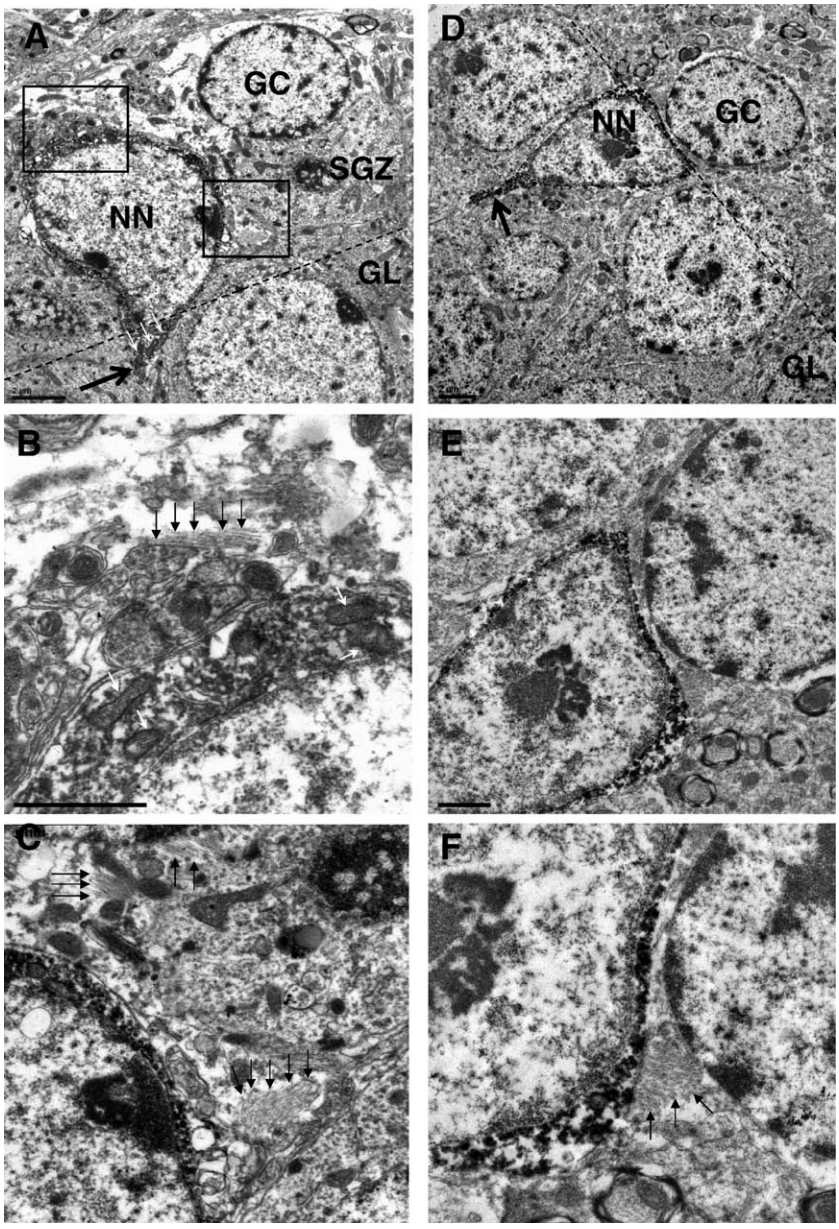

Fig. 5. Electron micrographs of DCX-positive NNs and astrocyte cell bodies (GC) at the base of the granule cell layer (GL). Panel A shows a DCX-immunolabeled NN with a labeled apical process (large arrow) extending into the GL and an adjacent glial cell with a watery cytoplasm. Note that within this rudimentary process is a congregation of mitochondria (white arrows). The left and right boxed areas in panel A are enlarged in panels B and C, respectively, to demonstrate the presence of bundles of glial filaments (small arrows) within the glial processes apposed to the surface of the DCX-positive cell body. Also note in panel B another congregation of mitochondria (white arrows) where a basal dendrite might arise. Panel D shows another DCX-labeled NN with a rudimentary process that is juxtaposed to the cell body of an astrocyte (GC). Panels E and F are enlargements of the pair of cell bodies in panel D to demonstrate the immunolabeling in the NN and the glial filaments (small arrows) within the perikaryal cytoplasm of the astrocyte. The dashed lines in panels A and D represent the border between the SGZ and GL. Scale bar = 2 μm in panels A and D, 0.5 μm in panels B and F, and 1 μm in panels C and E.

cells (Fig. 3D). It should also be noted that bundles of intermediate filaments, characteristic for astrocytes (Figs. 3B,C,E,F), are within the glial cytoplasm of the processes that cradle these DCX-labeled cells. In addition, these astrocyte–NN pairs are frequently located within 10 μm of a capillary (Fig. 3A).

3.5. NNs with a rudimentary apical process are also closely associated with an astrocyte

NNs that were found at the border between the SGZ and the GL had a rudimentary dendritic process and a thicker shell of perikaryal cytoplasm than those in the SGZ (Fig. 5).

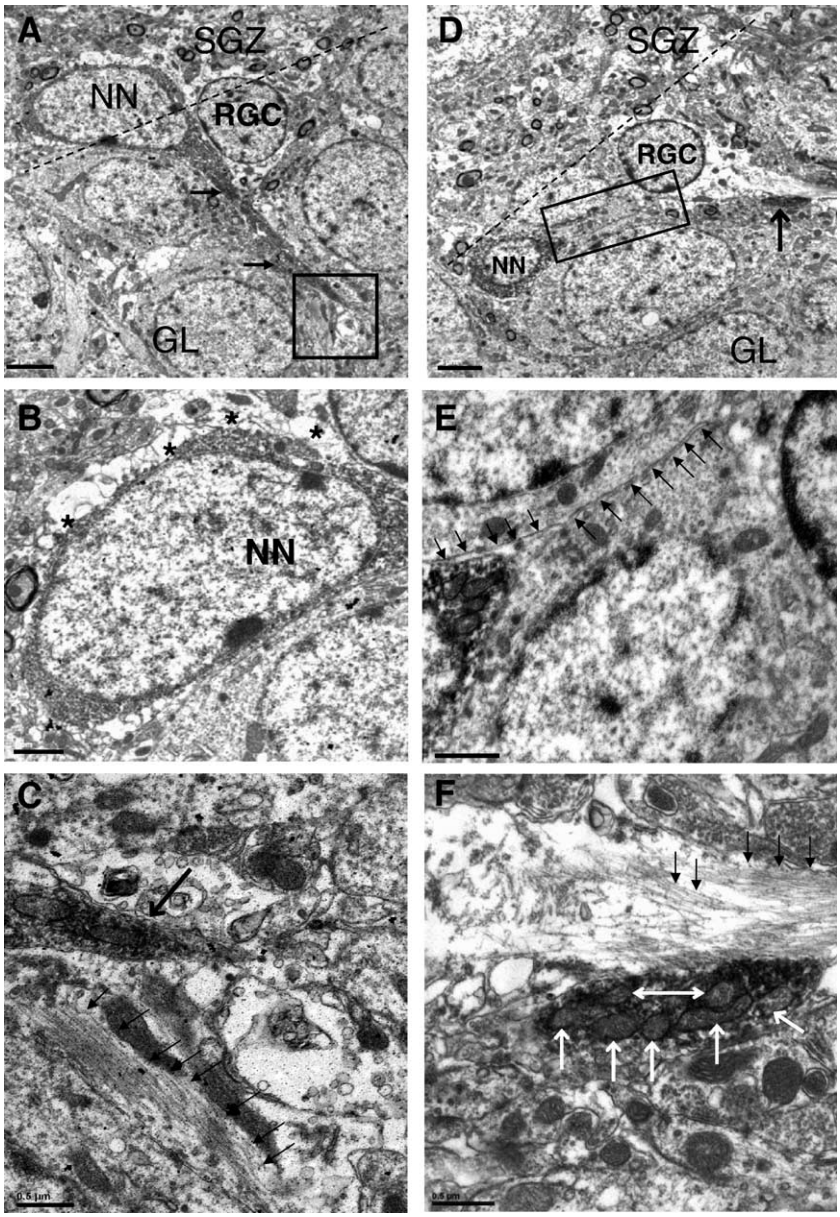

Fig. 6. Electron micrographs of DCX-positive NNs in the GL with apical processes juxtaposed to astrocyte cell bodies and their radial processes. In panel A, the DCX-positive NN extends an apical process (arrows) into the GL. Panel B shows an enlargement of the DCX-positive cell body (NN) and the cytoplasm (asterisks) of the radial astrocyte. Panel C is an enlargement of the boxed area in panel A and demonstrates that the apical tip of the DCX-labeled dendrite (large arrow) is adjacent to an astrocytic process that contains bundles of glial filaments (small arrows) and is continuous in other sections with the radial astrocyte in panel A. Panel D shows another DCX-positive NN with an associated growth cone (arrow) apposed to an astrocytic radial process. In panel E, the boxed area in panel D is enlarged to demonstrate a thin glial process (arrows) from the radial astrocyte that is continuous with the surface of the cell body of the NN. Also note that mitochondria are congregated in the soma of the NN at the site of origin of the growing apical process. Panel F is an enlargement of the process indicated by a black arrow in panel D to demonstrate the presence of glial filaments (black arrows) within the radial astrocyte and the many mitochondria (white arrows) within the dendritic growth cone. The dashed line in panels A and D represent the border between the SGZ and the GL. Scale bar = 2 μm in panels A and D, 1 μm in panels B and E, and 0.5 μm in panels C and F.

This rudimentary dendritic process had an accumulation of mitochondria at its origin from the cell body (Fig. 5A) and is consistent with the ultrastructural features of growth cones [21]. The nucleus of some of these labeled NNs was not as closely apposed to the nucleus of the astrocyte in the pair relative to that for NNs in the SGZ that lacked any apparent processes (Fig. 5A). These astrocytes are adjacent to the NNs at the border of the GL and are also identified by the presence of bundles of glial filaments in their processes (Figs. 5B,C). Consistent with the light microscopic results in Fig. 2G, NNs with rudimentary dendrites are partially enveloped by the cell bodies and processes of astrocytes (Figs. 5A,D).

3.6. NNs and their apical processes in the GL are apposed to radial glial processes

The NNs within the GL show elongated processes that appear to be apical dendrites and their growth cones (Figs. 6A,C,F), based on their orientation in the GL and the presence of many mitochondria [21], respectively. The cell bodies of these NNs display more perikaryal cytoplasm than the NNs in the SGZ (Fig. 6B). As a result, the nucleus of the NN in these pairs is separated by more cytoplasm from the nucleus of the adjacent astrocyte (Fig. 6D). However, as shown in Fig. 6E, the astrocytes continue to maintain glial processes apposed to the surface of DCX-labeled NN somata. Note that the NNs and their apical dendrites and growth cones are closely apposed to glial cells with radial processes (Figs. 6A,C,F). These astrocytes with radially aligned glial processes packed with intermediate filaments in the GL are similar to the radial astrocytes described by Seri et al. [20].

3.7. Analysis of synapses on DCX-labeled cells

A detailed analysis of the DCX-labeled cell bodies in the SGZ and the GL showed that none of them had axosomatic synapses (see Figs. 3–6), indicating that basket cells had not yet targeted them. In addition, DCX-labeled apical dendrites and growth cones that were apposed to the radially oriented processes of glial cells in the GL lacked synapses (Figs. 6C,F). Furthermore, an analysis of DCX-labeled processes in the SGZ (Fig. 3A) also showed no synapses.

4. Discussion

4.1. Light and electron microscopy identify GFAP-expressing radial glia-like cells involved in a one-to-one apposition to NNs in the dentate gyrus

The use of light and electron microscopy in this study provides evidence that NNs in the adult dentate gyrus are intimately associated with astrocytes from the site of their birth in the SGZ to their destination in the GL. The stages of NN development analyzed in this study using DCX as a marker are similar to stages 4 and 5 described by Kempermann et al. [8] and are not part of the neurogenic event because DCX is expressed about 3 h after birth of the NN. Thus, the somata of DCX-labeled NNs were examined with either no processes (stage 4) or a thick apical process (stage 5). GFAP-expressing radial glia-like cells in these pairs are probably radial glial cells because they displayed a process that was oriented radially through the GL (Fig. 2) and because Mignone et al. [10] showed that all of the nestin-positive cells with radial processes crossing the GL were also GFAP-positive. It should be noted that the non-radial processes of these GFAP-expressing radial glia-like cells envelope up to 75% of the cell body of the NN and that the radial processes are not apposing the NN cell body. Furthermore, NNs in the GL are observed to have apical dendrites and growth cones adjacent to radial astrocyte processes. Certainly, the wrapping of NNs by these extremely fine processes of astrocytes in the SGZ has escaped detection by previous studies at the light and confocal microscopic level by several investigators [7,12,16–18]. The use of serial thin sections for electron microscopy, DCX immunolabeling for NNs, GFAP-immunolabeling for astrocytes, and standard ultrastructural criteria for the identification of astrocytes facilitated the present study.

4.2. Technical considerations

The identification of the cradle formed by the GFAP-expressing radial glia-like cell around the NN is based on several types of anatomical preparations. First, Nissl preparations combined with DCX immunolabeling of NNs revealed a close apposition between a DCX-labeled cell body and an adjacent Nissl stained nucleus (Fig. 1). Based on the anatomical appearance of the Nissl stained cell nucleus, we hypothesized that it was a glial cell and used a second technique, double-immunolabeling for GFAP and DCX to identify the exact glial cell type in this pair. These double labeled preparations confirmed that the Nissl stained cell body adjacent to the DCX-labeled NN was a GFAP-expressing radial glia-like cell (Fig. 2). Note that only the bundles of glial filaments are observed when using GFAP-antibody, and they appear as horizontal bands around the NN cell body in light microscopy (Fig. 2). The interpretation of these bands as cytoskeletal elements within an astrocytic envelope surrounding the NN is from a third type of preparation, electron microscopy. Here, it is clearly demonstrated that glial processes containing bundles of glial filaments are forming a cradle around the NN cell body (Figs. 3–5).

4.3. A one-to-one relationship exists between GFAP-expressing radial glia-like cell bodies and NNs

In the adult dentate gyrus, NNs have been described to occur in clusters containing undifferentiated cells,

GFAP-positive astrocytes and cells undergoing apoptosis [16,17]. The present study shows a one-to-one relationship between a GFAP-expressing radial glia-like cell body and an NN. Because the NN's normal destination is the GL and the radial glial process extends through the GL, one interpretation of the data is that radial glial cells may guide the NN into the GL. In addition, radial glial cells in this region have been suggested to receive and transduce angiogenic cues to influence neuronal proliferation and differentiation [12]. Thus, we propose that the one-to-one relationship observed in this study represents a putative mechanism for NNs to migrate into the GL. A proposed scheme of this one-to-one relationship is found in Fig. 7. Note that, in Fig. 7A, the non-radial processes from a GFAP-expressing radial glia-like cell envelope most of the surface of the NN. In addition, a similar relationship is observed for the NNs that extend a rudimentary process. NNs and their apical processes in the GL remain apposed to GFAP-expressing radial glia-like cells and their processes and are now only partially enveloped by them. Thus, it appears as though the astrocytes and their processes cradle the NNs in the SGZ and contribute to the scaffold for process extension of the NN (see below).

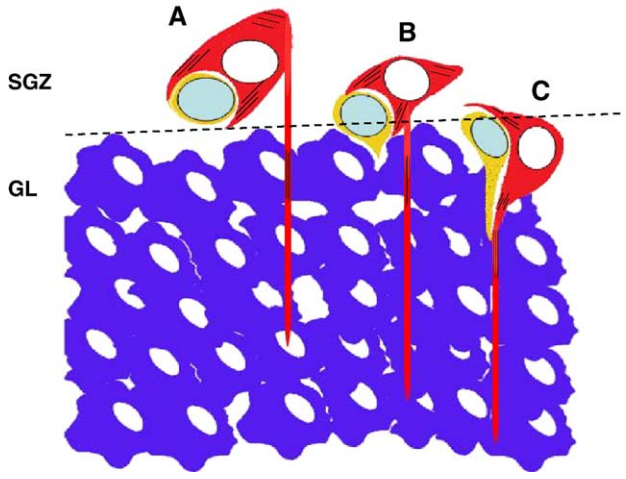

Fig. 7. A two-dimensional schematic diagram shows the described one-to-one GFAP-expressing radial glia-like cell–NN relationship in the adult dentate gyrus. In cell-pair A, an NN (yellow cytoplasm with light blue nucleus) in the SGZ is cradled by an astrocyte (red cytoplasm with white nucleus). Note that the NN lacks processes and the majority of its surface is enveloped by the astrocyte cell body and processes. This astrocyte also sends a radial process through the GL based on data shown in Figs. 2A–C. Both radial and non-radial processes contain glial filaments (black lines) that contribute to their cytoskeleton. In cell-pair B, the NN and its rudimentary process in the GL remain enveloped by some of the non-radial processes of the GFAP-expressing radial glia-like cell. Note the greater separation between the nuclei of the pair compared to cell-pair A. Cell-pair C shows an NN and apical dendrite extending deeper into the GL. Note that one side of its cell body and its apical process are apposed by a GFAP-expressing radial glia-like cell similar to the ones in Fig. 6 and that a radial process provides a scaffold for the apical dendrite to extend along (also observed in the light micrograph in Fig. 2H). The blue cells represent mature granule cells in the GL.

4.4. The role of radial glial processes for dendritic growth in the adult GL

Seki and Arai [18] demonstrated at the light microscopic level that developing granule cell dendrites are partly in contact with radial glial processes in the adult dentate gyrus. In fact, they describe some GFAP-expressing radial glia-like processes enveloping these dendritic processes. The data in the present study showing developing apical dendrites and their growth cones aligned on radial astrocyte processes (Fig. 6) provide ultrastructural evidence for the appositional relationship described by Seki and Arai [18]. Furthermore, the ability to identify dendritic growth cones on these dendrites suggests that radial glial cells provide a scaffold via their radial process for the growing apical dendrites of NNs. The lack of synapses on these growing apical dendrites indicates that synaptogenesis is probably not necessary for the initial apical dendritic outgrowth, unlike in the spinal cord where synaptogenesis plays a role for this function [23]. The presence of congregated mitochondria at the tip of the rudimentary apical process of the NNs suggests that process outgrowth has a high metabolic requirement. Therefore, it is likely that the radial glia-like cells adjacent to NNs provide nutrients and growth factors that promote their dendritic outgrowth.

4.5. Conclusions

The light and electron microscopic data in the present study show a one-to-one relationship between GFAP-expressing radial glia-like cell bodies and DCX-labeled NNs in the adult dentate gyrus. In this pair, the non-radial processes of a GFAP-expressing radial glia-like cell envelope up to 75% of the surface of the NN in the SGZ. Based on the observation of DCX-labeled dendrites and growth cones aligned on radial astrocyte processes, we suggest that the radial glial cell also provides a scaffold via its radial process for the NN to extend an apical dendrite into the GL. Future studies should address the lineage of these paired cells and factors that might disrupt this one-to-one relationship.

Acknowledgments

The authors gratefully acknowledge Drs. Mark Jacquin, Andre Obenaus, Michael Frotscher and Jurgen Wenzel for their meaningful discussions and suggestions about the data in this paper. We are also grateful for the support from NIH grant R01-NS38331 (to CER) and NIH training grant T32-NS045540 (for LAS).

References

[1] A. Alvarez-Buylla, D.A. Lim, For the long run: maintaining germinal niches in the adult brain, Neuron 41 (2004) 683–686.

[2] J.P. Brown, S. Couillard-Despres, C.M. Cooper-Kuhn, J. Winkler, L. Aigner, H.G. Kuhn, Transient expression of doublecortin during adult neurogenesis, J. Comp. Neurol. 467 (2003) 1–10.

[3] A.G. Dayer, A.A. Ford, K.M. Cleaver, M. Yassaee, H.A. Cameron, Short-term and long-term survival of new neurons in the rat dentate gyrus, J. Comp. Neurol. 460 (2003) 563–572.

[4] M.F. Eckenhoff, P. Rakic, Radial organization of the hippocampal dentate gyrus: a Golgi, ultrastructural, and immunocytochemical analysis in the developing rhesus monkey, J. Comp. Neurol. 223 (1984) 1–21.

[5] V. Filippov, G. Kronenberg, T. Pivneva, K. Reuter, B. Steiner, L.P. Wang, M. Yamaguchi, H. Kettenmann, G. Kempermann, Subpopulation of nestin-expressing progenitor cells in the adult murine hippocampus shows electrophysiological and morphological characteristics of astrocytes, Mol. Cell. Neurosci. (2003) 273–382.

[6] T.F. Freund, G. Buzsaki, Interneurons of the hippocampus, Hippocampus 6 (1996) 347–470.

[7] G. Kempermann, D. Gast, G. Kronenberg, M. Yamaguchi, F.H. Gage, Early determination and long-term persistence of adult-generated new neurons in the hippocampus of mice, Development 130 (2003) 391–399.

[8] G. Kempermann, S. Jessberger, B. Steiner, G. Kronenberg, Milestones of neuronal development in the adult hippocampus, Trends Neurosci. 27 (2004) 447–452.

[9] H.G. Kuhn, H. Dickinson-Anson, F.H. Gage, Neurogenesis in the dentate gyrus of the adult rat: age-related decrease of neuronal progenitor proliferation, J. Neurosci. 16 (1996) 2027–2033.

[10] J.L. Mignone, V. Kukekov, A.S. Chiang, D. Steindler, G. Enikolopov, Neural stem and progenitor cells in nestin-GFP transgenic mice, J. Comp. Neurol. 469 (2004) 311–324.

[11] J. Nacher, C. Crespo, B.S. McEwen, Doublecortin expression in the adult rat telencephalon, Eur. J. Neurosci. 14 (2001) 629–644.

[12] T.D. Palmer, A.R. Willhoite, F.H. Gage, Vascular niche for adult hippocampal neurogenesis, J. Comp. Neurol. 425 (2000) 479–494.

[13] A. Peters, S.L. Palay, H.F. deWebster, The Fine Structure of the Nervous System: Neurons and Their Supporting Cells, 3rd ed., Oxford Univ. Press, New York, 1991.

[14] M.S. Rao, A.K. Shetty, Efficacy of doublecortin as a marker to analyse the absolute number and dendritic growth of newly generated neurons in the adult dentate gyrus, Eur. J. Neurosci. 19 (2004) 234–246.

[15] C.E. Ribak, M.J. Korn, Z. Shan, A. Obenaus, Dendritic growth cones and recurrent basal dendrites are typical features of newly-generated dentate granule cells in the adult hippocampus, Brain Res. 1000 (2004) 195–199.

[16] T. Seki, Hippocampal adult neurogenesis occurs in a microenvironment provided by PSA-NCAM expressing immature neurons, J. Neurosci. Res. 69 (2002) 772–783.

[17] T. Seki, Expression patterns of immature neuronal markers PSA-NCAM, CRMP-4 and NeuroD in the hippocampus of young adult and aged rodents, J. Neurosci. Res. 70 (2002) 327–334.

[18] T. Seki, Y. Arai, Temporal and spatial relationships between PSA-NCAM expressing, newly generated granule cells, and radial glia-like cells in the adult dentate gyrus, J. Comp. Neurol. 410 (1999) 503–513.

[19] B. Seri, J.M. Garcia-Verdugo, B.S. McEwen, A. Alvarez-Buylla, Astrocytes give rise to new neurons in the adult mammalian hippocampus, J. Neurosci. 21 (2001) 7153–7160.

[20] B. Seri, J.M. Garcia-Verdugo, L. Collado-Morente, B.S. McEwen, A. Alvarez-Buylla, Cell types, lineage, and architecture of the germinal zone of the adult dentate gyrus, J. Comp. Neurol. 478 (2004) 359–378.

[21] C. Sotelo, S.L. Palay, The fine structure of the lateral vestibular nucleus in the rat. I. Neurons and neuroglial cells, J. Cell Biol. 36 (1968) 151–179.

[22] H. Van Praag, A.F. Schinder, B.R. Christie, N. Toni, T.D. Palmer, F.H. Gage, Functional neurogenesis in the adult hippocampus, Nature 415 (2002) 1030–1034.

[23] J.E. Vaughn, R.P. Barber, T.J. Sims, Dendritic development and preferential growth into synaptogenic fields: a quantitative study of Golgi-impregnated spinal motor neurons, Synapse 2 (1988) 69–78.