Neurogenic Radial Glial Cells in Reptile, Rodent and Human: from Mitosis to Migration

Tamily Weissman[1]*, Stephen C. Noctor[2]*, Brian K. Clinton[1], Lawrence S. Honig[2,4] and Arnold R. Kriegstein[1,2,3]

[1]Center for Neurobiology and Behavior, Departments of [2]Neurology and [3]Pathology and the [4]Sergievsky Center, Columbia University College of Physicians and Surgeons, 630 W. 168th Street, New York, NY 10032, USA

*The first two authors contributed equally to this work.

Radial glial cells play at least two crucial roles in cortical development: neuronal production in the ventricular zone (VZ) and the subsequent guidance of neuronal migration. There is evidence that radial glia-like cells are present not only during development but in the adult mammalian brain as well. In addition, radial glial cells appear to be neurogenic in the central nervous system of a number of vertebrate species. We demonstrate here that most dividing progenitor cells in the embryonic human VZ express radial glial proteins. Furthermore, we provide evidence that radial glial cells maintain a vimentin-positive radial fiber throughout each stage of cell division. Asymmetric inheritance of this fiber may be an important factor in determining how neuronal progeny will migrate into the developing cortical plate. Although radial glial cells have traditionally been characterized by their role in guiding migration, their role as neuronal progenitors may represent their defining characteristic throughout the vertebrate CNS.

Introduction

Since the term 'radial glia' was first used to describe the radial neuroglial cells of the developing CNS (Rakic, 1971a), radial glia were believed to be a specialized form of astroglial cell that served chiefly to support the migration of newborn neurons. Although evidence was obtained that radial glia were mitotically active and underwent interkinetic nuclear migration (Misson *et al*., 1988; Stagaard and Mollgard, 1989), it was assumed that radial glial divisions were producing additional radial glia, while a separate population of neuronal precursors residing in the same proliferative ventricular zone (VZ) were likely generating neurons (Levitt *et al*., 1981). This concept has changed recently due to the observation that radial glial cells themselves can undergo asymmetric division and produce neurons (Malatesta *et al*., 2000; Miyata *et al*., 2001; Noctor *et al*., 2001). Embryonic cortical radial glial cells isolated *in vitro* were capable of generating neurons (Malatesta *et al*., 2000), and individual radial glial cells *in vivo* (Noctor *et al*., 2001) or in slice cultures (Miyata *et al*., 2001; Noctor *et al*., 2001) were observed to generate neuronal daughter cells. Over time, individual radial glia could generate clones of neuronal progeny (Noctor *et al*., 2001).

The discovery of a neurogenic role for radial glia alters traditional views of cortical development. This does not rule out the possibility, however, that non-radial glial neuronal precursors may contribute to neurogenesis as well. This possibility was recently examined using several different strategies (Götz *et al*., 2002; Noctor *et al*., 2002). Since birthdate labeling experiments indicate that cortical neurons arise from mitotic cells within the VZ during a discrete developmental period (Bayer and Altman, 1995), we determined what proportion of the cells undergoing mitosis at these ages are radial glia. Cells in S-phase and M-phase were analyzed separately. S-phase cells were identified with BrdU pulse-labeling, and counts were made of how many mitotically active cells were labeled by the radial glial markers vimentin and RC2. At early, middle and late periods of rat cortical neurogenesis, essentially all S-phase cells expressed these markers (99.3 ± 0.3% at E12; 98.6 ± 0.5% at E15; and 98.3 ± 0.4% at E18). Moreover, using retrograde transport of fluorescently tagged microspheres we found that most cells (90.5 ± 2.5%) in S-phase also made contact with the pial surface, a morphological feature that helps to define radial glia. Furthermore, we determined the percentage of cells undergoing cytokinesis that also expressed a radial glia-specific mitotic marker, phosphorylated vimentin [4A4 antibody (Kamei *et al*., 1998)]. Once again, essentially all VZ cells in mitosis expressed this radial glial cell marker (98.7 ± 0.1% at E12; 98.3 ± 0.3% at E15; and 98.8 ± 0.1% at E18). Cells labeled by 4A4 were also observed to have radial fibers extending toward and in many cases reaching the pia, additionally confirming their identification as radial glia [see also Kamei *et al*. (Kamei *et al*., 1998)]. These data indicate that the majority of dividing VZ precursor cells in the rodent are radial glia. Together with work from Götz and colleagues (Götz *et al*., 2002), this leads to the hypothesis that most cortical neurons may therefore derive from radial glial cells. However, it is also likely that radial glia are heterogeneous in terms of the types of progeny that they may produce. Retroviral lineage experiments have suggested that progenitors may give rise to distinctly different clones of neuronal or glial progeny (Parnavelas *et al*., 1991; Luskin *et al*., 1993), and subsets of radial glia express different sets of molecular markers (Hartfuss *et al*., 2001). Therefore, while the majority of cortical neuronal progenitor cells may be radial glia, radial glia likely represent a diverse class of precursor cell, heterogeneous in terms of molecular expression and germative potential.

Neurogenic Radial Glia: a Common Theme among Vertebrates

There is compelling evidence that radial glial cells produce neurons in both the developing and regenerating adult vertebrate CNS. Unlike the adult mammalian brain where neurogenesis is spatially restricted, widespread adult neurogenesis occurs in the brains of adult fish (Birse *et al*., 1980; Raymond and Easter, 1983; Zupanc, 1999), amphibians (Polenov and Chetverukhin, 1993), reptiles (Lopez-Garcia *et al*., 1988; Font *et al*., 2001; Garcia-Verdugo *et al*., 2002) and birds (Paton and Nottebohm, 1984; Ling *et al*., 1997). In all of these species, adult neurons arise in the periventricular regions where the adult radial glia-like cells, known as ependymoglia, undergo active division (Polenov *et al*., 1972; Lopez-Garcia *et al*., 1988). In lower vertebrates (fish, amphibians, reptiles and birds), radial glia persist into adulthood, and appear to generate neurons throughout life. In adult and juvenile turtles, injections of BrdU initially label radial ependymoglial cell nuclei, but several months later BrdU labeling can be observed in telencephalic neurons (Perez-Canellas *et al*., 1997). For example, using a cellular marker and antibodies to BrdU and glial fibrillary acidic protein (GFAP), we show the morphology of periventricular

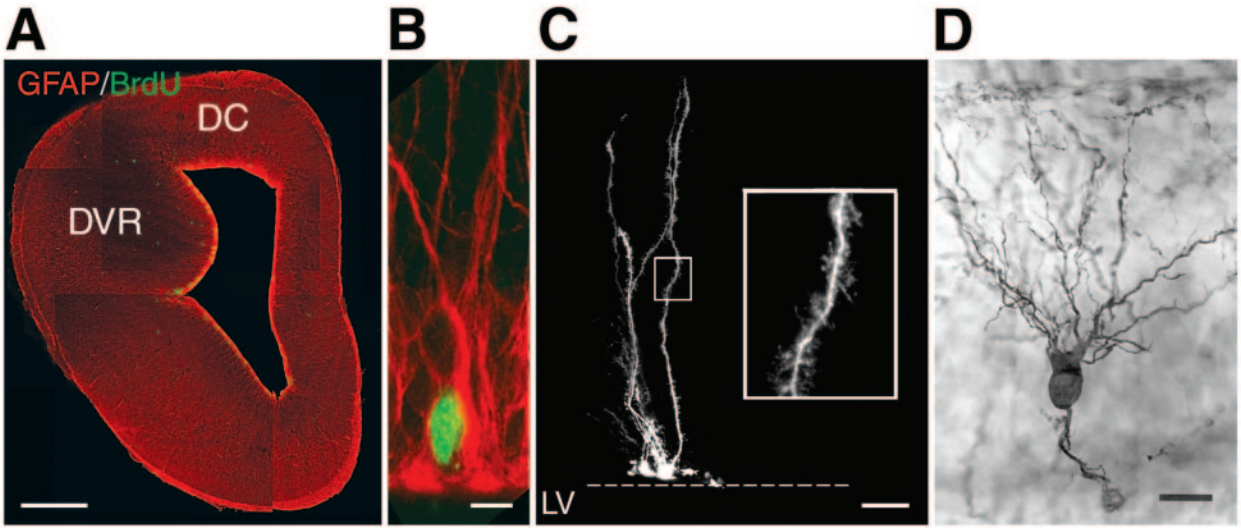

Figure 1. Mitotic radial glial cells in juvenile turtle forebrain. (*A*) Coronal section of juvenile turtle forebrain 7 days after intraperitoneal (IP)-injected BrdU (0.1 mg/kg, Sigma). BrdU-labeled nuclei (green) are visible lining the lateral ventricle, sparsely distributed within the dorsal cortex (DC) but particularly dense within the dorsal ventricular ridge (DVR). Glial fibrillary acidic protein (GFAP, Sigma) labeling is shown in red. Scale bar: 500 μm. (*B*) High power view of a radial ependymoglial cell double-labeled by GFAP (red) and BrdU (green). Radial ependymoglia continue to proliferate in juvenile and adult turtle forebrain. Scale bar: 10 μm. (*C*) Juvenile turtle radial ependymoglial cell labeled by Oregon Green–dextran conjugate (10 000 mol. wt, Molecular Probes), demonstrating characteristic spiny radial processes visible in the high-power inset. Scale bar: 50 μm. LV = lateral ventricle. (*D*). Weeks following BrdU labeling, turtle neurons including pyramidal cells with characteristic dendritic branching such as this example in the dorsal cortex, are BrdU-positive. Scale bar: 20 μm.

mitotic radial glia and mature pyramidal neurons in juvenile turtle cortex (Fig. 1). Radial glial cells also contribute to the remarkable ability of the CNS to regenerate following injury in the spinal cord of amphibians and salamanders (Margotta *et al.*, 1991) and in the cortex of reptiles (Molowny *et al.*, 1995; Font *et al.*, 2001). In addition, regeneration of the transplanted telencephalon of the adult amphibian occurs through increased mitotic activity of radial glia-like ependymoglial cells (Margotta *et al.*, 1992), and in the adult lizard, radial glia-like cells are able to regenerate an ablated hippocampus (Lopez-Garcia *et al.*, 1992; Font *et al.*, 2001). Furthermore, in adult songbirds, radial glia persist in brain regions where continued neurogenesis and migration occur (Goldman and Nottebohm, 1983; Alvarez-Buylla *et al.*, 1987, 1990). Therefore radial glial cells are likely neurogenic in a number of non-mammalian CNS regions, and in adults as well as developing animals.

In mammals there are two regions of adult neurogenesis: the subventricular zone (SVZ), where olfactory neurons arise (Altman, 1969; Lois *et al.*, 1996), and the dentate gyrus of the hippocampus, where granule neurons are generated (Altman and Das, 1965; Kaplan and Hinds, 1977; Cameron *et al.*, 1993). Other regions of the cortex have also been suggested to support adult neurogenesis (Gould *et al.*, 1999), but this claim is controversial (Rakic, 2002). In adult neurogenic regions, astrocytes and adult radial glia appear to serve as precursor cells. The neurons generated in the SVZ are destined for the olfactory bulb, and arise from precursor cells that express nestin, GFAP and vimentin (Doetsch *et al.*, 1997). The primary precursor cells are therefore identified as astrocytes, and have been shown to generate an intermediate precursor cell type that subsequently produces neurons (Doetsch *et al.*, 1999). Therefore, although the SVZ does not harbor radial glia *per se*, the neuronal stem cell appears to be an astroglial cell with a direct lineage relationship to embryonic radial glia (Alvarez-Buylla *et al.*, 2001). In the adult hippocampus, cells synthesizing DNA appear to be persistent GFAP-positive radial glia found in the subgranular layer of the dentate gyrus (Cameron *et al.*, 1993), and recently these cells have been shown to generate neurons, either directly or through an intermediate transient precursor cell (Seri *et al.*, 2001). Thus radial glial cells appear to be key neuronal precursor cells in most vertebrates throughout development and in areas of adult neurogenesis.

Radial Glial Cells May Also Be Neuronal Progenitors in Human Cortex

Evidence that radial glia are neuronal precursor cells during mammalian cortical development has been obtained primarily in rodents. The observations summarized above provide indirect support for the concept that radial glia may also function as neuronal precursors in forebrain regions of non-mammalian vertebrates, including fish, amphibians, reptiles and birds. In evolutionary terms, a multilaminar cortex probably arose in the common amniote ancestor of living reptiles and mammals (Nieuwenhuys, 1994; Reiner, 2000), but the neocortex has undergone its most elaborate expansion in the brains of primates. Consequently, the mode of origin of cortical neurons in primates may differ from that of other mammals. We have begun to examine human fetal cortex to ask whether radial glia give rise to neurons during primate cortical development.

Cortical neurogenesis occurs by mitosis of progenitor cells at the ventricular surface. We therefore examined the proportion of mitotic cells in the human VZ that express radial glial proteins. The intermediate filament marker, vimentin, is expressed by rodent radial glia and is phosphorylated during M-phase. Therefore, as mentioned above, anti-phosphorylated vimentin (4A4) antibody has been used as a marker of mitotic radial glia in rodent brain (Kamei *et al.*, 1998; Götz *et al.*, 2002; Noctor *et al.*, 2002). Because human radial glial cells also express vimentin at early stages of cortical development (Stagaard and Mollgard, 1989; Honig *et al.*, 1996), we examined whether the 4A4 antibody would label dividing human radial glia. We obtained fixed, coronal brain slices from gestational age 14 week human cortex and incubated them in anti-phosphorylated vimentin (4A4) with one of three nuclear markers: anti-Ki-67, anti-

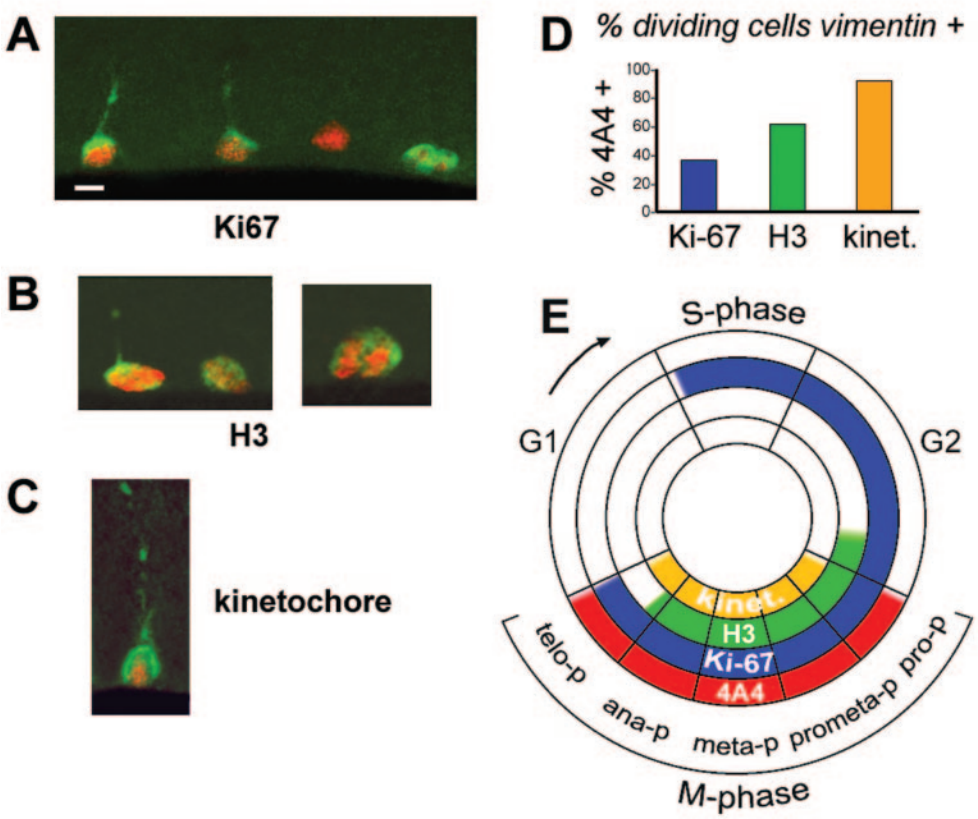

Figure 2. Dividing cells in the human embryonic VZ express radial glial markers. (*A–C*) Gestation week 14 human embryonic VZ co-stained with one of three markers for actively dividing cells. (*A*) Anti-Ki-67 (red, Chemicon) co-labeled with 4A4 (green, a kind gift from Dr K. Nagata, Aichi Cancer Center Research Institute, Nagoya, Japan). (*B*) Anti-phospho-histone-H3 (red, Upstate) co-labeled with 4A4 (green). (*C*). Human anti-kinetochore (red, a kind gift from Dr J.B. Rattner, University of Calgary) co-labeled with 4A4 (green). (*D*) A histogram comparing the co-expression of 4A4 among groups of cells that had been identified with one of three antibody markers for mitotic activity: Ki-67, H3 or kinetechore. (*E*) A cell cycle diagram comparing the expression patterns of the mitotic markers and 4A4 throughout the phases of the cell cycle. Mitotic cells identified with Ki-67 have the lowest percentage of 4A4 co-labeling because Ki-67 immunostaining is present through a longer portion of the total cell cycle. However, expression of the antigen recognized by the anti-kinetochore antibody closely matches the expression pattern of the 4A4 antibody. Ninety-two percent of dividing cells identified with the anti-kinetochore antibody were 4A4-positive. Note that the length of the cell cycle phases are not drawn to scale. Scale bar: 20 μm (*A*); 10 μm (*B*); 15 μm (*C*).

phospho-histone H3, or human anti-kinetochore antiserum. Anti-Ki-67 labels a nuclear transcription factor expressed from S-phase through M-phase; H3 labels a histone phosphorylated from G2 to early telophase; and the anti-kinetochore antibody labels condensed chromatin, thereby identifying cells exclusively within M-phase (Fig. 2*E*). We found that the 4A4 antibody was effective in labeling mitotic human radial glia. As expected, the 4A4-labeled cells were arrayed along the ventricular surface (Fig. 2*A–C*). We next identified M-phase cells at the ventricular surface using one of the three M-phase markers and examined whether these cells were double-labeled with the anti-phosphorylated vimentin antibody (4A4) using confocal microscopy. Ki-67 antigen expression begins during S-phase, continues through G2 and M-phase, and becomes nearly undetectable at the beginning of G1 (Fig. 2*E*) (Scholzen and Gerdes, 2000). Since 4A4 specifically labels cells in M-phase, we would expect to find double-labeled M-phase cells as well as Ki-67-positive cells that have not yet entered M-phase, and thus would not be 4A4-immunoreactive. Consistent with this prediction, we found that 38% of Ki-67-positive cells were labeled by the radial glial antibody 4A4 (Fig. 2*A*,*D*). Since we observed only partial overlap of these two markers, we next evaluated co-labeling of 4A4 with the phospho-histone H3, which has a more restricted expression pattern (Fig. 2*E*). H3 is phosphorylated during late G2 and dephosphorylated during mitosis [anaphase to telophase (Hendzel *et al.*, 1997)]. We therefore would expect a greater overlap with these two markers than that seen with Ki-67. We observed that 61% of H3-positive cells were 4A4-positive, consistent with the prediction that many dividing human VZ cells express the phosphorylated form of the radial glial vimentin protein during M-phase (Fig. 2*B*,*D*). We next determined how M-phase-specific 4A4 labeling overlaps with a more restricted kinetochore marker that is specific to M-phase (Fig. 2*E*). Since the anti-kinetochore antibody identifies the condensed chromatin of all cells in M-phase, we would expect a tight overlap with 4A4 labeling. We found that 92% of M-phase cells in the human VZ with condensed chromatin expressed the radial glial marker, 4A4 (Fig. 2*C*,*D*). These data indicate that in the human, as in the rodent, at an embryonic age when a large number of neurons are being born in the VZ, radial glia-specific proteins are expressed by most mitotically active cells. This expression pattern raises the possibility that radial glial cells may act as neuronal progenitors in the human VZ.

Radial Glial Fiber Morphology during Cell Division

Historically, the morphology of mitotic VZ cells has been the subject of considerable attention, with the overall conclusion that cells round up and lose their processes during division. Our labeling of mitotic radial glial cells with 4A4 (Fig. 2*A–C*) demonstrates that dividing human radial glial cells appear to maintain radial fibers during mitosis. These data are consistent

with several recent findings that radial glial cells maintain their pial contact during division (Malatesta *et al.*, 2000; Miyata *et al.*, 2001; Noctor *et al.*, 2001) as well as several earlier studies coming to similar conclusions (Berry and Rogers, 1965; Morest, 1970). However, this observation has been controversial during the last century. Early work used careful light and electron microscopy to show that mitotic VZ cells round up completely during division. While some findings suggested that these cells retained their radial processes (Berry and Rogers, 1965; Morest, 1970; Hinds and Ruffett, 1971; Seymour and Berry, 1975), others found no evidence for fiber retention (Sauer, 1935; Stensaas and Stensaas, 1968), possibly due to harsh fixation techniques.

Our own data suggest that a radial fiber is maintained through all stages of division. This is consistent with recent work from others as well (Miyata *et al.*, 2001; Götz *et al.*, 2002). We labeled rat VZ cells in fixed tissue (E16–E20) by applying DiI to the pia, and observed labeled cells in various mitotic stages, suggesting that cells may not lose contact with the pia during division (Fig. 3*A–C*). We have observed that when radial glial cells round up to divide, radial fibers become extremely thin and form small varicosities [Fig. 3; see also Miyata *et al.* (Miyata *et al.*, 2001)]. Interestingly, thin fibers labeled with DiI are often quite dim, suggesting that they remain connected to the pia but their contact may become limited during this stage. Thin fibers may therefore have been missed in previous EM studies, and may in fact remain extended to the pia throughout all stages of mitosis (Miyata *et al.*, 2001; Götz *et al.*, 2002; Noctor *et al.*, 2002).

What is the fate of the radial process during cell division? To answer this question, we used the 4A4 antibody to identify mitotic radial glia and label their basal (radial) processes. We were able to identify M-phase cells along the ventricular surface that corresponded to all stages of cytokinesis by examining fixed rat embryonic cortical slices labeled with anti-phosphorylated vimentin (4A4) antibodies and the chromatin marker Syto-11 (Fig. 4). The 4A4 antibody labels radial glial cells from prophase, when the chromatin begins to condense, until late telophase when the nascent cells separate from one another after division (Fig. 2*E*) (Kamei *et al.*, 1998). We found that the majority of cells divided with a vertical cleavage plane, as has been previously noted (Fig. 4) (Hinds and Ruffett, 1971; Smart, 1973; Landrieu and Goffinet, 1979; Zamenhof, 1987; Chenn and McConnell, 1995; Kamei *et al.*, 1998). Furthermore, from examining cortex at E12, E15 and E18, we found that the high percentage of vertical divisions did not decrease significantly from early to late stages of rat neurogenesis (80.0% at E12, 71.3% at E15, and 73.1% at E18; n = 149, 279, 208, respectively). During metaphase and anaphase, the pially directed vimentin-positive radial fiber condensed into a thick stub centered on the basal surface of dividing radial glial cells and extended radially a short distance from the cell (Fig. 4*C–E*). The thickened radial fiber thinned abruptly near the top of the VZ but continued towards the pia (Fig. 4*E*). During anaphase and telophase, nuclei dividing with a vertical cleavage plane contained dense phospho-vimentin-positive fibers stretching between the two bundles of chromatin (Fig. 4*D–M*). During telophase, a cleavage furrow developed on the basal surface of the dividing cell and the radial fiber often appeared to be centered on the mid-point of the cleavage furrow (Fig. 4*D*,*H*). Thus at this stage the fiber may not be clearly associated with either daughter cell (Fig. 4*H*,*J*). As cytokinesis progressed and the cleavage furrow deepened, the fiber most often became associated with one of the daughter cells in an asymmetric manner (Fig. 4*I*,*K*,*L*). In some instances it was not possible to trace the fiber to its precise site of origin (Fig. 4*G*,*J*,*M*). Most importantly, the pial fiber of the dividing radial glial cell did not appear to retract at any point throughout M-phase. A minority of mitotic radial glia along the ventricular surface appeared to divide with a horizontal cleavage plane (Fig. 4*N*) as has been previously noted (Chenn and McConnell, 1995; Kamei *et al.*, 1998). In contrast to radial glia dividing with a vertical cleavage plane, the horizontally dividing cells were not characterized by thick bundles of phosphorylated vimentin stretching between the pairs of chromatin. Rather, these cells possessed a thin short 4A4-positive region, or none at all, between daughter cells (Fig. 4*N*). These data indicate that a dividing radial glial cell maintains its basal process throughout all stages of mitosis, and suggest that the radial fiber becomes associated with one of the daughter cells during telophase.

Two Alternative Modes of Radial Glial Guided Neuronal Migration

If the radial fiber remains during division, which cell inherits the radial fiber? Given the traditional guidance role of radial glial fibers during neuronal migration, the simplest outcome would predict that the fiber remains associated with the radial glial mother cell during an asymmetric division. The daughter neuron would then migrate along the radial glial fiber into the cortex by traditional locomotion (Rakic, 1972; Nadarajah *et al.*, 2001). Alternatively, the radial glial fiber may be inherited by the daughter neuron during an asymmetric division. In this case, neuronal inheritance of a radial fiber could allow for somal translocation, an alternate mechanism that has been suggested for migration of neurons to the cortical plate (Morest, 1970; Brittis *et al.*, 1995; Miyata *et al.*, 2001; Nadarajah *et al.*, 2001). The radial glial cell would subsequently regrow a radial fiber. There is currently some question as to whether most neurons migrate using traditional locomotion or the alternate form of somal translocation, though a reasonable hypothesis is that translocation may predominate at early stages, while locomotion may occur later (Brittis *et al.*, 1995; Nadarajah and Parnavelas, 2002). We have begun to examine how prevalent these two forms of migration may be during rodent cortical development.

To examine the behavior of radial glial progeny we performed a lineage analysis using a green fluorescent protein (GFP)-expressing retrovirus that was injected *in utero* to infect mitotic cells (Noctor *et al.*, 2001). If a majority of neurons undergo somal translocation to the cortex, we reasoned that many of the progeny of a radial glia-derived clone should exhibit pial endfeet, since each neuron would remain in contact with the external limiting membrane while translocating to the top of the cortical plate. However, in our GFP-labeled radial clones, we typically observed only one radial fiber per clone, and that fiber appeared to belong to the radial glial cell based on intracellular dye filling (Noctor *et al.*, 2001). Therefore, in retrovirally labeled clones *in vivo*, we have not observed that a majority of neurons undergo translocation. Presumed migrating neurons have short leading and trailing processes, suggesting that these cells are locomoting rather than translocating. Figure 5*A* shows the typical morphology of migrating neurons observed in retrovirally labeled clones. There is a possibility, however, that our GFP labeling method fails to identify translocating clones, possibly as a result of the ages that we infect (E15–18), or by virtue of viral selectivity. We therefore used a different labeling technique to address whether or not a large number of neurons undergo somal translocation.

We reasoned that if a substantial number of neurons migrate using translocation, labeling of cells by pial endfeet would reveal multiple cells with translocating neuron morphology: a cell body located below the cortical plate and a radial process contacting the pia, but no remaining contact with the ventricle (Miyata *et*

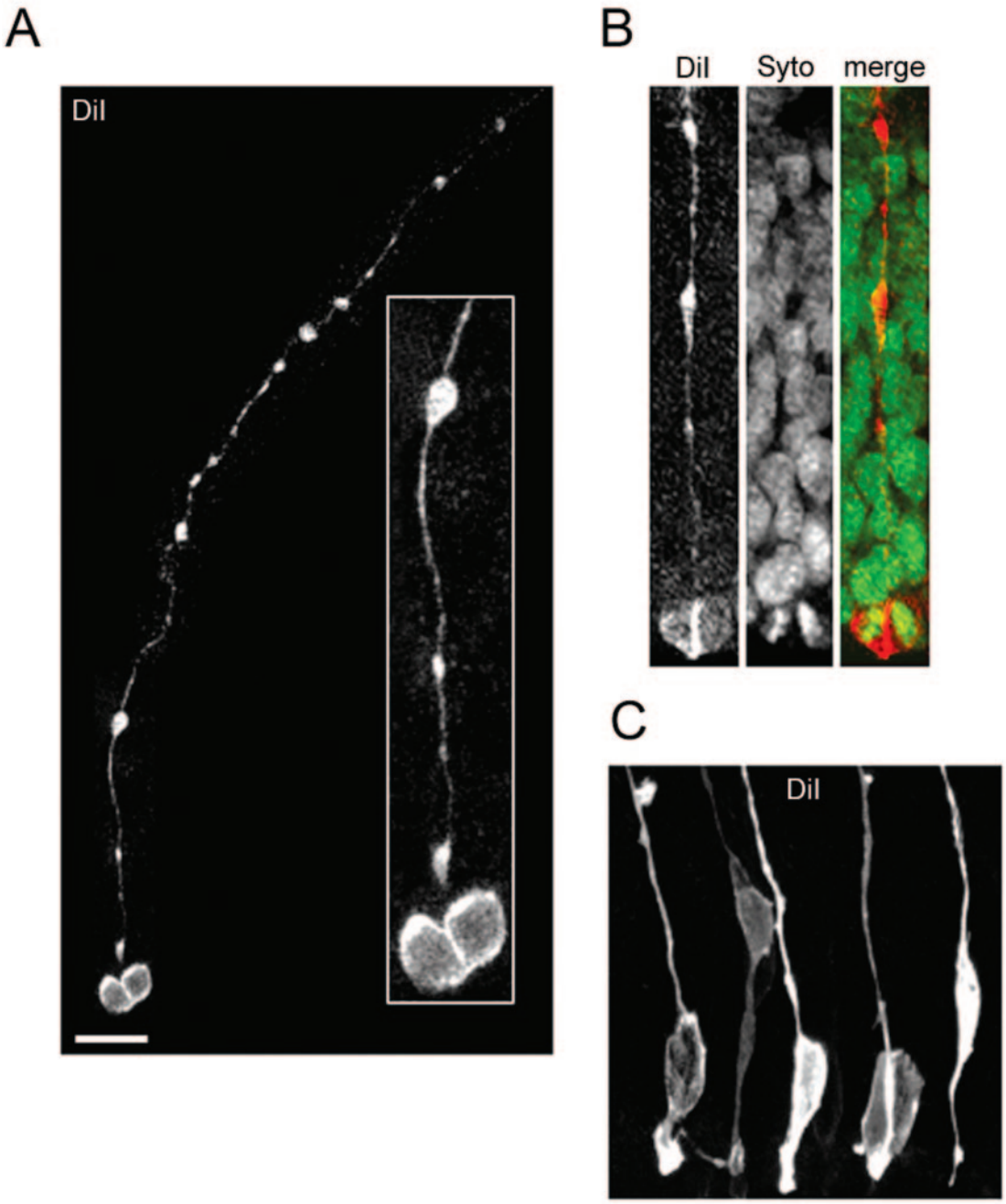

Figure 3. VZ cells in mitosis have radial processes that contact the pia. (*A*) This radial glial cell is undergoing mitosis and maintains contact with the pia. The radial fiber has thinned significantly, and small varicosities likely represent the streaming of cytoplasm toward or away from the cell body. Rat embryos aged E16 to E20 were transcardially perfused with 4% paraformaldehyde and the pia labeled with DiI (Molecular Probes). Brains were then coronally sectioned at 100 μm and imaged using confocal microscopy. (*B*) This radial glial cell is also DiI-labeled from the pial surface (red in merged image), and its chromatin is counterstained using the nuclear marker Syto-11 (green, Molecular Probes). (*C*) Additional radial glial cells that are DiI-labeled from the pial surface. One cell is undergoing division. Scale bar: 20 μm (*A*); 15 μm (*B*); 10 μm (*C*).

al., 2001; Götz *et al*., 2002). We therefore examined embryos at E16 and E20 by applying the lipophilic marker DiI to the pia and counting the number of labeled cells with this translocating neuron morphology (Fig. 5*B–D*, arrowheads). We also counted the number of pially labeled cells with radial glial morphology, that is with a radial process contacting the pia, a cell body within the VZ, and an endfoot clearly contacting the ventricle (Fig. 5*B*,*C*, small arrows). We found that at E16, 12.8% of labeled cells exhibited morphology consistent with translocating neurons, while 87.2% of labeled cells exhibited radial glial morphology (Fig. 5*B*,*D*; n = 376 cells). Cells with translocating neuronal morphology either possessed trailing processes directed toward the ventricle (Fig. 5*B*, left), or terminated in an isolated cell body (Fig. 5*B*, right). The overall ratio of migrating neurons to progenitor cells is known to increase from E16 to E20 (Takahashi *et al*., 1996). Therefore, if a significant number of migrating neurons undergo somal translocation (Miyata *et al*., 2001), one might expect to see an increase in the percentage of translocating neurons at E20. Furthermore, the thickness of the cortex increases dramatically from E16 to E20, also suggest-

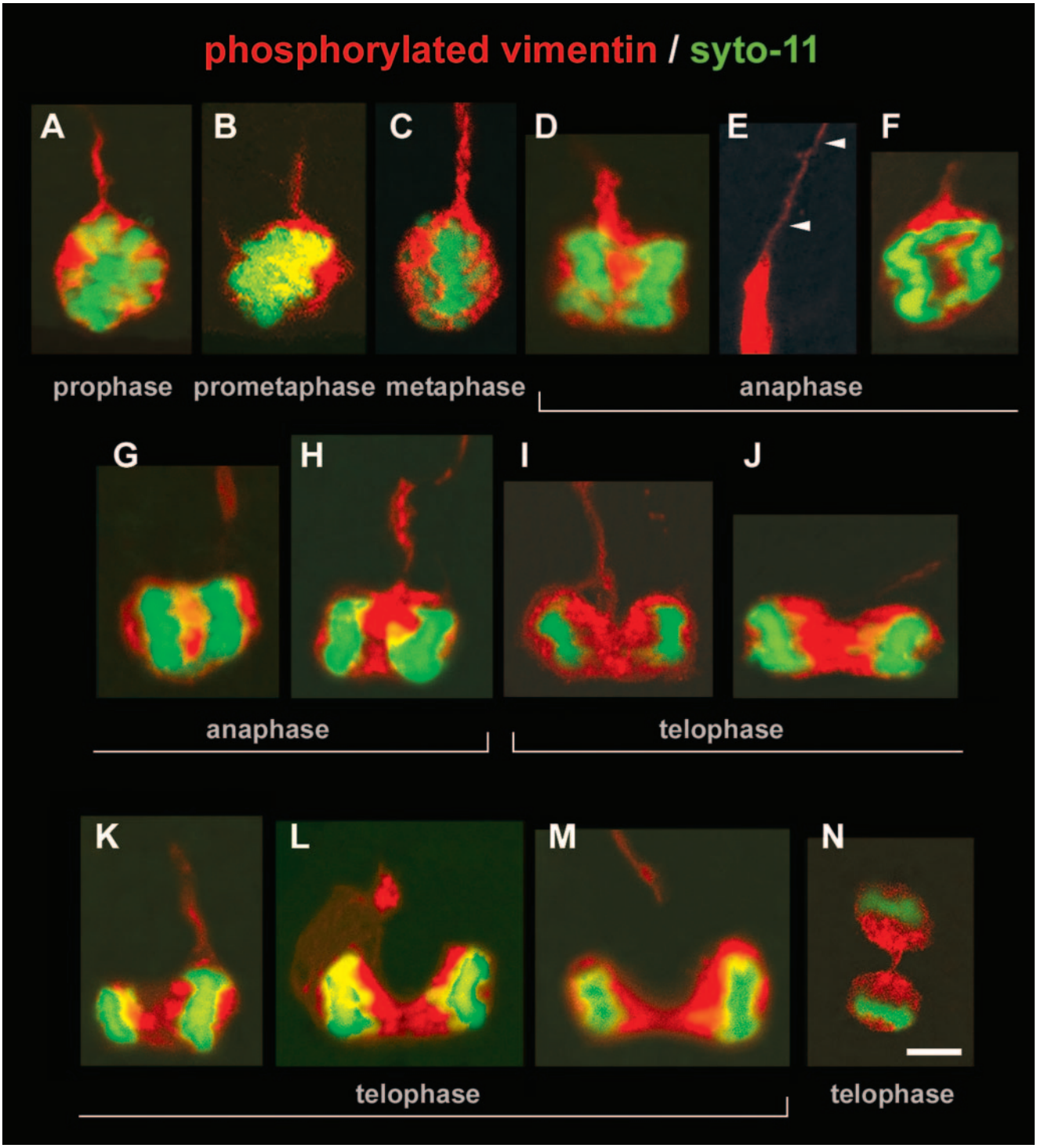

Figure 4. Radial glial cells retain their pially directed fiber throughout each stage of mitosis. Separate examples of 4A4-positive cells at the ventricular surface in thin sections of rat E15 neocortex. The cells have been arranged in a sequence to demonstrate the morphological changes that radial glial cells go through during M-phase. Mitotically active radial glial cells descend through the VZ during G2 phase, and reach the ventricular surface where they enter M-phase. (*A*) Prophase cells can be identified by their condensed chromatin (Syto-11, green). During prophase, vimentin is phosphorylated and radial glial cells become 4A4-positive (red), revealing large soma ~10 μm in diameter at the ventricular surface and a radial process extending from the center of the soma towards the pia. (*B*) During prometaphase the condensed chromatin (green) begins to contract, and the 4A4-positive radial process (red) is still visible. (*C*) During metaphase the condensed chromatin (green) contracts to the center of the soma to form a single plate, and the portion of the 4A4-labeled radial process (red) closest to the cell body thickens. (*D*) At the beginning of anaphase the chromatin (green) begins to separate, and the thickened portion of the radial process is still visible (red). (*E*) The thickened portion of the radial fiber extends a short distance from the soma, appears to pinch off, and a thin faintly 4A4-labeled radial process (white arrowheads) continues towards the pial surface. (*F–H*) As the cells progress through anaphase, the chromatin (green) continues to separate, but the 4A4-labeled radial process (red) appears thin, as seen in other cell cycle phases. (*I*) As the radial glial cells enter telophase the cleavage furrow can be visualized in the pattern of 4A4 staining (red). (*J–M*) As the bundles of chromatin (green) separate, the cleavage furrow becomes deeper, and a dense bundle of 4A4-positive fibers (red) stretches between the nascent daughter cells. As cytokinesis proceeds, the 4A4-labeled radial process (red) appears to associate with one of the two daughter cells in an asymmetric manner, although in some cases (panel *M*) it was not possible to trace the fiber's site of origin at the soma. N. Labeling with 4A4 (red) also revealed a minority of cell divisions occurring at the ventricular surface with a horizontal cleavage plane. Scale bar: 10 μm.

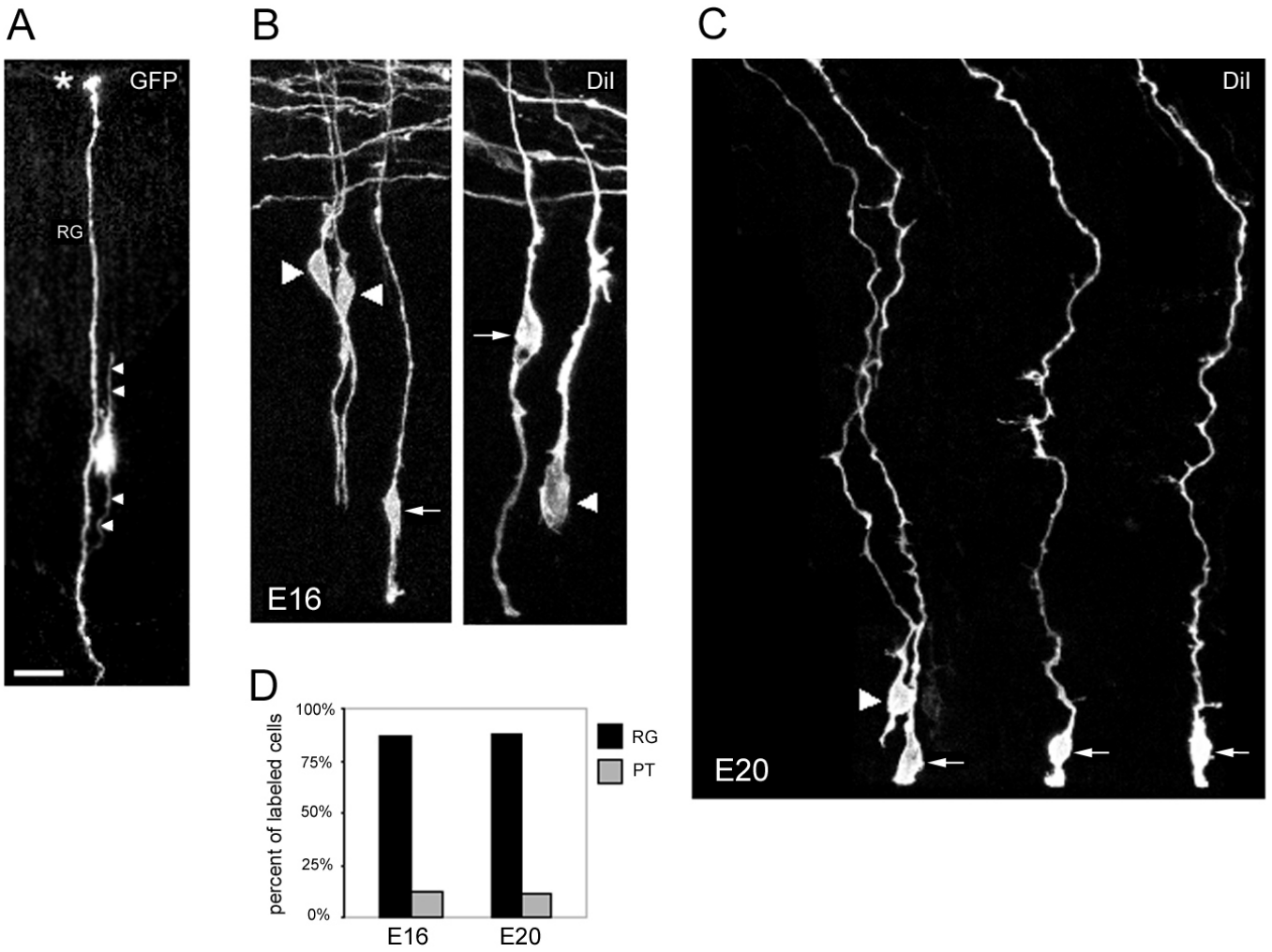

Figure 5. A minority of newborn neurons use translocation as a mechanism of migration. (*A*) An immature neuron migrates into the developing cortical plate at E18. The neuron's short leading and trailing processes can be seen (small arrowheads). This neuron does not contact the pia, and is likely using the 'locomotion' method of migration. The parental radial glial cell (RG), which can be seen contacting the pia (asterisk), was retrovirally infected with GFP following intraventricular *in utero* injection at E15 [adapted from Noctor *et al*. (Noctor *et al*., 2001)]. (*B*, *C*) VZ cells at E16 (*B*) and E20 (*C*) were labeled by pial application of DiI. Potentially translocating neurons (large arrowheads) appear next to cells with radial glial morphology (small arrows). The thickness of the cortex has expanded dramatically by E20. The pia of embryonic brains was labeled with DiI following fixation. Slices were sectioned at 100 μm and imaged using confocal microscopy. (*D*) VZ cells at E16 and E20 were quantified using the following criteria: (i) cells with a fiber contacting the pia, a nucleus within the VZ, but with no ventricular endfoot (large arrowheads in *B* and *D*) were counted as potentially translocating neurons (PT); (ii) cells with a fiber contacting the pia, a nucleus within the VZ, and a ventricular endfoot (small arrows in *B* and *D*) were counted as radial glial cells (RG). At E16, 12.8% of pially labeled cells with a nucleus in the VZ had the morphology of potentially translocating neurons (PT), while 87.2% resembled radial glial cells (RG). By E20, these percentages did not change significantly (11.5% PT; 88.5% RG). Scale bar: 15 μm (*A*); 15 μm (*B*, left); 10 μm (*B*, right); 15 μm (*C*).

ing that a larger number of translocating neurons should be observed at this stage. We therefore examined whether the ratio of potentially translocating neurons to radial glia changed from E16 to E20, and found no significant change. At E20, 11.5% of labeled cells exhibited the morphology of translocating neurons, while 88.5% appeared morphologically to be radial glia (Fig. 5*C*,*D*; n = 347 cells). Although simply a morphological analysis, our results suggest that translocating neurons may not represent the majority of migrating neurons from E16 to E20.

Conclusions

Our understanding of the role of radial glial cells in neuronal production and maturation has expanded in recent years. In addition to providing crucial support during neuronal migration, radial glial cells are now believed to produce neurons in several species, and in the adult brain as well as during development. Furthermore, radial glial cells are present in virtually all CNS regions where neurons are produced, underlining their critical role in neuronal production. We demonstrate here that radial glial cells may produce neurons in the human cortical VZ as well. In the fetal human VZ at 14 weeks, we show that the radial glial vimentin protein is expressed in 92% of M-phase cells, similar to observations in the rodent VZ (Noctor *et al*., 2002). These results suggest that radial glial cells are present in the human VZ during neurogenesis, and that they may constitute a large portion of the mitotically active cell population. Radial glial cells may thus function as important neuronal progenitors in the human brain.

Historically there has been some question as to whether radial glial cells maintain radial fibers during division. In general, it was not accepted that a differentiated cell type such as the radial glial cell could divide without retracting its elaborate pial process, although other cell types are known to undergo mitosis

without rounding up completely (Wolf *et al.*, 1997). Recent work suggests that radial glial fibers are maintained during cell division (Miyata *et al.*, 2001; Noctor *et al.*, 2001; Götz *et al.*, 2002), and we show here that in dividing VZ cells, a vimentin-positive radial fiber is present throughout each stage of mitosis. These data suggest that radial glial cells do not become completely spherical during mitosis, as was historically described for cells dividing at the ventricular surface. Rather, the somal portion of the cell, present at the ventricular edge, rounds and enlarges, likely due to cytoplasm streaming down from the radial fiber (Miyata *et al.*, 2001). The fiber remains elongated; however, it is 'pinched off' and dramatically thins distal to a short radial stub projecting from the soma (Fig. 4*E*). During telophase this stub appears to distribute asymmetrically by associating specifically with one of the two daughter cells (Fig. 4*I–M*). Following telophase, the thinned fiber quickly refills with cytoplasm that streams radially from the cell body (Miyata *et al.*, 2001; Noctor *et al.*, 2001). Retention of the radial fiber during cell division helps to explain how the radial fiber of a mitotically active cell can continually function in guiding neuronal migration.

Interestingly, the presence of a 'radial stub' begins to question the true nature of a symmetric division. Symmetric divisions in the VZ have been described as those in which the cleavage plane (plane of the metaphase plate) is roughly perpendicular to the surface of the ventricle ('vertical cleavage plane', Fig. 4*A–M*) (Chenn and McConnell, 1995). If radial fibers are maintained in the above manner during a vertical, 'symmetric' division, as we show in Figure 4, then the two daughter cells would not be truly symmetric, with one possessing the stub and the other completely round. Furthermore, McConnell and colleagues (Chenn and McConnell, 1995) observed that as neurogenesis proceeds in the developing ferret cortex, vertical cleavages (presumed to be symmetric divisions) decreased, while horizontal cleavages (presumed to be asymmetric divisions) increased. This was consistent with the idea that vertical cleavages are symmetric divisions producing two precursor cells, while horizontal cleavages are asymmetric divisions resulting in the production of one neuron and one precursor cell. Our results, however, do not show a significant decrease in vertical cleavages during the period of neurogenesis in the rat (E12–E18). Coupled with our observation of an asymmetric radial stub and fiber associated with one daughter cell during vertical division, this calls into question whether all of these divisions are truly symmetric. Our results suggest that at least some rat VZ cell divisions occurring in the vertical cleavage plane are asymmetric or result in neuronal production.

The establishment of a neurogenic role for radial glia could have implications for theories of neuronal migration. Radial migration is widely believed to involve neuronal locomotion along radial glial guides. A great deal of data has documented this mode of migration, including electron microscopic images of bipolar neurons clutching radial fibers (Rakic, 1971b), and *in vitro* time-lapse studies of neurons with leading and trailing processes migrating along radial glia (Edmondson and Hatten, 1987). However, an alternative mode of migration, whereby a newborn neuron resembling a radial glial cell simply undergoes intracytoplasmic nuclear translocation from the ventricular surface to the cortical plate, has also been repeatedly described (Berry *et al.*, 1964; Berry and Rogers, 1965; Morest, 1970; Brittis *et al.*, 1995; Miyata *et al.*, 2001; Nadarajah *et al.*, 2001). Based on the concept of radial glial neurogenesis, it is easy to imagine how the neuronal progeny of a radial glial cell could inherit a radial process of the parental radial glial cell and be ready to promptly translocate to the cortex.

Results from Miyata and colleagues (Miyata *et al.*, 2001), Götz and colleagues (Götz *et al.*, 2002), and those presented here suggest that translocation of newborn neurons appears to occur at least part of the time during cortical development. What factors might determine whether neurons migrate by translocation or locomotion? One possibility is that different modes of migration predominate at progressive stages of neurogenesis. For example, translocation has been discussed as a mode of migration at early stages of corticogenesis (Morest, 1970; Brittis *et al.*, 1995; Nadarajah *et al.*, 2001). However, our evidence suggests that translocation is not the predominant form of neuronal migration during mid to late stages of cortical development. At later stages of development, when the cortical mantle is greatly enlarged, newborn neurons may be more likely to migrate using the traditional locomotion mechanism.

Alternatively, whether a neuron undergoes translocation or locomotion neuronal migration and radial fiber inheritance may be dictated by extrinsic or intrinsic radial glial cell signals. Extracellular signals such as growth factors or neurotransmitters may bind to radial glial cell receptors and trigger downstream events that lead to asymmetric distribution of the radial fiber and critical cytoplasmic proteins. Alternatively, subpopulations of radial glial cells (Hartfuss *et al.*, 2001) may be committed to different modes of fiber inheritance, producing radial clones that consist entirely of either 'translocated' or 'locomoted' neurons. Some radial glial subtypes, for example, may consistently donate their fiber to daughter cells, generating translocating neurons, while other radial glial cells may exclusively retain their radial fibers, generating neurons that locomote. In fact, a subset of radial glia express brain lipid-binding protein (Hartfuss *et al.*, 2001), a factor that is induced by locomoting neurons (Feng *et al.*, 1994), and these radial glia may represent the latter class of fiber-retaining precursor cells.

The finding that radial glial cells can function as neuronal progenitors in a variety of contexts has implications for potential signaling mechanisms between proliferating precursor cells and neuronal daughter cells in the developing cortex. VZ precursor cells have previously been shown to respond to the neurotransmitters glutamate and GABA (LoTurco and Kriegstein, 1991; LoTurco *et al.*, 1995; Behar *et al.*, 1998). In fact, these transmitters can modulate rates of proliferation in the VZ (LoTurco *et al.*, 1995; Haydar *et al.*, 2000). Since VZ precursor cells were previously envisioned as short, VZ-contained cells, the source for GABA and glutamate was presumed to be immature neurons migrating within the VZ. However, the presence of a radial glial fiber that contacts neuronal progeny in the cortical plate raises the possibility that precursor cells may also be able to respond to neurotransmitters and other signaling factors released in developing cortical layers. Maturing neurons in the cortex may therefore signal to nearby radial glial fibers and thus modulate proliferation of their parental radial glial cells in the underlying VZ. Radial glial cells may concurrently provide trophic signals for maturing neurons. Radial glial cells have been shown to produce insulin-like growth factors (Jiang *et al.*, 1998) and estrogen (Forlano *et al.*, 2001); the release of such substances could potentially be involved in neuronal differentiation and/or the establishment of final cortical position. The role of radial glial cells may therefore extend beyond neuronal precursor and migrational guide, to also include the provision of trophic support during neuronal maturation. In this way, the parent radial glial cell would be responsible for not only

generating neurons and guiding their migration into the cortex, but also for nurturing neuronal development.

Notes

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Address correspondence to Arnold Kriegstein, 630 W. 168th St., P&S Bldg. Room 4-408/Box 31, New York, NY 10032, USA. Email: ark17@columbia.edu.

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