Development of Midline Glial Populations at the Corticoseptal Boundary

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ABSTRACT: Three midline glial populations are found at the corticoseptal boundary: the glial wedge (GW), glia within the indusium griseum (IGG), and the midline zipper glia (MG). Two of these glial populations are involved in axonal guidance at the cortical midline, specifically development of the corpus callosum. Here we investigate the phenotypic and molecular characteristics of each population and determine whether they are generated at the same developmental stage. We find that the GW is derived from the radial glial scaffold of the cortex. GW cells initially have long radial processes that extend from the ventricular surface to the pial surface, but by E15 loose their pial attachment and extend only part of the way to the pial surface. Later in development the radial morphology of cells within the GW is replaced by multipolar astrocytes, providing supportive evidence that radial glia can transform into astrocytes. IGG and MG do not have a radial morphology and do not label with the radial glial markers, Nestin and RC2. We conclude that the GW and IGG have different morphological and molecular characteristics and are born at different stages of development. IGG and MG have many phenotypic and molecular characteristics in common, indicating that they may represent a common population of glia that becomes spatially distinct by the formation of the corpus callosum. J Neurobiol 57: 81–94, 2003

Keywords: **axonal guidance; cortical development; astrocytes; radial glia; corpus callosum; commissure; BLBP; GLAST; RC2; Nestin; GFAP**

INTRODUCTION

During embryonic development, glia are required for the formation of the central nervous system (CNS) (Fitch and Silver, 1997). The earliest population of glia observed in the CNS is radial glia (Schmechel and Rakic, 1979). Until recently, radial glia were thought to only provide a scaffold for migrating neurons from the ventricular zone to the mantle region of the cortex and cerebellum (Rakic, 1972, 1995; Rakic and Sidman, 1973; Antonicek et al., 1987; Gasser and Hatten, 1990; Anton et al., 1997). Recently, radial glial cells have been shown to give rise to neurons and to have neuronal precursor characteristics (Hartfuss et al., 2001; Miyata et al., 2001; Noctor et al., 2001; Malatesta et al., 2000; Heins et al., 2002). Radial glia in the cortex also guide callosal axons that have already crossed the midline into their final target region (Norris and Kalil, 1991). Thus, radial glia may have a number of different functions in the developing CNS.

Glial cells also define boundaries between different brain areas or between functional subdomains within the same area (Cooper and Steindler, 1986; Silver, 1994; Silver et al., 1993; Garcia-Abreu et al., 1995; Mastick and Easter, 1996; Yoshida and Colman, 2000). These glial boundaries serve to prevent axons from straying from their correct path of growth, thereby acting as axon guidance barriers (Fitch and Silver, 1997). At the *Drosophila* midline, glia func-

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tion as guide post cells for commissural and ipsilaterally projecting axons to determine which axons cross the midline and which do not (Jacobs and Goodman, 1989; Hidalgo and Booth, 2000; Kidd et al., 1999). A similar function of sorting ipsilaterally from contralaterally projecting axons has been found for glia that make-up the glial palisade of the optic chiasm in mammals (Marcus et al., 1995; Erskine et al., 2000). In other regions of the mammalian nervous system, midline glial populations are associated with the formation of commissures such as the anterior commissure (Cummings et al., 1997; Pires-Neto et al., 1998), the corpus callosum (Silver et al., 1993; Shu and Richards, 2001), and decussating axons in the hindbrain, brain stem, and the corticospinal tract (Van Hartesveldt et al., 1986; Mori et al., 1990; Joosten and Gribnau, 1989).

Although a guidance function of glia has been suggested in many systems, most observations have been made by correlating the temporal and spatial formation of a specific glial structure with a particular fiber tract. However, little has been done to investigate whether these proposed guidance structures are actually required for the correct formation of a specific tract. An exception to this is the floor plate of the spinal cord, which is a transient structure made up of glial-like cells (Altman and Bayer, 1984) that guides commissural axons of the dorsal spinal cord (Tessier-Lavigne et al., 1988). Only within the last decade have the molecules underlying glial-mediated axon guidance been cloned. For example, by genetic analysis in *Drosophila,* midline glia were found to express a chemorepellent molecule called Slit (Kidd et al., 1999). The receptor for Slit, called Roundabout (Robo), is expressed on axons that are repelled from the midline. This Slit/Robo interaction prevents ipsilaterally projecting axons from crossing the midline and keeps contralaterally projecting axons from recrossing the midline (Kidd et al., 1998a, b, 1999; Rothberg et al., 1990). Slit2 is also expressed by midline glial populations in rodents and regulates axonal pathfinding in a number of different systems (Brose et al., 1999; Ba-Charvet et al., 1999; Li et al., 1999; Erskine et al., 2000; Ringstedt et al., 2000; Niclou et al., 2000; Zou et al., 2000; Shu and Richards, 2001; Plump et al., 2002; Bagri et al., 2002).

Here we investigate the development and interrelationships of three midline glial populations at the corticoseptal boundary: the glial wedge (GW), the indusium griseum glia (IGG), and the midline zipper glia (MG; Silver et al., 1993). An additional population of cells called the glial sling (Silver et al., 1982) exists at the corticoseptal boundary. These cells are thought to provide a barrier for axon growth that inhibits callosal axons from entering the septum and guides them across the midline (Silver et al., 1982, 1993). However, recent evidence suggests that the majority of the sling cells are neurons and not glia (Shu et al., 2003) and therefore these cells will not be further analyzed here as part of the midline glial populations at the corticoseptal boundary.

Little is known about the similarities and differences, particularly the antigen expression profiles, of these midline glial populations. A morphological description of these populations has been presented but no analysis has previously been performed on the antigen expression profiles, birth-dating, or postnatal development of these populations. Therefore, it was not known, for example, whether the GW was part of the radial glial population of the cortex or whether the IGG and the MG were part of the same population. Here we address these questions and provide further insight into these midline glial populations and their developmental relationships to one another.

MATERIALS AND METHODS

Animals

The embryos of timed pregnant C57BL/6J mice (Jackson Laboratories, Bar Harbor, ME) were used in this study. Embryos were staged based on a plug date of embryonic day (E) 0 and were used between E4 and postnatal day (P)10. E12 and E14 GFAP-LacZ transgenic embryos were used in some experiments (Brenner et al., 1994; obtained from Jackson Laboratories). To determine the genotype of transgenic mice, X-Gal reaction of the mouse spinal cord was performed during the perfusion procedure. Procedures related to animal use were approved by the animal care and use committee at the University of Maryland, Baltimore.

Immunohistochemistry

Embryos were perfused with 4% paraformaldehyde and brains were sectioned with a vibratome into 50 μm sections. Immunohistochemistry was performed as previously described (Shu et al., 2000). Primary antibodies used were:

1. Rabbit anticow glial fibrillary acidic protein (GFAP; DAKO, Denmark) 1:1000 for fluorescent Cy2 detection and 1:50,000 for nickel-DAB reaction.
2. Monoclonal RC2 (developed by Dr Miyuki Yamamoto and obtained from the Developmental Studies Hydrodoma Bank maintained under the auspices of the NICHD by the University of Iowa, Department of Biological Sciences, Iowa City) used at 1:200 for fluorescent Cy3 detection.
3. Monoclonal Rat-401 anti-Nestin (developed by Dr Susan Hockfield and obtained from the Developmen-

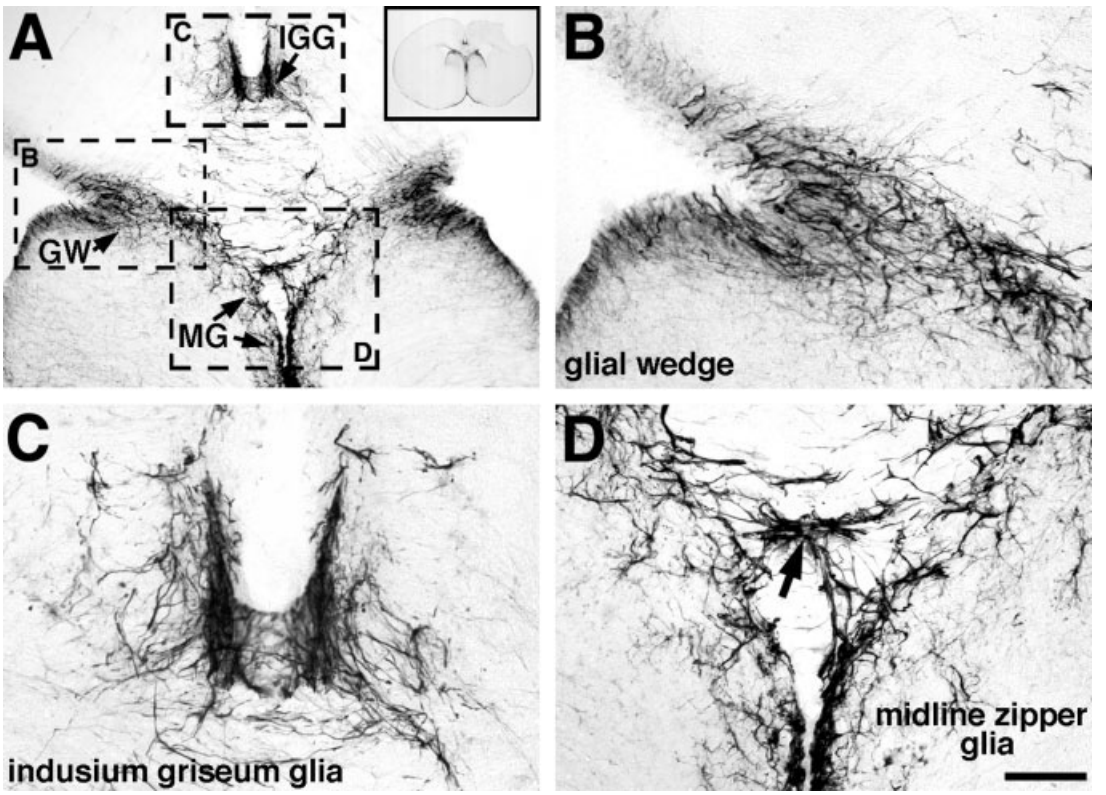

Figure 1 Location and morphology of three midline glial populations at the corticoseptal boundary. The GW, IGG, and MG were all are labeled with GFAP antibody at E18 (A). The inset in panel (A) (solid line) of a whole section demonstrates the high concentration of these glia at the midline and at the corticoseptal boundary in particular. The glial wedge [GW; demarcated area (dashed line) in panel (A) shown at higher magnification in panel (B)] is a bilaterally symmetrical structure that develops a wedge shape and is situated directly below the corpus callosum. Glia within the indusium griseum [IGG; demarcated area (dashed line) in panel (A) shown at higher magnification in panel (C)] are medially located at the pial surface on either side of the interhemispheric fissure. The IGG form a dense meshwork of GFAP-positive processes above the corpus callosum. The midline zipper glia [MG; demarcated area (dashed line) in panel (A) and shown at higher magnification in panel (D)] are concentrated at the midline below the corpus callosum and span the interhemispheric fissure [arrow in (D)] between the two GW on either side. Scale bar in (D) = 200 μm in (A) and 80 μm in (B, C, and D).

tal Studies Hydrodoma Bank) used at 1:200 for fluorescent Cy3.

4. Polyclonal anti-BLBP antibody (a gift from Dr N. Heintz, Rockefeller University, NY; Feng et al., 1994) used at 1:8000 for Cy2.
5. Polyclonal anti-GLAST (a gift from Dr. N. C. Danbolt, University of Oslo; Danbolt, 2001) used at 1:3000 for Cy3 detection.
6. Mouse antibromodeoxyuridine (BrdU; DAKO) used at 1:1000 for Cy3 detection.
7. Rabbit anti-*E. coli* β-galactosidase (5 prime $\rightarrow$ 3 prime, Inc.) used at 1:1000 for Cy2 detection.

Secondary antibodies used were: biotinylated donkey antimouse (Jackson ImmunoResearch Laboratories) used at 1:600; Cy2-conjugated donkey antirabbit IgG (H+L) (Jackson ImmunoResearch Laboratories) used at 1:400; and Cy3-conjugated donkey antimouse IgG (H+L) (Jackson ImmunoResearch Laboratories) used at 1:400. All primary antibodies were incubated on the sections overnight at room temperature with gentle agitation. Secondary antibodies were incubated with the sections for 2 h. For further details of the immunostaining protocol see Shu et al. (2000) and Shu and Richards (2001).

DiI Labeling of Radial Glia

Individual radial glia cells were labeled via previously published methods (Puche and Shipley, 2001). Briefly, a suspension of DiI "nanocrystals" was injected into the open ventricular space of one cortical hemisphere at E13 (n = 2

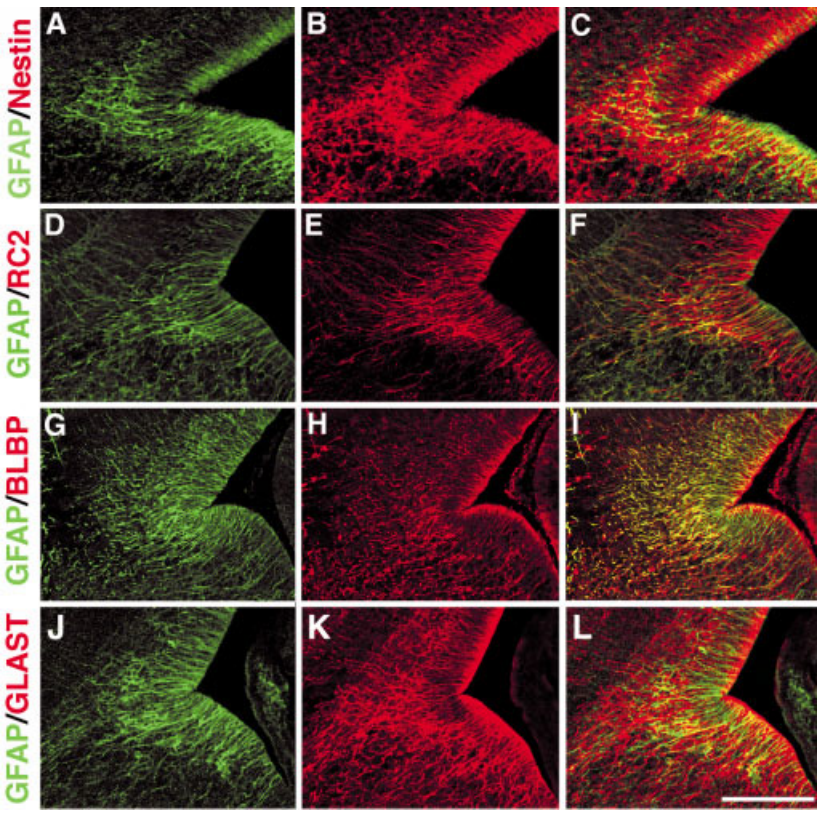

Figure 2

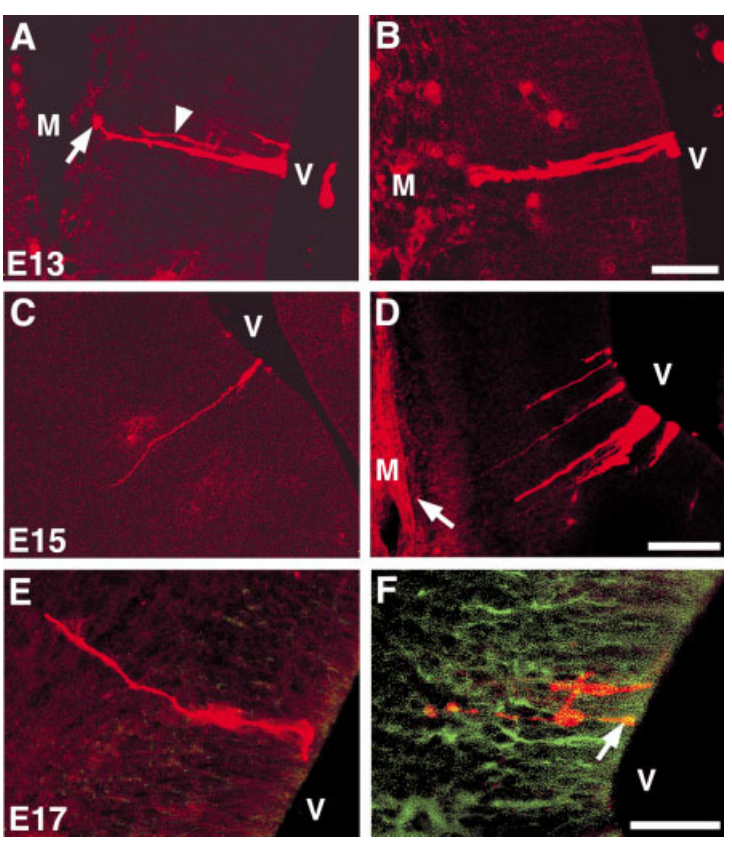

Figure 3

animals), E15 (n = 3 animals), or E17 (n = 2 animals). These crystals adhere randomly to the ventricular wall where they dissolve completely into the membrane of a single cell (either a ventricular endothelial cell, proliferating stem cell, or radial glia). The volume of DiI nanocrystals injected was empirically adjusted at each age such that two to seven midline radial glial cells were labeled in each brain. The embryos were incubated at 37°C for 24 h to allow transport of the DiI throughout the cell. Serial vibratome sections (50 μm) were cut, mounted in a DABCO (Sigma)-based antifade mounting media, and optically sectioned on a FluoView confocal microscope (Olympus Instruments, CA). The complete arbors of individual radial glia were reconstructed from these sections/optical stacks and are presented as Z-series photographs. In some animals double labeling for GFAP was performed to identify GW cells.

BrdU Labeling

BrdU was dissolved in sterile saline and injected intraperitoneally into pregnant dams at a concentration of 50 μg/g body weight on E12, E13, E14, E15, E17, E18, and P2. Embryos from E12- to E15-injected dams were collected on E17. E17, E18, and P2 injected animals were sacrificed on E18, P0, and P3, respectively. Brains were processed for immunohistochemistry as described above except that BrdU-labeled sections were incubated in 2 *N* HCL for 1 h before the immunostaining.

RESULTS

Midline Glial Populations at the Corticoseptal Boundary

Three midline glial populations have been described at the corticoseptal boundary: the GW, IGG, and the MG (Silver et al., 1993; Shu and Richards, 2001). Here we describe these populations in more detail and assess their phenotype by investigating the expression of different glial marker proteins. In contrast to a generally accepted theory that GFAP protein is not detected during early development in the rodent brain (Valentino et al., 1983; Boyer et al., 1990; Sancho-Tello et al., 1995), all three populations can be identified by GFAP immunohistochemistry as early as E13 (GW) and E17 (IGG and MG). The GW is one of the earliest GFAP-positive glial populations to arise in cortex, expressing GFAP prior to other regions of the dorsal telencephalon (Shu and Richards, 2001). A previous study, using a transgenic GFAP-lacZ (nuclear targeted) mouse (Brenner et al., 1994) showed that the cell bodies of the GW reside within the ventricular zone (Shu and Richards, 2001). GW cells send long radial glial-like processes toward the midline [Fig. 1(A,B)]. The IGG and the MG first begin to express GFAP on E17 and display a more mature, multipolar astrocyte-like morphology [Fig. 1(A,C) (IGG) and 1(A,D) (MG), shown at E18]. Both the IGG and MG have densely labeled GFAP-positive processes that line the medial pial surface of the corticoseptal region and have been hypothesized to participate in adhesion and closure of the interhemispheric fissure (Silver et al., 1993).

The GW Is Derived from the Radial Glial Scaffold

As previously described, the GW has long radial glial-like processes. Given that GW cells express GFAP much earlier than other regions of the radial

Figure 2 The GW labels with radial glial markers. Colabeling of the GW with GFAP (A, D, G, J) and either Nestin (B), RC2 (E), BLBP (H), or GLAST (K) demonstrates that all four radial glial markers label the GFAP-positive process of the GW (C, F, I, and L respectively). Although three of the markers label only the distal ends of the GFAP-positive processes [Nestin, (C); BLBP, (I); GLAST, (L)], RC2 labels the entire length of the processes (F). Scale bar in (L) = 200 μm in all panels.

Figure 3 Morphological development of individual cells in the GW. GW cells were labeled with DiI from the ventricular surface at either E13 (A and B), E15 (C and D), or E17 (E and F). At E13 cells within the GW that reached the pial surface at the midline (M) possessed morphological endfeet [arrow in (A)]. Some cells had processes that extended only part of the way to the pial surface [arrowhead in (A)]. At E15, one cell is seen extending to the pial surface [arrow in (D)] but most other cells have shorter processes that extend only part of the way. At E17, all cells analyzed at the cortical midline extended only part of the way to the pial surface. In panel (F), DiI-labeled cells were double-labeled with GFAP [yellow region of the cell labeled by an arrow in (F)]. The two panels at each age represent two different examples of labeled cells. V, ventricular zone. Scale bar in (B) = 50 μm in (A) and (B); scale bar in (D) = 100 μm in (C and D); Scale bar in (F) = 50 μm in (E) and (F).

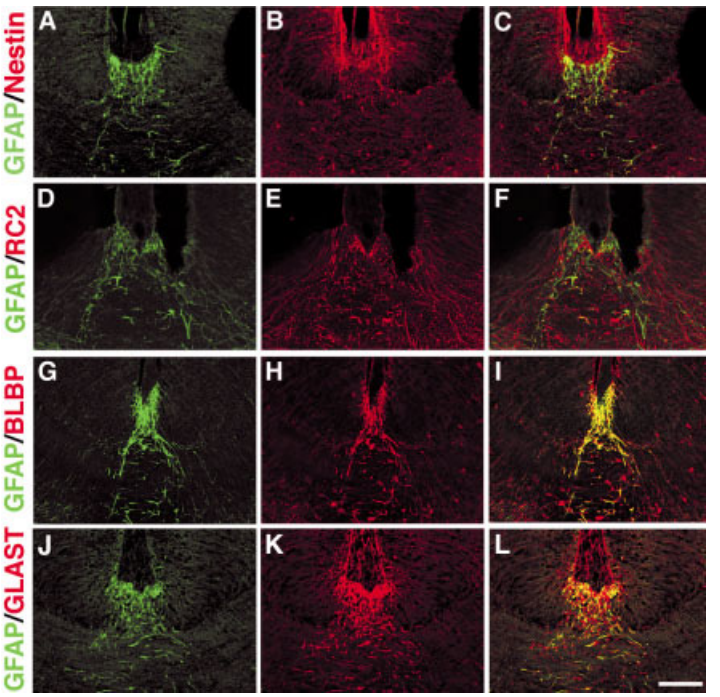

Figure 4 Immunohistochemical labeling of the IGG. Glia within the indusium griseum (IGG) express GFAP at E17 (A, D, G, J). Colabeling of the IGG with GFAP and either Nestin (B), RC2 (E), BLBP (H), or GLAST (K) reveals that only BLBP (I) and GLAST (L) colabel the IGG. Nestin (C) and RC2 (F) do not label the IGG. Scale bar in (L) = 120 μm in all panels.

glial scaffold, and molecules such as Slit2 are only expressed within this corticoseptal boundary region (Nguyen Ba-Charvet et al., 1999; Shu and Richards, 2001), are they part of the radial glial scaffold? To investigate this we labeled GW cells *in vivo* with radial glial markers and examined the morphology of individual cells by DiI labeling. Sections containing the GW were double-labeled with GFAP and with either Nestin (Hockfield and McKay, 1985), RC2 (Mission et al., 1988), astrocyte-specific glutamate transporter (GLAST; Danbolt, 2001; Ullensvang et al., 1997; Shibata et al., 1997), or brain lipid-binding protein (BLBP; Hartfuss et al., 2001; Feng et al., 1994; Kurtz et al., 1994). At E17, GW processes labeled with both GFAP and Nestin [Fig. 2(A–C)], GFAP and RC2 [Fig. 2(D–F)], GFAP and BLBP [Fig. 2(G–I)], and GFAP and GLAST [Fig. 2(J–L)]. Both Nestin and RC2 also labeled radial glia within the dorsal telencephalon. Nestin, BLBP, and GLAST expression was confined to the distal regions of the GW processes [Fig. 2(B) and (C), (H) and (I), and (K) and (L), respectively] and did not label the ventricular zone where the GW cell bodies reside (Shu and Richards, 2001). RC2 colabeled with GFAP along the entire length of the GW processes [Fig. 2(F)].

These data indicated that GW cells expressed molecular markers of radial glial cells. To examine their morphology we performed DiI labeling from the ventricular surface, using a technique that labels individual cells (Puche and Shipley, 2001). At E13 GW cells had long processes that spanned from the ventricular zone to the midline pial surface [Fig. 3(A,B)]. At the midline and ventricular surfaces cells displayed morphological "endfeet", which have been proposed to anchor radial glia in the cortex [arrow in Fig. 3(A); Bentivoglio and Mazzarello, 2000]. Of the 10 cells examined at E13, six cells spanned the entire ventricular to midline pial surface. Some cells had processes that only reached part of the way from the ventricular zone to the midline [arrowhead in Fig. 3(A)]. These cells were "anchored" to the ventricular surface. We performed the same experiment at E15 and E17 and found that the number of cells spanning the entire ventricular to pial surface at the midline declined with development. Fifteen cells were analyzed at E15 [Fig. 3(C,D)]; one cell was found spanning the ventricular to pial surface [arrow in Fig. 3(D)], and 14 cells had processes that only stretched part of the way. Six cells were analyzed at E17 and all cells spanned only part of the way to the pial surface [Fig. 3(E,F)]. These data indicated that during development GW cells lose their pial attachment at the time midline fusion is occurring.

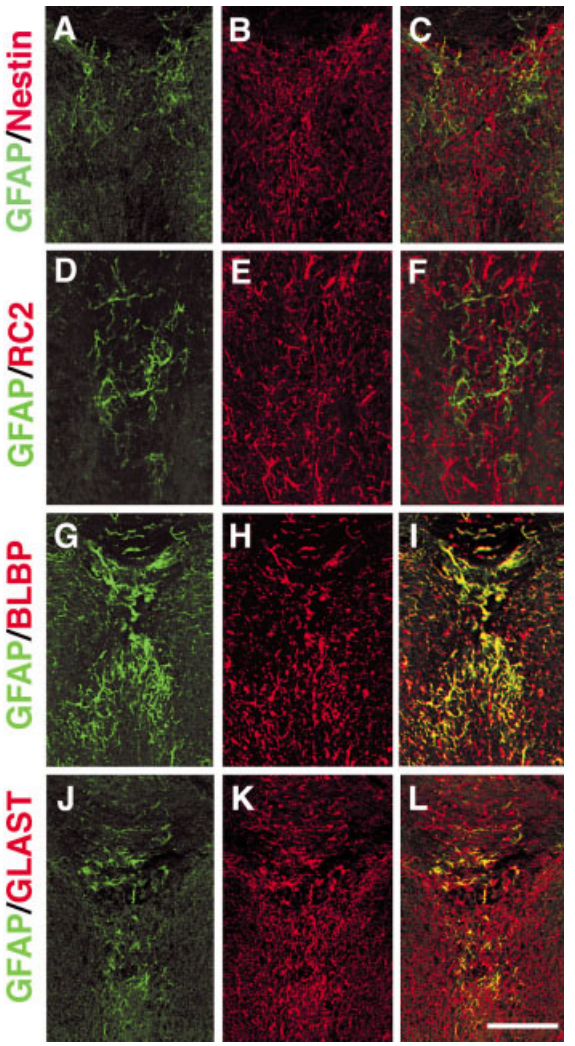

Figure 5 Immunohistochemical labeling of the MG. Midline zipper glia (MG) express GFAP at E17. MG were colabeled with GFAP (A, D, G, J) and either Nestin (B), RC2 (E), BLBP (H), or GLAST (K). The GFAP-positive processes of the MG were colabeled with BLBP (I) and GLAST (L) but not Nestin (C) or RC2 (F). Scale bar in (L) = 120 μm in all panels.

GFAP-Positive Glia within the IGG and the MG Do Not Label with RC2 or Nestin

To determine if other midline glial populations such as the IGG and the MG labeled with the same markers as the GW at E17 we colabeled sections with GFAP and either Nestin, RC2, BLBP, or GLAST. The staining profile was the same for both the IGG and MG (Figs. 4 and 5). Unlike the GW, neither population colabeled with Nestin or RC2 [Figs. 4(A–F) and 5(A–F)]. However, both populations labeled with BLBP and GLAST [Figs. 4(G–L) and 5(G–L); summarized in Fig.9]. These data indicate differences in the molecular expression of markers between the GW and the IGG and MG, and that the IGG and MG do not express typical radial or immature glial markers.

Midline Glial Populations Are Generated at Different Times during Embryogenesis

To determine if the three midline glial populations are generated at the same stage of development, BrdU was injected intraperitoneally into pregnant dams. GFAP-LacZ transgenic mice were used in some experiments (at E12 and E14) to identify GW cell bodies. The first GW cells were born on E13 [Fig. 6(B)]. BrdU labeling on E12 showed no GW cells labeled [Fig. 6(A)], but numerous dividing cells were present in the cortical subplate at this age (not shown). On E14, a large number of GW cells were born [Fig. 6(C)]. The colabeling of LacZ and BrdU demonstrated that the cells labeled with BrdU colabeled with the nuclear targeted LacZ transgene driven by the GFAP promoter [Fig. 6(D,E)]. GW cells continued to be colabeled with BrdU on E15 [Fig. 6(F)] and E18 [Fig. 6(G)], with a few cells generated at P2 [Fig. 6(H); summarized in Fig. 9].

The majority of IGG were generated on E14 [Fig. 7(A)] and E15 [Fig. 7(B)] with only a few still generated on E17, and none generated on P2 [Fig. 7(D); summarized in Fig.9]. MG were generated in small numbers between E14 and E17 [Fig. 7(E–G)], but a peak period of proliferation was not observed. The generation of MG ceased by P2 [Fig. 7(H); summarized in Fig. 9].

Postnatal Development of the Midline Glial Populations

Postnatally, when most axons have completed their pathfinding, there is a dramatic increase in the number of astrocytes in the CNS (Pixley and de Vellis, 1984; Sancho-Tello et al., 1995). On P0, the GW still maintained its wedge shape with long radial glial-like processes. However, some typical astrocytes also appeared in this region, possibly indicating that the GW cells were starting to transform into multipolar astrocytes [arrow in Fig. 8(B); (B) is a higher power view of the region demarcated in (A)]. On P3, a meshwork of GFAP-positive processes was present in the GW region. GW processes appeared to shorten and more multipolar astrocytes were present [arrows in Fig. 8(D); (D) is a higher power of view of the region demarcated in (C)]. On P10, GFAP expression increased and the GW had completely lost its distinctive shape. More GFAP-positive astrocytes were found

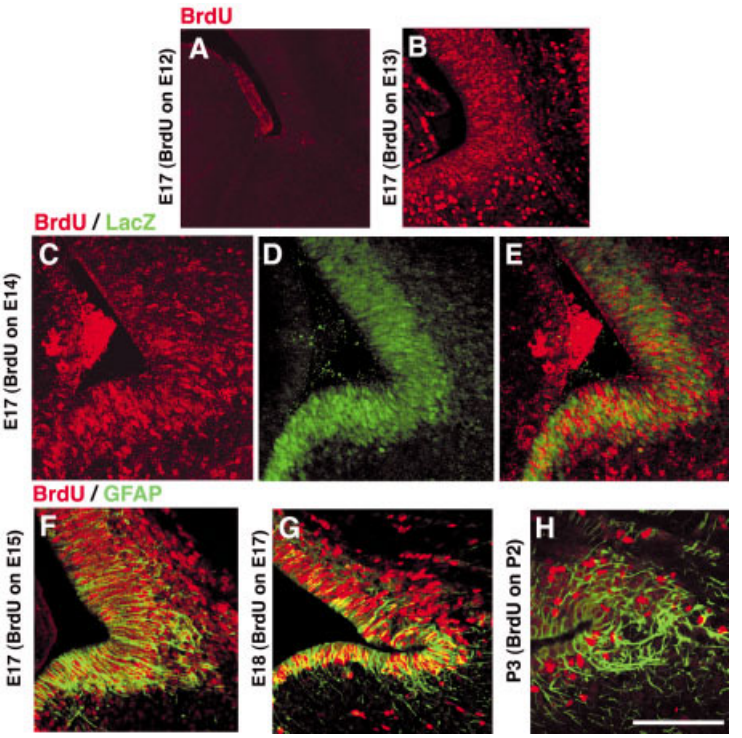

Figure 6

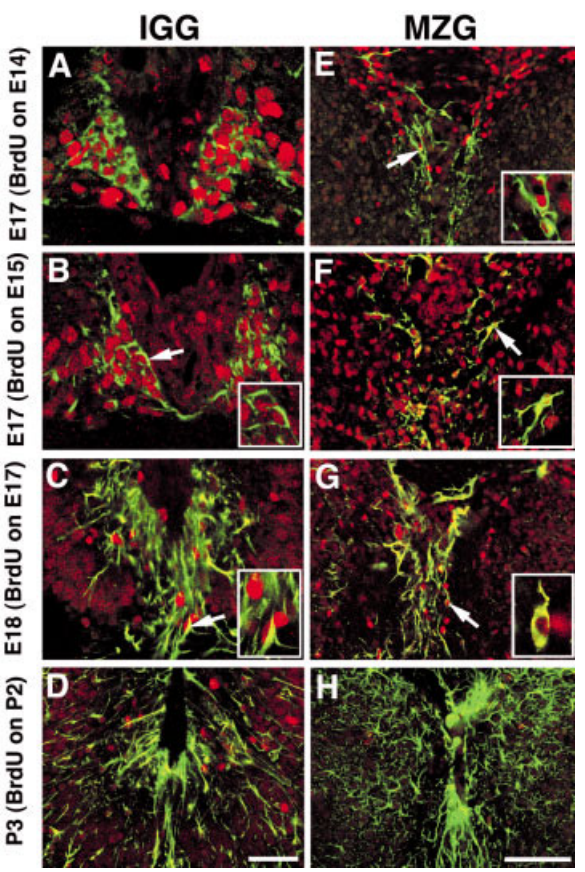

Figure 7

Figure 6 Birth-dating of the GW. BrdU was intraperitoneally injected into timed pregnant dams at either E12 (A), E13 (B), E14 (C, D, E), E15 (F), E17 (G), or P2 (H) and then sacrificed at the ages shown. BrdU labeling was detected by immunohistochemistry (red nuclear labeling in all panels). Cells within the GW were mostly generated between E13–17, with only scattered cells born at P2 (H). Colabeling with either a lacZ antibody (D and E) to detect the presence of the nuclear-targeted lacZ transgene driven by a GFAP promoter, or GFAP immunohistochemistry (F–H) was used to identify the GW. Scale bar in (H) = 200 μm in all panels.

Figure 7 Birth-dating of the IGG and MG. To determine when the IG and MG are generated, BrdU was injected into timed-pregnant dams at either E14 (A, E), E15 (B, F), E17 (C, G), or P2 (D, H) and then sacrificed at the ages shown. BrdU was detected by immunohistochemistry (red nuclei in all panels) and the IGG and MG populations were detected by GFAP immunohistochemistry (green processes and cytoplasm in all panels). Insets in (B, C, E, F, and G) show higher magnifications of double-labeled cells (arrows) within each panel. Scale bar in (D) = 50 μm in (D); 30 μm in (C); 25 μm in (A and B). Scale bar in (H) = 100 μm in (H) and 50 μm in (E, F, and G).

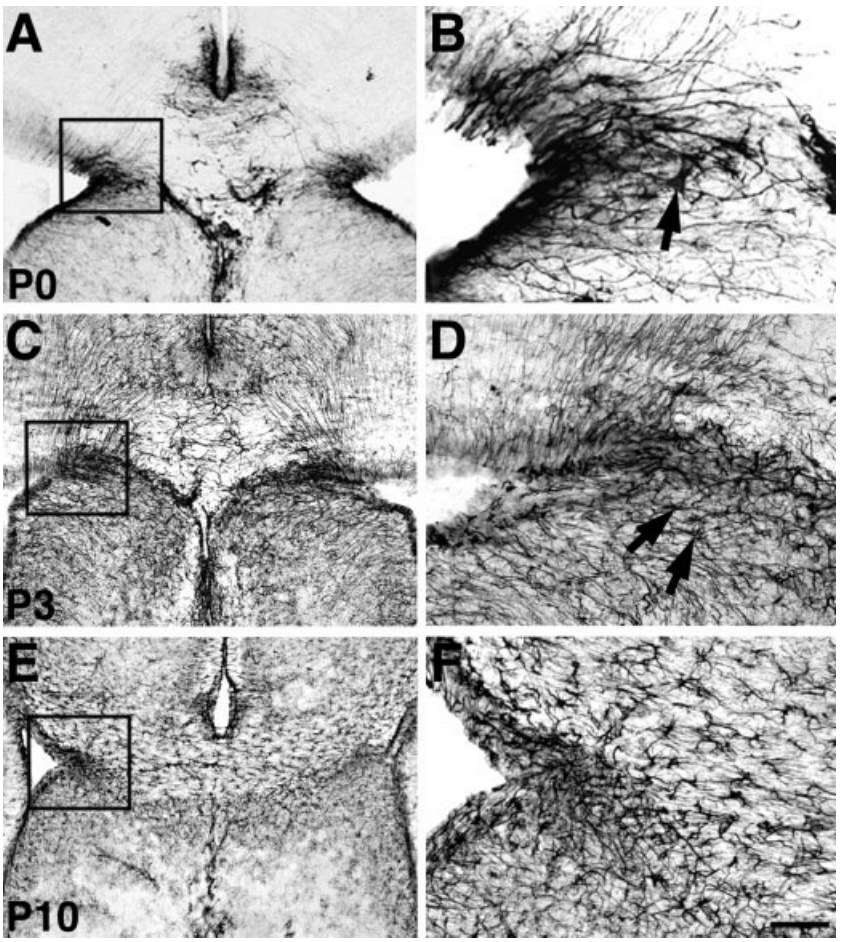

Figure 8 Postnatal development of the GW. At P0 the glial wedge still maintains its characteristic wedge-shaped structure with many processes still long and radial. However, some cells in the region resembled multipolar astrocytes [arrow in (B)]. By P3 the wedge became flatter and more astrocytes appeared [boxed region in (C) and panel (D)]. By P10 few cells with a radial morphology remained in the GW [boxed region in (E) and panel (F)]. All panels are coronal sections labeled with GFAP and nickel-DAB immunocytochemistry. Scale bar in (F) = 200 μm in (A, C, and E); 50 μm in (B, D, and F).

throughout the corticoseptal region at P10 and distinct populations were more difficult to identify [Fig. 8(E,F)]. The IGG and the MG persisted through P3 but were greatly decreased by P10 [Fig. 8(C,E)].

DISCUSSION

The corticoseptal boundary is a region were both callosal axons cross the midline and perforating axons project ipsilaterally from the medial septum and diagonal band of Broca complex into the cingulate cortex and vice versa (Silver et al., 1993; Hankin and Silver, 1988; Shu and Richards, 2001; Shu et al., 2001; Fig. 9). The pioneering axons of both the corpus callosum and the perforating pathway arrive at the corticoseptal boundary at E15–16 (in mouse: Rash and Richards, 2001; Shu et al., 2001). Thus, both the ipsilaterally projecting perforating axons and the contralaterally projecting callosal axons are differentially guided at the corticoseptal boundary at the same stage of development. At the *Drosophila* midline (Kidd et al., 1998a, 1999) and the mammalian optic chiasm (Marcus et al., 1995; Wang et al., 1995), midline glia are involved in sorting contralaterally and ipsilaterally projecting axons. Midline glia also provide important substrates and barriers for growing axons (Fitch and Silver, 1997). Four cellular populations have been described at the corticoseptal boundary: the GW, IGG, MG, and the glial sling [defined here as cells derived from the subventricular zone (SVZ) that form a sling structure between the two hemispheres by

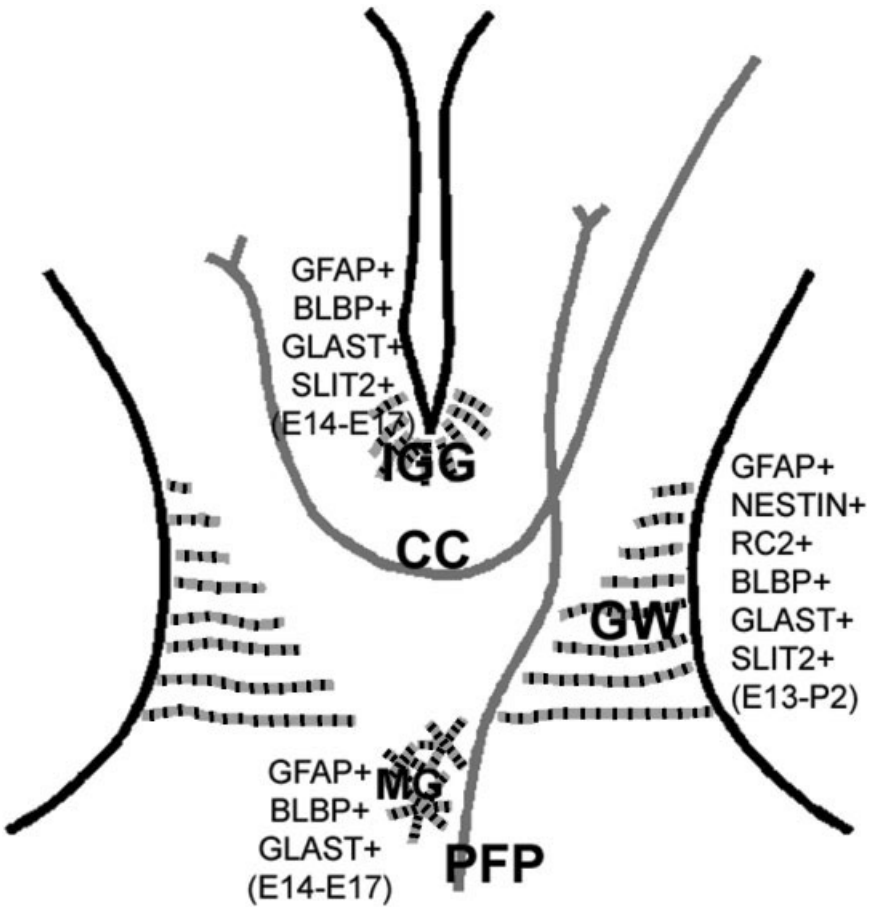

Figure 9 Development and gene expression of midline glial populations. The midline of the cerebral cortex is shown schematically. Grey axons show the position of the corpus callosum (cc) and the perforating pathway (PFP). The glial wedge (GW), indusium griseum glia (IGG), and the midline zipper glia (MG) are shown in lines with striping. The gene expression patterns described here are indicated for each glial population. The embryonic (E) and postnatal (P) stages at which each population is generated are shown in parentheses.

E17]. Although the glial sling was first described as a separate population (Silver et al., 1982), a later article grouped the GW and MG with the glial sling cells as one structure (Silver et al., 1993). A recent analysis of the cellular nature of the SVZ-derived sling cells has revealed that this population is largely composed of neurons and not glia (Shu et al., 2003), and therefore we did not include them in our analysis here. Here we investigated the GW, IGG, and MG as separate populations given their discrete anatomical locations within the corticoseptal boundary to determine what, if any, characteristics they had in common.

We found that GW cells express GFAP as early as E13, prior to other regions of the cortical plate. GW cells have long radial processes that reach the pial surface at E13 but during development appear to retract these processes away from the pial surface. The function of this retraction is not clear but may give way to a less cell-dense region to accommodate growing callosal axons. Alternatively, it may be the initial step in changing the morphology of the cells from radial to multipolar. GW processes appear shorter and thicker around P3 and by P10 cells within the GW have differentiated into astrocytes. This supports previous work showing that radial glia transform into astrocytes later in development (Schmechel and Rakic, 1979; Voigt, 1989). In addition, the timing of the retraction correlates with the timing of midline fusion. It is possible that these two processes are linked but further experiments are required to investigate this hypothesis. Thus at least part of the radial glial scaffold of the cerebral cortex that makes up the GW appears to transform into astrocytes.

Recent experiments have shown that radial glia also have the capacity to differentiate into neurons (Hartfuss et al., 2001; Malatesta et al., 2000; Miyata et

al., 2001; Noctor et al., 2001; Heins et al., 2002; reviewed in Parnavelas and Nadarajah, 2001). By cell-sorting and immunohistochemical labeling, Hartfuss et al. (2001) showed that radial-glia-derived cells that were $RC2^+$, $BLBP^+$, and $GLAST^+$ had the capacity to differentiate into both neurons and glia. However, glia that were only $BLBP^+$ and $GLAST^+$ differentiated into astrocytes. Here we find that the GW is $RC2^+$, $BLBP^+$, $GLAST^+$, and $Nestin^+$, whereas the IGG and the MG are only $BLBP^+$ and $GLAST^+$. This could indicate that the GW has the capacity to give rise to both neurons and glia, but this hypothesis remains to be tested. Based on the observation that cells within the GW initially have a radial morphology and then have the morphology of multipolar astrocytes, we hypothesize that radial glia within the GW may transform into mature astrocytes. Evidence for the transformation of radial glia into astrocytes has been previously described (Schmechel and Rakic, 1979; Pixley and de Vellis, 1984; Culican et al., 1990; Mission et al., 1991; Abd-el-Basset et al., 1992; Chanas-Sacre et al., 2000). Our data suggest that either the radial glia that make up the GW are multipotent or that the radial glial scaffold of the cerebral cortex may not be a homogeneous population of cells.

Although the GW is part of the radial glial scaffold, the IGG and MG do not label with Nestin and RC2 and do not have a radial morphology. These cells appear to differentiate directly into astrocytes. The IGG and MG had a number of immunohistochemical features in common and both arose at the midline along the interhemispheric fissure. However, one difference between these two populations is that the IGG, but not the MG, express Slit2 mRNA (Shu and Richards, 2001). The anatomical location of these two populations may also be significant in, for example, their difference in Slit2 expression. The corticoseptal boundary is not only an anatomical boundary but also a gene expression boundary. For example, a sharp boundary of expression exists in dorsally expressed patterning genes such as Emx1 and Emx2 at the corticoseptal boundary (Yoshida et al., 1997). Coincident with this expression boundary are the anatomical locations of the IGG and MG. The IGG lie on the dorsal or cortical side of this boundary and the MG lie on the ventral or septal side. Both populations are generated over the same developmental period from E14–18. The similarities of these glia suggest that the IGG and MG could initially be part of the same population of cells that was then split by the formation of the corticoseptal boundary and the corpus callosum. Differences in these two populations may then arise by their exposure to differentially expressed genes in the cortex or septum. The function of these two populations has not been thoroughly investigated. Morphological evidence suggests that the MG and possibly the IGG are responsible for midline fusion of the interhemispheric fissure (Hankin and Silver, 1988; Silver et al., 1993). An additional hypothesis is that the location of these glia specifies where the callosal axons cross the midline. Although the MG and the IGG share a number of similarities, *in vivo* clonal analysis is required to determine if they are derived from the same progenitor population that is split by the formation of the corpus callosum, or whether they are derived from distinct populations of cells.

An understanding of the development and molecular expression of these glial populations at the corticoseptal boundary is important for understanding the molecular regulation of callosal and perforating axon pathfinding. Recent experiments suggest that at least part of the guidance activity of the GW is due to the chemorepellent molecule, Slit2 (Shu and Richards, 2001). However, recent evidence suggests that callosal axon guidance is a multifactorial process. In the GAP-43 mutant the GW appears to form normally and cortical axons respond to Slit2 in culture, yet callosal axons do not cross the midline and instead form Probst bundles (Shen et al., 2002). These results suggest that additional molecules are required for the formation of the corpus callosum. These molecules may be derived from the GW or other midline glial structures described here or may be expressed on the callosal axons themselves.

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