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Mapping of Radial Glia and of a New Cell Type in Adult Canary Brain

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Frontal and coronal sections of adult male and female canary brain were stained with a monoclonal antibody to vimentin using an immunoperoxidase technique; some sections were counterstained with cresyl violet. The position of radial glia cells was mapped using a computer-linked microscope. The telencephalon was found to have a rich set of radial glia. The long processes of these radial glia showed a mediolateral orientation, and were much more abundant in some parts of the telencephalon (e.g., hyperstriatum, caudal neostriatum, and lobus parolfactorius) than in others (e.g., anterior neostriatum, archistriatum, and septum), which had few or no radial glia fibers. A small, elongated cell type not previously described in adult avian brain was frequently seen to be associated with the long processes of the radial glia, oriented in the same direction and often in close apposition. The position of these cells was also systematically mapped, and they were found to be virtually absent outside of the telencephalon. The relation between radial glia fibers and the small, elongated cells was most commonly seen close to the lateral ventricle of the forebrain, where the radial glia cells have their cell bodies. The above observations suggest that there may be a functional relation between radial glia and the small, elongated cells. We hypothesize that the latter cells are young migrating neurons. This hypothesis is tested in a separate publication (A. Alvarez-Buylla and F. Nottebohm, unpublished observations).

It has been customary to describe the anatomy of adult vertebrate brains in terms of clusters or layers of neurons and their connections, with little or no emphasis on whatever regularities might occur in the distribution of glia. The discovery that new neurons are added to the adult avian brain (Goldman and Nottebohm, 1983; Paton and Nottebohm, 1984; Burd and Nottebohm, 1985; Nottebohm, 1985) forced us to think about sources of these new neurons and pathways for their dispersal. This led us to discover the pervasiveness of radial glia in adult avian telencephalon (Nottebohm et al., 1985; Alvarez-Buylla et al., 1987).

Radial glia-like cells in adulthood had been reported by earlier workers (Van Gehuchten, 1897; Studnicka, 1900; Ramón y Cajal, 1911; Horstmann, 1954; Stensaas and Stensaas, 1968), who gave them various names [epithelial cell, faserglia (fiberglia), tanycytes, ependymoglia, typical ependimal cells]. These authors described the forms of these cells but did not attempt a systematic study of their distribution. Yet, it is only after one becomes aware of the patterns of distribution of adult radial glia that it is possible to address some of the questions concerning their potential function. The present report maps the position of radial glia and their association with a heretofore undescribed cell type in adult avian brain.

This is one of a series of 3 articles that deal with the relation between radial glia and neurogenesis in adult canaries. The other 2 articles in this series describe (1) the antibody used to characterize radial glia and their anatomy (Alvarez-Buylla et al., 1987), and (2) neuronal migration in adulthood (Alvarez-Buylla and Nottebohm, unpublished observations).

Materials and Methods

One-year-old canaries (*Serinus canaria*) of both sexes were used. Brains were cut transversely so as to match the plane of section of the stereotaxic atlas for canary brain (Stokes et al., 1974). The staining of radial glia was done in polyethylene glycol (PEG) sections as described before (Alvarez-Buylla et al., 1987). Briefly, the brain was fixed in 2% paraformaldehyde and 0.15% picric acid in 0.1 M phosphate buffer (pH 7.4), dehydrated in ethanol, impregnated in PEG M_r 1000–1500 at 46°C and embedded in PEG M_r 1500. PEG sections were cut on a rotary microtome at a thickness of 10 μm. Some brains were cut unembedded on the Vibratome at a section thickness of 25–30 μm.

Radial glia were visualized by reacting tissue sections for 36 hr with 40E-C hybridoma supernatant and the avidin–biotin–peroxidase technique (Vectostain; Vector Laboratories). The monoclonal antibody (40E-C) has been described previously and shown to recognize the intermediate filament protein vimentin (Alvarez-Buylla et al., 1987). This antibody, unlike others that also react with vimentin, gives a very weak or no reaction to blood vessels, thus rendering an uncluttered view of radial glia and their processes. Antibodies against glial fibrillary acidic protein (anti-GFAP) were the kind gift of Dr. L. Eng, and were used according to our protocol (Alvarez-Buylla et al., 1987). Some sections were counterstained with 0.5% cresyl violet, dehydrated in ethanol, cleared in xylene, and mounted in Diatex (Scientific Products), thus allowing us to see the relation of radial glia cells to other brain cells and to major anatomical landmarks (Stokes et al., 1974). PEG sections were used for mapping because, being thinner, they allowed for better anatomical resolution. In addition, the PEG sections were more homogeneous in thickness than were the Vibratome sections. However, radial glia cannot be followed for a very long distance in 10-μm-thick sections, and for this reason Vibratome sections were used to follow longer segments of the radial glia fibers. Sections were mounted so as to maintain overall section integrity.

Mapping. Fifteen 1–2-year-old male and female canaries were used to study the distribution of radial glia. However, on the basis of the preservation and the quality of the immunocytochemical staining, only the brains of one male and one female 1-year-old bird were used for detailed mapping.

Mapping was done by reconstructing individual microscopic fields of the tissue section onto the computer screen with the aid of camera lucida. These fields were pieced together using software developed in our laboratory. The final output was a plot of cells and fibers, the outlines of the section, ventricles, and laminae. Systematic scanning of the entire

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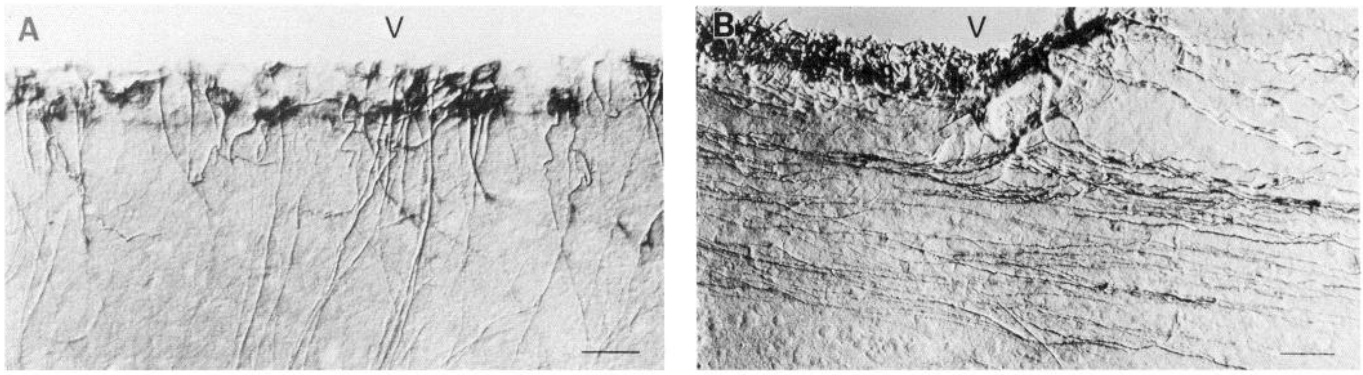

Figure 1. *A*, Differential interference contrast micrograph of a section of the ventricular wall facing the lateral ventricle of the forebrain (*V*). The dark cells and fibers belong to radial glia cells that reacted with antibody 40E-C. Calibration bar, 20 μm. *B*, Radial glia fibers stream laterally (to the right) from the lateral tip of the lateral ventricle of the forebrain, from which some of the fibers arise. Calibration bar, 50 μm.

section was done with a 40× oil-immersion objective. The microscope (Labophot; Nikon) was equipped with X–Y digital encoders (Mitutoyo) mounted onto the stage. These encoders provided positional information to the computer. This mapping technique is described elsewhere (Alvarez-Buylla and Vicario, 1988).

Results

Distribution of radial glia

The anatomy and distribution of radial glia were very similar in the 2 birds studied in detail. They were also very similar in the 13 other male and female adult canaries used in this study. The absence of radial glia in certain regions of the brain is reproducible from bird to bird. The drawings presented here are those for the male.

A typical radial glia of adult avian brain has its cell body in the ventricular zone lining the lateral ventricle and a fiber that penetrates the brain parenchyma for a variable distance (Fig. 1). We have been able to follow individual fibers for up to 2 mm and they are found as far as 5 mm from the ventricle. Figure 2 shows the appearance of a typical group of radial glia fibers in the hyperstriatum dorsalis (see Fig. 3) and their relation to cresyl violet-stained cell nuclei.

Figure 3 shows the position of radial glia in 3 rostrocaudally sequential transverse sections through the brain of an adult male canary. The occurrence of radial glia is not random. For example, they are abundant in the telencephalon, where they reach areas far removed from the ventricular wall, but are virtually absent from other areas, such as the septum and midbrain. A shorter type of fiber can be found coursing away from the midbrain ventricles; we have characterized the latter kind of fiber as belonging to tanycytes (Alvarez-Buylla et al., 1987). Most radial glia course from the lateral ventricle of the forebrain laterally, and many run parallel to the surface of the brain, penetrating into distant areas of the lateral forebrain.

There are some regions of telencephalic parenchyma that receive more radial glia fibers than others (Fig. 3). Areas rich in radial glia fibers are (1) hyperstriatum accessorium, dorsalis, and ventralis; (2) lobus parolfactorius (LPO); (3) ventromedial part of caudal neostriatum; and (4) dorsolateral caudal neostriatum. Areas with few, if any, radial glia fibers are (1) rostral neostriatum; (2) nucleus hyperstriatum ventralis, pars caudalis (HVc), thought to be part of neostriatum (Nottebohm et al., 1982); (3) archistriatum; and (4) septum. In some areas, discrete arrays of radial glia fibers seem to follow major anatomical laminae. For example, many fibers leave the lateral ventricle to travel above the lamina hyperstriatica (LH), which forms the dorsal boundary of the neostriatum. Radial glia fibers also seem to group in the dorsal border of LPO just under the lamina medullaris dorsalis (Fig. 3*A*, *B*).

The preceding description resulted from our maps of the distribution of fibers as seen on transverse sections of the brain. Coronal sections confirmed this distribution. For example, the mediolateral orientation of hyperstriatal fibers can be readily seen in a section cut very close to the roof of the brain (Fig. 4*A*). In deeper sections (Fig. 4*B*), a small subset of fibers is observed arising from the rostral edge of the lateral ventricle and coursing forward into the rostral tip of the telencephalon (hyperstriatum accessorium). Fibers from the caudolateral edge of the lateral ventricle also travel forward, in this case into lateral neostriatum. In summary, most radial glia fibers travel in the mediolateral plane, while a smaller number reach the most rostral and lateral forebrain, traveling in the rostrocaudal plane.

A new cell type for adult avian brain

Figure 2 shows a cell type often associated with the radial glia fibers. These cells show very little anatomical overlap with any other cell type of the adult avian brain. Typically they have an elongated nucleus that is 10 μm long and 3–4 μm across. This nucleus shows one or 2 relatively large and darkly staining nucleoli (Fig. 2, *C–E*). The cytoplasm is not clearly visible under the conditions we used. These cells do not react with our intermediate filament antibody, which stains radial glia, nor do they react with anti-GFAP antibodies. The only other cell type with which they could be confused is the endothelial cell, but the nuclei of these 2 cell types stain differently. The nucleus of endothelial cells shows a mottled distribution of basophilic substance, while that of the "new cell type" shows one or 2 dark-staining nucleoli (compare Fig. 2*B* with *C–E*). In addition, the relation of endothelial cells and a vascular lumen is usually sufficiently clear to prevent confusion between these 2 cell types. Except for their relation to vascular lumina, endothelial cell nuclei show no particular orientation.

The orientation of the nuclei of the new cell type is unusual in that it coincides very closely with the orientation of radial glia fibers and often shows close apposition to such fibers (Fig. 2). Despite this frequent association with radial glia fibers, the new cell type we describe here was also found, in smaller numbers, in parts of the telencephalon, such as ectostriatum and lateral part of neostriatum, where there were few, if any, radial glia fibers (Fig. 3).

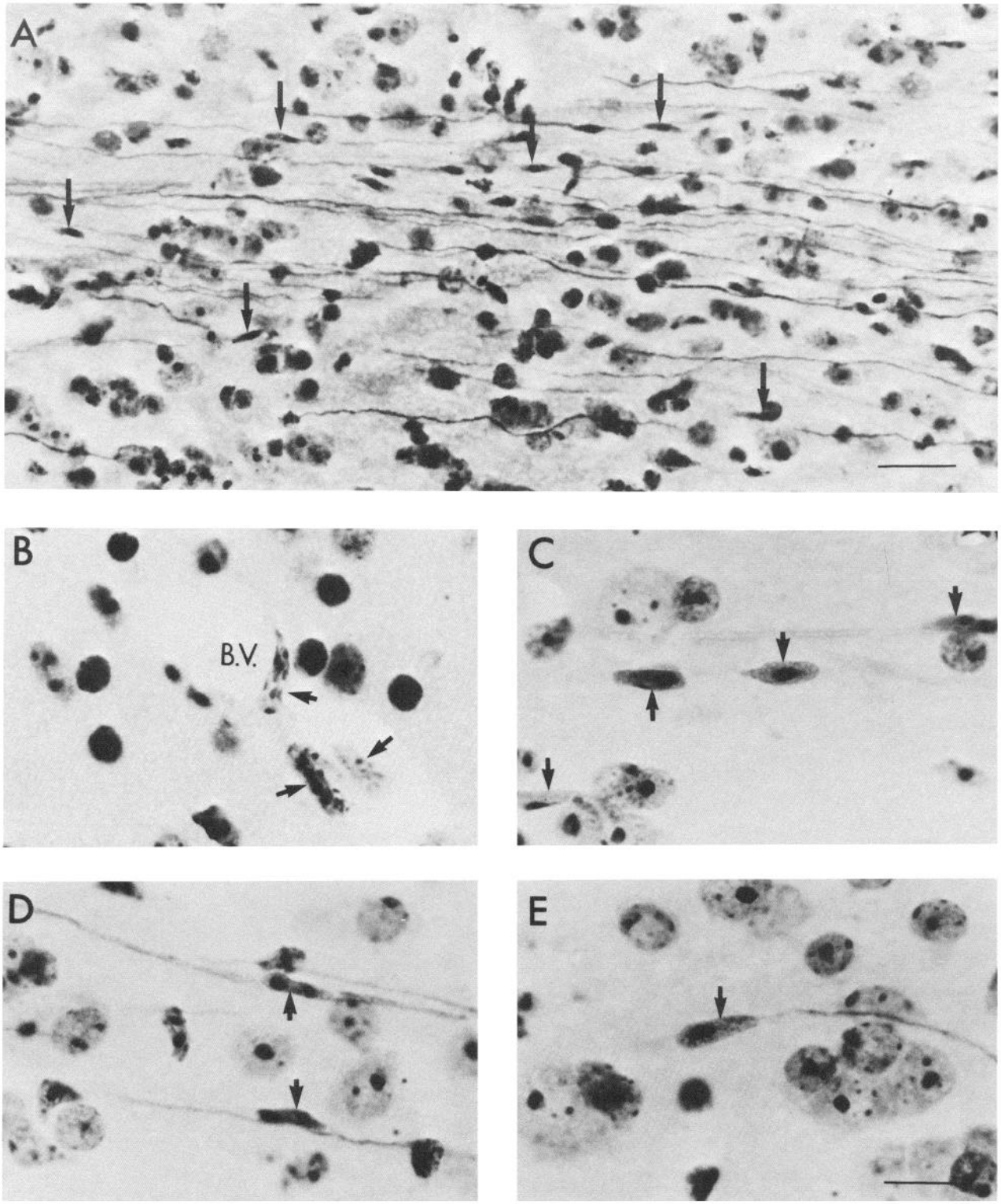

Figure 2. Photomicrographs of 40E-C-stained material counterstained with cresyl violet. *A,* Vibratome section, 25 μm thick at the level of the hyperstriatum dorsalis. Note the parallel arrangement of radial glia fibers and the many elongated cells oriented in the same direction (some indicated by *arrows*). Calibration bar, 20 μm. *B–E,* PEG sections, 10 μm thick, in *B,* nuclei of endothelial cells (*arrows*) on a tangentially cut blood vessel (*B.V.*). *C–E,* Examples of the nuclei of the new cell type (*arrows*). Note how some are closely apposed to radial glia fibers. Calibration bar, 10 μm.

Relative distributions of radial glia and new cell type

The new cell type is virtually unique to the telencephalon. For example, Figure 3 shows the position of 629 cells of the new type mapped in 3 brain sections. Of these, 625 were in the telencephalon, and the remaining 4 in the thalamus. In these 3 sections, as well as in 2 others not shown, the areas of telencephalon and non-telencephalon mapped were, respectively, 70.2

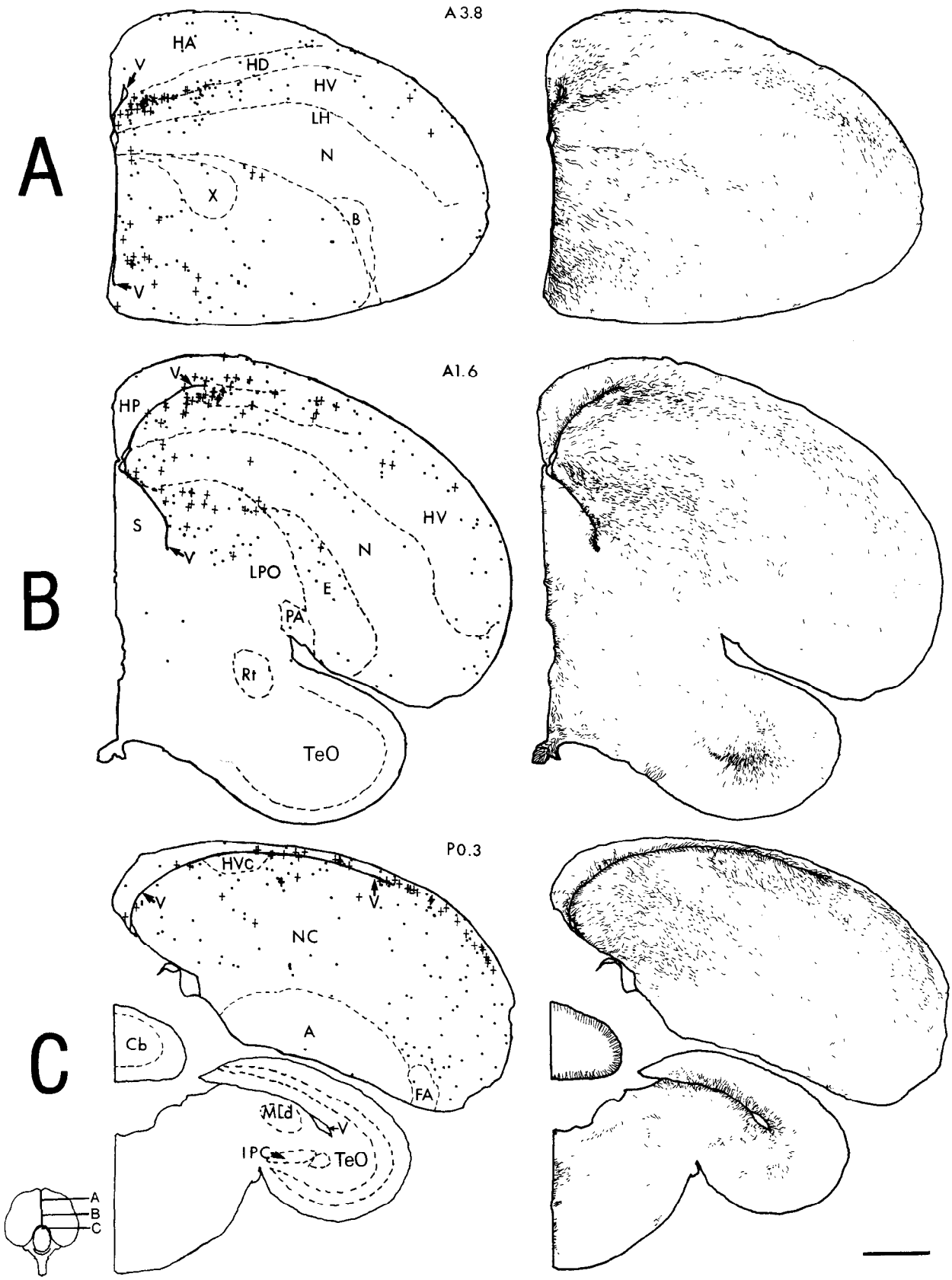

Figure 3. Maps of the positions of radial glia (*right*) and cells of the new type (*left*) reconstructed from 10 μm thick PEG sections of a male canary brain. Cells of the new type that appear attached to radial glia are indicated by a *cross*, and unattached ones by a *dot*. The *number* above each pair of sections indicates the position along the rostrocaudal plane corresponding to the canary atlas (Stokes et al., 1974). A key to this position is on the *lower left-hand corner*. On the *right panels* we show the position of radial glia on the same section. See text for details. Major laminae are indicated by *dashed lines*. *A*, archistriatum; *B*, nucleus basalis; *Cb*, cerebellum; *E*, ectostriatum; *FA*, tractus fronto-archistriatalis; *HA*, hyperstriatum

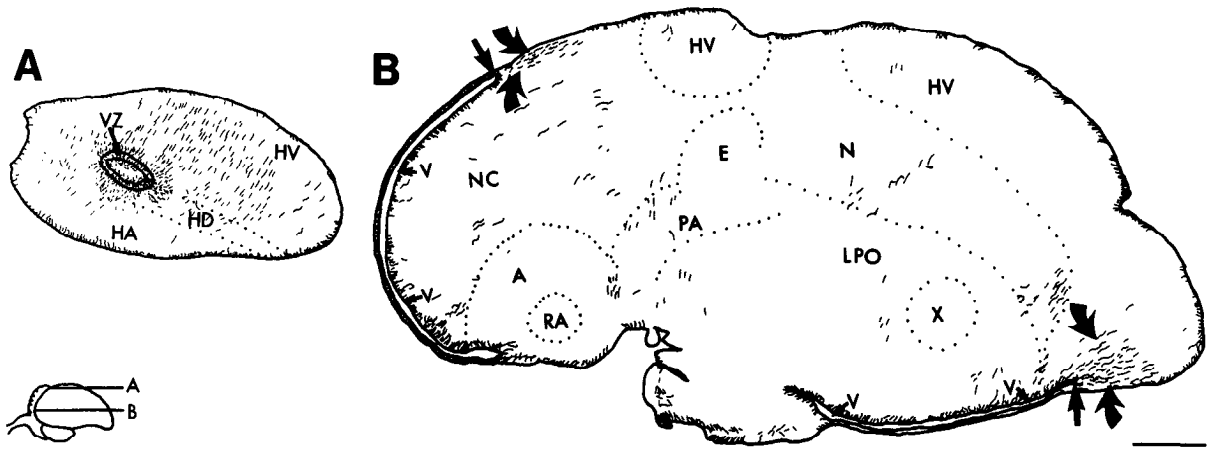

Figure 4. Map of position of radial glia reconstructed from horizontal PEG sections at the levels indicated on the *lower-left* key. Up is lateral and right is frontal. *Straight arrows* indicate the anterior and posterior ends of the lateral ventricle; *curved arrows* indicate sets of radial glia fibers that arise from these ventricular tips and then orient in a rostrocaudal direction. Major laminae are indicated by *dotted lines*, other abbreviations are as in Fig. 3. *RA*, nucleus robustus archistriatalis; VZ, ventricular zone. Calibration bar, 1 mm.

and 25.4 mm^2, and the total number of cells of the new type, 843 and 6, respectively. Thus, per unit area, cells of the new type were 51 times more common in telencephalon than in non-telencephalon. There were no instances of the new cell type in septum, optic tectum, cerebellum, or medulla. Recognition of exemplars of the new cell type is much aided by using sections of the brain that are parallel to the long axis of these cells. It could be argued that our failure to see the new cell type in non-telencephalic regions resulted from our use of the transverse plane of section. However, we also looked at coronal sections and found that cells of the new type were present in the telencephalon and absent in the rest of the brain.

Of the 629 cells of the new cell type that were mapped in the 3 sections of Figure 3, 158 were seen to be in close association with radial glia fibers. Those that were not seen to be apposed to radial glia fibers but that occurred in regions rich in radial glia fibers had, in most cases, the same orientation as fibers coursing in their vicinity.

Discussion

We believe that this is the first attempt to give a comprehensive account of the distribution of radial glia fibers in an adult vertebrate brain. We show that such fibers have their origin in the walls of the lateral ventricle and are very common in many parts of the telencephalon, but rare in others. Radial glia with long fibers are absent from regions outside the telencephalon, where tanycytes are common (Alvarez-Buylla et al., 1987).

The number of stained radial glia fibers decreases with distance from the ventricular wall. We cannot tell from our material whether this is because (1) the fibers get thinner at greater distances from the ventricle, (2) the amount of antigen decreases with distance, or (3) the fibers end in a staggered manner. Our representation of these fibers, e.g., in Figure 3, does not attempt to discriminate between these different interpretations.

The distribution of exemplars of what we call the "new cell type" tends to coincide with that of the radial glia fibers, to which they are often (in at least 25% of cases) apposed. Radial glia and exemplars of the new cell type occur in the telencephalon, but rarely, if at all, in the rest of the brain. It may not be coincidental that neurogenesis in adult avian brain is also thought to be a telencephalic property (Nottebohm, 1985). Furthermore, indirect evidence suggests that neurogenesis in adult avian brain occurs in the walls of the lateral ventricle of the forebrain (Goldman and Nottebohm, 1983), where radial glia fibers have their origin and from which the young neurons presumably migrate. The latter inference, together with the observations reported here, suggests that there is in the adult avian brain a functional relation between neuronal migration and radial glia, as has been described for the developing brain of mammals (Rakic 1971a, b, 1972). This hypothesis is tested in a subsequent publication, in which we show that the cells we have identified as members of a new cell type are migrating neurons (Alvarez-Buylla and Nottebohm, unpublished observations).

The distribution of radial glia may prove important in delineating the major migratory pathways that young neurons use in adult brain. Such migratory routes may also reveal the most likely site in the ventricular zone from which a differentiated new neuron originates ("glial coordinate system"; Smart, 1978). Given that most radial glia are oriented in the mediolateral plane (except for the rostral and caudal telencephalon; Fig. 4), we predict that, if they act as a distribution system for young neurons moving away from the ventricular zone, then most new neurons must originate from the ventricular zone within the same rostrocaudal level. Likewise, the trajectory of radial glia fibers suggests that neurons that are born in adulthood and take positions in the lateral hyperstriatum originate from ventricular zone lining dorsal reaches of the lateral ventricle. Neurons in the ventral telencephalon (e.g., LPO; Fig. 3) probably originate from ventricular zone lining the ventral walls of the lateral ventricle. The differential clustering of radial glia may also reflect

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accessorium; *HD*, hyperstriatum dorsalis; *HP*, hippocampus; *HVc*, hyperstriatum ventralis, pars caudalis; *IPC*, nucleus isthmi, pars parvocellularis; *LPO*, lobus parolfactorius; *MLd*, nucleus mesencephalicus lateralis, pars dorsalis; *N*, neostriatum; *NC*, neostriatum caudalis; *PA*, paleostriatum augmentatum; *Rt*, nucleus rotundus; *S*, septum; *TeO*, optic tectum; *V*, ventricle; *X*, area X. Calibration bar, 1 mm.

local differences in neurogenic potential within the ventricular zone.

Perhaps one of the more puzzling observations turned up by the present report is that the distribution of radial glia throughout the telencephalon is so uneven, with radial glia-rich and radial glia-poor areas. It would be interesting to know to what extent the complement of radial glia seen in adult avian brain differs from that seen in embryos, and whether the distribution seen in adulthood is similar to a subset or is a modification of the radial glia that occur during development. Interestingly, the fetal brain of mammals also shows local clustering of radial glia processes (Astrom, 1967; Sturrock and Smart, 1980).

The local distribution of radial glia fibers may be relevant to their possible role in substance transport from the ventricle to brain parenchyma and vascular system and vice versa. If there is such a role (e.g., Ivy and Killackey, 1978; Grafe and Schoenfeld, 1982), then it is unevenly distributed in the various parts of the telencephalon of birds. Recent research in turtles suggests that radial glia act as cellular mediators of K^+ redistribution following neural activity (Connors and Ransom, 1987). It is not known if this role also occurs in birds, but if it does, there may be interesting physiological differences between areas that are rich or poor in radial glia.

The occurrence of radial glia in adult brain has been described in cold-blooded vertebrates (Van Gehuchten, 1897; Studnicka, 1900; Ramón y Cajal, 1911; Horstmann, 1954), as well as in birds (Alvarez-Buylla et al., 1987, and the present report). Therefore, this may be an old vertebrate trait and so, too, may be neurogenesis and neuronal migration in adulthood, which are so poorly represented in mammals. As we come to better understand the various roles that radial glia play in adult vertebrate brain, we may better appreciate other ways in which the mammalian brain is special.

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