

# Migration of Newly Generated Neurons Upon Ependymally Derived Radial Guide Cells in Explant Cultures of the Adult Songbird Forebrain

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**ABSTRACT** The adult songbird forebrain undergoes neuronal production throughout adulthood, with the production of new neurons in discrete regions of the neostriatal ventricular zone. Upon mitogenesis, these new neurons migrate into the subjacent brain parenchyma along radially directed guide fibers. In long-term ventricular zone explant cultures, derived from the higher vocal center of the adult canary, newly migratory neurons were found to associate preferentially with a characteristic substrate cell type. These small, parvonuclear substrate cells formed tightly packed epithelioid sheets, in which ciliated ependymal cells were common, as recognized by both live observation and electron microscopy. A subpopulation of these cells was immunostained by monoclonal antibody 3A7, which preferentially stains the guide fiber network of the adult avian brain. These 3A7<sup>+</sup> cells included ependymal cells and bipolar radial cells, as well as morphologically defined astrocytes. As they matured in vitro, the 3A7<sup>+</sup> bipolar radial cells extended long, unbranching fibers, which ultimately traversed the culture substrate. Like ependymal cells, they supported neuronal migration. These cells were likely homologous to radial guide cells in vivo. Thus, neuronal migration in adult avian forebrain culture occurred upon guide cells of ependymal derivation. © 1993 Wiley-Liss, Inc.

## INTRODUCTION

The vocal control nucleus of the songbird brain [higher vocal center (HVC)] undergoes de novo neuronal production throughout adulthood, with the constant generation of new neurons from precursor cells located in the ventricular ependyma (Goldman and Nottebohm, 1982, 1983). Neuronal precursor cells reside not only in the ventricular zone overlying the adult HVC, but also in many other areas of the adult forebrain (Alvarez-Buylla et al., 1990; Nottebohm, 1985). These cells proliferate at the ventricular surface, after which a fraction of their daughter cells migrates into the subjacent brain and differentiates into functionally mature neurons.

The songbird is unusual among adult vertebrates in permitting neuronal migration through its forebrain parenchyma. Although neuronal migration is characteristic of ontogenesis, postmitotic neuroblasts introduced into adult mammalian brain typically fail to migrate into the host tissue (Bjorklund et al., 1983; Bjorklund and Gage, 1985). In contrast, the parenchyma of the adult songbird brain readily allows neuronal migration (Alvarez-Buylla and Nottebohm, 1988; Gold-

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man and Nottebohm, 1983), which occurs with the directed assistance of radial guide fibers arising from the ventricular zone layer (Alvarez-Buylla and Nottebohm, 1988; Alvarez-Buylla et al., 1990; Goldman, 1983).

The ontogeny and identity of these guide fibers has remained unclear. Although initially described as glial in nature (Alvarez-Buylla et al., 1988), their antigenicity, characterized by vimentin expression in the absence of immunoreactivity for glial fibrillary acidic protein, is more suggestive of either ependymocytes, ependymal tanycytes, or glial progenitors (Cameron and Rakic, 1991; Hirano and Goldman, 1988; Pixley and de Vellis, 1984; Sarnat, 1992). In the present study, we examined this issue using neurogenic explant cultures of the adult canary HVC ventricular zone, a preparation within which neuronal mitogenesis, migration, and functional development can be followed in vitro (Goldman, 1990; Goldman et al., 1992a; Goldman and Nedergaard, 1992). Using direct observation in vitro, in conjunction with ultrastructural and immunocytochemical verification of cell identity, we identified a cell type with which newly generated neurons preferentially associated. We determined that these cells were ependymal, and that they were able to support both neuronal migration and long-term survival in vitro. In addition, we observed the maturation of a subset of these cells into fiber-projecting cells, whose morphology and antigenicity suggested their phenotypic and functional homology to the radial guide fibers of the adult songbird brain.

## MATERIALS AND METHODS

### Culture Preparation

Explant cultures were prepared from two neurogenic regions of the adult canary forebrain, the HVC and its adjacent dorsomedio-caudal neostriatum (Goldman, 1990). Explants were prepared from thin (0.3–0.5 mm), hand-dissected strips of the neurogenic ventricular zone, whose area measured roughly 8 mm in length (spanning AP 0.0 to P 0.8 in the stereotaxic coordinates of Stokes et al., 1974) by 6 mm in width (medially from the lateral border of the HVC). This was done so as to enrich the cultures selectively in ventricular zone cells and their associated neuronal progenitors. The explants were plated onto layers of either human fibronectin or mouse laminin (Collaborative Research), which had been adsorbed onto the surfaces of 35 mm Primaria dishes (Falcon), within 24 h of culture preparation. Each dish surface was coated with 20  $\mu$ g of either substrate [each diluted in 0.2 ml Hanks' buffered salt solution (HBSS)], for a maximum surface deposition of 2.1  $\mu$ g/cm<sup>2</sup>. Cultures were otherwise prepared using previously described methods (Goldman, 1990).

### Transmission Electron Microscopy

A total of 110 HVC explants were raised upon fibronectin layers that had been plated onto carbon-coated

coverslips. After 8 days in vitro (8 DIV), the ten cultures with the most prolific neuron-like outgrowth were washed twice in warm HBSS, fixed for 1 h in 2% glutaraldehyde at 4°C, washed and post-fixed in 1% osmium tetroxide in 0.1 M cacodylate buffer for 1 h, and then dehydrated through graded alcohols into Spurr's embedding media (Ladd). Plastic-embedded samples were readily removed from the carbon-coated coverslips, and blocks trimmed to include the cellular outgrowth from each explant. Both 500 nm and adjacent 80 nm sections were cut on a Sorvall 5000 ultramicrotome; 500 nm sections were mounted on glass slides and stained with toluidine blue, while the thin sections were mounted onto Formvar-coated slot grids and counterstained with lead citrate/uranyl acetate. The 500 nm sections were then examined with a Leitz Laborlux 12 microscope equipped with a 63 $\times$  1.4 N.A. objective. Neuron-like cells were identified morphologically, and adjacent 80 nm sections were examined and photographed using a Jeol 100-CXII electron microscope at 80 kV.

### Scanning Electron Microscopy

Two adult HVC culture outgrowths were examined by SEM. The 8 DIV cultures were washed four times in warm HBSS, and then fixed in 2% glutaraldehyde (EM grade; Sigma) in HBSS for 6 weeks at 4°C. The cultures were then washed in 0.1 M phosphate buffer with 6% sucrose (PB-sucrose), and osmicated for 1 h in cold 1% OsO<sub>4</sub> (Pelco) in PB-sucrose. The samples were then dehydrated through ascending alcohols, incubated for 20 min in absolute ethanol, washed in cold absolute acetone, and dried in a Denton critical point dryer. The dried cultures were coated with a 10 nm layer of gold-palladium in a Technic sputter coater, and examined with a Jeol JSM-35 scanning electron microscope (SEM). The SEM operated on a secondary electron image mode, using a 25 kV beam and 15 mm working distance. Relevant areas were photographed at magnifications ranging from 460 $\times$  to 26,000 $\times$ , using a Polaroid camera and Polaroid type 55 positive/negative film.

### Immunocytochemistry

Radial guide fibers and ependymoglia were stained in both tissue sections and cultures of varying ages, using monoclonal antibody 3A7 (mAb 3A7), a mouse IgM that binds a vimentin-associated protein (Lemmon, 1985), and that preferentially labels the ventricular zone and guide fiber network of the adult canary brain (Alvarez-Buylla et al., 1987).

Tissue sections were fixed for 30 min in cold 4% paraformaldehyde in phosphate-buffered isotonic saline (PBS), washed in PBS, and permeabilized using 0.1% Triton X-100 in 0.1 M PB for 30 min. Samples were then blocked in 10% normal goat serum (NGS)/0.1 M PB for 30 min, and sequentially incubated in mouse 3A7 (1:1,000) for 18 h, biotinylated goat anti-mouse IgM (1:

200; Vector Labs) for 1 h, peroxidase-conjugated avidin-biotin complex (Vector) for 1 h, and 0.005% aminoethylcarbazole (AEC) in PB with 0.001% H<sub>2</sub>O<sub>2</sub> for 5 min. All antibody incubations were done at room temperature (23°C) except for that with mAb 3A7, which was done at 4°C overnight. All antibodies were prepared in a diluent of 1% NGS in PB, with 0.02% thimerosol (w/v) at pH 7.4. All washes were also done with this diluent. After immunocytochemistry, sections were mounted in PBS: glycerol (1:3) and examined by Nomarski optics using an Olympus IMT-2 photomicroscope.

Cultures were fixed in 4% paraformaldehyde in PBS for 10 min, permeabilized using 0.1% saponin in 0.1 M PB for 30 min, and blocked in 5% NGS/0.05% saponin in PB for 30 min. They were then incubated overnight in mAb 3A7 (1:300) at 4°C, followed by Texas Red-conjugated goat anti-mouse IgM (1:100) for 2 h at room temperature, mounted in SlowFade (Molecular Probes, Inc.), and observed by epifluorescence using the Olympus IMT-2.

In addition, in order to define the relationship of substrate cells to migrating neurons, selected cultures were also stained for the neuronal marker microtubule-associated protein-2 (MAP-2), which was localized using either rabbit anti-MAP2 antisera (kindly provided by Dr. Itzhak Fisher) or monoclonal mouse anti-MAP-2 IgG (Sigma, clone AP20). MAP-2 immunohistochemistry was done using our previously described protocols (Goldman, 1990; Goldman et al., 1992a).

## RESULTS

### Ependymal Cell Cultures Derived From the Adult HVC Ventricular Zone

The newly generated neurons migrated from adult HVC explants in association with a characteristic substrate cell type. We had previously noted that within the explant, neurons often continued to migrate in radially oriented arrays (Goldman, 1990, Fig. 2). However, upon leaving the explant, the neurons traveled upon a flat layer of morphologically primitive substrate cells, often longitudinally oriented with respect to one another. Areas of highest neuronal density were typically associated with ciliated substrate cells. These cells, whose motile cilia were pathognomonic for ependymal cells of the ventricular epithelium, were small and parvonuclear, and formed tightly packed epithelioid sheets, admixed with similar but non-ciliated cells (Fig. 1A). The cells were often noted to be ciliated upon initial migration from the explant, and remained so for several months *in vitro*. The presence of ciliated ependymal cells was predictive not only of those areas that would develop the densest congregations of neurons, but also of those regions in which neurons survived the longest *in vitro*: neurons found in these cultures after 2 weeks *in vitro* were almost uniformly associated with underlying ependymal cells (Fig. 1B).

### Morphological Development of Substrate Cell Types

Upon initial migration from the explants, the substrate cell layer was composed of flat epithelioid cells, some of which were ciliated, and a distinct and non-overlapping population of cells containing intracellular inclusions; these inclusions were most frequently noted in high glucose media, and were likely glycogen granules. After 1–2 weeks *in vitro*, three distinct types of substrate cells became apparent, including 1) ciliated ependymal cells; 2) non-ciliated parvonuclear cells of otherwise ependymal morphology; and 3) flat, well-spread cells bearing thick tapering processes, of astrocytic morphology. After 2–3 weeks in culture, the latter cells tended to migrate distantly from the confluent ependymal cell layer, resulting in the self-segregation of ependymal and glial cells in these cultures (Fig. 1C). Neurons migrating upon the ependymal layer accumulated upon reaching the astrocytic periphery (Fig. 1D), which did not support neuronal migration, often resulting in dense accumulations of neurons at the ependymal-astrocytic border (Fig. 1E,F). After 3–4 weeks in culture, cells within the ependymal layer developed polygonal borders apparent under phase microscopy (Fig. 4A,B). These cells retained the ability to support neuronal migration and adhesion for at least 2 months *in vitro* (Fig. 1G,H).

### Immunocytochemical Identification of Substrate Cell Types *In Vitro*

We found that those cells identified morphologically as astrocytes and radial cells were both immunoreactive for the cytoskeletal antigen recognized by mAb 3A7,

Fig. 1. A displays a layer of small, parvonuclear cells in a pseudo-synctial epithelium, which constituted the outgrowth from an adult zebra finch HVC ventricular zone explant after 8 days *in vitro* (DIV). These cells were ciliated, indicating their ependymal identity; the motile cilia cannot be appreciated in this still photo of a live substrate layer. Neuronal outgrowth from adult HVC explants was typically associated with this cell type, as in B, which displays a network of neurons lying upon a field of largely ciliated substrate cells after 8 DIV. C shows the outgrowth of another adult finch HVC explant (ex) after 27 DIV, in which ependymal cells (ep) and astroglia (gl) have partially segregated from one another, with glial emigration from the ependymal border. Neurons have accumulated at the ependymal-glial border (arrows; magnified view in E), having ceased migration upon reaching the peripheral astrocytes, which appeared relatively nonpermissive for neuronal migration. D shows the predominantly astrocytic morphology of those cells in the glial periphery (gl); no neurons were noted upon or among these glia. E, in contrast, shows the dense network of neurons lying upon the largely ependymal cell layer, extending to the ependymal-glial border. In F, a similar outgrowth from an adult canary HVC explant was immunoperoxidase stained after 8 DIV for MAP-2, in order to verify the identity of morphologically defined neurons. This population of MAP-2<sup>+</sup> neurons was widely dispersed upon the predominantly ependymal substrate cell layer. G displays a small aggregate of neurons overlying a field of substrate cells after 20 DIV, in the outgrowth from an adult finch HVC explant. The substrate cells of this layer were noted to be overwhelmingly ciliated during live observation. H reveals another group of neurons, found upon a largely ciliated ependymal substrate, after 45 DIV. Neurons were rarely evident on morphologically defined astrocytes after this duration of time *in vitro*. Scale bars = 50 μm, except in C (200 μm).

which targets vimentin-associated proteins (Alvarez-Buylla et al., 1987; Lemmon, 1985). In vivo, this antibody heavily and preferentially stains the guide fiber

network of the adult canary brain (Alvarez-Buylla and Nottebohm, 1988). However, we found in frozen sections of both adult canary and zebra finch brains that it

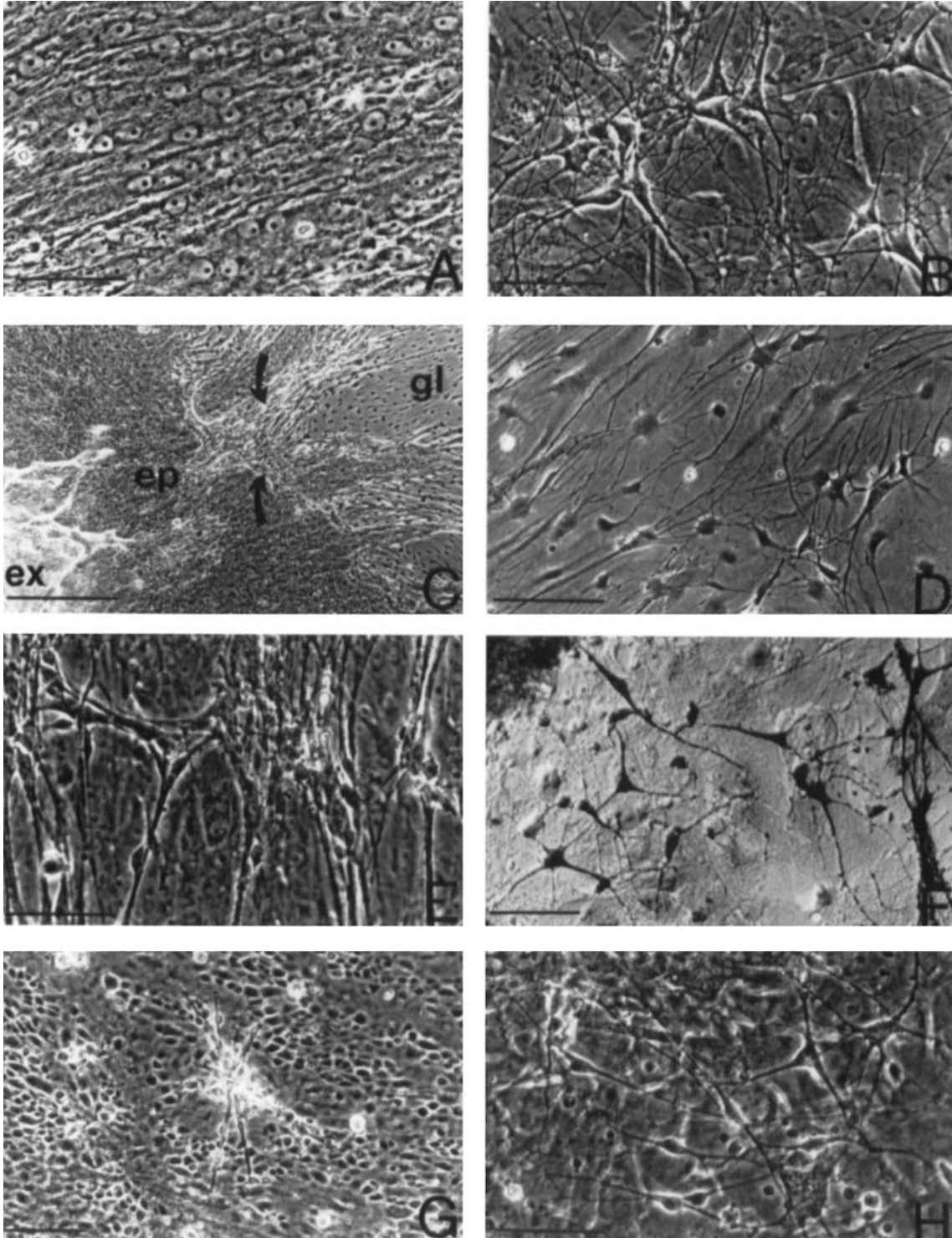


Fig. 1.

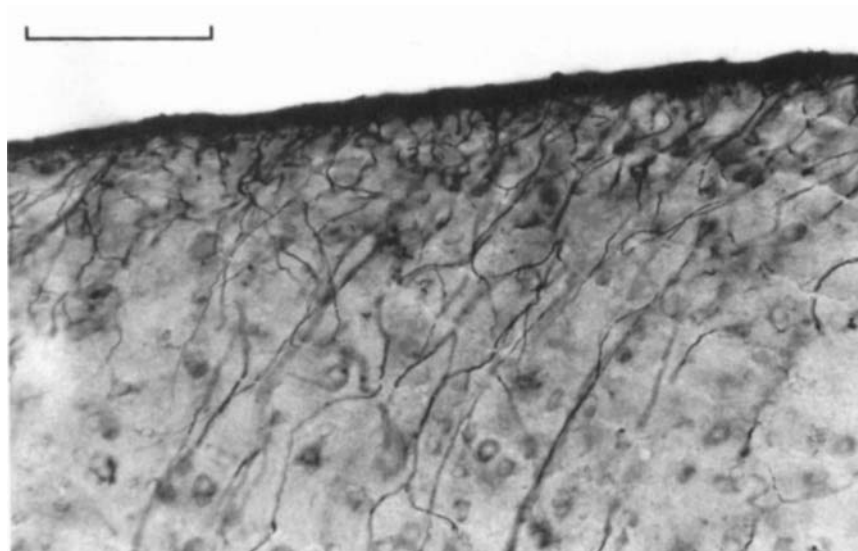


Fig. 2. This photo displays the radial guide fiber network of an adult female canary HVC, as defined using monoclonal antibody 3A7. Scale bar = 100  $\mu$ m.

also labeled ependymal cells, selected populations of astrocytes, and scattered populations of neurons, the latter restricted to the brainstem and cerebellum (Bahramian, Kirschenbaum, Lemmon and Goldman, unpublished data; see Fig. 2).

In vitro, 3A7<sup>+</sup> immunoreactivity was similarly seen among morphologically defined astrocytes, ependymal cells of the substrate pseudosyncytium (Fig. 4B), and their guide fibers (Fig. 6D). Neither neurons nor immature substrate cells initially leaving the explant expressed 3A7 immunoreactivity.

The 3A7<sup>+</sup> substrate cell population could be divided into ependymal and astrocytic subpopulations on ultrastructural as well as morphological grounds. In culture, a subpopulation of 3A7<sup>+</sup> substrate cells evolved processes of astrocytic morphology; ultrastructurally, these cells developed intracellular glycogen granules and cytosolic filament bundles, also characteristic of astrocytes (Goldman, unpublished data). Ependymal cells (see below) and astrocytes appeared to segregate from one another in these cultures, as the latter generally migrated from the periphery of the otherwise confluent explant outgrowth (Fig. 1C,D).

### Scanning EM

The frequent association of neurons with underlying ciliated cells was best revealed by scanning EM of these adult ventricular zone cell cultures. Figure 3A displays a young neuron, photographed within the outgrowth of an adult HVC ventricular zone explant after 5 DIV, as it lay upon an ependymal cell possessing both cilia and scattered microvilli. Figure 3B and C show other ciliated ependymal cells in the same culture, also located in areas of high neuronal density.

### Transmission EM

Serially sectioned culture outgrowths first revealed a substrate layer characterized by a mix of epithelioid ependymal cells, distinguished by their cilia, and glycogen-laden, highly filamentous astrocytes. Above this layer rested a monolayer of interconnected neurons and their fibers. Neurons were typically located either upon or in close association with substrate ependymal cells, rather than upon astrocytes. Neuronal cell bodies were characterized by large nuclei with dispersed heterochromatin and central nucleoli, with prominent Golgi apparatus, scattered multivesicular bodies, and a lack of glycogen or glycolipid inclusions. Neurons had one or more primary neurites, characterized by an abundance of polyribosomes and mitochondria, parallel arrays of microtubules, vesicles in transport, and multivesicular bodies [neuronal ultrastructure in these cultures has previously been described (Goldman and Nedergaard, 1992)]. The substrate cells subjacent to these neurons were characterized by loose arrays of intermediate filaments, with tightly packed filamentous arrays at their apical surfaces (Fig. 4); many of these cells possessed true cilia, a defining characteristic of ventricular zone ependymal cells (Fig. 5). Thus, neurons migrated and remained upon substrate cells that were highly enriched in ultrastructurally defined ependymal cells.

### Radial Guide Fiber Formation In Vitro

When substrate regions enriched in ciliated ependymal cells were maintained in long-term monolayer culture, relatively homogeneous layers of flat, pseudosyncytial parvonuclear cells with dense borders of membrane apposition developed (Fig. 4). After the first several

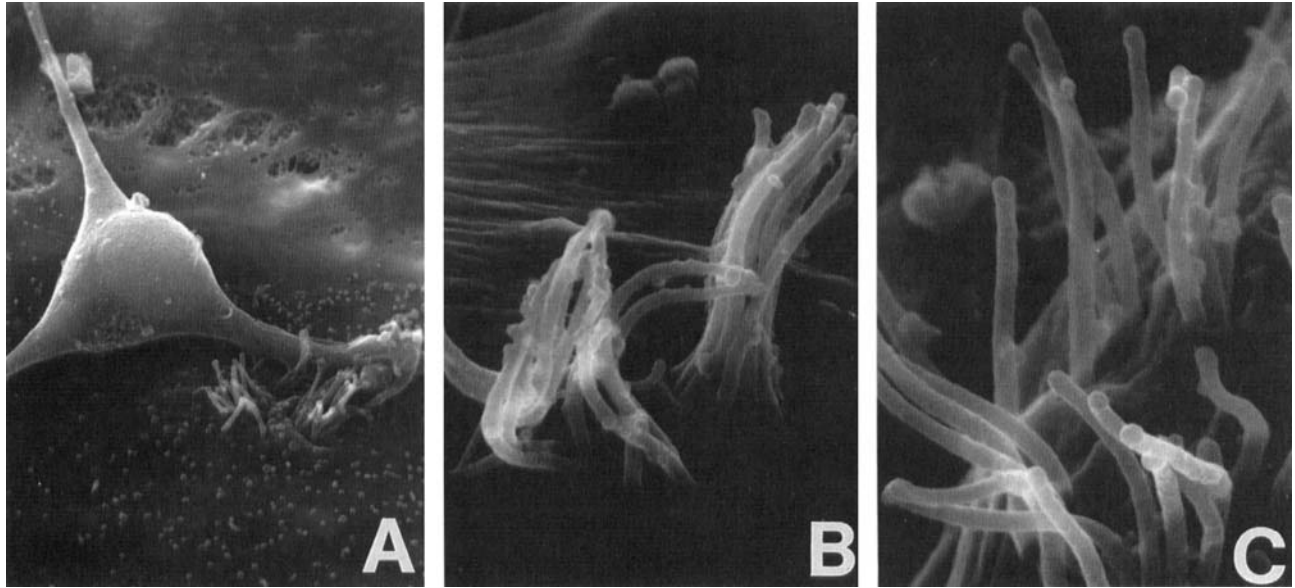


Fig. 3. After 5 DIV, scanning electron microscopy (SEM) of an adult HVC ventricular zone explant culture revealed the frequent association of emigrated neurons with ciliated ependymal cells of the substrate outgrowth. A displays a young neuron photographed in a 5 DIV

explant outgrowth, as it lay upon a subjacent cell possessing both a tuft of cilia and scattered microvilli ( $\times 3,000$ ). B ( $\times 11,000$ ) and C ( $\times 15,000$ ) show other ciliated ependymal cells in the same culture; both cells were located in an area of high neuronal density.

weeks in vitro, thin bipolar cells with fusiform cell bodies arose sporadically from this layer (Fig. 6). These cells projected an array of long ( $>1$  mm, and as long as 6–7 mm), thin, and parallel-oriented, generally unbranching fibers; over a period of several months, these fibers traversed wide areas of the substrate surface. These cells generally lay upon the surface of ependymal cells, and were not associated with astrocytes; they were morphologically reminiscent of radial cells, as described in vitro in other systems (Misson et al., 1991). Antigenically, both the cell somas and their fibers were 3A7<sup>+</sup> (Fig. 6C,D); this further suggested the structural and antigenic homology of these cells to the radial guide cells of the adult songbird brain in vivo.

## DISCUSSION

This report describes the in vitro migration of newly generated neurons of the adult songbird brain, upon ciliated ependymal cells of the forebrain ventricular zone. Within these explant cultures, derived from the neurogenic ventricular zone of the adult canary HVC, neurons preferentially localized upon ciliated ependymal cells, during initial migration and even more so after several weeks in vitro. Astrocytes, in contrast, were relatively non-permissive for neuronal migration in these cultures, to the extent that neurons segregated to regions of the substrate layer in which ependymal cells predominated. The neuro-ependymal association was particularly striking since the ependymal cells comprised only a minority of the total substrate outgrowth from these explants, in which morphologically defined astro-

cytes predominated. In this regard, the proportion of ependymal cells in each explant's outgrowth varied as a function of the ventricular zone region sampled as well as the time point of in vitro assessment. In addition, those neurons that emigrated upon ciliated ependymal cells appeared to display substantially longer survival times in culture: in cultures that initially displayed an abundance of migrating neurons, by the end of the second week in vitro, most—if not all—surviving neurons were located either directly upon or within 20–25  $\mu\text{m}$  of ciliated ependymal cells. The latter were identified readily, by their actively motile cilia, parvonuclear somata, and pseudo-syncytial epithelioid organization. However, given our prior observation of in vitro neuronal mitogenesis by these ventricular zone cells (Goldman et al., 1992a), we cannot exclude the possibility that the presence of neurons upon ependymal cells in culture may reflect the production in situ of new neurons from concurrently migrating neuroepithelial precursor cells (see below).

### Neuronal Association With Ependymal Cells In Vitro

Several explanations may be advanced to explain the preferential co-localization of neurons upon ependymal cells within these cultures. First, it is likely that specific neuroependymal adhesive mechanisms effect preferential neuronal migration upon ependymal cells in vitro, just as neuronal migration in the adult canary brain proceeds selectively along radial guide fibers in vivo (Alvarez-Buylla and Nottebohm, 1988; Alvarez-

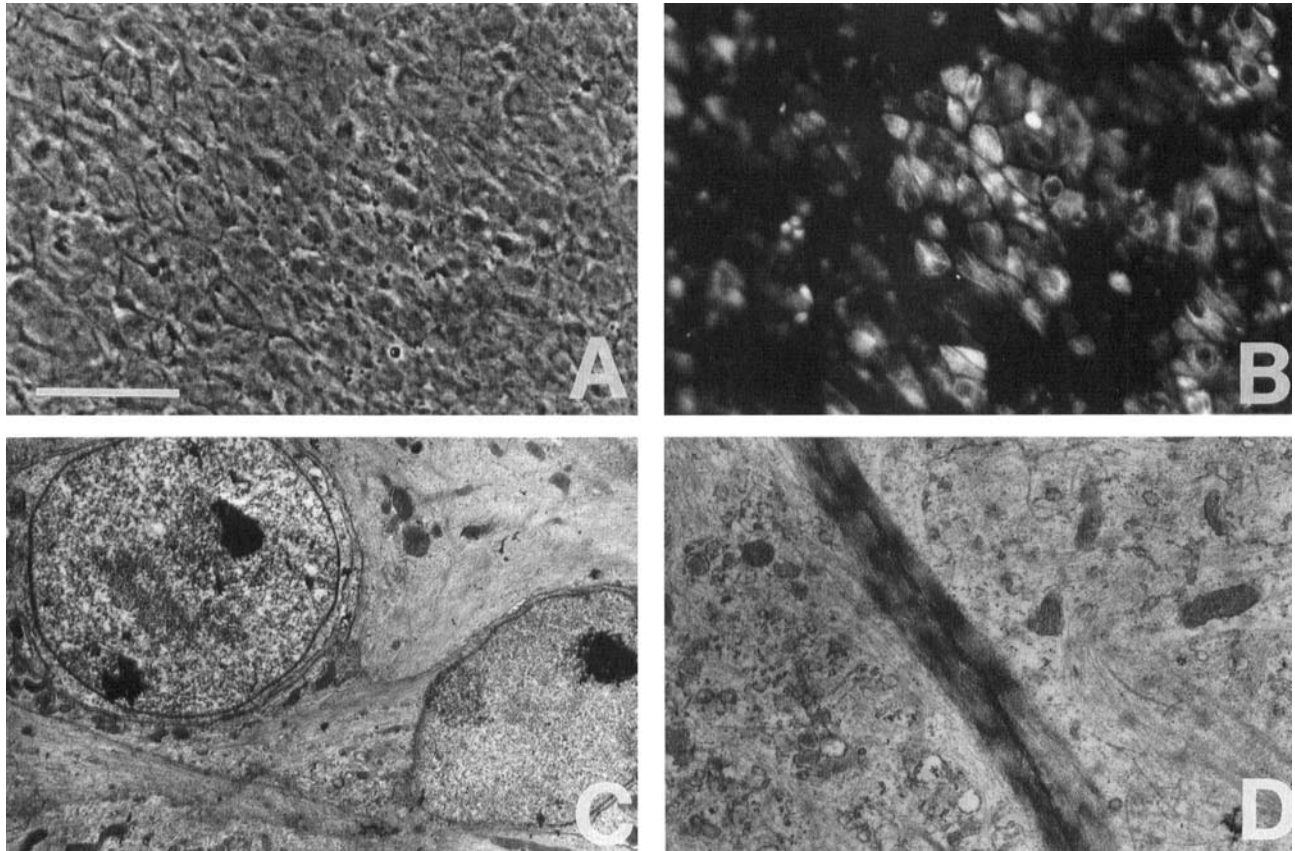


Fig. 4. Neurons migrated upon substrate cells that were highly enriched in ependymal cells. When regions of neuronal outgrowth were maintained in long-term monolayer culture, relatively homogeneous layers of flat, parvonuclear ependymal cells developed, including both ciliated and non-ciliated cells. A shows a region of ciliated ependymal cells after 45 DIV; by this time, polygonal intercellular borders have become visible within this pseudosyncytial epithelium. B shows the same field after immunostaining with mAb 3A7. The antigenic heterogeneity of the underlying substrate population is evident

here, with the predominantly ciliated ependymal cells in the center 3A7<sup>+</sup>. Ultrastructurally, these cells were characterized by nuclei with both dispersed euchromatin and clumped heterochromatin, as well as sharply defined nucleoli (C;  $\times 4,800$ ). They had loose arrays of intermediate filaments, with tightly packed parallel arrays of filaments at their apical surfaces and borders of membrane apposition (D;  $\times 14,000$ ). Many of these cells possessed cilia, characteristic of ventricular zone ependymal cells (see Fig. 5).

Buylla et al., 1990). Second, neurons and radial cells might arise from bipotential ventricular zone precursors (McKay, 1989), leading to co-localization of these two cell types *in vitro* by virtue of contemporaneous ancestry. In this regard, we have demonstrated that MAP-2<sup>+</sup> neurons and 3A7<sup>+</sup> radial cells may be co-derived from common precursor cells in cultures of postnatal HVC ventricular zone cells (Goldman et al., 1992b). Furthermore, recent reports have confirmed the proposition that neurons and radial glia may be derived from a common bipotential precursor cell *in vivo*, in mammalian striatal embryogeny (e.g., Halliday and Cepko, 1992). Thus, we cannot rule out the possibility that the neurons and their substrate cells are co-derived from a common precursor also migrating within the ventricular zone outgrowth. Indeed, a third possibility is that the neurons may be generated by the ependymal cells themselves, with similar resultant co-localization. Fourth,

neurons may preferentially survive upon or near ependymal cells by virtue of ependymally derived neurotrophic activity, whether soluble or membrane-bound. Finally, the mechanical action of ciliary motility within an otherwise unstirred surface media layer might promote neuronal survival simply on the basis of nutrient replenishment within these Petri dish cultures.

#### Morphological Characterization of Adult-Derived Ependymal Cells *In Vitro*

The vast majority of ciliated ependymal cells in these cultures were small, 3A7<sup>+</sup> polygonal cells, lying in pseudosyncytial epithelia (Fig. 4). However, occasional ciliated cells with large nuclei, flatly apposed cell bodies, and thick, tapering processes were also noted. These two phenotypes of ciliated ependyma may be roughly

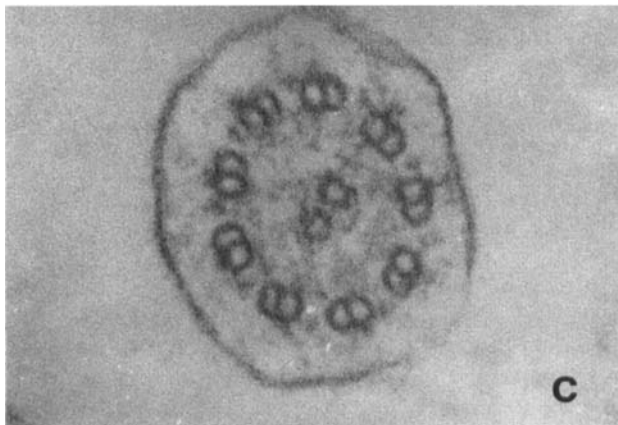
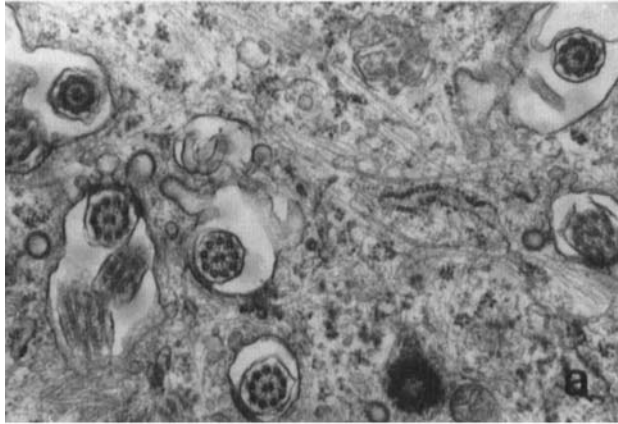


Fig. 5. In order to verify that the apparent cilia seen in both live cultures and by SEM were indeed cilia, and that their parent cells were ependymal, HVC ventricular zone culture outgrowths were examined by transmission EM. For these plates, an area of outgrowth was chosen that harbored a dense congregation of migrating neurons. When examined by TEM in a plane parallel to the plate surface, a layer was located at which cilia were identifiable in cross section, emanating from cells with dispersed filamentous arrays (a;  $\times 36,000$ ). In b, a cell was cut in a plane tangential to the surface of the substrate cell layer, yielding a view of a cilium projecting longitudinally from the ependymal cell surface ( $\times 19,000$ ). In the high power view of c, these cilia were seen to possess the characteristic  $9 \times 2$  arrangement of ciliary microtubules ( $\times 190,000$ ).

analogous to the cuboidal and tanycytic ependymocytes of the embryonic ventricular zone (Jordan et al., 1987). The preponderance of the small, polygonal ciliated cells in vitro may correspond to the overwhelming representation of cuboidal ependymocytes in the adult ventricular ependyma (Gould et al., 1990), but is also likely to reflect the preferential clonal expansion of this cell type under the culture conditions employed. As in previous studies (Gould et al., 1990), cells of tanycytic morphology appeared to lose their basal fibers and develop into common ependymocytes, integrating then into the ependymal epithelium.

#### Ependymally Derived Radial Guide Fiber Formation In Vitro

When populations of ciliated ependymal cells were maintained in long-term monolayer culture, they commonly developed networks of long, unbranching fibers ( $>1$  mm), often arranged in roughly parallel, longitudinal arrays (Fig. 6A,B). These fibers were strongly immunoreactive for the mAb 3A7 antigen (Fig. 6C,D), like the radial guide fibers in vivo (Fig. 2). Over a period of several months, these fibers traversed a wide area of the substrate surface; individual fibers were noted to extend as long as 6 mm in culture. It is likely that these fibers are homologous to the radial guide fibers of the adult brain in vivo; however, definitive evidence of this point must include lineage analysis and appropriate functional interaction with co-cultured migratory neuroblasts.

#### Molecular Regulation of Neuroependymal Adhesion

The ex vivo phenotypic development of the ependymal guide cells proceeded with some degree of antigenic fidelity, since the adhesive interaction of newly generated neurons with the ependymal cell surface was recapitulated in vitro. Nonetheless, the adhesive mechanisms by which newly generated neurons attach to their radial guide cells are unknown, as are the means by which adhesion is sequentially maintained during cell transit and terminated upon the completion of migration. A similar process of neuronal migration along radial glia has been studied in the postnatal rodent cerebellum, a system in which newly generated neurons migrate from the external granular layer into the sub-jacent brain (Rakic, 1971). The molecular basis of parenchymal neuronal migration, and its requisite neuroglial interaction, has been examined by several groups (e.g., Antonicek et al., 1987; Chuong et al., 1987; Edmondson et al., 1988; Hatten and Mason, 1990). In this system, neuronal migration coincides with the expression of the neuroglial adhesion molecules Ng-CAM and L1 (Daniloff et al., 1986; Lindner et al., 1986). Surface glycoproteins of the Ng-CAM/L1 class may be necessary for granule cell migration, as neuroglial adhesion can

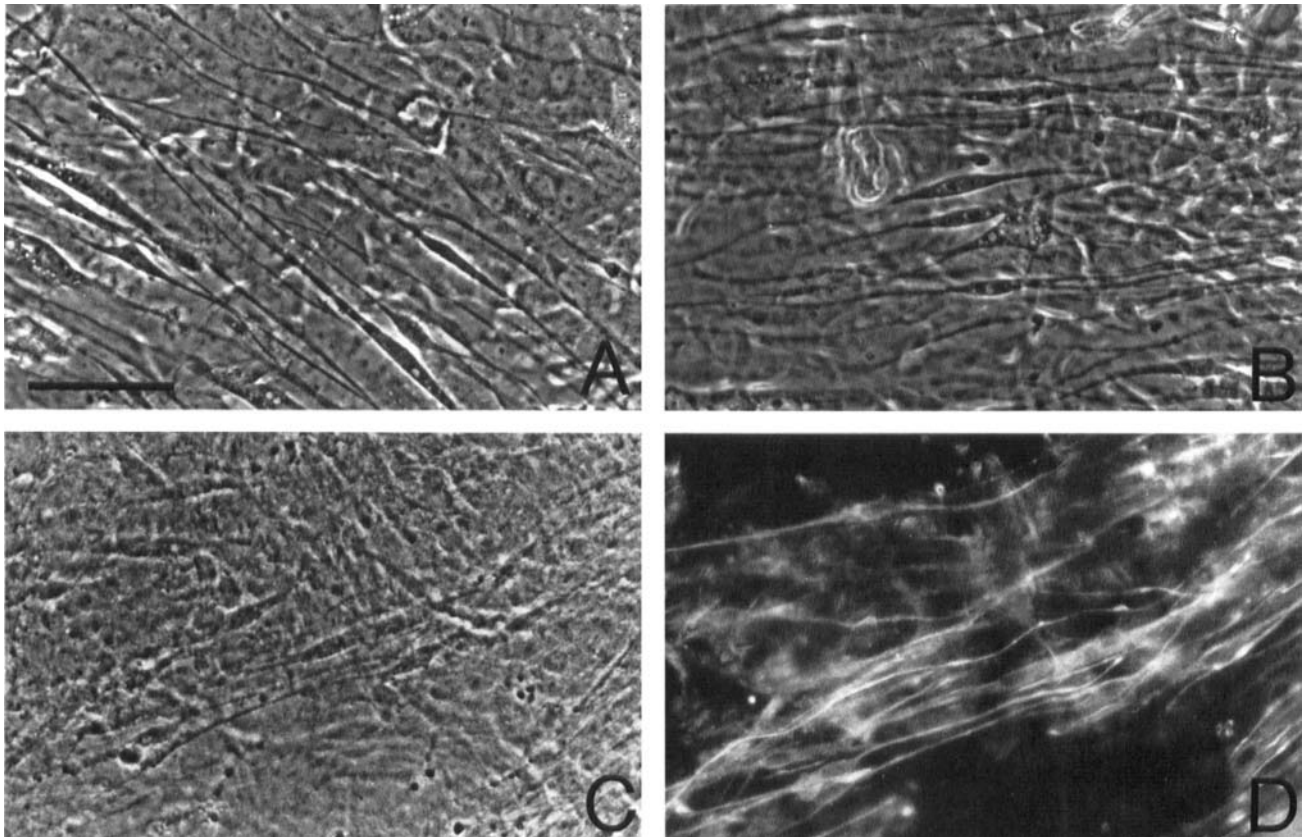


Fig. 6. When maintained in long-term culture, ependymal cell layers derived from adult HVC ventricular zone explants developed extensive arrays of long, radially arranged, rarely branching fibers. **A** and **B** display views of one such culture, derived from an explant highly enriched in ependymal cells, after 25 DIV. These photographs show cohorts of fusiform, radially oriented, and largely bipolar cells, which have arisen from the underlying substrate layer over several

weeks in culture. **C** and **D** show phase and fluorescent views, respectively, of a similar ependymal culture after 49 DIV, after immunostaining using mAb 3A7. These fibers were 3A7<sup>+</sup>, like the guide fibers of the adult canary brain in vivo (see Fig. 2) and did not express neuronal antigens such as MAP-2. These radial fibers were likely homologous to those of the adult avian brain in vivo. Scale bar = 50  $\mu$ m.

be inhibited *in vitro* with antibodies against Ng-CAM (Grumet et al., 1983), while granule cell migration is inhibited by antisera directed against L1 (Lindner et al., 1986), as is neuritic extension onto retinal Müller glia (Drazba and Lemmon, 1990). Since radial glia do not express Ng-CAM (Daniloff et al., 1986), and retinal Müller glia similarly fail to express L1 (Drazba and Lemmon, 1990; Lemmon and McLoon, 1986), it is likely that glial guide fibers express heterophilic cell-surface receptors for these closely related molecules. In addition, a neuronal surface antigen that appears to bind Bergmann glia, designated astrotactin, has been described, whose glial ligand is similarly unknown (Edmondson et al., 1988; Stitt and Hatten, 1990). Although the glial receptors for these various neuronal surface proteins have not been established, several glia-derived proteins with neuronal adhesive and/or repulsive properties have also been identified, including those of the J-1/cytotactin/tenascin class (Grumet et al., 1985; Hoffman et al., 1988; Kruse et al., 1985).

#### Cellular Ontogeny of Ependymally Derived Radial Cells

If the previously described mechanisms of embryonic neuroglial adhesion prove generally applicable, then one can predict that in the adult songbird brain ependymally derived radial cells might express heterophilic binding partners to one or more of the adhesion molecules expressed by the newly generated neurons (Goldman and Lemmon, unpublished data). One might thereby view the avian ependymal cell as having assumed in adulthood the neuronal guidance functions generally attributed to radial glia in embryogeny. If so, then the specific binding of neurons to their radial glia in fore-brain ontogeny might be paralleled by a specific neuroependymal interaction in the adult. The assumption of neuronal guidance functions by the adult ependymal cell may represent a conservation of function by two distinct cell types, ependymal and radial glial, at different points in ontogeny. Alternatively, adult ependymal

cells with projected fibers may represent a lineage-restricted subclass of radial glia, which have maintained their radial fibers while assuming other aspects of an ependymal phenotype, such as apical cilia (Cameron and Rakic, 1991). Similarly, adult ependymal cells may derive from a progenitor common to radial glia, type-I astrocytes, and ependymal cells, and might simply retain the potential for fiber elaboration displayed by their radial cell forebears.

In each of these possible derivations of the ependymal guide cell, the phenotypic expression of a radial fiber by an ependymal cell, whether in vivo or in vitro, might be dictated by the local environment, rather than by any autonomous cellular program. Whatever the ontogeny of the ependymal guide cell of the adult songbird brain, its function appears largely that of mediating the parenchymal migration of newly generated neurons (Alvarez-Buylla and Nottebohm, 1988). It remains to be seen which surface adhesion molecules are expressed by the ependymal guide cells, and how these ligands might contribute to neuronal migration in the adult avian brain.

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