Astrocytes in the adult cat retina were stained by immunocytochemical localization of glial fibrillary acidic protein (GFAP), a major constituent of the astrocytic intermediate filaments. Their density in whole mounted retinas showed a peak of about 2000 astrocytes/mm² at the optic nerve head and dropped to approximately 200 astrocytes/mm² in the far periphery. At the central area a prominent local minimum of astrocyte density was found. The shape of astrocytes changed from a stellate form in the outermost retinal periphery to an elongated form in the central part of the retina with the majority of astroglial processes aligned in parallel with the ganglion cell axons. Results in the cat retina suggest a close correlation between astrocytes and optic nerve fibers, the latter presumably being involved in the establishment of the astrocytic network. Invest Ophthalmol Vis Sci 27:828-831, 1986

Recent studies of astrocytes in the mammalian retina using classical anatomical methods showed some details of astroglial morphology and also gave evidence of a characteristic density distribution.1-4 Whereas most classes of retinal neurons have their maximum density in the central area, the number of astrocytes in the primate retina is roughly proportional to the thickness of the nerve fiber layer and peaks at the optic nerve head. Although the function of retinal astrocytes remains speculative, a definite correlation between astrocytes and axons in the retina might give further insight into their functional role. In contrast to Nissl- or selective Golgi-staining, applied in previous studies, we used immunocytochemical methods to label astrocytes. Application of antibodies directed against GFAP5 revealed, in whole-mount preparations, both the shape and density distribution of astrocytes in the entire retina.

Materials and Methods. Animal care and treatment in the present study conforms to the ARVO Resolution on the Use of Animals in Research. Twelve adult cats used for electrophysiological recording experiments were deeply anesthetized with 4% halothane and perfused through the heart with 0.9% saline followed by 0.1 M DL-lysine monohydrochloride and 0.01 M sodium-m-periodate in 0.1 M sodium phosphate buffer (PB) at pH 7.4. After enucleation, both retinas were removed from the eyecup and postfixed for another 2 hr. Immunohistochemical staining included the peroxidase antiperoxidase (PAP) method. All antibody dilutions were carried out in 0.5% Triton-X-100 in PB. After each step, free floating whole retinas were washed three times for 15 min in PB. In brief, the procedure is as follows: (1) Pretreatment with 3% hydrogen peroxide/10% methanol in PB for 30 min at room temperature. (2) Incubation for 1 hr at room temperature in 10% normal goat serum (NGS)/0.1 M DL-lysine monohydrochloride. (3) Rabbit antiserum directed against glial fibrillary acidic protein (GFAP; a gift from Drs. D. Dahl and A. Bignami) was used at a dilution of 1:150 followed by (5) incubation with a rabbit peroxidase antiperoxidase complex (Miles Scientific; Naperville, IL) at a dilution of 1:100. Demonstration of the antibody binding was carried out by (6) preincubation in 0.05% 3,3’-diaminobenzidine tetrahydrochloride (DAB; Sigma Chemical; St. Louis, MO) in PB for 5 min, followed by (7) incubation in 0.05% DAB/0.01% hydrogen peroxide in PB for an additional 10 min. Retinas were flat-mounted (ganglion cell-side up) on glass slides, dehydrated in ascending alcohols, cleared in xylene and mounted with Permount after counterstaining with the Lillie modification (1968) of Weigert Iron Hematoxylin. In addition we used the indirect immunofluorescence technique in which the GFAP antiserum was followed by goat anti-rabbit antibodies conjugated to fluorescein-isothiocyanate (FITC) (Cappel Laboratories; West Chester, PA). Cell counts were made using a Zeiss (Oberkochen, West Germany) drawing tube with a ×1/0.04 objective for constructing retinal maps and a ×40/0.75 (oil-immersion, Zeiss, Planapo) for scanning the whole mounts and counting the densities of all GFAP-positive astrocytes by means of a 10 × 10-mm grating in the eyepiece.
Results. GFAP-positive astrocytes in whole mount preparations of adult cat retina have differing shapes according to their retinal position. Astrocytes close to the ora serrata have many fine processes which leave the cell body radially and rarely branch (Figs. 1 & 2A). Similar star-shaped astrocytes are also seen in the gan-

![Fluorescence micrograph from a whole mount of the cat retina labeled with antibodies against GFAP. The field is from the peripheral retina with the focus at the ganglion cell layer. The labeled astrocytes are characteristically star-shaped (scale 100 μm).](image)

![Drawings of labeled astrocytes from different parts of the retina. The field in (A) was taken from peripheral retina, where only few optic nerve fibers are found. A few astrocytic branches were seen orienting towards the blood vessel (cross hatching). This tendency was common among astrocytes. (B) In the midretina the processes of astrocytes follow the direction of optic nerve fibers. (C) Close to the optic nerve head the high density of optic nerve fibers is reflected in the density and direction of astrocytic processes.](images)
Fig. 3. Isodensity lines of astrocyte distribution in a left cat retina. The numbers indicate densities in cells/mm². Densities steeply increase towards the optic nerve head (shown as black dot) up to approximately 2000 cells/mm². The local minimum of astrocyte counts in the central area is indicated by a star.

The other glial population of the cat retina, the Müller cells, have not been found to be convincingly GFAP-positive. However, in some whole mounts their pedicles appeared slightly immunoreactive. A striking feature of the astroglial density distribution is the lack of astrocytes in the area centralis. In peripheral retina astrocytes form a rather regular mosaic (Fig. 1), whereas in central retina astrocytes are arrayed in bundles that bypass the area centralis in successive arc-like formations and follow ganglion cell axon fascicles on their way to the optic disc. The quantitative analysis of astrocyte density in Figure 3 reveals a maximum of about 2000 astrocytes/mm² at the optic nerve head and densities scattering between 150–250 astrocytes/mm² close to the ora serrata. Furthermore there is a local minimum in the central area dropping to counts lower than 50 astrocytes/mm². Thus, morphologies and total number of astrocytes in the cat retina closely follow the topography of retinal axon bundles.

Discussion. The advantage of staining all astrocytes in a whole mount preparation is that it reveals the gradual change from stellate shape in peripheral retina to elongated shape close to the optic nerve head. From this material it is quite obvious that both are extreme morphological variations of one cell type. It is also apparent that the optic nerve fibers are the substrate that causes this change. The correlation between the number of astrocytes and thickness of the optic nerve fiber layer, as described in the primate,²,³ is confirmed here for the cat retina. Such a correlation or even a close apposition of glial and neuronal processes might indicate intercellular interactions as proved to be the case in axonal guidance during early brain development.⁸,⁹ It remains speculative as to whether astroglial processes, shown in vitro to serve as a template for outgrowing neurites and the neuronal pattern,¹⁰,¹¹ only provide mechanical support for neuronal elements or are involved in physiological functions. Although only Müllerian glia have been shown to take up and direct excess potassium into the vitreous body of the vertebrate retina,¹² astrocytes in general are thought to present an effective means of removing potassium released into the extracellular space during axonal activity.¹³ Bearing these functional aspects in mind, we consider cat retinal astrocytes to be a special glia for ganglion cell axons, as already suggested by Büsow.⁴

Key words: astrocytes, cat retina, glial fibrillary acidic protein, nerve fiber layer

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References

A monoclonal antibody that binds to cones has been produced. This antibody, 50-1B11, binds to the outer segments of cones in rhesus monkeys. Immunohistochemical experiments indicate that 50-1B11 binds to a subset of photoreceptors, probably cones, in all vertebrate species tested thus far, including man. In vitro experiments on chicken retina indicate that the antigen is intracellular and associated with the plasma membrane, while electronmicroscopic-immunohistochemical studies demonstrate that the antigen is contained in the lamellae of the outer segments of rhesus cones. Invest Ophthalmol Vis Sci 27:831–836, 1986

The ability to use immunohistochemical techniques to differentiate between different classes of cells has proven to be an invaluable approach in studying both normal cell function and pathological conditions. Over the past several years a number of monoclonal antibodies have been developed that bind to specific classes of cells in the retina of various species.1–3 Until now, there have been no antibodies produced that bind specifically to vertebrate cones. This, combined with the lack of conventional histochemical techniques to discriminate between rods and cones, has greatly hindered studies of retinal degeneration in both human pathological material and in animal models of photoreceptor degeneration. We have recently produced a monoclonal antibody that binds to cones in monkeys and also binds to cones in formalin fixed human retina. Therefore, we believe that this antibody will greatly facilitate studies of the cell biology and pathology of cones.

Materials and Methods. Mice were obtained from Jackson Labs (Bar Harbor, ME). White leghorn chicken eggs were obtained from S. Sacks and Son; Evans City, PA. All procedures used in this study conform to the ARVO Resolution on the Use of Animals in Research. To prepare material for immunization of mice, retinas were dissected from E19 chick embryos, and plasma membranes were prepared using discontinuous sucrose density gradients with steps of 0.32 M, 0.8 M, and 1.2 M sucrose.4 Plasma membranes were collected, solubilized in 0.5% NP-40 in 20 mM TRIS, saline, pH 7.2 and then centrifuged at 100,000g for 30 min. The supernatant was then incubated with Bio-beads (Biorad Laboratories; Richmond, CA) to remove the detergent. The supernatant was used to immunize Balb/C mice using Freund’s complete adjuvant. One month later the mice were boosted with antigen in Freund’s incomplete adjuvant and then rested for at least another 4 wk. Prior to fusion a mouse was injected with antigen via the tail vein on 3 successive days. All immunizations contained approximately 100 μg of protein.

Hybridomas were prepared following standard procedures using NS-1 cells.5 Supernatants were first screened using a dot blot immunoassay with the same material that was used to immunize the mice.6 Wells containing supernatants that were positive on the dot blot were subsequently tested using immunohistological procedures with cryostat sections of paraformaldehyde fixed E19 chick retina. After incubating the sections with hybridoma supernatant the sections were washed with phosphate buffered saline (PBS) and then incubated with a 1:500 dilution of fluorescein labeled goat anti-mouse-IgG (Cappel Labs). Wells containing supernatants that bound to restricted classes of retinal cells were cloned by limiting dilution.

In order to study the specificity of antibody 50-1B11 double label experiments were conducted using techniques developed by de Monasterio et al.7 Procion yellow (obtained from Dr. de Monasterio) was injected into the vitreous of rhesus monkeys that were being used in neuroanatomical experiments for other purposes and experimental studies, in vivo, on the role of preformed gial pathways. J Comp Neurol 210:10, 1982.