Displaced ganglion cells were studied in the rabbit retina after filling them with horseradish peroxidase via injections into the optic nerve. These cells are primarily in areas outside of the visual streak. They are larger than other inner nuclear layer cells and are the size of the smallest neurons in the ganglion cell layer. Large labeled cells (>20 μm) are found only in the ganglion cell layer. These findings further illustrate the wide variability in the size and distribution of displaced ganglion cells in the retinas of different mammals. Invest Ophthalmol Vis Sci 25:1376–1381, 1984

In nonmammalian vertebrates it has long been known that some retinal ganglion cells are not located in the ganglion cell layer.1–6 These “displaced ganglion cells” or Dogiel cells3 are located in the inner half of the inner nuclear layer, adjacent to the inner plexiform layer. They vary in size and frequency in different species and, at least in the pigeon, they have been shown to project preferentially to part of the accessory optic system, the nucleus of the basal optic root.6

In mammals, descriptions of displaced ganglion cells are less common although they have been described in both rodents7,8 and primates,9,10 where their distribution and size differ. In rats and monkeys displaced ganglion cells are concentrated in the central retina and tend to have large somata (rat, see reference 7) or medium-sized somata (monkey, see reference 10). In the mouse they are concentrated in the peripheral retina and have somata ranging from small to large.8

In rabbits, displaced ganglion cells have not been well characterized. Hughes and Vaney11 reported that some cells in the inner nuclear layer are retrogradely labeled if the optic nerve is cut and soaked in horseradish peroxidase. Vaney et al12 also showed that some of these displaced ganglion cells project to the contralateral superior colliculus. However, neither study describes the size, frequency, or distribution of these cells. Oyster et al13 injected horseradish peroxi-

Materials and Methods

The results of this study are based on experiments with nine rabbits of the chinchilla strain Chbb:Ch (Thomae, Biberach), and all procedures adhered to the ARVO Resolution on the Use of Animals in Research. For the injections of horseradish peroxidase (HRP), each animal was initially anesthetized with a mixture of ketamine and nembutal and anesthesia was maintained with halothane. The optic nerve was exposed by making an incision above the orbit, cutting the superior rectus muscle, and rotating the eye down. A small cut then was made in the nerve and the tip of a Hamilton syringe needle was inserted. Three to 4 μl of a solution containing HRP (30%), DMSO (2%), and polyornithine (0.5%) was injected into the nerve over 30 min using an infusion pump. After an additional 10 min, the needle was withdrawn and the incision closed. Postoperative survival periods were 10 hr (one rabbit), 24 hr (seven rabbits) and 40 hr (1 rabbit). Each animal was then given an overdose of nembutal and perfused. Five rabbits were perfused only with saline and the others were perfused with fixative (1.25% glutaraldehyde and 1% paraformal-
dehyde in 0.1 M phosphate buffer; followed by 3% glutaraldehyde and 2% paraformaldehyde in 0.1 M phosphate buffer). The eyes were removed immediately and the retinas detached and submerged in fixative. The retinas then were washed in phosphate buffer, preincubated in cobalt chloride and nickel ammonium sulfate (V. H. Perry, personal communication), and incubated in Hanks-Yates reagent. They then were dehydrated in a glycerin series and mounted onto glass slides in 100% glycerin.

The retinas then were examined with the light microscope. Areas containing labeled cells were studied, drawn, and photographed. Selected areas of 2–10 mm² containing labeled ganglion cells then were cut out, rehydrated into phosphate buffer, treated with OsO₄, dehydrated with ethanol, and embedded in plastic (Polarbed 812). During the final polymerization, the pieces were flattened onto beam capsule blanks. The embedded pieces were sectioned at 5–7 μm using an ultramicrotome and a dry glass knife. The sections were dried onto glass slides in serial order and covered with immersion oil and a coverglass.

**Light Microscopy and Quantitative Measurements**

All counts and measurements of displaced ganglion cells were made from unstained plastic sections. In different regions of the retina an approximation of the proportion of ganglion cells that were displaced was made by counting the number of displaced ganglion cells and dividing that number by the total number of labeled cells (displaced and normal ganglion cells).

Cell measurements were made from two plastic embedded pieces of retina. From these sections the outlines of all displaced ganglion cells in which the nucleus was visible were drawn at ×1940 (n = 45). The same was done for 94 labeled neurons in the ganglion cell layer and for 68 unlabeled cells in the inner plexiform layer. Staining of these sections was unnecessary because the glial cytoplasm was very dark and outlined the somata (Figs. 1B, C and 2). The outlines were traced using a digitizing tablet interfaced with a DEC 20 computer and a graphics terminal. Cell areas and average diameters were calculated and displayed as bar histograms.

**Results**

The injections of HRP into the optic nerve produced varying degrees and patterns of labeling in the retina. Usually a wedge of labeled axons and somata fans out from the optic disc. In some cases the wedge is quite narrow but in others it is as large as half of the retina. Within the wedge of label, many axons are solidly filled with HRP. In comparison, most labeled somata contain HRP granules although this granular labeling is often very dense and frequently extends into peripheral dendrites, especially in larger cells within the ganglion cell layer. A postoperative survival of 24 hr produced the best results. With a shorter survival (10 hr) less labeling is found and
with a longer survival (40 hr) degenerative changes are seen.

Unfortunately, the dendrites of displaced ganglion cells are not generally stained past their primary branch points. Furthermore, these cells are very difficult to identify in whole-mount preparations because the inner plexiform layer is quite thin in the rabbit (<15 μm) and because the whole mounts are not perfectly flat. Thus a labeled ganglion cell only can be identified as displaced if it is at least 15 μm directly beneath another labeled cell which is in the ganglion cell layer.

In 5-7 μm sections, however, displaced ganglion cells are easy to identify. In cross-sections of the retina (Fig. 1A), displaced ganglion cells are seen in the inner half of the inner nuclear layer, bordering the inner plexiform layer. In comparison, Figure 1C is a tangential section through the inner nuclear layer and shows three labeled displaced ganglion cells scattered among unlabeled inner nuclear layer cells. In such tangential sections very few labeled dendrites of displaced ganglion cells are seen for two reasons: (1) these dendrites are distributed primarily to the inner plexiform layer and therefore are cut by the plane of section; (2) the dendrites of these cells are not well labeled in our material.

Certain structural features of these cells are apparent. For example, Figure 2 is a high magnification photomicrograph of one of the labeled neurons in Figure 1C (arrow). The perikaryon is ovoid and densely labeled with HRP. It is larger than the surrounding unlabeled inner nuclear layer cells, which also have more rounded perikarya. Although they are not well labeled, three primary dendrites (arrows) can be seen radiating away from the labeled cell. They are of large diameter and appear to run tangential to the inner plexiform layer.

Distribution and Frequency of Displaced Ganglion Cells

As mentioned above, displaced ganglion cells are difficult to identify in our whole-mount preparations. Therefore, we have relied on tangentially cut, plastic embedded sections in order to determine the distribution and frequency of these cells relative to other ganglion cells. The different regions of the retina sampled are shown in Figure 3.

Fig. 2. Photomicrograph of a labeled displaced ganglion cell. This is a high magnification, phase-contrast micrograph of the cell indicated by the arrow in Figure 1C. The cell body is densely labeled with HRP. Two dendrites are seen attached to the soma (large arrows) and a third dendrite is visible (small arrow) which joins the soma in a different plane of focus. None of the dendrites are densely labeled with HRP. Scale bar = 25 μm.

Fig. 3. Schematic representation of a whole mount of the rabbit retina to show the areas used to determine the distribution and size of labeled displaced ganglion cells. The outlined areas a-g indicate the retinal loci sampled (see text for details) and the thinly outlined, elongated oval indicates the approximate location of the visual streak. The percentages of labeled ganglion cells that were displaced in each of the areas counted are listed beneath the drawing (a-g). D = dorsal; V = ventral; N = nasal; T = temporal; OD = optic disc; scale bar = 5 mm.

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The results of this survey indicate that displaced ganglion cells in the rabbit are concentrated in areas outside of the visual streak. For example, two pieces of tissue were taken from the visual streak of a rabbit in which the density of labeled cells is extremely high (Fig. 3, sites a and b). These two pieces (total area: 4-5 mm²) contain a total of only two labeled displaced ganglion cells. More peripherally, but near the lateral edge of the visual streak in the same animal (Fig. 3, site c), the number of labeled displaced ganglion cells increases, but still represents much less than 1% of the total number of labeled cells in that region.

In comparison, tissue pieces farther away from the visual streak contain a higher proportion of displaced ganglion cells (Fig. 3, sites d-g). In these areas 2-5% of the labeled cells are in the inner nuclear layer. Figures 1B, C are intended to illustrate the frequency of displaced ganglion cells relative to other ganglion cells and other cells in the inner nuclear layer. These micrographs are from an area with a high proportion of displaced ganglion cells (Fig. 3, site f). Both micrographs are from the same retinal region but one section is through the ganglion cell layer (Fig. 1B) and the other is through the inner nuclear layer (Fig. 1C). In Figure 1B about 40 cells are labeled. In Figure 1C only three are seen, and they are scattered among hundreds of unlabeled cells. Thus, displaced ganglion cells are restricted almost totally to areas outside of the visual streak. However, even in the peripheral retina where they are most common and the density of neurons in the ganglion cell layer is relatively low,16-19 displaced ganglion cells constitute only a small proportion of the cells projecting into the optic nerve.

Size of Displaced Ganglion Cells

As mentioned above, displaced ganglion cells appear to be larger than most of the unlabeled cells in the inner nuclear layer. They also appear to be smaller than most of the labeled cells in the ganglion cell layer. To test this we measured labeled displaced ganglion cells, unlabeled inner nuclear layer cells, and labeled cells in the ganglion cell layer from two pieces of retina (Fig. 3, sites d and e).

The results confirm that labeled displaced ganglion cells are larger than unlabeled inner nuclear layer cells (Fig. 4). Their mean average diameter is nearly twice as great (14.9 μm compared to 8.6 μm). Compared with labeled neurons in the ganglion cell layer, however, the displaced cells are small (Fig. 4). The largest labeled displaced ganglion cell that we measured has an average diameter of 17.7 μm, whereas the median average diameter of labeled neurons in the ganglion cell layer is 18.4 μm. Thus both layers contain small labeled cells but in our sample more than one-half of the labeled neurons in the ganglion cell layer are larger than the largest labeled displaced ganglion cell. Some labeled neurons in the ganglion cell layer are as large as 33 μm (Fig. 4).

Discussion

The results of this study demonstrate several features of displaced ganglion cells in the rabbit. These cells appear to be the largest neurons in the inner nuclear layer, but they are among the smallest of the total ganglion cell population. Further, they are concentrated outside of the visual streak, although even in regions of relatively high frequency they comprise only a small proportion of the total number of ganglion cells. In the peripheral retina, approximately 5% of the labeled ganglion cells are displaced compared with less than 0.1% in the central part of the visual streak. The magnitude of this change is due, in part, to a decrease in the concentration of cells in the ganglion cell layer,16-19 but it also reflects a real increase in the density of displaced ganglion cells in the peripheral retina.

We could not measure directly the increase in density of displaced ganglion cells in the peripheral retina because we were not certain that we labeled all of the ganglion cells in each case. Previous studies have indicated that in the ganglion cell layer, approximately 15% of the neurons in the visual streak and
30% in the peripheral retina are displaced amacrine cells, or "coronate" cells,11,17 that can not be labeled retrogradely by injecting HRP into the optic nerve. Thus, even though we find unlabeled cells in the ganglion cell layer in every case, it seems reasonable to conclude that most of the ganglion cells in this layer have been labeled, especially in the regions used for quantitative measures. The measurements of Oyster et al19 and Vaney17 indicate that ganglion cell density can be greater than 5000 cells/mm² in the visual streak and less than 500 cells/mm² in the peripheral retina. Based upon these measurements, calculations using our percentages for displaced ganglion cells suggest that the density of displaced ganglion cells in the visual streak may be less than 5 cells/mm² while in the peripheral retina it may be greater than 25 cells/mm².

Technical Considerations

The labeling technique used in the present report involves injections of HRP into the optic nerve near the optic disc. Such injections have the advantage of labeling a large number of ganglion cells. However, no single injection labeled ganglion cells throughout the entire retina, and these injections provide no information identifying the central targets of the labeled cells, although previous evidence indicates that at least some of these neurons project to the superior colliculus.12

Our analysis of the regional distribution of displaced ganglion cells assumes that the axons of displaced and normal ganglion cells are intermixed in the optic nerve in corresponding topographic patterns. If this assumption is false, the results showing very few displaced ganglion cells in the visual streak could be misleading since those counts were made from a single animal. However, the counts of displaced ganglion cells in the peripheral retina were from three different retinas and for each the results are similar. Furthermore, labeled displaced ganglion cells were found only in regions also containing labeled neurons in the ganglion cell layer. This overlap in the distribution of labeled displaced and normal ganglion cells suggests that their axons are intermingled in the optic nerve.

A limitation of our results concerns the dorsal-ventral distribution of displaced ganglion cells. None of the rabbits used in this study showed dense labeling of ganglion cells in the ventral half of the retina. Therefore we could not determine directly the proportion of displaced ganglion cells below the visual streak and dorsal-ventral comparisons were not possible. Previous studies of the size and distribution of neurons in the rabbit's ganglion cell layer have suggested differences between these areas,16,17,19 but there has been some disagreement on the nature of the differences.

Comparisons with Other Species

The first descriptions of displaced ganglion cells were made in nonmammalian vertebrates and showed that many of these cells are quite large.1-6 In the pigeon, Karten et al6 have shown that a portion of these large cells project preferentially to the accessory optic system (to the nucleus of the basal optic root).

In mammals the distribution and size of displaced ganglion cells differs with each species that has been studied. For example, in the monkey displaced ganglion cells are concentrated around the optic disc and range in diameter from 10–13 μm.10,20 This is intermediate in size relative to other ganglion cells in this region of the monkey retina, most of which have diameters of 8–20 μm.10,20 In comparison, two different patterns for displaced ganglion cells are found in the rat and mouse. The rat’s displaced ganglion cells are located in the central retina and are uniformly large, while in the mouse they are restricted to the peripheral retina and include cells with small, as well as large, somata.

The rabbit shows still another pattern. Our evidence suggests that displaced ganglion cells have a distribution similar to that seen in the mouse in that they are rare in the central retina. However, unlike the mouse, other animals that have been described, displaced ganglion cells in the rabbit are uniformly small.

Assigning some distinct functional role to displaced ganglion cells in mammals continues to be difficult and this problem is only made worse by interspecies variability. Dräger and Olson10 have shown that these cells are associated preferentially with ipsilaterally projecting axons in the mouse, while Bunt and Minckler10 have raised the possibility that they merely represent an anomaly of primate development.

In the pigeon there is evidence that large displaced ganglion cells are functionally linked to visual input to the cerebellum via the nucleus of the basal optic root,5,21,22 but this does not appear to be true for mammals, at least for the mouse6 and the rabbit. In the rabbit the present results demonstrate a lack of large displaced ganglion cells. Further, injections of HRP into the medial terminal nucleus (the homologue to the pigeon's nucleus of the basal optic root) retrogradely label large neurons in the ganglion cell layer but no cells in the inner nuclear layer.13

Recently, Amthor et al23 classified ganglion cells in the rabbit's retina based on a variety of morphologic criteria including soma size and dendritic branching.
pattern. They also suggested some correlations between morphologic and functional groups, but their analysis did not include displaced ganglion cells. Unfortunately the dendrites of displaced ganglion cells were not sufficiently labeled in the present study to allow a comparison with the results of Amthor et al. 23

Thus, displaced ganglion cells are a common feature in the retinas of a disparate variety of vertebrate species. However, the variability in the morphology and distribution of these cells suggests that they may not be functionally related across species lines. Perhaps the interspecies variations that have been reported for displaced ganglion cells in mammals are related to differences in experimental techniques or perhaps they reflect variations in function.

Key words: displaced ganglion cells, retina, optic nerve, rabbit, horseradish peroxidase, quantitative measurements

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