Topography of Pig Retinal Ganglion Cells

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ABSTRACT

In the present work we analyzed the distribution of retinal ganglion cells (RGCs) in the pig retina. RGCs were retrogradely labeled in vivo by injecting Fluoro-Gold into the optic nerve. RGC density and the distribution of RGCs in terms of soma size were analyzed. Different regions of the porcine retina were identified following analysis of the distribution of RGCs in terms of cell density and soma size: in the central retina, we found a high-density horizontal RGC band lying dorsal to the optic disc. Moreover, in this region, a high proportion of RGCs with small soma size was observed. From the central to the more peripheral retina, we observed a decrease in RGC density, together with a greater presence of RGCs with larger somas. The results of this study should prove to be useful as a foundation for future studies with the porcine retina as a model in ophthalmic research. The study also highlights the necessity to label the RGC population specifically with retrograde tracers in order to quantify precisely alterations in the cell population associated with experimental treatments. J. Comp. Neurol. 486:361–372, 2005. © 2005 Wiley-Liss, Inc.

Indexing terms: porcine; neuronal tracing; Fluoro-Gold; RGC; cell size; cell density; retina

Retinal ganglion cells (RGCs) are the output neurons of the retina, decoding and conveying visual information along the optic nerve to the brain. These cells are specifically affected in pathologies such as glaucoma, retinal ischemia, and diabetic retinopathy (Glovinsky et al., 1991; Vickers et al., 1995; Wygnanski et al., 1995; Selles-Navarro et al., 1996; Zhang et al., 1998; Joo et al., 1999; Lieth et al., 2000). However, the mechanisms that lead to selective RGC death associated with these pathologies remain unknown.

To study the etiology of such pathologies affecting human vision, it is essential to use an adequate model, which is relevant to the human visual system. Although nonhuman primates are considered the best substitutes for humans in visual science experimentation, both the expense and the availability of these species limit their use. In the present work, we have focused on the pig as an appropriate model, on the basis of the following interesting features: 1) the anatomy of the eye and the structure of the pig retina are very similar to those of humans (Prince et al., 1960; Beauchemin, 1974; De Schaepdrijver et al., 1990; McMenamin and Steptoe, 1991; Olsen et al., 2002; Ruiz-Ederra et al., 2003, 2004); 2) the immune system is well characterized; 3) tools employed for diagnostics in ophthalmology, such as optical coherence tomography, corneal topography imaging, or multifocal electroretinogram, can be applied to pig eye, supporting the use of this animal as a good model for ophthalmological studies (Kyhn et al., 2004; Maverick et al., 2004; van Velthoven et al., 2004); and 4) it is possible to reproduce ocular diseases in pigs similar to those found in humans (Li et al., 1998), and the porcine retina seems to be a good model for study of the pathophysiology of diseases affecting RGCs (Komáromy et al., 2003). Despite its obvious interest, there are few studies describing the distribution of RGCs in the...
porcine retina and comparing this distribution with that found in the human retina.

Despite it not being feasible to classify RGCs exclusively in terms of soma size, evidence indicates that, in the cat retina, RGC soma size is closely related to cellular properties, such as receptive field organization, number and distribution of dendrites, morphology, and central projections (Boycott and Wässle, 1974; Stone and Clarke, 1980; Rowe and Dreher, 1982). The presence of subgroups of RGCs based on soma size has been reported for a variety of mammalian retinas, such as the cat (Stone, 1965, 1978; Boycott and Wässle, 1974; Wässle and Illing, 1980; Chen and Weber, 2001) and the primate retina, including that of humans (Stone and Johnston, 1981; Yamada et al., 1996).

However, the presence of these size-based subgroups in the rat retina remains controversial (Fukuda, 1977; Luo et al., 2001).

Even though some studies point toward a nonselective loss of cells in terms of RGC size (Kalloniatis et al., 1993; Graham et al., 1996; Morgan et al., 2000), it has been widely documented that RGCs are differentially affected during ocular pathologies, in a size-dependent manner. Thus, large RGCs have been found to be more susceptible to death in human glaucoma (Quigley et al., 1987, 1988, 1991) and in monkey experimental glaucoma (Glovinsky et al., 1996). Moreover, previous in vitro studies performed in our laboratory have demonstrated that, in the pig retina, a differential survival capacity was associated with three different RGC sizes (García et al., 2002). In addition, large porcine RGCs in vitro have been reported to be more susceptible to excitotoxic insults induced by glutamate (Luo et al., 2001).

The object of the present work was to characterize in detail the density and soma size distribution of Fluoro-Gold-prelabeled porcine RGCs. In addition, we compared the number of RGCs stained with the classical Nissl staining method vs. the specific RGC retrograde labeled Fluoro-Gold-prelabeled porcine RGCs. In addition, large porcine RGCs in vitro have been reported to be more susceptible to excitotoxic insults induced by glutamate (Luo et al., 2001).

The object of the present work was to characterize in detail the density and soma size distribution of Fluoro-Gold-prelabeled porcine RGCs. In addition, we compared the number of RGCs stained with the classical Nissl staining method vs. the specific RGC retrograde labeled Fluoro-Gold method. Knowledge of the precise topographic distribution of adult porcine RGCs will facilitate the development of new models of retinal injuries affecting RGCs, involving a large mammal that closely resembles the human being.

**MATERIALS AND METHODS**

**Tissue collection**

Retinas from the left eyes of seven adult pigs (Sus scrofa) were used in the present work. All experimental methods and animal care procedures adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and were approved by the local Institutional Animal Care and Use Committee (IACUC). Pigs were deeply anesthetized with an intramuscular injection of ketamine hydrochloride (Ketolar) + Diazepam (20 mg/kg each). Once the animal was anesthetized, an intravenous cannula was applied to the ear to provide the animal with Propofol (1 ml every 15 minutes), an additional anesthetic, which maintained deep anesthesia throughout the operation. A life-support machine was used to facilitate breathing and to monitor vital functions during the operation.

**RGC back-filling**

RGCs from five left eyes were back-filled with 3% Fluoro-Gold (Fluorochrome, Englewood, CO) diluted in a solution containing 0.9% NaCl and 0.1% dimethylsulfoxide. Forty microliters of Fluoro-Gold was injected into the optic nerve about 4 mm from the optic nerve head. Pigs were kept alive for 2 days postoperation to allow Fluoro-Gold to fill the entire population of RGCs. Then animals were euthanized with an overdose of anesthesia, the eyes were enucleated, and the lens and vitreous were extracted by cutting the anterior chamber at the level of the ora serrata. The eyecups were fixed with 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS; pH 7.4) for 4 hours at 4°C, and then retinas were removed and flat-mounted with the RGC layer being uppermost. They were then coverslipped with phosphate-buffered saline (PBS)/glycerin (1:1), so that shrinkage did not occur during the processing of the tissue.

**Nissl staining**

Two porcine retinas were stained with 1% toluidine blue following the Nissl method as described by Hebel (1976) for the pig retina.

**Image capture**

Images from each retina were obtained by using an epifluorescence microscope (Axioskop 2; Zeiss, Jena, Germany) coupled to a digital camera (CoolSnap; RS Photometrics, Tucson, AZ). The images were captured in a systematic way, by using the optic disc (OD) as a reference point. Two imaginary perpendicular lines, through the X and Y axis, both of them crossing the OD, were drawn, thus dividing the pig retina into different subregions: dorsal (D), ventral (V), temporal (T), and nasal (N), and quadrants resulting from the combination of these (nasal-dorsal, ND; temporal-dorsal, TD; nasal-ventral, NV; and temporal-ventral, TV). We recorded one of four ×40 microscopic fields following the imaginary X and Y lines as references. We recorded 100–130 fields/retina, and the distance between each sampling point was 2.3 mm. We captured each retinal field at different sampling points, shown as open circles in Figure 1, resulting from running the entire retina through the X axis (letters) and Y axis (numbers; from 1A at the OD to 8H at the periphery). The sampling area recorded represented 1.4% of the mean area of the five retinas analyzed. This sample size has previously been reported to represent a significant percentage of the retina (Peinado-Ramón et al., 1996). Moreover, we captured additional samples from retinal areas with an especially high density of RGCs in order to have a statistically significant sample from this region. Drawings of pig retinas representing isodensity profiles and the distribution of RGCs were made in Adobe Photoshop 5.0. Measurement of the total retinal area, retinal regions, and other parameters were performed with the aid of Spot Advanced software (Diagnostic Instruments Inc., Sterling Heights, MI), in order to 1) estimate the number of RGCs present in different retinal regions or the total number of RGCs (by multiplying the area of a particular retinal region by its corresponding RGC mean density) and 2) to measure retinal distances such as the distance different retinal regions and the OD.

**Morphometric analysis**

For each recorded retinal field, we quantified the number of RGCs and measured the soma major axis length and soma area of each of the RGCs present. Analysis of these parameters was performed with a digital palette.
that RGCs with similar soma sizes can have different functional roles. Nevertheless, we employed this classification scheme to facilitate the handling of the huge volume of data analyzed in the present work (45,800 RGCs).

Data concerning RGC parameters were collected from five retinas, and corresponding mean values were obtained with SPSS software (SPSS, Chicago, IL). Subsequently, we plotted out the mean density and RGC soma size distribution on color-coded or size-distribution maps representing the pig retina. RGC density, mean soma area, and percentage of size groups were expressed as mean ± SEM. Mean data from different retinas, regions, and subregions were compared with one-way ANOVA followed by the Scheffé test to compare two groups. Comparison of results obtained from Nissl staining and Fluoro-Gold methods was performed by using the Student’s t-test. The minimal level of significant difference was defined as \( P < 0.05 \).

RESULTS

Gross anatomy of the pig retina

The mean area of the porcine retina (781 ± 40 mm\(^2\)) is similar to that of the human retina (Stone and Johnston, 1981). However, the pig OD is not located in the center of the retina (Fig. 2), so the mean area of the dorsal retina (462 ± 27 mm\(^2\)) was found to be about threefold larger than that of the ventral retina (172 ± 23 mm\(^2\)), whereas the mean area of the nasal retina was 1.6-fold larger (480 ± 32 mm\(^2\)) than that of the temporal retina (301 ± 30 mm\(^2\)).

Distribution of RGCs (Fluoro-Gold method)

In the present study, we counted and measured a total of 45,800 RGCs from five pig retinas. The mean number of RGCs per retina was estimated to be 583,871 ± 42,658. However, RGCs were not distributed homogeneously throughout the retina. Thus, by attending to RGC density, mean soma area, and soma size distribution, we distinguished three regions in the retina: the visual streak, the midperiphery, and the periphery.

The visual streak consisted of a high-density RGC horizontal band, which constantly lies dorsal to the OD, running from the nasal to the temporal extreme above the OD. This retinal landmark of 2.5 mm width was easily discernible at low magnification. In the visual streak, we found the highest RGC density, the lowest mean soma area, and a normal distribution of RGCs, by attending to soma sizes. This band, which represented only 7% of the total area of the retina, contained 24.5% of all RGCs (143,243 ± 13,243; Table 1, Fig. 3).

We considered the midperiphery as being a vast region located between the visual streak and the periphery. Here we found medium RGC density and medium soma mean area, whereas soma sizes were distributed in a unimodal fashion. This region represented 78% of the total retinal area and contained 70.5% of RGCs (411,439 ± 36,715; Table 1, Fig. 3).

Finally, we defined the periphery as a ring in the outer edge of the retina measuring ~1.5 mm width in the dorsal retina and ~3.5 mm width in the ventral retina. This was found to be the region with the lowest RGC density and the highest mean soma area and showed a plurimodal distribution of soma sizes. Thus, this region, making up

![Fig. 1. Schematic representation of a whole-mounted pig retina, showing the localization of the different retinal fields analyzed. Two imaginary perpendicular axes, the X axis (represented by letters) and the Y axis (represented by numbers), both of them intersecting at the optic disc, were used as references in order to perform the systematic capture of 100–130 fields by running the entire retina through both axes. The distance between one sampling point and the next was 2.3 mm. Sample points are represented by open circles, whereas solid circles represent the retinal points from which histograms shown in Figure 4 were obtained. d, dorsal; n, nasal; t, temporal; v, ventral. Scale bar = 2.3 mm.](image-url)
15% of the retinal area, contained only 5% of RGCs (29,189 ± 3,931; Table 1, Fig. 3).

RGC density

Variability in the density of RGCs among the five porcine retinas was found to be low. Thus, the mean value of RGC density was 1,133 ± 57 RGCs/mm², with no significant differences among retinas (P = 0.113). However, statistical analysis showed that RGC density was significantly different (P < 0.01) among different regions of the pig retina. The region with highest RGC density was the visual streak (2,641 ± 149 RGCs/mm²), followed by the midperiphery (678 ± 20 RGCs/mm²) and finally the periphery, with the lowest density of RGCs (241 ± 19 RGCs/mm²; Table 2).

Within the visual streak, different subregions can be identified on the basis of RGC density. At its central region (about 1.2 mm from the optic disc), we found a subband with a high mean RGC density of 4,331 ± 231 RGCs/mm². About 8.5 mm temporally to the OD, we found a small circular area (with a diameter of ~250 μm), where RGCs density peaked to 5,735 ± 1,066 RGCs/mm². Additionally, we found an additional peak in RGC density in the nasal arm of the visual streak, located about 6.5 mm nasally to the OD, with a similar mean density of 5,658 ± 290 RGCs/mm² (Fig. 3A). We will refer to these areas as the area centralis.

The distribution of RGCs along the midperiphery was not homogeneous. Thus, when we compared RGC density in the different subregions of the midperiphery retina, we found significant differences between them. Similarly, RGC density in the periphery was heterogeneous (Table 2, Fig. 3A).

RGC mean soma area

The mean soma area (in square micrometers) of RGCs was larger in regions of lower RGC density and smaller in regions of higher RGC density. This cellular parameter was significantly different (P < 0.01) among the three retinal regions. Thus, we found the smallest values of mean RGC area in the visual streak (214 ± 5 μm²), fol-
**Fig. 3.** Representations of pig retina whole mounts, showing retinal ganglion cell (RGC) densities in the different analyzed fields. **A:** Mean values of RGC density (in RGCs/mm²) in the different fields of the five retinas analyzed. The five gray values represent RGC isodensity groups (see key). VS, visual streak; MP, midperiphery; P, periphery. **B:** Map of the pig retina showing the three main retinal regions: the visual streak (black), the midperiphery (gray), and the periphery (pale gray). The images to the right illustrate Fluoro-Gold-prelabeled RGCs in the visual streak (1), the midperiphery (2), and the periphery (3) of the retina. A representation of the typical retinal vascular pattern is also shown. d, dorsal; n, nasal; t, temporal; v, ventral. Scale bars = 5 mm in A (applies to A,B); 20 μm in B1-3.
TABLE 2. Porcine Retinal Ganglion Cell Mean Density1 (RGCs/mm²)

<table>
<thead>
<tr>
<th>Region</th>
<th>Subregion</th>
<th>Quadrants</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visual streak</td>
<td>Dorsal</td>
<td>823 ± 24</td>
<td>ND 763 ± 29</td>
</tr>
<tr>
<td></td>
<td>Ventral</td>
<td>422 ± 18½</td>
<td>TD 887 ± 39</td>
</tr>
<tr>
<td></td>
<td>Nasal</td>
<td>616 ± 22</td>
<td>TV 422 ± 19</td>
</tr>
<tr>
<td></td>
<td>Temporal</td>
<td>762 ± 35½</td>
<td>TV 422 ± 38</td>
</tr>
<tr>
<td>Midperiphery</td>
<td>Dorsal</td>
<td>292 ± 25</td>
<td>ND 344 ± 42</td>
</tr>
<tr>
<td></td>
<td>Ventral</td>
<td>166 ± 25½</td>
<td>TD 256 ± 28</td>
</tr>
<tr>
<td></td>
<td>Nasal</td>
<td>262 ± 30</td>
<td>NV 181 ± 32</td>
</tr>
<tr>
<td></td>
<td>Temporal</td>
<td>218 ± 23</td>
<td>TV 149 ± 28</td>
</tr>
<tr>
<td>Periphery</td>
<td>Dorsal</td>
<td>321</td>
<td>WD 678 ± 20**</td>
</tr>
<tr>
<td></td>
<td>Ventral</td>
<td>574</td>
<td>TD 3,043</td>
</tr>
<tr>
<td></td>
<td>Nasal</td>
<td>1,492</td>
<td>ND 3,386</td>
</tr>
<tr>
<td></td>
<td>Temporal</td>
<td>1,472</td>
<td>TV 3,043</td>
</tr>
</tbody>
</table>

1Mean RGC density in the different subregions and quadrants of regions of the porcine retina. To the right, mean RGC density in the whole region is shown. Values are expressed as mean ± SEM of the number of RGCs mm². ND, nasodorsal; TD, temporal-dorsal; NV, nasoventral; TV, temporal-ventral.

TABLE 3. Mean Porcine RGC Soma Area1 (µm²)

<table>
<thead>
<tr>
<th>Region</th>
<th>Subregion</th>
<th>Quadrants</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visual streak</td>
<td>Dorsal</td>
<td>266 ± 3</td>
<td>ND 258 ± 3</td>
</tr>
<tr>
<td></td>
<td>Ventral</td>
<td>289 ± 4½</td>
<td>TD 275 ± 6</td>
</tr>
<tr>
<td></td>
<td>Nasal</td>
<td>267 ± 3</td>
<td>NV 290 ± 5</td>
</tr>
<tr>
<td></td>
<td>Temporal</td>
<td>284 ± 5½</td>
<td>TV 310 ± 9</td>
</tr>
<tr>
<td>Midperiphery</td>
<td>Dorsal</td>
<td>321 ± 8</td>
<td>ND 289 ± 9</td>
</tr>
<tr>
<td></td>
<td>Ventral</td>
<td>333 ± 14</td>
<td>TD 347 ± 9</td>
</tr>
<tr>
<td></td>
<td>Nasal</td>
<td>302 ± 10</td>
<td>NV 315 ± 18</td>
</tr>
<tr>
<td></td>
<td>Temporal</td>
<td>353 ± 9½</td>
<td>TV 367 ± 20</td>
</tr>
</tbody>
</table>

1Mean RGC soma area values in the different subregions and quadrants of regions of the porcine retina. In the right column, the mean RGC area in the whole retinal region is shown. Values are expressed as mean ± SEM of the soma area of RGCs in µm². ND, nasodorsal; TD, temporal-dorsal; NV, nasoventral; TV, temporal-ventral.

Distribution of RGCs (Nissl staining method)

We measured the same parameters in Nissl-stained RGCs as those measured in the RGCs back-filled with Fluoro-Gold. The results obtained when analyzing the distribution of presumptive RGCs stained with toluidine blue were significantly different from those obtained when we specifically back-filled the RGCs with Fluoro-Gold. Thus, the mean RGC density estimated from Nissl-stained retinas in the midperiphery (1,192 ± 54 RGCs/mm²) and periphery (689 ± 89 RGCs/mm²) was significantly higher than that estimated from retinas labeled with Fluoro-Gold (699 ± 38 and 315 ± 25, respectively). In contrast, RGC density estimated in the visual streak after Nissl staining was similar to that observed with Fluoro-Gold (3,386 ± 472 vs. 3,043 ± 344 RGCs/mm²; Table 4).
The distribution of the three RGC size groups is summarized in Table 5.

**DISCUSSION**

In the present paper, we describe the distribution of RGCs within the pig retina. The porcine retina was chosen as a suitable model, principally because of its many similarities with the human retina and because of its accessibility in comparison with nonsimian primate retinas.

For RGC density, three main regions can be distinguished in the porcine retina; the region of highest density was the visual streak, and that with the lowest density of RGCs was the periphery. This distribution has also been described for the human retina (Stone and Johnston, 1981; Curcio and Allen, 1990). The presence of the visual streak specialization may be a general feature of mammalian topography; it has been described in nonprimates and also in primates (Stone and Johnston, 1981). Thus, although the primate retina shows...
a clear foveal specialization, it has been reported that isodensity lines in ganglion cell density maps from different species of primates are horizontally elongated, suggesting the presence of a visual streak specialization. This situation is clearly apparent even in the human retina (Stone and Johnston, 1981; Curcio and Allen, 1990). Another similar feature between this proposed streak region in primates and the porcine visual streak is that, in both cases, only small branches and capillary plexuses, and not large vessels, cross the structure.

Finally, not all species of primates possess a well-developed fovea specialization; often it is relatively small (Stone and Johnston, 1981). This may be equivalent to the high density peak we observed in the porcine visual streak.

**RGC density**

We found that the mean density of RGCs was about 50% higher in the dorsal than in the ventral retina. Previous studies of human retina have reported that RGC density

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**Fig. 5.** Percentages of small (A), medium-sized (B), and large (C) retinal ganglion cells. The percentages of different-sized RGCs are represented by four gray scales, ranging from 100% (black) to less than 15% (white; see key). n, nasal; d, dorsal; t, temporal; v, ventral. Scale bar = 5 mm.
pig are more difficult to evaluate, because different retinal landmarks (as reference points) have been considered, and, consequently, the nomenclature used in the present work does not match that used for the human retina. Thus, previous works on human RGCs (Stone and Johnston, 1981; Curcio and Allen, 1990) use the terms nasal and temporal retina to refer to the hemiretina located nasally and temporally with respect to the foveal center, respectively, whereas we define nasal and temporal with respect to the OD (Fig. 2). Authors have described a higher mean density in nasal retina in comparison with the temporal retina (Curcio and Allen, 1990), whereas we measured a greater mean value of RGC density in the temporal than in the nasal retina. These differences probably are due to differences in the reference points used.

The fact that pig retina is endowed with a smaller number of RGCs in comparison with the human retina does not rule out the pig retina as being a good model for eye research. In fact, it has been reported that some non-human primate retinas, such as those of the macaque (Wassle et al., 1989), baboon (Fischer and Kirby, 1991), or cebus monkey (Silveira et al., 1989), have more RGCs than the human retina. Finally, we found that the mean area of the pig retina (781 ± 45 mm²) is more similar to that of the human retina (883 mm²) than that described for other primates, whose retinas have areas ranging between 319 and 527 mm² (Stone and Johnston, 1981).

**RGC soma sizes**

The trends in RGC soma size distribution among different parts of the retina described in the present work have also been reported for other species. Thus, mean RGC soma size is smaller in the central than in the peripheral retina in different species of primates. The presence of smaller cells in the central retina could be a response to the mechanical need to pack many ganglion cells into a

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**TABLE 4. Comparison of Mean RGC Densities (RGCs/mm²) in the Different Retinal Regions Obtained by Fluoro-Gold and Nissl Staining Methods**

<table>
<thead>
<tr>
<th>Region</th>
<th>Fluoro-Gold</th>
<th>Nissl</th>
<th>Nissl (Hebel, 1976)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visual streak</td>
<td>3.043 ± 0.344</td>
<td>3.386 ± 0.472</td>
<td>4.084</td>
</tr>
<tr>
<td>Midperiphery</td>
<td>689 ± 38</td>
<td>1.192 ± 0.54**</td>
<td>1.990</td>
</tr>
<tr>
<td>Periphery</td>
<td>315 ± 25</td>
<td>689 ± 89*</td>
<td>1.006</td>
</tr>
</tbody>
</table>

*Values are expressed as mean ± SEM of the number of RGCs mm².
**P < 0.05, ***P < 0.01 with respect to Fluoro-Gold staining.

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**TABLE 5. Comparison of the Percentage of Small, Medium-Sized, and Large RGCs Obtained With Fluoro-Gold and Nissl Staining Methods in the Different Retinal Regions**

<table>
<thead>
<tr>
<th>Region</th>
<th>Fluoro-Gold</th>
<th>Nissl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visual streak</td>
<td>33.06 ± 2.59</td>
<td>28.58 ± 3.4</td>
</tr>
<tr>
<td>Midperiphery</td>
<td>53.03 ± 2.02</td>
<td>51.36 ± 2.5</td>
</tr>
<tr>
<td>Periphery</td>
<td>8.86 ± 0.71</td>
<td>19.34 ± 1.91**</td>
</tr>
</tbody>
</table>

*Values are expressed as mean ± SEM of the percentage of RGCs.
**P < 0.05, ***P < 0.01 with respect to Fluoro-Gold staining.

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**Fig. 6.** Percentages of small, medium-sized, and large RGCs in different regions of the retina. A: RGC size distribution in the three main retinal regions: the visual streak, the midperiphery, and the periphery. **P < 0.01, significantly different with respect to visual streak. B: Comparison of the RGC size distribution between the dorsal and the ventral midperipheral retina. **P < 0.01, significantly different with respect to the dorsal retina. C: Comparison of the RGC size distribution between the nasal and the temporal peripheral retina. **P < 0.01, significantly different with respect to the nasal retina. Values are expressed as the mean ± SEM of the percentages of each RGC size group.
small region of retina, as has also been described for primates (Stone and Johnston, 1981).

Upon comparison of the nasal and temporal retina, a greater mean soma size was observed in the temporal retina than in its nasal counterpart, as has been described for the cat (Stone et al., 1980), opossum (Tancred et al., 1977; Rowe et al., 1978), fox (Rapaport et al., 1979), dog (Osmotherly, 1979), monkey, and human (Stone and Johnston, 1981) retinas. It has been suggested that these differences may reflect the different phylogenetic histories of temporal and nasal regions of the retina and that these differences may reflect different physiological types of RGCs with these two methodologies. If we were to assume that most of the cells that were stained with the Nissl method but were not back-filled with Fluoro-Gold in the ganglion cell layer correspond to displaced amacrine cells, we could estimate the following: 343 displaced amacrine cells/mm² in the visual streak, 493 displaced amacrine cells/mm² in the midperiphery, and 374 displaced amacrine cells/mm² in the periphery. However, we cannot rule out the possibility that some of the cells stained with Nissl but not with Fluoro-Gold in the RGC layer correspond to astrocytes, which are quite abundant in this layer (Ruiz-Ederra et al., 2003).

Hebel (1976), using the Nissl staining method, obtained a higher RGC density than our estimate. These differences probably are due to tissue shrinkage, because Hebel dehydrated the retina, whereas we used PBS/glycerin-mounted retinas.

CONCLUSIONS

The distribution of porcine RGCs was found to be similar to that reported for primates in terms of cell size and distribution. Moreover, the variability in RGC distribution between individuals was found to be low. The porcine retina may be considered, on the basis of these findings, to be a useful model for ophthalmic research. The detailed characterization of the distribution of RGCs within the normal porcine retina reported in the present work will facilitate a more precise evaluation of RGC death in future studies concerning experimentally induced ocular pathologies, using the pig as a novel model.

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