Topography of Ganglion Cells in the Retina of the Horse

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ABSTRACT. Topography of ganglion cells in the retina of the horse (*Thoroughbred*) was analyzed in the wholemount retina stained with cresyl violet, and a total number of ganglion cells were estimated. Sizes of ganglion cells were also measured and size spectra were analysed. The main results showed that: (1) a common point in 4 wholemount retinæ, based on cell densities and retinal locations, was that a retina could be divided into 5 regions, namely visual streak, nasal, temporal, dorsal and ventral region to the visual streak. A maximum cell density of 4,000 cells/mm² was found in the visual streak. And a total number of ganglion cells was estimated in a range of $398 \times 10^3 - 469 \times 10^3$, with a mean of $441 \times 10^3 \pm 31 \times 10^3$ (n=4). (2) cell sizes were measured as the mean lengths of the major and minor axes of the somas, and were in a range of 5–53.8 μm . The lowest mean diameter was 14.0 μm (± 3.7) in the visual streak and the highest was 25.9 μm (± 7.6) in the ventral region. Cell size spectra were unimodal and positively skewed. It is expected that these analyses will provide an anatomical and physiological background for further study of the visual system in the horse.

KEY WORDS: equine, ganglion cell, retina, topography, wholemount.

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Retinal ganglion cell represents the only connection between the retina and higher visual centers, and its density can be regarded as the number of paths of output per unit area of retina. It is significant to investigate the distribution of ganglion cells in the retina for understanding how the retina sends the outside image information to higher visual centers. By now, a large amount of observations have been done on the distribution of retinal ganglion cells of comparatively small mammals, such as cats [8, 14], rabbits [7, 12], marsupials [4, 19] and primates [13, 16, 18]. Some major retinal specializations, such as area or fovea centralis [1, 3, 14, 18] and visual streak [8, 12, 14], have been found and quantitated in a lot of animals. There were also a few literatures on retinal ganglion cells of the horse [5, 6]. Hebel [6] demonstrated a well demarcated narrow linear band of high cell density, and Harman *et al.* [5] estimated the total ganglion cell number after mapping the horse's retina. However, no detailed reports were given for the distribution of retinal ganglion cells of the horse as a large animal. Furthermore, there are no observations on ganglion cell sizes.

Therefore, the purposes of this study were firstly, to provide a detailed analysis of ganglion cell distribution in the horse's retina, and secondly, to investigate sizes of ganglion cells. It is expected that these analyses will provide an anatomical and physiological background for further study of the visual system in the horse.

MATERIALS AND METHODS

Eyeballs of 9 adult horses (*Thoroughbred*), weighing 350–450 kg, were used in this study. Most eyeballs were derived from the Equine Research Institute of Japan Racing Associa-

tion and the others were from a local slaughter house. After horses were killed, eyeballs were enucleated quickly and the connective tissue, muscles and blood from the exteriors of eyeballs were removed as soon as possible. Eyeballs were hemisected and immersed in 10% phosphate buffered formalin (pH 7.4) for at least two days.

Topography of retinal ganglion cell

(1) Preparation of retinal wholemounts

The retinæ were excised free from the eyecups and cut in several radial ways for flat mounting on gelatin-coated glass slides (Fig. 1). After being de-fatted, the retinæ were stained with cresyl violet. Areas of retinal wholemounts were measured by an area-measuring software (Area properties 3.2). Altogether 17 wholemounts were prepared and 4 best-stained wholemounts of which, named 1L, 1R, 2R, 3L (where L is left eye, R is right eye), were used for mapping ganglion cells. Since the retinal shrinkage was only about 1.8% in this study, no correction for shrinkage has been applied.

(2) Identification of ganglion cells

Retinal ganglion cell layer consists of three kinds of cells: glia cells, displaced amacrine cells and ganglion cells. Glia cells were distinguished easily from the two others because of tiny soma sizes and pale staining (arrowhead in Fig. 2f). Ganglion cells were identified by the standard criteria [14] of clumped Nissl substance in the cytoplasm and large somas which were often irregular (large arrow in Fig. 2f). On the other hand, displaced amacrine cells (small arrow in Fig. 2f) were recognized by the lack of clumped Nissl substance, small soma sizes and smooth outline [10].

(3) Mapping of the retina

The details for mapping ganglion cells in the retina were as follows: (a) a piece of overhead project sheet containing a regular pattern of rectangle 2 × 4 mm, was pasted on the wholemount's cover-glass, with an intersection located in the middle of the optic disc (Fig. 1). (b) the retina was sampled

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Fig. 1. The method to decide sampling areas. A piece of overhead project sheet containing a regular pattern of rectangle 2×4 mm, was pasted on the whole-mount's cover-glass, with an intersection located in the middle of the optic disc. The scale bar represents 1 cm.

for every rectangle in regions with high or higher ganglion cell densities, while sampled for every other rectangle in regions with low ganglion cell densities. In each rectangle sampled, the numbers of cells within 3 or 4 sites (0.8×0.8 mm), which were decided by an eye-piece graticule, were counted. (c) the ganglion cell number was calculated in every region by multiplying a region's area by its average cell density. And the total number of ganglion cells in the retina was estimated as a sum of the ganglion cell number in every region.

Measurement of ganglion cell sizes

Wholemount 1L was also used for measuring ganglion cell sizes after mapped. Microphotographs of ganglion cells in different regions were taken, and cell sizes were measured as the lengths of the major and minor axes of the somas. The mean of these two diameters was recorded as its soma diameter for each cell. Altogether 3,939 ganglion cells were measured. Cell size spectra were analysed and mean cell sizes were calculated for every region.

RESULTS

Ganglion cells in the horse's retina

Ganglion cells in the horse's retina were found as cells with large (or medium) somas and stained clumps of Nissl substance in the abundant cytoplasm (large arrow in Fig. 2f), or cells with small somas and thin ringed Nissl substance surrounding nuclei (arrow in Fig. 2e). The large ganglion cells were either rounded, or more commonly elongated or angu-

lar in shape. The medium and small size ganglion cells were rounded or elongated (Fig. 2).

Map of ganglion cell distribution

Here we showed the map of ganglion cell distribution in wholemount 1L as an example (Fig. 3). No ganglion cells were found around the optic disc because of the thick nerve fiber layer. Based on cell densities and retinal locations, a retina could be divided into 5 regions, namely visual streak, nasal, temporal, dorsal and ventral region to the visual streak. The most striking feature in the map was a well demarcated visual streak with relatively high ganglion cell densities and locating 2–3 mm above the optic disc. A maximum cell density of 4,000 cells/mm² was found at the temporal end of the visual streak. From the visual streak to retinal periphery, the ganglion cell density decreased markedly and reached a minimum of 6 cells/mm² both in the dorsal and ventral region. Furthermore, there were two regions in nasal and temporal to the visual streak, where ganglion cell densities were slightly higher than the rest of the periphery (Fig. 3). Distributions of ganglion cells in wholemount 1R, 2R and 3L were similar to that in 1L except of the maximal cell densities.

Microphotographs of ganglion cells in five regions of wholemount 1L were shown in Fig. 2. Ganglion cells were quite scattered and fairly large in the dorsal and ventral region to the visual streak (Fig. 2a, c). However, in the visual streak (Fig. 2e), ganglion cells were densely packed, more homogeneous in size and smaller than those of other regions (although a few relatively large cells could be picked out).

Fig. 2. Microphotographs of a wholemount. a, b, c, d and e were taken from regions showed in the lower right figure respectively. Arrow in e pointed a ganglion cell with small soma and thin ringed Nissl substance surrounding nuclei. Three kinds of cells were showed in f: the large arrow pointed to a ganglion cell, the small arrow pointed to a displaced amacrine cell and the arrowhead pointed to a glia. T: temporal; N: nasal; D: dorsal; V: ventral. The scale bar in a was $100\ \mu\text{m}$ and applied to b, c, d, e. The scale bar in f was $70\ \mu\text{m}$.

Total number of ganglion cells

The area and the mean cell density of every region in wholemount 1L, 1R, 2R, 3R were tabulated in Table 1. The total number of ganglion cells was estimated in a range of 398×10^3 – 469×10^3 , with a mean of $441 \times 10^3 \pm 31 \times 10^3$ ($n=4$).

Ganglion cell sizes

We divided visual streak, dorsal and ventral regions into two areas respectively for analysing ganglion cell sizes. The results of ganglion cell sizes in 8 areas (dorsal-nasal, dorsal-temporal, ventral-nasal, ventral-temporal, nasal of the visual streak, temporal of the visual streak, nasal region to the visual streak, temporal region to the visual streak), were summarized in Fig. 4. Instead of ganglion cell numbers, cell percentages were used in size spectra because of different numbers of cells measured among areas. Ganglion cell somas ranged from 5 – $53.8\ \mu\text{m}$ in diameter, and their spectra were unimodal and positively skewed. Cells in the visual streak were significantly smaller than those of other regions.

Some small ganglion cells with diameters ranging from 5 – $10\ \mu\text{m}$ presented in the visual streak, while not existing in other regions. Furthermore, in the visual streak, ganglion cells with diameters in 10 – $15\ \mu\text{m}$ owned the largest percentage, while in other regions, the largest percentage was belonged to cells with diameters more than $15\ \mu\text{m}$. The lowest mean diameter was $14.0\ \mu\text{m} (\pm 3.7)$ in the visual streak and the highest was $25.9\ \mu\text{m} (\pm 7.6)$ in the ventral region (Fig. 4).

DISCUSSION

Distribution of ganglion cells in the horse's retina

(1) The division of the retina into five regions

Five region pattern found in the retina of the horse was quite different with the common concentric pattern found in the retinae of animals as cat [8, 14], rabbit [7, 12] and etc. The existences of the nasal and temporal regions in the horse's retina were supposed to induce the discrepancy. The non-concentric pattern was also present in the pig, sheep, ox

and dog [6]. The nasal region to the visual streak which we found probably corresponded with the field of relatively high density continuing along the nasal arm of the visual streak in the horse [6]. On the other hand, an upper temporal concentration was reported in the retina of the elephant [17], which might be similar to our temporal region to the visual streak.

In the visual streak, a ganglion cell was supposed to receive information only from one or few photoreceptors for detailed analyses of image, while in the periphery, a ganglion cell was supposed to receive information from several photoreceptors, which was the reason of regional differences of ganglion cell densities. A visual streak was also necessary to herbivore to have a large visual field.

(2) Visual streak and area centralis

As in the retina of the rabbit or the cat [8, 12, 14], a visual streak was also found in the retina of the horse. However, we could not find an area centralis. Was there an area centralis in the horse's retina? A second area, which was supposed to be utilized in forward binocular, was found to lie at the lateral extremity of the central area in the retina of the horse [11]. A weak area centralis at the temporal end of the visual streak was demonstrated in the retina of the horse by Harman *et al.* [5], while nothing about area centralis was indicated in the retina of the horse [6]. Stone [15] extended Hughes' observations [9] and concluded that an area centralis might be present in all mammals. In our study, the region at the temporal end of the visual streak presumably was the area centralis of the horse.

Ganglion cell densities and the total cell number

The maximum ganglion cell density in Hebel's study [6] (more than 6,500 cells/mm²) or in Harman's [5] (mean 5600 cells/mm²) was significantly higher than ours (4,000 cells/mm²). Two factors may account for this discrepancy in the different maximum cell density. First, individual variation among horses, or more consistent differences between horse breeds, may account for much, perhaps all, of the difference. Second, neither ganglion cell microphotographs nor criteria for the identification of ganglion cells appeared in Hebel's [6] paper, so his criteria for the identification may be somewhat different from ours.

Our total number of ganglion cells (mean 441,158) was less than Harman's [5] (mean 615,000). Besides the different maximum cell density, the method to estimate the total number of ganglion cells might induce this difference. A common way, which was also adopted by Harman *et al.* [5], is estimating by proportionality, namely counting cell in sampled retinal area firstly, and then estimating the total number by proportionality [4]. Our method, which considered cell density and area variations among regions, should be more accurate than that.

One problem in calculating cell totals was to estimate the number of ganglion cells in the blank area around the optic disc (Fig. 3). Ganglion cells here were not included in the study because they were covered with the thick nerve fiber layer. An examination of corresponding area of the rabbit's retina in radial sections indicated that ganglion cell density there was similar to that found in peripheral retina [12]. So

Fig. 3. Topography of ganglion cells in wholemount 1L. Based on cell densities and retinal locations, the wholemount could be divided into five regions. Meaning of symbols was listed in upper right of the map. OD: optic disc. Meaning of other abbreviations as in Fig. 2. The scale bar represents 1 cm.

the number of ganglion cells in the region around the optic disc in the horse (a mean area of 60 mm²) was also thought to have little effect on the total ganglion cell number.

Ganglion cell sizes

In the present study, the mean soma size in the visual streak (temporal side: 14.0 μ m, nasal side: 15.5 μ m) was significantly smaller than those in the peripheral regions (for instance, even the minimum mean soma size was 18.6 μ m in the peripheral regions). A similar trend was also found in the cat's retina [2]. A significant increase in perikaryal diameter with distance from the central area was reported for α -ganglion cells stained with the Golgi-rapid method in the retina of the cat [2]. Our present result, with the result in the cat's retina [2], provided evidence for the existence of an inverse relationship between soma diameter and cell density. However, this relationship was not present in the retina of the rabbit [12], elephant [17] and honey possum [4]. In the rabbit's retina [12], mean soma diameter fell only slightly between, for example, nasal periphery (at density = 300 cells/mm², soma diameter = 12 ± 3 μ m) and nasal streak (at density = 3,800 cells/mm², soma diameter = 11 ± 3 μ m). With a Kruskal-Wallis H test, Stone and Halasz [17] reported that the differences of soma sizes between the regions of relatively high density and low density, were not significant ($P = 0.15$) in the elephant's retina. And in the retina of the honey possum [4], mean diameters differed little across the retina.

A wide range of 5–53.8 μ m in diameter of ganglion cell somas was the other peculiarity of ganglion cells in the horse's retina. The range was much broader than a range of 13–37 μ m for ganglion cells in the elephant [17], 5–32 μ m in

Fig. 4. Size spectra of ganglion cells in 8 areas of wholemount 1L. Meaning of the abbreviations as in Fig. 2. The scale bar represents 1 cm.

Table 1. Total ganglion cell numbers in four wholemounts. The areas and average densities (mean $\pm$ sd) of five regions in each wholemount were listed

Region		1L	1R	2R	3L
Visual streak	A	88.8	64.53	60.73	55.63
	D	2300 $\pm$ 929	2150 $\pm$ 525	1884 $\pm$ 571	1950 $\pm$ 459
Temporal	A	262.5	176.4	157.4	250.6
	D	213 $\pm$ 136	242 $\pm$ 142	266 $\pm$ 139	252 $\pm$ 113
Nasal	A	120.7	177.4	171.2	153.6
	D	216 $\pm$ 92	271 $\pm$ 118	339 $\pm$ 207	295 $\pm$ 135
Dorsal	A	1612	1664	1753	1811
	D	81 $\pm$ 46	96 $\pm$ 53	80 $\pm$ 40	107 $\pm$ 75
Ventral	A	949	823.7	963.2	898.4
	D	46 $\pm$ 47	57 $\pm$ 47	46 $\pm$ 33	65 $\pm$ 33
Total cell No.		460,450	436,198	398,868	469,115
Average cell No.		441,158 $\pm$ 31,447 (n=4)			

A: Area (mm²); D: Density (cells/mm²).

the rabbit [12], 5–30 μ m in the cat [8] and 8–20 μ m in the honey possum [4]. Since the properties of ganglion cells (receptive field organization, central projections, numbers of dendrite distribution) vary significantly with soma size, the range of soma size observed in this study suggests a substantial differentiation of properties among ganglion cells in the horse. More research such as staining of ganglion cell axons, dendrites, are necessary for classifying cells morphologically.

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