Retinal Ganglion Cell Topography and Spatial Resolving Power of Eye in the Native Chicken of Bangladesh

Topografía de Células Ganglionares de la Retina y Poder de Resolución Espacial del Ojo en el Pollo Nativo de Bangladesh

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SUMMARY: The distribution of retinal ganglion cells (RGCs) was observed in the retinal wholemount of native chicken (*Gallus gallus domestricus*) of Bangladesh by using light microscopy. We considered five different anatomic regions (central, nasal, temporal, dorsal, and ventral) of Nissl stained wholemount, and the RGCs were counted, plotted, and measured accordingly. The average area of the retina was 431.75 mm^2 while the total number of ganglion cells was 2124431 on average. Only the central area of the retina was the peak density (10400 cells/mm^2) area, signifying the acute visual area, whilst the maximum spatial resolving power was 11 cycles/degree. The overall concentration of RGCs gradually declined towards the periphery but the size of cells generally decreased towards centrally. The size of ganglion cell was not uniform (12 to 180 μm^2), specifically the central retina, just above the optic disc was packed with tiny-sized cells. The number, topographic distribution, and size of RGCs in native chicken signified their domesticated or terrestrial characters, uneven visual acuteness, and possibly only the central retina was the area for fine vision as the function of RGCs.

KEY WORDS: Area centralis; Native chicken; Peak density; Retinal ganglion cell; Spatial resolving power.

INTRODUCTION

Many traits of the eye of vertebrates are adapted with the visual atmosphere where they develop gradually (Land & Nilsson, 2002). For example, the density of retinal ganglion cells varies with the habitat and style of food gathering (Rahman *et al*., 2007; Hodos & Erichsen, 1990; Inzunza *et al*., 1991). In particular, the pattern of ganglion cell distribution in terrestrial or domesticated birds is typically differentiated by the presence of only one area of highly packed ganglion cells, known as area centralis (Ikushima *et al*., 1986; Rahman *et al*., 2010; Uddin *et al*., 2013). Conversely, some aerial or long-ranged birds have been stated to have area centralis and area dorsalis with the highest ganglion cell density (Hodos & Erichsen; Rahman *et al*., 2008). Besides, the predatory birds are frequently described with an elongated part of peak ganglion cell concentration which is mapping horizontally in retinal wholemount, known as a visual streak and nasal fovea (Inzunza *et al*.). Bird responses to their surroundings and cope with food and predator could be evaluated by using the topography of retinal ganglion cells (Coimbra *et al*.,

2006). The central part of retina that possesses the peak density of ganglion cells percept the stimuli from lateral visual fields, while the temporal retina with the peak density of ganglion cells increases the detection of the stimuli from frontal visual fields. Retinal ganglion cells are concentrated in central and dorsal areas that assign birds toward a head-up arrangement to elevate the confined perception and sharpness, and the feasibility to identify predators and also looking over the ground objects and foods concurrently (Hodos & Erichsen). By using the density of RGCs, the spatial resolving power of the retina or the maximum limit of visual acuteness could be determined (Collin & Pettigrew, 1989). The visual acuteness of birds is very important from an ecological point of view because it gives an idea about the proper distance from which birds can recognize objects (predators or food items) with enough resolution to make behavioral settlements (Kiltie, 2000). The native chickens of Bangladesh are mostly terrestrial, semi-scavenger in nature, and forage preferentially on the ground, but visual system especially, the topography of RGCs and the visual

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acuity has not been reported yet. Hence, we have explored the visual acuity of native chickens to clarify the habitat and style of food gathering by estimating retinal ganglion cell density, cell size, gradients of variation in cell density across the retina, and spatial resolving power.

MATERIAL AND METHOD

Handling of all experimental animals and events were executed as per the guidelines for the care and use of Laboratory Animals at Chittagong Veterinary and Animal Sciences University; the methodology was approved by the Ethics Committee of the University where the work was carried out and followed to the rations of the Declaration of Helsinki (as revised in Edinburgh, 2000).

Obtaining retina from the eyeball: Ten mature native chickens (2-2.5 years old) of both sexes, equal in number, were used in the present study. Eyeballs were enucleated from anesthesia-overdosed native chickens (Sodium pentobarbital, 30 mg/kg) and cleaned of their external connective tissues, muscles, and blood. Then the eye-globes were placed in phosphate-buffered saline (PBS) and the cornea of the eyes was cut and the internal lens was removed from the globes. By giving subsequent incision into the globe, the orientation of the retina such as appearance, the position of pecten, blood vessels, etc. were observed.

Preparation of the whole mount for Nissl staining: The posterior eyecup containing the retina was fixed in 4 % paraformaldehyde solution (0.1 M phosphate buffer and pH 7.2–7.4) for 12 hours at room temperature. Subsequently, the eyecup was immersed in PBS to prepare the wholemount with a regular protocol (Coimbra *et al*., 2006). The different anatomical positions of the retinal wholemount were clear-cut as the location of the pectin is in the ventral part of the retina and the tip of the pectin is in the temporal region. The pigment epithelium which was strongly attached just beneath the retina was removed with a solution of 3 % hydrogen peroxide in PBS for 24 hours at room temperature. Soon after, the vitreous body was removed softly from the inner surface of the retina to flatten and expand it on a gelatinized slide. The retina without a vitreous body was also facilitated in the quality staining of ganglion cells. Subsequently, the flattened retina was carefully washed in PBS before placing it on a gelatinized slide and then exposed to formaldehyde vapors at room temperature overnight.

To enhance the fixation to the slide, the retina was treated with formaldehyde vapors at about 60°C for 2 hours and it also boosts up the discrimination of neurons in the

staining. On the following day, the retina was defatted by a solution containing chloroform and ethanol, and the defatted retina was kept overnight at room temperature for staining purposes. Then, for staining, an aqueous solution of 0.1 % cresyl violet was used applying the Nissl method as described elsewhere (Coimbra *et al*., 2006) and coverslipped using Canada balsum.

Identification of RGCs: Since the topography and size of ganglion cells were not uniform across the retina, so different anatomical regions were considered for this study namely the central region, temporal region, nasal region, ventral region, and dorsal region of the retina (Fig. 1).
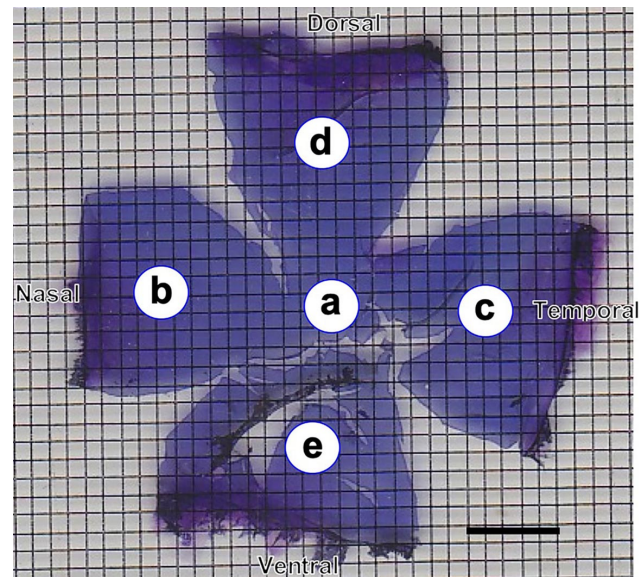

Fig. 1. A photograph of a right retinal specimen of native chicken stained with cresyl violet. The areas (a= central, b= nasal, c= temporal, d= dorsal, e= ventral) were chosen for measuring the sizes of the ganglion cells. Scale bar = 5 mm.

Based on some unique characteristics the RGCs were recognized. In Nissl staining, the large soma size of ganglion cells along accumulated Nissl substances was observed under a microscope. Besides, large and prominent nucleolus (Rahman *et al*., 2008, 2010) and clear nucleoplasm were also observed in ganglion cells (Fig. 2). In the ganglion cell layer, the displaced amacrine cells were also observed and their microscopic features were fairly different than that of ganglion cells. The amacrine cells were diagnosed by their round or oval-shaped soma, comparatively smaller in size, and lack of clumped Nissl substance in the cytoplasm (Hart, 2002) (Fig. 2). Glial cells were also observed in the ganglion cell layer and these cells were differentiated by their dark staining, small size, and elongated spindle or cigar-like shape (Lisney *et al*., 2012) (Fig. 2).
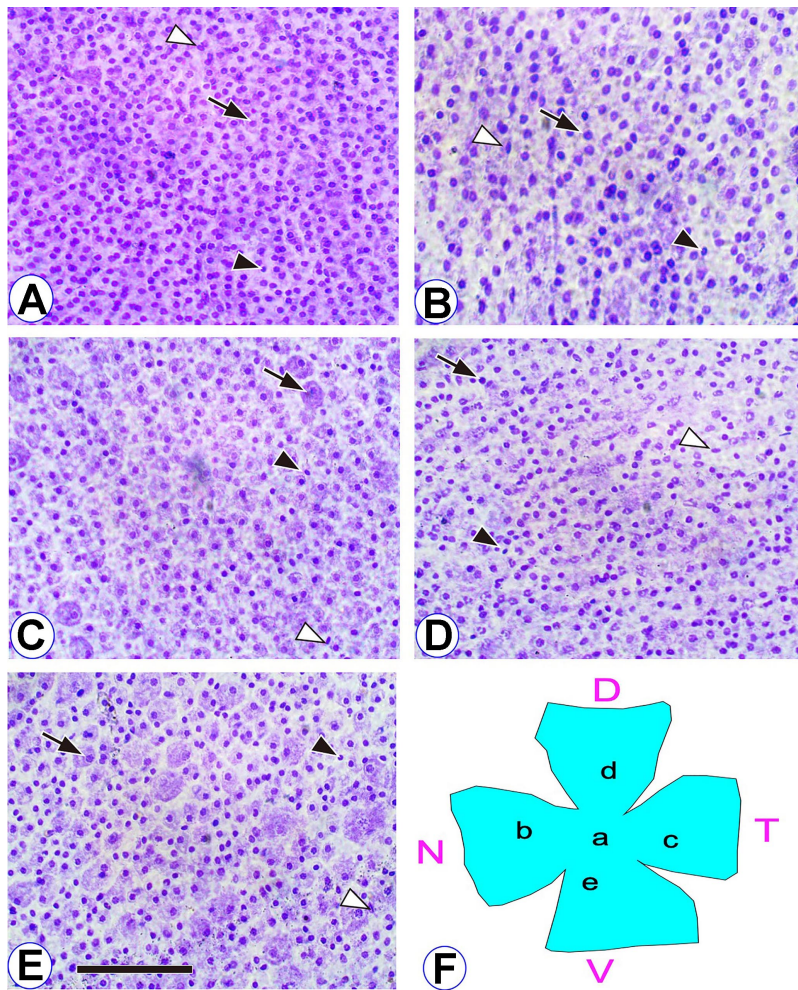
Fig. 2. Photomicrograph of the retinal ganglion cell layer (a, b, c, d, e) of the native chicken illustrating the cytological features used to distinguish ganglion cells (arrow) from amacrine (black arrowhead) and glial cells (white arrowhead) during counting and quantification. The key (F) indicates the areas: mid-central (a), mid-nasal (b), mid-temporal (c), mid-dorsal (d) and mid-ventral (e). D=Dorsal, N=Nasal, V=Ventral, T=Temporal; Bar in E represents 50 µm, which applies to all panels.

Total number and density of ganglion cell: Before placing under a microscope, the retinal specimen was pasted over with a transparent net of 1 mm x 1 mm unit size to facilitate the counting of cells. In addition, an eyepiece graticule was set in the eyepiece of the microscope which marked 0.1 mm x 0.1 mm areas on the retina with 100x objective. Ganglion cells were counted with 100x objective using unbiased counting unit area which was 0.01 mm². The cells counted in each counting area were converted into cells/mm².

The coefficients of error were calculated using the following formula Schaeffer *et al*. (1996).

$$\text{Coefficient of error (CE)} = \frac{1}{\sqrt{Q}}$$

Where Q is the number of cells counted within the sample fractions.

The CE was less than 0.056 in every specimen which indicated that the estimated total cell numbers have a high degree of accuracy (Coimbra *et al*., 2009; Ullmann *et al*., 2012).

Quantification of ganglion cell size: Digital photomicrographs of the retinal ganglion cells layer were taken at regular intervals using a 100× oil immersion objective on the AmScope compound microscope with a USB Camera (California, USA). The size of the ganglion cells was measured by using Canvas software (ACD Systems International, Saanichton, BC, Canada). During the measurement of cell size, ganglion cells were pulled out by their large soma, the gathering of Nissl elements in the body, well-defined nucleoplasm with a prominent nucleolus (Fig. 2). The data was recorded to analyze the mean and standard deviation (SD) by using STATA/IC-12.0 software.

Evaluation of spatial resolving power: The peak spatial resolving power (SRP) of native chicken was calculated by using the value of the peak density of ganglion cells along with the retinal magnification factor (RMF). Considering

the retina as a hexagonal mosaic (Hart), SRP and RMF were estimated (Ullmann *et al*.) as:

$$SRP = 0.5 \times RMF \times (2D/\sqrt{3})1/2$$
$$RMF = \frac{2\pi F}{360}$$

Where D is the highest density of ganglion cells in mm^2 and F is the focal length of the eye. The focal length (F) of the eye was indirectly calculated by multiplying the axial length by 0.60 (Ullmann *et al*.).

RESULTS

Orientation of the retina: The native chicken retina has a dark, velvety appearance and no definite indicators. Moreover, has no blood vessels coursing across the retina. The corrugated pecten was placed in the inferotemporal field and trespassing into the vitreous humor which aids in orientation when preparing the whole mounts.

Morphometric features of retinal ganglion cells: The size and the shape of retinal ganglion cells exhibited extreme variation in different anatomical areas where they were distributed (Fig. 3). For example, in the high density (>8000 cells/mm^2) area, the neurons were very small, rounded, and uniform in size. The ganglion cells are accommodated with a deeply stained nucleus encircled by an excellent margin. The cytoplasm of the cells was lightly stained and Nissl granules were distributed throughout the whole nucleus (Fig. 3a). Besides, in the intermediate density (4000–8000 cells/mm2) areas, cells were oval and comparatively larger than the highly dense area. Besides, the cells were somewhat paler and the nucleus was eccentric with a prominent nucleolus. The Nissl positive particles were dispensed heterogeneously in the cytoplasm; the cellular periphery was strongly stained, whereas the cytoplasm at the side of the nucleus was pale (Fig. 3b). In the low density (1–4000 cells/mm^2) areas, cells were oval, and the largest containing eccentric nuclei and prominent nucleolus. Fascinatingly, the Nissl granules are distributed heterogeneously within the cytoplasm of the peripheral ganglion cells (Fig. 3c).
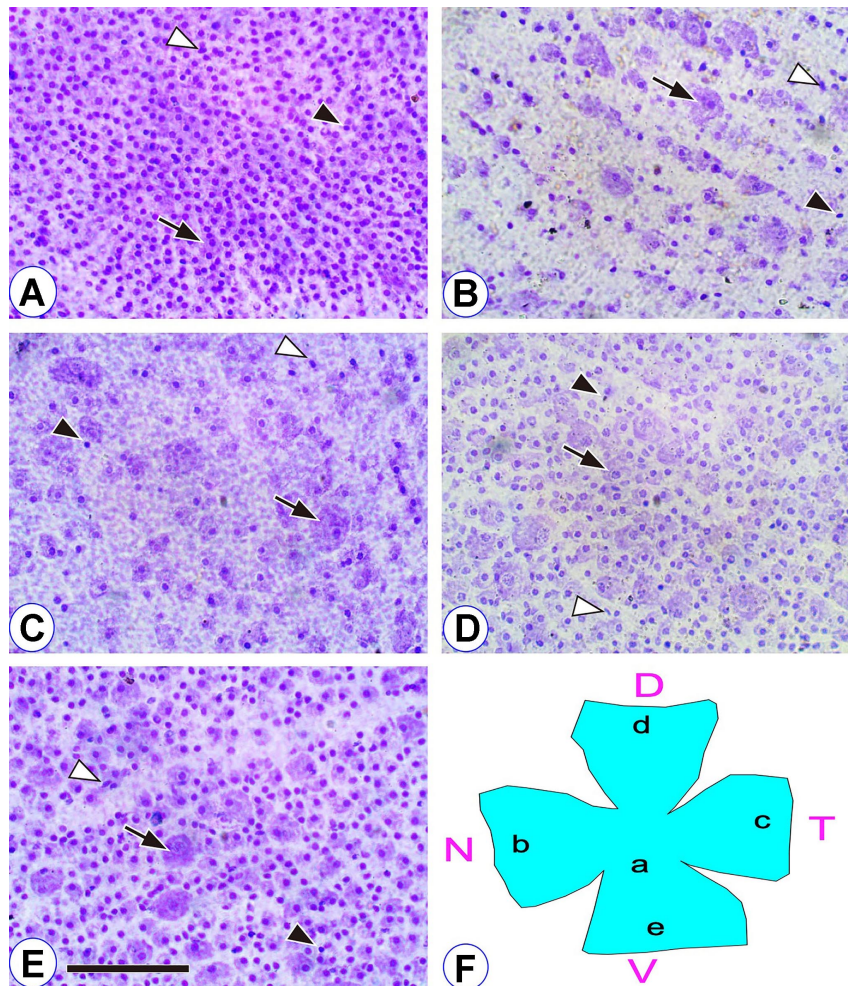

Fig. 3. Photomicrograph of ganglion cells in the central periphery (a), nasal periphery (b), temporal periphery (c), dorsal periphery (d), and ventral periphery (e) areas of the retina of native chicken for counting and identification of ganglion cells (arrow) from amacrine (black arrowhead) and glial cells (white arrowhead). The key (f) indicates the areas: D=Dorsal, N=Nasal, V=Ventral, T=Temporal; Scale bar in e represents 50 μm, which applies to all panels.

Ganglion cell number, density, and mapping: The whole retinal area and the sum of ganglion cells in that area were shown in Table I. The estimated ganglion cells in four retinal specimens were ranged from 1905875 to 2264725 (mean: 2124431 ± 174106 cells). The distribution of ganglion cells of the retinal specimen was in Figure 4. Based on the location of ganglion cells, a retina was divided into five regions: central, nasal, temporal, dorsal, and ventral regions. A well-distinguished central area with a relatively high density of ganglion cells was the most salient feature in the designed map. The peak concentration of ganglion cells located in the central retina (10400 cells/mm^2) while from central to the retinal periphery, the concentration of ganglion cells declined gradually. Besides, in the dorsal and the ventral region, cell densities were slightly greater than temporal and nasal regions. These patterns of cell densities in all retinal specimens were almost similar.

Ganglion cell size: A total of four areas (central, nasal, temporal, dorsal, and ventral) of each retinal specimen were selected to measure the size of the ganglion cell. The findings of these computations were summarized in Table II and Figure 5. In size spectra, cell percentages were considered, and mean value (MV), standard deviation (SD) were presented for each chosen area. Size of ganglion cells varied from 12 to 180 µm^2. Few ganglion cells perceived in the central area were notably smaller (with a soma size of 12 µm^2) than those of other regions, although the cell sizes in the central area were differing from 12 to 57 µm^2. Besides,
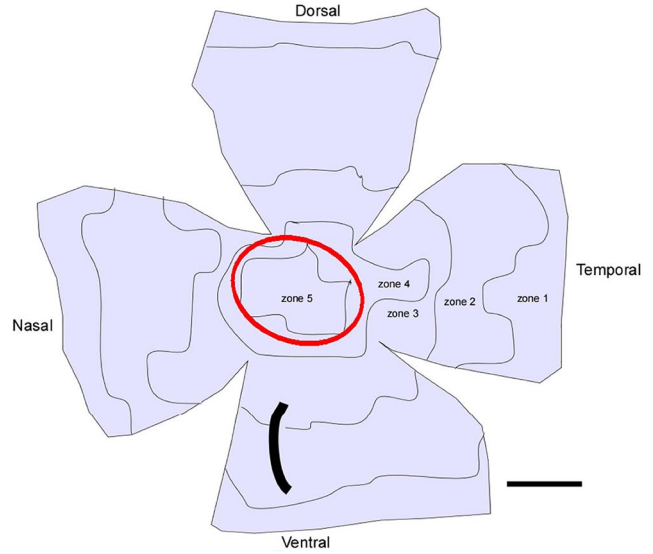

Fig. 4. The map showing the iso-density of ganglion cells of the retina of native chicken. Areas of retina categories as zone 1, zone 2, zone 3, zone 4, and zone 5, where zone 5 is the peak density area. Scale bar denotes 5 mm.

in temporal and in nasal areas, most of the cell sizes fluctuated within 25 to 35 µm^2. In dorsal and in ventral areas, most of the cell sizes varied from 15 to 25 µm^2. The smallest cell size (12 µm^2) was noticed in the central area, while the largest cell size (180 µm^2) was in temporal and in nasal regions (Fig. 5).

Table I. Overview of ganglion cells distribution in four retinal cases.

SL No	Eye	Total specimen area (mm^2)	Average number of cells/mm^2	Total number of cells
1	Right-1	495	4575	2264625
2	Right-2	462	4902	2264724
3	Left-1	375	5500	2062500
4	Left-2	395	5500	1905875
Average ± SD		431.75 ± 56	4950 ± 392	2124431 ± 174106

Table II. Outline of the size of ganglion cells in five retinal areas of sample right-1.

Ganglion cell size (µm^2)	Number of ganglion cells				
	Central	Nasal	Temporal	Dorsal	Ventral
1-20	70	15	10	5	20
21-40	22	61	59	66	44
41-60	8	6	8	10	12
61-80	0	5	11	7	6
81-100	0	4	3	5	13
101-120	0	4	3	4	2
121-140	0	2	1	2	2
141-160	0	2	2	1	1
161-180	0	1	3	0	0

Spatial Resolving Power: We obtained the axial length of the native chicken eye varied from 16.8-21 mm (19.2 ± 1.3 mm) and the mean posterior nodal distance was 11.5 ± 0.8 mm. This resulted in a mean retinal magnification factor of 0.20/degree. We determined the upper limits of spatial resolving power in the chicken eye using peak density (10400 cells/mm^2) of retinal ganglion cells assuming that the cells at the peak region are arranged in a hexagonal lattice. We estimated a maximum resolving power of 11 cycles/degree by using the hexagonal lattice algorithms.
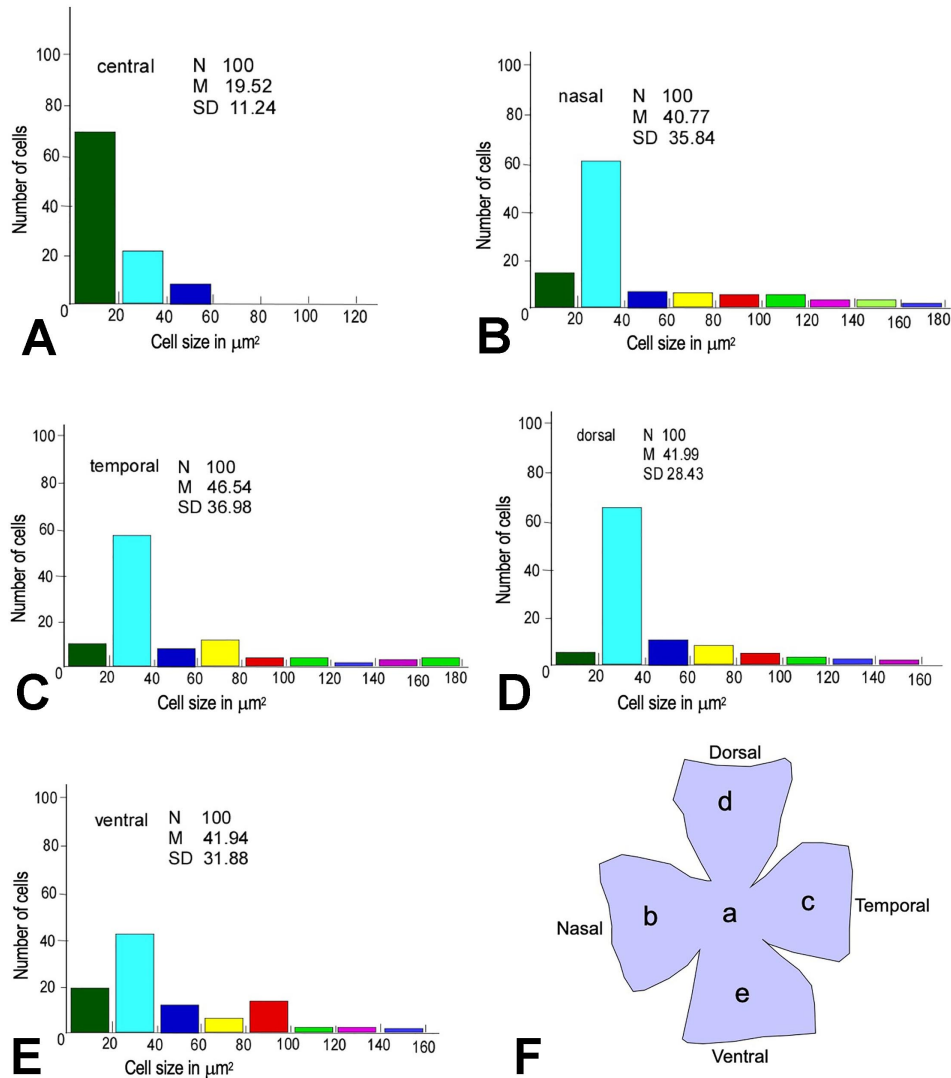
Fig. 5. Size ranges of ganglion cells in five areas of the right-1 retina of native chicken, where M=Mean; N=Number; SD= Standard deviation. The key (F) indicates the areas: central (a), nasal (b), temporal (c), dorsal (d), and ventral (e).a

DISCUSSION

The density map of RGCs reveals the variation in visual acuity across the retina as a function of RGCs. While the spatial resolving power of the eye was controlled by the concentration of retinal photoreceptor cells at the input stage, but the concentration of ganglion cells, each with an axon projecting immediately to the higher visual center, which confines spatial resolving power and maintains the key visual axis (Collin, 1999). The density map in our present study designates the central area as the peak density area of ganglion cells, indicating the area of maximum spatial resolving power and dictates the fine visual acuity.

Some terrestrial birds i.e. native duck (Uddin *et al.*), ostrich (Rahman *et al.*, 2010), quail (Ikushima *et al.*) have

only one retinal peak density area known as area centralis which is similar to our present findings in native chicken. Many diurnal aerial birds possess two retinal peak density areas, referred to as area centralis and area dorsalis (Hodos & Erichsen) or area centralis and area dorso-temporalis (Rahman *et al*., 2008) or either a temporal area or a temporal fovea (Coimbra *et al*., 2009). Whereas, predatory birds e.g. eagle, hawk, and chimango have been investigated to have nasal fovea and a horizontal streak with high ganglion cell densities (Inzunza *et al*.). These findings recommend that the specialties in different regions of the avian retina are not only associated with zoological groupings but also correlate with the habitat and methods of food gathering in the environment.

The highest density of retinal ganglion cells in native chicken has been reported to be in 10400 cells/mm^2 which was lower than semiaquatic birds such as mallard duck [15820 cells/mm^2, Rahman *et al*. (2007); native duck (11200/mm^2), Uddin *et al*.]. The pattern is also similar in the cases when we compared the peak density of native chicken with some other predatory birds (such as eagle 62000 cells/mm^2 and sparrow hawk 65000 cells/mm^2; Inzunza *et al*.). These observations indicate that the visual acuity of birds is also related to the style of food gathering in their environment. Our findings coincide with the earlier reports, ground scavenging groups that feed on stationary prey items, such as duck, and ostrich tends to have a lower concentration of ganglion cells than diurnal and aerial predatory birds that feed on moving prey, such as raptors and kingfishers.

A huge number of RGCs translate to a huge number of central nervous system projections per unit area. We estimated that there are on average 2124431 (n = 4) ganglion cells in the native chicken retina. The fact that the total number of ganglion cells, we have obtained from the chicken retina was more than that of quail (1986000 cells; Ikushima *et al*.) and ostrich (1435052 cells; Rahman *et al*., 2010) suggesting that the chicken retina conducts greater numbers of electrical signals to the higher visual centers via ganglion cell axons. The chicken retina provides the best vision as a function of RGCs among these birds.

Our study revealed that there is a striking fashion for RGCs size, the larger ganglion cells were in the less populated retinal periphery while the smaller cells were in the densely populated central retina. Similar findings, regarding the relation of ganglion cell size and their density, has been investigated for the retina of the native duck (Uddin *et al*.), pigeon (Hayes, 1982), crow (Rahman *et al*., 2006), and owl (Wathey & Pettigrew, 1989). The diversification in the size of ganglion cells throughout the retina seems to be of noble importance for retinal function.

The central part of the retina is recognized as the highest density area for certain cells such as in the native chicken, small and homogenous ganglion cells prevail in the central area. Considering the total number of RGCs and their distribution pattern, this study recommended that the central area of the native chicken retina transmits more visual cues towards the central nervous system as a function of RGCs and similarly holds a fine quality area for vision.

The central part of the retina is used to sight the distant items during monocular fixation, and high spatial resolution has a great significance for both predator and prey revelation. The ground-foraging birds tend to have lower spatial resolving powers than predatory birds (Boire *et al*., 2001). We obtained peak anatomical spatial resolving power to be approximately 11.0 cycles/degree in the native chicken. The ground-foraging species that feed on stationary prey items, such as ostrich (19.32 cycles/degree; Boire *et al*.) tend to have lower spatial resolving powers than diurnal predatory birds that feed on moving prey, like kingfishers (40 cycles/degree; Moroney & Pettigrew, 1987), raptors (70-140 cycles/degree; Reymond (1985, 1987). There is evidence that spatial resolving power is related to foraging in birds. For example, our results are consistent with previous reports that ground-foraging birds tend to have lower spatial resolving powers than diurnal predatory birds.

UDDIN, M.; AL FARUQ, A.; SOHEL, M. S. H. & RAHMAN, M. L. Topografía de células ganglionares de la retina y poder de resolución espacial del ojo en el pollo nativo de Bangladesh. *Int. J. Morphol., 40 (2)*:289-296, 2022.

RESUMEN: En este studio se observó la distribución de las células ganglionares en la retina (CGR) de pollo nativo (*Gallus gallus domesticus*) de Bangladesh mediante el uso de microscopía óptica. Consideramos cinco regiones anatómicas diferentes (central, nasal, temporal, dorsal y ventral). Las muestras de CGR se tiñeron con Nissl, posteriormente, se midieron y contó el número de células totales. El área promedio de la retina fue de 431,75 mm^2, mientras que el promedio del número total de células ganglionares fue de 2124431. El área central de la retina fue el área de densidad máxima (10400 células / mm^2), señalando el área visual aguda, mientras que el poder de resolución espacial máximo fue de 11 ciclos / grado. La concentración general de CGR disminuyó gradualmente hacia la periferia, sin embargo, el tamaño de las células disminuyó hacia el centro. El tamaño de las CGR no fue uniforme (12 a 180 mm^2), específicamente en la retina central, por encima del disco óptico, aumentaron significa-tivamente las células pequeñas. El número, la distribución topográfica y el tamaño de las CGR en pollos nativos determinaron las características domésticas o terrestres, agudeza visual desigual y, posiblemente, la función de las CGR, en la retina central era el área de visión fina.

PALABRAS CLAVE: Área central; Pollo nativo; Densidad maxima; Células ganglionares de la retina; Poder de resolución especial.

REFERENCES

Boire, D.; Dufour, J. S.; Théoret, H.; & Ptito, M. Quantitative analysis of the retinal ganglion cell layer in the ostrich, *Struthio camelus. Brain Behav. Evol., 58(6)*:343-55, 2001.

Coimbra, J. P.; Marceliano, M. L. V.; da Silveira Andrade-da, B. L. & Yamada, E. S. The retina of tyrant flycatchers: topographic organization of neuronal density and size in the ganglion cell layer of the great kiskadee *Pitangus sulphuratus* and the rusty margined flycatcher *Myiozetetes cayanensis* (Aves: Tyrannidae). *Brain Behav. Evol., 68(1)*:15-25, 2006.

Coimbra, J. P.; Trevia, N.; Marceliano, M. L. V.; da Silveira Andrade-da, B. L.; Picanço-Diniz, C. W. & Yamada, E. S. Number and distribution of neurons in the retinal ganglion cell layer in relation to foraging behaviors of tyrant flycatchers. *J. Com. Neurol., 514(1)*:66-73, 2009.

Collin, S. P. & Pettigrew, J. D. Quantitative comparison of the limits on visual spatial resolution set by the ganglion cell layer in twelve species of reef teleosts. *Brain Behav. Evol., 34(3)*:184-92, 1989.

Collin, S. P. *Behavioural Ecology and Retinal Cell Topography.* In Adaptive Mechanisms in the Ecology of Vision. Dordrecht, Springer, 1999. pp.509-35.

Hart, N. S. Vision in the peafowl (Aves: *Pavo cristatus*). *J. Exp. Biol., 205(24)*:3925-35, 2002.

Hayes, B. P. *Chapter 7 The structural organization of the pigeon retina.* In: Progress Retinal Research, Vol. 1, 1982. pp.197-226.

Hodos, W. & Erichsen, J. T. Lower-field myopia in birds: an adaptation that keeps the ground in focus. *Vision Res., 30(5)*:653-7, 1990.

Ikushima, M.; Watanabe, M. & Ito, H. Distribution and morphology of retinal ganglion cells in the Japanese quail. *Brain Res., 376 (2)*:320-34, 1986.

Inzunza, O.; Bravo, H.; Smith, R. L. & Angel, M. Topography and morphology of retinal ganglion cells in Falconiforms: a study on predatory and carrion-eating birds. *Anat. Rec., 229(2)*:271-7, 1991.

Kiltie, R. A. Scaling of visual acuity with body size in mammals and birds. *Funct. Ecol., 14(2)*:226-34, 2000.

Land, M. F. & Nilsson, D. E. *Animal Eyes.* Oxford, Oxford University Press, 2002.

Lisney, T. J.; Iwaniuk, A. N.; Bandet, M. V. & Wylie, D. R. Eye shape and retinal topography in owls (Aves: Strigiformes). *Brain Behav. Evol., 79(4)*:218-36, 2012.

Moroney, M. K. & Pettigrew, J. D. Some observations on the visual optics of kingfishers (Aves, Coraciformes, Alcedinidae). *J. Comp. Physiol., 160(2)*:137-49, 1987.

Rahman, M. L.; Aoyama, M. & Sugita, S. Ganglion cell density and oil droplet distribution in the retina of brown-eared bulbul (*Hysipetes amaurotis*). *Anat. Sci. Int., 83(4)*:239-46, 2008.

Rahman, M. L.; Aoyama, M. & Sugita, S. Number, distribution and size of retinal ganglion cells in the jungle crow (*Corvus macrorhynchos*). *Anat. Sci. Int., 81(4)*:253-9, 2006.

Rahman, M. L.; Aoyama, M. & Sugita, S. Topography of ganglion cells in the retina of the duck (*Anas platyrhynchos* var. *domesticus*). *Anim. Sci. J., 78(3)*:286-92, 2007.

Rahman, M. L.; Kuroda, K.; Aoyama, M. & Sugita, S. Regional specialization of the ganglion cell density in the retina of the ostrich (*Struthio camelus*). *Anim. Sci. J., 81(1)*:108-15, 2010.

Reymond, L. Spatial visual acuity of the eagle (Aquila audax): a behavioural, optical and anatomical investigation. *Vision Res., 25(10)*:1477-91, 1985.

Reymond, L. Spatial visual acuity of the falcon, *Falco berigora*: a behavioural, optical, and anatomical investigation. *Vision Res., 27(10)*:1859-74, 1987.

Schaeffer, R. L.; Mendenhall, W. & Ott, L. *Elementary Survey Sampling.* 5th ed. Boston, PWS Kent, 1996.

Uddin, M.; Quasem, M. A. & Rhaman, M. L. Regional specialisation of the ganglion cell density in the retina of the native duck (*Anas platyrhynchos*) of Bangladesh. *Anat. Histol. Embryol., 42(6)*:471-8, 2013.

Ullmann, J. F.; Moore, B. A.; Temple, S. E.; Fernández-Juricic, E. & Collin, S. P. The retinal wholemount technique: a window to understanding the brain and behaviour. *Brain Behav. Evol., 79(1)*:26-44, 2012.

Wathey, J. C. & Pettigrew, J. D. Quantitative analysis of the retinal ganglion cell layer and optic nerve of the barn owl, Tyto alba. *Brain Behav. Evol., 33(5)*:279-92, 1989.

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