Retinal ganglion cells in *Strigidae* raptors: distribution and morphometry

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Summary

The Authors studied the morphometry and the topographical distribution of Retinal Ganglion Cells (RGCs) in four nocturnal raptors of the order of *Strigiformes*, family of *Strigidae*: little owl, tawny owl, scops owl, eared owl. In order to recognize specialized retinal vision areas (fovea and visual streak), the number of RGCs/mm^2 and the soma size in the four retinal fields (dorsal, ventral, temporal and nasal) by the histological analysis of retinal radial sections were recorded. A temporal fovea was identified in little owl, tawny owl and eared owl while in scops owl this visual area was localized near the *fundus oculi*. A radial visual streak ventrally directed was pointed out in the retinas of the four raptors with different shape according to its width. The Authors linked the obtained data with the predatory behavior of nocturnal raptors in their habitat.

Key words: retinal ganglion cells, morphometry, topographical distribution, nocturnal raptors, *Strigidae*.

Introduction

Strigidae are a family of raptors belonging to the order of *Strigiformes*, which includes over 200 living species and 25 genera. Also defined as “true owls” or “typical owls”, they are found world-wide, covering nearly all types of terrestrial

habitats. As all the *Strigiformes*, they have eyes placed frontally and surrounded by feathers arranged in concentric circles, strong hooked beak, feet armed with claws, soft and abundant plumage that extends to cover also the tarsal bones and sometimes the fingers. As the other raptors, they have a very developed sense of sight with large elongated eyes and slightly thickened corneas. Large pupils allow to increase the amount of light that stimulates the photoreceptors. The number of rods in their retina is high allowing them an increased sense of vision in twilight even if they cannot see in darkness. The eyes are fixed in their orbits by a bony sclerotic ring therefore they need to turn head to see the surrounding environment. An excellent sense of hearing allows them an increased hunting skills towards prey that cannot be seen.

Few studies investigate the differences in eye shape and retinal organization of *Strigiformes* in relation to their type of predation. Even if they are nocturnal birds, only about 30% of all species are strictly nocturnal and the other species are from crepuscular or catemerals to diurnal (Gutierrez-Ibanez, 2012). Moreover, the different activity pattern is linked not only to photoreceptor density and rod/cone ratio, but also to shape and deep of the fovea and number and distribution of Retinal Ganglion Cells (RGCs) (Oehme, 1961). *Strigiformes* have only a temporal fovea (Jones et al., 2007) which is associated with the frontal position of the eyes and thus improving the binocular vision. At the retinal level, the "visual streak" assumes a radial and symmetrical shape (Lisney et al., 2012; Coli et al., 2018), confirming the observation that many *Strigiformes* follow prey using the lower part of their visual field, and therefore they do not have the lower-field myopia demonstrated in many other birds.

In the present work we studied the Retinal Ganglion Cells (RGCs) from eyeballs of four *Strigidae* genera, focusing on the relationship between their topographical retinal distribution and soma size, then comparing the morphometrical data among the four raptors.

Materials and Methods

The retinae were taken from eyeballs of four *Strigidae* genera: little owl (*Athene noctua*), tawny owl (*Strix aluco*), scops owl (*Otus scops*) and eared owl (*Asio*

otus). All specimens came from a center of recovery of birds (Lipu, Livorno, Italy) after systemic diseases or impact trauma. The raptors were euthanized following a certified avian protocol (Embutramide, 0.5-3mg/Kg intrapulmonary) and the retinal samples were collected by trans conjunctival enucleation. All data were reported from the study of left eyeball. Each sample was cut cranially to ora serrata and fixed in 4% paraformaldehyde in 0.1 M phosphate buffered saline (PBS, pH 7.4). After several washing in PBS, samples were cryoprotected in a solution of 20% sucrose in PBS, orientated taking the pecten position as reference point, frozen at -80°C and stored at -20°C. Retinal radial serial sections (10µm thick) were obtained by a cryomicrotome (Kryostat 1720, Leitz Wetzlar) to localize the RGCs, excluding the displaced amacrine cells of the inner plexiform layer. The slices were mounted on gelatin-coated slides and stained with thionine blue at 0.1% (Nissl method) to highlight Nissl body. For the topographical study, RGCs from dorsal (D), ventral (V), temporal (T) and nasal (N) fields in each section were studied (Fig.1a).Ten slices at 100µm distance were taken at the level of ora serrata (level 1), at the level the optic disc (level 3) and at the level of intermediate position (level 2) (Fig.1b). RGCs were identified as showing the following cytological features (Duke-Elder, 1958; Cunnigham, 2006):

- Polygonal cell body
- Nissl body in the cytoplasm
- Euchromatic oval nucleus and round nucleolus.

These parameters enabled to distinguish RGCs from glial cells which have small, slightly elongated cell body, reduced or not detectable cytoplasm and negative to Nissl staining (Fig.2). Nikon ECLIPSE Ni microscope, connected to Nikon-Digital.Sight DS-U1 digital image acquisition system, was used to analyze each slice from the three levels. The number of RGCs/mm^2 (cell density) and the soma size of RGCs were obtained analyzing a reference area (0,06mm^2) into the thickness of the RGCs layer by a NIS-Elements Basic Research software, at a magnification of 20x. The cell density in each reference area was reported and the RGCs were classified on the basis of four different

range of median soma area (<50mμ^2, 50-100mμ^2, 100-150mμ^2 and >150mμ^2). The results are given without corrections for shrinkage.

Results

Figure 3 shows the RGCs density related to the retinal fields (D, T, V, N) and to the three level section. The lowest values of density in all the retinal fields are found in the retina of eared owl. In all the raptors the highest RGCs number/mm^2 is detectable in the ventral retinal field, even if it is not homogeneously distributed among section levels: indeed, the little owl shows the highest cell density at the level of optic disc (level 3) while in the other raptors the highest density is highlighted at the level of the ora serrata (level 1).

Figure 4 shows the RGCs density in temporo-nasal and dorso-ventral directions to identify specialized retinal vision areas.

The study of the RGCs density in the temporo-nasal direction in the retina of the little owl points out lower values toward central temporal field (T3). In the retina of tawny owl this decrease reaches also external/intermediate nasal fields (N1/N2). A Gaussian trend, with higher values in the central retinal fields (T3/N3) and lower values to the peripheral fields (T1/N1) is evidenced in the retina of the scops owl. The RGCs density in the eared owl retina is rather steady with a reduction only in the intermediate nasal field (N2).

Regarding the dorso-ventral direction, in all the *Strigidae* the ventral retinal fields show an higher density than the dorsal fields. In the little owl retina the higher values are found in the central peripheral field (V3) while in the tawny owl and the scops owl this parameter is highlighted in the ventral peripheral fields (V1). The RGCs density in the eared owl retina is almost steady in dorso-ventral direction too.

The percentage of distribution of the RGCs according to four different ranges of median soma area (<50mμ^2, 50-100mμ^2, 100-150mμ^2 and >150mμ^2) in each retinal field and section level are showed in Table 1. The RGCs with <50mμ^2 of median soma area are only recorded in the retinal fields at each section level in the little owl and in the peripheral nasal field (N1) and central ventral field (V3) in the scops owl retina. In all the *Strigidae* analyzed the most represented population of RGCs in all the section levels and retinal fields is in the range of

50-100mμ^2 of median soma area (ranging from 20% to 100%) with the exception of those fields where the RGCs with <50mμ^2 of median soma are recorded. The percentage of the RGCs in the range of 100-150mμ^2 of median soma area is high in the tawny owl and in the eared owl retinas (ranging from 9% to 35%). Conversely in the little owl retina this range does not exceed 9% in all the retinal fields and in the scops owl retina reaches 24% in D1. The largest RGCs (>150mμ^2) lack in the little owl retina and only the eared owl retina shows an high percentage of these cells, particularly in the ventral field, near the optic disc (V3) where this value reached 40%. The largest RGCs are the least represented in the retinas of tawny owl and scops owl where the percentage is lower than 11%.

Discussion

Many authors reported on the number and distribution of RGCs in *Strigiformes* raptors which differ with activity patterns and habitat (Oehme et al., 1961; Bravo & Pettigrew,1981; Güntürkün, 2000; Lisney et al., 2012): all these studies are carried out using retinal wholemounts. Since in the highest density areas of avian retinas (Bravo & Pettigrew, 1981) the RGCs become smaller and distributed into multiple sublayers, as also reported previously (Coli et al., 2018), the Authors refer to the study of RGCs density by radial retinal sections to identify also the deeper RGCs and to distinguish them from the displaced amacrine cells.

The evaluation of RGCs density in temporo-nasal and dorso-ventral directions points out retinal areas with high RGCs density that might be justified by the presence of specialized areas like fovea or visual streak. In little owl, tawny owl and eared owl retinas the localization of a temporal fovea, as reported by Lisney et al. (2012) in nine species of owls, might be linked with the high density found in the temporal field. While in eared owl retina the RGCs distribution is quite steady, in the other two raptors a decrease in temporal *fundus oculi* (T3) is evident. In scops owl retina it is not possible to localize a temporal fovea, as in the other raptors, because an high RGCs density is identified in temporal and

nasal *fundus oculi* (T3 and N3). An high RGCs density in nasal fields related to the presence of a streak in the nasal direction is pointed out in little owl retina.

A particular outline of a radial visual streak better detectable ventrally, as reported by Lisney et al. (2012) in other *Strigidae* (barred owl, northern saw-whet owl) is also identified by the Authors in the ventral fields of the four raptors, even if with different distribution according to the section level. With the exception of little owl, where the most RGCs are in ventral *fundus oculi* (V3), in the other three raptors the outline of the visual streak reaches the peripheral ventral fields (V1). As reported by Lisney (2012) an increase of RGCs number in ventral fields might be responsible for a greater image projection on the visual cortex. The particular outline of the radial visual streak might also be correlated with the predatory behavior of the four *Strigidae* analyzed. As summarized in Table 2, these typical owls are generally nocturnal and spend much of the day roosting. Their preys are small and generally moves on the ground, so it is necessary that the best visual acuity be linked in the ventral visual fields. Moreover, in all four analyzed *Strigidae* a *tapetum lucidum* in the dorsal fields at the opening of the eyeballs is visible, so confirming its role in the amplifying the light in the dark, as reported in a previous study (Coli et al., 2018).

The study of percentage distribution of RGCs according to the ranges of medium soma area points out that the most represented RGCs population (50-100mμ^2 of median soma area) is localized in the areas of greater visual acuity where the RGCs density increases, thus confirming the bibliographic data (Ikushima et al., 1986). Considering the close correlation between the cell soma size and their morphological type (Ikushima et al., 1986, in quail retina; Binggeli & Paule, 1969 in pigeon retina) the cells most represented in the retina of the *Strigidae* studied, and related to the medium-large range, might be definable as the alpha cells in the classification of cat retinal ganglion cells (Boycott & Wassle, 1974). Our data suggest to extend the study of RGCs to other families of the order of *Strigiformes* with the same procedure investigation.

Acknowledgments

We are grateful to Renato Ceccherelli, DVM, Health Director of the Center of Recovery Seabirds and Waterfowl (Lipu, Livorno, Italy) for his assistance and collaboration in the sample collection.

References

Binngeli, R.L. & Paule, W.J. The pigeon retina: quantitative aspects of optic nerve and ganglion cell layer. J. Comp. Neurol., 137:1-18, 1969.

Boycott, B.B. & Wassle, H. The morphological types of ganglion cells of the domestic cat's retina. J. Physiol. (London) , 240: 397-419, 1974.

Bravo, H. & Pettigrew, J.D. The distribution of neurons projecting from the retina and visual cortex to the thalamus and tectum opticum of the barn owl, Tyto alba, and the burrowing owl, Speotyto cunicularia. J Comp Neurol., 199(3): 419-444, 1981.

Coli, A.; Stornelli, M.R.; Lenzi, C. & Giannessi, E. Number and topographical distribution of retinal ganglion cells in diurnal and nocturnal raptors. Int. J. Morphol. 36 (in press), 2018.

Cunningham, J.G. Manuale di Fisiologia Veterinaria. 3st ed. Rome, Antonio Delfino Editore, 2006.

Duke-Elder, S. The Emergence of Vision in the Animal World. Ann. R. Coll. Surg. Engl., 23(1):1-24,1958.

Gutiérrez-Ibáñez, C.; Iwaniuk, A.N.; Lisney, T.J. & Wylie, D.R. Comparative Study of Visual Pathways in Owls (Aves: Strigiformes). Brain Behav. Evol., 81(1): 27-39, 2013.

Ikushima, M.; Watanabe, M. & Ito, H. Distribution and morphology of retinal ganglion cells in the Japanese quail. Brain Res, 376: 320-334, 1986.

Jones, M.P.; Kenneth, E. P. Jr & Ward, D. Avian Vision: A Review of Form and Function with Special Consideration to Birds of Prey. Journal of Exotic Pet Medicine, 16(2): 69-87, 2007.

Lisney, T.J.; Iwaniuk, A.N.; Bandet, M.V. & Wylie, D.W. Eye shape and retinal topography in owls (Aves: Strigiformes). Brain. Behav. Evol., 79(4): 218-236, 2012.

Oehme, H. Vergleichend-histologische Untersuchungen an der Retina von Eulen. Zool. Jb. Anat. Ontol., 79: 439-478,1961.

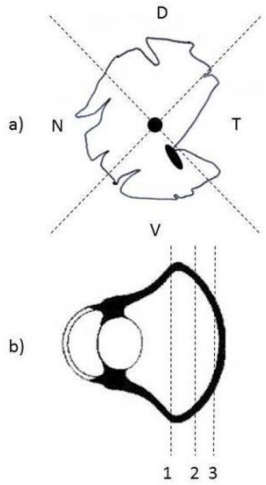

Fig 1

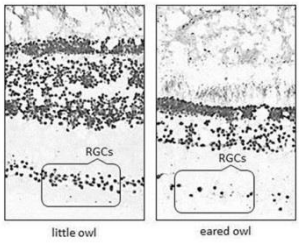

Fig. 2

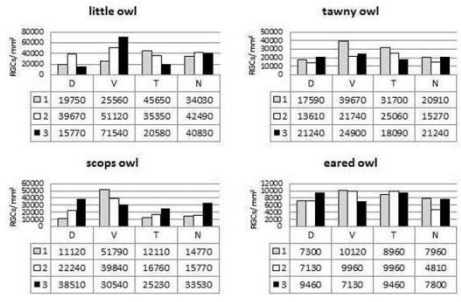

Fig. 3

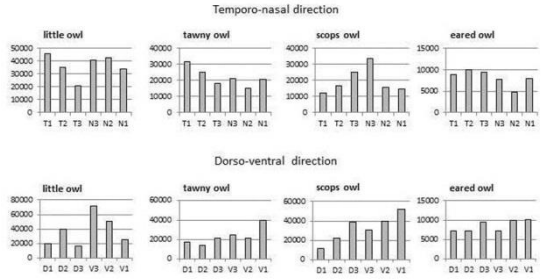

Fig. 4

Little owl	*<50 mμ²*	*50-100 mμ²*	*100-150 mμ²*	*>150 mμ²*	*Tawny owl*	*<50 mμ²*	*50-100 mμ²*	*100-150 mμ²*	*>150 mμ²*
D1	8	87	5		***D1***		78	17	5
V1	60	38	2		***V1***		82	16	2
T1	9	90	1		***T1***		79	17	4
N1	4	94	2		***N1***		86	12	3
D2	21	75	4		***D2***		72	23	5
V2	15	83	2		***V2***		81	14	5
T2	4	90	6		***T2***		70	25	5
N2	12	87	1		***N2***		73	16	11
D3	7	87	6		***D3***		69	23	8
V3	17	81	2		***V3***		87	9	4
T3	4	87	9		***T3***		77	19	4
N3	4	92	4		***N3***		59	35	6
Scops owl	***<50 mμ²***	***50-100 mμ²***	***100-150 mμ²***	***>150 mμ²***	***Eared owl***	***<50 mμ²***	***50-100 mμ²***	***100-150 mμ²***	***>150 mμ²***
D1		72	24		***D1***		75	25	
V1		98	2		***V1***		72	16	12
T1		85	11		***T1***		91	9	
N1	77	20	3		***N1***		67	21	12
D2		80	19		***D2***		53	35	5
V2		99	1		***V2***		78	17	
T2		84	16		***T2***		82	18	
N2		100			***N2***		100		
D3		99	1		***D3***		84	16	
V3	89	9	2		***V3***		30	30	40
T3		94	6		***T3***		72	19	9
N3		98	2		***N3***		49	28	23

Table 1

Common name	***Species***	***Predatory behavior***
Little owl	***Athene noctua***	Eats small vertebrates, predominantly in open spaces, hunting on the ground after a short flight
Tawny owl	***Strix aluco***	Eats small and medium sized mammals, amphibians and birds, hunting the prey quickly
Scops owl	***Otus scops***	Eats insects, reptiles, small mammals and birds, hunting from perches in semi-open landscapes
Eared owl	***Asio otus***	Eats rodents, small mammals and birds, hunting over open country by night

Table 2

Figure legends

Fig. 1: a) Four retinal fields (Dorsal; Temporal; Ventral; Nasal) in *Strigidae* retinal fundus. Optic disk (black round shape); pecten (black oval shape). b) Scheme of *Strigidae* eyeball: retinal radial section at three levels (1: ora serrata, 2: half distance between levels 1 and 3, 3: optic disc).

Fig. 2: Radial section of little owl and eared owl retina: the RGCs are highlighted in the insert (Nissl method).

Fig. 3: RGCs density (number of cells/mm^2) in *Strigidae* raptors (D, T, V, N: retinal fields; 1, 2, 3: section levels).

Fig. 4: RGCs density in Temporo-nasal and Dorso-ventral directions in *Strigidae* raptors. (T1: external temporal; T2: intermediate temporal; T3: central temporal; N3: central nasal; N2: intermediate nasal; N1: external nasal; D1: external dorsal; D2: intermediate dorsal; D3: central dorsal; V3: central ventral; V2: intermediate ventral; V1: external ventral).

Table 1: Percentage distribution of the RGCs according to range of medium soma area in each retinal fields (D,V,T,N) and section level (1,2,3) in *Strigidae* raptors.

Table 2: Predatory behavior of the four *Strigidae* raptors.