Distribution of Parvalbumin-Immunoreactive Retinal Ganglion Cells in the Greater Horseshoe Bat, *Rhinolophus ferrumequinum*

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Parvalbumin occurs in various types of cells in the retina. We previously reported parvalbumin distribution in the inner nuclear layer of bat retina. In the present study, we identified the parvalbumin-immunoreactive neurons in the ganglion cell layer of the retina of a bat, *Rhinolophus ferrumequinum*, and investigated the distribution pattern of the labeled neurons. Parvalbumin immunoreactivity was found in numerous cell bodies in the ganglion cell layer. Quantitative analysis showed that these cells had medium to large-sized somas. The soma diameter of the parvalbumin-immunoreactive cells in the ganglion cell layer ranged from 12.35 to 19.12 μm (n=166). As the fibers in the nerve fiber layer were also stained, the majority of parvalbumin-immunoreactive cells in the ganglion cell layer should be medium to large-sized retinal ganglion cells. The mean nearest neighbor distance of the parvalbumin-immunoreactive cells in the ganglion cell layer of the bat retina ranged from 59.57 to 62.45 μm and the average regularity index was 2.95 ± 0.3 (n=4). The present results demonstrate that parvalbumin is expressed in medium to large-sized retinal ganglion cells in bat retina, and they have a well-organized distributional pattern with regular mosaics. These results should be important as they are applicable to a better understanding of the unsolved issue of a bat vision. This data will help to provide fundamental knowledge for the better understanding of the unique behavioral aspects of bat flight maneuverability.

Key words − Bat, distribution, immunocytochemistry, parvalbumin, retinal ganglion cell

Introduction

Amazingly, bats rely upon echolocation in order to fly speedily to inhabit a dark environment, especially those of the suborder microchiroptera consisting of about 825 species [14,18,19]. However, all bats have eyes. The suborder megachiroptera have large eyes with excellent eyesight while microchiroptera have mostly small eyes with poor eyesight. The greater horseshoe bat (Microchiroptera: *Rhinolophus ferrumequinum*), is an insectivorous, cave-dwelling, and typically nocturnal species. This species inhabits a wide area over the world, ranging from Europe and northern Africa in the west, to China, Korea, and Japan in the east. Though microchiroptera bats have tiny eyes that may be capable of functional roles, their visual abilities and the functional organization of the retina are poorly understood.

Calcium plays a critical role in maintaining a wide variety of cellular mechanisms such as signal transduction, neurotransmission, learning and memory, and cell proliferation. Calcium-binding proteins are known to control and modulate the actions of calcium. Parvalbumin, a member of the EF-hand calcium-binding proteins family, consists of a single, unbranched chain of linked amino acids and has a molecular weight near 12,000 Daltons [2,7,8]. Although the exact physiological role of parvalbumin has not been established, it has been suggested that it plays a major role in buffering the intracellular calcium level [6,8].

Parvalbumin occurs abundantly in many neurons of the central nervous system, including the retina. Although there is some degree of species-specific variation, subpopulations of amacrine, bipolar, horizontal and ganglion cells in the vertebrate retina are immunoreactive for parvalbumin [3,5,21]. The vertebrate retina contains diverse retinal ganglion cells classified physiologically, anatomically, and neurochemically [4,16,20,22,23]. Our previous report demonstrated that parvalbumin occurred in a specific subpopulation of the retinal ganglion cells in mouse [15].

Recently, we reported the distributional pattern and the cell type of parvalbumin-immunoreactive cells in the inner

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nuclear layer of bat [13]. Parvalbumin was specifically localized in AII amacrine cells in the bat retina. As AII cells are critically involved in both rod- and cone-driven signals by sending a vertical flow of information within the On- and Off-layers, the existence of AII cells suggests that bats have retinas involved in both rod- and cone-driven signals. For a better understanding of the unsolved issue of the bat vision, the present study is undertaken to investigate the parvalbumin-containing cells in the ganglion cell layer of the greater horseshoe bat retina. Ganglion cells are the final output neurons of the retina and the investigation of the expression and cellular distribution of neurochemical substances in the retinal ganglion cells is the basic critical information to understand a specific function of the retinal ganglion cell. Our primary goal was to localize the neurochemical specific parvalbumin-expressing retinal ganglion cells to determine whether parvalbumin specifically labels certain neuronal populations in the ganglion cell layer and to analyze the distributional pattern of parvalbumin expressing cells in bat retina.

Materials and Methods

Animals and tissue preparation

Adult greater horseshoe bats (*Rhinolophus ferrumequinum*) were used in this study. The bats were anesthetized with a mixture of ketamine hydrochloride (30-40 mg/kg) and xylazine (3-6 mg/kg). A local anesthetic, proparacaine hydrochloride (100-200 µl), was applied to the cornea of the bats to suppress blink reflexes. Their eyes were enucleated, and the animals were euthanatized by an overdose of the same anesthetics. The anterior segments of the eyes were removed, and the retinas were dissected from the eyecups. The retinas were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4) for 2hr at 4-5°C, and then they were rinsed 3 x 10 min in 0.1 M PB. The guidelines of the National Institute of Health regarding the Care and Use of Laboratory Animals were followed in all experimental procedures.

Immunocytochemistry

A monoclonal antibody against parvalbumin was obtained from Sigma Chemical (St. Louis, USA). The immunocytochemical methods were described in detail in our previous reports [10-13,17]. For immunocytochemistry, the retinas were incubated in 1% sodium borohydride

(NaBH₄) for 30 min. Subsequently, these tissues were rinsed for 3 x 10 min in 0.1 M PB, and incubated in 0.1M PB with 4% normal horse serum for 24 hr with 0.5 % Triton X-100 added. Retinal whole mounts were processed free floating in small vials at 4-5°C with gentle agitation. The retinas were then incubated in the primary antiserum in 0.1 M PB with 4% normal horse serum for 72 hr with 0.5% Triton X-100 added. The primary antiserum was diluted 1:1000-1:2000. Following 3 x 10 min rinses in 0.1 M PB, the retinas were incubated in a 1:200 dilution of biotinylated anti-mouse IgG in 0.1 M PB with 4% normal horse serum for 24 hr with 0.5% Triton X-100 added. The retinas were then rinsed for 3 x 10min in 0.1 M PB and incubated in a 1:50 dilution of avidin-biotinylated horseradish peroxidase complex (ABC, Vector lab, USA) in 0.1M PB for 2 hr. The tissues were again rinsed in 0.25 M Tris buffer for 3 x 10 min. Finally, the staining was visualized by reacting with 3, 3'-diaminobenzidine tetrahydrochloride and hydrogen peroxide in 0.25 M Tris buffer for 3-5 min using a DAB reagent set (Kirkegaard & Perry, USA). The retinas were then rinsed in 0.25 M Tris buffer before mounting. The whole mounts were coverslipped in glycerol without dehydration procedure. The tissue was examined and photographed on a Zeiss Axioplan microscope using either conventional or differential interference contrast (DIC) optics.

Quantitative analysis

Cell density was expressed as the number of parvalbumin-immunoreactive cells/mm² of retinal surface. In three DAB-reacted wholemount retinas, parvalbumin-immunoreactive cells in the ganglion cell layer were viewed on a computer monitor using a Zeiss Plan-Apochromat 20x objective, and a Zeiss AxioCam HRc digital camera at 200 µm intervals along the central dorsoventral and nasotemporal axes. The sample area was 100 x 100 µm². A transparency sheet was placed on the computer monitor, and labeled cells were circled with a pen. To measure the soma diameter, area, and nearest neighbor distance, we used a Zeiss Axiovision 4 program. They were performed on the cells located in the mid-peripheral region from the one nasal, temporal, dorsal, and ventral of a bat retina. The sample area was 500 x 500 µm² wide. The nearest neighbor analytical method to determine the regularity index (mean nearest neighbor distance/standard deviation) has been previously well-reported [25].

Results

Parvalbumin-immunoreactivity in the ganglion cell layer

Fig. 1 shows a well labeled parvalbumin-immunoreactive whole mount of bat retina taken from the mid-periphery retina. Through the differential interference contrast (DIC) micrograph, strongly labeled, medium to large-sized cells are seen in the ganglion cell layer. As the fibers in the nerve fiber layer and the axonal processes in the nerve fiber layer are also stained, the majority of parvalbumin-immunoreactive cells in the ganglion cell layer should be ganglion cells. In our study, we occasionally encountered some small-sized cells that were also labeled by the parvalbumin antibody (Fig. 1 arrow). These small cells were stained more strongly than other medium to large cells and did not contain axons. The lack of axonal staining suggests that these small darkly labeled cells in the ganglion cell layer are displaced amacrine cells. In consistent with the present study, displaced parvalbumin-immunoreactive amacrine cells are clearly distinguishable as small and more darkly labeled neurons in the ganglion cell layer of the rabbit retina [17]. These observations suggest that the majority of parvalbumin-immunoreactive cells in the ganglion cell layer should be retinal ganglion cells, and small populations of cells (< 5%) are displaced amacrine cells.

Diameter and area of parvalbumin-immunoreactive cells

We analyzed the cell diameter and area of parvalbumin-labeled retinal ganglion cells using the AxioVision 4
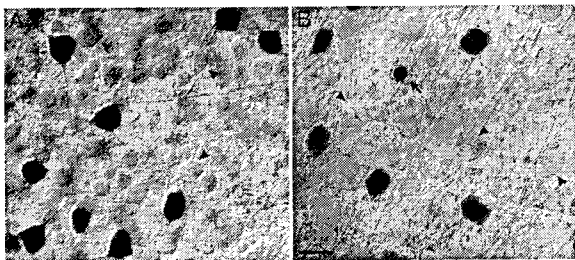

Fig. 1. Parvalbumin-immunoreactive cells of the ganglion cell layer in the whole mount of DAB-reacted bat retina from the mid-peripheral region (A, B). In DIC micrograph, both labeled and unlabeled cells and nerve fibers can be seen. Note that a small displaced amacrine cell is labeled (arrow). Arrowheads indicate some unlabeled medium to large-sized cells. Scale bar = 20 µm.

program (Carl Zeiss Meditec, Inc.). The small cells could not be included in the present analysis as these cells are displaced amacrine cells. In the mid-peripheral regions of the nasal (n=39), temporal (n=42), dorsal (n=44), and ventral (n=41) retinas, a total of 166 parvalbumin-immunoreactive cells were sampled. Parvalbumin-immunoreactive ganglion cells varied from about 12.35 to 19.12 µm in diameter. In the nasal retina, the mean of the soma diameter and area were 15.99 ± 1.53 µm and 189.56 ± 36.49 µm^2 (mean ± s.d.; n=39), respectively. In the temporal retina, the mean of the soma diameter and area were 14.93 ± 1.16 µm and 163.88 ± 26.23 µm^2 (mean ± s.d.; n=42), respectively. In the dorsal retina, the mean of the soma diameter and area were 15.34 ± 1.22 µm and 173.54 ± 28.21 µm^2 (mean ± s.d.; n=44), respectively. In the ventral retina, the mean of the soma diameter and area were 15.55 ± 1.42 µm and 178.48 ± 33.45 µm^2 (mean ± s.d.; n=41), respectively (Fig. 2). The results indicate that parvalbumin is mainly expressed in the medium to large-sized ganglion cells within the retinal ganglion cell population of bat retina.

Distributional pattern of parvalbumin-immunoreactive cells

The distributional pattern of parvalbumin-immunoreactive cells in the ganglion cell layer of bat retina is shown in Fig. 3. In three DAB-reacted whole-mount retinas, the parvalbumin-immunoreactive ganglion cells in the ganglion cell layer were counted. The estimated total number of parvalbumin-immunoreactive ganglion cells varied from 844 to 986 cells among the three sampled in this
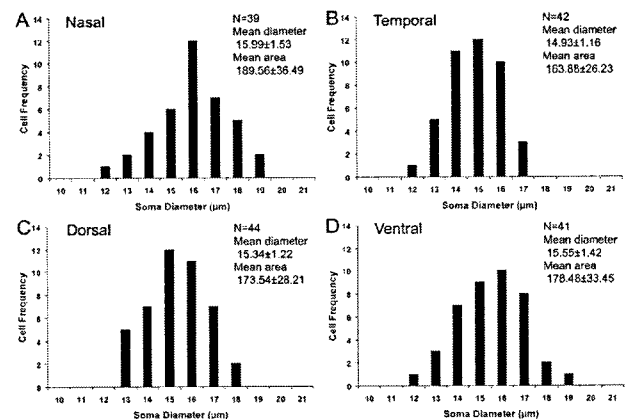

Fig. 2. Cell frequency histogram of parvalbumin-immunoreactive cell diameters in the ganglion cell layer. The sample sites are the mid-peripheral regions of the nasal (A), temporal (B), dorsal (C), ventral (D) retina.
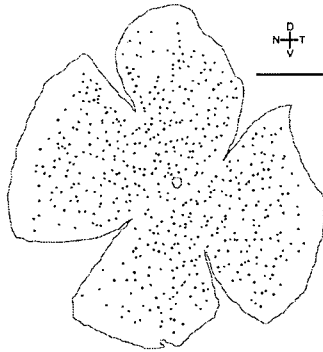
Fig. 3. Retinal maps from a bat retina indicating the position of each parvalbumin-immunoreactive cell within the ganglion cell layer. Overall, parvalbumin-immunoreactive cells in the ganglion cell layer show a distribution pattern with regular arrangements. Scale bar = 500 μm.

study. Fig. 4 and Table 1 show the results; there were 957 cells in retina 1L, 986 cells in retina 2R, and 844 cells in retina 3R; therefore, the average number of parvalbumin-immunoreactive ganglion cells per retina was 929 ± 75 (mean ± s.d.; n=3). The mean density of parvalbumin-immunoreactive ganglion cells in the ganglion cell layer was 254 ± 29 cells/mm^2 in the three retinas (Table 1). The distribution of total parvalbumin-immunoreactive ganglion cells is shown in Fig. 4. The two graphs (Fig. 4A and 4B) show the numbers of cells encountered along the dorsoventral and nasotemporal axes intersecting the optic nerve head. Although there were some differences among the three retinas, the peak and the lowest density was found near 0.4 mm from the optic disk and in the peripheral regions of the retinas, respectively.

Nearest neighbor analysis of parvalbumin-immunoreactive cells

The retinal mosaic of the parvalbumin-immunoreactive retinal ganglion cells in the ganglion cell layer was measured by nearest neighbor analysis [25]. The mean nearest

neighbor distance of parvalbumin-immunoreactive retinal ganglion cells in the ganglion cell layer of bat retina ranged from 59.57 μm to 62.45 μm. The regularity index in the four mid-periphery regions ranged from 2.60 to 3.33, and its average was 2.95 ± 0.3 (Fig. 5). The histograms are relatively well matched by a Gaussian curve (solid line),
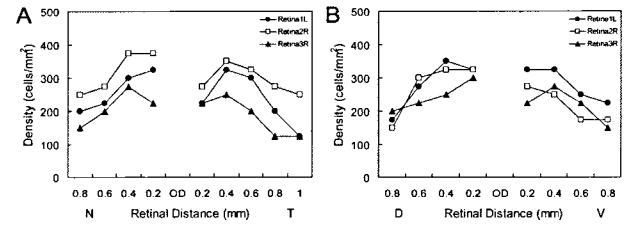

Fig. 4. Parvalbumin-immunoreactive ganglion cells in the ganglion cell layer. (A, B) The two graphs show the numbers of cells encountered along the two axes intersecting the optic nerve head. N, nasal; T, temporal; D, dorsal; V, ventral.
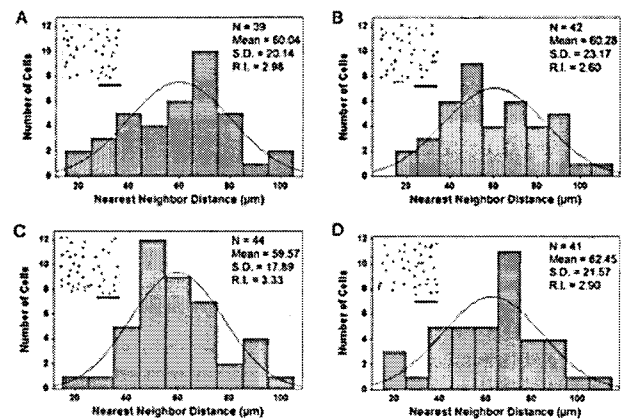

Fig. 5. Nearest neighbor analysis of parvalbumin-immunoreactive cells in the ganglion cell layer of bat retina. The histogram for parvalbumin-immunoreactive cells in the mid-peripheral regions of the nasal (A), temporal (B), dorsal (C), ventral (D) retina shows the number of cells in the sample (N), the mean distance between cells (Mean), the standard deviation (S.D.), and the regularity index (R.I.). The location of cells within the sample area is shown in the upper left. Scale bar = 200 μm.

Table 1. Total parvalbumin-immunoreactive ganglion cells in the ganglion cell layer of bat retina.

Retina	Sampled area*	Sampled area(μm^2)	Neurons counted	Total retinal area(mm^2)	Mean density (cells/mm^2)	Total parvalbumin-IR neurons
Retina1L	68	680,000	179	3.638518	263	957
Retina2R	68	680,000	189	3.547171	278	986
Retina3R	228	2,280,000	507	3.796553	222	844
Mean±S.D.					254 ± 29	929 ± 75

*One sampled area = 100 x 100 μm^2
IR, immunoreactive; L, left; R, right

which describes a regular cell distribution. As a random distribution is indicated by a ratio of 1 and a regular mosaic is higher than a ratio of 1, the data support the idea that the parvalbumin-immunoreactive retinal ganglion cells in the ganglion cell layer show regular arrangements.

Discussion

In the present study, we identified the selective localization of parvalbumin-immunoreactive retinal ganglion cells in the bat retina. These labeled cells were medium to large-sized ganglion cells and they showed a regular mosaic in the greater horseshoe bat retina.

Generally, it is known that megabats employ their functional eyes to find prey and monitor their environment in order to protect themselves from predators. However, it is not well known whether their retinas of microbats, such as *Rhinolophus ferrumequinum* play functional roles or not. In our previous study about greater horseshoe bats [13], for the first time we presented the existence of AII amacrine cells in the inner nuclear layer of the retina using parvalbumin immunocytochemistry. As AII cells are critically involved in both rod- and cone-driven signals by sending a vertical flow of information within the On- and Off-layers, the existence of AII cells suggests that bat retina has versatile synaptic connectivity and diverse functional physiology for vision. In this study, we identified the existence of well-organized parvalbumin-immunoreactive retinal ganglion cells in the ganglion cell layer of the greater horseshoe bats. With respect to the distribution of parvalbumin, at least one solid conclusion can be drawn from the previous and present data. This protein is expressed in specific types of retinal cells in bat retina. These combined results of the existence of AII amacrine and the subpopulation of parvalbumin-containing retinal ganglion cells strongly suggest that microbats not only rely on echolocation but also have functional eyes to help flight maneuverability.

Retinal ganglion cells collect visual information in the eyes and finally send it to the brain for visual perception. At least 11 different types of retinal ganglion cells have been identified in rabbit retina using a combination of modern anatomical techniques [20]. More than 10 morphologically distinct ganglion cell types have been revealed in mouse retina, by the introduction of particle-mediated gene transfer [16,22] and by the expression of the gene en-

coding an alkaline phosphatase [1]. Although there is some degree of species-specific variations, subpopulations of ganglion cells in the vertebrate retina were immunoreactive for parvalbumin [5,21,24]. The previous studies show that the majority of parvalbumin-immunoreactive cells in the ganglion cell layer are medium to large-sized cells. In our previous study, using a newly developed single cell injection after immunocytochemistry technique, for the first time we demonstrated that at least eight different types of mouse retinal ganglion cells express parvalbumin [15]. Our previous results [15] support the idea that parvalbumin-immunoreactive retinal ganglion cells likely belong to a different chemical subpopulation from each type. In the present study, we found that the vast majority of parvalbumin-immunoreactive neurons in bat retina are medium to large-sized neurons. However, we also found many unlabeled medium to large-sized cells in the retinal ganglion cell layer (The DIC pictures in Fig. 1 show many unlabeled medium to large-sized cells). In accordance with the previous study [15], the present study strongly suggests that parvalbumin is expressed to a different subpopulation of retinal ganglion cells in bat retina. Studies on the expression and cellular distribution of neurochemical substances in retinal ganglion cells are the basic information to understand a specific function of retinal ganglion cells. A previous study has shown that somatostatin is found only in a subpopulation of alpha ganglion cells [26]. In addition, Hutsler *et al* [9] have shown that neuropeptide Y immunoreactivity recognizes a subpopulation of about 2,000 gamma-type ganglion cells. In our previous study, we have reported that calretinin is not found in the alpha retinal ganglion cells in rabbit and cat retina [11]. The different proportional localization of these proteins along with the present results may reflect their specific functional characteristics in retina and visual behaviors.

The nearest neighbor analysis was performed to quantify the regularity of the parvalbumin-immunoreactive retinal ganglion cell mosaic in the ganglion cell layer. The regularity of the cell mosaic is represented by the regularity index, which is the ratio of the mean nearest neighbor distance to the standard deviation in the distribution of a cell population. A regular arrangement would mean that there would not be blind spots with respect to the distribution of cells by covering each retinal position. Thus, it is more economic for regularly arranged cells to cover the retina

than for randomly arranged cells to do. A further profit of cells with a regular arrangement is that this would allow synaptic connections to facilitate between the different layers in the retina [25]. In our study, the regularity index of parvalbumin-immunoreactive cells in the ganglion cell layer of bat retina varied from 2.60 to 3.33 according to the different regions, and the average regularity index was 2.95. So far no regularity index of parvalbumin-immunoreactive retinal ganglion cells has been reported in any other animals and our data is the first report on this matter. Although we cannot compare the present results with other results, the results indicate that the parvalbumin-immunoreactive retinal ganglion cells in the ganglion cell layer have regular arrangements.

In conclusion, greater horseshoe bats (*Rhinolophus ferrumequinum*) possess well-organized parvalbumin-immunoreactive retinal ganglion cells in the ganglion cell layer of the retina. The majority of these cells are medium to large-sized cells and they have regular arrangements in a mosaic pattern. Our study demonstrates that there is immunocytochemical evidence of retinal ganglion cells expressing a specific protein in a microbat retina. These results should be important as they are applicable to a better understanding of the unsolved issue of a bat vision.

Acknowledgments

References

1. Badea, T. C. and J. Nathans. 2004. Quantitative analysis of neuronal morphologies in the mouse retina visualized by using a genetically directed reporter. *J. Comp. Neurol.* **480**, 331-351.
2. Baimbridge, K. G., M. R. Celio and J. H. Rogers. 1992. Calcium-binding proteins in the nervous system. *Trends Neurosci.* **15**, 303-308.
3. Casini, G., D. W. Rickman and N. C. Brecha. 1995. AII amacrine cell population in the rabbit retina: identification by parvalbumin immunoreactivity. *J. Comp. Neurol.* **356**, 132-142.
4. Coombs, J., D. van der List., G. Y. Wang and L. M. Chalupa. 2006. Morphological properties of mouse retinal ganglion cells. *Neuroscience* **140**, 123-136.
5. Haverkamp, S. and H. Wässle. 2000. Immunocytochemical analysis of the mouse retina. *J. Comp. Neurol.* **424**, 1-23.
6. Heizmann, C. W. 1993. Calcium signaling in the brain. *Acta Neurobiol. Exp.* **53**, 15-23.
7. Heizmann, C. W. and U. Kagi. 1989. Structure and function of parvalbumin. *Adv. Exp. Med. Biol.* **255**, 215-222.
8. Heizmann, C. W., J. Röhrenbeck and W. Kamphuis. 1990. Parvalbumin, molecular and functional aspects. *Adv. Exp. Med. Biol.* **269**, 57-66.
9. Hutsler, J. J., C. A. White and L. M. Chalupa. 1993. Neuropeptide Y immunoreactivity identifies a group of gamma-type retinal ganglion cells in the cat. *J. Comp. Neurol.* **336**, 468-480.
10. Jeon, C. J., E. Strettoi and R. H. Masland. 1998. The major cell populations of the mouse retina. *J. Neurosci.* **18**, 8936-8946.
11. Jeon, M. H. and C. J. Jeon. 1998. Immunocytochemical localization of calretinin containing neurons in retina from rabbit, cat, and dog. *Neurosci. Res.* **32**, 75-84.
12. Jeon, Y. K., S. Y. Kim and C. J. Jeon. 2001. Morphology of calretinin and tyrosine hydroxylase-immunoreactive neurons in the pig retina. *Mol. Cells* **11**, 250-256.
13. Jeon, Y. K., T. J. Kim., J. Y. Lee., J. S. Choi and C. J. Jeon. 2007. AII amacrine cells in the inner nuclear layer of bat retina: identification by parvalbumin immunoreactivity. *Neuroreport* **16**, 1095-1099.
14. Jones, G. and J. M. V. Rayner. 1989. Foraging behavior and echolocation of wild horseshoe bats *Rhinolophus ferrumequinum* and *R. hipposideros* (Chiroptera, Rhinolophidae). *Behav. Ecol. Sociobiol.* **25**, 183-191.
15. Kim, T. J. and C. J. Jeon. 2006. Morphological classification of parvalbumin-containing retinal ganglion cells in mouse: single-cell injection after immunocytochemistry. *Invest. Ophthalmol. Vis. Sci.* **47**, 2757-2764.
16. Kong, J. H., D. R. Fish., R. L. Rockhill and R. H. Masland. 2005. Diversity of ganglion cells in the mouse retina: Unsupervised morphological classification and its limits. *J. Comp. Neurol.* **489**, 293-310.
17. Kwon, O. J., J. Y. Kim., S. Y. Kim and C. J. Jeon. 2005. Alterations in the localization of calbindin D28K-, calretinin-, and parvalbumin-immunoreactive neurons of rabbit retinal ganglion cell layer from ischemia and reperfusion. *Mol. Cells* **19**, 382-390.
18. Ransome, R. D. 1968. The distribution of the Greater horseshoe bat, *Rhinolophus ferrumequinum*, during hibernation, in relation to environmental factors. *J. Zool.* **154**, 77-112.
19. Ransome, R. D. and A. M. Hutson. 2000. Action plan for the conservation of the greater horseshoe bat in Europe (*Rhinolophus ferrumequinum*), *Nature and Environment*, **109**, 7-52.
20. Rockhill, R. L., F. J. Daly., M. A. MacNeil., S. P. Brown and R. H. Masland. 2002. The diversity of ganglion cells in mammalian retina. *J. Neurosci.* **22**, 3831-3843.
21. Sanna, P. P., K. T. Keyser., M. R. Celio., H. J. Karten and F. E. Bloom. 1993. Distribution of parvalbumin immunoreactivity in the vertebrate retina. *Brain Res.* **600**, 141-150.
22. Sun, W., N. Li and S. He. 2002. Large-scale morphological

survey of mouse retinal ganglion cells. *J. Comp. Neurol.* **451**, 115-126.

23. Sun, W., N. Li and S. He. 2002. Large-scale morphological survey of rat retinal ganglion cells. *Vis. Neurosci.* **19**, 483-493.

24. Wässle, H., U. Grünert and J. Röhrenbeck. 1993. Immunocytochemical staining of AII-amacrine cells in the rat retina with antibodies against parvalbumin. *J. Comp. Neurol.* **332**, 407-420.

25. Wässle, H. and H. J. Riemann. 1978. The mosaic of nerve cells in the mammalian retina. *Proc. R. Soc. Lond. B. Biol. Sci.* **200**, 441-461.

26. White, C. A. and L. M. Chalupa. 1991. Subgroup of alpha ganglion cells in the adult cat retina is immunoreactive for somatostatin. *J. Comp. Neurol.* **304**, 1-13.

초록 : 한국관박쥐 망막에서 파브알부민 면역반응성 망막신경절세포의 분포 양상

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파브알부민(Parvalbumin)은 망막의 다양한 세포타입에서 분포하고 있다. 본 연구팀은 이전연구에서 박쥐 망막의 내핵층에서의 파브알부민의 분포를 보고하였다. 현재 연구에서 본 연구팀은 한국관박쥐 (*Rhinolophus ferrumequinum*) 망막의 신경절세포층에 존재하는 파브알부민을 함유하는 신경세포를 규명하였고, 이들 세포의 분포양상을 조사하였다. 실험 결과, 파브알부민의 면역반응성은 신경절세포층의 다수 세포에서 발견되었으며, 이들 세포는 주로 중간형 이상 크기의 세포체를 가지고 있었다. 조사된 세포체의 직경은 12.35 - 19.12 μm 의 범위를 가지며 (n=166), 신경섬유층의 섬유 역시 염색되는 것으로 보아, 파브알부민을 함유하는 신경절세포는 대부분이 중간형 이상 크기의 신경절세포임을 뒷받침하고 있다. NND (nearest neighbor distance) 분석을 통해서 본, 평균 NND는 59.57 에서 62.45 μm 로 나타났으며, 평균 RI (regularity index) 는 2.95 ± 0.3 (mean±s.d., n=4) 으로 계산되었다. 이를 종합해보면, 파브알부민은 한국관박쥐 망막의 신경절세포층에서 중간형이상 크기의 신경절세포에서 주로 발현하고 있으며, 이들은 규칙적인 배열을 가진 채 잘 조직화된 분포양상을 보여주고 있음을 알 수 있었다. 이러한 결과들은, 아직까지 명확하게 규명되어 있지 못한 박쥐의 시각에 대한 이해에 중요하게 적용될 수 있을 것이라고 사료된다.