Postnatal development of the retina in root vole Microtus oeconomus *

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Abstract   Vision plays an important role in the living habits of animals , especially in feeding. We investigated the postnatal development of retina in root vole *Microtus oeconomus*. The result shows that the retina of the *M. oeconomus* is very primitive before postnatal day (PD) 3. The neuroblastic layer does not differentiate and makes up more than half of the retina layer. The outer plexiform layer (OPL) first comes into existence at PD5. At PD6 , as the presence of the OPL becomes obvious , the outer nuclear layer (ONL) and inner nuclear layer (INL) are much clearer. At PD18 , the retina is similar to an adult retina and each layer becomes distinct. The thickness and cell density of the ganglion cell layer (GCL) and ONL during different postnatal days were also examined. These results show that the thickness and density of ONL increase during ontogeny , while the thickness and density of GCL decrease. Compared with *Rattus norvegicus* , *Apodemus agrarius* , *Cricetulus triton* , *Microtus mandarinus* , *Myospalax cansus* , *Spermophilus dauricus* and *Sciurotamias davidianus* , the histological structure of the retina of *M. oeconomus* is between that of nocturnal and diurnal rodents [*Acta Zoologica Sinica* 52 (2) : 376 - 382 , 2006].

Key words   *Microtus oeconomus* , Retina , Histological structure , Postnatal development , Differentiate

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Microtus oeconomus is widely distributed throughout the northern regions of Europe and Asia. It is the dominant rodent in the *Potentilla freticosa* shrub of the ecosystem of alpine meadows in Haibei , Qinghai provinces of China. It is a phytophagous rodent , and it doesn't hibernate (Sun et al. , 1982).

Its population , productivity and behavior have been studied thoroughly. Molecular and cellular details of its bioenergetics have also been elucidated (Hou , 1996).

There are two contrary viewpoints about the activity time of *M. oeconomus*. One viewpoint is that

the *M. oeconomus* is active both day and night, but mostly at night. Its peak of activity is at midnight. Very few activities occur in the afternoon from 12 to 4 pm. The active period is very short and the rhythm is not very obvious in the daytime (Zeng et al. , 1981). Another viewpoint is that the activity on the ground of *M. oeconomus* is chiefly concentrated to the daytime, and activity time is between 5 30 am to 8 45 pm. The rhythm of the activity on the ground is very obvious, which belongs to diurnal-ultradian rhythm (Wang et al. , 1991). However, a reason for the contrary conclusion may be that inside the laboratory and under man-made conditions, the temperature is suitable, and food is sufficient.

There are differences in the animal retina structure between nocturnal animals and diurnal animals. The ratio of the photoreceptor cell numbers and the ganglion cell numbers is a typical indication (Wang et al. , 1980; Li and Wu, 1989). The typical diurnal retina has the same number of cells in the ONL and the GCL. However, the typical nocturnal animal retina has far more ONL cells than GCL cells (Zhang and Jia, 2003).

The retina structure of *Rattus norvegicus*, *Apodemus agrarius*, *Cricetulus triton*, *Microtus mandarinus*, *Myospalax cansus*, *Spermophilus dauricus* and *Sciurotamias davidianus* were compared in a previous study (Zhang and Liu, 1994). In this study, the postnatal development of the retina in *M. oeconomus* was investigated, and the histological structure of the retina was compared with that of the *R. norvegicus*, *A. agrarius*, *C. triton*, *M. mandarinus*, *M. cansus*, *S. dauricus* and *S. davidianus*. Whether *M. oeconomus* is a diurnal or nocturnal rodent can be distinguished through comparisons. We also investigated the photoreceptors of the retina in *M. oeconomus* using a scanning electron microscope.

1   Material and methods

1.1   Animals

All *M. oeconomus* came from Northwest Plateau Institute of Biology, Xining, China, and were reared in the laboratory. They were fed with a diet of carrot and cabbage. The postnatal development of the eyeball and lens was analyzed. Six individuals were taken every week from postnatal day (PD) 7 to PD70, six PD3 were also taken. The postnatal development of the retina was also investigated. Six individuals were taken every 3 days from PD3 to PD30. Two adult *M. oeconomus* were used to examine the photoreceptors by a scanning electron microscope.

1.2   Method

1.2.1   Retinal development

All *M. oeconomus* at different postnatal days were killed, their eyes were removed, the lens and vitreous gels were wiped off. The retinas were fixed with Bouin's fixative for 24 - 48 h, dehydrated in ethanol, and then embedded in paraffin. The paraffin sections were cut from sagittal direction serially at 6 μm, and stained with hematoxylin and eosin. Then they were observed through Nikon YS2-H light microscope, using a 40 × and 4 × objective.

1.2.2   Retina photoreceptor in adult *M. oeconomus*

M. oeconomus was deeply anesthetized and perfused through the heart with saline (NaCl 0.7%) followed by 2.5% glutaraldehyde in 0.1 mol/L phosphate buffer (PB), pH 7.2. The eyes were removed quickly, together with the vitreous gels and the pigmented epithelium of the retina (RPE), and postfixed with 2.5% glutaraldehyde in 0.1 mol/L PB for 2 h, rinsed in 0.1 mol/L phosphate buffer, postfixed with 1% OsO_4 in 0.1 mol/L PB, followed by two washes in 0.1 mol/L PB, dehydrated in ethanol, placed in ethyl acetate, dried at 31.5, 72.8 kg/cm^2, fixed to an iron plate, then plated with gold. Then they were observed through a scanning electron microscope.

1.2.3   Statistical analyses

The anteroposterior diameter of the eyeball and equatorial diameter of the lens were measured using a vernier. Each sample was measured from 5 different directions. The average diameter of the eyeball and lens were computed by Statistical Package for the Social Sciences (SPSS). We tested the means. Then the graph was made by Excel.

The thickness and the cell density of ONL and GCL were examined in an area of 2 mm away from the optic disc on 5 parallel sections including the meridian of the eye bulb, using a microscope reticule (Produced by Shanghai optical instruments plant 3, China). Cells of ONL and GCL were counted in the same area in one square, using the microscope reticule. Then the density was calculated. The average thickness and the cell density of ONL and GCL were computed by SPSS.

2   Results

2.1   Development of the eyeballs and lens

The diameter of the eyeball increases during ontogeny from only 2.11 mm to 3.27 mm by PD70 (Fig. 1). The greatest increase in eyeball diameter occurs from PD3 to PD7. Fig. 1 also shows that the growth of the lens in *M. oeconomus* is similar to that of the eyeball. The diameter of the lens in *M. oeconomus* at PD3 is only 1.20 mm and increases to 2.10 mm by PD70. The greatest increase is also from PD3 to PD7.

In general, both the diameters of the lens and eyeball increase during postnatal development in *M.*
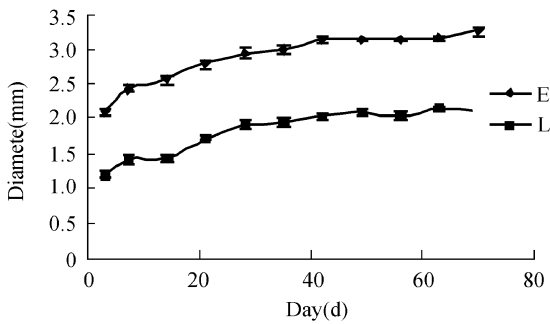
Fig. 1   The average (Mean ± SD) diameter of the eyeballs and lens during postnatal development in Microtus oeconomus.

oeconomus.

2. 2   Retina development

The retina of the *M . oeconomus* is mostly undifferentiated before postnatal 3 days. Only RPE, inner plexiform layer (IPL), GCL and the neuroblastic layer can be observed. The photoreceptor out segment is not clear. The neuroblastic layer makes up more than half of the retina layer. There is no differentiation in the neuroblastic layer. In contrast with the scattered cells of the inner part of the neuroblastic layer, the cells in the outer part are very dense. The nucleus is very clear. The ganglion cells of GCL are sparse. There are about three to six horizontal rows of cells in GCL (Plate : A).

At PD5, the OPL first comes into existence. The ONL and the INL can be observed. However, the stratification is not very clear (Plate : B). At PD6, as the presence of OPL becomes obvious, the ONL and the INL are much clearer. Two or three horizontal rows of ganglion cells can be observed. The photoreceptor out segment is short from PD5 to PD6 (Plate : C).

As the *M . oeconomus* develops, each layer becomes much clearer. From PD9 to PD12, the ONL and INL are quite distinguishable, and the photoreceptor out segment becomes clear. The cell density of the ONL, which also has clear nuclei, is greater than that of INL. However, the retina is not mature, since each layer has not developed completely (Plate : D).

In PD18 retina (Plate : E), each layer is similar to the adult retina (Plate : F). Each layer can be observed clearly. The ganglion cells have been gradually arranged into a single row. The photoreceptor out segment is longer.

2. 3   The cell thickness and density of ONL and GCL

At PD3, the thickness of GCL is 29.83 μm (Fig. 2). However, at PD6, the thickness decreases to 20.33 μm. The greatest decrease in thickness is from PD3 to PD6. Fig. 2 also shows that the thick-

ness of GCL reduces sharply during ontogeny. The cell density of GCL shows the similar trends (Fig. 3). At PD3, the density of GCL is only 18.09 $\times 10^3/ mm^2$, while it is just 12.92 $\times 10^3/ mm^2$ at PD6 (Fig. 3). It shows that the density also decreases fastest from PD3 to PD6. Fig. 3 also shows that the cell density reduces sharply during ontogeny. Consequently, the ganglion cells decrease sharply in numbers during ontogeny.

On the contrary, the thickness of ONL is 32.59 μm at PD6, while it is only 39.74 μm at PD30 (Fig. 2). The thickness of ONL during ontogeny increases slowly. At PD6, the cell density of ONL is 37.94 $\times 10^3/ mm^2$, while it is only 44.88 $\times 10^3/ mm^2$ at PD30 (Fig. 3). Thus, the cell density of ONL also increases slowly. Consequently, compared with the rapidly reducing ganglion cells, the numbers of the outer nuclear cells increase slowly during ontogeny.
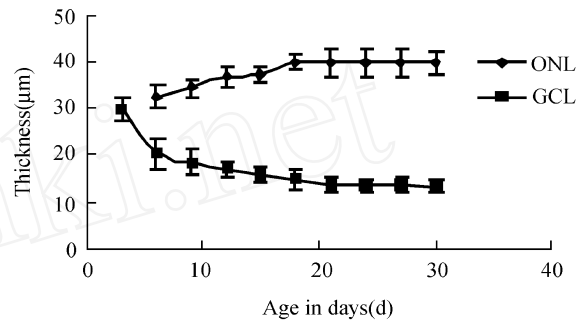

Fig. 2   The average (Mean ± SD) thickness of the ganglion cell layer (GCL) and the outer nuclear layer (ONL) during postnatal development
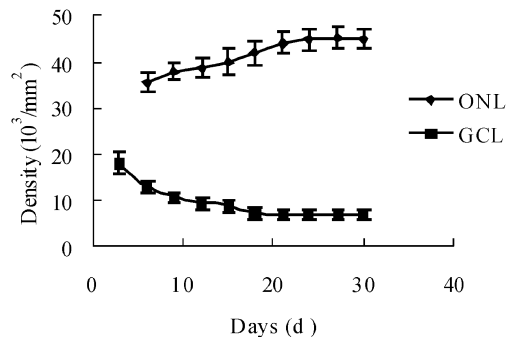

Fig. 3   The average (Mean ± SD) cell density of the ganglion cell layer (GCL) and the outer nuclear layer (ONL) during postnatal development

In general, both the thickness and cell density of the ONL increase during postnatal development. Therefore, the number of outer nuclear cells increases. On the contrary, both the thickness and cell density of GCL decrease during ontogeny. So the ganglion cell numbers decrease during ontogeny. Therefore, those data indicate that the ganglion cell numbers decrease rapidly and the outer nuclear cell num-

bers increase slowly during postnatal development.

2. 4　The photoreceptor of retina in adult M. oec-onomus

　　With the aid of a scanning electron microscope , we can see each layer in adult *M. oeconomus* (Plate Ⅱ : H). In photoreceptor out segment layer , most part of the retina was occupied by rods , but some areas were occupied by a few cones (Plate Ⅱ : I). Fine processes on the outer segment of the cones and rods were observed (Plate Ⅱ : H and I).

3　Discussion

　　The eye originates as a bilateral organ from a single field in the anterior neural plate (Varga et al. , 1999 ; Stefan et al. , 2003). Proliferation and evagination gives rise to the optic vesicles. Their infolding into optic cups and their progressive determination originates the optic stalk , the neural retina , and the RPE (Stefan et al. , 2003). Retinal neurons are born in two waves of cytogenesis. The ganglion cells , cones , horizontal cells and certain amarcrine cells differentiate first. In the second wave , rods , bipolar cells and more amarcrine cells are added (Rajesh et al. , 2003 ; Sharma and Ehinger , 1997 ; Lavail et al. , 1991 ; Reichenbach et al. , 1994 ; Reichenbach and Robinson , 1995). The retinas of Kun Ming mice are immature before PD20 (Peng et al. , 1999) , so they can be used as transplantation donors. When the Sprague-Dawley (SD) rat opens its eyes at PD15 , the retina is the same as the adult retina (Peng et al. , 1999 ; Casini et al. , 1994). In this study , we observed the histological structure of retina in *M. oeconomus* during postnatal development stages. The retina at PD3 is very primitive. The neuroblastic layer does not differentiate and makes up more than half of the retina layer. PD5 - 6 is the key period when the neuroblastic layer differentiates. During PD5 to PD7 , the elements of the OPL come together to form a histologically distinguishable OPL , where they show staining for synaptophysin , perhaps imply synaptogenesis (Rajesh et al. , 2003). From PD9 - 12 , the *M. oeconomus* opens its eyes (Liang et al. , 1982) , while the SD rat opens its eyes at P15. Compared with the SD rat , *M. oeconomus* opens its eyes earlier. However , the histological structure of retina of the SD rat when it opens its eyes is the same as the adult retina. In contrast , when the *M. oeconomus* opens its eyes , the retina is different from the retina of the adult *M. oeconomus*. Although the ONL and the GCL are very clear , the OPL and the photoreceptor layer have not developed completely. This demonstrates that although *M. oeconomus* opens its eyes earlier than the SD rat , the retina develops slower than the SD rat. At PD18 , the retina of *M. oeconomus* becomes the same as the adult retina ,

and each layer has developed completely.

　　The ratio of the number of photoreceptor cells to the ganglion cells is one of the typical indications that distinguish the nocturnal animal or diurnal animal (Wang et al. , 1980). The typical diurnal animal retina has the same ratio of the outer nuclear cells to ganglion cells , nearly 1∶1 ; while the typical nocturnal animal retina has a higher ratio of the outer nuclear cells to ganglion cells , nearly 100∶1 (Zhang et al. , 2003 ; Gao and Zhang , 1996). This finding is applicable to both amphibians and rodents. In the adult *M. oeconomus* , the ONL is thinner than that of *R. norvegicus* , *A. agrarius* , *C. triton* , but it is thicker than that of *S. dauricus* and *S. davidianus*. The ONL in adult *M. oeconomus* is thinner than diurnal mice , and it is thicker than nocturnal mice. This suggests that the retina structure of *M. oeconomus* is between that of the nocturnal mice and diurnal mice. This structure is consistent with its living behavior , since its activity occurs both at day and night.

　　Jeon et al. (1998) reported that the photoreceptors in mouse are mostly rods ; cones comprise less than 3 % of the cells in the cells in ONL. We found that in the retina of *M. oeconomus* , most photoreceptors were rods. Fine processes on the outer segment of the cones and rods were observed. These processes may connect with other channels of the photoreceptors , which may increase the visual acuity when *M. oeconomus* searches for food at night.

　　Within the brain , retinal ganglion cells are the only neurons connecting to the midbrain targets , especially to the superior colliculus and the lateral geniculate nucleus. It has been reported that ganglion cells of the mammalian retina die following section of the optic nerve. It also been has reported that cell apoptosis in the retina (including ganglion cells) is the key mechanism of cell death during development (Chen , 1999). This effect has been shown both in adult mammals (Francisco et al. , 2004 ; Misantone et al. , 1984 ; Villegas-Pérez et al. , 1993) and during development (Francisco et al. , 2004 ; Cowan , 1970 ; Allcut et al. , 1984). In the retina of the chick embryo , two waves of programmed cellular death (PCD) have been described : one occurring between embryonic day 5 (E5) and E7 (Stefan et al. , 2003 ; Garcia-Porrero , 1979 ; Rager and Rager , 1978) , and a later one at E10-14 (Stefan et al. , 2003 ; Rager and Rager , 1978). In the rat , estimates derived from counts of ON axons indicate that about 50 % of newly generated RGCs die soon after reaching their targets (Stefan et al. , 2003 ; Perry et al. , 1983). By studying the elimination of RGCs generated at defined embryonic ages , it has been estimated that as many as 90 % of the RGCs die during the first postnatal week in rats

（Reichenbach and Robinson , 1995 ; Galli-Resta and Ensini , 1996）. We have demonstrated that the ganglion cell decreases in numbers during ontogeny. At birth , there are three to six horizontal rows of ganglion cell , while it reduces to about a single row in adult retina. Quantification of cellular loss in the retina of the rat , cat and mouse （Chen , 1999）, shows that the ganglion cells decrease in the retina of *M. oeconomus*. Further examination of RGC loss along retinal development and the number of rods and cones by the immunocytochemical methods is currently under study and will show elsewhere.
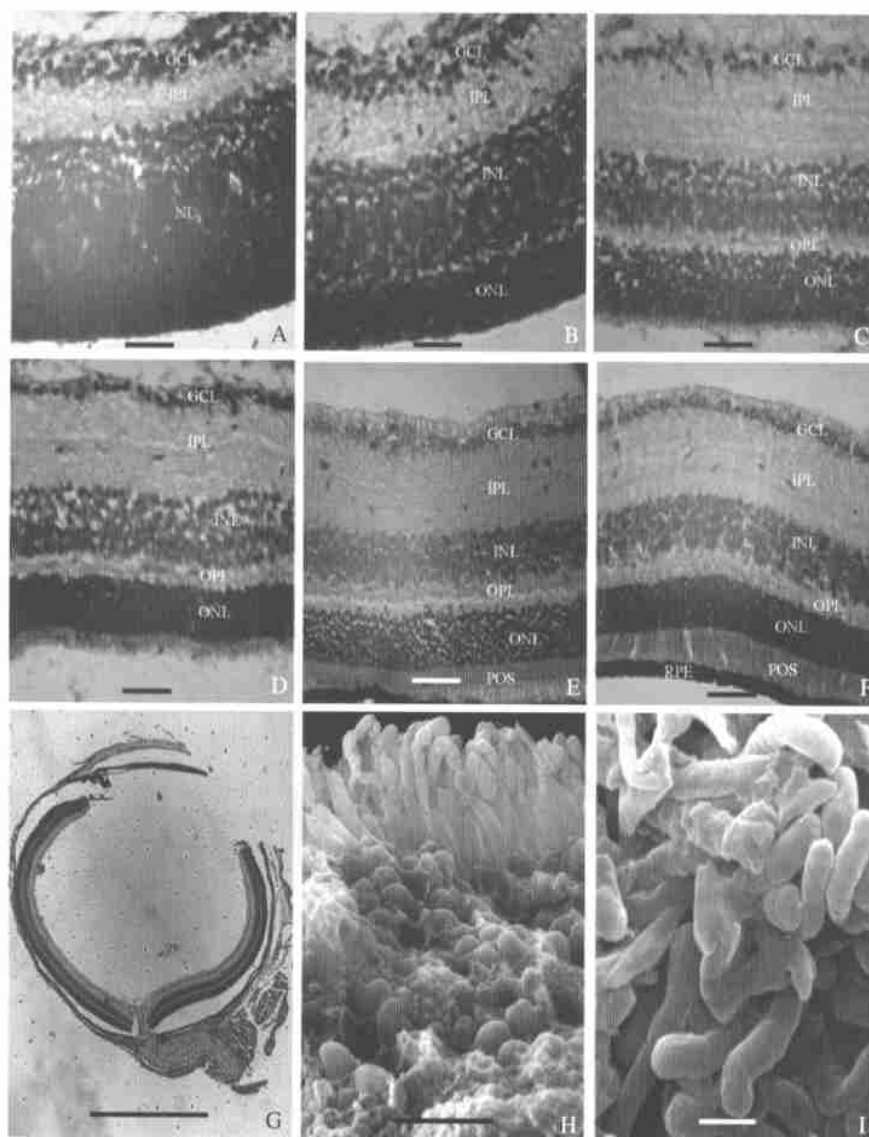
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A. The retina is primitive at PD3. The neuroblastic layer (NL) has not differentiated. Bar $= 50 \mu$m.

B. The neuroblastic layer begins to differentiate at PD5. The outer plexiform layer (OPL) first comes into existence. Bar $= 50 \mu$m.

C. The outer nuclear layer (ONL) and inner nuclear layer (INL) become distinct at PD6. Bar $= 50 \mu$m.

D. The retina is not mature when *M. oeconomus* opens its eyes. Bar $= 50 \mu$m.

E. In P18 retina , each layer is similar to the adult retina. Bar $= 50 \mu$m.

F. In the adult retina , the ganglion cells have been arranged into a single row. Bar $= 50 \mu$m.

G. The photomicrograph of meridional of the eye of *M. oeconomus*. Bar $= 250 \mu$m.

H. Each layer of the *M. oeconomus* retina with SEM , Bar $= 100 \mu$m.

I. The photoreceptor of the *M. oeconomus* retina with SEM. Bar $= 20 \mu$m.