Histological studies on the telencephalon of *Hynobius leechii* at the metamorphosis phase and the adult phase

Ying-Ying ZHOU¹, Ran SHAO¹, Chuan-Cheng LIANG¹, Yong WANG², Li-Wen WANG¹

¹College of Chemistry and Life Science, ²Experimental Center, Shenyang Normal University, Shenyang 110034, China

Abstract: Objective To investigate the telencephalon developmental characteristics of *Hynobius leechii*, and enrich the research data of comparable neurobiology and nervous system development of amphibian. **Methods** HE staining and Nissl staining methods were used to study the telencephalon histological structure of *Hynobius leechii* at both the metamorphosis and the adult phases, and to explore the developmental phases of telencephalon. **Results** The olfactory bulb could be roughly divided into 6 layers from lateral to medial. The lateral cerebral ventricles at the metamorphosis phase were smaller than those at the adult phase, and there were no clear borderlines between the primordial pallium and the primordial hippocampus, or between the primordial pallium and the primordial piriform area. Moreover, the cells in the primordial piriform area were more closely distributed than those in the primordial hippocampus or the primordial pallium. Compared with those at the adult phase, cells in nucleuses at the metamorphosis phase were larger in number and more closely distributed. **Conclusion** The telencephalon of *Hynobius leechii* at the metamorphosis phase has generally formed the adult structure, however, it is still at a transition state of differentiation to maturity during the development of *Hynobius leechii*.

Keywords: *Hynobius leechii*; telencephalon; histology; metamorphosis phase; adult phase

1 Introduction

The development of the nervous system is an important part of organism growth. The development of brain, which is crucial in the central nervous system, can demonstrate the evolution of species to some extent. Currently, considerable studies on telencephalon of the vertebrates have been reported. These investigations include research on fish, such as *Sinipeca chuatsi*^[1], *Schizothorax prenanti*^[2], and *Onchorhynchus keta*^[3]; the histological studies on the telencephalon of tailed amphibians, such as *Onychodactylus fischeri*^[4], the Chinese giant salamander^[5], and *Batrachuperus*

tibetanus^[6]; the correlative mode between cytoarchitecture of the ventral telencephalon and the axons in *Plethodon shermani*^[7]; the telencephalic efferents of *tiger salamander*^[8]; the olfactory bulb, accessory olfactory bulb and cerebral hemisphere of Anuran amphibian^[9,10]; the olfactory bulbs, olfactory tract and central nervous telencephalon of the reptiles, such as new-Hatching *Alligator sinensis*^[11-14]; and the Golgi-Cox of anterior dorsal ventricular and the rostralateral area of anterior dorsal ventricular (ADVR) of telencephalon in Gekko gecko^[15,16]. However, few studies have been reported on the developmental process of the central nervous system, except on that of *trituberculatus*^[17], *goats*^[18], and *Xenopus*^[19,20]. *Hynobius leechii* is an amphibian belonging to *Hynobiidae*, *Caudata*, and distributes specifically in the northeast of China and in Korean Peninsula^[21]. However, little information on the development of the nervous system of *Hynobius leechii* can be found due to regional restric-

Corresponding author: Li-Wen WANG
Tel: +86-024-86593307
E-mail: wangliwen1956@163.com
Article ID: 1673-7067(2009)04-0196-07
CLC number: Q954.67
Document code: A
Received date: 2009-02-13

tions and limited research conditions. In the present study, the histological characteristics of telencephalon of *Hynobius leechii* at both the metamorphosis phase and the adult phase were investigated using HE staining and Nissl staining, to reveal the developmental processes of telencephalon of *Hynobius leechii* and to enrich the research data on comparable neurobiology, comparative histology and nervous system development of amphibian.

2 Materials and methods

The adult *Hynobius leechii* and the egg sacs of *Hynobius leechii* were obtained from Dashihu Natural Conservation Area in Benxi City, Liaoning Province, China. The egg sacs were then cultured to develop to the metamorphosis phase.

A total number of 5 *Hynobius leechii* at the metamorphosis phase and the adult phase were selected, respectively. The animals were anesthetized with aether prepared in 10% formaldehyde solution, and then the intact brain tissue was isolated under Olympus CX21 anatomic microscope. The tissue was then embedded in olefin and transections were cut at 8 μm , followed by HE staining and Nissl staining. In the sequential sections of telencephalon, every other 5 samples were selected for histological analysis. The telencephalon structure was observed using Olympus CX21 and photos were taken by BX-50 Olympus photomicroscope system. The organizational model diagram was drawn with computer software. The sections were measured with micrometer under microscope. Data were analyzed by Microsoft Excel and presented as mean $\pm$ SD.

3 Results

The telencephalon of *Hynobius leechii* which is located at the anterior part of the brain, is composed of olfactory bulb and hemisphere. Results showed that the structures of telencephalon at the metamorphosis phase and the adult phase were generally similar. However, there were some differences in neuron quantity and density, and the cell scope of nucleus.

3.1 Olfactory bulb The olfactory bulb in the central nervous system was located at rostra and ventral lateral position of telencephalon (Fig. 1A, 2A). At the metamorphosis phase,

no distinct borderline existed between the olfactory bulb and the hemisphere, and the olfactory bulb could be divided into 6 layers from lateral to medial without clear boundaries, including the stratum nervosum, glomerular layer, external plexiform layer, mitral cell layer, internal plexiform layer and granule cell layer. Mitral cells are specifically located within olfactory bulb, and named from their mitral shapes and large sizes. As observed under the microscope, mitral cells at the metamorphosis phase were almost round in shape with the diameter of about $11.0\pm0.5\ \mu\text{m}$. They were lightly stained and located between the internal and the external plexiform layers, and there were crosses between the internal and external plexiform layers and the glomerular layer and between the internal and external plexiform layers and the mitral cell layer. No distinct dividing line existed between different cells. Meanwhile, although granule cells were similar in shape with mitral cells, they were smaller than mitral cells with a diameter of about $0.6\pm0.2\ \mu\text{m}$, more frequently located at medial positions and stained heavily. At medial anterior ledge, there were only 2-3 rows of granule cells, whose thickness increased gradually in the caudal position. The olfactory bulb of the telencephalon of *Hynobius leechii* at the adult phase exhibited similar structural characteristics with that at the metamorphosis phase. However, at the adult phase, the boundary between the olfactory bulb and the hemisphere was relatively clearer and the cell layer of the olfactory bulb was more distinct. Besides, both mitral and granule cells were similar in size with those at the metamorphosis phase, but they were larger in quantity and higher in density as compared with those at the metamorphosis phase.

3.2 Hemisphere The cerebral hemisphere of *Hynobius leechii* consists of the lateral cerebral ventricle, the primordial pallium, the primordial hippocampus, the primordial piriform area, the septal area, the striatal complex and the amygdala.

3.2.1 Lateral cerebral ventricle As shown in Fig. 1B, the lateral cerebral ventricles of *Hynobius leechii* at the metamorphosis phase were 2 narrow cavums in the medial of hemisphere. From rostra to caudal, the 2 crescent-shaped lateral cerebral ventricles gradually became wider, with the presence of the choroids plexus anterior in transversal posi-

Fig. 1 HE staining images of sequential sections of the telencephalon of *Hynobius leechii* at metamorphosis phase arranged in the rostrocaudal sequence. A: olfactory bulb, B-E: hemisphere. an: amygdale nucleus; cp: choroids plexus; lv: lateral ventricle; o: olfactory bulb; pal: primordial pallium; ph: primordial hippocampus; pr: primordial piriform; se: septum; sp: striatal complex; tv: third ventricle.

Fig. 2 Nissl staining images of sequential sections of the telencephalon of *Hynobius leechii* at adult phase arranged in the rostrocaudal sequence. A: olfactory bulb, B-E: hemisphere. an: amygdale nucleus; cp: choroids plexus; lv: lateral ventricle; o: olfactory bulb; pal: primordial pallium; ph: primordial hippocampus; pr: primordial piriform; se: septum; sp: striatal complex; tv: third ventricle.

tion (Fig. 1C, D). At the metamorphosis phase, the largest transversal width ($50.0 \pm 1.7 \mu\text{m}$) of the lateral cerebral ventricle was located at the middle of rostra-caudal line, and its length was $220.2 \pm 2.8 \mu\text{m}$. Moreover, the cavums of lateral cerebral ventricle of *Hynobius leechii* at the metamorphosis phase were smaller than those at the adult phase, in which the largest transversal width was $200.3 \pm 4.6 \mu\text{m}$, and its length was $580.6 \pm 3.0 \mu\text{m}$. Although there were more choroids plexus at the adult phase (Fig. 2C), the location of the largest transversal width was similar to that at the metamorphosis phase. At both the metamorphosis and the adult phases, the lateral cerebral ventricles were 2 separated cavums in the anterior position of the hemisphere, corresponding to the separated cerebral hemispheres, while in the caudal position of the telencephalon, the 2 separated cavums were connected with each other.

3.2.2 Primordial pallium The primordial pallium in most anterior position was located at dorsal position of the lateral cerebral ventricle (Fig. 1B-E, Fig. 2B-E). Cells were round or oval in shape and distributed densely. As revealed by HE staining, the nucleus was stained into heavy blue, and generally one or two nucleolus were stained into heavy color in each cell. With the appearances of primordial hippocampus and the septal area, the primordial pallium zone gradually centralized on the dorsolateral of the hemisphere. There was no obvious terminal line between the primordial pallium and the dorsal part of primordial hippocampus, nor between the primordial pallium and the superior part of primordial piriform area. Moreover, there was no significant difference in the primordial pallium zone between the metamorphosis phase and the adult phase except the greater cell density and larger cell size at the metamorphosis phase. The sequential sections of primordial pallium zone of telencephalon at the metamorphosis phase revealed that the cells were initially concentrated on the dorsal part of hemisphere and then gradually migrated to the ependyma of lateral cerebral ventricle.

3.2.3 Primordial hippocampus As shown in Fig. 1 and Fig. 2, the primordial hippocampus was located at the medial wall of ventricles, extending to the third ventricle. The structural characteristics of the primordial hippocampus at the metamorphosis phase were similar to those at the adult phase.

Primordial hippocampus in the anterior portion of the hemisphere was poorly developed, while its size was gradually increased in caudal orientation but decreased in the third ventricle. Primordial hippocampal cells were larger than primordial pallium or piriform cells, appearing in spherical or oval shapes. Moreover, compared with those at the metamorphosis phase, primordial hippocampal cells at the adult phase were smaller in number and distributed more sparsely, but in larger sizes.

3.2.4 Primordial piriform area The primordial piriform area was located at the lateral position of ventricle (Fig. 1B-E, Fig. 2B-E). Compared with that at the adult phase, the boundary between the primordial piriform area and the olfactory bulb at the metamorphosis phase was more distinct in rostral sections of telencephalon. There was no obvious borderline between the primordial piriform area and the primordial pallium at the metamorphosis phase. Moreover, the cells in the primordial piriform area at the metamorphosis phase were not only larger and denser than those at the adult phase, but also larger than those in the primordial hippocampus zone.

3.2.5 Septal area Located at the ventromedial part of the primordial hippocampus, the septal area at the metamorphosis phase shared structural similarities with that at the adult phase (Fig. 1C, 2C). In the anterior sections of telencephalon, there was no obvious boundary between the septal area and the primordial hippocampus. In the middle sections of telencephalon, the cell number was increased and the cells began to laminate, structure of which at the metamorphosis phase was distinct, and the cell number and the area were both larger than those at the adult phase.

3.2.6 Striatal complex Located in the ventral part of the primordial piriform (Fig. 1C, D, Fig. 2C, D), the striatal complex was a relatively bigger nucleus at the ventrolateral of hemisphere and was located at the bottom of the cerebral ventricle, especially at the connecting positions of the ventricles. The main function of the striatal complex is to coordinate the actions of the organism. In vertebrates except the mammals, pallium is poorly developed, thus the striatal complex becomes the topmost kinetic nerve central instead^[22]. The striatal complex of *Hynobius leechii* belongs to paleostriatum in evolution. Our results showed that the striatal

complex had appeared at the metamorphosis phase, during which cells in the striatal complex were larger and denser, and the cell number was also larger, compared with those at the adult phase.

3.2.7 Amygdala As shown in Fig. 1E and Fig. 2E, the amygdala consisted of 2 cell groups that were located in 2 ventrolateral positions of the third ventricle. There were no differences in the location or structure of the amygdala between the metamorphosis phase and the adult phase. However, the number and the size of cells in the amygdala at the metamorphosis phase were both larger compared with those at the adult phase.

4 Discussion

The olfactory bulb is one of the vital sensory organs by which lower animals perceive the world. The olfactory bulb of *Hynobius leechii* is located in ventrolateral of hemisphere. Instead of developing into a special olfactory central structure, the olfactory bulb is separated from the hemisphere. The cell layers of the olfactory bulb of *Hynobius leechii* are similar to those of tailed amphibians, such as *Chinese giant salamander*^[5], *Batrachuperus tibetanus*^[6] and *Onychodactylus fischeri*^[4], and their olfactory bulbs can be roughly divided into 6 layers with no obvious borderlines. However, in some anurans, the olfactory bulb is located in rostra tip of hemisphere, connecting olfactory tract to the hemisphere, and the olfactory bulb has 7 cell layers with distinct accessory olfactory bulbs^[9,10]. The olfactory bulb of Yangzi crocodile, which belongs to crawling animals, has 8 cell layers with distinct structures and the mitral cells are tightly and evenly arranged^[11]. Lower animals, such as fish^[2], have only 3 cell layers in the olfactory bulb, including the epithelium, the layer of the olfactory nervous fibers and the small cellular layer. Observations of the structures of olfactory bulb in fish^[1-3], amphibians^[9,10], reptiles^[11,13] and mammals^[23] indicate that the olfactory bulb of *Hynobius leechii* is poorly developed, however, the olfactory bulb of *Hynobius leechii* at the metamorphosis phase has cells of all the styles, just like the adult olfactory bulb, which gives us a hint that the olfactory function of *Hynobius leechii* at the metamorphosis phase has been formed, but it is inferior to that at the

adult phase.

The primordial pallium of *Hynobius leechii* which belongs to tailed amphibians, is part of archicortex, and the primordium hippocampi of *Hynobius leechii* is lower than that of mammals, for example, the adult rat^[24]. The primordial pallium of fish, such as *Onchorhynchus keta*^[3], is less developed than that of tailed amphibians, and there are few or even no nerve tissues in the primordial pallium. For example, the cerebral cortex in new-Hatching *alligator sinensi* in reptiles belongs to neocortex, occupying the whole dorsal hemisphere except the anterior hemisphere. There are more cells in the cerebral cortex than in the hippocampus or the piriform. Besides, compared with the archicortex, the neocortex becomes thicker and can be divided into 3 parts: outer plexiform layer, cell layer and inner plexiform layer. The cerebral cortex of *Phrynocephalus frontalis* and *Eremias arguta* are better developed, divided into 3 parts—the medical cortex layer, the dorsal cortex layer and the lateral cortex layer^[25]. The primordial pallium of *Hynobius leechii* at the metamorphosis phase is similar to that at the adult phase, however, cells are denser and larger than those at the adult phase. At the metamorphosis phase, the lateral cerebral ventricle is much smaller and the choroids plexus in the lateral cerebral ventricle is less abundant, compared with that at the adult phase. Although there are no differentiations between the primordial piriform area and the primordial pallium in the transverse sections of the lateral cerebral ventricle, they can be distinguished by histological characteristics that the cells in the dorsolateral of the lateral cerebral ventricle transfer towards upper side portion and form the primordial pallium. In some anurans, the medial limiting sulcus separates the primordial hippocampus from the septal area, the latter of which consists of medial septal nucleus and lateral septal nucleus, while the lateral limiting sulcus separates the primordial piriform from the corpus striatum^[9]. The septal area and the lateral septal nucleus of *Hynobius leechii* are not obvious, while medial limiting sulcus appears in subadults of *Onychodactylus fischeri*, but neither medial limiting sulcus nor lateral limiting sulcus is present in adults^[26]. The septal area of *Hynobius leechii* is less developed than that of anurans. The difference in caudata between *Onychodactylus fischeri* and *Hynobius leechii* needs

to be further investigated. The histological results indicate that neuron quantity and density in the transversal sections of telencephalon at the metamorphosis phase are greater than those at the adult phase, which may result from division of neuroblasts, hyperplasia and differentiation, and neuron apoptosis in the process of neural system maturation.

Based on the analysis above, it can be concluded that the telencephalon of *Hynobius leechii* at the metamorphosis phase is not mature and the metamorphosis phase is still the interim towards maturation although some structures similar to those at the adult phase have been formed during this period.

Acknowledgement: This work was supported by the Sciences Foundation of Department of Education, Liaoning Province, China (No. 20060840).

References:

- [1] Huang XZ, Yang XP, Wang Q. Histological study of the telencephalon of mandarin fish *sinipeca chuatsi*. J Huazhong Agric Univ 1996, 15(2): 168-171.
- [2] Pan KC, Fang J. Study on the Telecephlon Morphology and Histology of *Schizothorax prenati*(Tchang). J Sichuan Agric Univ 2002, 20(2): 144-147.
- [3] Pushchina EV, Varaksin AA, Romanov NS. Neuronal organization of the telencephalon in the Chum Salmon *Onchorhynchus keta*. Russ J Mar Biol 2003, 29(6): 362-367.
- [4] Wang HH, Li LY, Wang LW, Liang CC. Preliminary studies on morphology and histology of telencephalon of *Onychodactylus fischeri*. Neurosci Bull 2007, 23(3): 170-174.
- [5] Zhang YH, Liang G. Study on the telecephalon of Chinese Giant Salamander. Study of Amphibia and Reptilia 1995, 4-5: 30-34.
- [6] Jiang YM, Zhang YH. Histological observation on cytoarchitecture of telencephalon in stream salamander (*Batrachuperus tibetanus*). J Shaanxi Norm Univ (Nat Sci Ed) 2003, 31(4): 80-83.
- [7] Laberge F, Roth G. Connectivity and cytoarchitecture of the ventral telencephalon in the salamander *Plethodon shermani*. J Comp Neurol 2005, 482(2): 176-200.
- [8] Kokoros JJ, Northcutt RG. Telencephalic efferents of the tiger salamander *Ambystoma tigrinum tigrinum* (Green). J Comp Neurol 1977, 173(4): 613-628.
- [9] Hoffman HH. The olfactory bulb, accessory olfactory bulb, and hemisphere of some anurans. J Comp Neurol 1963, 120: 317-368.
- [10] Fritz A, Gorlick DL, Burd GD. Neurogenesis in the olfactory bulb of the frog *Xenopus laevis* shows unique patterns during embryonic development and metamorphosis. Int J Dev Neurosci 1996, 14(7-8): 931-943.
- [11] Yang CX, Yang C, Chen BH. A histologic study of olfactory bulb and olfactory tractus in new-hatching *Alligator sinensis*. J Anhui Norm Univ (Nat Sci Ed) 1999, 22(4): 321-323.
- [12] Yang CX, Yang C, Chen BH. The histologic study of the cerebral cortex in new-hatching *Alligator sinensis*. J Anhui Norm Univ (Nat Sci Ed) 2000, 23(1): 43-46.
- [13] Yang CX, Chen BH. The ultrastructural study of olfactory bulb in *Alligator sinensis*. Life Sci Res 1999, 3(3): 239-245.
- [14] Yang CX, Yang C, Chen BH. The histologic study of the basal center in Telencephalon of new-hatching *Alligator sinensis*. J Anhui Norm Univ (Nat Sci Ed) 2002, 25(3): 270-273.
- [15] Tang ZX, Jiang SY, Shi M, Lan Y, Pang SW. The Golgi-Cox research of anterior dorsal ventricular in Gekko gekko. Anat Res 2000, 22(1): 1-4.
- [16] Shen DW, Zhang XH. Electron microscopic study on synapses in The rostralateral area of ADVR of telencephen of Gekko gekko. J Chin Electr Microsc Soc 1998, 17(3): 213-219.
- [17] Xue JZ. Neurogenesis and development of the central nervous system in the embryo stage of swimming crabs, *Portunus trituberculatus*. Zoolog Res 2005, 26 (2): 184-189.
- [18] Xu YP, Qing SZ, Zhao HY. The histogenesis and development of cerebral cortex in goat fetus. Chin J Vet Sci 2006, 26(5): 551-560.
- [19] David Tay, Charles Straznick. The development of the dien-cephalon in *Xenopus*. An Autoradiographic Study. Anat Embryol 1982, 163: 371-388.
- [20] Gaze RM. and Grant P. Development of the tectum and dien-cephalon in relation to the time of arrival of the earliest optic fibres in *Xenopus*. Anat Embryol 1992, 185: 599-612.
- [21] Zhao EM, Hu QX. Studies of Chinese tailed amphibian. Sichuan. Publication House of Science and Technology, 1984,p1.
- [22] Yang AF, Cheng H. Comparative anatomy of Vertebrate. Beijing: Peking University Press, 1999, p238-265.
- [23] Hamburger V, Hamilton HL. A series of normal stages in the development of the chick embryo. J Morphol 1951, 88(1): 49-92.
- [24] Chen L, Gong S, Shan LD, Xu WP, Zhang YJ, Guo SY, *et al.* Effects of exercise on neurogenesis in the dentate gyrus and ability of learning and memory after hippocampus lesion in adult rats. Neurosci Bull 2006, 22 (1):1-6.
- [25] Zhang FJ, Pang JQ, Wang YZ. The histologic study of telencephalon of two Species of Lizards. Acta Herpetol Sinica 1988, 6: 81-86.
- [26] Wang HH, Li LY, Wang LW, Liang CC. Preliminary study on histology of telencephalon of adult and subadult *Onychodactylus fischeri*. Sichuan J Zool 2008, 27(2): 223-226.

变态期与成体东北小鲦的端脑组织学研究

周莹莹¹, 邵然¹, 梁传成¹, 王勇², 王丽文¹

¹沈阳师范大学化学与生命科学学院 沈阳 110034

²沈阳师范大学实验中心 沈阳 110034

摘要: **目的** 探讨东北小鲦端脑发育的阶段性特点, 充实两栖类比较神经生物学和神经系统发育的研究资料。**方法** 采用HE染色法和尼氏染色法, 初步研究变态期和成体东北小鲦端脑的组织学结构特征, 探讨其端脑的部分发育过程。**结果** 嗅球结构由外侧向内侧大致分为6层。变态期东北小鲦端脑侧脑室腔小于成体东北小鲦, 并且其原始大脑皮层, 原始梨状区和原始海马的界限都不很清晰。原始梨状区的细胞分布与原始大脑皮层细胞和原始海马细胞相比, 更为密集。变态期各核团的细胞数量和细胞密集程度均显著大于成体水平。**结论** 变态期东北小鲦的端脑大致具备了成体结构, 但仍处于脑发育分化走向成熟的过渡阶段。

关键词: 东北小鲦; 端脑; 组织学; 变态期; 成体