Morphological and histological studies on the telencephalon of the salamander *Onychodactylus fischeri*

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Abstract: Objective In order to probe into the morphological and histological characteristics of the telencephalon of *Onychodactylus fischeri*, and to enrich the comparable neurobiology. **Method** HE-staining method was used to describe the characters of the telencephalon of *Onychodactylus fischeri*. **Results** The olfactory bulb of *Onychodactylus fischeri* locates in the rostral and lateral to the cerebral hemisphere, and six distinct layers can be identified from the lateral to the medial, quite similar to *Batrachuperus tibetanus* and *Hynobius leechii*. In the cerebrum, the primordial hippocampus developed better than the primordial piriform. The former belongs to archipallium and the latter is paleopallium. Ventral to the primordial hippocampus there is a septal area which cannot be divided into medial and lateral parts. In the ventricular wall, there is neither medial limiting sulcus nor lateral limiting sulcus to separate the primordial hippocampus and the septal area, or the primordial piriform and the corpus striatum. The corpus striatum of *Onychodactylus fischeri* is paleostriatum. There is choroids plexus anterior in the lateral ventricle. The cell group that located at two sides of the third ventricle is the amygdale. Besides, the shape and size of neurons within the telencephalon are poorly differentiated. **Conclusion** *Onychodactylus fischeri* is a relatively primitive type in the amphibian. The present data will help us to further understand the nerve system of tailed amphibian.

Keywords: salamander; telencephalon; morphology; histology

1 Introduction

Onychodactylus fischeri, belonging to the *Onychodactylus Tschudi* (Amphibia: Caudata: Hynobiidae), is a very rare and special kind of tailed amphibious animal. Lung of the mature *Onychodactylus fischeri* degenerates and disappears. There are corneous talons on their fingers and toes. It takes the young about three years to complete metamorphism in natural environment, which is a very long time. Fortunately the young with lamella can come through winter. It has reported that there are two kinds of *Onychodactylus fischeri* in other countries, including Korea, Japan, and the far east region of Russia. In China only one kind of *Onychodactylus fischeri* was distributed in Tonghua, Jilin province, and Xiuyan, Liaoning province, both in the northeast of China^[1]. Imputing to the limited

zone of distribution, the languishing amount, the contaminative environment around, and the gradually warmed climate, *Onychodactylus fischeri* are lined in the list of endangered animals^[2]. At present, the research concerning *Onychodactylus fischeri* is still at primary phase, involving its skeleton system, digestive system and the ultrastructure of its skin^[3-5]. However, the morphological and histological studies concerning the neural system of *Onychodactylus fischeri* is still a blank. Even data of the neural system of other relative tailed amphibian animals are insufficient, though some primary research on the telencephalon of Chinese giant salamander, *Batrachuperus tibetanus*, and *Hynobius leechii* has been reported^[7,9,10]. In this work, we made vertebrate nerve samples and used HE-staining to observe the characteristics of external configurations and the histological structures of the telencephalon of *Onychodactylus fischeri* on the basis of its intact brain dissection, and compared these structures with those of other tailed amphibian with a purpose to enrich the information about the central nerve system of tailed amphibian animals, to probe into the evolutionary degree of *Onychod-*

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Article ID: 1673-7067(2007)03-0170-05
CLC number: Q954
Document code: A
Received date: 2007-01-19

actylus fischeri, and to settle a base for the further study.

2 Materials and methods

Three mature *Onychodactylus fischeri* (ignoring the sex, length 11.5–16.7 cm) used in our experiment were obtained from Sanjiazi village, Xiuyan county of Liaoning province, China. .

The animals were anesthetized with aether and fixed in 10% formaldehyde solution. The intact brain was separated under Olympus CX21 anatomic microscope, then embedded with olefin and sectioned transversely at 8 μ m. The sections were stained by HE-staining. The photos of the sections were taken by Moticam1300 photomicroscope system. In the serial sections of telencephalon, we selected samples every other five sections to make histological analysis.

3 Results

3.1 Morphology of telencephalon

The telencephalon of *Onychodactylus fischeri* is located at the rostral part of its brain, covered with dura mater membrane and pia mater membrane. It can be separated into the olfactory bulb and the hemisphere. There are other structures of central nervous system such as diencephalons, midbrain, myelencephalon and spinal cord located caudally to the telencephalon (Fig. 1).

3.1.1 Olfactory bulb The olfactory bulb of *Onychodactylus fischeri* is an olfactory nerve center, which locates at ventrolateral of the rostral hemisphere. From ventral view of the brain, there is a clear dividing line between the olfactory bulb and the hemisphere, while the line is obscure in dorsal view. The olfactory bulb is an expended portion of rostral hemisphere (Fig. 1B), where the olfactory nerve terminates.

3.1.2 Hemisphere Caudal to the olfactory, the hemisphere is divided into left and right portion, both of which have oval shape. Approximately the rostral one third hemisphere is separated into two parts by midline and the posterior two thirds portion links together. In dorsal view, the midline is a relatively deep sulcus, which terminates at the caudal telencephalon. In ventral views, the midline is obscure. The cavity formed in left and right hemisphere are called ventricle (Fig. 1).

3.2 Histology of telencephalon In transverse sections of telencephalon of *Onychodactylus fischeri*, most of neurons are round, oval or approximately round in shape.

Nucleoli of the neurons were stained into amethyst and cytoplasm were stained into pink. The density of neurons is different in different areas of the brain.

3.2.1 Olfactory bulb The olfactory bulb of *Onychodactylus fischeri* is typically laminated, and six layers can be identified from the lateral to the medial. They are stratum nervosum, glomerular layer, external plexiform layer, mitral cell layer, internal plexiform layer, and granule cell layer. The dividing lines between them are somewhat faint. In general, the distribution of cells in ventrolateral olfactory bulb is disperser than that in the medial portion (Fig. 2).

The stratum nervosum is composed of the terminal branches of olfactory nerve fibers that encircles the olfactory bulb, and in microscope, this layer is relatively equal in the surface of the olfactory bulb.

The glomerular layer is a tight wrap formed by the terminal of olfactory nerve and mitral cells, around which there are periglomerular cells with small size.

The mitral cell layer contained the most characteristic cells of the olfactory bulb, which are named after their mitral shape, located between the external plexiform layer and the internal plexiform layer, but there are crosses between these layers.

The granule cell layer is concentrated in the inner part with small size and round or starlike shape.

3.2.2 Hemisphere In the caudalward preparations, two crescent-like cavums called ventricles of the hemisphere (Fig. 3A) appear. In the frontier portion of the telencephalon, the two ventricles separate from each other and so do the left and the right hemisphere, but in rear preparations, the ventricles appear wider and longer gradually, and the choroids plexus anterior appears (Fig. 3B). The separated ventricles connect with each other in the end of the telencephalon (Fig. 3C). In different transverse sections of the hemisphere, the neuronal cytoarchitectures are as follows.

3.2.2.1 Primordial hippocampus This part locates at the inboard wall of the ventricle, extending to a position where the third ventricle appears (Fig. 3D). Neurons of the primordial hippocampus are of bigger size and lightly stained. The cells distribute more and more intensive from the midline to the ventricle wall in the most developed portion, where it pops out toward the ventricle (Fig. 3C).

3.2.2.2 Septal area Septal area locates at the ventromedial in the primordial hippocampus, and it cannot be separated from the primordial hippocampus at rostral telencephalon. In the middle portion of telencephalon, cells

Fig. 1 Brain and the spinal cord. A: dorsal view; B: ventral view. Scale bar, 0.6 mm.

Fig. 2 Photomicrograph of a transverse section through the olfactory bulb of *Onychodactylus fischeri* (HE staining). gc, granule cell layer; g, glomerular layer; m, mistral cell layer; nf, nfstrum nervosum; o, olfactory bulb. Scale bar, 110 μ m.

of septal area are more scatter than those of primordial hippocampus, but a dividing structure to separate this area into medial and lateral nucleus cannot be seen (Fig. 3B).

3.2.2.3 Primordial piriform area It locates at laterals of the ventricle. The neurons of this part are separated clearly from those of olfactory bulb in most rostral part of the telencephalon (Fig. 3A). Cells distribution becomes disperse from the ventricle wall to the dorsolateral. The neurons of this portion are stained heavily in cytoplasm. Two or more cells are connected with each other, and some of them are overlapped (Fig. 3B).

3.2.2.4 Striatal complex At the ventral to the primordial piriform and the bottom of the ventricle, a cell group called striatal complex can be identified, especially at the portion where two ventricles connected (Fig. 3C). Both nucleolus and cytoplasm of the neurons here are stained heavily. Striatal complex is a relatively bigger nucleus at the fundus of the hemisphere, whose function is to harmonize the action of organism. In all kinds of vertebrate except mammalian, the pallium is poorly developed, and the corpus striatum becomes the topmost kinetic nerve central instead^[8]. The striatum of *Onychodactylus fischeri* belongs to the paleostriatum in revolution.

3.2.2.5 Primordial pallium Primordial pallium locates at the most dorsal extent of the ventricle and connects with both the pirimordial hippocampus and the piriform primordium. The cells in this area are stained heavily and their distribution is greatly dense (Fig. 3A, B).

3.2.2.6 Choroids plexus anterior In the successive HE transverse sections through the hemisphere of the telencephalon, choroids plexus anterior is stained into pink (Fig. 3B, C). It is composed of epithelium and pia mater membrane, which is rich in blood vessel. The choroids

Fig. 3 Four transverse sections through the cerebral hemisphere from the rostral part to the caudal part (HE staining). am, amygdale; cp, choroids plexus; lv, lateral ventricle; o, olfactory bulb; pal, primordial general pallium; ph, primordial hippocampus; pr, primordial piriform; se, septum; sp, striatal complex; tv, third ventricle. Scale bar, 146 μ m.

plexus in the diencephalon crosses forwardly through interventricle bore and terminates in the ventricle to form the choroids plexus anterior.

3.2.2.7 Amygdala The cell group that located at two sides of the third ventricle is amygdala, both cell nucleolus and cell cytoplasm of which are stained heavily (Fig 3D). The function of this nucleus complex is to accept the nerve fibers mainly from the olfactory and then transfers them to the hypothalamus^[14].

4 Discussion

The olfactory of *Onychodactylus fischeri* locates at the ventrolateral of rostral hemisphere. It cannot be divided clearly from the hemisphere, while in some anurans, the olfactory bulb is in the most rostral part of the telencephalon contacting with hemisphere through an olfactory tract^[6,13]. It indicates that the telencephalon of *Onychodactylus fischeri* is primitive to some extent, and it does not differentiate an independent olfactory nerve centre from the hemisphere. In some advanced vertebrates, with the augmentation of the hemisphere in bulk, the proportion of olfactory structure decreases. It recedes to the ventral part of rostral hemisphere, playing a subordinate role. In histological study, *Onychodactylus fischeri*, *Batrachuperus tibetanus*^[7] and Chinese giant salamander^[10] have similar laminated structures in their olfactory bulb, which is different from those of amphibious and reptile ones. For instance, cells in the olfactory bulb of Chinese alligator are divided into eight layers, and the dividing zone can be clearly identified. The mitral cells distribute densely and equally^[11]. The olfactory bulb of anurans can be divided into seven layers and an obvious accessory olfactory bulb can be identified^[6]. While in *Onychodactylus fischeri* and *Batrachuperus tibetanus*, there are no accessory olfactory bulbs. Its cells are divided into six layers, and no specific limit can be seen. Its mitral cells distribute dispersely. All of these show that the olfactory function of *Onychodactylus fischeri* is not developed.

Similar to *Hynobius leechii*^[9] and *Batrachuperus tibetanus*^[7], the most developed structure in the telencephalon of *Onychodactylus fischeri* is also the primordium hippocampi. Its neurons begin to migrate to the surface from the rostral hemisphere to the third ventricle. Both the area they occupied and the thickness are obviously larger than those of the primordial piriform, so the primordium hippocampi of *Onychodactylus fischeri* belongs to

archipallium, but the shape and size of cells do not have obvious difference. In anurans, the primordium hippocampi can be distinguished to the dorsal part and the dorsalmedial part. The function of the primordium hippocampi of *Onychodactylus fischeri* is somewhat similar to anurans, but its higher function can be determined in adult rats by hippocampus lesion^[15]. Besides, there is a medial limit sulcus to separate primordium hippocampi and septal area. And the latter is distinguished from the lateral septal nucleus and the medial septal nucleus^[6]. But in *Onychodactylus fischeri*, there is not a clear limit between the primordium hippocampi and the septal area, and the septal area cannot be distinguished from different nucleus complexes. It indicates that *Onychodactylus fischeri*, as one kind of tailed amphibians, is relatively primitive in amphibious animals.

In the primordial piriform area of *Onychodactylus fischeri*, the nerve cells muster in the medial portion is near to the ventricle. According to the less layers and the smaller area they occupied, the primordial piriform area belongs to the paleopallium. In preparations of the telencephalon of some anurans, it can be seen that a lateral limit sulcus separates the primordial piriform area and the striatal complex^[6], but this limit cannot be identified in the telencephalon of *Onychodactylus fischeri*.

The hemisphere of amphibious animals is controlled by the olfactory stimulation, and its dominating function is to feel smells. The pallium is poorly developed. Striatal complex becomes the topmost centrum to harmonize the movement of organism. As far as its volume, striatal complex is a small group relatively, and only locates at the ventral of primordial piriform area and the bottom of ventricle. While in fish, the telencephalon is composed mainly by the left and right striatal complexes, which occupies the most part of its hemisphere^[12]. This may be an evidence to estimate the involution position of *Onychodactylus fischeri*.

In conclusion, this work has analyzed the telencephalon of *Onychodactylus fischeri* in both morphology and histology. The results have revealed that the configuration of the telencephalon of *Onychodactylus fischeri* is simple and neurons construct originally in the histological transection. In a word, *Onychodactylus fischeri* is a primary species in the amphibious animals.

References:

- [1] Liu MY, Xie YH, Ji DM. Vertebrate of China. 1st ed. Shenyang:

- Liaoning University Press, 2000, 463-465.
- [2] Wang S, Zhao EM. China Red Data Book of Endangered Animals (Amphibia and Reptilia). Beijing: Science Press, 1998, 20-21.
- [3] Wang LW, Zhao YY, Liang CC. Studies on the Skeletal System of *Onychodactylus fischeri*. Sichuan J Zool 2004, 23: 172-177. (Chinese)
- [4] Zhao YY, Wang LW, Liang CC, Zhang JX. Preliminary studies on anatomy and histology of digestive system of *Onychodactylus fischeri*. Sichuan J Zool 2005, 24: 290-294. (Chinese)
- [5] Lu YY, Wang J, Liu XH, Zhou ZY, Chen XZ, Wang XA, *et al.* Microstructure of skin of fischer's clawed salamander (*Onychodactylus fischeri*). Sichuan J Zool, 2004, 23: 178-183. (Chinese)
- [6] Hoffman HH. The olfactory bulb, accessory olfactory bulb, and hemisphere of some anurans. J Comp Neurol 1963, 120: 317-368.
- [7] Jiang YM, Zhang YH. Histological observation on cytoarchitecture of telencephalon in stream salamander (*Batrachuperus tibetanus*). J Shaanxi Norm Univ Nat Sci Ed 2003, 31: 80-83. (Chinese)
- [8] Yang AF, Cheng H. Comparative Anatomy of Vertebrate. 1st ed. Beijing: Peking University Press, 1999, 238-265.
- [9] Li LY, Wang LW, Na J, Yu HX. Preliminary studies on morphology and histology of central nervous system of *Hynobius leechii*. Sichuan J Zool 2005, 24: 287-289. (Chinese)
- [10] Zhang YH. Study on the telencephalon of Chinese giant salamander. Study of Amphibia and Reptilia, 1995, 4-5: 30-34. (Chinese)
- [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: 321-323. (Chinese)
- [12] Pan KC, Fang J. Study on the Telencephalon Morphology and Histology of Schizothorax prenat (Tchang). J Sichuan Agric Univ 2002, 2: 144-147. (Chinese)
- [13] 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 Devl Neurosci 1996, 14: 931-943.
- [14] Moreno N, Morona R, Lopez JM, Munoz M, Gonzalez A. Lateral and medial amygdala of anuran amphibians and their relation to olfactory and vomeronasal information. Brain Res Bull 2005, 66: 332-336.
- [15] 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: 1-6.

爪鲵端脑的形态学与组织学研究

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摘要: **目的** 探讨爪鲵端脑的形态学和组织学特点。**方法** 采用脊椎动物神经标本制作技术和常规HE染色法。**结果** 爪鲵嗅球与北方山溪鲵和东北小鲵的嗅球极为相似, 位于大脑半球的腹外侧缘, 细胞从外到内大致可分为6层结构。大脑内原始海马较原始梨状区发达, 前者属于原脑皮, 后者属古脑皮。隔区位于原始海马的下方, 没有外侧隔核和内侧隔核之分。侧脑室的侧壁没有内侧界沟区分原始海马和隔区, 也没有外侧界沟作为原始梨状区和纹状体的分界。爪鲵的纹状体属于古纹状体。有前脉络丛伸入侧脑室。爪鲵的杏仁核是位于第三脑室两侧的两个细胞团。此外, 爪鲵端脑内细胞形态和大小分化较为单一。**结论** 爪鲵在两栖纲动物中属于较原始的种类。本研究有助于更深入地了解有尾两栖类动物的神经系统。

关键词: 爪鲵; 端脑; 形态学; 组织学