

Histology and Immunohistochemical Analyses in the Brain of Juvenile Chinese Alligator (*Alligator sinensis*)

Análisis Histológico e Inmunohistoquímico en el Encéfalo de Caimanes Chinos Juveniles (*Alligator sinensis*)

Yu Wang¹; Ruiyun Liu¹; Ranran Hu¹; Hui Chen¹; Zimo Li³; Xueyi Yin² & Zaiqun Liu¹

WANG, Y.; LIU, R.; HU, R.; CHEN, H.; LI, Z.; YIN, X. & LIU, Z. Histology and immunohistochemical analyses in the brain of juvenile Chinese alligator (*Alligator sinensis*). *Int. J. Morphol.*, 42(5):1181-1188, 2024.

SUMMARY: In current study, we used Nissl staining to examine the histological structure of the juvenile Chinese alligator brain, and immunohistochemistry (IHC) staining to detail the serotonin (5-hydroxytryptamine, 5-HT) localization and morphology of neurons belonging to the serotonergic system. Nissl staining revealed that the Chinese alligator brain was divided into four regions (telencephalon, diencephalon, brainstem, and cerebellum) and a connected ventricular system (containing the pair of lateral ventricles, third ventricle, fourth ventricle and the aqueduct). According to the IHC results, 5-HT immunoreactive (5-HT-IR) neurons were primarily observed in the cerebral cortex, the hypothalamic paraventricular nucleus (PH), tectum and tegmentum of the optic lobes, the medial longitudinal fasciculus (Flm) of medulla oblongata and the cerebellar cortex. 5-HT-IR fibers were mostly located lateral to the hypothalamic paraventricular nucleus (PH), in the medial vestibular nucleus (MVe) of the medulla oblongata, and in the choroid plexus (CP) of the lateral ventricles. Additionally, our findings paralleled those results of other reptiles; nevertheless, some distinctions in terms of both histological architecture and function were identified. The layering of the cerebral cortex and tectum, as well as the arrangement of Purkinje cells, differed between reptile brains. The distribution of 5-HT-IR neurons varied in the layer of cerebral and cerebellar cortex when compared to other reptiles. Within the diencephalon, 5-HT-IR neurons formed distinct nuclei in the hypothalamic paraventricular nucleus (PH), but few in the infundibular recess.

KEY WORDS: Chinese Alligator brain; Nissl staining; Immunohistochemistry (IHC); 5-HT-IR neurons.

INTRODUCTION

Serotonin (5-hydroxytryptamine, 5-HT), an indole derivative, is a crucial neurotransmitter that can modulate neural activity and neuropsychological processes (Xu *et al.*, 2021). The 5-HT system is one of the most significant and conserved neurotransmitter systems in all vertebrates (Rodrigues *et al.*, 2008).

Currently, there were several papers detailing the location and morphology of the serotonin system in the reptilian brain. Smeets & Steinbusch (1988) showed a distribution of 5-HT immunoreactive (5-HT-IR) cells and fibers in the forebrain and midbrain of geckoes. Wolters *et al.* (1985), refined the distribution of serotonin-containing nerve cells, fibers, and terminals in the brain of the lizard. Martinez-Marcos *et al.* (2005), reported that garter snakes exhibit dense serotonin immunoreactive positivity in the

olfactory striatum. Challet *et al.* (1991), concretized the distribution pattern of serotonergic cell bodies and fibers in the whole brain of the *Vipera aspis*. Ueda *et al.* (1983), described the distribution of 5-HT neuronal bodies and fibers in the central nervous system of turtles. Rodrigues *et al.* (2008), showed that the presence of serotonergic neurons was found in the diencephalon and brainstem of the Nile crocodile. These researches demonstrated that the neurotransmitter 5-HT in the brain of the reptiles was widely distributed, but there were species-specific differences. Notably, these previous researches essentially examined members of the orders Squamata or Testudines, with few studies of the order Crocodylia.

The Chinese alligator (*Alligator sinensis*), which is an endangered endemic species, belongs to the Crocodylian

¹ College of Life Sciences, Anhui Normal University, Wuhu, Anhui 241000, China.

² Wuhu Blood Center, Wuhu, Anhui 241000, China.

³ Wuhu No 11 Middle School, Wuhu, Anhui 241000, China.

FUNDING. This study was funded by Anhui Provincial Key Laboratory of the Conservation and Exploitation of Biological Resources, the Anhui Provincial Key Laboratory of Molecular Enzymology and Mechanism of Major Diseases (No. KJ2020A0083) and the National Student Innovation and Entrepreneurship Training Program (No. 202310370175).

(Xie *et al.*, 2023). At present, histological studies mainly conducted on the dorsal telencephalon (Yang & Chen, 2001) and the optic lobe of the midbrain (Zaiqun *et al.*, 2002). While previous researches have served as the basis, our understanding of its anatomical structure remain rudimentary. Meanwhile, regarding the immunohistochemistry investigations, Ren *et al.* (2019), have provided a thorough analysis of serotonin and dopamine in the cerebellar cortex of Chinese alligator. However, this research was restricted to a single brain region, the Chinese alligator cerebellum.

Herein, SABC immunohistochemistry (IHC) staining was utilized to analyze the expression and location of 5-HT in the Chinese alligator brain, meanwhile Nissl staining was employed to examine the Chinese alligator brain's histological structure.

MATERIAL AND METHOD

Animals. Four juvenile Chinese alligators (*Alligator sinensis*) (at first post-hatched week) collected from Xuancheng Alligator Culturing Centre were used in the current study (Fig. 1A). The alligators were around 15 cm in total length and 24.7 g to 28.3 g weight, and had brain weights ranging from 0.29g to 0.34 g. Sex was not taken into consideration here because their sexes were determined by incubation temperature of eggs during the temperature-sensitive period (TSP) (Chen *et al.*, 2003). All procedures were in compliance with the regulations of the forestry authorities of China and the Animal Care and Welfare Committee of Anhui Normal University. Authorization to use Chinese alligator tissues was granted by Animal Ethics Committee of Anhui Normal University (Certificate No. 2018012).



Fig. 1. The juvenile Chinese alligator (A) and its brain (B). (c: caudal; v: ventral).

Perfusion and Tissue Processing. Two animals were used for Nissl staining and two were used for IHC. All animals were deeply anesthetized with Pentobarbital by intraperitoneal injection, and the entire brains were dissected out of the skull carefully. Tissues were cleaned with 0.70 % physiological saline, fixed in Bouin's fluid for 72 h. Then, they dehydrated in ascending series of ethanol, cleared in xylene, and embedded in paraffin. Finally, the tissues were serially sectioned in the coronal and sagittal planes (7 μ m thick serial sections) on an Ultra-Thin Semiautomatic Microtome.

Nissl Staining. Nissl staining protocol has been previously described (Liao *et al.*, 2023), and we won't introduce it here.

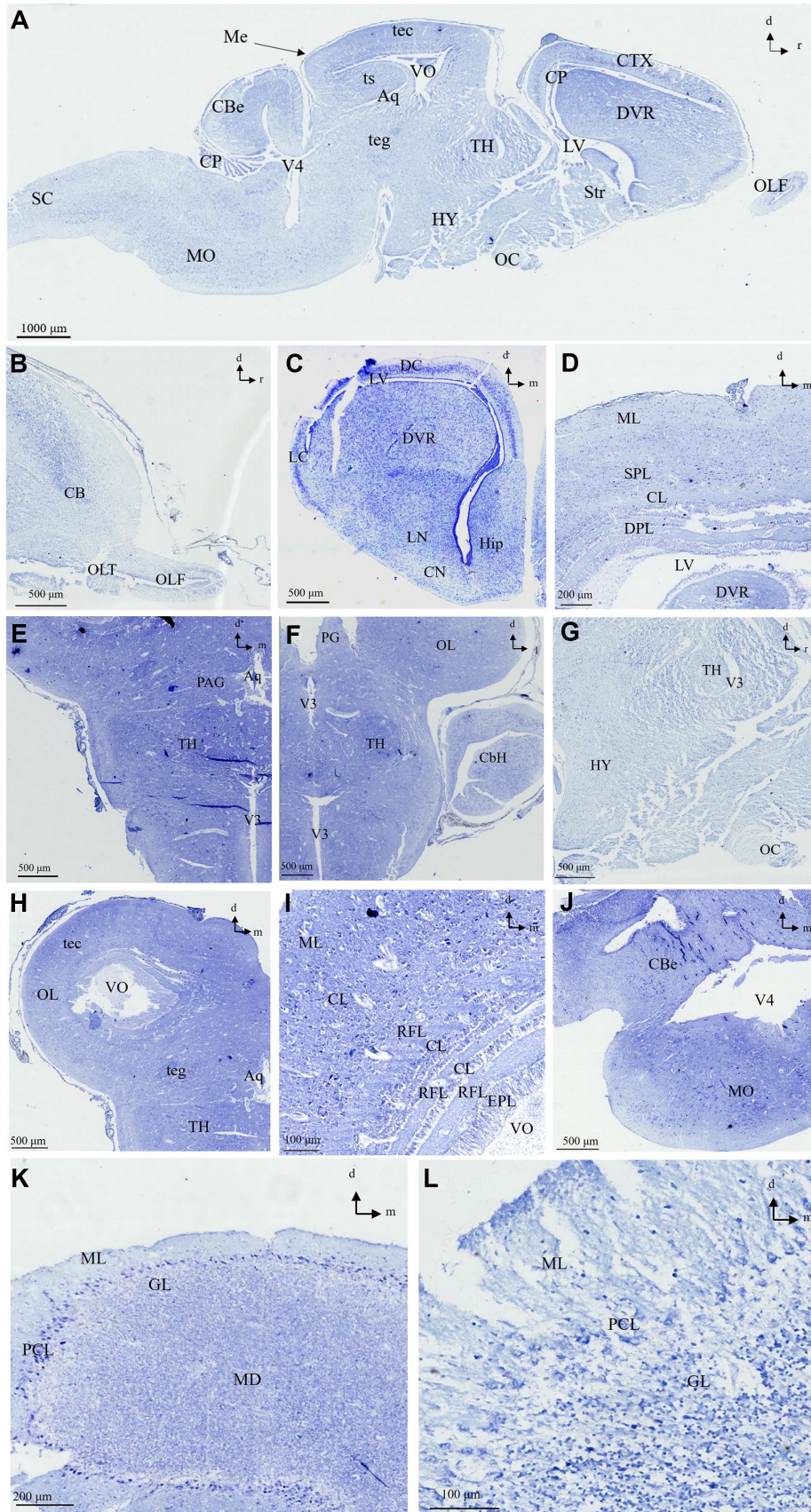
Antisera and Reagents. The 5-HT primary antibody (BA-0121-1) (raised in rabbit) and concentrated Streptavidin-Biotin-enzyme Complex Peroxidase (SABC POD) (Goat anti-Rabbit IgG) kit (SA 1029) were purchased from Wuhan Boster Biotech (Wuhan, China).

SABC Immunohistochemical Staining. The sections were initially deparaffinized and rehydrated in preparation for immunohistochemistry. Immunohistochemical staining was carried out as follows: sections were rinsed in 0.1 M PBS (PH = 7.4) 3 times and treated with 3 % H₂O₂ (30 % H₂O₂ in methanol) for 15 min to reduce endogenous peroxidase activity, and rinsed three times again with PBS. Then, sections were blocked with nonspecific antigen binding using 5 % BSA (Servicebio, GC305010) while gently shaking for 15 min at room temperature. Sections were followed by an overnight incubation with primary antibody, a 5-HT antibody (BA-0121-1) diluted 1:100 in PBS, at 4 °C. Sections were next immersed in PBS for two min and incubated for two h at room temperature with the secondary antibody from the ready-to-use Streptavidin-Biotin-enzyme Complex Peroxidase (SABC-POD) (Goat anti-Rabbit IgG) kit (SA 1029). After three further PBS rinses, sections were placed in the SABC for a two-hour period. After the last incubation, sections were rinse three times with PBS, then treated with 3,3-diaminobenzidine (DAB, Boster, AR1027) for 3–5 min away from light. The development was followed visually and checked under a microscope. Finally, sections were rinsed, dehydrated in 95 %, 100 % ethanol, cleared in xylene and coverslipped with Rhamsan gum (Servicebio, WG10004160).

Observation and Photomicrography. All sections were examined and imaged with an Olymplus BX61 microscope and a Motic BA600-4 figure microscope software. Images were cropped and corrected for brightness using Photoshop CS6. No other changes were made to images. The nomenclature used in this paper conforms as much as possible to that provided by Briscoe & Ragsdale (2018), Chen *et al.* (2003), and Rodrigues *et al.* (2008), for ease of comparison.

RESULTS

The Histological Structure of Brain in Juvenile Chinese Alligator by Nissl Staining. The brain of the juvenile Chinese alligator measured about 1.60 cm in length and 0.7 cm high (Fig. 1B). From the rostral to the caudal side, it was divided into the telencephalon, diencephalon, midbrain, cerebellum, and medulla oblongata (Fig. 2A). Generally, the



midbrain and the medulla oblongata were commonly referred to as the brainstem. Moreover, surrounding the exterior of the brain was a thin layer of tissue which named meninges.

Fig. 2. Histological slices through the juvenile Chinese alligator brain (Nissl staining). (A) The sagittal section of the juvenile Chinese alligator brain. Scale bar: 1000 μm ; (B) The olfactory bulb was connected to the cerebrum (CB); scale bar: 500 μm ; (C) The coronal section through one half of the cerebral hemisphere; scale bar: 500 μm ; (D) The cellular hierarchy of the cerebral cortex; scale bar: 200 μm ; (E) The coronal section through one half of the diencephalon; scale bar: 500 μm ; (F) The pineal gland (PG) was connected to the diencephalon; scale bar: 500 μm ; (G) The relative position of the thalamus (TH) and hypothalamus (HY); scale bar: 500 μm .; (H) One half of the midbrain optic lobes (OL); scale bar: 500 μm ; (I) The cellular hierarchy of the tectum cortex; scale bar: 100 μm ; (J) The cerebellum (CBe) and medulla oblongata (MO) surround the fourth ventricle (V4); scale bar: 500 μm ; (K) The cellular hierarchy of the cerebellar cortex; scale bar: 100 μm ; (L) Cerebellar cortex and medulla of the cerebellum; scale bar: 200 μm ; (r: rostral; c: caudal; d: dorsal; v: ventral; m: medial; l: lateral).

The connected ventricular system, including the lateral ventricles, the third ventricle and the fourth ventricle, was revealed within the brain region. The pair of C-shaped lateral ventricles were located in the cerebral hemisphere, and communicated with the third ventricle through the interventricular foramen (Fig. 2A, C). The third ventricle was a narrow space located between the two sides of the diencephalon (Fig. 2H, I), which opened caudally into the midbrain aqueduct. Then, the aqueduct passed through the midbrain and communicated with the fourth ventricle. The tent-shaped fourth ventricle was located between the brainstem and cerebellum (Fig. 2A, J). Ventricles contained choroid plexus (CP), which was the site for secretion of cerebrospinal fluid (Fig. 2A, E).

The telencephalon consisted of the olfactory bulb and cerebrum. The olfactory bulb, with a smooth surface, was oval and connected to the brain by a slender olfactory tract (Fig. 2B). The cerebrum included the cerebral cortex, the underlying cerebral white matter, and the basal ganglia (Fig. 2A, B, C). The cerebral cortex could be divided into three parts by region: the medial cortex (MC), the lateral cortex (LC), and the dorsal cortex (DC) (Fig. 2C). From outside to inside, the four layers of cerebral cortex were the molecular layer (ML), superficial plexiform layer (SPL), cellular layer (CL), and deep plexiform layer (DPL) (Fig. 2D). The tissue that bulges into the lateral ventricle was called dorsal ventricular ridge (DVR) (Fig. 2C). The lateral ventricle (LV), which also had a rich choroid plexus (CP), was situated in the white matter of the cerebral hemisphere (Fig. 2A, 3A). Additionally, the striatum (Str) of the basal ganglia was visible in the sagittal section in juvenile Chinese alligators (Fig. 2A).

The diencephalon was situated between the telencephalon and midbrain (Fig. 2A), connected to the pineal gland dorsally (Fig. 2F), and was mainly composed of the thalamus and hypothalamus (Fig. 2G). Each half of the brain contained a thalamus, a large, ovoid, gray mass of nuclei (Fig. 2F, G). The hypothalamus lied beneath the thalamus (Fig. 2A, G). The optic chiasm was a caudal marker in the hypothalamus, ventral to the diencephalon (Fig. 2G). Furthermore, the third ventricle (V3) lies between the halves of the diencephalon (Fig. 2E, F). This ventricle was connected to the aqueduct, which surrounded by the periaqueductal gray matter (PAG) (Fig. 2E).

The brainstem included the midbrain (mesencephalon) and medulla (medulla oblongata), located ventral to the cerebellum (Fig. 2A). The midbrain formed a transition to the cerebrum. The midbrain lobes were known as the optic lobes (OL), and the middle cavity was commonly referred to as the ventriculus opticus (VO) (Fig. 2A, H). The three internal longitudinal divisions of the midbrain were the tectum, tegmentum, and basis. The ventral side was referred to as the tegmentum (teg), and the apex as the tectum (tec) (Fig. 2A, H). From outside to inside, the cortex of tectum consisted of eight layers. Layer I was the molecular layer (ML), layer II, IV, and VI were the cell layer (CL), layer III, V, and VII were the reticular fiber layer (RFL) (Fig. 2I). At the tegmentum (teg), the cellular hierarchy was not clear. The division was based on the absence or presence of the lower fourth ventricle (V4). The fourth ventricle (V4) was the cavity produced by the cerebellum and dorsal side of the medulla oblongata (Fig. 2J). The fourth ventricle was joined to the midbrain aqueduct rostrally and to the central canal of the spinal cord caudally.

The elliptical cerebellum was located just dorsal to the brainstem, played a major role in motor coordination (Chen *et al.*, 2003). Together with the brainstem, the cerebellum formed the fourth ventricle (V4) (Fig. 2A). The cerebellum consists of the cerebellar cortex and the underlying cerebellar white matter (Fig. 2K). From

Table I. The structures were listed alphabetically, followed by their abbreviations.

aqueduct	Aq
cellular layer	CL
cerebellum	CBe
cerebral hemisphere	CbH
cerebrum	CB
choroid plexus	CP
cortex	CTX
deep plexiform layer	DPL
dorsal ventricular ridge	DVR
dorsal cortex	DC
ependymal layer	EPL
fourth ventricle	V4
granular layer	GL
hypothalamic paraventricular	PH
hypothalamus	HY
lateral cortex	LC
lateral ventricle	LV
medial cortex	MC
medial longitudinal fasciculus	Fim
medial vestibular nucleus	Mve
medulla	MD
medulla oblongata	MO
meninges	Me
midbrain	MB
molecular layer	ML
olfactory bulb	OLF
olfactory tract	OLT
optic chiasm	OC
optic lobe	OL
optic tectum	tec
periaqueductal gray	PAG
pineal gland	PG
Purkinje's cellular layer	PCL
raphe inferior nucleus	Rai
reticular fiber layer	RFL
reticular formation	ReF
reticularis inferior nucleus	Ri
spinal cord	SC
striatum	Str
superficial plexiform layer	SPL
tegmentum	teg
thalamus	TH
third ventricle	V3
torus semicircularis	ts
ventriculus opticus	VO

outside to inside, the cerebellar cortex was composed of three layers: the molecular layer (ML), the Purkinje cell layer (PCL), and the granular layer (GL) (Fig. 2L).

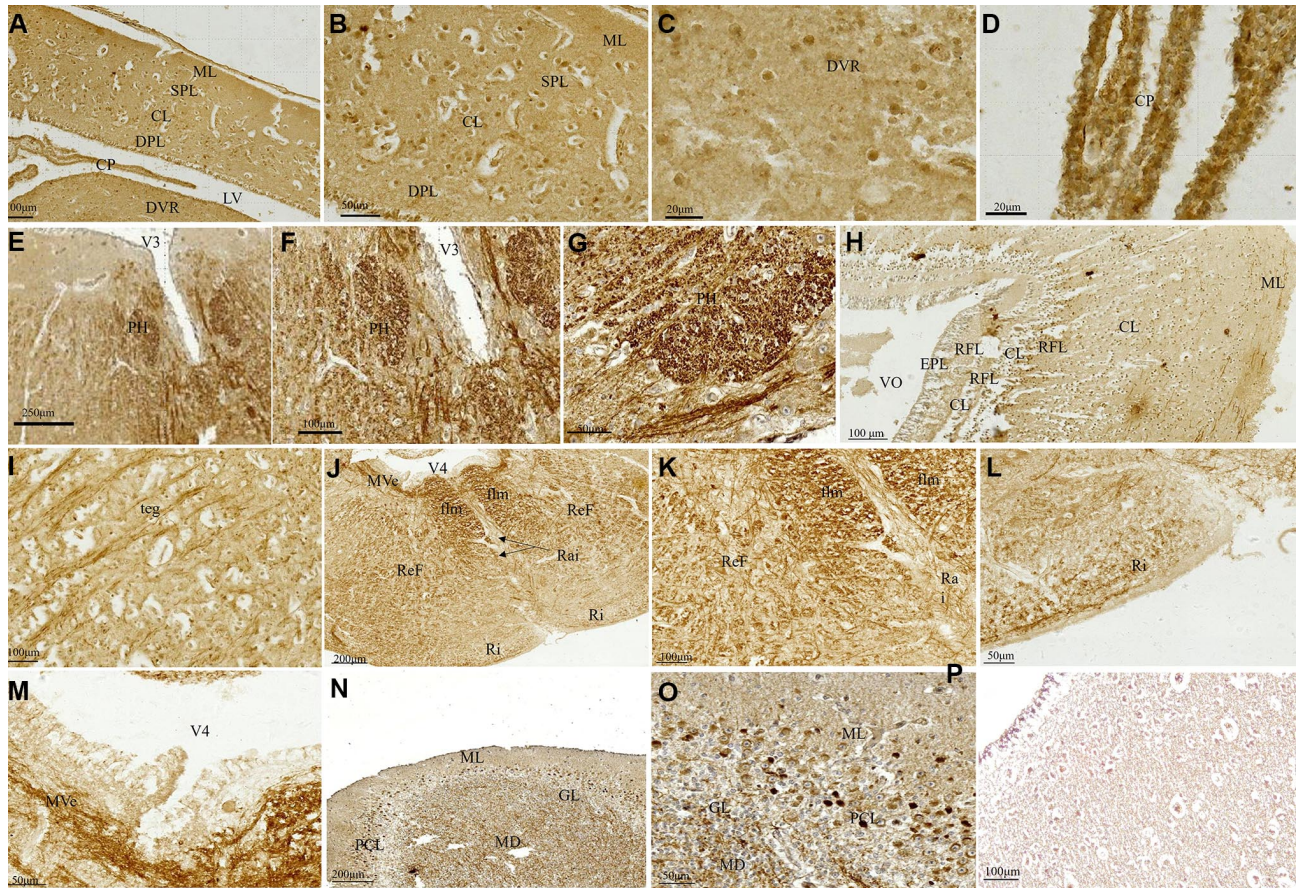


Fig. 3. Immunohistochemical slices through the juvenile Chinese alligator brain (IHC staining). (A) 5-HT-IR neurons in the cerebral cortex and the dorsal ventricular ridge (DVR); Scale bar: 100 μm ; (B) 5-HT-IR neurons in the cerebral cortex; Scale bar: 20 μm ; (C) 5-HT-IR neurons in the dorsal ventricular ridge (DVR); Scale bar: 20 μm ; (D) 5-HT-IR neurons and fibers in choroid plexus (CP); Scale bar: 20 μm ; (E) The overall distribution of 5-HT-IR neurons and fibers in PH; scale bar: 400 μm ; (F) The partially enlarged view of 5-HT-IR neurons and fibers in the hypothalamic paraventricular nucleus (PH); scale bar: 200 μm ; (G) The 5-HT-IR neurons and fibers in the hypothalamic paraventricular nucleus (PH); scale bar: 50 μm ; (H) 5-HT-IR neurons in the cortex of optic tectum; scale bar: 100 μm ; (I) 5-HT-IR neurons and fibers in the tegmentum (teg); scale bar: 100 μm ; (J) 5-HT-IR neurons and fibers in the medulla oblongata; scale bar: 200 μm ; (K) 5-HT-IR neurons and fibers in medial longitudinal fasciculus (flm), raphe inferior nucleus (Rai) and reticular formation (Ref); scale bar: 100 μm ; (L) 5-HT-IR neurons and fibers in reticularis inferior nucleus (Ri); scale bar: 50 μm ; (M) 5-HT-IR neurons and fibers in medial vestibular nucleus (MVe); scale bar: 50 μm ; (N) 5-HT-IR neurons in the cerebellar cortex and medulla; scale bar: 200 μm ; (O) The 5-HT-IR neurons were larger and round in PCL; scale bar: 50 μm ; (P) The negative control for the IHC procedure; scale bar: 100 μm .

5-HT Neurons and Fibers in Juvenile Chinese Alligator by IHC Staining. Within the cerebral cortex of the telencephalon, the majority of 5-HT-IR neurons were found in the superficial plexiform layer (SPL) and the cellular layer (CL), with a smaller number in the deep plexiform layer (DPL) but none in the molecular layer (ML) (Fig. 3A, B). 5-HT-IR fibers were extensively distributed and highly immunoreactive, particularly in the cellular layer (CL) (Fig. 3B). Within the subcortical area, a profusion of 5-HT-IR neurons and fibers were noticed in the choroid plexus (CP)

(Fig. 3A, D), while scattered round 5-HT-IR neurons were discovered in the DVR (Fig. 3C).

In the diencephalon, a large number of 5-HT neurons and fibers with strong immunopositive were located in the hypothalamic paraventricular nucleus (PH), which was found on both sides of the third ventricle (V3) (Fig. 3E, F, G). The majority of these neurons had round or oval nuclei, with a few irregular shapes (Fig. 3F). Furthermore, numerous 5-HT-IR fibers formed a distinct and high-density cluster in a

position immediately lateral to the hypothalamic paraventricular nucleus (PH) (Fig. 3G).

Within the midbrain, tectum and tegmentum both had a large number of 5-HT-IR cells (Fig. 3H, I). In the optic tectum, 5-HT-IR cells were mostly concentrated in the cellular layer (layer II, IV, and VI), with few in the molecular layer and reticular layers (layer III, V, and VII) (Fig. 3H). Several fibers were distributed in the molecular layer and cellular layer (layer II) (Fig. 3H). In the tegmentum, 5-HT-IR cells were scattered in it, and some 5-HT-IR fibers were gathered into small bundles (Fig. 3I). In the medulla oblongata, the medial longitudinal fasciculus (Flm) of the contained the highest density of 5-HT-IR cells and the majority of their cytoplasm was oval or irregular (Fig. 3J, K). Besides, the reticular formation (ReF), raphe inferior nucleus (Rai), reticularis inferior nucleus (Ri) and medial vestibular nucleus (MVe) were the primary locations for 5-HT-IR fibers. The fibers of medial vestibular nucleus (MVe) were the most highly positive among them, followed by the reticular formation (ReF), while nucleus raphe inferior (Rai) and reticularis inferior nucleus (Ri) was fewer positively affected (Fig. 3K, L, M).

In the cerebellum, 5-HT-IR neurons typically resided in the Purkinje cell layer (PCL) of the cortex, with only a few detectable in the molecular layer (ML) and granular layer (GL) (Fig. 3N, O). Comparatively, neurons in the Purkinje cell layer were larger and spherical, whereas neurons in the molecular layer (ML) were smaller. The medulla was the primary location where nerve fibers congregated, and it was barely present in the cortex (Fig. 3O).

DISCUSSION

The current study of alligator brain structure and serotonergic system shared much in common with previous studies in reptiles, but certain discrepancies were noted. In order to provide some new perspectives on reptilian brain, we will compare our findings in detail with other reptiles.

The Histological Structure Compared with Other Reptile Brain. For the cerebral cortex of reptiles, two concepts of layering (three-layer or four-layer) were observed. Based on some researches, the cerebral cortex of lizards (*Tropidurus hispidus*) (de Carvalho Pimentel *et al.*, 2011), turtles (*Pseudemys scripta elegans*) (Schmolke & Künzle, 1997), and alligators (*Alligator mississippiensis*) (Briscoe & Ragsdale, 2018) presented a three-layer structure. These results led some academics to hypothesize that reptiles' cerebral cortex consists of only three layers, which they compared to the layers I, V, and VI of mammals (Cheung *et al.*, 2007). Nevertheless, some researchers

discovered that the cerebral brain of lizards (*Gekko gekko*) (Smeets *et al.*, 1986) and Chinese alligators (Wang *et al.*, 2017) contained four layers. In present study, the cerebral cortex also showed a 4-layer structure, similar to the above. We hypothesize that this disparity is related to regional and species differences.

The hierarchical structure of the midbrain tectum was also debated. Ueda *et al.* (1983), believed that the midbrain tectum of turtle (*Clemmys japonica*) was 11 layers. Smeets & Steinbusch (1988) found that the midbrain tectum of lizard (*Gewio gekko*) was a 14-layer structure, while the lizard (*Gallotia galloti*) has seventh floor (del Mar Romero-Alemán *et al.*, 2003). In birds, the midbrain tectum of the chick (*Gallus gallus*) was divided into 10 layers (Britto *et al.*, 1992). The results of this experiment showed that the Chinese alligator's midbrain tectum was a seven-layer structure. These experiments were all performed using Nissl or IHC staining. Therefore, the reason for this controversy can be ruled out as the possibility of different experimental methods. We speculate that this result is due to age difference and species differences.

The Serotonergic Systems Compared with Other Reptile Brain. Serotonergic neurons were widely distributed in the cerebral cortex of reptiles, but the density in the same location varies. It was demonstrated that the plexus of serotonin fibers was primarily located in the molecular layer of the turtle cerebral cortex (Ueda *et al.*, 1983). Guirado *et al.* (1989), showed that the distribution of serotonin fibers in the cerebral cortex of lizards was mainly concentrated in the molecular layer, followed by the inner plexiform layer, and a small number of positive cells existed in the outer plexiform layer. Another study discovered that the cerebral cortex of *Vipera aspis* had a majority of 5-HT-IR fibers in the outer and inner plexiform layers (Challet *et al.*, 1991). However, in the current study, 5-HT cells in the cerebral cortex of Chinese alligators were primarily found in the superficial plexiform layer and cell layer, with a negligible number being present in the deep plexiform layer. Generally, there were some differences in the distribution of serotonergic nerves in the cerebral cortex of different species of reptiles. We speculate that this is caused by species differences.

Serotonergic nerves were distributed differently in the thalamus of different species. In reptiles, such as turtles (Ueda *et al.*, 1983), lizards (Smeets & Steinbusch, 1988), and ophidian (Challet *et al.*, 1991), the paraventricular organ of the third ventricle and the infundibular recess were found 5-HT-IR cells. However, the chameleon was an exception, and Bennis (1990) detected no positive reaction for 5-HT in its corresponding position. In Nile crocodiles,

5-HT-IR neurons found in paraventricular organ, absent in infundibular recess (Rodrigues *et al.*, 2008). According to our findings, the paraventricular nucleus of Chinese alligator had a high number of 5-HT-IR neurons and fibers. In general, the distribution pattern of serotonin in the diencephalon is quite distinct, even among animals of the same order.

CONCLUSION

In summary, this paper suggested the juvenile Chinese alligator's brain anatomy and histology but also first captured the distribution of 5-HT system in the whole brain. 5-HT-IR neurons and fibers were found extensively throughout the Chinese alligator's brain. We also discovered that 5-HT has varied impacts on different areas of the brain. This research will increase the background data onto the study of the Alligator brain atlas and enhance understanding the brain of Chinese alligator.

WANG, Y.; LIU, R.; HU, R.; CHEN, H.; LI, Z.; YIN, X. & LIU, Z. Análisis histológico e inmunohistoquímico en el cerebro de caimanes chinos juveniles (*Alligator sinensis*) *Int. J. Morphol.*, 42(5):1181-1188, 2024.

RESUMEN: Utilizamos la tinción de Nissl para examinar la estructura histológica del cerebro juvenil del caimán chino y la tinción inmunohistoquímica (IHC) para detallar la localización y morfología de la serotonina (5-hidroxitriptamina, 5-HT) de las neuronas que pertenecen al sistema serotoninérgico. La tinción de Nissl reveló que el cerebro del caimán chino estaba dividido en cuatro regiones (telencéfalo, diencefalo, tronco encefálico y cerebelo) y un sistema ventricular conectado (que contiene el par de ventrículos laterales, el tercer ventrículo, el cuarto ventrículo y el acueducto). Según los resultados de IHC, las neuronas inmunorreactivas 5-HT (5-HT-IR) se observaron principalmente en la corteza cerebral, el núcleo paraventricular (PH) hipotalámico, el tectum y tegmento de los lóbulos ópticos, el fascículo longitudinal medial (Flm) de médula oblonga y la corteza cerebelosa. Las fibras 5-HT-IR se ubicaron principalmente lateral al núcleo paraventricular (PH) hipotalámico, en el núcleo vestibular medial (MVe) de la médula oblonga y en el plexo coroideo (CP) de los ventrículos laterales. Además, nuestros hallazgos fueron semejantes a los resultados obtenidos de otros reptiles; sin embargo, se identificaron algunas distinciones en términos tanto de arquitectura histológica como de función. Las capas de la corteza cerebral y el tectum, así como la disposición de las neuronas purkinjenses, diferían entre los cerebros de los reptiles. La distribución de las neuronas 5-HT-IR varió en la capa de la corteza cerebral y cerebelosa en comparación con otros reptiles. Dentro del diencefalo, las neuronas 5-HT-IR formaron núcleos distintos en el núcleo paraventricular (PH) hipotalámico, pero pocos en el recesso infundibular.

PALABRAS CLAVE: Cerebro de caimán chino; Tinción de Nissl; Inmunohistoquímica (IHC); Neuronas 5-HT-IR.

REFERENCES

- Bennis, M.; Gamrani, H.; Geffard, M.; Calas, A. & Kah, O. The distribution of 5-HT immunoreactive systems in the brain of a saurian, the chameleon. *J. Hirnforsch.*, 31(5):563-74, 1990.
- Briscoe, S. D. & Ragsdale, C. W. Molecular anatomy of the alligator dorsal telencephalon. *J. Comp. Neurol.*, 526(10):1613-46, 2018.
- Britto, L. R.; Keyser, K. T.; Lindstrom, J. M. & Karten, H. J. Immunohistochemical localization of nicotinic acetylcholine receptor subunits in the mesencephalon and diencephalon of the chick (*Gallus gallus*). *J. Comp. Neurol.*, 317(4):325-40, 1992.
- Challet, E.; Pierre, J.; Repérant, J.; Ward, R. & Miceli, D. The serotonergic system of the brain of the viper, *Vipera aspis*. An immunohistochemical study. *J. Chem. Neuroanat.*, 4(4):233-48, 1991.
- Chen B. H.; Hua T. M.; Wu X. B. & Wang C. L. *Research of the Chinese Alligator*. Shanghai, Shanghai Scientific and Technical Publishers, 2003.
- Cheung, A. F.; Pollen, A. A.; Tavare, A.; DeProto, J. & Molnár, Z. Comparative aspects of cortical neurogenesis in vertebrates. *J. Anat.*, 211(2):164-76, 2007.
- de Carvalho Pimentel, H.; Dos Santos, J. R.; Macêdo-Lima, M.; de Almeida, F. T. C.; Santos, M. L.; Molowny, A.; Ponsoda, X.; Lopez-García, C. & Marchioro, M. Structural organization of the cerebral cortex of the neotropical lizard *Tropidurus hispidus*. *Cell Tissue Res.*, 343(2):319-30, 2011.
- del Mar Romero-Alemán, M.; Monzón-Mayor, M.; Yanes, C.; Arbelo-Galván, J. F.; Lang, D.; Renau-Piqueras, J. & Negrín-Martínez, C. S100 immunoreactive glial cells in the forebrain and midbrain of the lizard *Gallotia galloti* during ontogeny. *J. Neurobiol.*, 57(1):54-66, 2003.
- Guirado, S.; de la Calle, A.; Gutiérrez, A. & Dávila, J. Serotonin innervation of the cerebral cortex in lizards. *Brain Res.*, 488(1-2):213-20, 1989.
- Liao, L.; Chai, B.; Zhao, T.; Liu, W.; Zhang, X.; Xu, X.; Tian, X.; Lv, M.; Yao, X. & Bai, S. HDAC2 inhibits hippocampal neurogenesis and impairs the cognitive function in prenatally stressed adult offspring. *Int. J. Morphol.*, 41(3):811-8, 2023.
- Martinez-Marcos, A.; Ubeda-Bañon, I.; Lanuza, E. & Halpern, M. Chemoarchitecture and afferent connections of the "olfactostriatum": a specialized vomeronasal structure within the basal ganglia of snakes. *J. Chem. Neuroanat.*, 29(1):49-69, 2005.
- Ren, S.; Chen, M.; Yang, L. & Liu, Z. 5-hydroxytryptamine and dopamine neurons in the cerebellum of the new-hatching Yangtze Alligator *Alligator sinensis*. *Anat. Rec. (Hoboken)*, 302(6):861-8, 2019.
- Rodrigues, S. L.; Maseko, B. C.; Ihunwo, A. O.; Fuxe, K. & Manger, P. R. Nuclear organization and morphology of serotonergic neurons in the brain of the Nile crocodile, *Crocodylus niloticus*. *J. Chem. Neuroanat.*, 35(1):133-45, 2008.
- Schmolke, C. & Künzle, H. On the presence of dendrite bundles in the cerebral cortex of the Madagascan lesser hedgehog tenrec and the red-eared pond turtle. *Anat. Embryol.*, 196(3):195-213, 1997.
- Smeets, W. J. & Steinbusch, H. W. Distribution of serotonin immunoreactivity in the forebrain and midbrain of the lizard *Gekko gekko*. *J. Comp. Neurol.*, 271(3):419-34, 1988.
- Smeets, W. J.; Hoogland, P. V. & Lohman, A. H. A forebrain atlas of the lizard *Gekko gekko*. *J. Comp. Neurol.*, 254(1):1-19, 1986.
- Ueda, S.; Takeuchi, Y. & Sano, Y. Immunohistochemical demonstration of serotonin neurons in the central nervous system of the turtle (*Clemmys japonica*). *Anat. Embryol.*, 168(1):1-19, 1983.
- Wang, H.; Zhang, R.; Zhang, S.; Zhou, Y. & Wu, X. Immunohistochemical localization of somatostatin in the brain of Chinese alligator *Alligator sinensis*. *Anat. Rec. (Hoboken)* 300(3):507-19, 2017.
- Wolters, J. G.; Ten Donkelaar, H. J.; Steinbusch, H. W. M. & Verhofstad, A. A. J. Distribution of serotonin in the brain stem and spinal cord of the lizard *Varanus exanthematicus*: an immunohistochemical study. *Neuroscience*, 14(1):169-93, 1985.

- Xie, Z.; Shi, P.; Yang, X. & Lu, M. Morphological Study in Heart of Wild Chinese Alligator (*Alligator sinensis*). *Int. J. Morphol.*, 41(1):324-30, 2023.
- Xu, P.; Huang, S.; Zhang, H.; Mao, C.; Zhou, X. E.; Cheng, X.; Simon, I. A.; Shen, D. D.; Yen, H. Y.; Robinson, C. V. & Xu, H. E. Structural insights into the lipid and ligand regulation of serotonin receptors. *Nature*, 592(7854):469-73, 2021.
- Yang, C. X. & Chen, B. H. The later embryonic development of dorsal-lateral area in *Alligator sinensis* telencephalon: an ultrastructural study. *Acta Hydrobiol. Sin.*, 25(1):83-7, 2001.
- Zaiqun, L.; Qingyan, S.; Kun, L.; Haitao, W.; Tianmiao, H. & Dongsheng, C. The histologic study of the optic lobe in new-hatching *Alligator sinensis*. *J. Anhui Norm. Univ. Nat. Sci.*, 25(4):368-70, 2002.

Corresponding author:
Zaiqun Liu
College of Life Sciences
Anhui Normal University
No 2 Beijing East Road
Wuhu
Anhui 24100
CHINA
Tel: +86-13635531809
E-mail: liuzaiqun@126.com

Corresponding author:
Xueyi Yin
Wuhu Blood Center
No. 29 Huajin South Road
Wuhu
Anhui 24100
CHINA
E-mail: 18119876977@163.com