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Immunocytochemical localization of serotonin and neuropeptide Y in the brain of *Scylla serrata*

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Abstract: To explore the locations of serotonin (5-HT) and neuropeptide Y (NPY) in the brain of *Scylla serrata*, immunocytochemical method of Strept Avidin-Biotin-Complex was applied to observe the immunoreaction to two antibodies. Among all the 12 somal clusters and 11 neuropils of the brain in *S. serrata*, 5-HT-like immunoreactivity occurs in 4 somal clusters and 6 neuropils in the protocerebrum and deutocerebrum; while NPY-like immunoreactivity is present in 7 somal clusters and 3 neuropils in the deutocerebrum and tritocerebrum. The specific distribution patterns of these two immunoreactive substances may thus provide morphological proofs for their different neurophysiological functions.

Key words: *Scylla serrata*; brain; serotonin; neuropeptide Y; immunocytochemistry
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1 Introduction

Researches on crustacean neurology have been mostly focused on the optic ganglia, such as detailed studies on the morphology, the cytology and the molecular biology of neuropeptides from CHH-family. However, investigations on the brain of crustaceans have only been limited to anatomy and histology. Little has been known about the locations of bioactive substances in the brain as well as their functions[1-3]. So far, studies on *Scylla serrata*, one kind of commercial crabs in China, have been mainly focused on the aquacultural biology. Sandman *et al* have clearly depicted the anatomical locations of somal clusters as well as the neuropiles in the brain[4]. Huang *et al* have investigated histological characters of neurosecretory cells in the brain[5,6]. 5-HT and NPY are important neurobioactive substances of crustaceans, exerting in activities like molting, feeding, circulation and metabolism[7,8]. In the present study, antibodies of 5-HT and NPY, and immuocytological techniques of Strept Avidin-Biotin-Complex (SABC), were applied to distinguish and locate 5-HT and NPY immunoreactivity in the brain of *S. serrata*. The specific distribution patterns of these two immunoreactive substances may probably provide morphological proofs for their function on neurobiology.

2 Materials and Methods

2.1 Tissue preparation

Adult crabs (*Scylla serrata*; body length of 6.0 - 8.8 cm) were obtained from local vendors in Xiamen. A total of 20 crabs were used in this study. The brains were dissected free, and fixed in Bouin's solution for 10- 12 h at 4℃. The tissues were then embedded in paraffin after routine dehydration in alcohol and clearing in xylene. Serial sagittal and cross sections of 6 μm were mounted on clear glass slides.

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2.2 Main reagents

Primary antisera generated in rabbits against 5-HT (1∶100 dilution) were produced by ZYMED Company. Primary antisera generated in rabbits against NPY (ready-to-use) and 3', 3'-diaminobenzidine (DAB) were produced by Sigma Company. Strept Avidin-Biotin-Complex (SABC) kit was purchased from Wuhan Boster Biological technology LTD.

2.3 SABC immunocytochemistry method

To detect 5-HT and NPY, the sections were immunocytochemically stained by SABC method. The following procedures were performed. Tissues were: (1) incubated in 3% H_2O_2/PBS for 10 min to inactivate endogenous peroxidase at room temperature, then rinsed in PBS (pH 7.4) for 5 min; (2) incubated in normal goat serum (1∶10) for 10 min to reduce non-specific binding at room temperature; (3) incubated in primary antisera for 1.5 h at 37 ℃, then rinsed three times in PBS for 15 min; (4) incubated in biotinylated goat anti-rabbit IgG for 0.5 h at 37 ℃, then rinsed three times in PBS for 15 min; (5) incubated in Strept Avidin-Biotin-Complex (SABC) for 0.5 h at 37 ℃, then rinsed three times in PBS for 15 min; (6) after 5-10 min in 0.06% DAB and 0.03% H_2O_2, sections were rinsed thoroughly in tap water, they were dyed in hematoxylin, dehydrated in alcohol, cleared in xylene and coverslipped; (7) preparations were viewed and photographed with an Olympus BH-2 microscope. Control sections were prepared simultaneously by substituting normal goat serum (1∶10) in place of the primary antibodies.

3 Results

Based on the study of Sandeman *et al*[4], the brain of *S. serrata* is divided into 11 neuropils and 12 somal clusters (cluster 6-17), those locations in the brain are listed in Fig. 1 and Tab. 1.

Tab. 1 The distribution of 5-HT and NPY immunoreactivity in the brain of *Scylla serrata*

locations of somal clusters and neuropils in the brain		5-HT	NPY
protocerebrum	cluster 6	+	+
	cluster 7	+	+
	cluster 8	-	-
	protocerebral bridge (PB)	+	-
	central body (CB)	+	-
	anterior medial protocerebral neuropil (AMPN)	+	-
	posterior medial protocerebral neuropil (PMPN)	+	-
deutocerebrum	cluster 9	+	+
	cluster 10	-	-
	cluster 11	+	+
	cluster 12, 13	-	-
	olfactory lobe (ON)	+	+
	accessory lobe (AcN)	-	-
	olfactory globular tract neuropil (OGTN)	-	-
	median antennaⅠneuropil (MAN)	+	-
	lateral antennaⅠneuropil (LAN)	-	+
tritocerebrum	cluster 14, 15	-	+
	cluster 16	-	-
	cluster 17	-	+
	tegumentary neuropil (TN)	-	+
	antennaⅡneuropil (AnN)	-	-

Notes: 1) cluster 12 converges with 13, and cluster 14 converges with 15; 2)"+" means immunoreactivity,"-" means no immunoreactivity

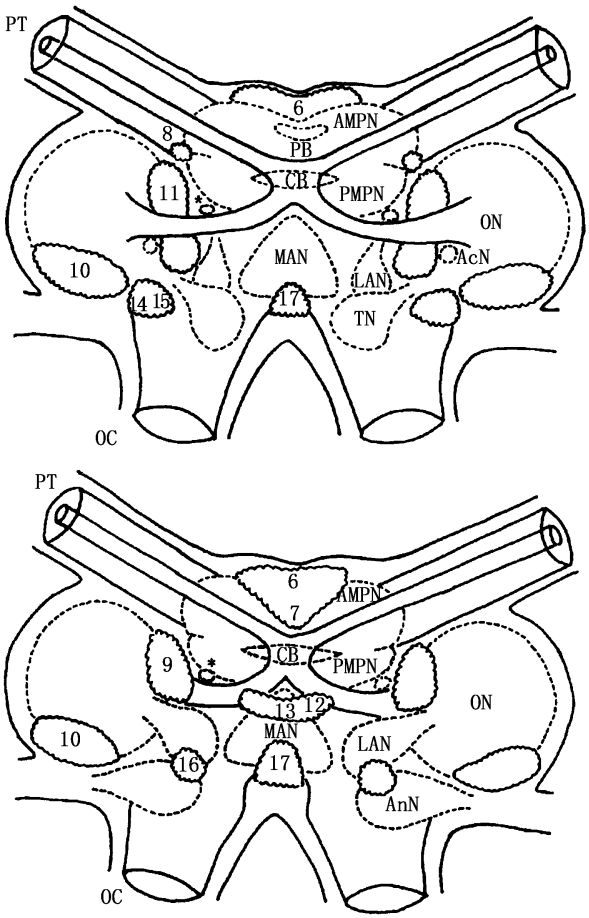

Fig. 1 The brain structure of *Scylla serrata* (from Sandeman *et al*.)

the upper picture is the dorsal side, while the other is the ventral side; 6 – 17 represents somal cluster 6 – 17; * represents OGTN; PT represents the protocerebrum tract, OC represents esophagus nerve

3.1 Immunoreactivity of 5-HT

In the protocerebrum, there are many immunoreactive cells in clusters 6 and 7, some with long cytoplasmic projections (Plate-1, 2). Strong immunoreactive staining is observed in CB, PB, AMPN and PMPN. Bits and pieces of immunoreactive substances are broadly distributed among these neuropils (Plate-3, 4). There are some immunoreactive fibers between AMPN and PMPN. Immunoreactive cells in cluster 9 of the deutocerebrum are all small (Plate-5). Glomeruli in ON shows strong immunoreactivity (Plate-6). There are many large immunoreactive cells in cluster 11 (Plate-7). Immunoreactivity is observed in the central MAN. No immunoreactivity is detected in the tritocerebrum.

3.2 Immunoreactivity of NPY

Immunoreactive cells in clusters 6 and 7 of the protocerebrum are distributed sporadically (Plate-8). Cluster 9 of the deutocerebrum has many immunoreactive cells, which assemble into clusters, sometimes over 100 immunoreactive cells were observed in one paraffin section (Plate-9). Glomerulis in ON show weak immunoreactivity (Plate-10). There are also many immunoreactive cells in cluster 11 (Plate-11). Immunoreactivity shows patches-like in LAN (Plate-12). A few immunoreactive cells are detected in clusters 14, 15 and 17 of the tritocerebrum (Plate-13). TN also shows immuoreactivity in patches' shape. The distribution of 5-HT and NPY immunoreactivity in the brain of *Scylla serrata* is listed in Tab. 1.

4 Discussion

4.1 Immunoreactivity of 5-HT

In *Artemia salina*, 5-HT immunoreactive cells are distributed only in clusters 6 and 8[9]. In *S. serrata*, 5-HT immunoreactive cells are detected in clusters 6 and 7, PB, CB, AMPN and PMPN of the protocerebrum, and cell cluster 9, 11, ON and MAN of the deutocerebrum. There are many similarities between the 5-HT immunoreaction of *S. serrata* and *Homarus americanus*[10]. Meanwhile, their difference lies in that, in *H. americanus*, PB showed no 5-HT immunoreactivity and there are many 5-HT immunoreactive cells in cluster 17. In crustaceans, as we know, the protocerebrum is the nerve center of vision and behavior, the deutocerebrum, olfaction; and the tritocerebrum, the visceral activities[11]. So the different location mode of 5-HT in the brain might reflect the complexity of the cerebral structure and functions. The wide distribution of 5-HT in the protocerebrum and deutocerebrum might be owing to the benthic ecology of *S. serrata* which requires quick regulation of vision and olfaction to pass in and out mud holes frequently. It was reported that in *Procambarus clarkii* and *Uca pugilater*, 5-HT can stimulate the brain and thoracic ganglia to secrete gonad-stimulating hormone (GSH), which can

accelerate the gonad development[12]. Ye *et al*[13] found that in *S. serrata*, 5-HT can stimulate the neuroendocrine of the brain, thus promote the ovarian development. This study provides the morphological proofs of possible function of 5-HT in stimulating the secretion of GSH in the brain of *S. serrata*.

4.2 Immunoreactivity of NPY

NPY belongs to the Pancreatic Polypeptide Family, and modulates the feeding, sexual activity, blood pressure and physiological rhythm of vertebrates[14]. Researches on the distribution of NPY in crustaceans are quite limited. The immunoreactivity of NPY was found in the anterior medial cluster (*i. e* clusters 6 and 7) and AMPN of *Litopenaeus vannamei*[15]. In *S. serrata*, NPY-immunoreactivity is present in many clusters in the protocerebrum and deutocerebrum. These results coincide with the terrestrial crab, *Chiromantes haematocheir*[16]. In the deutocerebrum of decapoda, the intermediate olfactory neurons in cluster 9 project fibers into ON[17]. In *S. serrata*, both cluster 9 and ON show NPY-immunoreactivity, which probably participate in the formation of olfaction, feeding activity and other functions.

4.3 Comparison of the locations of immunoreactive substance with 5-HT and NPY

It may be concluded from Tab. 1 that both NPY-immunoreactive neurons and 5-HT immunoreactive neurons are present in clusters 6, 7, 9 and 11, but there is much difference in the case of immunoreactive neuropiles with 5-HT and NPY: 5-HT immunoreactive neuropiles are mostly distributed in the protocerebrum and deutocerebrum (e. g. PB, CB, AMPN, PMPN, ON and MAN), which modulate the vision and olfaction; and NPY-immunoreactive neuropiles are present in the deutocerebrum and tritocerebrum (e. g. ON, LAN and TN), which regulate the olfaction and visceral activities. On the other hand, strong 5-HT immunoreactive neuropiles turn out in bits and pieces, dramatically different from the patches-like NPY-immunoreactive neuropiles, which might imply that 5-HT has broad regulation in the protocerebrum and deutocerebrum, while NPY probably exerts regulation only in local areas. The different location modes of 5-HT and NPY may reveal their specific physiological functions and modulatory manner. Both 5-HT and NPY appear in ON, which probably imply that they might coordinate or function independently in olfactory formation. In the study on *Chiromantes haematocheir*, NPY-like peptide and FMRFamide-like peptide (cardioexcitatory neuropeptide) coexist in neurons, and exert as neurotransmitter or neuromodulator in the processing of brain[16]. It is interesting to explore whether there is coexistence of NPY and 5-HT in neurons of the brain of *S. serrata*.

References:

[1] Keller R. Crustacean neuropeptides: structure, functions and comparative aspects[J]. Experientia, 1992, 48: 439-448.

[2] Fingerman M. Crustacean endocrinology: a retrospective, prospective and introspective analysis [J]. Physiological Zoology, 1997, 70(3): 257-269.

[3] Huberman A. Shrimp endocrinology: a review [J]. Aquaculture, 2000, 191 (1-3): 191-208.

[4] Sandeman D C, Sandeman R E, Derby C, *et al*. Morphology of the brain of crayfish, crabs, and spiny lobsters: a common nomenclature for homologous structures [J]. Biological Bulletin, 1992, 183: 304-326.

[5] Huang H Y, Li S J, Wang G Z, *et al*. A preliminary study on the histology of the neurosecretory cells in the brain of *Scylla serrata*[J]. J Xiamen University (Natural Science), 2001, 40 (3): 793-797.

[6] Huang H Y, Li S J, Ye H H, *et al*. Ultrastructure of neurosecretory cells in the protocerebrum of *Scylla serrata*[J]. Marine Science, 2003, 27(1): 17-19.

[7] Fingerman M, Nagabhushanam R, Sarojini R. Vertebrate-type Hormones in crustaceans: localization, identification and functional significance [J]. Zoological Science, 1993, 10(1): 13-29.

[8] Fingerman M, Nagabhushanam R, Sarojini R, *et al*. Biogenic amines in crustaceans: identification, location and roles [J]. J Crustacean Biology, 1994, 14(3): 413-437.

[9] Harzsch S, Glötzner J. An immunohistochemical study of structure and development of the nervous system in the brine shrimp *Artemia salina* Linnaeus, 1758 (Branchiopoda, Anostraca) with remarks on the evolution of the arthropod brain [J]. Arthropod Structure & Development, 2002, 30: 251-270.

[10] Langworthy K, Helluy S, Benton J, *et al*. Amines and peptides in the brain of the American lobster: immunocytochemical

localization patterns and implications for brain function [J]. Cell and Tissue Research, 1997, 288: (1): 191– 206.

[11] Atwood H L, Sandman D C. The biology of crustacea, vol.3, Neurobiology structure and function[M]. New York: Academic Press, 1982.

[12] Fingerman M. Roles of neurotransmitters in regulating reproductive hormone release and gonadal in decapod crustaceans [J]. Invertebrate Reproduction Development, 1997, 31(1): 47 – 54.

[13] Ye H H, Li S J, Li Q F, *et al*. Regulation of biogenic amines over the reproductive neuroendocrine activity of female *Scylla serrata*[J]. Oceanol et Limnol Sin, 2003, 34(3): 329– 333.

[14] Hoyle C H. Neuropeptide families: evolutionary perspectives [J]. Regulatory Peptides, 1998, 73: 1– 33.

[15] Ye H H, Wang G Z, Jin Z X, *et al*. Immunocytochemical study of the optic ganglion and the brain of *Penaeus vannamei*. [J]. Oceanol et Limnol Sin, 2004, 35(1): 93– 98.

[16] Yoshiharu H, Keiko T, Akira C, *et al*. Immunohistochemical localization of neuropeptides in the cephalic ganglion of the land crab *Chiromantes haematocheir* [J]. Fisheries Science Tokyo, 1996, 62(6): 909– 913.

[17] Schmidt M, Ache B W. Antennular projections to the midbrain of the spiny lobster Ⅱ. Sensory innervation of the olfactory lobe [J]. J Comparative Neurology, 1992, 318: 291– 303.

锯缘青蟹脑内 5-HT 和 NPY 的免疫细胞化学定位

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摘要: 为探讨 5– 羟色胺和神经肽 Y 在锯缘青蟹脑内的存在与否和分布状况, 应用免疫细胞化学技术, 在光学显微镜下观察 5– 羟色胺和神经肽 Y 阳性细胞和神经髓质的形态和分布。结果表明: 在锯缘青蟹脑中共 12 个胞体群和 11 个神经髓质中, 前脑和中脑中有 4 个细胞群和 6 个神经髓质检出 5– HT 免疫阳性反应; 中脑和后脑中有 7 个细胞群和 3 个神经髓质具有 NPY 免疫阳性反应。5– 羟色胺和神经肽 Y 在锯缘青蟹脑内的特异性分布, 为其参与神经生理活动提供了形态学证据。

关键词: 锯缘青蟹; 脑; 5– 羟色胺; 神经肽 Y; 免疫细胞化学

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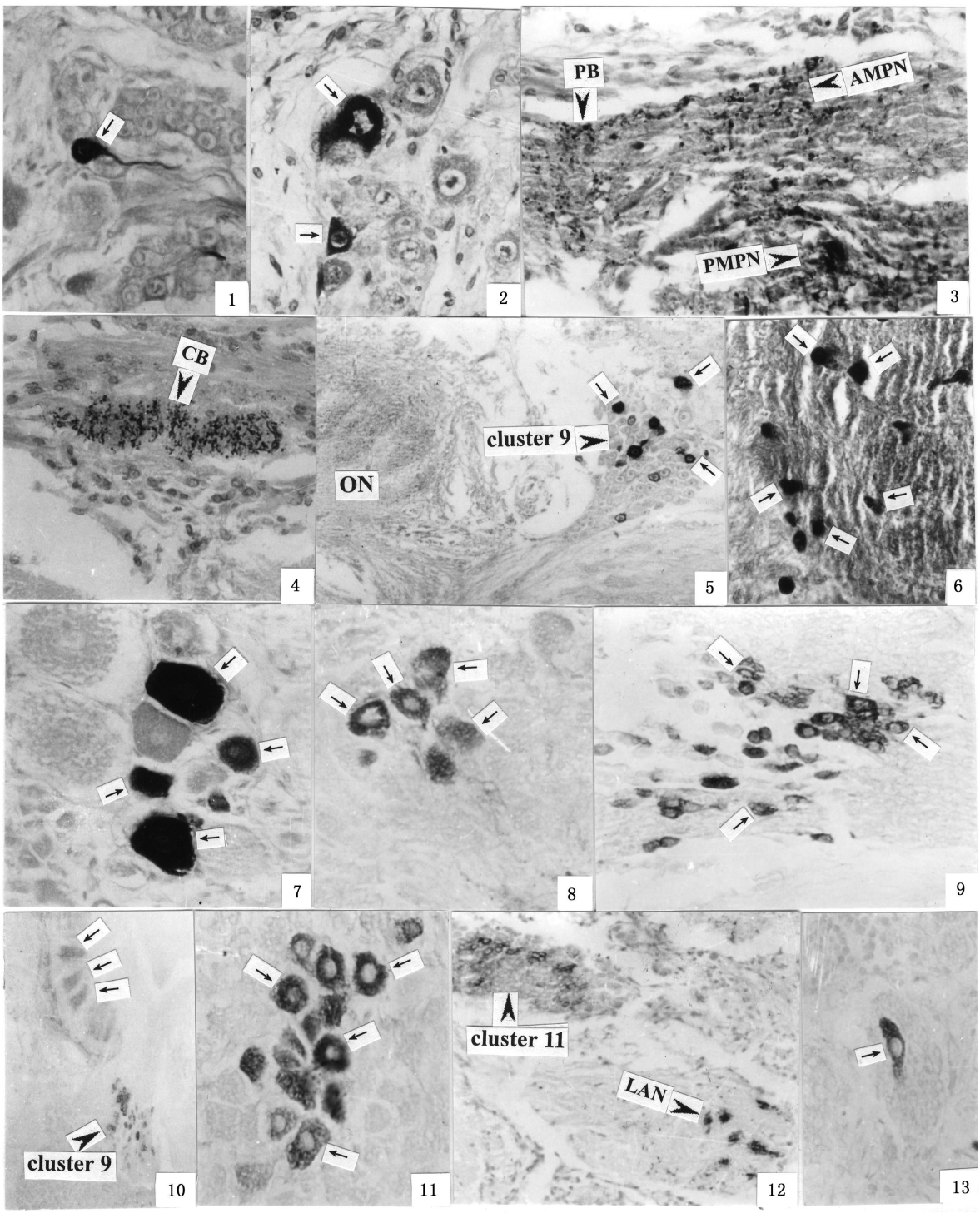

Plate

1. 5-HT-immunoreactive cell in cluster 6 with long cytoplasmic projections(↑), × 330; 2. 5-HT-immunoreactive cells(↑) in cluster 7, × 330; 3. Bits and pieces of 5-HT-immunoreactive substances broadly distribute among PB, AMPN and PMPN, × 330; 4. CB(⬆) show 5-HT-immunoreactivity, × 330; 5. small 5-HT- immunoreactive cells(↑) in cluster 9, showing cluster 9 project fibers(⬆) into ON, × 330; 6. 5-HT-immunoreactive glomeruli(↑) in ON, × 330; 7. 5-HT- immunoreactive cells(↑) in cluster 11, × 330; 8. NPY-immunoreactive cells(↑) in cluster 6, × 330; 9. NPY-immunoreactive cells(↑) in cluster 9, × 330; 10. weak NPY-immunoreactive glomeruli(↑) in ON, showing NPY-immunoreactivy in cluster 9, × 82.5; 11. NPY-immunoreactive cells(↑) in cluster 11, × 330; 12. patches-like NPY-immunoreactivity in LAN(⬆), showing NPY- immunoreactivy in cluster 11, × 165; 13. NPY- mmunoreactivity cell(↑) in cluster 14 and 15, × 330