

available at www.sciencedirect.comwww.elsevier.com/locate/brainres**BRAIN
RESEARCH****Research Report****Discovery of specific tryptophan hydroxylase in the brain of the beetle *Harmonia axyridis*****Xuexiang Bao^{a,*}, Ximei Tian^a, Xihan Hu^a, Zhifu Zhao^a, Yutang Qu^a, Chuantao Song^{b,c}**^aLaboratory of Insect Brain Neurobiology, School of Life Sciences, Northeast Normal University, Changchun 130024, P.R. China^bCollege of Environment and Resources, Jilin University, Changchun 130012, P.R. China^cCollege of Urban and Environmental Sciences, Northeast Normal University, Changchun 130024, P.R. China

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ABSTRACT

Rabbit anti-serotonin and mouse monoclonal anti-tryptophan hydroxylase antisera were applied on the brain sections of the beetle *Harmonia axyridis*, butterfly *Chilodactylus zenobia*, moth *Antheraea pernyi* and ant *Camponotus japonicus*, using the Streptavidin–Peroxidase immunohistochemical method and Colophony–Paraffin embedded section technique. Results revealed that all the experimental insects showed notable serotonin-like immunoreactivity in the brain. However, only the brain sections of the beetle *H. axyridis* were strongly labeled by mouse monoclonal anti-tryptophan hydroxylase antiserum, with the distribution pattern matching that of serotonin. These results demonstrate that specific tryptophan hydroxylase may exist in the brain of the beetle and likely reflect the diversity of serotonin synthetic mechanisms as well as the evolution of aromatic amino acid hydroxylase genes.

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1. Introduction

The indolealkylamine serotonin (5-hydroxytryptamine, 5-HT) is widely distributed throughout the central and peripheral nervous systems of vertebrates and invertebrates. Although 5-HT has entirely different physiological functions in these two distinct systems, the synthetic mechanisms and metabolic pathways are consistent (Wan et al., 1999). The 5-HT usually serves as a neurotransmitter and/or neuromodulator in the insect central nervous system and is also released in the hemolymph, acting as a neurohormone away from the releasing site (Homberg and Hildebrand, 1989). Studies have shown that, in insects, 5-HT is present in a small population of neurons with certain distribution patterns that are involved in numerous neural signaling and behavior variations (Nässel,

1988). Therefore, it is an important signal molecule in insects and is essential to both behavior and development processes (Manastiriotti, 1999).

Two enzymatic steps are necessary for the synthesis of 5-HT from its natural precursor tryptophan, an essential amino acid in mammals. After being taken up in 5-HTergic neurons by a high-affinity carrier, tryptophan is converted into 5-hydroxytryptophan (5-HTP) by tryptophan hydroxylase (TPH) in cytoplasm. The 5-HTP is then transported to axon terminals and decarboxylated into 5-HT by 5-HTP-decarboxylase (Bender et al., 1987). TPH is the rate-limiting enzyme in the biosynthesis of 5-HT and possesses a high specificity. TPH activity is exclusively required for the 5-HT synthesis despite the low levels of presence in tissues and the instability of the enzyme (Hufton et al., 1995). Therefore, TPH represents a specific

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property of 5-HTergic neurons and can be considered as an important differentiation character as well. Furthermore, the presence of TPH is more reliable than 5-HT concentration in identifying the character of neurocyte (Hufton et al., 1995).

TPH is the key enzyme in the biosynthesis of 5-HT, and it indirectly plays an important role in the process of development and behavior modulation. In recent years, TPH has been a focus of growing international interest, though its study presents significant challenges. In mammals, the aromatic amino acid hydroxylase (AAAH) family comprises three genes that code for the enzymes respectively responsible for the hydroxylation of tryptophan (TPH, EC 1.14.16.4), phenylalanine (PAH, EC 1.14.16.1) and tyrosine (TH, EC 1.14.16.2) (Alcañiz and Silva, 1997). TPH and PAH are two distinct enzymes with non-overlap distribution regions. However, the characterization of AAAHs in insects is still preliminary. It was originally thought that there were only two AAAHs in the fruit fly *Drosophila melanogaster*: DTH (*Drosophila* tyrosine hydroxylase), which hydroxylates tyrosine, and DTPH (*Drosophila* tryptophan-phenylalanine hydroxylase), a dual function enzyme responsible for the hydroxylation of both phenylalanine and tryptophan (Neckameyer and White, 1992). According to the sequence comparison analysis, the PAH function outweighs the TPH and the DTPH gene is more likely to be a *Pah* gene with the capacity of hydroxylate tryptophan rather than a *Pah/TPH* gene. Thus, it is postulated that the existence of a specific *Tph* gene in *Drosophila* and other insects is most unlikely (Neckameyer and White, 1992). Recently, however, Coleman and Neckameyer (2005) had provided sufficient evidence to the presence of an additional enzyme in *Drosophila*, DTRH (*Drosophila* tryptophan hydroxylase), distinct from DTPH, with extensive homology to mammalian TPH. DTRH may be primarily neuronal in function and expression, while DTPH may be only the peripheral TPH (Coleman and Neckameyer, 2005). Therefore, it can be concluded that, at least in *Drosophila*, two enzymes (DTRH and DTPH) must be regulated to synthesize 5-HT in the presence of the appropriate substrate, which is consistent with recent work from mammalian studies that have demonstrated the presence of at least two TPH enzymes (Coleman and Neckameyer, 2005).

However, the knowledge of TPH in insects other than *Drosophila* is still incomplete and premature. In the present experiment, using the Streptavidin–Peroxidase immunohistochemical method (Bao et al., 1999) combined with Colophony–Paraffin embedded section technique, the brain sections of more insects (beetle, butterfly, moth and ant) were immunolabeled by rabbit anti-5-HT antiserum and mouse monoclonal anti-TPH antiserum. The results provide insights into the presence style, regulatory mechanism and evolutionary history of TPH in insects and thus provide information to support the studies of neurocyte types, physiological functions and molecular mechanisms of 5-HT.

2. Results

In the present study, about 20 preparations of each insect species were immunostained, and the stainings of all the four insect species were carried out in parallel in order to reduce

variability. The immunostaining results among every tested animal in each experiment were consistent.

2.1. 5-HT- and TPH-like immunoreactivity

The immunohistochemical studies showed that the brain of all experimental insect species displayed notable 5-HT-like immunoreactivity. 5-HT-like immunoreactive (5-HT-IR) neurons had an extensive and stable distribution pattern (Niu et al., 2004; Tian et al., 2005; Zhang et al., 2003). 5-HT-IR fibers were resolved in all areas of the brain, which displayed brown after immunostaining. The number of 5-HT-IR neurons was relatively small, but the positive somata had extensive varicose arborizations. Most of 5-HT-IR neurons were tangential neurons and bilaterally symmetrical distributed mainly in the optic lobe, midbrain and suboesophageal ganglion.

Only the brain of the beetle *Harmonia axyridis* demonstrated strong TPH-like immunoreactivity; the other experimental insects were all negatively labeled by mouse monoclonal anti-TPH antiserum. The regional distribution of TPH in the brain of *H. axyridis* approximately corresponded to that of 5-HT. All major neuropil regions, in general, showed low level background after TPH immunostaining for no TPH substance releasing into synaptic gaps, but TPH-like immunoreactive (TPH-IR) processes were still prominent. The neurocyte type, number, distribution and projection of TPH-IR neurons in *H. axyridis* were similar to that of 5-HT, which are rendered as follows.

2.2. 5-HT/TPH-IR neurons in *H. axyridis*

In the optic lobe, 5-HT/TPH-IR neurons (ca. 35 per hemisphere) were mainly situated between the optic lobe and the protocerebrum. The neurites of these cells either descended into the optic neuropils or ascended into the protocerebrum, thus possibly integrating the optic lobes and the other brain

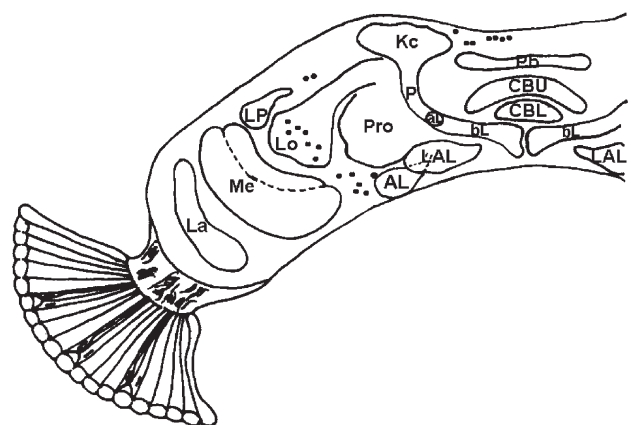


Fig. 1 – Basic organization of brain structures of the beetle and the localization of 5-HT/TPH-IR neurons. La, lamina; Me, medulla; Lo, lobula; LP, lobula plate; Pro, protocerebrum; LAL, lateral accessory lobe; Kc, Kenyon cells; P, pedunculus; al, α -lobe; bL, β -lobe; Pb, protocerebral bridge; CBU, upper central body; CBL, lower central body; AL, antennal lobe.

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