

## Distribution of GABA and 5-HTergic immunoreactive neurons in the visual system of butterfly *Mimathyma schrenckii* \*

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**Abstract** -aminobutyric acid (GABA) and serotonin (5-HT) immunoreactivity in the visual system (compound eyes and optic lobes) of the butterfly *Minathyma schrenckii* were studied with the CP (Colophony-paraffin) embedding serial section technique and the SP (Streptavidin-peroxidase) immunohistochemical method. Contrary to other insects reported previously, certain photoreceptors of this taxon react with GABA and 5-HT antiserum. In each optic lobe, about 2 600 GABA-like immunoreactive neurons cluster into six groups; three (M1 - 3) are located near the medulla, the other three (LC1 - 3) are confined to the lobula complex. Processes from GABA-like neurons supply all the three neuropiles of optic lobes. However, weak 5-HT immunoreactivity in the optic lobes was detected compared to the strong labeling of GABA. The neuropiles are devoid of classical thick 5-HT immunoreactive varicose processes as reported in other insects, and only fine fibres with a regular pattern can be identified. There are only 25 5-HT positive neurons per hemisphere, which share a similar position with M3 GABA-like immunoreactive neurons. The possible involvement of 5-HT and GABA in control of neuronal activity in the visual system is discussed [*Acta Zoologica Sinica* 50 (5): 770 - 777, 2004].

**Key words** *Minathyma schrenckii*, Visual system, -aminobutyric acid (GABA), Serotonin (5-HT), Immunohistochemistry

## 白斑迷蛱蝶视觉系统中 GABA 和 5-HT 能神经元的分布 \*

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**摘 要** 采用树脂石蜡 (Colophony-Paraffin, CP) 组织包埋切片技术和链霉菌抗生物素蛋白-过氧化物酶 (Streptavidin-peroxidase, SP) 免疫组织化学方法, 首次报道了 GABA 和 5-HT 两种神经递质在白斑迷蛱蝶视觉系统 (复眼及视叶) 中的分布。与以往所报道的昆虫不同, 白斑迷蛱蝶复眼中部分感光细胞对 GABA 和 5-HT 抗血清产生免疫反应。每侧视叶中约有 2 600 多个 GABA 能阳性神经元, 它们共分为 6 群。其中 3 群位于外髓附近 (M1 - 3), 另外三群位于内髓复合体边缘 (LC1 - 3)。GABA 能神经元发出的轴突在整个视叶的 3 个神经纤维网中都有分布。相比之下, 视叶对 5-HT 抗血清的反应较弱, 视叶神经纤维网中不存在代表 5-HT 阳性反应的粗大静脉曲张状纤维, 只有一些排列规则的细小纤维。每侧视叶只有位于外髓附近的 25 个神经元呈现阳性反应, 它们的分布位置与部分 M3 群的 GABA 能样神经元相同。本文还探讨了 5-HT 和 GABA 在调节视觉信息时可能发挥的作用 [*动物学报* 50 (5): 770 - 777, 2004]。

**关键词** 白斑迷蛱蝶 视觉系统 -氨基丁酸 (GABA) 5 羟色胺 (5-HT) 免疫组织化学

Two classical neurotransmitters, GABA and 5-HT, whose functions have been studied in the physiological, biochemical, pharmacological and pathological investigations (Strausfeld and Miller, 1980; Homberg, 1994; Pineyro and Blier, 1999), are widely distributed in insect visual system (Homberg, 1994;

Leitinger et al., 1999). Considerable evidence indicates that GABA acts as an inhibitory transmitter in processing of visual inputs (Homberg et al., 1987; Homberg, 1994; Dang et al., 2002; Tian et al., 2003). However, the physiological actions of 5-HT in insect visual system are largely unknown (Zhang et

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al. , 2003). Anatomical , physiological and pharmacological findings suggest that 5-HT might influence the diurnal rhythm of the sensitivity of insect visual neurons ( Ichikawa , 1994a; Cuttle et al. , 1995; Leitinger et al. , 1999).

*M. schrenckii* belongs to the Nymphalidae of Lepidoptera (Zhou and Zhu , 2003). Like most other butterflies, they are predominantly diurnal and rely on a well-developed visual system for communication and predator detection (Yack and Fullard , 1999). The brain structure and function of butterflies have been studied in a few conventional anatomical (Singh and Maurya , 1977; Hämmerle and Klob , 1997) and physiological studies (Ichikawa , 1994a , b). So far no immunohistochemical data has been published related to GABA and 5-HT distribution in the brain of this taxon. In contrast , the sphinx moth *Manduca sexta* a nocturnal Lepidopteran , has frequently been studied as a model for insect neurobiology and physiology. The distribution of GABA and 5-HT in its brain have previously been reported (Homberg et al. , 1987; Homberg and Hildbrand , 1989; Homberg , 1994).

Here , we describe the distribution , number and projections of 5-HT and GABA-like immunoreactive neurons in the compound eyes and optic lobes of the butterfly *M. schrenckii* , using the colophony-paraffin (CP) embedding serial section technique (Bao et al. , 1999) and streptavidin-peroxidase (SP) immunohistochemical detection method. Our aim is to provide a basis for understanding the insect vision mechanism.

## 1 Material and methods

### 1.1 Experimental animals

The investigation was carried out on 40 (20 for each antiserum immunoreactive group ) adult *M. schrenckii* caught from the wild in Huinan County , Jilin Province , China.

### 1.2 Preparation of the specimen

Heads (with integrated exoskeleton) of live *M. schrenckii* adults were decapitated and fixed in 4 % paraformaldehyde at room temperature for 3.5 - 4 hours , thoroughly rinsed in 0.1 mol phosphate buffer (PBS , pH 7.4 , 3 × 15 min) , dehydrated through ethanol in ascending concentrations (70 % , 80 % , 90 % , 95 % , 100 % ) , cleared in tert-butanol at 30 °C for 24 hours and then embedded in CP embedding reagent ( + , + , at 53 °C for one hour each) (Bao et al. , 1999). The specimens were then sectioned at a thickness of 6 μm , and mounted on polylysine-coated glass slides.

### 1.3 Antibodies

Anti-serotonin antibody was obtained from Sigma (Product No. S5545) , it was a polyclonal rabbit antiserum against serotonin and had been characterized in cockroach (Baumann et al. , 2002). The rab-

bit polyclonal antiserum against GABA was purchased from Sigma too (Product No. A2052) , and its specificity had been characterized in locust (Wildman et al. , 2002). Ultra-sensitive SP kit (Product No. KIT-9706) , including endogenous peroxidase blocking solution , normal non-immune serum , biotin conjugated goat anti rabbit IgG (secondary antibody) and streptavidin-peroxidase (SP) complex , were purchased from Maixin Bio-Company in Fuzhou , China.

### 1.4 Immunohistochemistry

Sections were (1) deparaffinized in xylene , rehydrated in graded ethanol ; (2) incubated with 50 μl endogenous peroxides blocking solution for 10 minutes at room temperature ; (3) incubated with 50 μl non-immune serum for 10 minutes at room temperature ; (4) incubated with 50 μl rabbit 5-HT antiserum (diluted 1 : 1 800 with 0.1 mol PBS) or GABA (diluted 1 : 3 000 with 0.1 mol PBS) antiserum at 4 °C overnight ; (5) incubated with 50 μl biotin-conjugated secondary antibody for 10 minutes at room temperature the next day ; (6) incubated with 50 μl streptavidin-peroxidase complex for 10 minutes at room temperature ; (7) treated for 2 minutes with a solution of 3 , 3'-diaminobenzidine (DAB) as a chromogen with H<sub>2</sub>O<sub>2</sub> as a substrate (Maixin-Bio , China) (PBS was used to rinse sections 15 min between every two procedures , except for the interval between 3 and 4) ; (8) counter-stained with Ehrlich-hematoxylin (Chemical Reagent Factory of Shanghai , China) dehydrated , cleared and mounted with neutral balsam. (9) observed and photographed under an Olympus microscope.

### 1.5 Controls

Sections were processed as above but treated with non-immune serum or PBS instead of primary antibodies.

## 2 Results

### 2.1 Distribution of GABA-like immunoreactive neurons in the compound eyes and optic lobes of *M. schrenckii*

*M. schrenckii* has two juxtaposed eyes (Plate 1) , which comprise numerous identical ommatidia. The ommatidia show a regular pattern , with each of them consisting of two major functional regions , (a) the light harvesting part and (b) the light sensitive part. The former is composed of cornea , corneal cells , crystalline cells , crystalline cone and pigment cells (Plate 2) , while the latter comprises pigment cells , photoreceptors (also termed as retinula cells) and retinal rod (Plate 3). Pigments are distributed widely in the pigment cells and photoreceptors. Under living conditions , pigments appear purplish red ; but after SP procedures , they become Oxford blue. Some photoreceptors are weakly labeled by GABA an-

tiserum (Plate :3). Their axons project into the lamina through crossed post-retina fibres.

The optic lobe consists of three major neuropil regions, the distalmost lamina, the medulla, and the innermost lobula complex (Plate : 1). The lamina can be divided into two strata: distal cell body layer and proximal nerve fibre layer. The former contains the somata of the monopolar cells, which do not exhibit GABA immunoreactivity. The latter shows a diffuse and homogeneous meshwork of GABA immunoreactive fibres. The lamina is organized into columns corresponding to the ommatidia of the retina. (Fig. 1A).

All the 7 layers of the medulla exhibit a positive GABA-like immunoreactivity. The distalmost layer 1 is characterized by relatively few and mostly perpendicularly column-like arborizations. The same pattern, but with a higher density, can be seen in layer 2. In layer 3, the serpentine layer, the GABA immunoreactivity is weak and predominantly tangentially oriented. Layer 4 exhibits fine granular staining, which is weaker in layer 5. Fine perpendicularly oriented GABA containing arborizations are seen in layer 6. Layer 7 is characterized by large immunoreactive fibre profiles, which are connected with the inner chiasma and branch tangentially (Plate : 4). Some GABA immunoreactive fibres join the lobula complex originated anterior optic tract and project into anterior optic tubercle (Plate : 5). There are some GABA-like immunoreactive fibres in both the outer and inner chiasm.

GABA-immunoreactive perikarya in three groups (M1 - M3) send fibres toward the medulla (Fig. 1A). Group M1 lies in the cell-body cortex near lamina between lamina and outer chiasm, and comprises of 400 - 500 large trans-the medullary columnar cells (10 - 15  $\mu\text{m}$ ), which project perpendicularly neurites toward the medulla and send side arborizations in several layers of the medulla (Plate : 6). About 500<sup>3</sup>/600 columnar neurons (14  $\mu\text{m}$ ) and amacrine cells (8  $\mu\text{m}$ ) in group M2 send primary neurites toward the outer surface of the medulla. Their somata scatter in

the cell-body cortex dorsally and frontally from the outer surface of the medulla (Fig. 2C). Group M3 is located in the anterior cortex and especially in the dorsal and ventral margins between the medulla and protocerebrum. Most of the neurons in M3 are elliptical-shaped large tangential cells (9 - 16  $\mu\text{m}$ ), which project tangentially into the middle parts of the medulla (Plate : 5, 7).

In *M. schrenckii*, the lobula complex is subdivided into the lobula and the lobula plate. Three strata can be observed in both of these neuropiles. The GABA immunoreactive staining in outer and inner layer is denser than that in the middle layer (Plate : 4, Fig. 1A). Although some lobula fibres project via the anterior optic tract into anterior optic tubercle (Plate : 5), no GABA containing fibres can be traced from the medulla and the lobula complex into the posterior optic commissure. The same situation is found in both the serpentine optic commissure, which projects from middle layer of the lobula into the lateral accessory lobe, and the superior optic tract, which project from inner layer of lobula into superior lateral protocerebrum. The lobula plate appears to be an obtuse triangle-shaped structure in horizontal sections (Plate : 1, 4). There are some immunoreactive fibres running between the outer surface of the lobula and lobula plate.

Three groups of GABA-like neurons could be distinguished near the lobula complex (LC1-LC3, Fig. 1A). LC1 comprises of 800 small amacrine cells (4.5  $\mu\text{m}$ ), which scatter in the posterior cell cortex of the lobula plate. These cells send neurites toward the inner face of the medulla or the lobula plate (Fig. 2A). A group (LC2) of about 80 tangential cells (9  $\mu\text{m}$ ) lies between lobula and protocerebrum, some of which send neurites projecting toward the middle layer of the lobula; others give rise fascicles of fibres running toward the middle protocerebrum (Plate : 8). In addition, about 60 amacrine cells (9  $\mu\text{m}$ ) with perikarya in the anterior margin of the lobula send primary neurites toward the outer layers of the lobula and the lobula plate (Plate : 9).

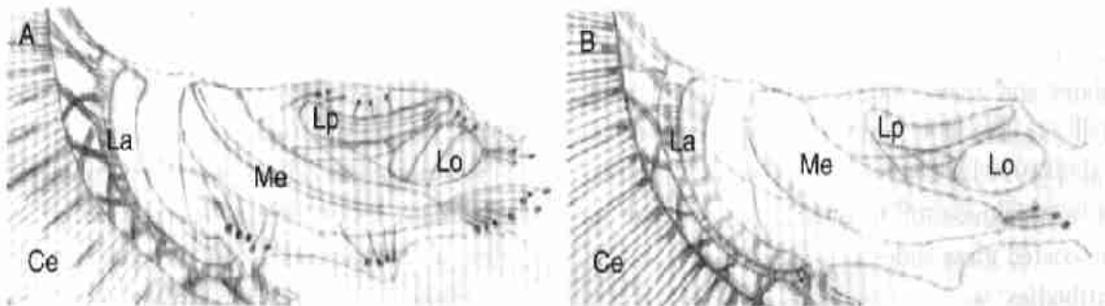
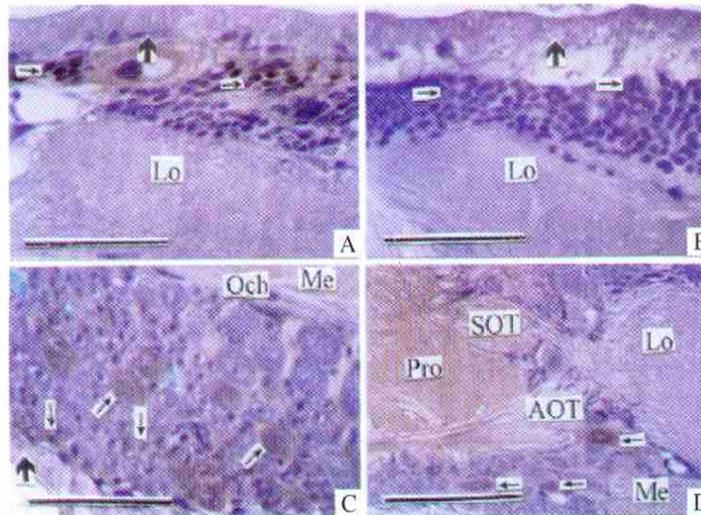


Fig. 1 Principal localization of GABA (A) and 5-HT (B) -ergic immunoreactive neurons in the visual system of the butterfly *M. schrenckii*

Ce: Compound eyes. La: Lamina. Lo: Lobula. Lp: Lobula plate. Me: Medulla.

Two accessory structures that modulate circadian rhythm (similar to those of cockroach, (Reischig and Stengl, 2002) are found in the optic lobes of *M. schrenckii*, including the accessory medulla, which is ventro-medial to the medulla, and the extraretinal photoreceptors, which lie poster-ventrally toward the lamina and appear purplish red under living conditions. The accessory medulla reacts with GABA antiserum (Plate : 4), while extraretinal photoreceptors are not labeled under the same conditions. These photoreceptors turn to Oxford blue after SP immunoreactive procedures.



**Fig. 2 Original color figures of GABA and 5-HT immunoreactivity in the visual system of *M. schrenckii***

A. Tested section showing Group LC<sub>1</sub> (○) neurons react with GABA antiserum. B. Group LC<sub>1</sub> (○) neurons are not labeled in control section. C. Group M<sub>2</sub> contain certain large columnar neurons (△) and small amacrine cells (○). D. Horizontal section showing anterior optic tract (AOT), superior optic tract (SOT), and some tangential neurons (○) exhibit 5-HT immunoreactivity, however the optic lobe (Me, medulla and Lo, lobula) is almost unlabeled by 5-HT antiserum. Och: Outer optic chiasm. Pro: Protocerebrum. ◻: Perineurium. Scale bar: A - C = 50 μm, D = 125 μm.

## 2.2 Distribution of 5-HT-like immunoreactive neurons in the compound eyes and optic lobes of *M. schrenckii*

Each ommatidium in the compound eye of *M. schrenckii* contains some 5-HT immunoreactive photoreceptors. They extend through the column of ommatidia, and are stained with higher density in the proximal part. 5-HT positive photoreceptors send primary neurites projecting toward the lamina through post-retinal fibres after passing through the basement membrane and the perineurium (Plate : 10, Fig. 1B). Some of their neurites even extend into the proximal parts of the medulla.

There are some weak 5-HT immunoreactivities within the optic lobes of *M. schrenckii*. No classical thick 5-HT immunoreactive varicose processes are found in the three neuropiles as reported in other insects, and only fine fibres with a regular pattern can be identified (Plate : 11). Only one group of 5-HT neurons, about 25 tangential neurons per hemisphere, which share a similar position with M3 GA-

BA-like neurons, is found in the anterior cortex between the medulla and protocerebrum (Fig. 1B, Fig. 2D). All the four optic tracts, the anterior optic tract, the posterior optic commissure, the serpentine optic commissure, and the superior optic tract (Reischig and Stengl, 2002), contain some 5-HT immunoreactive fibres, which are labeled denser in protocerebrum (Fig. 2D). Thus, in addition to the projections from photoreceptors and tangential neurons, the optic lobes of *M. schrenckii* also contain 5-HT immunoreactive fibres coming from the protocerebrum. Furthermore, the extraretinal photoreceptors

react with 5-HT antiserum and show homogeneous dense immunoreactivity in the middle parts (brown, plate : 12), whereas there is no 5-HT immunoreactivity in the accessory the medulla.

Control: sections were unlabeled when treated with non-immune serum or PBS (Fig. 2B) instead of primary antibodies.

## 3 Discussion

### 3.1 Distribution of GABA and 5-HT-like immunoreactive photoreceptors in insect compound eyes

In the compound eyes of *M. schrenckii*, some photoreceptors react with GABA or 5-HT antiserum. Immuno-positive photoreceptors send primary axons projecting toward the lamina through post-retinal fibres after passing through the basement membrane. Some of the axons might even extend into the proximal parts of the medulla. Actually, the same situation is found in beetle *Hanmonia axyridis* (Tian et al., 2003). Although Datum et al. (1986) reported that the GABA-like photoreceptors in blowfly *Calliphora*

*erythrocephala*, other investigations on bee *Apis mellifera* (Schäfer and Bicker, 1986), moth *M. sexta* (Homberg et al., 1987) and cockroach *Periplaneta americana* (Füller et al., 1989) did not support it. These different observations mentioned above are probably due to the inter-specific difference of various insects (Füller et al., 1989). However, in our opinion, it is more likely due to different methods. In the present investigation, the sections are stained with Ehrlich-hematoxylin after SP detection method (Zhang<sup>\*</sup>). The counter staining of hematoxylin, with the immunoreaction are brought up from the blue background. Compared with other immunohistochemical techniques that are widely applied in foreign entomological researches, this method provides broader visible areas and higher sensitivity with the visualization of the unlabeled parts on the same sections. Thus SP detecting method provided an alternative way to detect the low level antigens in tissue sections. Our results suggest that GABA and 5-HT-like photoreceptors in insect compound eyes are involved in some unknown visual modulating processes. Since GABA and 5-HT immunoreactivity can be found in the same photoreceptors or adjoining ones, they might also act as co-transmitters in these unknown processes.

### 3.2 Distribution of GABA and 5-HT-like immunoreactive neurons in insect optic lobes

GABA is a primary inhibitory neurotransmitter in the insect visual system (Leake and Walker, 1980). The distribution of GABA-like immunoreactive neurons in insect optic lobes has been reported in various species such as moth *M. sexta* (Homberg et al., 1987), cockroach *P. americana* (Füller et al., 1989), ant *Camponotus japonicus* (Dang et al., 2002), beetle *H. axyridis* (Tian et al., 2003), bee *Apis sinensis* (Xu et al., 1994) and *A. mellifera* (Schäfer and Bicker, 1986). A similar distribution pattern of GABA immunoreactive neurons is found in the optic lobes of *M. schrenckii*. For instance, all the three neuropiles exhibit strong GABA immunoreactivity and can be subdivided into several layers; significant distribution, number and projections of GABA-like immunoreactive neurons can be seen in all species; the neurons under the same categories are clustered into groups and their projections are visible at microscopical level. Thus, GABA in the optic lobes of *M. schrenckii* shares the same function with that in other insects, which mainly suppresses local neural activity during visual inputs processing (Homberg, 1994; Dang et al., 2002; Tian et al., 2003).

In contrast, the distribution pattern of 5-HT varies greatly among species (Nässel and Klemm, 1983), but there are still some general aspects we could draw from different insects. As reported in blowfly *Calliphora erythrocephala*, locust *Schistocerca gregaria*, cockroach *P. americana* (Nässel and Klemm, 1983), bee *A. mellifera* (Schürmann and Klemm, 1984), moth *M. sexta* (Homberg and Hildebrand, 1989), mantis *Tenoderma sinensis* (Leitinger et al., 1999) and ant *Camponotus japonicus* (Zhang et al., 2003), 5-HT-like immunoreactive processes originate from a relatively small number of cell bodies but distribute over a large volume in the optic lobes. The optic lobes contain varicose 5-HT positive fibres, which are distinct in morphology and degree of staining. All of them show pronounced stratification. However, the optic lobes of *M. schrenckii* show a different immunoreactive pattern. As they show weak 5-HT immunoreactivity, no classical stratification and varicose fibres are found in this taxon. Actually, the physiological actions of 5-HT in the insect visual system are largely unknown (Zhang et al., 2003). The distribution of 5-HT immunoreactivity in the optic lobes of *M. schrenckii* might be a special phenomenon. Although the similar weak immunoreactivity is also found in the optic lobes of other butterflies, such as *C. zenobia*, *Pieris melete* (forma aestivalis), and *Papilio bianor* (forma aestivalis), both of the pronounced stratification and varicose fibres could be detected in all species mentioned above (unpublished data). In Jilin province (northeast China), the adults of *M. schrenckii* mainly emerge in midsummer (June - July), which is the longest daylight in the whole year, whereas the other butterflies develop later (July - September) when the daytime gradually become shorter than that in midsummer. Thus, the distribution of 5-HT immunoreactivity in the optic lobes of butterflies might be involved in insect photoperiodic adaptation: the longer the daylight, the weaker the 5-HT immunoreactivity.

### 3.3 Comparison of GABA and 5-HT immunoreactivity in the optic lobes between butterfly *M. schrenckii* and moth *M. sexta*

No obvious difference of GABA immunoreactivity is found in the optic lobes between these two species, except that the lamina of *M. sexta* is divided into three layers (Homberg et al., 1987). However, the number of GABA immunoreactive neurons varies greatly between *M. schrenckii* and *M. sexta*. In the optic lobes of the moth *M. sexta*, 9 000 neurons per

\* Zhang SJ, 1993. Introduction of a new immunohistochemistry-SP method. The Third Academic Session of Pathological Technique in Guangdong Province. 41 (In Chinese).

张素娟, 1993. 介绍一种新的免疫组化方法 第三次广东省病理技术学术会议论文集. 41.

hemisphere are immunoreactive with GABA antiserum (Homberg et al., 1987), but in *M. schrenckii*, only 2 600 neurons in there.

On the contrary, distinct 5-HT immunoreactivity exists between these two species. The optic lobes of *M. sexta* show relatively strong 5-HT immunoreactivity. All the four optic ganglia in *M. sexta*, the lamina, the medulla, lobula and lobula plate, exhibit stratification and varicose-like immunoreactive fibres (Homberg and Hildebrand, 1989), whereas no such 5-HT immunoreactive pattern is detected in *M. schrenckii*. In contrast to the large number of 5-HT immunoreactive cells in *M. sexta* (300 - 350 per hemisphere), only 25 neurons exhibit 5-HT immunoreactivity in each optic lobe of *M. schrenckii*.

To test the specificity of the discrepancy between *M. schrenckii* and *M. sexta*, we used the same method to detect the level of GABA and 5-HT in the brain of other Lepidoptera. The results testified our finding that GABA and 5-HT-like immunoreactive neurons in moth always outnumber that of butterflies, and the difference with regard to 5-HT immunoreactivity is quite remarkable. So, there may be two different visual modulation mechanisms in moths and butterflies. 5-HT involving in adjusting the sensitivity of sensory systems often exhibit a circadian rhythm that reflects changing environmental conditions as well as the behavioral demands of the animal (Kloppenborg et al., 1999). As we all know, most butterflies are diurnally active, and depend on well-developed visual senses for communication and orientation (Yack and Fullard, 1999). In contrast, moths are predominantly nocturnal and sensitive to faint light in the dark. Thus, the differences of GABA and 5-HT immunoreactivity in the optic lobes between this two species are probably due to the distinct circadian rhythm of butterflies and moths.

### 3.4 Potential physiological actions of 5-HT and cooperation with GABA in insect visual system

The classical neurotransmitter, 5-HT, is widely distributed throughout the nervous system of vertebrates and invertebrates (Homberg and Hildebrand, 1989; Pineyro and Blier, 1999). Its role in development, reproduction, feeding behavior, and diurnal rhythm is well established (Homberg, 1994). Most insects show strong 5-HT immunoreactivity in the visual system (Homberg, 1994). 5-HT modulates visual sensitivity by regulating the activity of insect optic interneurons (Nässel, 1988). Anatomical, physiological and pharmacological findings suggest that 5-HT influences the diurnal rhythm of the sensitivity of insect visual neurons (e.g., Homberg, 1994; Ichikawa, 1994a; Cuttle et al., 1995; Kloppenburg et al., 1999). In particular, the cells reported to be influenced by the application of 5-HT could be pho-

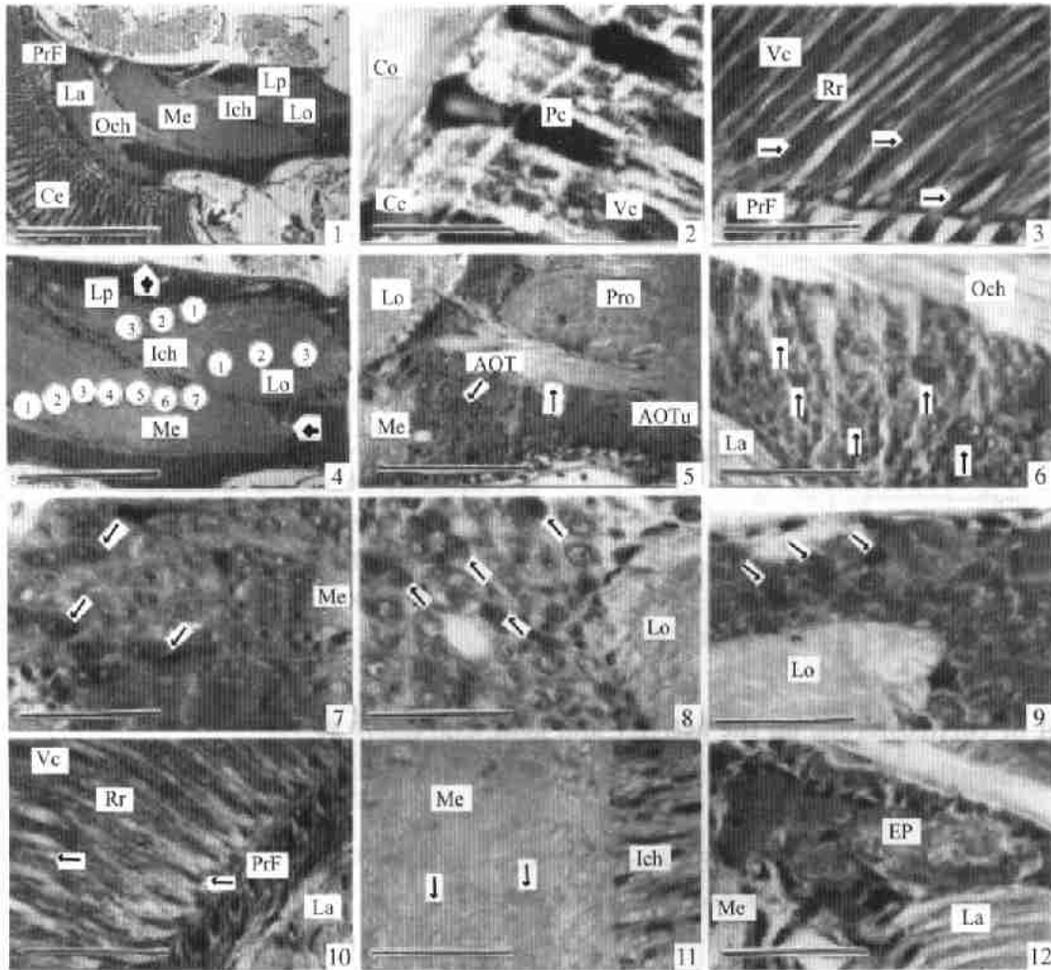
toceptors, optic lobe interneurons, and surrounding glial cells (Leitinger et al., 1999). 5-HT immunoreactive neurons at the anterior edge of the medulla discharge impulses in the dark, and these process is inhibited by illumination of the ipsilateral eye. Thus, these neurons may have a role in controlling light-dark adaptation or overall activity of the optic lobe and midbrain (Ichikawa, 1994a). In addition, 5-HT has been implicated in switching insect photoreceptors from a high-acuity, low-sensitivity day state to a low-acuity, high-sensitivity night state (Kloppenborg et al., 1999). Thus the difference of 5-HT immunoreactive neurons between butterflies and moths as mentioned above suggests that 5-HT could improve the insect's ability in response to the faint light.

Two accessory structures that modulate circadian rhythm (Reischig and Stengl, 2002) are found in the optic lobes of *M. schrenckii*, the accessory medulla and extraretinal photoreceptors, the latter were not described in the early reports on the visual system of moth *M. sexta* (Homberg et al., 1987; Homberg and Hildebrand, 1989; Homberg, 1994). Actually, most Lepidoptera possess one or more extraretinal photoreceptors in their visual system (Singh and Maurya, 1977). However, the accessory medulla was only found in some large butterflies such as *M. schrenckii* and *Papilio xuthus* (Ichikawa, 1994). In the optic lobes of *M. schrenckii*, the accessory medulla reacts with GABA antiserum, whereas the extraretinal photoreceptors show 5-HT immunoreactivity. Thus, 5-HT might cooperate with GABA in modulating insect circadian rhythm.

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1. Horizontal section through the visual system of *M. schrenckii* showing the compound eye (Ce) and all optic neuropiles of the optic lobe are labeled by GABA antiserum.
2. Horizontal section demonstrates the distal part of compound eye.
3. Certain photoreceptors react with GABA antiserum ( ).
4. GABA immunoreactive section showing the stratification of medulla (Me), lobula (Lo), and lobula plate (Lp).
5. Some fibers ( ) in anterior optic tract (AOT) exhibit GABA positive immunoreactivity.
- 6-9. Horizontal sections demonstrate 4 groups of GABA-ergic neurons: Group M1 ( ), Group M3 ( / ), Group LC2 ( \ ), and Group LC3 ( \ ).
10. Certain photoreceptors ( ) react with 5-HT antiserum and are labeled strongly in proximal parts.
11. Thin fibers ( ) in medulla react with 5-HT antiserum.
12. Extraretinal photoreceptors (EP) react with 5-HT antiserum.

The icons “ ” in Plate : 4 refer to the different layers of neuropiles. Other abbreviations and icons: AOTu, anterior optic tubercle; Ce, crystalline cone; Co, cornea; Ich, inner optic chiasm; La, lamina; Och, outer optic chiasm; Pc, pigment cells; PrF, post-retina fibers; Pro, protocerebrum; Rr, retinal rod; Vc, visual column; , accessory medulla; /, orbital skeleton; , perineurium. Scale bar: Plate : 1 = 500  $\mu$ m; Plate : 4 = 250  $\mu$ m; Plate : 5 = 125  $\mu$ m; others = 50  $\mu$ m.