氏　　名	Pei-Ju CHEN
学位(専攻分野)	博士(理学)
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学 位 論 文 題 目	Histaminergic signaling underlies the early stages of color vision in the butterfly, *Papilio xuthus*
論 文 審 査 委 員	主　査　講師　木下　充代 講師　大槻　久 教授　蟻川　謙太郎 准教授　Gregor Belusic University of Ljubljana,Biotechnical Faculty

Summary of Doctoral Thesis

Name in full Pei-Ju Chen

Title Histaminergic signaling underlies the early stages of color vision in the butterfly, *Papilio xuthus*
（ナミアゲハ *Papilio xuthus* の色覚初期過程におけるヒスタミン依存性情報処理）

Spectrally opponent responses, i.e. wavelength-dependent response-polarity inversions, have been observed at the level of photoreceptors quite frequently in butterflies. The origin of the inhibition in these butterflies can be due to extracellular return currents, real synaptic transmission or by both. I propose that the observed opponency is not due to extracellular return currents because these photoreceptors show fast-onset hyperpolarization when stimulated with specific wavelengths. Similar fast, on-transient hyperpolarizing responses that are found in the insect second-order visual neurons (lamina monopolar cells, LMCs), are mediated by histamine-gated chloride channels. In the lamina of *Papilio*, the photoreceptors are not only presynaptic to LMCs, but also pre- and post-synaptic to other photoreceptors. Thus, I hypothesized that histaminergic sign-inverting synapses exist between different spectral receptors as a mechanism for the spectral opponency in butterfly photoreceptors.

Does the “histamine hypothesis” explain the recorded spectral opponency of butterfly photoreceptors? If the inhibition is synaptic, which spectral classes of photoreceptors are involved, and how are their spectral sensitivities modified by the synaptic interaction? What are the roles of histaminergic channels in color processing in the *Papilio* visual system? This thesis attempts to answer these questions by examining the physiological properties of histamine-gated chloride channels, their distribution in visual synapses, and the function in shaping spectral sensitivities of visual neurons. I performed immunohistochemistry and several electrophysiological experiments including patch clamp, single electrode switching clamp, and intracellular recording followed by dye injection to address the above questions.

I first immunolocalized two candidates of histamine-gated chloride channels, PxHCLA and PxHCLB, in the visual system of the butterfly *Papilio xuthus* (Chapter 2). The anti-PxHCLA labeling was associated with the plasma membrane of non-photoreceptor neurons that are postsynaptic to photoreceptors, suggesting that PxHCLA is located in the LMCs at least in the lamina, the first optic ganglion. The anti-PxHCLB labeling overlapped with photoreceptor axons, indicating the PxHCLB is expressed at the inter-photoreceptor synapses.

I further analyzed physiological properties of these channel candidates by whole-cell patch-clamp using those expressed in cultured cells to confirm whether these molecules really form histamine-gated chloride channels (Chapter 3). I studied the responses of PxHCLA and PxHCLB channels to histamine as well as to other neurotransmitter candidates. I found that histamine and GABA activated both PxHCLA and PxHCLB, while the other molecules did not. The sensitivity of both channels to histamine was about 500-fold more than that of GABA. In other words, the patch-clamp experiments confirmed that both PxHCLA and PxHCLB are indeed activated by histamine and permeate chloride ions. The sensitivity to histamine and GABA was consistently higher in PxHCLB than in PxHCLA.

Using sharp microelectrode recording with current injections, I found that the responses of both LMCs (Chapter 4) and spectrally opponent photoreceptors (Chapter 5) could be reversed when the membrane potential was close to the equilibrium potential of chloride ions. The results provide an *in vivo* electrophysiological evidence for the presence of chloride channels in these cells, which is consistent with my results of immunohistochemistry of histamine-gated chloride channels in the *Papilio* lamina.

The combination of different approaches has provided a support for the “histamine hypothesis”: spectral opponency in butterfly photoreceptors is attributed to direct inhibition between photoreceptors, mediated by the histaminergic chloride channel PxHCLB.

To understand how the histaminergic synapses mediate signal processing in the early visual system, I recorded responses of LMCs and photoreceptors in the *Papilio* lamina. The recorded LMCs can be divided into non-spectrally opponent and spectrally opponent ones (Chapter 4). The former includes three spectrally heterogeneous classes, which are ommatidial type dependent. LMCs seem to integrate chromatic signals from the photoreceptors in the same ommatidium. This suggests that PxHCLA channels most likely implement the primary visual processing from photoreceptors to secondary neurons. The findings of spectrally opponent LMCs provide a clue that *Papilio* LMCs do contribute to the chromatic information processing. I propose that both spectrally opponent and non-opponent LMCs feed signals into motion detection circuitry and in parallel into the color vision system.

On the other hand, I recorded eight classes of spectrally opponent photoreceptors in the *Papilio* lamina (Chapter 5). The recorded opponent responses are well explained by the ommatidial type-specific inter-photoreceptor synaptic connections via PxHCLB. The present study brings a closer view on the underlying histaminergic signaling and neural circuits of color vision at the initial stage in *Papilio*.

The findings presented in this thesis provides new insights into the complexity of chromatic processing of *Papilio*. My electrophysiological recordings will be a springboard for future studies of early chromatic processing in insect visual systems.

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Results of the doctoral thesis screening

博士論文審査結果

Name in Full
氏　　名　　Pei-Ju CHEN

Title
論文題目　　Histaminergic signaling underlies the early stages of color vision in the butterfly, *Papilio xuthus*

Pei-Ju CHEN, the candidate, studied the possible histaminergic signaling in the visual system of the Japanese yellow swallowtail butterfly, *Papilio xuthus*. The thesis starts with the General Introduction, which is followed by four main chapters (Chapter 2-5), and concluded by the General Discussion. A number of original discoveries are described in great detail in generally well-written English. The results are highly convincing because several different approaches were employed in almost equal weights: the experimental techniques include molecular biology, light and electron microscopic anatomy and electrophysiology.

General Introduction (Chapter 1) explains the history of color vision study in insects, the recent progress in the butterfly vision research, and the histamine signaling mainly studied in flies. The candidate then explains the research questions about possible spectral opponency of butterfly photoreceptors using her own data.

Chapter 2 is devoted to the immunohistochemical localization of two candidate proteins of histamine-gated chloride channel, PxHCLA and B. PxHCLA was found at the membrane of second visual neurons, LMCs, which receive inputs from photoreceptors. PxHCLB was found at the interphotoreceptor synapses. Published in the Journal of Comparative Neurology.

Chapter 3 shows that the PxHCLA and B proteins in fact function as histamine-gated chloride channels by patch-clamp electrophysiology. The candidate successfully measured and analyzed the membrane currents in cultured cells expressing the proteins and reached the above conclusion. Published in the Journal of Experimental Biology.

Chapter 4 describes the response characteristics of LMCs recorded in the first optic ganglion, the lamina. The candidate recorded about 200 LMC-like units, and identified 130 as those from LMCs. The LMC responses were categorized into spectrally non-opponent and opponent classes based on their spectral sensitivities.

Chapter 5 describes the response characteristics of photoreceptors recorded in the lamina. Many of the units exhibit spectral opponency, whose origin was analysed based on some criteria including reversal potential, polarization sensitivity as well as angular sensitivity.

The thesis is concluded by the General Conclusion (Chapter 6), which briefly overviews the obtained results. The candidate has proposed the “histamine hypothesis” for the photoreceptor spectral opponency: the opponency should be attributed to the antagonistic interaction between photoreceptors with distinct spectral sensitivities via the activation of PxHCLB. The candidate’s conclusion that the hypothesis has been basically confirmed is quite

reasonable. Such a thorough physiological and anatomical analysis about insect lamina is very unique, only preceded by the research on flies. However, color vision of flies is rather poor, and therefore the impact of the candidate's work would add substantial body of knowledge to the field of comparative physiology, sensory ecology, visual neuroscience, evolutionary biology and some related areas. Because the thesis is written in English, the committee concluded that the candidate's ability of English is satisfactory. The value of the thesis should be highly evaluated, and all members of the committee agreed that this was certainly more than enough for a PhD.