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Short Note

Study on the Development of the Ocellar L-neurons of the Cockroach *Periplaneta americana*

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Jia-Ling Chang, Jin-Tun Lin, Tzay-Show Tsai and Chin-Yih Wu (1994) Study on the development of the ocellar L-neurons of the cockroach *Periplaneta americana*. *Zoological Studies* **33**(4): 310-313. Cobalt staining techniques were used to compare the amounts and sizes of ocellar L-neurons of the American cockroach *Periplaneta americana* at various stages of development. Molting was observed; the head width, brain size, and ocellar corneal diameter were measured to investigate their relationship to body growth. The results show that after the sixth instar (body length 12-15 mm), the head width, brain width, and ocellar morphological changes are progressively related to body length. Individuals of body length shorter than 12 mm have no ocellar cornea. The ocellar cornea diameter in adults was double that of nymph instars. The results also showed that except for those individuals of less than 10 mm in body length, each ocellus contains three or four L-neurons at various stages. Their cell bodies are oval, and the diameter increases as the individual grows, about 10 μm in early stages and 30 μm in the adult stage. The cell bodies are distributed irregularly in the forebrain near the protocerebral bridge, some are strung together and some are dispersed.

Key words: *Periplaneta americana*, Ocellar L-neurons, Cobalt staining method.

Adult American cockroaches have two compound eyes and two ocelli. The former are mainly for photoreception, but the functions of the latter are not fully understood; they may be related to escape or mating behavior (Carlson and Chi 1979). Each adult ocellus consists of 10,000 photoreceptors. Each ocellar optic nerve consists of 30-40 neurons; three or four of these are the largest-diameter neurons in the insect brain, and are called "L-neurons" (Webber and Renner 1976). L-neurons have been shown to be the main sensory neurons of ocelli; they have been stained in vivo and classified by some researchers (Mizunami et al. 1982, Mizunami and Tateda 1986). Still there remain the nymphal ocellar L-neurons. Until now there have been few reports concerning the morphogenesis of insect ocelli with the exception of grasshoppers (Mobbs 1976 1979).

To compare L-neurons at various developmental stages, a cobalt staining method was used to identify the L-neurons at various nymphal instars. Additional relationships among body length, head width, brain width, and ocellar corneal diameter in cockroach development were also investigated to better understand the structural and physiological differences between nymph and adult ocelli.

Materials and Methods—American cockroach *Periplaneta americana* were kept at 25 $\pm$ 2°C, relative humidity of 60 $\pm$ 10%, in a 12 hr light/12 hr dark photoperiodic regime. Sixty ootheca were chosen and put in a series of cages. After hatching, nymph molts were recorded every day. Nymphs were then lightly anesthetized with CO_2 and their body length, head width and ocellar corneal diameters were measured.

To study L-neuron insects in various growth stages were immobilized by waxing the bodies onto a petri dish. A small square opening was cut on the surface of the ocellar cornea and a cotton pad soaked with 200 mM $CoCl_2$ was placed on the opening. After two or three hours the cotton pad was taken away and the ocelli were rinsed with saline solution. The heads were dissected and the brains isolated in a vial; then some ammonium sulphide was added. After rinsing with saline the brain width and length were measured; then the brain was fixed in Carnoy's solution, followed by silver intensified solution (Chang and Lin 1992). Finally, a permanent slide was made. The L-neurons and cell bodies at various stages were examined under a microscope.

Results—The nymphal stage of the American cockroach lasts for half a year. The nymph usually develops into adulthood through eight to ten molts. Though judging nymph instar on the basis of individual development was the ideal it proved troublesome in application. After statistical analysis of body length it was found that after the sixth instar, body length is a more appropriate criterion for deciding instar status (Fig. 1). Ratios of head width and brain width vs body length analysis also showed that the relation of head width to body length was similar to that of brain width to body length; both were

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close to a linear relationship. In our studies, the brain grew more slowly, longitudinally than transversely. This is the result of an increase of ommatidia; there are 100 ommatidia at the first instar and 3,500 at adulthood. Usually the ommatidia grow forward or outward so that the head of the first instar resemble a "fat triangle" instead of the adult "flat triangle" (transverse axis larger than longitudinal axis).

The ocellar corneal diameter of the American cockroach showed a close relationship to body length (Fig. 2). Before the fourth instar, they never had formative ocelli. From the ninth instar on (25 mm), ocellar corneal diameters increase with body growth. However, the boundary line around the cornea was still not sharp until the nymph metamorphosed to adult. After reaching adulthood, the ocelli are rather concrete and their diameter approximately 600 μm.

Each the adult cockroach ocellus contains three or four L-neurons. Because all neurons are monopolar, after injecting dyes from ocellus to follow L-neurons we easily found that the three or four globular cell bodies were irregularly arranged in the forebrain near the protocerebral bridge, each with a diameter of about 30 μm. Because staining nymph stage L-neurons is difficult, some individuals were dyed only in the optic nerve. Results of counting successful samples (n = 38), indicated that the distribution status of cell bodies in the nymph stage brain were similar to those at the adult stage (Fig. 3). The number of ocellar L-neurons at various nymphal stages was not very different from that of the adult. However, they were obviously smaller (Figs. 3, 4). The ocelli containing three or four L-neurons comprise 39.47% and 47.32%, respectively.

In cockroaches with body lengths shorter than 10 mm, even when treated with repeated staining (n = 35), cell bodies of L-neurons were not found (Figs. 2, 4). Observation of sliced paraffin samples has shown that the L-neurons did not fully develop before this stage. Thus the nymph brain before this stage does not stain, except for the ocellar optic nerves. Cell bodies of L-neurons after this stage enlarge as nymphs grow, especially at the final instar, when they are about 30 μm in diameter (Figs. 2, 3). From this, one may speculate that the critical time of development of ocellar L-neurons in cockroaches may be at the fifth or sixth instar (body length at 12-15 mm). At that time, ocellar L-neuron cell bodies are approximately 10 μm in diameter (Fig. 2).

Discussion—In *Periplaneta americana*, ocelli may be related to the mating behavior of the adult (Toh and Yokohari 1988). However, some studies on the ocellar physiology in other insects report that ocelli act in antagonism and summation with compound eyes (Lall and Trouth 1989). From other studies of organogenesis, it was also found that the time of ocellar development coincided with wing development; thus some researchers have inferred that ocelli were flight related (Goodman 1981). Cockroaches are nocturnal and are not skilled in flying even though they possess wings as adults so ocelli might not relate significantly to flight. If they relate to escape, then the ocellar structure at adulthood must be similar to that of the nymph stage.

To date grasshopper ocellar development has been studied most thoroughly. They hatch with developed ocelli and their ocellar optic nerve contains L-neurons even 48 hours before hatching. Furthermore, the basic structure of immature ocelli is almost the same as that of adults (Mobbs 1976). However, in cockroach nymphs with a body length shorter than 5 mm, there are no ocelli on the frons. The time of development of L-neurons in cockroaches also has been shown to be unique.

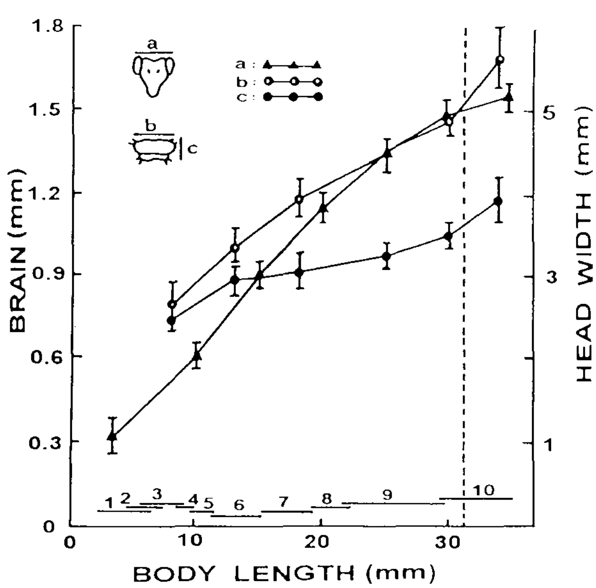

Fig. 1. Head width (a), brain size (b and c), and body length of American cockroach at various stage. Ordinates indicate head width (right) and brain size (left), abscissa is body length. Each spot on curves represents the average of seven samples. Short upright lines are standard deviation. The numerals (1-10) indicate nymphal instar. Right side of the dashed line represents the adult stage.

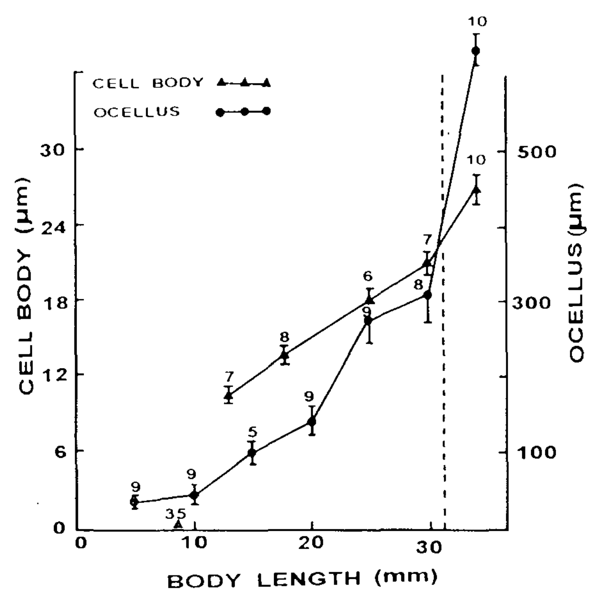

Fig. 2. The diameter of ocellar cornea and L-neuron cell bodies and body length at various stages. Ordinates represent the diameter of ocellar cornea (right) and cell bodies of L-neurons (left). Abscissa is body length at various stages. Sample number for average are above each spot. The short upright lines are standard deviation. Right side of the dashed line represents the adult stage.

L-neurons have been found at the fifth or sixth instar; the ocellar cornea also becomes obvious only in the adult. From the above, it may be concluded that the functions of adult ocelli are not same as those of nymphal ocelli.

Cockroach ocelli of the first nymph stage contain some undeveloped pre-retinula cells: at 5 mm body length, they contain 18-40 retinula cells and at 10 mm they increase to 100 but at adult they contain over 10,000 cells (Toh and Hara 1984, Toh and Sagara 1984). Because there are different numbers of retinula cells at various developmental stages, it can be suggested that the function of ocelli at immature stages is different from that of the adult.

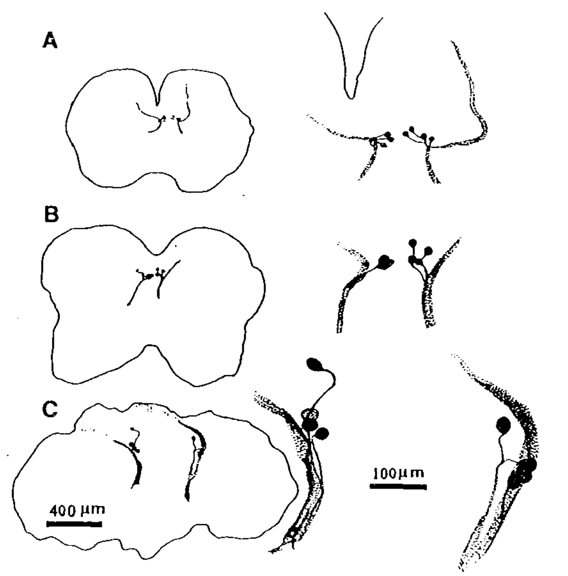

Fig. 3. The brain width and cell bodies of L-neurons at nymphal stages of body length 13 mm (A), 30 mm (B), and adult stage (C). Left side locates the L-neurons in brain and right side shows the magnifications of L-neurons.

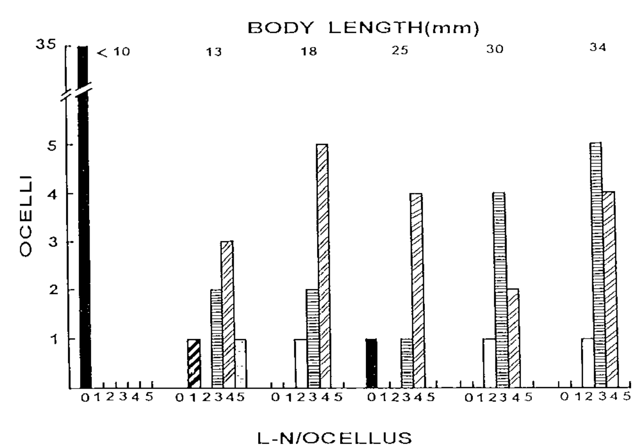

Fig. 4. The number of ocellar L-neurons in each ocellus at various stages. L-neurons are typically monopolar neurons so that the number of L-neurons can be decided on the number of cell bodies. Ordinate represents the number of samples (observed number of ocelli).

Cockroach adult males show complex mating behavior (Roth and Willis 1952), whereas the nymphs do not. When behavioral changes of the adult cockroach and its ocellar development are considered together, they suggest that cockroach ocelli may be characteristic of adult life. But the quantity of L-neurons in the immature cockroach, and their distribution in the brain, are similar to those of adults. If the function of ocelli is related to light-avoiding behavior, then the avoidance mechanism may be very simple. This behavior could be developed by coordinating ocelli with compound eyes at the nymph stage. However, the complex mating behavior of adults involves an interaction of hormones and odors which may need the fully developed L-neurons to coordinate with other interneurons to organize a communication network for signal transmission. Increase of the signal-noise ratio of impulses could enable the ocelli to perceive the changes in the environment. Perhaps, this is why the ocellar cornea, the cell bodies of L-neurons, and the brain volume of cockroach adults are larger than those of immature cockroaches.

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美洲蟑螂稚蟲單眼巨大神經元之研究

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本研究先長期觀察蟑螂個體成長過程的蛻皮情形，並測量不同發育時期個體之頭部，腦部的大小，單眼角膜直徑與體長的關係，再以鈷離子染色法比較不同時期個體之單眼巨大神經元細胞體的數目、形狀、大小及在腦部的分佈和排列情形。結果得知蟑螂共蛻皮十次左右，第六齡（體長12-15 mm)之後，各齡間體長涵蓋範圍較寬，重疊較少，如以體長表示齡期，誤差較少。頭寬、腦寬和單眼的形態變化與體長均有漸進關係，在長出翅變爲成蟲的時期，其變化最顯著。體長小於12 mm的個體，沒有單眼角膜的構造，成蟲時單眼角膜直徑增加爲稚蟲期的兩倍。除體長小於10 mm個體的巨大神經元尚未發育完成，故染不出細胞體之外，其餘各齡的每個單眼均有三個(占39.47％)或四個(占47.32％)的巨大神經元，其細胞體均呈球狀，是典型的單極神經元，直徑則隨體長變化而漸漸增加，最早期的巨大神經元細胞體直徑約10 μm，成蟲時期增爲30 μm左右。稚蟲和成蟲一樣，細胞體均分佈於前腦，而且排列不規則，有些並排成串，有些則分散開。

關鍵詞：美洲蟑螂, 單眼巨大神經元, 鈷染色法。

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