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Intracellular Stainings of the Large Ocellar Second Order Neurons in the Cockroach

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Summary. The large ocellar second order neurons (L-neurons) in the cockroach, *Periplaneta americana* have been studied physiologically by intracellular recordings and morphologically by intracellular and whole nerve cobalt stainings. All the recorded L-neurons showed similar light responses, i.e., light on-hyperpolarization and a small number of off-spikes. All the stained L-neurons had an ocellar arborization covering the whole region of the ocellar neuropile and an central arborization in the region posterior to the protocerebral bridge.

Introduction

Most insects have two or three ocelli, in addition to compound eyes. The ocellar nerve which connects the ocellus to the brain consists of a small number of large axons and a larger number of smaller axons. In analysis of the characteristics of the ocellar visual system, the large second order neurons (L-neurons) of several insects have been studied electrophysiologically and morphologically (review by Goodman 1981; Taylor 1981; Milde 1981; Mobbs et al. 1981).

The cockroach has two ocelli. It has been shown that four large axons in the ocellar nerve of the cockroach are second order neurons (Ruck 1957, 1961; Weber and Renner 1976; Toh, personal communication). The central projections of the ocellar neurons were reported by Cooter (1975) and Bernard (1976) using whole nerve cobalt impregnation. However, the anatomy and physiology of individual L-neurons have not been reported.

In the present study, cockroach ocellar L-neurons have been studied physiologically and morphologically.

Materials and Methods

Adult males of the cockroach, *Periplaneta americana*, reared in the laboratory, were used throughout this study. The neck of the animal was put into a slit in a lucite case and the head was projected into the case. The head and thorax of the animal were fixed with bee's wax to the lucite wall. The lucite case was filled with a physiological saline solution (Yamasaki and Narahashi 1959). The ocellar nerves were exposed by removing a small piece of cuticle from the front of the head.

Intracellular potentials were recorded by means of glass-pipette microelectrodes (ca. 50 MOhm) filled with a solution of 1.5 mol/l KCl and 0.5 mol/l $CoCl_2$. Electrodes were inserted in the various parts of the ocellar nerves. A small piece of platinum metal placed in the saline served as an indifferent electrode.

White light emitted by a 6–8 V tungsten filament lamp was passed through a heat-absorbing filter. The duration of illumination was controlled by a mechanical shutter, and the intensity was adjusted by calibrated neutral density filters. The intensity of the light without filters is referred to as unit intensity ($\log I = 0.0$), and has a value of about 3×10^3 lux at the preparation.

For intracellular stainings, cobalt was introduced to the neurons via intracellular recording electrodes by applying positive current pulses (50 nA, 0.4 s, 1 Hz) for about 40 min. For whole nerve impregnations, the cuticlar lens of the ocellus of the intact animal was pierced by a sharpened needle and a drop of 0.25 mol/l $CoCl_2$ solution was placed over the hole of the cuticular lens. In most cases cobalt was impregnated from both right and left ocelli simultaneously. The animal was placed in a moist chamber at room temperature for about 2 h. Following cobalt impregnation, the ocelli, the ocellar nerves, and the brain were removed from the head capsule, stained, fixed in Carnoy's solution and intensified as whole mounts (Bacon and Altman 1977). Drawings were made with the aid of Abbe's drawing camera.

Results

Electrophysiological Responses

All recorded L-neurons had resting potentials of about 50–60 mV and no spontaneous action poten-

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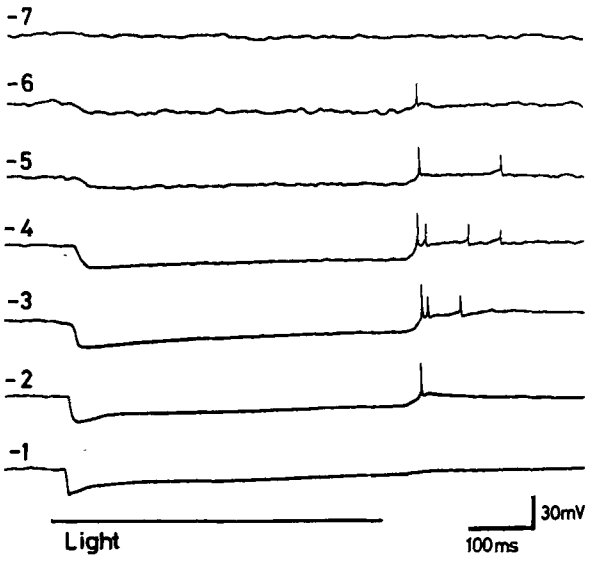

Fig. 1. Intracellular responses of the ocellar L-neuron to white light stimuli of 0.5 s duration at various intensities. Light intensities are indicated to the left of each trace

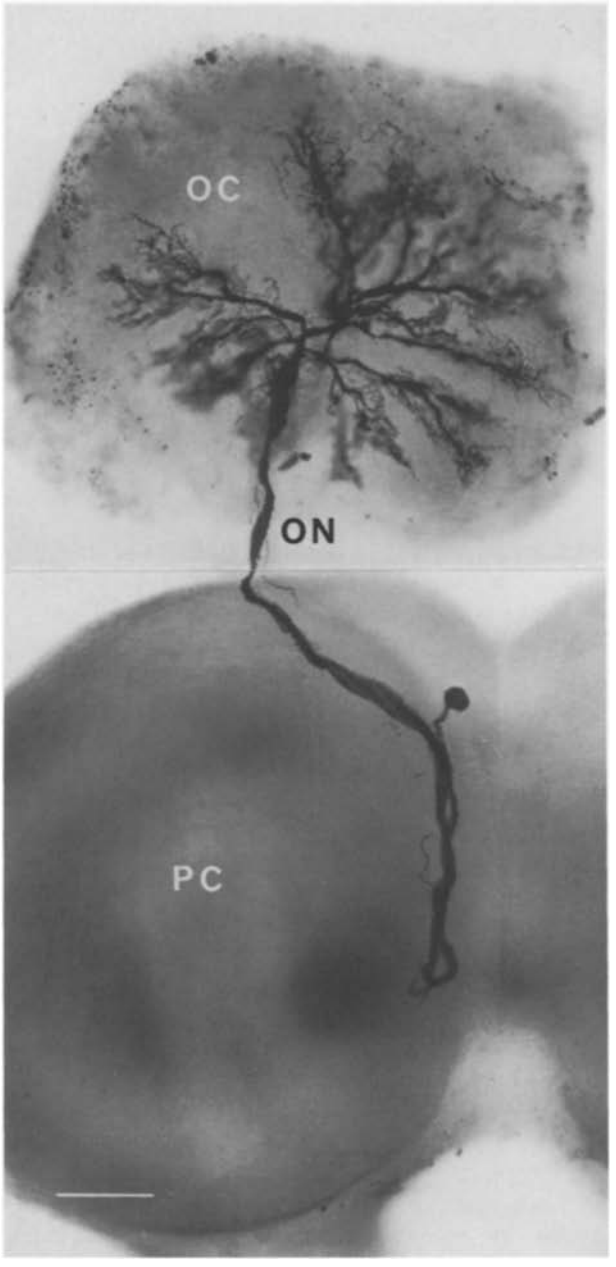

Fig. 2. An intracellularly stained ocellar L-neuron. *OC* ocellus; *ON* ocellar nerve; *PC* protocerebrum. *Scale*: 100 μm. Viewed postero-dorsally

tials. All the L-neurons responded to illumination with a graded on-hyperpolarization and a small number of off-spikes, and significant difference was not observed among light responses of individual L-neurons. The most common example of light responses of the L-neuron is shown in Fig. 1. At high intensity illuminations the hyperpolarizing response consists of a rapid initial phase and a following slow phase, while at low intensity it consists of only a slow phase. At the end of illumination, a small number of spikes occurred, with no prominent graded off-depolarization as reported for L-neurons in the locust (Patterson and Goodman 1974; Wilson 1978) and in the dragonfly (Chappell and Dowling 1972; Klingman and Chappell 1978). In all cases, a maximum number of off-spikes was elecited by an intermediate intensity of about −4 log unit. However, the number was often unstable, and varied between one and four to five during recordings made for 2–3 h. No further investigation was carried out on this matter.

Anatomy

The anatomy of the ocellar L-neurons was revealed by injection of cobalt via intracellular recording electrodes. 22 L-neurons were stained successfully in different preparations. One representative example is shown in Fig. 2. The cell body is located in the pars intercerebralis. We found that all of the stained L-neurons have an ocellar arborization covering the entire region of the ocellar neuropile and a central arborization in the region posterior to the ipsilateral margin of the protocerebral bridge (cf. Figs. 3, 4). From this observation, we conclude that all L-neurons have a more or less similar input area in the ocellus and a similar terminal area in the brain. However, the L-neurons showed some variations in their process anatomy. In the case shown in Fig. 3a, the process extending from the cell body runs postero-ventrally for a short distance, then bifurcates at a certain region

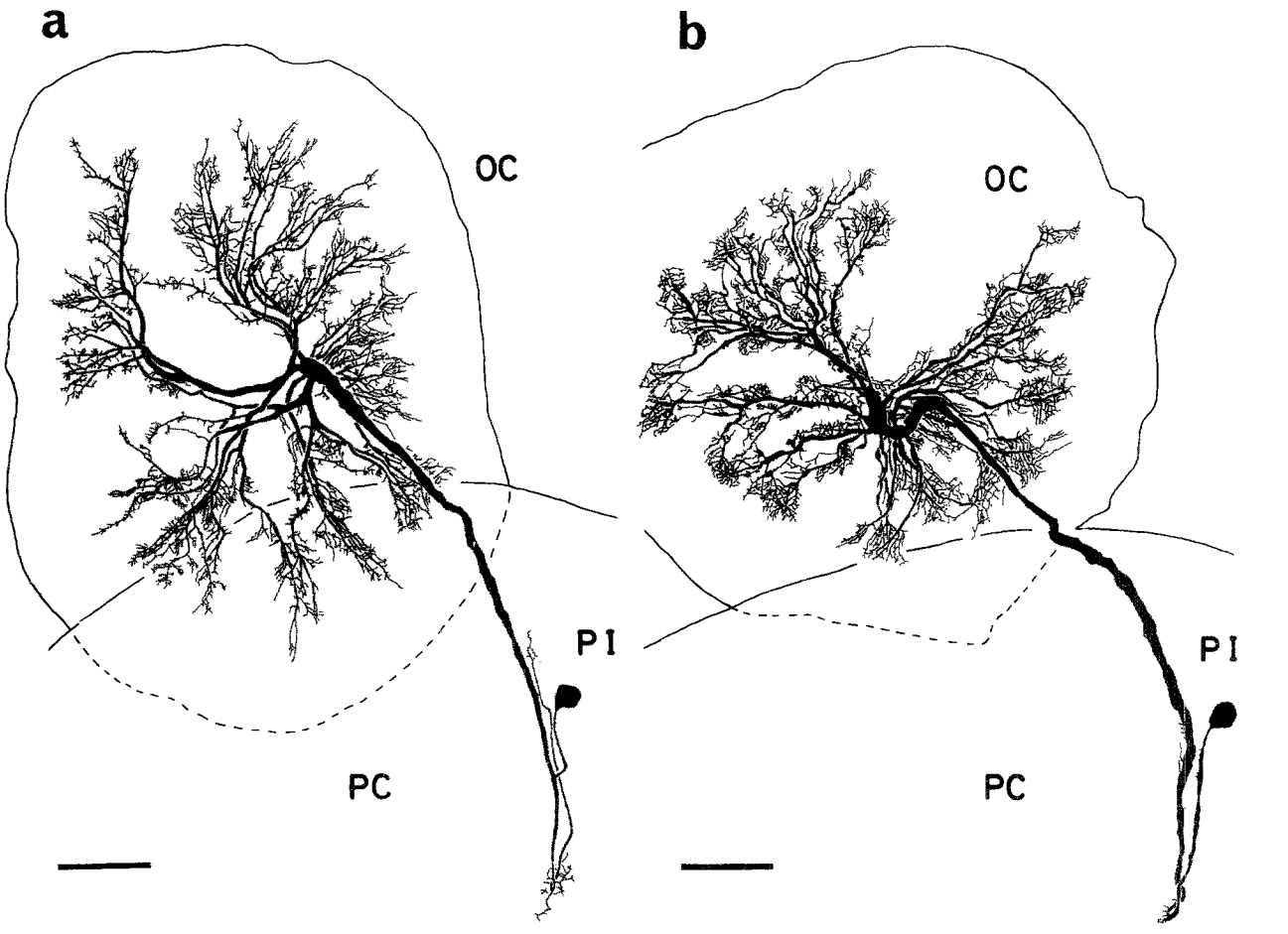

Fig. 3a, b. Drawings of the ocellar L-neuron. The process extending from the cell body bifurcated T-shaped in **a**, but not in **b**. Viewed postero-dorsally. *OC* ocellus; *PC* protocerebrum; *PI* pars intercerebralis. *Scales:* 100 μm

along the ocellar tract, with one of the branches running antero-dorsally toward the ocellus and the other postero-ventrally toward the terminal region. In another case shown in Fig. 3b, the process from the cell body first runs postero-ventrally toward the terminal area, then turns toward the ocellus.

To determine how many of the L-neurons in single ocellar nerves can be categorized as bifurcated or non-bifurcated L-neurons, whole nerve impregnation was performed. When the duration of cobalt impregnation without any current was about two hours, only L-neurons were selectively stained. In nine ocellar nerve fills, all the thick processes of four L-neurons could be identified. The results are listed in Table 1 and two examples are shown in Fig. 4. In six cases out of nine ocellar nerve fills, the L-neurons consist of two bifurcated and two non-bifurcated L-neurons (Fig. 4a), and in three cases, they consist of one bifurcated and three non-bifurcated L-neurons (Fig. 4b). The variation in the ratio of the bifurcated and non-bifurcated L-neurons suggests that the difference in the process anatomy of the L-neurons has little meaning physiologically.

Table 1. The number of bifurcated and non-bifurcated L-neurons in single ocellar nerves. I–VI indicate individual animals. *R* right ocellar nerve. *L* left ocellar nerve

Preparations	Number of bifurcated L-neurons	Number of non-bifurcated L-neurons
I (L)	2	2
I (R)	2	2
II (L)	2	2
II (R)	2	2
III (R)	2	2
IV (L)	2	2
V (L)	1	3
V (R)	1	3
VI (R)	1	3

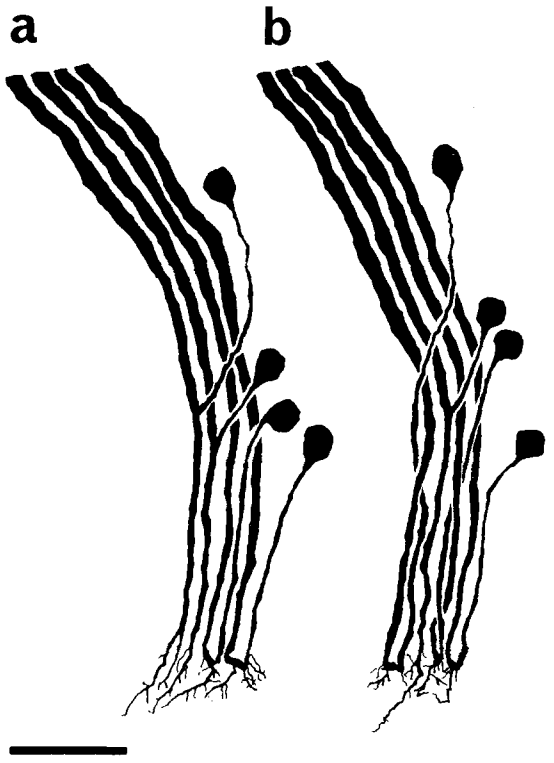

Fig. 4. Variations between individuals (**a** and **b**) in the number of bifurcated and non-bifurcated L-neurons in single ocellar nerves. The positions of individual L-neurons were somewhat modified. Whole nerve impregnations. Viewed postero-dorsally. *Scale:* 100 μm

Discussion

The light response of the L-neurons has been studied intracellularly in dragonfly, locust and bee ocelli (review by Goodman 1981; Milde 1981). In all cases L-neurons responded to illumination with a hyperpolarization. Mobbs et al. (1981) reported that all L-neurons of the median ocellus of the dragonfly showed similar light responses, although the L-neurons were classified anatomically into four classes. Recently, in the median ocellar nerve of the locust, Taylor (1981) reported that three anatomical types of L-neurons showed different light responses, since they received different effects from lateral ocelli. In the present study, all the cockroach ocellar L-neurons showed similar light responses and we could not find any significant difference among L-neuron responses. We also could not find, as yet, any effects produced by contralateral ocellar illumination.

The projection of the ocellar interneurons in the brain was studied in the cockroach by Cooter (1975) and Bernard (1976) using whole nerve impregnation. These authors reported four large cell bodies of about 25–35 μm in the pars intercerebralis. These cell bodies may correspond to those of the L-neurons in the present study. In addition to the four large cell bodies, both Cooter (1975) and Bernard (1976) revealed that one large cell body of about 50–60 μm lies in the more posteroventral part near the protocerebral bridge. Cooter (1975) thought the large cell body was also that of an L-neuron. However, Bernard (1976) showed that the large cell body was stained when cobalt was impregnated using a current from the ocellus, but not stained during impregnation without any current. He also observed that the large cell body was stained when cobalt was impregnated from a cervical connective back into the brain. He concluded, therefore, that the large cell body was of a higher order interneuron. In the present study, cobalt impregnation from the ocellus for about two hours without any current did not stain the large cell body. However, when the cobalt impregnation was done for 8–12 h without any current, in addition to four L-neurons, the large cell body was frequently stained together with other several smaller cell bodies as reported by Cooter (1975) and Bernard (1976). Furthermore, in our intracellular stainings in the ocellar nerves, we never encountered such a large cell body. These observations support the conclusion of Bernard (1976) that the large cell body is of a higher order interneuron.

In most insects, ocellar L-neurons can be classified into two to five types according to their central and ocellar arborizations (review by Goodman 1981; Taylor 1981). The present study, however, showed that the cockroach ocellar L-neurons consisted of only one type, i.e., all the L-neurons had almost the same ocellar and central arborization. Therefore, we suggest that signal processing via the L-neurons in the cockroach ocellar system is of a less complex type than that found in other, more advanced species of insects.

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