SHORT COMMUNICATION

MULTIMODALITY OF OCELLAR INTERNEURONES OF THE AMERICAN COCKROACH

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Many insects possess two or three dorsal ocelli in addition to the paired compound eyes. The dorsal ocellus is characterized by a high convergent ratio of many retinular axons upon several thick second-order neurones in the posterior region of the ocellus. The thick second-order neurones extend towards the brain as an ocellar nerve together with other thin processes, and their central projections have been well demonstrated by cobalt backfills in many insects (reviewed by Goodman, 1981). However, our knowledge about how information is processed in the ocellar system is limited (but see Chappell & Dowling, 1972; Wilson, 1978*a,b*; Goodman, 1981; Simmons, 1981). In the present study we present some evidence that in the cockroach, *Periplaneta americana*, the ocellar second-order neurones function as CNS integrative multimodal neurones.

The cockroach *Periplaneta americana* possesses two ocelli, each occurring near the base of the antenna. In the *Periplaneta* ocellus more than 10 000 retinular axons synapse with only three or four thick second-order neurones (10–15 μm in diameter) which are referred to here as L-neurones (Cooter, 1975; Bernard, 1976; Weber & Renner, 1976; Toh & Sagara, 1984). The L-neurones are hyperpolarized by the ocellar illumination, and respond with a few off-spikes at the cessation of the illumination (Fig. 3A) (Mizunami, Yamashita & Tateda, 1982). In addition to these thick afferent neurones, several thin processes, which respond to various sensory stimuli other than ocellar illumination, are included in the *Periplaneta* ocellar nerve and are referred to here as small multimodal ocellar interneurones (SM-neurones). The SM-neurones respond with spike discharges to the following stimuli; illumination to compound eyes, movement of antennae including tactile stimuli and air puffs to antennae, air puffs to cerci, vibration to legs, and spontaneous/forced wing beats (Fig. 1). Details of stimulus conditions are given in the figure legends.

Recordings from suction electrodes attached to a mid-region of the ocellar nerve revealed spikes originating in more than two SM-neurones (Fig. 1A–E). Whether each SM-neurone responds with spikes to all of the five stimuli used here has
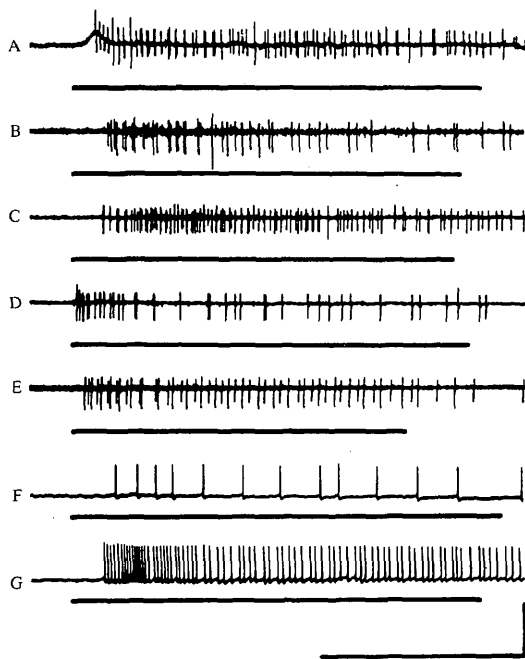
Fig. 1. Responses of multimodal ocellar units to various stimuli recorded in mid-regions of the ocellar nerves by suction electrodes (A–E), and by a glass microelectrode (F,G). Stimuli are as follows: (A) and (F) illumination of compound eyes (1 lx); (B) an air puff to an antenna (3 m s^{-1}); (C) a mechanical stimulus to legs by vibration of floor (amplitude 100 μm, 200 Hz); (D) and (G) an air puff to cerci (3 m s^{-1}); (E) rise and fall of a wing by external manipulation, frequency and amplitude not being exactly controlled. Stimulus duration is indicated by a bar under each recording. Recordings B and C are from the same nerve. Recordings F and G are intracellular recordings from the same SM-neurone. Time scale, 500 ms; calibration of amplitude, 250 μV for B–D, 100 μV for A and E, 30 mV for G and F.

therefore not been determined. However, each SM-neurone is likely to respond to all five stimuli because intracellular recordings from a single SM-neurone with a glass microelectrode showed that all recorded SM-neurones respond to two or three sequentially applied stimuli of the five (F,G in Fig. 1). Sequential application of all the five stimuli was difficult due to the present experimental arrangement.

The five types of sensory stimulation that cause spikes in SM-neurones also produce graded depolarizations in L-neurones. Responses were recorded intracellularly with cobalt-filled microelectrodes, and all recorded neurones were identified, by subsequent cobalt fillings, to be L-neurones reported by previous workers (Bernard, 1976; Goodman, 1981; Mizunami et al. 1982; Toh & Hara, 1984). The following

data suggest that a train of SM-neurone spikes causes depolarization in L-neurones. (1) A train of SM-neurone spikes always preceded depolarization of L-neurones (Fig. 2A). (2) Replacement of normal saline (Yamasaki & Narahashi, 1959) around the ocellar neuropile with a Ca^{2+}-free and $10\,mmol\,l^{-1}$ $CoCl_2$-containing saline resulted in a large reduction of depolarization of the L-neurone without detectable changes in impulse frequencies of SM-neurones to cercal stimulation (Fig. 2). This result may suggest that the SM-neurones have excitatory effects upon the L-neurones, and that their interaction may be extensive in the ocellar neuropile. Failure of complete inhibition of depolarization of L-neurones even 25 min after replacement of the saline is suggestive of their interaction even in the ocellar nerve and ocellar tract in the brain. Blocking of SM-neurone spikes at the ocellar nerve by tetrodotoxin also confirms this assumption, but it will be dealt with elsewhere. It is not known whether SM-neurones are monosynaptically connected with L-neurones or indirectly connected with them *via* unknown interneurones.

Hyperpolarization of the L-neurone by ocellar illumination was reduced by subsequently superimposed spikes of SM-neurones (Fig. 3). The membrane potential of the L-neurone may be an algebraic sum of the two inputs (ocellar retinular cells and SM-neurones), but their effects for spike generation in the L-neurone are not simple summations. A train of SM-neurone spikes caused by an air puff alone usually generated a few spikes in L-neurones under light-adapted conditions (more than $0.015\,lx$), but rarely generated spikes in completely dark condition. If the L-neurone was hyperpolarized by ocellar illumination in advance, a subsequent train of
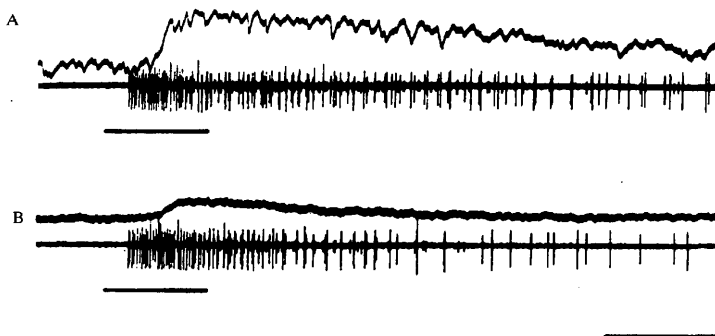

Fig. 2. Simultaneous recordings of membrane potential of an L-neurone (upper trace) and multimodal ocellar (SM-) units by a suction electrode (lower trace) in response to an air puff to the cerci ($2\,m\,s^{-1}$). The stimulus duration is indicated by a horizontal bar. In A the ocellus (including ocellar neuropile) is bathed in a normal saline, whereas in B the responses are recorded 25 min after replacement of the normal saline bathing the ocellus by a Ca^{2+}-free and $10\,mmol\,l^{-1}$ $CoCl_2$-containing saline. The L-neurone responds with graded depolarization to cercal stimulation (A), but the depolarization reduces in amplitude in B. Time scale, 500 ms; calibration of amplitude, 5 mV for upper recordings and $100\,\mu V$ for lower recordings.
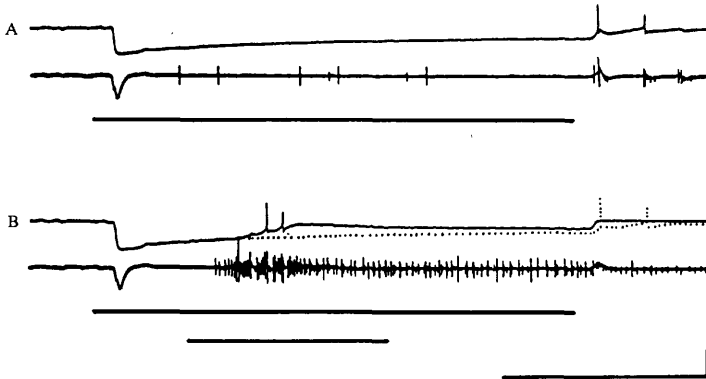
Fig. 3. Simultaneous recordings of a membrane potential of an L-neurone (upper trace) and multimodal ocellar (SM-) units (lower trace) by a suction electrode in response to ocellar illumination (1·5 lx, stimulus marker) alone (A), and to a combination of ocellar illumination (1·5 lx, upper stimulus marker) and an air puff to cerci (3 ms^{-1}, lower stimulus marker) (B). A and B are continuous recordings. Ocellar off-spikes in the L-neurone appearing in A, disappear in B, but two spikes appear in response to the air puff in B. To compare membrane potentials, a part of trace A is superimposed on the trace B (dotted line). Time scale, 500 ms; calibration of amplitude, 30 mV for upper recordings and 125 µV for lower recordings.

SM-neurone spikes generated one or two spikes in the L-neurone, but spikes at the cessation of the ocellar illumination often failed to discharge (Fig. 3).

The SM-neurones found in this study may be homologous to the ocellar efferent fibres of dragonflies: the efferent fibres receive inputs from the compound eyes and wing sensory system, and their spikes increase dark discharges of L-neurones (Kondo, 1978). The SM-neurones of *Periplaneta* appear to have more sensory inputs from various body appendages, including the most anterior and posterior sense organs, antennae and cerci, respectively.

The functional role of SM-neurones in the ocellar system is not properly known, because the ocellar contribution to behaviour itself is not known in *Periplaneta*. However, some possible function may be inferred by referring to ocellar function reported in other insects. The ocellar system is known to play auxiliary roles to the compound eye under dim light conditions during flight and locomotion in locusts, dragonflies and flies (Jander & Barry, 1968; Wilson, 1978a; Stange & Howard, 1979; Miller, Hansen & Stark, 1981; Taylor, 1981). Sensory stimuli which cause spikes in the SM-neurones result in reduction of the ocellar photic response of the L-neurones (hyperpolarization). If one supposes an auxiliary role of the ocelli in phototactic behaviour in cockroaches, outputs from wing and leg mechanoreceptors may serve a feedback system to the ocellar outputs for smooth performance of the behaviour pattern. Outputs from antennal and cercal mechanoreceptors as well as those from the compound eyes may temporarily reduce or cut off the ocellar output in order to

respond exclusively to (e.g. to orientate to or escape from) a source of mechanical stimuli or visual stimuli to compound eyes. These assumptions will be examined by recordings of responses of both SM-neurones and L-neurones to more elaborately controlled mechano- and light stimuli, and by related behavioural experiments.

Spike generations of the L-neurones are more influenced by sensory stimuli other than ocelli than are graded potentials. The L-neurones usually discharge a few spikes at the cessation of the ocellar illumination, but they also discharge a few spikes in response to other sensory stimuli under light (more than 0·015 lx) conditions. As shown in Fig. 3, off-responses of L-neurones are often cancelled by other sensory stimuli. It is not known at present what information this small number of spikes deliver to the CNS. They may send the CNS information about total change of the environment including light condition.

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