J Comp Physiol A (1984) 155:151–160

Journal of Comparative Physiology A Sensory, Neural, and Behavioral Physiology

Ocellar interneurons in the honeybee

Characteristics of spiking L-neurons

Jürgen J. Milde* and Uwe Homberg**
Institut für Tierphysiologie, AG Neurobiologie, Freie Universität Berlin, Germany

Accepted May 21, 1984

Summary. Morphologically identified spiking ocellar interneurons (L_B and L_D-neurons) of the honeybee (*Apis mellifera*) were investigated by combined intracellular recording and staining techniques using multimodal stimulus programs.

Response patterns containing both graded and action potentials (mixed response), and pure spiking responses were analysed. Mixed responses allow a comparison of information coded simultaneously by graded and action potentials in one neuron. In most cases the intensity dependence coded by spikes was found to be similar to the intensity dependence coded by one of two different parameters evaluated from the graded signal. L-neurons with mixed responses were unimodal, i.e. they reacted exclusively to stationary illumination of the ocelli, as do nonspiking L-neurons.

In contrast, spiking L-neurons that lacked a graded response component could also respond to stimuli of other sensory modalities: moving patterns, compound eye illumination, airstreams, mechanical and gustatory stimulation. One L_D-neuron was also excited by the wing beat.

Recordings from the same type of neuron in different individuals demonstrate that the input modalities and response patterns of L-neurons vary remarkably. Consequently many recordings are required to properly characterise the physiological properties of these neurons even though anatomically they are identified.

The existence of graded and action potentials in the same cell and the fact that these two signals carry different information is discussed in the context of a possible role for information transmission from L-neurons to postsynaptic cells.

Abbreviation: *R/I* response/intensity

* Present address: European Molecular Biology Laboratory, Postfach 10.2209, D-6900 Heidelberg, Federal Republic of Germany

** Present address: Technische Universität Berlin, Institut für Biologie, Franklinstraße 28/29, D-1000 Berlin 10, Federal Republic of Germany

Introduction

The first-order interneurons (L-neurons) connecting the ocellar retina to the central nervous system (CNS) in bees are amongst the largest neurons in insect brains and are readily accessible for intracellular study. They are often referred to as 'nonspiking' because analyses have shown L-neurons to have graded responses to light in common with other species (dragonfly: Chappell and Dowling 1972; Patterson and Chappell 1980; Mobbs et al. 1981; locusts: Patterson and Goodman 1974; Wilson 1978a, b, c; Taylor 1981; Simmons 1981, 1982; cockroach: Mizunami et al. 1982; honeybee: Guy et al. 1979).

However, Hengstenberg (1977) showed that large 'nonspiking' neurons in the fly could generate spikes under certain conditions. This was later supported by recordings of identified honeybee L-neurons (Milde 1981) whose responses ranged from graded potentials to inhibition of spontaneous action potentials. Some bee L-neurons could switch from graded to action potentials during an experiment without any observable change in the recording situation, suggesting that a single L-neuron might have two coding mechanisms for signal transmission.

In the honeybee the 30 L-neurons of the ocellar nerve can be subdivided into 20 neurons terminating in the brain (L_B-neurons) and 10 neurons with branches in the brain but terminating in the thoracic ganglia (L_D-neurons: for review see Goodman 1981). A recent analysis demonstrated that the

electrophysiological response patterns of these subgroups can differ (Milde 1984). Only L_B-neurons exhibit the whole range of nonspiking to spiking responses whereas L_D-neurons do not show pure nonspiking responses.

The present paper shows that spiking L-neurons can be multimodal in contrast to non-spiking L_B-neurons, which have been shown to respond only to ocellar illumination. Intracellular recording and staining of the same type of neuron during multimodal stimulation, is used to characterize the interindividual variability of the responses of single identified cells.

Materials and methods

Worker bees, *Apis mellifera*, were caught at the hive entrance and prepared in two different ways for the experiment.

Preparation 1. The bee was fixed in a metal tube, and the brain exposed by cutting a small window in the front of the head capsule, leaving tissue as intact as possible (Milde 1984). Bees were stimulated by: (1) illumination of all three ocelli; (2) visual stimuli to compound eyes; (3) airflow over the front of the head with or without the presence of isoamyl acetate, an alarm pheromone.

Stationary light stimuli for ocelli and compound eyes were produced by a 450 W xenon lamp. Wavelength, intensity, and duration were varied with chromatic and neutral density filters controlled by electrical shutters. Light stimulation to all three ocelli was through a light guide (active diameter = 5 mm) placed close to the ocellar lenses providing wide-field illumination. Stray light to the dorsal parts of both compound eyes was eliminated by attaching an opaque tube to the tip of the light guide.

A second light guide, used to stimulate a small median area of the compound eye, was attached to a long opaque tube combined with a diaphragm to prevent simultaneous illumination of the ocelli.

Back-lighted, black and white gratings were driven by a DC motor with a constant velocity of 45°/s (Erber and Schildberger 1980). The pattern was presented to the ocelli and had a spatial wavelength of 25 °. Gratings could also be seen by the dorsal parts of the compound eyes. Only two directions could be tested: from anterior to posterior (progressive) and posterior to anterior (regressive).

Preparation 2. The bee's head was fixed with wax to a metal holder leaving the thorax and abdomen free to move. To achieve stable recordings the brain was supported on a small metal platform which also served as the indifferent electrode. Insertion of the platform required removal of the dorsal head cuticle, including the three ocellar lenses but leaving most of the ocellar retinae intact (Homberg 1982).

In this second type of setup the qualitative rather than the quantitative properties of ocellar interneurons were tested using multimodal stimulation. Compound eye stimulation to each eye was by flexible light guides from a 100 W halogen lamp (Spindler and Hoyer). White light had an intensity of 5.75×10^{-4} W/cm^2 measured at the eye with a photomultiplier (International Light IL 700/IL 760) and subtended an angle of 11–15 °. Weak simultaneous illumination of the ocelli could not be prevented.

A 150-W halogen lamp connected to a light guide (Schott 150 B: intensity 32.5×10^{-2} W/cm^2), subtending an angle of 7.6 °, was used to illuminate the ocellar retinae, as well as the frontal and dorsal part of the compound eyes.

Rose oil, lavender oil and isoamyl acetate were used for olfactory stimulation. A piece of filter paper, soaked with odour, was placed in a small tube which ended in front of the bee's head. The stimulus was in parallel to a second air stream not containing odour. This control also served as a mechanical stimulus. The air stream velocity (3.2 m/s) was controlled by magnetic valves. Other antennal stimuli were applied by touching the tip of one antenna with a glass rod with or without a drop of sugar water suspended from it.

Air puffs onto the thorax and abdomen were also used as mechanical stimulation. The bee's tarsi normally rested on a piece of styrofoam mounted on a metal lever, which could be suddenly removed electromagnetically (Homberg 1982) to cause active movements of legs, thorax and abdomen and sometimes flight movements of the wings.

Recording. All recordings were from L-neuron axons located in the posterior median protocerebrum. Tips of glass microelectrodes (Ultratip, Frederick Haer and Co) were filled with a 3% aqueous solution of Lucifer yellow backed up with 0.1 ml LiCl. Resistances, measured after brain penetration, ranged from 80 to 200 MOhm. After intracellular recording dye was injected by applying a negative (3–10 nA) DC pulse for 3–8 min. The recorded signals were amplified and stored on magnetic tape for data evaluation.

Histology. Injected brains were dissected and processed histologically. Whole mounts (Stewart 1978) were photographed with a fluorescence microscope onto Kodak Ektachrome 400 and some were embedded later in Spurr's medium (Strausfeld et al. 1983) and cut at 20 µm to resolve fine details.

Data evaluation. L-neurons were reconstructed from projected colour slides. The most recent nomenclature (Goodman 1981) is here used for their description. 'L-neuron' is always used as a general term in the text and embraces both L_B (brain)- and L_D (descending)-neurons.

Stored signals were fed from tape into an oscilloscope and selected areas filmed. Analysis of various parameters of the graded response was with a Kontron Digiplan image analyser (Milde 1984). Spike responses were analysed by counting the number of action potentials over a given period of time. If not indicated otherwise the response/intensity (R/I) functions of spiking L-neurons were measured by evaluating the difference between the frequency before (background activity) and the frequency during stimulation. To facilitate comparison each R/I function is given in terms of the maximum response of the cell.

Results

Thirty-nine spontaneously spiking, large ocellar first-order interneurons were recorded and identified.

Responses to ocellar illumination. The responses to ocellar illumination of the 26 L_B- and L_D-neurons recorded in preparations with intact ocelli were generally the same, i.e. the spontaneous spike discharge was inhibited (Figs. 1–5). The spontaneous

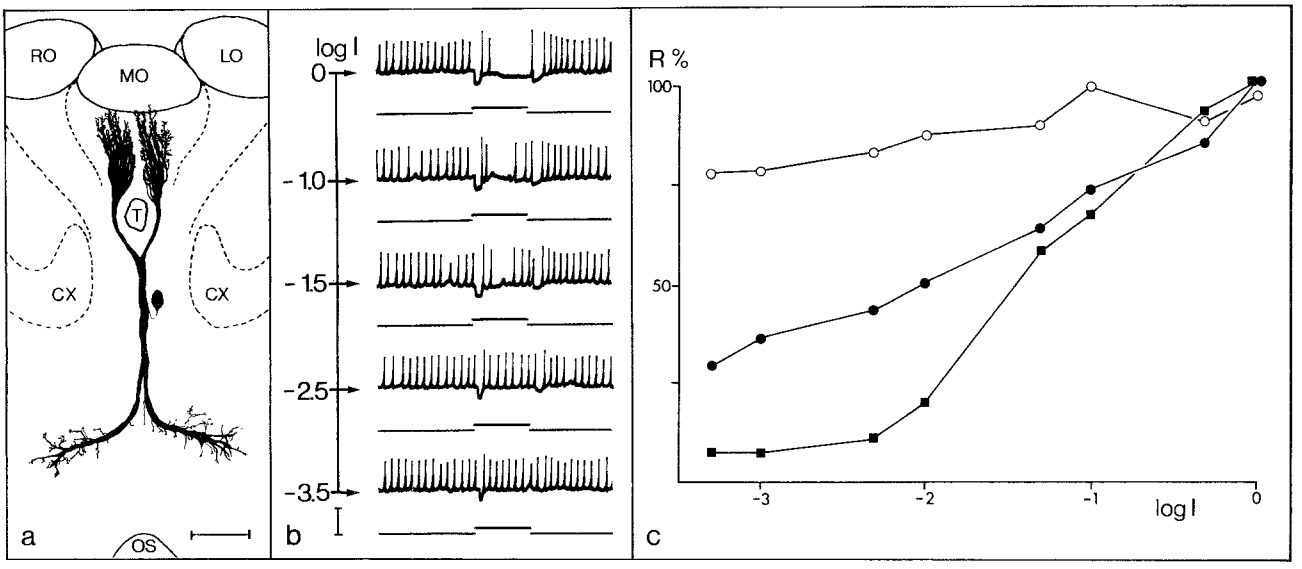

Fig. 1a–c. Median ocellar L_B-neuron (MOC 7) with mixed responses. **a** Reconstruction of the Lucifer-stained neuron, anterior view. CX mushroom body calyces; LO left, MO median, RO right ocellus; OS oesophagus; T central trachea. Scale = 100 µm. **b** Responses to different intensities of white light stimuli. At log I = 0 the stimulus inhibits the spontaneous discharge and elicits hyperpolarising on- and off-transients and a plateau. At lower light intensities spontaneous discharge is less affected and the plateau and the off-hyperpolarisation are smaller. Stimulus duration = 300 ms; vertical scale = 30 mV. **c** Response/intensity functions for three different parameters of the light response. *Squares* represent inhibition of the spontaneous spike discharge; *closed circles*, integral of the graded on-transient; *open circles*, amplitude of the graded on-transient

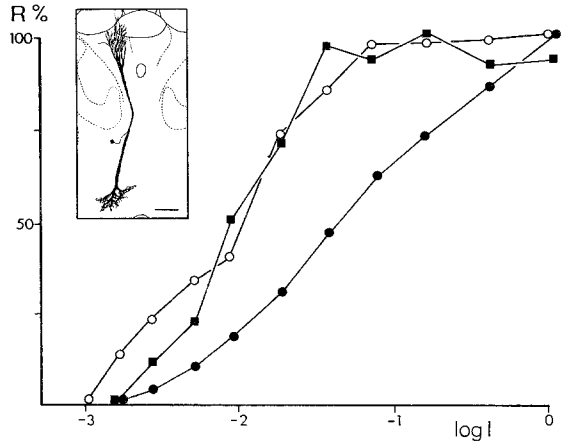

Fig. 2. Response/intensity functions for a unilateral ocellar L_B-neuron (*inset*) with mixed responses to white light stimulation. *Squares* represent inhibition of spontaneous spike discharge; *open circles*, amplitude of on-transient; *closed circles*, integral of the on-transient. Scale bar = 100 µm

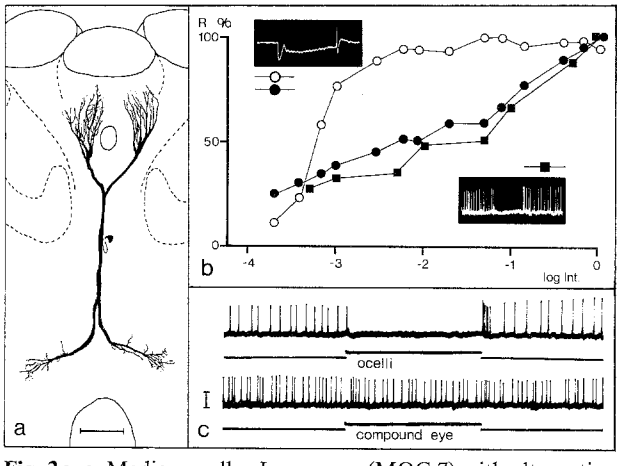

Fig. 3a–c. Median ocellar L_B-neuron (MOC 7) with alternating graded or spike responses during recording. **a** Anterior view of reconstructed neuron. Background brain structures are as in Fig. 1. Scale = 100 µm. **b** R/I functions for the amplitude (*open circles*) and integral (*closed circles*) of the graded on-transient (upper left inset) and the inhibition (*squares*) of the spike discharge (lower right inset). **c** *Upper row:* typical response to ocellar light stimulation. The spontaneous frequency is inhibited by light and an off-burst appears. *Lower row:* light stimulation of a compound eye leads to extra spikes at stimulus on and off. Stimulus duration = 300 ms; vertical scale = 10 mV. The movement-sensitive response of this cell is shown in Fig. 5

firing frequency ranged from 0 to 120 Hz and often varied during a recording.

Of these 26 spiking interneurons 17 also responded with graded components – an on- and off-transient and a small hyperpolarising plateau at high light intensities (Fig. 1b). This allowed comparison between intensity dependencies of the graded and spiking response of the same L-neuron. The graded response pattern of spiking L-neurons matched that of nonspiking L_B-neurons. Furthermore all spontaneously spiking L-neurons that also exhibited graded components (mixed response) reacted only to ocellar illumination as did nonspiking L_B-neurons (Milde 1984).

Figure 1 shows the R/I functions of a median

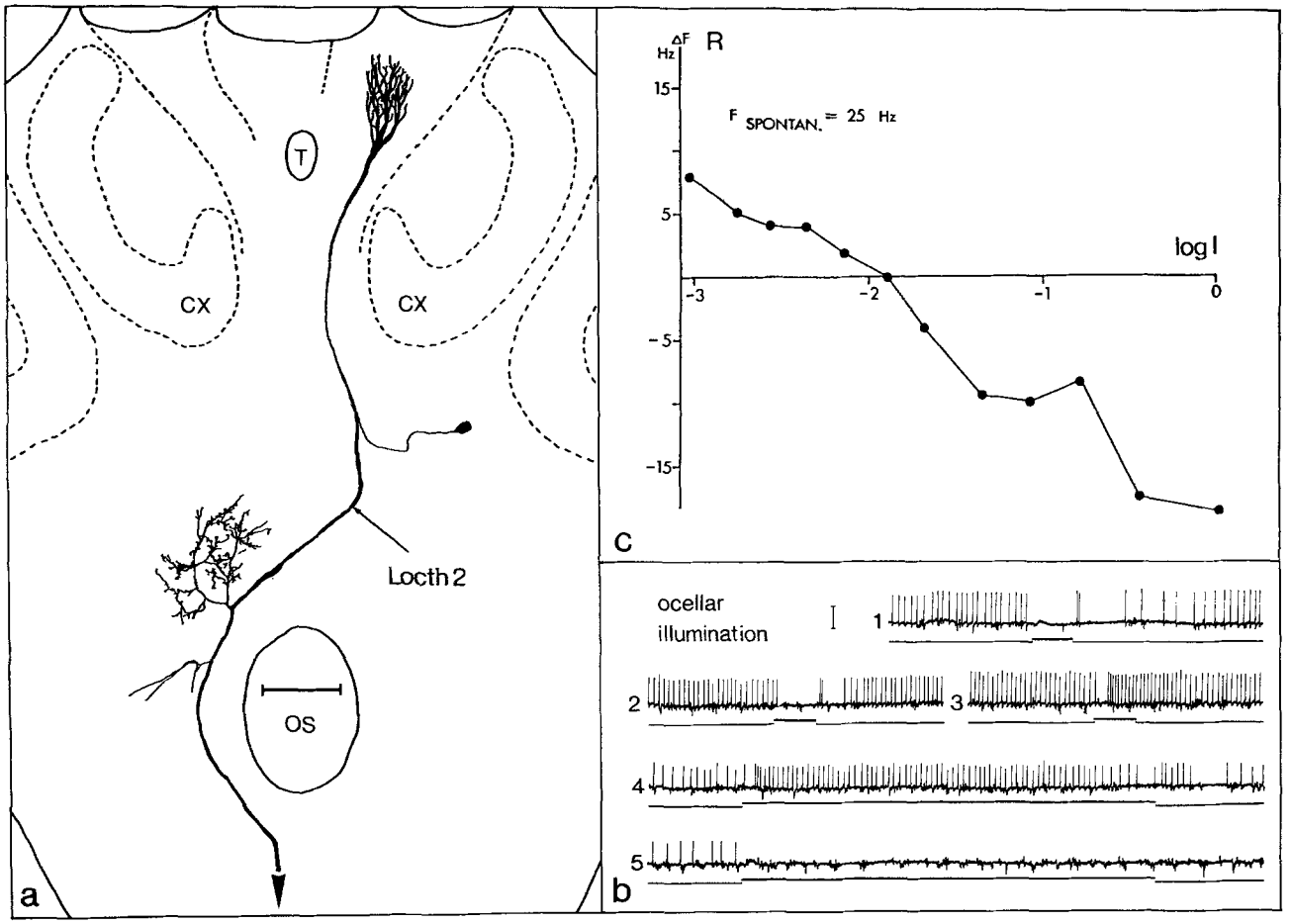

Fig. 4. **a** L_D-neuron (Locth 2). Reconstruction. *Arrowhead* indicates axon to the thoracic ganglia; brain structures labelled as in Fig. 1. Scale = 100 µm. **b** Responses to illumination of the ocelli at log I = 0 (*1*), −0.9 (*2*), −3.0 (*3*), −3.6 (*4*) and 0 (*5*). Duration was 300 ms for short stimuli and 3 s for long ones. Vertical scale = 20 mV. **c** R/I function for 300-ms light flashes. With decreasing intensity the inhibition disappears and below log I = −1.9 excitatory responses can be observed. This curve was derived from the difference between spike frequency before stimulus onset and in a 300 ms time window after the end of the stimulus

ocellar L_B-neuron with a mixed response. Graded responses increase as light intensity increases. The spontaneous spike discharge is unaffected by dim light; however, above a light intensity of log I = −2 the spike discharge is inhibited with increasing illumination.

Different response parameters of nonspiking neurons (e.g. amplitude or integral of the on-transient) have different relationships to light intensity (Milde 1984; see also Figs. 1–3). The L_B-neuron of Fig. 1 exhibits no linear correlation between the graded response and spike inhibition. However, usually the intensity dependence of spike inhibition is similar to that of one of the graded response parameters. One example (Fig. 2) from a lateral ocellar L_B-neuron shows the R/I function of the spike response to be like the R/I function of the on-transient amplitude but not that of its integral. The converse can be observed in the responses of a median ocellar L_B-neuron (Fig. 3) that change from spiking to graded during the recording. The R/I function of the spike inhibition is very similar to that of the on-transient integral but unlike that of the on-transient amplitude (Fig. 3b). The spike response of this neuron to ocellar illumination showed none of the graded components known from nonspiking L-neurons (Fig. 3c). The ocellar light stimulus inhibits the spontaneous spike discharge, and there is an increase in spike rate at light-off.

In the L_D-neuron (Locth 2) shown in Fig. 4a a flash of high-intensity white light completely inhibited the spikes (Fig. 4b, *1*). Light-off was accompanied by an off-burst and a prolonged, intensity-dependent after-inhibition (Fig. 4b, *2*). Illumination at lower intensities resulted in a brief inhibition followed by excitation which set in during the stimulus and was sustained some time after light-

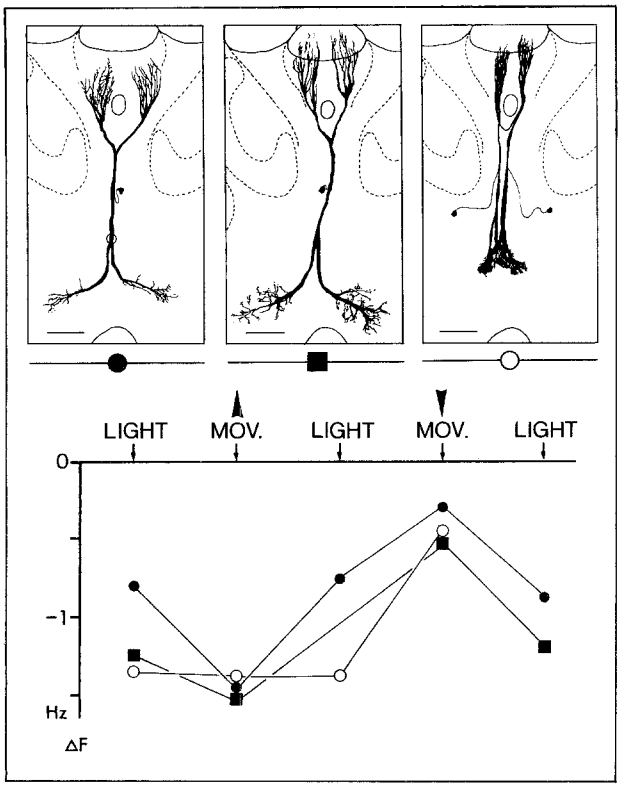

Fig. 5. Spiking L_B-neuron responses to an illuminated moving grating. The ordinate gives the frequency change (ΔF) in Hz. The abscissa represents the background frequency in the dark, which is inhibited by illumination. *Left arrowhead* indicates regressive pattern movement from posterior to anterior; *right arrowhead*, progressive movement from anterior to posterior. The background impulse frequencies of the three types of neurons (shown in *insets*) were 2.4, 3.8 and 3.0 Hz. Scale bars = 100 µm

off (Fig. 4b, *3*). Illumination at still lower intensities elicited excitation exclusively throughout its duration (Fig. 4b, *4*). In contrast a stimulus of equal length but maximum intensity resulted in strong inhibition (Fig. 4b, *5*) for up to 1 min after light-off (cf. Fig. 4b, *1*) when background activity resumed. Occasionally during a recording, noisy background signals appeared that were uncorrelated with any stimulus. The origin of these signals is unknown, it cannot be excluded that they represent postsynaptic potentials. R/I functions were measured for 300 ms illumination by measuring differences between background frequency and the light-off frequency. Figure 4c shows that the inhibition decreased when the light intensity was decreased. At log $I = -1.9$ the spike rate equals the background frequency. This does not necessarily mean that stimulated and unstimulated state are equivalent. The spontaneous frequency was unchanged because the illumination caused equal inhibition and excitation in the time window chosen for data evaluation, and their effects canceled each other out. As intensity was reduced further, the excitation became more dominant.

Spectral sensitivities of spiking L-neurons have not been investigated quantitatively. However, all tested neurons showed responses to both short- and long-wave stimuli within a spectral range of 340–600 nm.

Responses to compound eye illumination. L-neurons lacking graded responses were also stimulated by illumination of the compound eyes. White light presented to a compound eye could elicit weak on- and off-bursts (Fig. 3c), which may have been ill-defined to the small-field characteristic of the stimulus. Responses to compound eye stimulation were shown by three of seven spiking L-neurons.

Movement-sensitivity. Moving gratings presented to the ocelli elicited no response in nonspiking or mixed response L-neurons, but could result in direction-sensitive responses in spiking L-neurons (Fig. 5). For example, the median ocellar L_B-neuron (same as in Fig. 3), showed a reduced spike rate to the onset of the ambient light used to illuminate the grating. Inhibition was slightly enhanced when the grating was moved regressively (from posterior to anterior), and the firing rate returned to the ambient level when movement stopped. Progressive movement reduced the inhibition incurred by ambient illumination, thus increasing the spike rate. When movement stopped the spike rate again returned to the level for ambient illumination. If all lights were switched off, the original background activity returned. A similar motion-sensitive response was seen in the two other cells shown in Fig. 5. Responses to moving stimuli appeared as slow, sustained changes of the spike rate without phasic events. Compound eye stimulation with stationary light failed to elicit responses in all but one movement-sensitive L-neuron (Fig. 3) which showed weak on- and off-excitations.

Bimodal responses in L-neurons. Certain L_D-neurons responded to light by inhibition of spike discharge and to air streams by excitation (Fig. 6). The R/I function of one of these cells to ocellar illumination can be seen in Fig. 6c. This neuron (LMocth 1) is the largest L-neuron recorded and was filled eleven times. Seven LMocth 1 neurons had mixed responses to ocellar illumination similar to these of the cell shown in Fig. 1b. Three of the four spiking cells reacted to light and wind in the same way as the cell in Fig. 6b. The fourth cell did not respond to air stimuli.

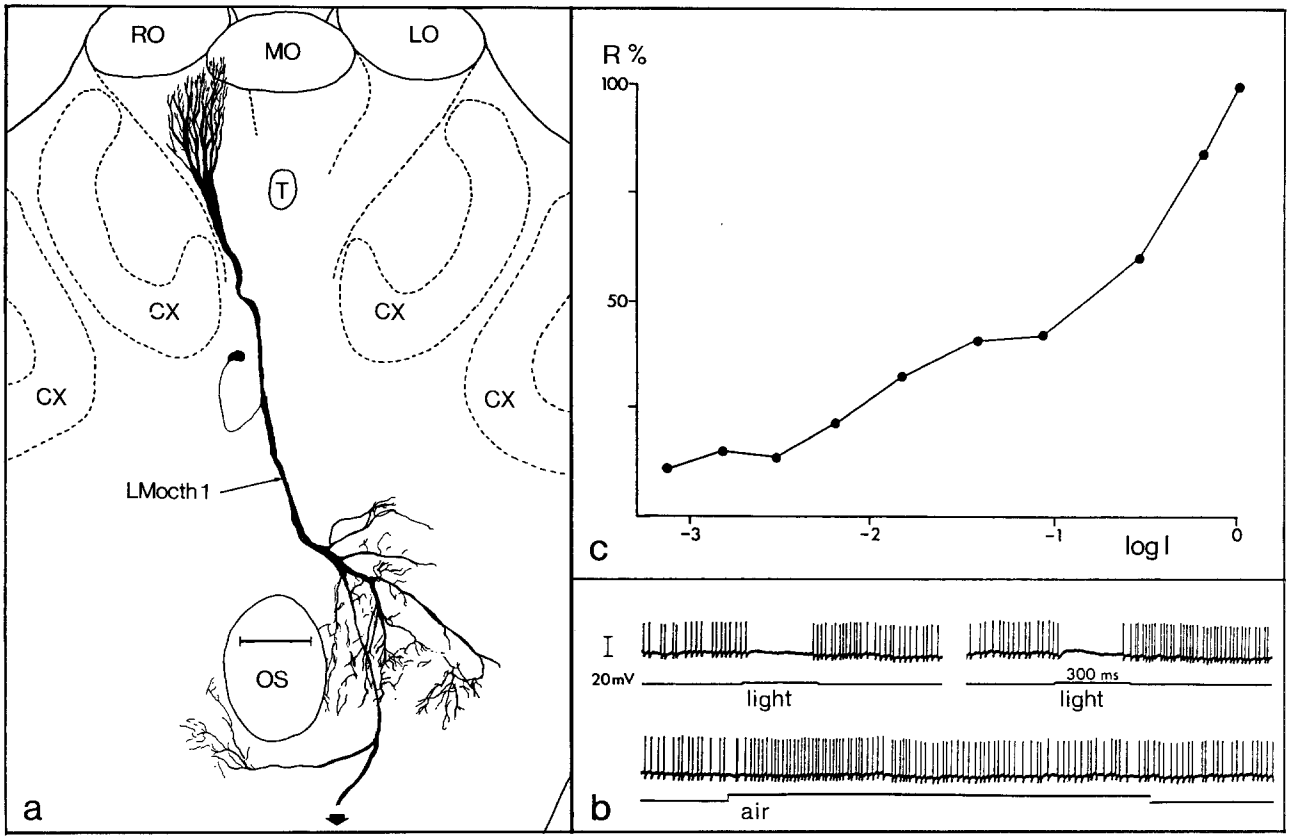

Fig. 6. a Lateral L_D-neuron (LMocth 1). A reconstruction demonstrating the extensive arborisations in the posterior protocerebrum and the suboesophageal ganglion. *Arrowhead* indicates axon leaving the suboesophageal ganglion for the thoracic ganglia; brain structures labelled as in Fig. 1. Scale = 100 (μm). **b** Responses to light and wind stimulation. *Upper trace*: flashes of white light totally inhibit firing; *lower trace*: a wind stimulus excites the neuron. **c** R/I function for the ocellar light response

Multimodal sensory inputs to L-neurons

Multimodal properties of spiking ocellar L-neurons were characterized in 13 L-neurons, tested by preparation method 2 (see Materials and methods). In these experiments all recorded L-neurons showed pure spiking responses with no graded components (Figs. 7 and 9). Of the 13 cells, 10 were L_D-neurons (LMocth 1, Locth 2, Locth 5) and 3 were L_B-neurons. Eight cells responded to other sensory stimuli in addition to light.

The LMocth 1 was recorded and filled eight times (Figs. 7 and 8). Its response properties are illustrated in Fig. 7. Apart from responding to visual stimuli, the cell was weakly excited by an air stream over the head and strongly excited by mechanical stimulation of the thorax and abdomen. It did not respond to sugar water stimulation of the antennae.

The response of the morphologically identified LMocth 1s from eight individuals are summarized in Fig. 8, which along with Fig. 7 shows that: (1) There is remarkable variation in background activity, with the discharge rate ranging from 0 to 47 Hz. (2) All cells responded to light. Compound eye illumination led to weak phasic excitations (see Fig. 7 light right and left). (3) Responses to ocellar stimulation (light frontal) were similar but more distinct. In addition to excitatory phasic responses four cells exhibited tonic inhibition lasting up to 20 ms (Fig. 7). (4) Five neurons responded to either mechanical stimuli or sugar water stimulation of the antennae or both. No response to olfactory stimuli was found.

The response of a neuron to the same stimulus could vary markedly during an experiment (Fig. 9): Removal of the styrofoam substrate only led to excitatory responses when flight movements of the wings also occurred demonstrating that this cell received information about the wing beat. Responses to illumination were similar to those of some LMocth 1 neurons. For example, the response to ocellar illumination (light frontal) was a phasic on- and off-excitation followed by a long-lasting inhibition (Fig. 9). Compound eye illumination elicited weak on- and off-excitations. Al-

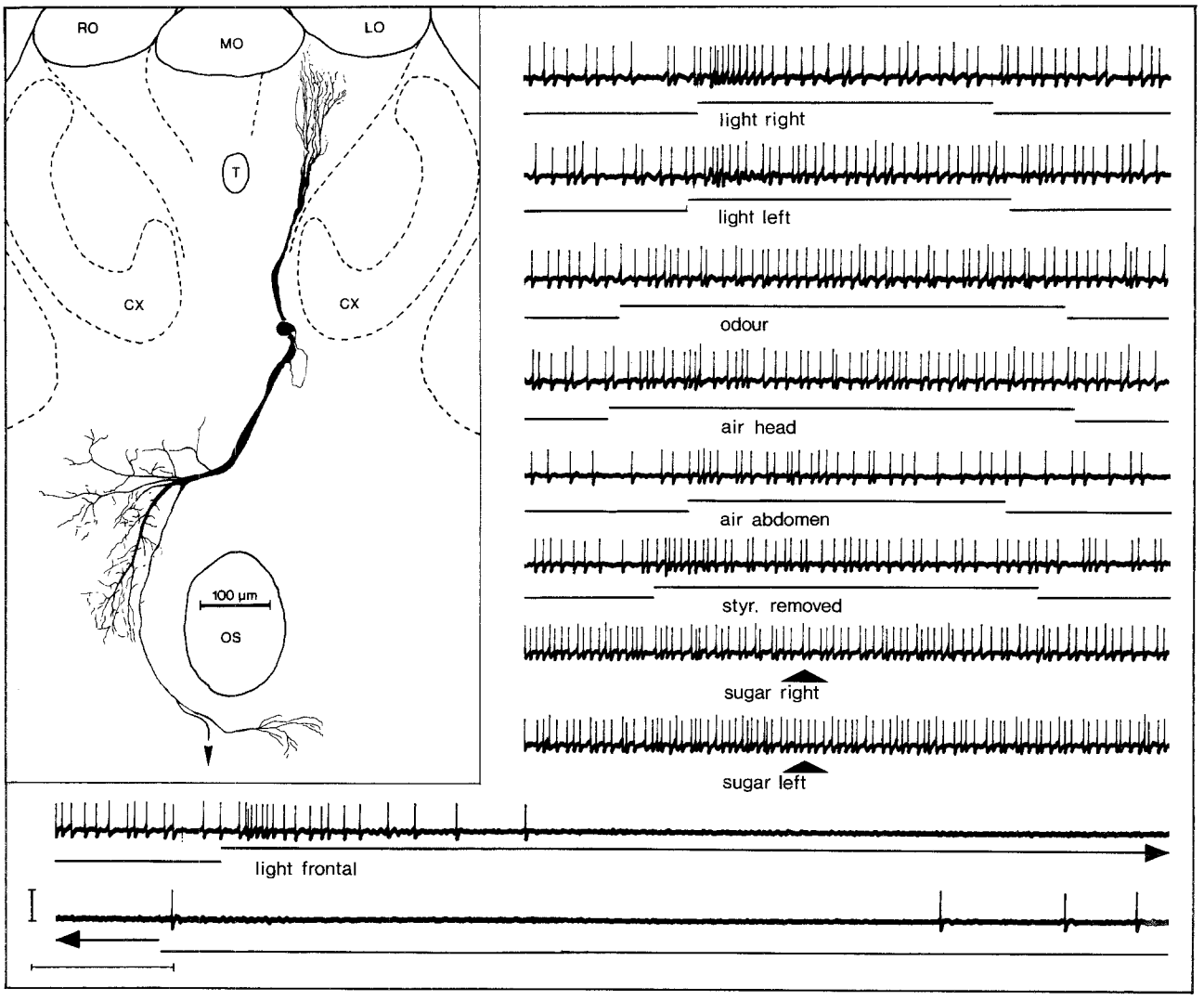

Fig. 7. Reconstruction and recording of an LMocth 1 neuron. The cell responds with phasic excitation to light stimuli presented to the compound eyes (light right and left), and airstream over the head (air head) and mechanical stimuli to abdomen (air abdomen) and thorax (styrofoam removed). There is no response to olfactory stimulation (odour), which elicits the same response as an odourless air stream, or to touching the antennae with a drop of sugar water (sugar right and left). Illumination of the ocelli and frontal parts of the compound eyes (light frontal) leads to an on- and off-excitation and inhibition that outlasts the stimulus. Horizontal bar = 1 s, vertical bar = 10 mV

though the Lucifer yellow fill of this cell was incomplete, it resembles an ocellar L-neuron.

Discussion

Visual responses of L-neurons. The responses of certain large ocellar interneurons have graded and spiking components. The usual response of spiking L-neurons to illumination is an inhibition of the spike discharge accompanied by a more or less pronounced light-off excitation. This has been also described from extracellular studies of locusts (Hoyle 1955), dragonflies (Ruck 1961; Rosser 1974) and flies (Autrum and Metschl 1963). This kind of response pattern can be attributed to graded hyperpolarisations reflecting the synaptic activity of ocellar photoreceptors onto L-neurons. However, our recordings demonstrate more than a simple translation of DC potentials to spike frequency. We show that L-neurons can have complex spike response patterns, such as minute-long spike inhibition after high-intensity stimulation. These observations are difficult to explain simply on the basis of phasic graded potentials transmitted from the ocellar retina. Long-term changes of spike activity were also found in extracellular studies on dragonfly ocellar neurons (Rosser 1974).

However, the underlying mechanisms for this effect are unknown.

The R/I functions of neurons having complex spike responses are unusual in that they have both excitatory and inhibitory components. We suggest that such an intensity-dependent response might be used for adjusting the sensitivity of other visual interneurons postsynaptic to an L-neuron. At high intensities, the postsynaptic cells might be inhibited and at low intensities excited, thus maintaining their defined working range at different intensities.

n	Light	Styr. rem.	Air Head	Air Abd.	Sugar	Odour	F (Hz)
1	●		○	○	○	○	24.8
2	●		○	○	○	○	46.8
3	●		○	○	○	○	0
4	●		○	○	●	○	27.2
5	●	●	●	○	●	○	11.6
6	●	●	○	●	○	○	27.4
7	●	●	●	●	●	○	23.6
8	●	●	●	●	○	○	20.6

Fig. 8. Responses to different stimulus modalities (explained in Fig. 7) of eight LMocth 1 neurons and their background activities in Hz. *Closed circle* indicates response; *open circle*, no response, *blank*, not tested. All cells responded to light stimuli and the removal of the styrofoam substrate when it was tested; none responded to odour. Other stimuli (air head, air abdomen and sugar water) elicited responses only in some recordings. There is no correlation between the background spike frequency (*F*) and the responses

Movement sensitivity. An important distinction between L-neurons with and without graded response components is in their response to moving optical gratings. The optical properties of ocelli (Wilson 1978a) and the high convergence of information onto L-neurons do not support participation of the ocelli in the detection of moving patterns. Furthermore behavioural analyses of head movements demonstrated that in bees optomotor reactions are mediated exclusively by a small ventrolateral region of the compound eyes (Moore et al. 1981). Different movement stimuli presented to the dorsal field of view (ocelli and compound eyes) failed to elicit head movements (Haring and Hertel, pers. communication). However, stimuli similar to those we used resulted in a non-direction-selective antennal movement, which became direction-sensitive when dorsal parts of the compound eyes were stimulated simultaneously (Erber, pers. communication). In our recordings, where the stimuli could be seen by ocelli and compound eyes as well, regressive movement of gratings increased, progressive movement decreases a L-neu-

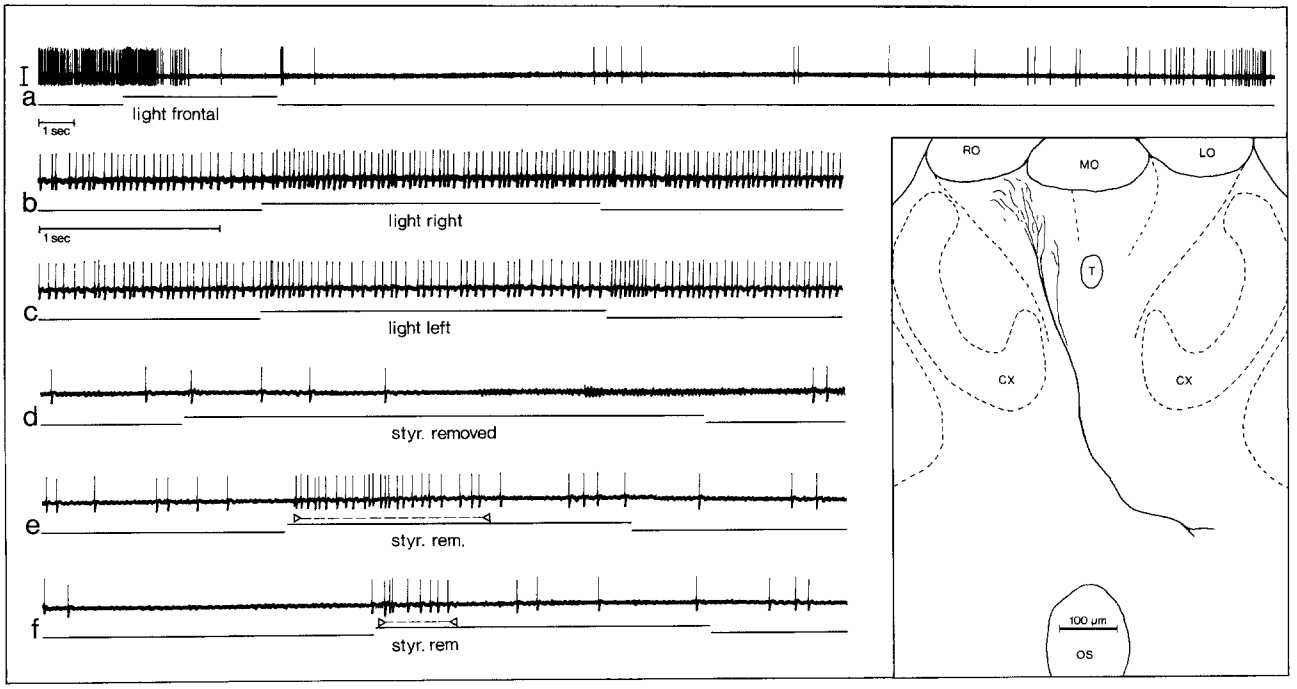

Fig. 9a–f. Recording and staining of an ocellar L-neuron. Since the fill was poor, the reconstruction is incomplete. The cell could be LOC 1 or 3, LMocth 1, or Locth 2. Recording shows light responses as in Fig. 7: Phasic on- and off-excitation and long-lasting inhibition in response to frontal illumination (**a**) and weak on- and off-excitations for stimulation of the right (**b**) and left (**c**) compound eye. The responses to the removing of the styrofoam substrate (**d**, **e** and **f**) differ. No response can be seen in d; in e and f, the stimulus elicits wing movements (indicated by *arrowheads* and dotted lines), which are correlated with an excitatory response. Vertical bar = 10 mV

ron's spike rate. This may indicate a participation of the L-neuron in the observed antennal behaviour.

Interaction of ocelli and compound eyes. Excitatory inputs from the compound eye onto spiking L-neurons demonstrate that there is already interaction between the two optical systems at the level of ocellar L-neurons. Structural correlates for direct synaptic contact between optic lobe neurons and L-neurons have not yet been demonstrated in bees. However, local interneurons in the median protocerebrum have been recorded that react to stimulation of ocelli and compound eyes. These neurons arborise amongst L-neuron terminals and amongst outgoing axons from the lobula that end in the brain (Milde, in prep.). Interaction between the two visual systems could also be mediated by the class of small ocellar first-order interneurons (S-neurons) that project directly from the ocellar retina to areas of the medulla and lobula (Pan 1981; Milde 1982). There is also anatomical evidence that L-neurons and neurons leaving the lobula converge onto common descending neurons (Guy et al. 1979). In flies, certain ocellar L-neurons and giant vertical-motion-sensitive cells from the lobula plate (VS-cells) converge and synapse onto characteristic clusters of descending neurons that terminate in the thoracic ganglia (Strausfeld et al. 1984; Bassemir and Strausfeld, in prep.).

Thoracic input to L-neurons. We have shown that different recording preparations (see Materials and methods) influence the physiological properties of ocellar L-neurons. Removal of the ocellar lenses and supporting the brain with a platform eliminated graded responses suggesting that the ocellar receptors, normally responsible for graded responses, or the L-neuron dendrites, were damaged. The advantage of the second of the two experimental procedures was that the bee was less restrained and the thorax and abdomen were able to move freely. The observed correlation between L_D-neuron excitation and wingbeat could indicate either recruitment of thoracic mechanosensory inputs presumably via ascending interneurons or efferences from the flight motor system to ocellar units. This additional input could contribute or even be solely responsible for the change from nonspiking to spiking mode. Inputs from wing proprioceptors to the ocellar nerve have been described from the dragonfly (Kondo 1978).

Visual and air current interactions in L-neurons. Convergence of wind-sensitive and optic inputs to certain L_D-neurons is comparable to findings from locusts where L-neurons do not leave the brain but input onto descending neurons arising in the brain and ending in the thoracic ganglia (Simmons 1980, 1981). Wind stimuli excite these descending cells and ocellar illumination inhibits them as is the case for the LMocth 1 neuron of the honeybee. Possibly, in bees, an equivalent pathway is realized by L_D-neurons projecting directly from the ocelli to the thoracic ganglia. Wind and visual information relayed by the L_Ds suggests a role in flight control. Spiking ocellar interneurons represent yet another example of the convergence of different modalities onto peripheral units of the CNS as reported for neurons in the medulla (Hertel 1980) and antenno-cerebral tract (ACT) of bees (Homberg 1981, 1984).

Transmission in L-neurons. Different physiological properties of anatomically identified cells do not depend only on the type of preparation. This is demonstrated by differences in the response of eight LMocth 1s (Fig. 7) and by the switchover of the MOC 7 from pure graded to pure spiking responses.

Electron microscopy has demonstrated the presence of numerous presynaptic terminals onto L-neurons in the locust (Goodman 1981). In the bee, small-diameter arborizations enwrap the axons of L-neurons, and are presumably presynaptic to them (Guy et al. 1979). Possibly these efferents modulate signal transmission in L-neurons dependent on the state of the animal at any given moment. This is supported by data summarized in Fig. 9, where an L-neuron monitors information about wing movement. We suggest that the discharge rate of this cell is much higher during flight than at rest and that this modifies responses of the L-neuron to ocellar illumination.

Do the various states (graded, mixed and spiking) of L-neurons reflect a multifunctional nature of the ocellar system and are all these features of functional significance? The respective roles of graded and regenerative potentials are difficult to establish without more knowledge of the biophysical properties and the pre- and postsynaptic characteristics of L-neurons. Although is has not yet been shown that graded signals in bee L-neurons can be transmitted to the postsynaptic cell, Simmons (1981) has demonstrated that in locusts graded L-neuron potentials transmit information to third-order neurons via chemical synapses. In bees, a single neuron can exhibit graded or action potentials or mixed signals at one and the same recording site. Presumably this requires cell mem-

brane properties such as those described by Wilson (1978b) for locusts where complex membrane characteristics are caused by the existence of active and passive membrane compartments. If graded and spiking signals reach the L-neuron terminals in the brain, then at least two modes of transmission may exist between the L-neuron and its postsynaptic neuron, given an appropriate presynaptic membrane structure that can select between the two modes. For example, terminals that are selective for spikes or graded potentials could be presynaptic to two distinct sets of postsynaptic units. This arrangement could allow the transmission of unimodal information to one target by graded potentials and multimodal information to another by spiking signals. Such a mechanism would enlarge the abilities of single nerve cells in information processing in the CNS.

Acknowledgements. We wish to thank Camilla and Nick Strausfeld for editing the manuscript, Randolf Menzel and Joachim Erber for their helpful comments, Renate Weisskirchen for processing of the figures and Wendy Moses and Heide Seifert for word processing. This work was supported by a DFG grant to R. Menzel and J. Erber.

References

Autrum H, Metschl N (1963) Die Arbeitsweise der Ocellen der Insekten. Z Vergl Physiol 47:256–273

Chappell RL, Dowling JE (1972) Neural organization of the median ocellus of the dragonfly. I. Intracellular electrical activity. J Gen Physiol 60:121–147

Erber J, Schildberger K (1980) Conditioning of an antennal reflex to visual stimuli in bees (*Apis mellifera* L.). J Comp Physiol 135:217–225

Goodman LJ (1981) Organisation and physiology of the insect dorsal ocellar system. In: Autrum H (ed) Handbook of sensory physiology, vol VII/6C. Springer, Berlin Heidelberg New York, pp 201–286

Guy RG, Goodman LJ, Mobbs PG (1979) Visual interneurons in the bee brain: Synaptic organization and transmission by graded potentials. J Comp Physiol 134:253–264

Hengstenberg R (1977) Spike responses of 'nonspiking' visual interneurone. Nature 270:338–340

Hertel H (1980) Chromatic properties of identified interneurons in the optic lobes of the bee. J Comp Physiol 137:215–231

Homberg U (1981) Recordings and Lucifer yellow stainings of neurons from the tractus olfacto-globularis in the bee brain. Verh Dtsch Zool Ges 74:176

Homberg U (1982) Das mediane Protocerebrum der Honigbiene (*Apis mellifera*) im Bereich des Zentralkörpers: Physiologische und morphologische Charakterisierung. Ph D Thesis, Freie Universität Berlin

Homberg U (1984) Processing of antennal information in mushroom body neurons of the bee brain. J Comp Physiol A 154:825–836

Hoyle G (1955) Functioning of the insect ocellar nerve. J Exp Biol 32:397–407

Kondo H (1978) Efferent system of the lateral ocellus in the dragonfly: Its relationship with the ocellar afferent units, the compound eyes and the wing sensory system. J Comp Physiol 125:341–349

Milde JJ (1981) Graded potentials and action potentials in the large ocellar interneurons of the bee. J Comp Physiol 143:427–434

Milde JJ (1982) Elektrophysiologische und anatomische Untersuchungen an Interneuronen erster und höherer Ordnung des Ocellensystems der Biene. Ph D thesis, Freie Universität Berlin

Milde JJ (1984) Ocellar interneurons in the honeybee. Structure and signals of L-neurons. J Comp Physiol A 154:683–693

Mizunami M, Yamashita S, Tateda H (1982) Intracellular stainings of the large ocellar second-order neurons in the cockroach. J Comp Physiol 149:215–219

Mobbs PG, Guy RG, Goodman LJ, Chappell RL (1981) Relative spectral sensitivity and reverse Purkinje shift in identified L neurons of the ocellar retina. J Comp Physiol 144:91–97

Moore D, Penikas J, Rankin MA (1981) Regional specialization for an optomotor response in the honeybee compound eye. Physiol Entomol 6:61–69

Pan KC (1981) The neural organization of the ocellar system and associated pathways in the central nervous system of the worker honeybee, *Apis mellifera*. Ph D thesis, University of London

Patterson JA, Chappell RL (1980) Intracellular responses of procion filled cells and whole nerve cobalt impregnation in the dragonfly median ocellus. J Comp Physiol 139:25–39

Patterson JA, Goodman LJ (1974) Intracellular responses of receptor cells and second order cells in the ocelli of the desert locust *Schistocerca gregaria*. J Comp Physiol 95:237–250

Rosser BL (1974) A study of the afferent pathways of the dragonfly lateral ocellus from extracellularly recorded spike discharges. J Exp Biol 60:135–160

Ruck P (1961) Elektrophysiology of the insect dorsal ocellus. II. Mechanisms of generation and inhibition of impulses in the ocellar nerve of dragonflies. J Gen Physiol 44:629–639

Simmons P (1980) A locust wind and ocellar brain neurone. J Exp Biol 8:281–294

Simmons PJ (1981) Synaptic transmission between second- and third-order neurones of a locust ocellus. J Comp Physiol 145:265–276

Simmons PJ (1982) Transmission mediated with and without spikes at connexions between larger second-order neurones of locust ocelli. J Comp Physiol 147:401–414

Stewart WW (1978) Functional connections between cells as revealed by dye-coupling with a highly fluorescent naphthalmide tracer. Cell 14:741–759

Strausfeld NJ, Seyan HS, Wohlers D, Bacon JP (1983) Lucifer yellow histology. In: Strausfeld NJ (ed) Functional neuroanatomy. Springer, Berlin Heidelberg New York, pp 132–155

Strausfeld NJ, Bassemir UK, Singh RN, Bacon JP (1984) Organizational principles of outputs from dipteran brains. J Insect Physiol 30:73–93

Taylor CP (1981) Graded interactions between identified neurons from the simple eyes of an insect. Brain Res 215:382–387

Wilson M (1978a) The functional organization of locust ocelli. J Comp Physiol 124:297–316

Wilson M (1978b) Generation of graded potential signals in the second-order cells of locust ocellus. J Comp Physiol 124:317–331

Wilson M (1978c) The origin and properties of discrete hyperpolarizing potentials in the second-order cells of locust ocellus. J Comp Physiol 128:347–358