Response Characteristics and Identification of Extrinsic Mushroom Body Neurons of the Bee

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The activity of single neurons with constant discharge frequencies in the area around the α-lobe of the mushroom bodies of the bee was recorded intracellularly. The spontaneous discharge frequency of these neurons ranged between 5 and 95 impulses per second. When stimulated, about 80 percent of the neurons responded to at least one of five different sensory modalities: scent; light; air current to the antennae; sugar water applied to the antennae and to the proboscis. 45 percent of the neurons responded to two or more modalities, these multimodal neurons are common in the median protocerebrum of the bee. The differentiated response pattern of the cells does not allow a simple classification. Some of the neurons were identified after the injection of the fluorescent dye Procion yellow. We found 4 neurons with arborizations in the α-lobe and the calyces of the mushroom bodies.

Introduction

The mushroom bodies are paired neuropile structures in the median protocerebrum of insects [1 – 3]. They are formed by so called intrinsic fibres which do not leave the mushroom bodies. Extrinsic fibres provide the information input and output of the system, there are no fibres known to project through the mushroom bodies. The main input region is located in the calyx area, the main output region are the α- and β-lobes [4 – 7]. The structure of most extrinsic neurons is unknown, there is one anatomical description of an extrinsic neuron connecting the different parts (α-lobe, β-lobe, calyces) of the mushroom bodies [8].

In spite of a number of physiological experiments the function of the mushroom bodies is not clearly understood. Electrical stimulation and lesion experiments [9–11] indicate that they act as an "inhibitory higher centre" of the brain, together with the central body they possibly control complex sequences of behavior. In the bee local cooling experiments suggest that the mushroom bodies play a decisive role during short-term memory formation after olfactory conditioning [12].

The responses of neurons of the mushroom body area to olfactory, gustatory and optical stimuli have been described by different authors [13 – 15]. Many neurons are multimodal, quite often we found cells with a constant spontaneous frequency of spikes. The statistical variation in the background frequency of these neurons is relatively small over long periods. Most of them respond to more than one modality [13]. Because we can find these neurons reliably in the area around the α-lobes, we set out to characterize them. After the general description of response characteristics of single neurons in the bee brain [13, 14] this analysis with identified neural elements is a first step towards a better understanding of the interaction of neural architecture and physiological function in the mushroom body system.

Methods

Recordings from single neurons were made in the median protocerebrum of the honey bee *(Apis mellifera)* with glass capillary electrodes (2.5 M KCl, 50 – 120 MOhm). We always recorded from one defined location, lateral to the α-lobe of one mushroom body (details of preparation and recording can be found in ref. 13). The depth of the tip of the electrode relative to the surface of the brain was always registered. We recorded at this location from over 70 neurons with constant spontaneous discharge frequency.

Five different sensory modalities were applied whenever a neuron was found. The application of different modalities was controlled by a programmable stimulator with defined durations and sequences of stimulation. Sugar water (30% solution) was ap-

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plied to the antennae and the proboscis manually. The light stimulus (from an incandescent lamp, 8 V, 6 A) was applied to the ipsilateral complex eye with a light guide which substended 15° at the bee's eye. The scent stimulus was a mixture of thyme, rosemary and lavender oil. Air current was presented frontally to the bee via a pipe mounted beside the scent tube [13]. After recording, the fluorescent dye Procion yellow was injected into some of the neurons. Marked cells were photographed with a fluorescence microscope and color slides were used for reconstructing the structure of the cell.

Results

The spontaneous rate of spiking by the neurons varied between 5 and 95 impulses/s, the median of this distribution is 30 impulses/s. We did not find a correlation between constant spontaneous frequency and the depth of the recordings. The spontaneous frequency of the neurons was very constant. An example is shown in Fig. 1 a. The interval histogram was measured over 60 s, and the medium value of the symmetrical interval histogram was 13.9 ms with a standard deviation of ± 2.9 ms. This medium interval duration is equivalent to a spontaneous frequency of 72 impulses/s. As we are able to record the activity of these neurons for up to 30 minutes, we think it is very unlikely that the constant background discharge is due to an artifact such as injuring the membrane of the cell.

The next question we wanted to answer was whether neurons with constant spike frequencies have similar response patterns. We found that the neurons responded to the variety of stimuli quite differently with excitation or inhibition, and that some responded to only one modality whereas others had clear multimodal response patterns.

A high proportion of the neurons reacted to scent (46%) and light (36%), only some neurons responded to an air current (16%). When we tested the neurons described in this paper with sugar water, we found that more than a quarter of them responded to sugar water applied to the antenna (27%) or to the proboscis (28%). 21% of the cells did not react to the stimuli we presented. From this finding we conclude that we either did not find the appropriate stimuli for these cells or that these neurons are not engaged in processing sensory information. 34% of the neurons responded only to one of the tested modalities, whereas
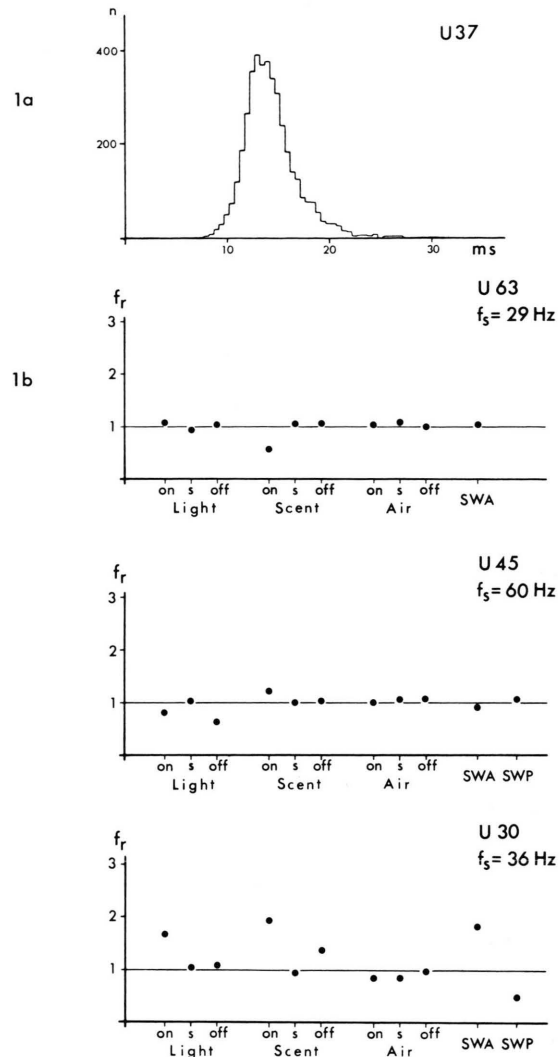

Fig. 1. Interval histogram and reaction spectra of interneurons with constant spontaneous discharge frequency. (a) The interval histogram of neuron U 37 shows the spontaneous rate of action potentials measured over 60 s; abscissa: interval classes; ordinate: number of intervals in the different classes. (b) The reaction spectra show the reaction of three neurons (U 63, U 45, U 30) to the stimuli indicated on the abscissae: light (on, sustained (s), off), scent (on, sustained (s), off), air current (on, sustained (s), off), sugar water to the antennae (SWA), and to the proboscis (SWP). The ordinates denote the relative frequency f_r, *i. e.* the actual frequency during or after a stimulation divided by the spontaneous frequency (f_s) before the stimulus.

45% of the neurons responded to more than one stimulus. In most cases the reaction of the neurons consisted of an increase of the frequency of action potentials, only in 29% of the stimulations we found inhibitory responses.
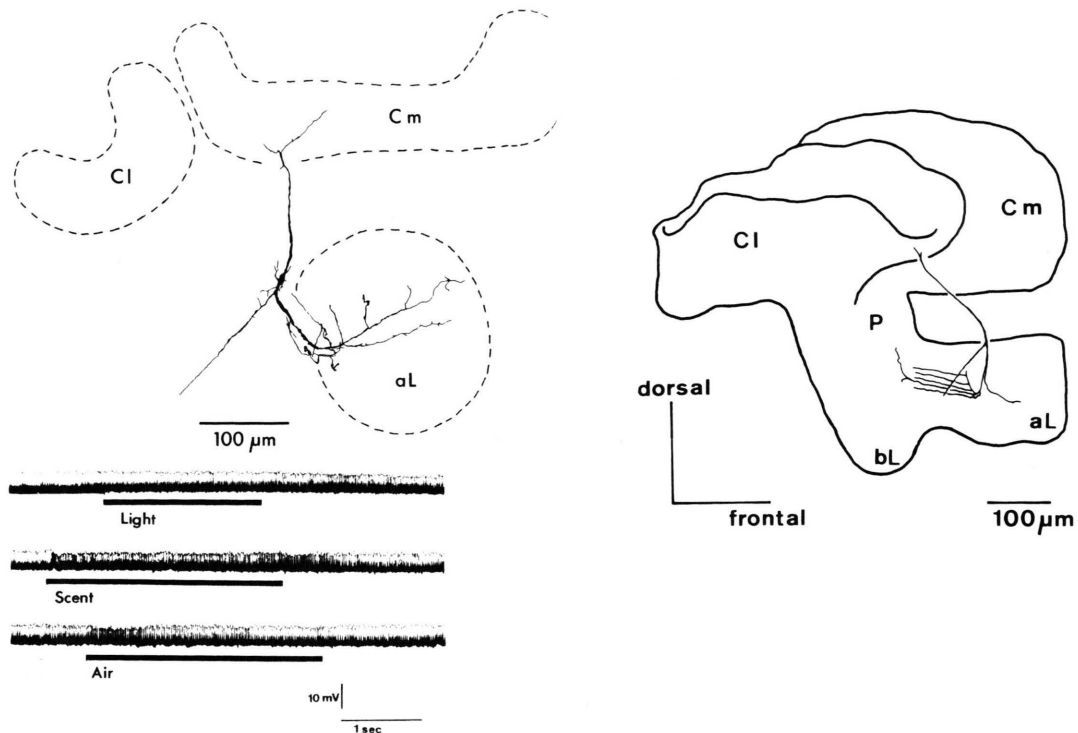
Fig. 2. Recording and reconstruction of a Procion injected neuron in the mushroom body area. The recording shows the spontaneous discharge rate ($f_s = 62$ Hz) and the reaction of the neuron, the duration of the light, scent and air stimulation are marked. The reconstruction on the left shows the frontal projection of the neuron, the schematic drawing on the right gives the sagittal view. The neuron has arborisations in the α-lobe (aL) and the median calyx (Cm) of the mushroom body. Cl, lateral calyx; P, pedunculus; bL, β-lobe.

Fig. 1 b shows the response patterns of three neurons with constant spontaneous frequencies of spiking. The diagrams show the relative frequency of action potentials during (on or sustained) and after (off) stimulation by different modalities. The relative frequency is the stimulus dependent impulse rate divided by the spontaneous frequency before the stimulus. The modulation of the spontaneous discharge rate by a stimulus is often rather small in constantly firing neurons compared to neurons with irregular and low spontaneous frequencies [13]. The three examples represent a unimodal neuron (U 63) which responds with inhibition to the onset of a scent stimulus; cell U 45 is a multimodal neuron which shows inhibition when a light is switched on or off and excitation when a scent stimulus begins. Neuron U 30 shows a typical multimodal response pattern with excitation and inhibition to four different stimulus modalities. This last example also makes it clear why it is nearly impossible to classify the multimodal neurons in a limited number of categories. If one classifies the responses in excitation, inhibition and no response and if one tests 11 different multimodal response parameters, like in the last example, then there are nearly 200 000 different possible outcomes of the experiment. If one keeps in mind that the neurons of the median protocerebrum can change their response characteristics during the experiment due to unknown occurences it does not seem reasonable to attempt a simple classification of the neurons.

Confronted with these difficulties we wanted to find out whether neurons with constant spontaneous frequency perhaps had common neuroanatomical features. With the Procion injections we surprisingly found four neurons with very similar neuroanatomical structure. These neurons had arborizations in the α-lobe and calyces of a mushroom body. The four neurons had different physiological response patterns, ranging from no response to multimodal responses. They had in common constant spontaneous frequencies and similar neural structures. Fig. 2

shows one example of these neurons. The cell responds to a scent stimulus with excitation, to an air stimulus with a small and short increase of the frequency of action potentials. The spontaneous frequency of this neuron was 62 impulses/s. The reconstruction shows a fibre with projections in the α-lobe and the median calyx. A branch of the fibre projects to the optic tubercle. The cell body was not marked in this recording.

Discussion

Neurons with constant spontaneous discharge frequency exist in different depth around the α-lobe of the mushroom bodies in the bee. The physiological response patterns of these neurons vary widely, on the basis of physiological findings a classification of these neurons is not possible. As we expected from other electrophysiological analyses of the bee brain [13 – 16], many of the neurons were multimodal.

Cells with constant spontaneous frequency seem to be widely distributed in insect brains. Schümperli [17] found them in the protocerebrum of Lepidoptera, Erber [13] describes this physiological property in neurons around the mushroom bodies of the bee, Hertel and Riehle recorded from these neurons in the optic ganglia of the bee (personal communication). At the moment the significance and physiological function of neurons with constant spontaneous discharge is quite uncertain. As the variation of the spontaneous discharge rate is often rather small and as the modulation of the background discharge is not very significant in many of these neurons (sometimes only very few action potentials are added or subtracted from the spontaneous discharge), these cells could act as synchronizing and gating elements between different neuropiles [13].

Given the wide physiological response range of the recorded neurons, it is rather astonishing to find similar neural structures in four of the neurons. These cells were extrinsic neurons of the mushroom body system connecting the α-lobes with the calyces. They represent a new class of extrinsic fibres, which have not been described by other authors. Electron-microscopic analyses [4 – 7] on the input — output relationships of the mushroom body system suggest that the neurons described here are part of a feedback loop, transfering information from the α-lobe to the calyces.

We can only speculate about the function of such feedback loops. If we assume that the mushroom bodies are engaged in the control of complex behavioral sequences [6, 8 – 10], the neurons described here can have different functions. By feeding back the excitation or inhibition of a certain group of intrinsic fibres onto another group of intrinsic fibres, a sequence of excitation, inhibition or both could be generated in the mushroom bodies. The feedback loops could be an essential prerequisite for long lasting after effects which were found after different sensory stimuli [13, 18], in this case they would participate in reverberating circuits in the mushroom body system. Another possible function of the neurons with constant spontaneous frequency could be the selective inhibition of certain groups of intrinsic neurons whenever another group of intrinsic fibres is active. The constancy of the spontaneous discharge rate could define with accuracy a level of excitation or inhibition of certain intrinsic fibres. It is possible that both mechanisms, lateral interaction and sequential processing of information, are realized in the mushroom body system. The astonishing correlation of a rather specialized physiological parameter (constant spontaneous frequency) with the structure of these neurons is a further step towards a better understanding of the mushroom body system.

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