The Number and Structure of Giant Vertical Cells (VS) in the Lobula Plate of the Blowfly Calliphora erythrocephala

R. Hengstenberg, K. Hausen, and B. Hengstenberg
Max-Planck-Institut für Biologische Kybernetik, Spemannstrasse 38, D-7400 Tübingen 1, Federal Republic of Germany

Accepted June 21, 1982

Summary. 1. The structure of one class of giant tangential neurons in the lobula plate of Calliphora, the ‘Vertical System (VS)’ has been investigated by light microscopy. Different staining and reconstruction procedures were employed to ensure that all existing VS-neurons are revealed.

2. There are 11 VS-cells in a characteristic, and constant arrangement (Fig. 2). Each cell covers a particular area of the lobula plate, i.e., a distinct area of the retinotopic input array (Table 2), and therefore has a distinct receptive field.

3. Although VS-cells in general tend to occupy the posterior surface of the lobula plate, only three of them (VS 2–VS 5) reside exclusively in this layer. The other cells (VS1 and VS6–VS10) have bifurcated dendritic arborizations (Fig. 6), whose dorsal parts are apposed to the anterior surface of the lobula plate.

4. The arrangement, territory and stratification of any given VS-cell is largely invariant in different individuals, whereas the branching pattern may vary considerably (Fig. 3).

5. The present results provide the framework for physiological studies of the role of individual VS-cells in movement perception, and their involvement in the control of particular locomotor behaviour.

Introduction

The visual system of Diptera has been studied intensively in the past, because basic processes of visual perception, and their influence upon the control of different locomotor behaviour can be studied in freely moving flies, and under strictly controlled experimental conditions (reviews: Götz 1980; Kirschfeld 1979; Poggio and Reichardt 1976; Reichardt and Poggio 1976; Wehner 1981). The orderly structure of the visual nervous system has also been extensively studied, so the structure and arrangement of a large number of interneurons, their projections, and some of their synaptic connectivity are known (reviews: Strausfeld 1976; Strausfeld and Nässel 1981). Recently it has also become possible to investigate the response properties of individual interneurons, either by extracellular recording or by intracellular penetration, combined with dye injection, which allows the unambiguous identification of the neurons under investigation (review: Hausen 1981). Finally, the neural control of power- and steering muscles during flight has extensively been investigated (Nachtigall and Wilson 1967; Heide 1982).

This work reports on the structure of one particular class of giant visual interneurons: the ‘Vertical System (VS)’ in the lobula plate of the blowfly Calliphora. This type of neuron has been investigated anatomically in the housefly Musca (Braitenberg 1972; Pierantoni 1975, 1976), in the blowflies Calliphora, Phaenicia and Sarcophaga (Eckert and Bishop 1978; Hausen 1976a; Hausen et al. 1980; Nässel 1981; Strausfeld 1976; Strausfeld and Obermayer 1976), and in the fruitfly Drosophila (Heisenberg et al. 1978). Striking similarities in the structure of these neurons have been revealed in the different species. Physiological investigations have so far only been carried out in Calliphoridae, showing that these cells respond predominantly to vertical movements in the visual field (Dvorak et al. 1975a, b; Eckert 1979; Eckert and Bishop 1978; Hausen 1976a, b, 1981; Hengstenberg 1977, 1981; Soohoo and Bishop 1980).

The present account attempts to answer the following questions: (a) How many VS-cells are present in the lobula plate of normal flies? (b) Are they arranged in a stereotyped manner? (c) Can each...
VS-cell be specified by a characteristic structure? (d) How similar are these characteristic features in different individuals of the species? The principal aim of this work is to define the characteristics of VS-cells as a class of neurons, and to establish the uniqueness of individual VS-cells in order to provide the framework for physiological investigations of their particular functions.

Materials and Methods

Animals. Average sized females of wild type Calliphora erythrocephala Meig. (Diptera) 4-10 days post emergence have been taken from laboratory cultures, which were refreshed by flies, caught in the wild, every few generations.

Histological Procedures

Semithin Sections. Specimen were fixed in 2% formaldehyde, and 2.5% glutaraldehyde, in phosphate buffer pH 7.3 (Karnovsky 1965) overnight at 4°C. Dehydration: graded ethanol, intermedium: propylenoxide, embedding medium: Araldite. Serial sections of 3 µm thickness were cut in a frontal plane with glass knives on a rotary microtome (Jung, Autocut), and stained on a hot plate (ca. 85°C) with 1% toluidine blue in 1% Na2B4O7 at pH 10-11 for 1-5 min. Excess dye was removed by washing in cold water (Pease 1964). A flawless series of 85 sections was used for the three dimensional reconstruction of the large cells in the lobula plate.

Cobalt-Impregnation and Silver-Intensification. Procedures for mass staining of VS-cells by retrograde uptake of cobalt, and for silver-intensification of the precipitated CoS en bloc have been previously described in detail (Strausfeld and Obermayer 1976; Hausen and Wolburg-Buchholz 1980; Hausen et al. 1980).

Procion Yellow Injection. Procion Yellow M4-RAN (Serva, Heidelberg, FRG) was injected into VS-cells by dc-iontophoresis (≤ 5 nA; ≥ 5 min) via fine-tipped, fused-fibre microelectrodes (200 < R < 500 MΩ) filled with a 3% aqueous dye solution. Overnight diffusion at 4°C guaranteed homogeneous distribution of dye in VS-cells. Specimen were fixed for 2 h in a mixture of 4% formaldehyde, and 5% acetic acid in 85% ethanol, embedded in paraffin and serially sectioned at 7 µm. Details of cell penetration, dye injection, and histological procedures are given in Hengstenberg and Hengstenberg (1980).

Microscopy and Microphotography

Sections were scrutinized and photographed with a Zeiss ‘Universal’ compound microscope, fitted with high resolution, low power fluorescent lenses (Zeiss Plan Neofluar 16 ×, 25 ×), and equipped for bright field, phase contrast, and incident light fluorescence microscopy. Photographs were taken by a 24 × 36 mm camera on color transparency film. Details on fluorescence microscopy and microphotography are found in Hengstenberg and Hengstenberg (1980).

Cell Reconstruction

Neurons were reconstructed from serial sections by sequential projection of slides onto white paper, and manual drawing of the outlines of stained profiles and of auxiliary structures with pens of different color. Alignment of successive sections was achieved by shifting, and turning the drawing paper such as to obtain a ‘best fit’ of stained profiles and of auxiliary structures. For a discussion of different reconstruction procedures see Hengstenberg and Hengstenberg (1980). Three-dimensional reconstructions of neurons, together with their environment have been obtained recently by feeding the outlines of different structures via a graphics digitizer into a minicomputer, and by computation of stereograms, which, when viewed with a stereoscope unambiguously display the three dimensional structure of neurons, and of their environment (Hengstenberg and Hengstenberg 1980; Hengstenberg et al., in press).

Valuation of Histological Procedures

Three different histological procedures are employed in this work, in order to display the number, location and characteristic structure of VS-cells. These procedures reveal in part complementary aspects of VS-cells: (a) Semithin serial sections show definitely all existing cells. Large fibres (> 5 µm) can be traced, but it is difficult or even impossible to follow fine processes (<3 µm) unambiguously. (b) Mass impregnation with cobalt has the tremendous advantage of displaying most of the VS-cells in a fly. With silver-intensification, very fine details can be seen. In most cases, however, not all of the existing cells will be labelled, and some possibly never. The retrograde staining usually obscures the terminal arborizations of VS-cells. (c) Procion yellow, when injected in sufficient quantity, invariably reveals even the finest details of one or a few cells per animal. Neighbouring cells are, however, either invisible or only in coarse outline. Therefore small cells may be missed entirely.

By parallel application of these three procedures and by cross comparison of their results, it should be possible to specify the total number of VS-cells that are present in the lobula plate with minimal ambiguity, to describe their arrangement within the neuropil, and to resolve the characteristic structure of particular neurons of this class.

Results

Architecture of the Lobula Plate

Numerous studies have been devoted to the structural and functional organization of the compound eyes, and the visual nervous system of Diptera (reviews: Hausen 1981, Strausfeld 1976). Therefore only a brief sketch of the architecture of the lobula plate is given:

The lobula complex is the last stage in the chain of three visual neuropils constituting the optic lobe. In Diptera it is divided into two parts: the anterior ‘lobula’, and the posterior ‘lobula plate’. Both receive input from the ipsilateral eye via numerous types of retinotopically arranged columnar fibres of the medulla (Strausfeld 1976). They are further connected to one another in a retinotopic manner by small field neurons. Lobula and lobula plate receive input from the contralateral optic lobes via heterolaterally projecting neurons. The lobula is characterized by its conspicuous columnar structure, whereas the architecture of the