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Postsynaptic Potentials from a Single Monopolar Neuron of the Ganglion Opticum I of the Blowfly *Calliphora**

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Summary. Hyperpolarizing potentials were recorded from second order monopolar neurons of the ganglion opticum I of *Calliphora* by means of glass microcapillaries. The eye was stimulated with a spotlike light source. Potentials were recorded in relation to the intensity of the light stimulus. The recording site was identified by marking the cell with a dye and by localizing the tip of the electrode.

Introduction

Morphological studies from Cajal and Sánchez in 1915 show that the retinula cells of the compound eyes of insects send processes to the first optic layer, the Lamina ganglionaris. There, together with neuronal elements, they form ommatidium-like structures, the cartridges. Investigations with the electron microscope in this region (Trujillo-Cenóz and Melamed, 1963; Trujillo-Cenóz, 1965) show typical synaptic structures. The processes of the retinula cells were seen as presynaptic elements, the axons of the monopolar neurons as postsynaptic ones. Furthermore in the lamina there were seen glia cells, horizontal cells, amacrine cells and various types of centrifugal fibres (Cajal and Sánchez, 1915; Strausfeld, 1970). Recently Boschek (1970) discovered series of further synaptic connections.

Although the morphology of the nervous tracts in the optic lobe of the insect eye is well known, knowledge about the functional relations between cells is very scarce. Electrophysiological recordings were made in most cases with metal electrodes without a precise localization of the source of recorded potentials. Scholes (1969) first reported intracellular potentials from single cells in the lamina region. These potential recordings could be confirmed by Zettler and Järvilehto (1970) and were correlated to the retinula cell processes by means of a single cell marking technique (Järvilehto and Zettler, 1970). In these processes of retinula cells only slow potentials without superimposed action potentials were found. So the presynaptic site delivers slow potentials and not action potentials like usual in the nervous system.

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Up to now no recordings of postsynaptic potentials from the first stage of information processing in the insect visual system have been made. As mentioned already, there are a lot of neuronal cell types, which can act as postsynaptic elements in this region. The monopolar neurons represent one of the most striking types because of their magnitude and their position. One tends to suppose that these cells play an important role in processing the optical information.

Method

All the experiments discussed here were made with the blowfly *Calliphora erythrocephala* (normal type), which is bred in our institute. Preparation and design of the experiment are the same as described by Zettler (1969). The electronic equipment for recording cell potentials was improved in some respect and was expanded by a constant current source. The possibility of giving a defined and constant current through the glass capillary is of essential importance for the injection of dye into the cell. The circuit of the cathode follower, combined with the constant current source is shown in Fig. 1. Included in this figure is a diagram, which shows the transmission quality of the recording device.

The first stage of this recording device is represented by an electrometer pentode, which has the advantage that no grid current compensation is necessary. This could also be done by using a field effect transistor, but this element is easily damaged by influenced voltages. Any arrangement to prevent this lowers either the input resistance and band width or increases the noise level.

The second stage is a follower-circuited operational amplifier. It establishes a second impedance transmission. By adder I the dc output level can be compensated to zero. Adder II delivers the voltage for the screening grid of the input pentode.

The relay Rel 3 enables the experimenter to put a constant current source in contact with the control grid leading. A Reed-relay is used as Rel 3 to guarantee a high resistance in the opened state. The principle of the constant current source is built up according to Philbrick/Nexus Research (1968), modified by two field effect transistors. They are necessary to lower the magnitude of the applied current down to 10^{-9} amperes. By means of S_2 and Pot_2 the experimenter can choose any current in the range of 10^{-9} to 10^{-6} amperes. By S_3 the polarity of the current can be determined. At last the current, preselected in magnitude and polarity by the experimenter, can be driven through the recording electrode by pressing T. This current is not only available for the dye injection, but also for the control of the electrode resistance. The recording electrodes are micro glass capillaries, filled with an aqueous solution

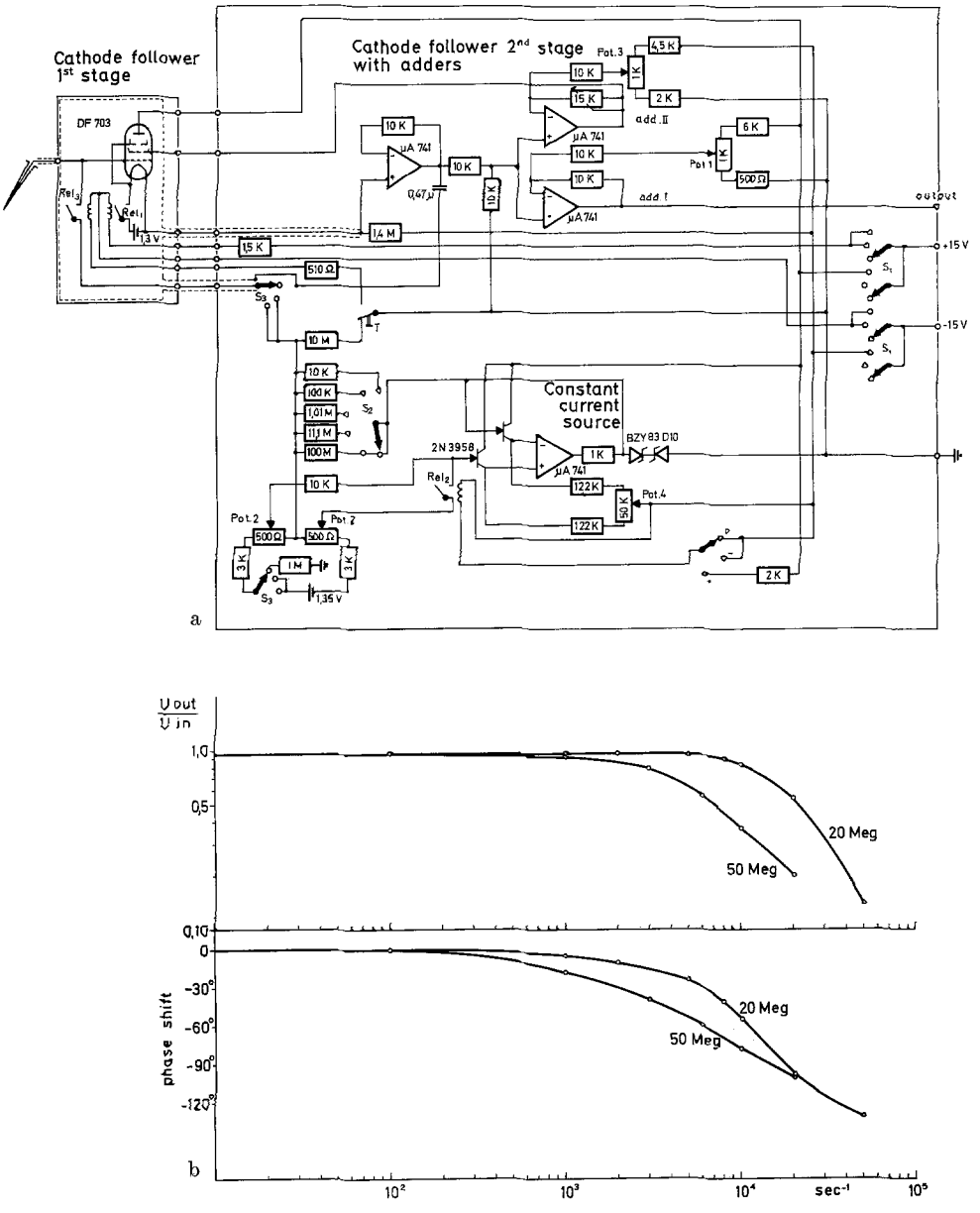

Fig. 1. Circuit of the cathode follower used here, with a constant current source for dye injection. The input-output ratio and the phaselag vs. frequency is shown in the diagram below. The different curves are obtained by using different resistances of the input source. These resistances are mainly represented in the experiment by the electrode resistance

of 10% Navy Blue H-3R and 3% $NaHCO_3$ (Stretton and Kravitz, 1968). The capillaries have tip diameters of 0.1 μm and are filled at room temperature (Zettler, 1970). Their resistance in the tissue fluid is about 50 megohms.

After a light sensitive cell in the fly's retina has been penetrated, maximum answer is found by moving a spotlike light source. This position of the light source is retained during the further experiments, where the answer is recorded in relation to the intensity of the light stimulus. Thereafter a dye is injected into the cell by means of an electrical current, whose magnitude is 3×10^{-8} amperes and whose polarity gives the capillary a negative voltage. Duration of injection is about 20 minutes, while the current is applied in pulses of about 1 second duration each.

Histological fixation of the preparation is done by rapid freezing of the whole fly's head in the recording position. Thus the tip of the electrode can be broken off and can be identified later in the histological sections (Zettler and Järvilehto, 1970). The preparation is kept in the frozen state and dried at $-40°$ C in the vacuum. Thereupon it is embedded in Esterwax and cut in 10 μm sections. The wax is removed with xylene and no further treatment is applied. Thus the tissue remains in a native state without shrinking. This method makes it possible to identify both the cell of interest and the tip of the recording electrode, which means the position within the cell, from where the potentials were recorded. Thus the experimenter is enabled to give a subcell localisation of the recorded potentials.

Results and their Discussion

Identification of the Recording Site

Fig. 2a shows the dye-marked monopolar neuron from which the recordings of Fig. 3 were made. The plate is a composition from five successive 10 μm sections. The tip of the electrode came unfortunately within a cutting plane and was damaged while cutting. Nevertheless its position could be localized very exactly in the bloc just before cutting. The very tip of the capillary touched the dye-marked neuron in the position as shown in Fig. 2b. The injected dye diffused from the tip both directions into the axon, up to the soma and down to the chiasm. The chiasm shows a fracture which is probably due to freezing of the preparation.

Electrophysiological Results

In most cases where the lamina ganglionaris is penetrated by a micro glass capillary, the recorded potentials originate from the process of a retinula cell. Steplike light stimuli induce positive going monophasic potentials. In some rarer cases one obtains intracellular recordings

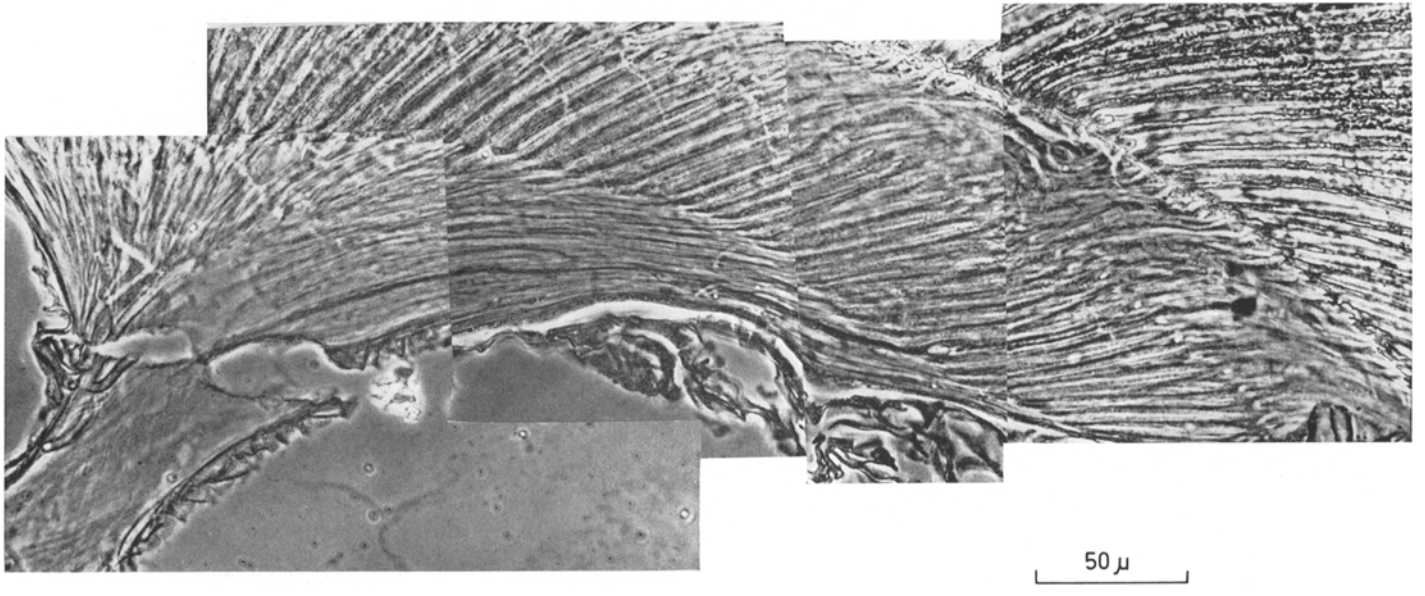

Fig. 2a. Horizontal section of a compound eye of *Calliphora* with a dye-marked monopolar neuron

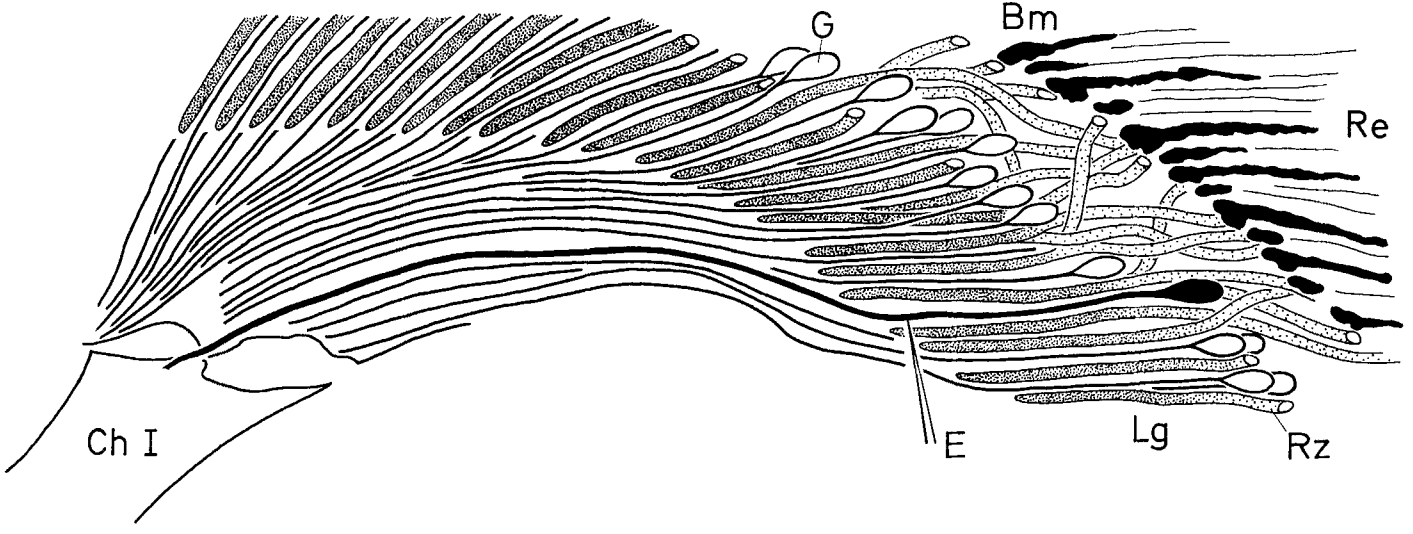

Fig. 2b. The tracing shows the morphological arrangement of the cells from the first optical ganglion in a somewhat idealized way. *Bm* basement membrane, *Ch* chiasma, *E* electrode, *G* monopolar neuron, *Lg* Lamina ganglionaris, *Re* outer retina, *Rz* retinula cell process

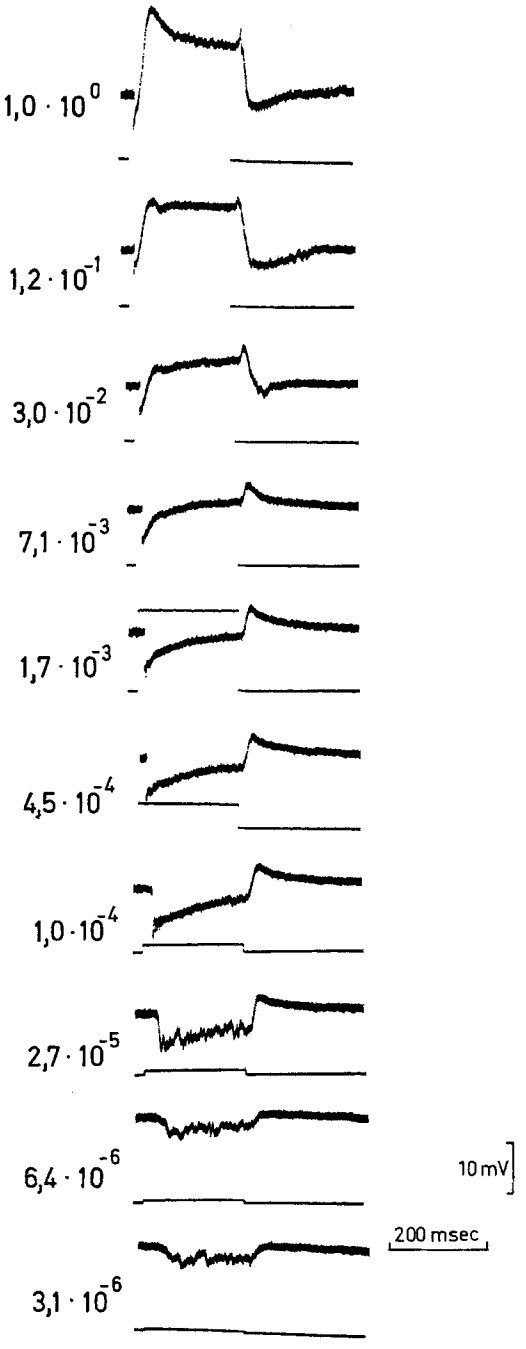

Fig. 3. Light-evoked potentials, recorded from the monopolar neuron, marked in Fig. 2. Stimulus duration 200 ms from a spotlike light source. The numbers mean relative intensities

with negative going monophasic potentials. These recordings usually are very instable and diminish in most cases within a few minutes. With very fine tapered capillaries one sometimes obtains potential recordings lasting over half an hour. Fig. 3 shows the responses of those cells following steplike light stimuli. The intensities were varied in a range of about 6 decades. At lower intensities the potentials are negative monophasic with big noise. At somewhat higher intensities the noise disappears and the potentials are acquiring more and more a diphasic character. At high intensities the potentials are essentially positive with a negative on- and a positive off-effect.

These results lead to the supposition that the recorded potentials are made out of two superimposed monophasic parts, a negative one and a positive one. Hereby the negative part has a much faster on- and off-flank than the positive one. Furthermore the positive part of the potential seems to increase faster with increasing light intensity than the negative one.

This supposition is supported by the following observation. A bad stability is characteristical for all recordings from monopolar neurons. That means, the negative component of the recorded potentials decreases in the course of time and disappears completely after a certain time of experimentation. On the other hand the positive component is not affected by the time of experimentation, so that after a certain time the recorded potentials are monophasic positive ones. These surviving positive potentials have the same shape as the potentials recorded from the presynaptic site, the retinula cell processes (Järvilehto and Zettler, 1970), but they don't reach their magnitudes. The dye marking of the recording site shows with striking evidence that these observed potentials originated from the interior of a monopolar neuron.

Therefore one can argue that the negative component of the potentials has its origin in some active properties of the postsynaptic membrane, and that on the other hand the positive component spreads electrotonically from the visual cell terminals. The neuron begins to die slowly as soon as the microcapillary is thrust into it, and its membrane looses its active properties. Its passive properties, necessary for electrotonical spread, remain unaffected and so do the positive components of the recorded potentials.

Fig. 4 first row (I) shows the same potentials as fig. 3. The potentials of the second row (II) originated at the same recording site as those from the first row, but are recorded 30 minutes later. At this time the negative component vanished. By subtracting the corresponding potentials of these two series (I–II), the pure negative phase of the first row potentials should result. This indeed is the case, as shown

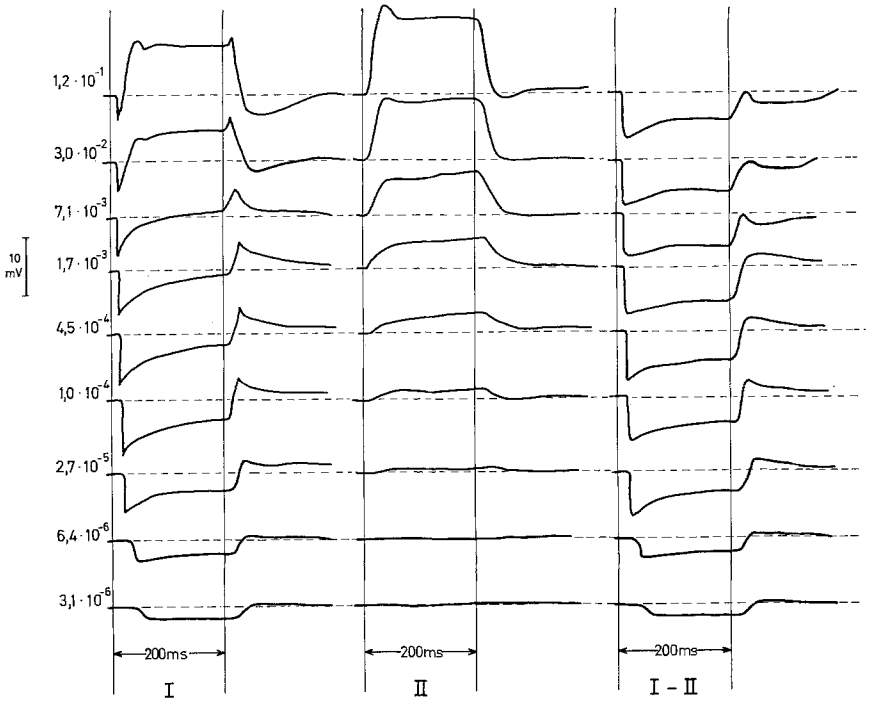

Fig. 4. I Potentials from a monopolar neuron at different stimulus intensities, immediately after penetration. II Potentials from the same neuron, 30 minutes after penetration. The negative phase has vanished. I–II Difference of the potentials I and II. Pure negative phase

in the third row of fig. 4. Remarkable, however, is the fact that the negative phase has a saturation value at relative low intensities of the light stimulus.

Furthermore, at low intensities, the measured potentials are superimposed by a large noise activity. We interpret this noise as a postsynaptic effect, provoked by a quantal release of a transmitter substance. By means of electronmicroscopy Trujillo-Cenóz (1965) found ribbons and vesicles in the visual cell processes, typical for a presynaptic site. The corresponding postsynaptic site is a monopolar neuron. Since the light induced potentials on the presynaptic site are never superimposed by noise, one is inclined to interpret the noise from monopolar cells as a postsynaptic effect.

In the common terminology of synapses, a negative postsynaptic potential would be explained as an inhibitory postsynaptic potential (IPSP). One would suggest that the monopolar neurons produce a

spontaneous spike activity which is suppressed by the negative postsynaptic potential. But untill now our recordings have never shown any spike activity.

A possible reason for the absence of spike activity could be seen in a band width of the recording system, which is too low to indicate the fast going action potentials. The band width of our cathode follower in Fig. 1 shows about 6 kc/s when the resistance of the glass capillary is 50 Megohm. On the other hand the electrode itself has a band width determined by its resistance and its capacity across the wall (about 1 pF per mm penetration depth). As our penetration depth is maximal 1 mm, a band width of about 3 kc/s results. This indeed is not sufficient to give an undistorted recording of action potentials, but it should be good enough to see any marks of them when the electrode is located intraaxonal.

Another interpretation for the absence of spike activity in the monopolar neurons may be seen in cell lesions. The possibility can not be excluded that the active membrane mechanisms are disturbed by the penetration of the microcapillary into the axon's membrane. Our previously discussed observation that the negative phase of the recorded potentials decrease in the course of time already led us to suppose cell lesions. So it can not be totally excluded that the potentials recorded here do not yet represent the true situation in the undistorted neuron.

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