Action Potentials in "Non-Spiking" Visual Interneurones

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Z. Naturforsch. **36 c,** 470–474 (1981); received August 7, 1980/January 21, 1981

Vision, Diptera, Central Nervous System, Identified Neurones, Graded Signal Transmission

The response of the homolateral "horizontal cells" (H-cells) of the third visual neuropile of the blowfly, *Phaenicia sericata*, has been investigated by intracellular recordings coupled with dye injections (Procion Yellow, cobaltous chloride). Responses were obtained from their terminal axon endings in the central protocerebrum. Under natural conditions, the horizontal cells respond not with graded potentials as reported previously, but with action potentials upon stimulation by regressive pattern motion within the contralateral receptive field. The stimulus-induced responses change from action potentials to graded signals within a few minutes after opening the head capsule. The possible causes of this change, namely hypoxic conditions and a consequent depletion of the ATP-supply, are consistent with the experimental data: a change in the resting potential as well a reduction of the spike amplitude. Furthermore, the possibility of two functionally different output regions of the H-cells is discussed, namely, (1) the terminal axon endings appear to conduct action potentials induced by the contralateral input (to the H-cells). Thus, these endings provide a solely contralateral, *i.e.* "*monocular output*". Whereas (2) the axonal arborisation appears to conduct graded signals elicited by the ipsilateral input onto which the contralateral input is superimposed, thus providing a "*binocular output*".

The means of signal transmission by graded potentials has recently been reported for certain directionally selective, motion sensitive cells in the third optic neuropile of flies, namely the vertical cells (VH, V1–V9), the horizontal cells (NH, EH, SH) and the centrifugal horizontal cells (DCH, VCH) [1–3]. However, this concept had already been challenged when a complex potential behaviour was described for the horizontal cells consisting of action potentials and graded potentials, respectively: the conduction of graded potentials was allocated to the axon connecting the dendritic tree in the lobula plate with the central protocerebrum; whereas action potentials appeared to be generated by the terminal axon endings in the ventrolateral protocerebrum [1, 4]. In addition, it was shown that action potentials may be induced in vertical and horizontal cells by imposing hyperpolarizing currents [3, 5]. Recent experiments suggest, that the concept of graded signal transmission does not hold, neither for the vertical nor for the horizontal cells [3, 6]. We show here that the previously reported graded potential behaviour of the horizontal cells is caused by a time-dependent "degeneration" process and that they are capable of generating action potentials under natural conditions. We shall discuss (the most likely) source of these potential changes and their possible ionic basis.

In dipterans, the third optic neuropile is divided into a rostral lobula and a caudal lobula plate. The vertical cells comprise a set of 10 cells whose axons traverse along the caudal surface of the lobula plate and enter the central protocerebrum (midbrain) where they terminate lateral to the oesophageal canal [7]. Within the lobula plate, the axons bifurcate giving rise to two main dendritic branches oriented dorso-ventrally with respect to the retinotopic projection of this neuropile [7, 8]. These main dendrites give off second and higher order dendritic branches penetrating into the lobula plate and terminating in the caudal part of this neuropile. An example of this cell type (VH-cell) is shown in Fig. 1 A. The horizontal cells (Fig. 1 B) are located at the frontal surface of the lobula plate: their axons pass between the two parts of this neuropile, enter the central protocerebrum where they give rise to an axonal arborisation (aa) and terminate in the ventrolateral protocerebrum [1, 2, 4, 7, 8].

Intracellular recordings were combined with dye injection (Procion Yellow M4RAN and cobaltous chloride, respectively; [1, 4, 6] allowing anatomical identification as well as allocation of the measured

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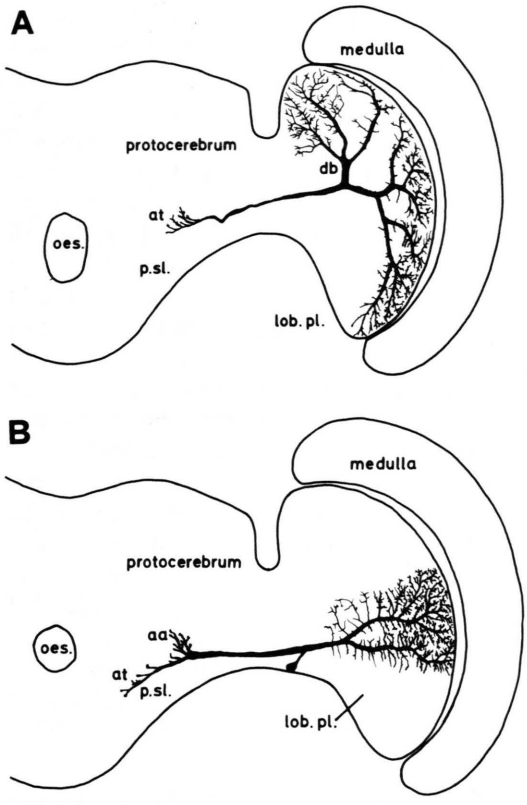

Fig. 1. Graphic reconstruction of motion sensitive neurones of the lobula plate of dipterans after serial sections of Procion Yellow stained (EH 1) and cobalt stained (VH) cells. *Phaenicia*♀; marker corresponds to 100 µm. *A*: VH-cell (Eckert [7]) of the right side of the fly's brain is shown in a caudal view. *db*: dorsal branch of the cell whose dendritic endings terminate at the level of those of the horizontal cells, *i.e.* close to the rostral surface of the lobula plate. All other dendritic endings terminate at a more caudal layer within the lobula plate at the level of the dentritic endings of the vertical cells. p. sl.: posterior slope of the ventrolateral protocerebrum. *B*: Equatorial horizontal cell as shown in caudal view; the cell lies at the rostral surface of the lobula plate. *aa*: axonal arborisation; *at*: terminal axon endings; *lob. pl.*: lobula plate; *oes*: oesophageal canal.

response characteristics to the anatomically identified neurones. The details of the dye injection technique have already been described elsewhere [5, 6].

Female blowflies, *Phaenicia sericata*, 6–10 days old were the principal species used in this study. Details of the experimental preparation are given elsewhere [5, 6]. The most important deviation of the experimental procedure from the one quoted above, was the very short time between the opening of the head capsule (by slicing off a posterior, triangular section) and the impaling of a cell. Furthermore, no Ringer solution was applied at all.

For the sake of clarity we shall briefly describe the two types of stimulus. A "motion" stimulus was provided either by a pattern projector or by a series of light bulbs turned on sequentially and thus, providing the "illusion" of motion (ficticious or apparent motion). The pattern projector consisted of a dc-powered quartz iodine light bulb whose rays projected through a metal cylinder onto a milky glass screen. This cylinder was driven by a dc-motor whose angular velocity could be varied between 0.1 and 164 deg/s.

Excitatory responses of the horizontal cells are induced by regressive motion presented to the contralateral eye and by progressive motion presented to the contralateral eye [1, 2, 6]: progressive motion stimulates the ommatidia successively from the anterior to the posterior region of the eye, regressively in the opposite direction. Fig. 2A–H shows the response of an equatorial horizontal cell (EH 1, [6]) to an excitatory visual stimulus moving horizontally through the binocular receptive field. The cell was penetrated in the axon terminals (at) located in the protocerebrum proximal to the axonal arborisation (aa) (Fig. 1B). In Fig. 2A the response of the EH-cell to regressive movements presented to the contralateral eye is shown: the neurone responds with a short series of spikes which are not accompanied by a dc-shift of the membrane potential. In contrast, progressive movement presented to the ipsilateral eye does not elicit any responses at all in this physiological condition of the cell (Fig. 2B). It should be remembered, however, that the recording site is in the terminal axon ending and thus, the response measured in this part of the cell does not necessarily reflect the cell's response to ipsilateral progressive motion in other parts of the cell. Occasionally, noise-like potentials could be observed occurring spontaneously (Fig. 2C). These "noise potentials" were accompanied by dc-membrane potential shifts characteristic for the final potential behaviour (compare to Fig. 2G, H). Subsequently, the membrane returned to its original value (−64 mV) and the cell responded in a similar fashion as prior to these occurrences (Fig. 2D). However, a relatively short time after impalement of the cell, the response behaviour changed drastically within a short time: two minutes after penetration we find that now the stimulus-induced action potentials are accompanied

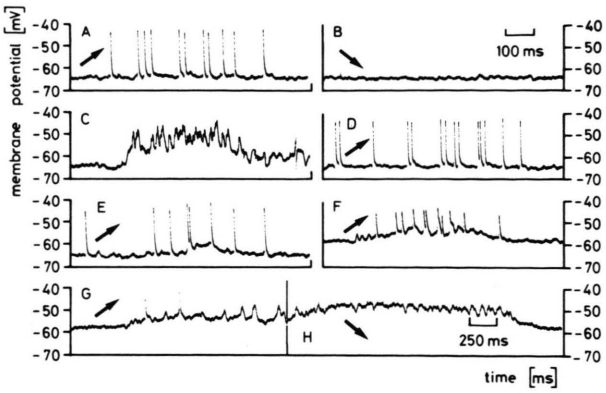

Fig. 2. Intracellularly measured response of an EH cell elicited by regressive motion (↗) presented to the contralateral eye (*A*, *D*–*G*). In *B* and *H*, regressive stimulation of the contralateral eye was followed by progressive movement (↘) presented to the ipsilateral eye. Time marker for *A*–*F* is shown in *B* (100 ms) and that for *G*, *H* is shown in *H* (250 ms), respectively. The recording was obtained from the terminal axon part (*at* of Fig. 1 B). *A*, *B*: stimulus-induced response immediately after impalement. *C*: noise-like potentials occurring spontaneously without stimulation. Recording obtained 2 min after penetration. *D*: Response as in *A* but 2 min after impalement. *E*: stimulus-induced action potentials are now accompanied by a small depolarization (3 min after penetration). *F*: stimulus-induced action potentials of reduced amplitude are accompanied by a small depolarization. Note that prior to stimulation the resting potential is already shifted by 6 mV towards depolarized levels (−64 mV to −58 mV; 4 min after impalement). *G*: the resting potential is shifted by 6 mV in the depolarizing direction. Contralateral regressive motion now causes an *additional* depolarization. Note the superimposed "local action potentials" (Hausen [2]) (6 min after impalement). *H*: ipsilateral stimulation by progressive movement also causes an additional depolarization (6 min after penetration). The graded potential behaviour shown in *G*, *H* represents the "final" potential behaviour which does not change any more. Details see text. *Phaenicia* ♀.

by a small dc-membrane potential shift (Fig. 2 E) which after three minutes becomes more prominent (Fig. 2 F). In addition, we notice for the latter recording that the resting potential (*i.e.* without stimulation) has changed with respect to that measured immediately after penetration of the cell: at this time, a depolarizing shift of 6 mV can be noticed which is permanent. Upon stimulation, an additional depolarization with superimposed action potentials is observed. These action potentials, however, are reduced in amplitude (compare with Fig. 2 A). In Fig. 2 G and 2 H, the final potential behaviour which does not change any more is depicted: stimulation induces almost exclusively graded potentials of "noisy" appearance upon *ipsi- as well as contralateral stimulation.* Thus, if we compare Fig. 2 A and 2 G, and Fig. 2 B and 2 H, we find two major changes in the cell's response upon stimulation: (1) the stimulus-induced response changes from action potentials to graded potentials; only two "spike-like" potentials of strongly reduced amplitude can be recognized. These "local action potentials" were already described by Hausen [2]. (2) At first, the cell responds *only* to contralateral stimulation, *i.e.* at first, the cell possesses a *monocular* receptive field and then, in the physiological condition depicted in Fig. 2 G and 2 H, it possesses a *binocular* receptive field. Similar potential changes were observed in recordings from the H-cells in the brain of Calliphora (Hengstenberg, pers. communication).

Therefore, the signal transmission by graded potentials reported previously [1–3] may have to be modified for the horizontal cells, and, most probably, for the vertical cells, too. An analysis of the "noise-like" potentials of these cells had already led to the conclusion that "the non-spiking state may, therefore, not represent the natural conditions .... in these neurons" and that, in the physiological condition depicted in Fig. 2 G and 2 H, the cell is already in a permanent "refractory state" [3].

A simple explanation for the change in the observed potential behaviour is based on the physiological changes we have to expect due to the experimental procedure: following the opening of the head capsule, the hemolymph which is pumped into the head by abdominal movements cannot compress the large air sacs and tracheal tubes as in the intact animal; thus, these means of passive expiration are hindered or may even be blocked, and we have to expect hypoxic or even anoxic conditions for neurones such as, *e.g.* the horizontal cells, which do not lie at the surface of the ganglion. The vertical cells may be less affected, since they are located at the caudal surface of the lobula plate and thus, oxygen diffusion into the tissue may partially compensate for the reduced oxygen transport via the tracheal system. The oxygen supplied to the brain tissue is, moreover, mainly used for the synthesis of ATP ([9], Rivera, pers. communication) which in turn provides the "fuel" for the Na^+/K^+-pump. Consequently, the lack of oxygen will reduce the activity of the ion pump or even block it, causing a reduction of the Na^+- and K^+-gradients across the membrane, because the ion gradients are not restored to their original levels.

Thus, we could expect the following alterations as a consequence of the changed ion gradients: (1) the resting potential should be shifted towards depolarizing values due to the diminished K^+-gradient and (2) a reduction of the spike amplitude should be noticeable, caused by the reduced Na^+- and K^+-gradients across the membrane. Fig. 2F supplies experimental evidence for these expectations. Therefore, the changes in potential behaviour which have been confirmed by altogether four recordings support our interpretation that the graded potentials reported previously [1–3, 5, 6] can be explained by a depletion of the oxygen supplied to these neurones. We do not infer this to be the only possible explanation but rather the most likely one. We do not think it likely that the observed potential changes from action to graded potentials could be explained by "injury potentials" due to penetration of the cell. Such injury potentials are observed frequently in this neuropile region while searching for motion sensitive cells; however, they are characterized by a sharp increase in the frequency of spikes of a penetrated cell, followed by a gradual – sometimes rapid – decrease in spike frequency and finally, the neurone stops firing. Such a behaviour was never observed in the recordings from horizontal or vertical cells.

Additional experiments were performed trying to restore the presumed original activity of these cells: intracellular injection of energy providing compounds (10 mM glucose in 150 mM KCl) induced a hyperpolarization which, according to the interpretation given above, may be looked upon as a "repolarization" of the membrane. However, this effect may have been due to the injected K^+-ions rather than to the injected glucose. In addition, fast potential changes were observed that probably indicated partially restored mechanisms of spike generation. Additional experiments in which the brain tissue was perfused with oxygen-enriched Ringer solution did not induce any changes, as had been found in *Calliphora* (Hengstenberg, pers. communication). That the membranes of the vertical and the horizontal cells are, in principle, electrically excitable has already been demonstrated by means of hyperpolarizing the membrane artificially: by injecting small hyperpolarizing currents of 1–5 nA action potentials were induced in both cell types [3, 5].

The experiments described above make it likely that the graded potential behaviour of the horizontal cells does not reflect the potential behaviour under *in vivo* conditions: in the intact animal, these cells appear to be capable of spike generation in the terminal axon endings (in the ventrolateral protocerebrum); this capability seems to be gradually diminished because of experimental procedures. It cannot be decided, yet, whether only the terminal axon part in the protocerebrum is capable of spiking or whether the main axon, residing at the frontal surface of the lobula plate, is also generating spikes under natural conditions. If the latter is also generating action potentials the question arises why these cannot be measured in the terminal axon endings (compare Fig. 2B). We would have to postulate a very effective block preventing the spread of such action potentials into the terminal axon endings. This does not appear very likely, even if it may be possible, since it requires, in addition, a unidirectional block: spikes generated in the terminal axon part can still be recorded in the main axon in the lobula plate [1, 4, 6]. Therefore, the more likely alternative is that under *in vivo* conditions the main axon conducts graded potentials.

Taking this likely possibility one step further it implies

(1) the horizontal cells have two *functionally different output regions:* action potentials are propagated into the terminal axon endings (*at* of Fig. 1B); whereas graded potentials spread into the axonal arborisation (*aa* of Fig. 1B). Ultrastructural investigations have already shown that both cell regions possess presynaptic structures [6, 7, 10].

(2) The terminal endings of the H-cells provide the contralateral output of the H-cells; whereas the axonal arborisation provides a mixed ipsi-/contralateral output: superimposed onto the graded signals elicited by stimulation within the ipsilateral receptive field are the signals induced by a heterolateral, spike conducting element which appears to make contact with the horizontal cells at the terminal axon endings as well as the axonal arborisation. Thus, the output signals in the axonal arborisation should be derived from an interaction of both, ipsi- as well as contralateral signals. This consequence is born out of an inference reported by Hausen *et al.* [10] that the contralateral input to the H-cells is provided by a spike-generating element (termed H2-cell), whose telodendritic endings envelope tightly the terminal axon endings as well as the axonal arborisation of the horizontal cells (Hengstenberg, quoted after Hausen

[2]): thus, the H2-cell appears to make contact at both these cell sites according with ultrastructural investigations demonstrating *pre- as well as post-synaptic* structures at these sites (*aa, at*) [6, 7, 10]. Consequently, the terminal axon endings provide a *monocular (contralateral) output* and the axonal arborisation a *binocular output* computed from ipsi- as well as contralateral contributions.

Acknowledgements

This research was supported by grant Ec 56/1b and a Heisenberg stipend (Ec 56/3) by the German Research Foundation (DFG) as well as funds from the SFB 114 (Bionach). We wish to express our gratitude to Prof. W. Broughton who kindly corrected the English style, and to him and Dr. J. Ostwald for critical reading of the manuscript.

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