Spectral Sensitivity of Monopolar Cells in the Bee Lamina

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Summary. 1. The intracellularly recorded response of the lamina monopolar cells (MC’s) to retinal illumination is a triphasic hyperpolarisation (Fig. 1). Recordings stable enough to allow measurement of a whole series of intensity and spectral runs were obtained from 3 cells.

2. The gain between retinal input and MC output is calculated by comparison of normalised response/log intensity functions, and is found to be about 7 over most of the MC's dynamic range (Fig. 2, 3).

3. The spectral sensitivity of MC’s has its maximum at 482 nm and a broad shoulder around 550 nm. Sensitivity is low (20%) in the UV. However, in some MC’s there is an increase in sensitivity to the shortest wavelengths tested (316 nm). One MC (No. 7) responded to UV wavelengths at only high intensities and then with a depolarising potential.

4. The interpretation of the MC’s spectral sensitivity function is that blue and green receptors supply excitatory inputs to the MC with about equal weighting. UV receptors may supply inhibitory inputs with less weight than those from the blue and green receptors.

5. These spectral sensitivity functions of dark adapted MC’s are calculated for an intensity range which is near the threshold of the receptors. For an intensity range which lies around 50% response of the receptors the spectral sensitivity function of the MC’s is flat between 300 and 600 nm (if there is no or only small inhibition by the UV receptors) or between 400 and 600 (if the UV inhibition increases strongly with intensity). It is discussed that the spectral response in light adapted MC’s may be very different from those of dark adapted MC’s.

Introduction

In insects with fused rhabdons all the retinula axons of one ommatidium project to the same synaptic region (the cartridge) in the first optic ganglion (the lamina), where they synapse with the first interneurons (the lamina monopolar cells, MC) (Cajal and Sánchez, 1915; Straußfeld, 1970; Varela, 1970; Meinertzhagen, person. commun.). In the bee two or three of the nine retinula axons travel through the lamina down to the second optic ganglion, the medulla, but these throughrunning fibres have spines in the proximal layer of the lamina cartridge (Straußfeld, 1970) and one can assume that they too make synaptic contact on to the MC’s and an unknown number of small cells in the lamina.

During the last three years it has become possible to record intercellularly from MC’s (Locusta: Shaw, 1968; Calliphora: Autrum, Zettler and Jürgen, 1970; Zettler and Jürgen, 1971, 1972; Jürgen and Zettler, 1971; dragonflies: Laughlin, 1973, 1974; Phoenicia: Arnett, 1972), thus making possible the study of the first steps of information processing in the insect visual system. It was found that MC respond to retinal illumination with a triphasic hyperpolarisation, with an initial hyperpolarising "on"-transient, a sustained but slowly
falling plateau hyperpolarisation and a depolarising "off"-transient. MC responses are characterised as follows: (1) They have more low frequency noise (thought to be synaptic noise) than with retinula cells. (2) Dark adapted MC's have a response/log intensity function (referred to in future as the intensity function), which is steeper and elicited by lower intensities than the intensity function of retinula cells. The gain between retinula input and MC output is in the range of 5–15. (3) MC may receive inhibitory inputs from neighbouring ommatidia, because their visual field is narrower than that of the retinula cells, and one can record depolarising potentials with point light sources off axis (Review: Laughlin, 1974).

It is not known how the MC integrate the colour coded information from the receptors and what role the MC play in the insect's colour coding system. The bee's colour vision, which has been intensively studied at different levels since von Frisch's famous colour training experiments, is a trichromatic colour vision (Daumer, 1956) enabling it to distinguish very precisely between different wavelengths in the violet and blue/green (Menzel, 1967; von Helversen, 1972). Three colour receptor types (UV, blue and green receptors) have been demonstrated by single cell recording (Antrum and v. Zech, 1964; Snyder, Menzel and Laughlin, 1973), and each ommatidium most probably contains 4 green receptors symmetrically arranged, 2 UV, 2 blue receptors and a short proximal UV-cell (Gribakin, 1969, 1972; Menzel and Snyder, 1974).

A first step in analysing colour integration in the visual ganglia should be an examination of the first interneurones. Here I report a preliminary analysis of the spectral properties of the MC's to see if and how they are involved in colour coding.

Method

Young honey bee workers (Apis mellifera linguets) were taken from the hive and mounted on a ball joint in such a way that a glass microelectrode could be inserted vertically into the left lamina through a hole in the frontal head capsule. Electrodes were glass pipettes filled with 2.5 M KCl (180–300 M2). Conventional recording and data storage methods were used. The stimulus was a point light source which subtended 45° at the eye and which could be moved around the eye with a perimeter device. When an MC was found the light source was very carefully positioned at the centre of the receptive field of the receptor input. Monochromatic light was produced by a 900 W Xenolamp, and a grating monochromator (Schoeffel GM 290). Broadband stray light filters reduced the off-peak transmittance of the monochromator. All light measurements and calculation of spectral sensitivity were made as described in detail in Menzel (1974).

Results

Intensity Dependence and Gain

In the lamina one can record positive going (depolarising) potentials to retinal illumination, either intracellularly from retinula axons or extracellularly from within the cartridge. However, one can also record hyperpolarising, graded potentials (Fig. 1). The potentials always appear after a negative jump in the DC potential level of 5 to 15 mV and are difficult to keep as stable potentials for longer than several minutes.

Of many recordings only 9 were stable enough to measure intensity dependence for at least one wavelength or white light (Xenon light) and spectral dependence for 22 wavelengths between 316 and 666 nm. In contrast to retinula cells, marking experiments with Precion Yellow have not yet been successful. However, it is concluded that these are intracellular recordings from MC's for the following reasons: (1) The resting potential contains much more low frequency noise than shown on intracellular recordings from retinula axons (Fig. 1). The amplitude of noise varies in different recordings, but a low amplitude always
indicates a poor recording. (2) With increasing retinal illumination the graded hyperpolarising potential increases. At very low intensities the potential is monophasic, then it becomes triphasic with a rapid "on"-transient, a sustained plateau and a small "off"-peak. In some cells the amplitude of the "on"-peak can reach 20 mV, but mostly it was 8–12 mV. The plateau potential is maximally 10 mV. (3) Very similar potentials are picked up when the electrode is in the first chiasma between lamina and medulla.

The comparison with the potentials recorded from large monopolar cells (LMCs) in flies and dragonflies (see Introduction) shows that these hyperpolarising potentials are those of MC possibly of LMCs. The results reported here come from 9 MCs which were recorded in the cartridge region of the lamina and had a stable resting potential for at least 15 min (1 cell 35 min).

The response/log intensity function (intensity function) is shown in Fig. 2 with the averaged and graphically smoothed intensity function of 9 retinula cells. The retina cells were recorded in the retina of the same animal shortly after a successful MC recording had been made. All 9 retinula cells are green receptors.

For comparison of the MCs both with each other and with the retinula cells the responses are normalised in Fig. 2 as follows: Firstly all responses are expressed in percent of the maximal response amplitude. As some MCs were tested with monochromatic light a comparison of absolute sensitivity is not possible. Therefore the intensity functions are moved parallel to the abscissa till the 50% response value of each function lies at the relative intensity value of $10^{-4}$. This value is chosen as it approximates the 50% response of MCs tested with 540 nm (M 1.3 and 9 see below). In quantal content the relative intensity value of $10^{-4}$ is equivalent to $2 \cdot 10^6$ h·cm⁻²·sec at 549 nm.

The average intensity function of the retinula cells is obtained by moving each intensity function by the same amount as the MC function obtained a short time before in the same animal. The standard deviation of the retinula cells 50% value is given in Fig. 2.

The MCs have a steeper intensity function than the retinula cells. Contrast efficiency (percentage increase of the normalised intensity function per 1 log intensity step) is 60–70% in MCs and 35% in retinula cells. The MC's potential rises from 0 to 100% within 1.5 log intensity, that of the retinula cells within about 3 log intensity steps. The MC's 50% response value is reached at 1.5 log intensity lower than retinula cells' 50% response value. Fig. 2 also shows that the 50% response value of the MC is produced when the retinula cells give only about 8% of their maximal response. Therefore the retinula cells' signal must be amplified in the MC.

For an estimation of the gain between retinula cells and MCs the normalised intensity functions (Fig. 2) can be used. This gain is defined as $Gr = \%_{R_{MC}}/\%_{R_{Ret}}$, where $\%_{R_{MC}}$ and $\%_{R_{Ret}}$ are the normalised response values of MC or retinula cell. $Gr$ increases rapidly to 7.0 with increasing intensity and decreases again above $10^{-4}$ relative intensity. As the normalised response values are the basis for the calculation of the gain a more accurate determination of the voltage input-output relationship between retinula cells and MC is not yet possible.

**Spectral Sensitivity**

An assumption for the calculation of spectral sensitivity from one intensity function and one spectral run through the 22 test wavelengths is the parallelity of the intensity functions for all wavelengths. With 2 MCs (M5 and M9) it was possible to measure the intensity function at different wavelengths. Some other MCs which did not last long enough for an additional spectral run were tested for this intensity dependence with different wavelengths. In all cases the intensity functions were parallel, including the wavelengths 540 nm, 596 nm. There is only one exception, M7, which will be discussed later. Either the on-transient or the plateau potential can be used for the calculation of spectral sensitivity. As the on-transient is bigger and less dependent on the low frequency noise, the on-transient was used for the majority of measurements.

All 9 MCs are maximally sensitive to 484 nm or 500 nm (Figs. 3–5). In contrast to the spectral sensitivity of the receptors the major sensitivity maximum is very narrow: the half band width is about 35 nm (compare with the average function Fig. 5), that of the green receptors is 120 nm, of the blue receptors 80 nm and of the UV receptors 40–60 nm. Further more all spectral sensitivity functions of the MCs have a secondary peak or shoulder around 550 nm and there is sometimes a peak or shoulder in the blue region (Fig. 4a, b). However, the greatest differences are found in the UV region. In three cells (M4, 6, 8; Fig. 4a) sensitivity to short wavelengths increases below a minimum around 360 nm. In all these MCs the spectral run was started with the UV wavelengths. It cannot be excluded that the test flashes produced an increasing UV adaptation, despite a 10 sec interval between the flashes and a resting potential, which came back to the dark adapted state within a fraction of a second. If there is adaptation the first test flashes would reduce sensitivity and the sensitivity function would give higher values for the shortest wavelengths. In all other MCs (with the exception of M7, see below) spectral sensitivity between 300 and 400 nm is around 20%. A low side band at 349 nm was found only once (M9, Fig. 4b).
steps higher than at 500 nm) small depolarising potentials were found. This result could be interpreted as an indication for an inhibitory input from the UV-receptors.

With the exception of M7 all other MC's can be regarded as belonging to one group with respect to their spectral sensitivity. An average spectral sensitivity function is given in Fig. 5. The scatter of the data is not too great if one keeps in mind that the low frequency noise in MC's is considerable, that only one spectral run was carried out with each MC, and that MC's have very steep intensity functions. One of the MC's (M5, Fig. 3) has a spectral sensitivity function which is nearly identical with the average function.

As discussed above the responses of some MC's to retinal illumination decrease at high intensities (Fig. 1, 2). It is possible that the falling intensity function is caused by inhibitory inputs and these could be colour specific. To test this hypothesis spectral runs in 2 MC’s (M1 and M9) were also carried out at high intensities on the falling part of the intensity function (e.g. M9 in Fig. 4b). The spectral sensitivity functions of both cells measured by the rising or the falling part of the intensity function are generally similar. This result indicates that the falling part of the intensity function is dominated by the same colour receptor inputs as the rising parts. Therefore colour specific inhibitory inputs can be excluded as the mechanisms for the decreasing intensity function of the MC’s at high intensities.

Discussion

The recordings presented here are in agreement with what we already know of the function of the monopolar cells in the first optic ganglion, the lamina. The intracellularly recorded potential is a graded hyperpolarisation as was found in Locusta (Shaw, 1968), Calliphora (Autrum, Zettler and Järvielto, 1970; Zettler and Järvielto, 1971), Phaenicia (Arnett, 1972) and the dragonfly Hemicordulia (Laughlin, 1973). No spikes were recorded. Synaptic transmission between retinula axons and MC produces an amplification of the signal in the dark adapted state as in the large monopolar cells of flies and dragonflies (see discussion in Laughlin, 1973, 1974). The gain was found to be 7 times in the rising parts of the MC intensity function. This value is very similar to that found in Calliphora (8 times; Zettler and Järvielto, 1971, 1972) and about half as high as in dragonflies (12–14 times; Laughlin, 1973). These values for the gain in MC’s, however, are not directly comparable, because different methods were used for stimulation and calculation.

In some of the MC’s the intensity function was found to decline at high intensities. Such falling intensity functions were also measured in Calliphora (Zettler and Järvielto, 1971) and were indicated in dragonflies (Laughlin, 1973). It is not known what causes the decrease, but it seems more likely to be the result of physiological processes rather than a recording artefact.

As the membrane resistance in MC decreases to retinal illumination (Laughlin, in press) extracellular positive potentials may invade the MC. The source of the positive extracellular potential is retinal and therefore increases with increasing intensity, whereas the MC potential reaches saturation very early. Thus the invading extracellular potential progressively dominates the MC potential, and the intensity function decreases. A second factor could be
lateral inhibition. As the point source intensity increases the surround stimulation also increases, while the response to the axial stimulation cannot rise above saturation level. Therefore further input from the surround will cause a decline of the recorded response.

The spectral sensitivity of the tested MC's indicates that they receive inputs from more than one colour receptor type. The position of the maximum (483 nm, some MC's 500 nm) and the shoulder or lower peak at 549 nm suggest that the green and blue receptors are positive inputs. The peak value for green receptors show a broad scatter between 500 and 550 nm (Autrum and v. Zwehl, 1964; Snyder, Menzel and Laughlin, 1973) with most of the green receptors having their maximum at 525 nm and a shoulder or lower peak at about 545 nm or alternatively a maximum at 545 nm and a shoulder at 525 nm. The first group (λ_max = 525 nm) is somewhat more frequent. The position of the spectral maximum of the MC's at shorter wavelengths than that of the green receptors must be caused by a receptor type with a maximum at lower wavelengths but not too far from that of the green receptors, i.e. the blue receptor. Blue receptors have a maximum at about 440 nm or 460 nm, those with λ_max = 460 nm are most frequently recorded. As the spectral sensitivity of the MC's in the UV region is only about 25% and displays a small minimum at around 360 nm (Fig. 5) it is very unlikely that the UV receptors supply positive inputs to this type of MC.

If our assumptions are correct it should be possible to reconstruct the MC's spectral sensitivity function from the receptor's spectral functions (Fig. 6). The spectral sensitivity functions of the different colour receptors contain sidebands which are thought to be caused by the partial electrical coupling between the retinal cells of each ommatidium (Snyder, Menzel and Laughlin, 1973; Menzel and Snyder, 1974; see Fig. 6a). The height of these sidebands and the exact position of the maximum differ considerably in different retinal cells of the same colour receptor types. To simplify our modelling only the average functions of a large number of receptor recordings were used here. Thus the blue receptors with λ_max = 440 nm and the green receptors with λ_max = 545 nm are not considered.

The position of the spectral maximum of the MC and the shoulder in the green region is reconstructed best if one assumes an input from blue and green receptors with equal weight.

There is only qualitative agreement between the model in Fig. 6b and the average spectral sensitivity function in Fig. 5. The shoulder in the green region has the right position but is much higher than that recorded. The difference in the UV region is more important, however. Even without input from UV receptors the electrical coupling between the receptors causes a low UV peak in the model (Fig. 6b). Such a UV peak was not found. Assuming the UV receptors make inhibitory input, e.g., with half the weight of that of the blue and green receptors, the secondary peak in UV disappears. The result is a spectral function which is in good agreement with that of the MC M7 (Fig. 3). Therefore a spectral function comparable to the average function in Fig. 5 can be constructed assuming an inhibitory input from the UV receptors with less weight.

All recordings were carried out with dark adapted animals, and short test flashes were separated by long intervals. Therefore our conclusions on the MC's function in the intensity and chromaticity coding system are restricted to the dark adapted state. The spectral sensitivity of the MC is calculated for a 50% response of the MC. Such a response is elicited by a receptor response of only 8% (see Fig. 2). Test flashes with an intensity which causes 50% depolarisation of the retinal cells would saturate the dark adapted MC and therefore would produce a flat spectral sensitivity function. This means that over most of the dynamic range of the retinal cell the MC's show a flat spectral sensitivity response, which is directly attributable to the high gain between dark adapted retinal cells and MC's. A peak in the bluish green region can be demonstrated in the dark adapted state only at intensities close to the retinal cells' threshold. As the MC's shift their intensity function with adaptation (Laughlin, pers. comm.) the contrast detection and spectral sensitivity of MC's may be very different in the light adapted state.

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