J Comp Physiol A (1991) 168:631–637

Journal of Comparative Physiology A Sensory, Neural, and Behavioral Physiology

Fast temporal adaptation of on-off units in the first optic chiasm of the blowfly

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Accepted April 3, 1991

Summary. 1. We recorded from spiking units in the first optic chiasm between lamina and medulla in the brain of the blowfly *(Calliphora vicina)*. Both previously characterized neuron types, on-off units and sustaining units, were encountered. On-off units had a temporal frequency response with a lower cut-off frequency than blowfly photoreceptors. This low cut-off frequency is related to a fast temporal adaptation of the on-off units to trains of short light pulses. Temporal adaptation occurred independently for short on- and off-pulses.

2. On-off units only responded to stimuli of relatively large contrast. Contrasts of less than 10% gave little or no response.

Key words: On-off units – Blowfly – Lamina – Temporal adaptation – Signal rectification

Introduction

About 20 years ago Arnett (1971, 1972) and McCann and Arnett (1972) reported on two spiking units in the chiasm between lamina and medulla in the fly brain. One of these neurons, the on-off unit, produces a transient response when a light is switched on or off in its receptive field. The unit is essentially silent when the stimulus intensity (or position) is not changed. The other neuron, the sustaining unit, is silent in the dark and produces a steady spike train to a steady light in the centre of its receptive field. Its spike rate increases with light intensity, and it has two inhibitory regions flanking the central, excitatory region. On the basis of lesion experiments (Arnett 1972), both on-off and sustaining units are believed to carry spikes from lamina to medulla (centripetally). The on-off and sustaining units might correspond to lamina neurons L5 and L4, respectively (see Shaw 1981, and Laughlin 1981, 1984 for discussions).

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As the knowledge of other cells in the fly retina and lamina has greatly increased in the past 20 years (reviews: Laughlin 1981, 1989; Hardie 1985; Shaw 1989), we decided that another look at these spiking units was long overdue. Moreover, research in higher neural processes, such as movement detection, has also made much progress (reviews: Hausen and Egelhaaf 1989; Franceschini et al. 1989). For assessing the possible role of peripheral neurons in these central processes (see e.g. Srinivasan and Dvorak 1980; Coombe et al. 1989) a more detailed knowledge of the units reported by Arnett is valuable as well.

We were able to extend the results already reported on these units (Arnett 1971, 1972; McCann and Arnett 1972). In this article we will confine ourselves mainly to the temporal properties of on-off units, which we will show to have unusual characteristics that to our knowledge have not been reported before for lamina neurons, but have been reported for medulla neurons in the locust (Osorio 1987, 1991).

Materials and methods

Animals and preparation. For all experiments we used the blowfly *Calliphora vicina*, also called *Calliphora erythrocephala*, taken from a laboratory culture (F1) raised on a vitamin A rich diet. Female flies, 2–4 weeks old, were immobilized with wax and mounted in the setup. We removed a small piece of chitin at the posterior surface of the head, opening a dorsal air sac. Through this hole we could see the first chiasm lying between the retina/lamina on the one side, and the medulla/lobula complex on the other side.

Electrophysiology. We made extracellular single unit recordings using tungsten microelectrodes (A–M Systems, type 5760, impedance at 1 kHz about 12 MΩ), taking great care to place the electrodes in the chiasm, and not in the lamina or medulla. Spiking units were encountered throughout the chiasm. The reference electrode, a silver wire, was gently pressed either against the ventrolateral part of the chiasm, or against the lateral edge of the hole cut in the chitin. The signal was amplified (amplifier type DAM–50, World Precision Instruments), and was both low-pass and high-pass filtered at 300 Hz and 3 kHz, respectively. Noise amplitude was

typically 10 μV peak to peak, spike amplitude in different recordings between approximately 30 and 100 μV. We recorded from a total of 13 on-off units, and 4 sustaining units. Recordings were generally very stable, and could be continued regularly for more than 24 h.

Stimulus generation and data acquisition. The ipsilateral (right) eye of the fly was illuminated with a wide-field stimulus, consisting of an LED (Siemens LD57C, peak wavelength 560 nm, half width 25 nm). The stimulus had an extent of about 45°, and its intensity I produced in the photoreceptors a plateau depolarization of about 10 mV. This LED was driven by a voltage-current converter connected to a DAC (CED1401, Cambridge Electronic Design). An ADC of the same CED1401 recorded the amplified spike signals, sampling at 5 kHz. From this sampled signal spikes were discriminated on line by an IBM PC/XT-compatible computer, connected to the CED1401 and running ASYST (Macmillan Software). Also on line a post stimulus time histogram with a bin-width of 1 ms was compiled from typically 100 stimulus presentations, and stored on disk. Time between successive stimulus presentations was typically 5 s. Further analysis of the data was performed off line. All data presented in the figures below were obtained with this setup. Additional measurements with narrow-field stimuli (LED) and moving edges and gratings on a CRT were performed in another setup (described in van Hateren 1986, 1990).

Results

On the basis of their response, two neuron types were distinguished. Figure 1A shows the response of one unit to a 500 ms light pulse of intensity I on a constant intensity 0 (dark). This neuron type responds with a short spike train to both the onset and cessation of the flash, and the response decays to a zero spike rate typically within 500 ms. It does not respond to steady illumination, irrespectively of the intensity. The other neuron type (Fig. 1B) responds with an increase in spike rate to the onset of the flash, then rapidly adapts to a lower spike rate, and responds with a decrease in spike rate to the cessation of the flash. This unit keeps spiking during prolonged illumination. Neither of the two types of neuron spikes in darkness.

These two units appear to correspond to the units described by Arnett (1971, 1972): the on-off unit (Fig. 1A) and the sustaining unit (Fig. 1B). We could confirm many of the results obtained by Arnett (mainly in the fly *Phaenicia sericata*). As he reports, spikes from the on-off unit were in general larger than spikes from the sustaining unit. On a few occasions we recorded simultaneously from both units, with large spikes from the on-off unit and smaller ones from the sustaining unit. We also found that the on-off unit has a higher threshold than the sustaining unit. We could extend this result, obtained by Arnett (1972) on the dark-adapted eye, to the light-adapted eye. We found that on-off units give no or very little response to contrasts less than 10% (see also below), while we did not observe any clear contrast threshold for the sustaining units. As well as dark-adapted ones, light-adapted on-off units respond transiently to contrast steps of both polarities (Fig. 1C and D).

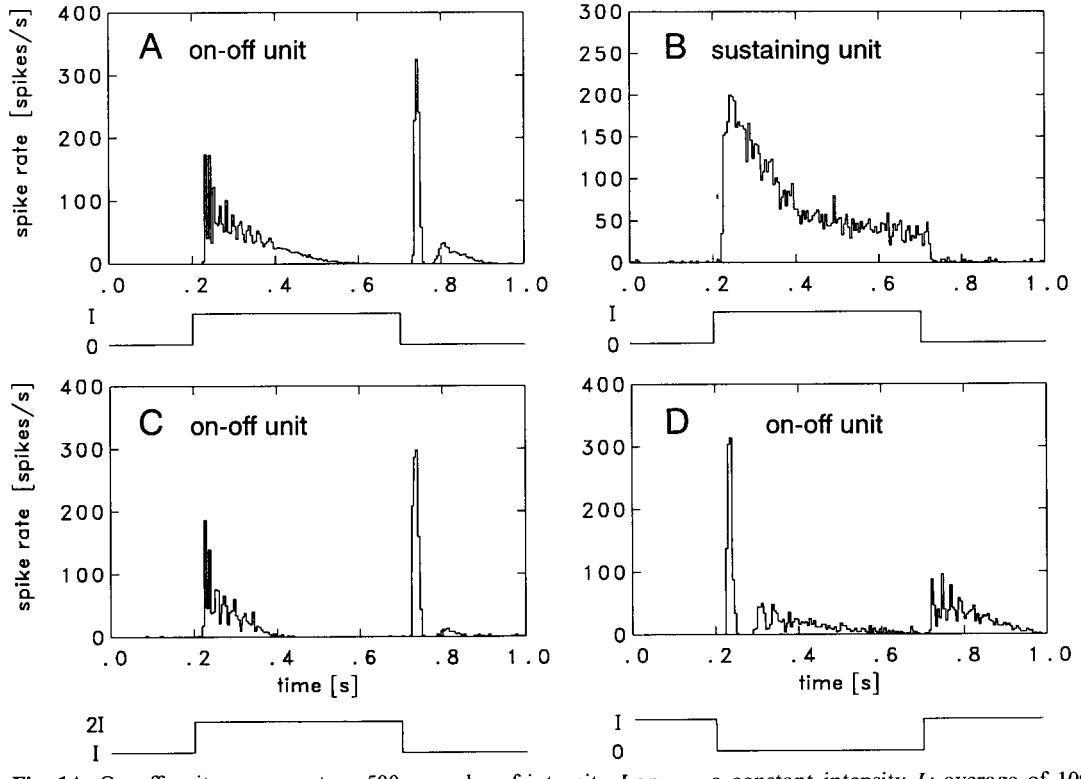

Fig. 1A. On-off unit; response to a 500 ms pulse of intensity I on a constant intensity 0; average of 1000 stimulus cycles, cycle repetition time 5 s, bin-width 5 ms. **B.** Sustaining unit; stimulus as in **A**; average of 100 cycles, repetition time 5 s, bin-width 5 ms. **C.** On-off unit, same as in **A**; response to a 500 ms pulse of intensity $2I$ during a constant intensity I; average of 100 cycles, repetition time 5 s, bin-width 5 ms. **D.** On-off unit, same as in **A**; response to a 500 ms pulse of intensity 0 during a constant intensity I; average of 100 cycles, repetition time 5 s, bin-width 5 ms. The traces beneath each figure denote the timing and intensity of the stimulus

Although we did not systematically investigate the spatial properties of the on-off units, experiments on two cells with moving edges and gratings indicate that the receptive field is elongated horizontally (see Arnett 1971, 1972). The vertical extent of the receptive field is close to that of a single photoreceptor (or neuro-ommatidium). As can be seen in Fig. 1A, C, and D, on-off units often show oscillations in their response (see also Fig. 2B). Similar oscillations can be observed elsewhere in the visual system (e.g. LMCs, H1), and may well be a property of the photoreceptor synapse (van Hateren 1987).

A novel and surprising property was encountered when we investigated the temporal behaviour of the on-off unit, and it is to this that most of this article is devoted. We investigated the frequency response of the on-off unit by exposing the unit to a stimulus with an intensity sinusoidally modulated in time, using frequencies between 1 and 50 Hz (100% modulation). Figure 2A presents the response of a unit to 1 Hz stimulation. As expected, the unit shows a highly nonlinear response: as it responds to both light on and light off, its response effectively doubles the frequency of the stimulus (see bottom trace on the left). This frequency doubling disappears at higher frequencies, in most units somewhere between 5 and 10 Hz. Figure 2B shows the response to 10 Hz stimulation. Figure 2C gives the spike rate (in spikes per second, mean of the total stimulus period) as a function of frequency for frequencies between 1 and 20 Hz, averaged over 6 units after normalization to the highest spike rate. Responses of all units became negligible when stimulated with frequencies of 50 Hz and higher (all gave less than 0.5 spikes/s to a 50 Hz sinusoid of 100% modulation). Figure 2D shows the delay of the first peak in the response relative to the positive peak of the sinusoidal stimulus, normalized to the stimulus period; in a linear system this would be equivalent to the phase of the response. At low frequencies the normalized delay is about -0.25 (i.e., the phase of the response leads the stimulus 90°), which is in agreement with the differentiating nature of the unit. The normalized delay at higher frequencies is consistent with a delay between stimulus and response of 20–30 ms.

The frequency response of these units clearly differs from that of the photoreceptors. At the applied intensity, a frequency of 50 Hz is not responded to by the on-off unit, but yields a quite distinct photoreceptor response of about 18% of the photoreceptor response at 1 Hz, and 25% of the photoreceptor response at 10 Hz (de Ruyter van Steveninck 1986, p.19). The frequency response of the on-off units thus appears low-pass filtered as compared with the frequency response of the photoreceptors. This conclusion is also valid if we define the response of the on-off unit in a different way, namely as the am-

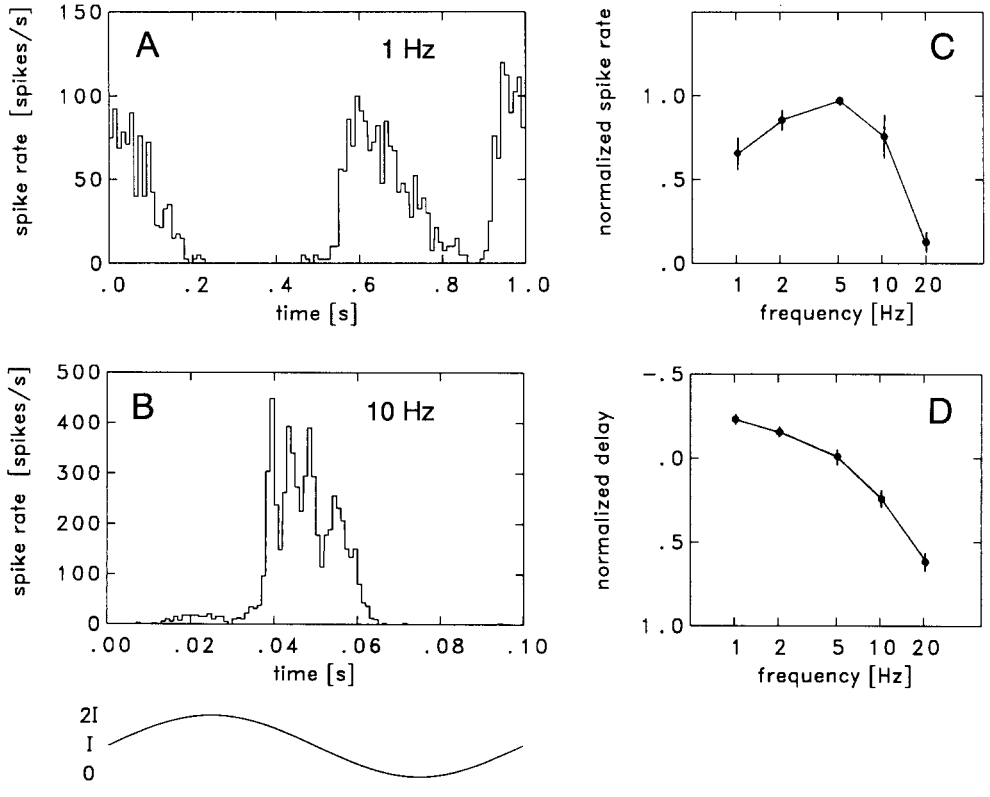

Fig. 2A–D. Response of on-off units to stimuli modulated sinusoidally in time. **A.** Response to a sinusoid (see bottom trace) of frequency 1 Hz and modulation 100%; note the frequency doubling; average of 100 contiguous cycles, bin-width 10 ms. **B.** Response to a 10 Hz sinusoid, modulation 100%; average of 1000 contiguous cycles, bin-width 1 ms. **C.** Response (normalized average spike rate) as a function of frequency, average of 6 on-off units. **D.** Delay (normalized to the period of the sinusoid) of the first peak of the response relative to the positive peak of the stimulus (see bottom trace to the left); average of the same on-off units as in **C.** A normalized delay of -0.25 would in a linear system correspond to a phase lead of 90°

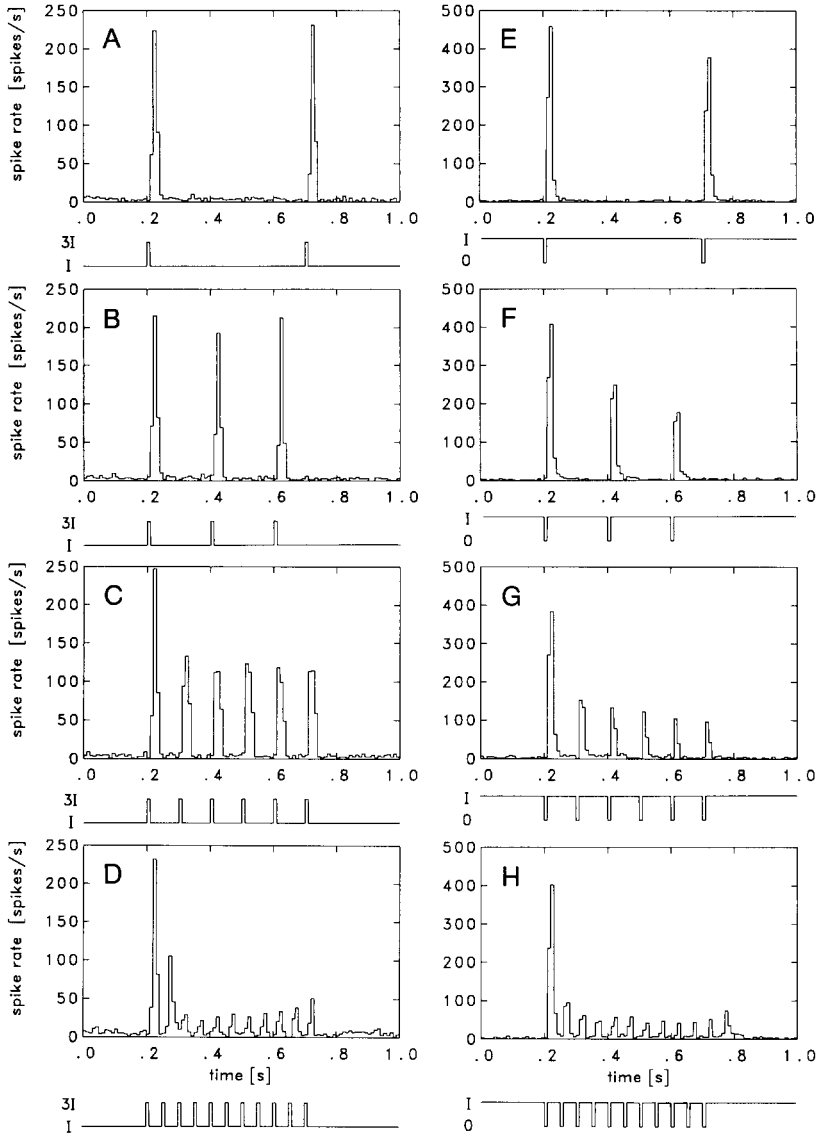

Fig. 3A–H. Responses of an on-off unit to trains of 10 ms pulses of intensity $3I$ (**A, B, C, D**) and 0 (**E, F, G, H**) during a constant intensity I. **A, E:** two pulses separated by 500 ms (i.e., 500 ms between the onset of each pulse). **B, F:** 3 pulses separated by 200 ms. **C, G:** 6 pulses separated by 100 ms. **D, H:** 11 pulses separated by 50 ms. The extra response peak at the end of the trace in **H** is often observed after the cessation of a fast series of on- or off-pulses. Each stimulus condition is the average of 100 cycles, repetition time 5 s, bin-width 10 ms

plitude of the first harmonic component in the response. This component obviously vanishes at 50 Hz, as there is no response at all.

An interesting property of the on-off units related to this apparent low-pass filtering was revealed by the following experiment. We stimulated the eye with short pulse trains with different intervals between successive pulses: 500, 200, 100, and 50 ms. Both on-pulses (10 ms intensity $3I$ during a constant intensity I) and off-pulses (10 ms intensity 0 during a constant intensity I) were used (Fig. 3). Whereas the response amplitude is about constant with intervals 500 and 200 ms (Fig. 3A and B), both at 100 ms (Fig. 3C) and 50 ms intervals (Fig. 3D) the response decreases from the second pulse onwards. This temporal adaptation is clearly related to the poor response of on-off units to continuous sinusoidal stimulation at higher frequencies: high temporal frequencies can be considered as a fast train of short light pulses. In most of the recorded units, the unit adapted to off-pulses already at longer intervals (from 200 ms downward; compare e.g. Fig. 3B and F). Most experiments with

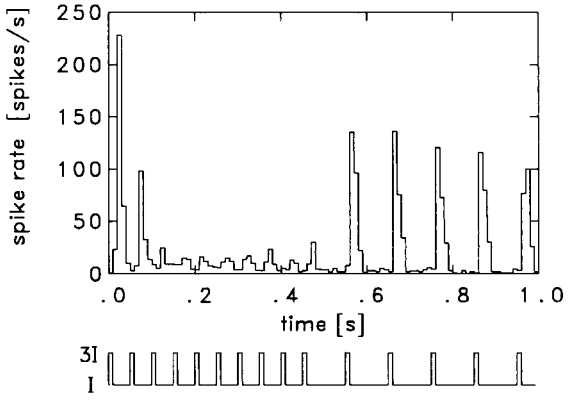

Fig. 4. Response of the same cell as in Fig. 3 to a train of 10 ms pulses of intensity $3I$ (during a constant intensity I) separated by 50 ms, immediately followed by a train of identical pulses separated by 100 ms. Average of 100 cycles, repetition time 5 s, bin-width 10 ms

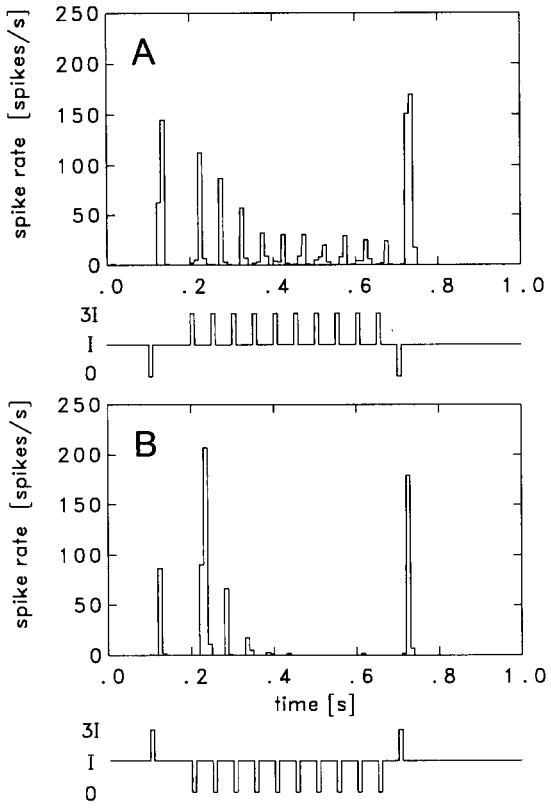

Fig. 5A, B. Interaction between pulses of opposite polarities. **A.** Two 10 ms off-pulses (intensity 0) preceding and succeeding a train of on-pulses (intensity $3I$, separation 50 ms) during a constant intensity I. Average of 100 cycles, repetition time 5 s, bin-width 10 ms. **B.** Two 10 ms on-pulses (intensity $3I$) preceding and succeeding a train of off-pulses (intensity 0, separation 50 ms) during a constant intensity I. Same cell as **A**, average of 100 cycles, repetition time 5 s, bin-width 10 ms

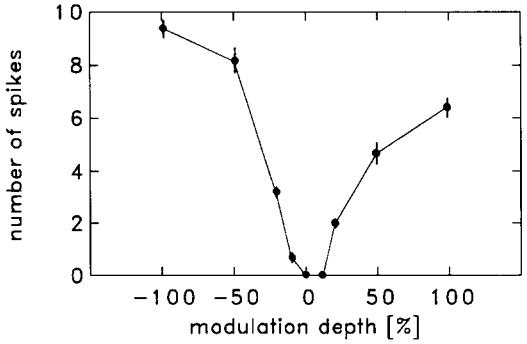

Fig. 6. Response of an on-off unit to steps of different intensities during a constant intensity I. The average number of spikes elicited per step is shown, not the spike rate. Modulation depths of −100%, −50%, −20%, −10%, 10%, 20%, 50%, 100% correspond to steps of intensities 0, $0.5I$, $0.8I$, $0.9I$, $1.1I$, $1.2I$, $1.5I$, $2I$, respectively. Each datapoint average of 300 cycles, repetition time 5 s, step width 500 ms

pulse trains were performed with a wide-field stimulus (on a total of 5 units), but we observed the same phenomenon in 3 on-off units where we presented narrow-field stimuli of a size less than the receptive field of a single neuro-ommatidium.

Two additional aspects of this temporal adaptation were investigated: recovery and on-off interdependence. In Fig. 4 the response is shown to a 50 ms pulse train of on-pulses, followed by the same pulses with 100 ms interval: the response is already recovered at the first flash (compare with Fig. 3C and D). Figure 5 presents the response to a stimulus paradigm adapted from Osorio (1991): a 50 ms pulse train of on-pulses preceded and succeeded by an off-pulse (A) and vice versa (B). Clearly, adaptation to on-pulses does not inhibit the response to off-pulses (the response to off is even increased), and vice versa.

Apart from the poor frequency response as compared with the photoreceptor cell, the on-off unit shows another striking difference: poor contrast sensitivity. Figure 6 gives the average number of spikes in response to a step from a constant intensity I to a range of intensities between 0 and $2I$. This unit hardly responds to contrasts of 10% and less. Similar low contrast sensitivities were obtained in 3 other on-off units using the LED as a wide-field stimulus, and in 2 more on-off units using moving edges of various contrasts.

Discussion

The two main results reported in this article are the following. Firstly, we found that the on-off units fail to follow temporal frequencies readily followed by the photoreceptors. This low temporal resolution is related to a fast temporal adaptation, occurring essentially independently for positive and negative contrasts. Secondly, we showed that these units have a rather low contrast sensitivity, with negligible response to contrasts smaller than about 10%. However, this will not prevent the neuron from responding when the animal moves around, as contrasts greater than 10% are quite common in natural scenes (average contrast 40%, see Laughlin 1983).

The low contrast sensitivity may partly explain the poor response of on-off units to continuous sinusoidal stimulation at higher frequencies. Although the photoreceptor transmits higher temporal frequencies, the effective transmitted contrast will decrease compared with lower temporal frequencies (due to temporal smearing, i.e. low-pass filtering). It is possible that this effect is enhanced by low-pass filtering of the photoreceptor response before the spike generation of the on-off units themselves. Low-pass filtering might be due to e.g. passive transmission through narrow dendrites, such as amacrines (see Shaw 1981), or to synaptic processes.

The low temporal resolution and low contrast sensitivity of the on-off units stand in marked contrast to the properties of another, parallel channel transmitting information from lamina to medulla, namely the Large Monopolar Cells (LMCs, see e.g. Laughlin and Hardie 1978; Laughlin 1981, 1989; van Hateren and Laughlin 1990). The LMCs have a temporal cut-off frequency even higher than that of the photoreceptors (Laughlin et al. 1987) due to a selective boost of high frequencies, and a high contrast sensitivity due to a high gain synapse (Laughlin et al. 1987). It seems that the on-off units

deliberately ignore much more of the information present in the photoreceptor signals than the LMCs do. By doing this, they are able to maintain very low spontaneous spike rates (often less than 1 min). This means that even a single spike, rather than one or a series of spike intervals, obtains significance. It can already transmit information single-handedly, namely that something has definitely changed in its receptive field. Thus the on-off unit could be a very fast channel (depending on the latency of the first spike), involved in behaviour requiring very short reaction times. It remains to be seen, however, whether it can really compete in this respect with a high quality graded potential channel, as the LMC's (see also Shaw 1981 for a discussion).

Yet another information channel between lamina and medulla are the sustaining units. Although we have recorded from only 4 of these cells, we consistently found them to have no clear contrast threshold. In two of these cells we measured the temporal frequency response, and found them to respond readily to 50 Hz stimulation, even at only 10% modulation of the sinusoid.

Neurons with a very similar behaviour to the on-off units described here were recently reported by Osorio (1987, 1991) in the locust medulla. These transient units also respond with fast adaptation to light flashes, independently to positive and negative contrasts. After adapting to a series of light flashes of a given contrast, these units still respond well to flashes of a higher contrast. Osorio argues that they will tend to respond to object boundaries, and not to the interior of objects, as they will quickly adapt to the interior's lower contrast when scanning a visual scene. A similar behaviour may be expected from the on-off units investigated in the present study: their low contrast sensitivity and low temporal resolution will lead them to respond mainly to boundaries between relatively large segments of a visual scene, e.g. those coinciding with object boundaries.

The neural circuitry shaping the response of the on-off unit is not known. If the unit corresponds to L5 (see discussions by Shaw 1981; Laughlin 1981, 1984), it is mainly driven by the amacrines (e.g. Shaw 1981), which are directly postsynaptic to the photoreceptors. If we furthermore assume that the sustaining unit corresponds to L4 (see also Shaw 1981; Laughlin 1981, 1984), also primarily driven by the amacrines, then we must conclude that the amacrines do not rectify the photoreceptor signal (i.e. full-wave rectification as in the on-off units). This is because otherwise the sustaining units would display full-wave rectification, which they do not. Therefore, the rectification observed in the on-off units has to originate from possibly two types of synaptic processes from amacrines to L5, one sign-conserving and one sign-reversing. Alternatively there could be two classes of amacrines. This splitting in separate on- and off-pathways (see also Öğmen and Gagné 1990a) would be consistent with the independent adaptation to on- and off-pulses (Fig. 5). All this remains highly speculative, however, until physiological and anatomical types are firmly linked. Unfortunately, both L4 and L5 seem to be too thin for conventional intracellular recording and staining.

The on-off and/or sustaining units have been implicated repeatedly in movement detection (McCann and Arnett 1972; Srinivasan and Dvorak 1980; Franceschini 1985; Coombe et al. 1989; Öğmen and Gagné 1990b). One of the arguments of Coombe et al. (1989) for rejecting the LMCs in the fly lamina as the sole precursors of the movement detecting system, is that their temporal cut-off frequency is higher than that of movement detecting neurons in the lobula plate, such as H1. Interestingly, the frequency response of the on-off unit (Fig. 2C) matches that of H1 more closely (e.g. Mastebroek et al. 1980; Coombe et al. 1989). On the other hand, the on-off unit rectifies the signal, thus its response does not allow discriminating positive and negative contrasts. This seems difficult to reconcile with conventional movement detection models, both of the correlator and gradient varieties. These models assume sign conservation at least until the nonlinear interaction producing the directional selectivity.

Acknowledgements. We thank D.G. Stavenga for valuable comments. This research was supported by the Netherlands Organization for Scientific Research (NWO), partly through the Foundation for Biophysics.

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