Graded Responses and Spiking Properties of Identified First-Order Visual Interneurons of the Fly Compound Eye

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SUMMARY AND CONCLUSIONS
1. We studied the graded and spiking properties of the "non-spike" first-order visual interneurons of the fly compound eye in situ with the use of intracellular recordings. Iontophoretical QX-314 injections, Lucifer yellow marking, and (discontinuous) current-clamp method together with transfer function analysis were used to characterize the neural signal processing mechanisms in these neurons.

2. A light-OFF spike was seen in one identified anatomic subtype (L1, n = 6) of the three first order visual interneurons (L1, L2, and L3, or LMCs) when recorded from synaptic region (i.e., in the 1 st visual ganglion, lamina ganglionaris) in dark-adapted conditions. Hyperpolarization of the membrane potential by current caused the identified L1 (n = 4), as well as L3 (n = 6), to produce an OFF spike, a number of action potentials, and some subthreshold depolarizations after the light-ON response. In L2 the OFF spike or action potentials could not be elicited.

3. To produce action potentials in L1 and L3, it was found to be necessary to hyperpolarize the cells ~35-45 mV (n = 43) below the resting potential (RP) in the synaptic zone. Recordings from the axons of these cells revealed that near the second neuropil (chiasma) the threshold of these spikes was near to (~10 mV below, n = 16) or even at the RP when an ON spike was also produced (n = 4).

4. The recorded spikes were up to 54 mV in amplitude, appeared with a maximum frequency of up to 120 impulses/s, and had a duration of ~8 ms. In L1 and L3 the spikes were elicited either after a light pulse (L3) or after a negative current step that was superimposed on a hyperpolarizing steady-state current (L2 and L3). A positive current step (similarly superimposed on a hyperpolarizing steady-state current) also triggered the spikes during the step.

5. Iontophoretic injection of a potent intracellularly effective blocker of voltage-gated sodium channels, QX-314, irreversibly eradicated the spikes and subthreshold depolarizations (n = 5). In addition, further injections elongated the light-ON responses and decreased or even abolished the light OFF response.

6. Negative prepulses followed by positive current steps were applied from the RP, to test the activation-inactivation properties of the channels responsible for the OFF spike. During this experiment, the increase of the negative prepulse (removal of the inactivation) increased the amplitude of the OFF spike from 7 to 21 mV, whereas the decrease of the positive test pulse (activation test) led to the increase of the spike from 5 to 51 mV.

7. These results indicate that voltage-gated sodium channels that are normally highly inactivated are responsible for the OFF-spoke generation in L1 and L3, and that probably a component of the light OFF response is mediated via the same conductance as well. The fact that L3 did not show any spiking properties suggests that this subtype is responsible of feeding visual information to a different functional subsystem than L1 or L3.

8. Frequency domain analysis suggested that the putative sodium channels in LMC axons considerably increase the gain of the signals at high frequencies and produce a resonance. Thus in these neurons the OFF spike is probably used to compensate the gain loss and to improve the signal-to-noise ratio (SNR) during the passive propagation through the long and thin axon. All three interneurons therefore regulate the gain in different manner and could be considered as parallel pathways with differently modulated responses.

INTRODUCTION
The well-known anatomy and physiology of the fly's retina and first synaptic region, Lamina ganglionaris, makes it an excellent model for studying the neural signal processing in visual systems (Meinertzhagen and Fröhlich 1983; Shaw 1984; Strausfeld 1984). The first synaptic region is constructed of uniform structures (cartridges) that form a homogeneous organization throughout the lamina (Braitenberg 1967; Strausfeld 1971, 1984; Zettler et al. 1994). The light-OFF response is partly amplified and inverted to form a hyperpolarizing response with a strongly transient character (Autrum et al. 1970; French and Järvilehto 1978; Järvilehto and Zettler 1971, 1973, 1987; Zettler and Järvilehto 1971). A part of this process takes place already in the presynaptic photoreceptor axons (Weckström et al. 1992). The typical dark-adapted light-ON response in the LMCs is thus a hyperpolarization caused by histamine-activated chloride channels (Hardie 1987-1989; Nässet et al. 1988; Zettler and Straka 1987). The transmitter has been reported to be released tonically, even in darkness (Laughlin et al. 1987; Laughlin and Orsino 1989; Weckström et al. 1989). This tonic release causes a considerable Cl- load on the photoreceptor neurons, requiring an efficient Cl- extrusion mechanism (Uusitalo and Weckström 1994). The light-OFF response is partly caused by closing of the Cl- channels, during which the histamine release may be smaller than its tonic, background value (Laughlin and Orsino 1989; Uusitalo and Weckström 1994; Weckström et al. 1989). A small depolarization of unknown origin accompanies the end of hyperpolarization on which an OFF spike may be superimposed (Guy and Srinivasan 1988; Hardie et al. 1989; Hardie and Weckström 1990; Järvilehto and Zettler 1971, 1973; Zettler and Järvilehto 1971). Anatomically all LMC axons pass through the optic chiasma to finally synapse within the second optic neuropil, medulla.
The physiological and anatomic differences (Hardie and Weckström 1990; Strausfeld 1971) suggest that L3 and L1 could be responsible of feeding visual information into a different anatomic subsystem than L2 (Strausfeld 1989).

Recently, it has been reported (Hardie and Weckström 1990) that L3 responds to a light OFF in the end of a light pulse with an OFF spike superimposed on the OFF response. In L1 and L2 only an OFF response (without a spike) is observed when recorded from the synaptic region. The OFF spike has also been reported to be part of a signal coding mechanism in other invertebrate visual neurons: visual movement sensitive cells (Hengstenberg 1977), locust L neurons (Ammermüller and Zettler 1986; Wilson 1978), cockroach ocelli (Mizunami et al. 1986; Mizunami and Tatada 1988), honeybee monopolar cells (de Souza et al. 1992), monopolar cells of the droneyfly Eristalis (Guy and Srinivasan 1988), and ocellus interneuron of the bee (Milde 1981, 1984). In some of them, the actual generation mechanism and in all cases the functional significance still seems to be unclear.

In this study we will demonstrate that the OFF spike is a constant finding not only in L1 but also in L2 when hyperpolarized and that the OFF spike is elicited most probably through a voltage-dependent sodium conductance that is highly inactivated in dark-adapted conditions. Furthermore, the actual location of these voltage-gated sodium channels seems to be in the axon, and the OFF spike, especially in L3, is likely to be conducted to the synaptic region (lamina) by backward propagation. The role of the OFF spike in information processing is analyzed.

**METHODS**

Experiments were performed with the use of standard intracellular recording techniques (Laughlin and Hardie 1978) in situ with the use of intact adult blowflies (Calliphora vicina). The first-order interneurons (LMCs) were approached via a hole made in the marginal area of the cornea or via a hole made in the head capsule behind the eye. In each case the hole was filled with high-vacuum grease. The microelectrodes were in normal recordings filled with a cocktail containing potassium acetate (3 M) and potassium chloride (2.5 mM) to facilitate the current passing and to reduce the resistance of the electrodes (R, ~100–200 MΩ). Also 5% Lucifer yellow in 5% LiCl (R, ~ 200–400 MΩ) or 50 mM QX-314 in 2.5 mM KC1 (R, ~ 400–600 MΩ) were used. The fly was glued to a holder and grounded with a Ag/AgCl wire that was placed inside the head capsule. The recording electrode was attached to the amplifier via a Ag/AgCl wire and the tip and asymmetric potentials were compensated before measurements. The eye was stimulated by 10-μs light flashes delivered by a xenon tube (C552, Cathodeon, England) or by a light-emitting diode (LED) stimulus (Stanley HBG3660X) driven by a controlled current source, and calibrated with a PIN-diode and the 80-X Opt-O-Meter (United Technology).

The recorded signals were amplified by NPI intracellular amplifier (SEC 1L, NPI, Germany). Digital (DAT) recorder (DTR-1800, Bio-Logic, France) and/or IBM-compatible computer using ASYST (Keithley ASYST) program was used to store, analyze, and reproduce the responses (Juusola 1993).

The chiasma recordings, i.e., recordings from the LMC axons, were performed by adjusting the microelectrode angle to impale the region between lamina and medulla, as verified by high input resistance of axon impalements (Guy and Srinivasan 1988) and by monitoring the optical axes of adjacent cells that show large variations of the optical axes of successive cells as a typical finding in chiasma region.

To identify the different LMC subtypes, Lucifer yellow (Sigma) was introduced into the cells by means of negative current pulses (~0.5 nA) concomitantly with a negative steady-state current (~1 nA) for 1–5 min. After fixation the tissue was cut into ~35-μm slices, which were studied and photographed with the use of confocal microscopy. The marked cells recorded with Lucifer yellow-containing microelectrodes were not used to determine the threshold of the channels responsible for the OFF spike or input resistance of the LMCs because of the much higher electrode tip resistance (200–400 MΩ).

To determine the conductance responsible for the OFF spike, we used ionophoretic injections of QX-314 (Alomone Labs, Israel), a charged lidocaine analogue that is a potent intracellular voltage-gated sodium channel blocker. Injections were performed by positive current pulses (2 nA) for 1–5 min or by passive diffusion (Andrade 1991). The responses were recorded to a DAT tape.

To study the membrane properties of LMCs, we used discontinuous current-clamp method according to standard techniques (Finkel and Redman 1984; Laughlin and Osorio 1989; Wilson and Goldner 1975). Negative and positive steady-state current (0.1–5 nA) or a current step series containing a negative prepulse and a positive test pulse were introduced to the cells. This was done to investigate the threshold of the OFF spike and to study the activation-inactivation properties of the responsible channels. Careful attention was paid to critical complete compensation of the capacitance. Potassium acetate (2 M, with 5 mM KCl) buffered to pH of 6.8 with phosphate-buffered saline was used in the electrodes while using the prepulse-test pulse stimulus to minimize the diodelike behavior of the electrode.

Pseudorandomly modulated white noise stimulus from the green 555-nm LED (see above) was introduced to the cells tested for spectral analysis as described previously (Juusola et al. 1994; Kouvalainen et al. 1994). Shortly, a pseudorandom signal trace, generated by the computer program, was fed to the LED via the D/A-converter and the LED current control unit. The power spectra of the intracellular responses to the stimulus were calculated via fast Fourier transform by the use of standard methods including time domain and ensemble averaging (Bendat and Piersol 1971; Kouvalainen et al. 1994) using Blackman Harris 4 term window (Harris 1978).

**RESULTS**

The results in this paper are based on recordings from 60 LMCs, tested with current-clamp method, from which 43 exhibited spiking properties and 17 did not. Successful markings with Lucifer yellow injections were made in 15, and QX-314 injections to 30 LMCs. Axon recordings were performed from 30 LMCs.

We used only cells selected by electrophysiological criteria, i.e., input resistance (in L1 and L2 ~10–20 MΩ and in L1 ~20–30 MΩ), resting potential (L1 and L2 ~30 to ~40 mV and L3 ~45 to ~60 mV), and dark-adapted light-on response at least 35 mV. These criteria were not always possible to achieve with pharmacological interventions or for other obvious reasons (e.g., QX-314 experiments, Lucifer yellow injections, and chiasma recordings with small axons).

**LMC responses to light on and light off in the synaptic region**

Generally, the LMCs, when dark adapted, responded to on-axis light-step stimulus with a hyperpolarization transient...
light stimulus. The RP was approximately -42 mV.

We could thus divide the cells' spiking properties into three classes when recorded from the synaptic region, lamina (Table 1).

**Characteristics of the light-off spike in the synaptic region**

Intracellular recordings and current injections (with the discontinuous current-clamp method) were used to investigate the threshold of the off spike (L1 and L3) in the synaptic region. Steady-state current was applied to hyperpolarize or depolarize the membrane potential to a value at which maximum spiking frequency appeared. This methodology was chosen because of the unsuitability of these neurons for voltage clamping (too thin cells, φ 1–2 μm, poor space-clamp properties). Both in L1 and L3 a threshold for the spikes was found to be ~35–45 mV below the membrane potential. When the resting potential (RP) of these cells was current clamped near this potential, the off spike and additional spikes, at a frequency of up to 120 Hz, could be triggered either by a light flash, by a hyperpolarizing current step (spikes at the end), or by a depolarizing current pulse (spikes during the pulse; Fig. 3, A–C). The spikes that appeared in L1 and L3 were ~10–50 mV of amplitude and were quite fast, duration ~8 ms. Sometimes additional small depolarizations, probably subthreshold spikes, were also observed (Fig. 3, arrowheads).

Clearly these action potentials in the synaptic region could only be triggered with hyperpolarized cells by a depolarizing step produced with light or current. No spontaneous spiking was seen by merely hyperpolarizing the RP near the estimated threshold without any depolarizing stimuli. Depolarizing steady-state current injection (not shown) had only the effect of decreasing the amplitude of the off spike (in L3) and did not by itself trigger spikes. These results demonstrate that the threshold of the off spike was normally ~35–45 mV negative to the dark-adapted RP in the synaptic region. The absolute amplitude of the threshold was not identical in all LMCs, however, because the normal RP of these two subclasses was not the same. The RP in L1 was approximately ~40 mV and in L3 approximately ~60 mV as reported earlier (Hardie and Weckström 1990) and also found in this study.

In the experiments where the membrane potential was hyperpolarized to a level of 35–45 mV lower than the normal RP, the input resistance obtained by negative current pulse in L1 was about two times larger (~60 MΩ) than in resting conditions (~30 MΩ; Fig. 3C). This was not seen by applying positive current steps with the same hyperpolarization.
**FIG. 2.** Axonal endings of the 3 types of LMCs, as recovered in confocal microscopy from Lucifer yellow-injected cells. $L_1$: note the typical shape of the axon terminal and the long distance from the medulla surface (doubling of the top part of the axon is an optical artifact); $L_2$: the axon terminal was more spread out and located much nearer to the medulla surface (note the different scale); $L_3$: the ending has a typical shape and insertion depth as well (Strausfeld 1976).

**Activation-inactivation properties of the off-spike channels**

To test the activation-inactivation properties of the channels generating the action potentials and/or off spikes, we manipulated the cells with current steps delivered at RP in dark. The steps included both a negative prepulse and a positive test pulse (Fig. 4). By increasing the amplitude of the negative prepulse (0 to -1 nA) and keeping the positive test pulse constant (+0.3 nA), we found that the amplitude of the spike or (the initial depolarization) as measured from the RP increased from 7 to 21 mV (Fig. 4A). When the negative prepulse was kept constant (-1 nA) and the depolarizing test pulse increased (0-3 nA), the amplitude of the spike increased from 5 to 52 mV (Fig. 4B). Thus the channels responsible for the off-spike generation in these cells are highly inactivated in resting conditions. Further, the inactivation of the channels can be removed increasingly by briefly (for 200 ms) shifting the membrane potential to more negative values.

**Iontophoretical QX-314 injections**

To investigate the possibility that the conductance causing the spike is caused by voltage-gated sodium channels, we injected a lidocaine analogue, QX-314, iontophoretically into the intracellular medium. The drug was injected with the use of positive current (+2 nA) or by means of passive diffusion (Andrade 1991). Before QX-314 injections, a steady-state current of -2.5 nA was used to hyperpolarize the RP and thus to test for the spiking properties. After 1-5 min of iontophoretical QX-314 injection, the spikes and subthreshold depolarizations regularly vanished (Fig. 5). This finding was repeatable in all experiments where the...
FIG. 3. Action-potential-like responses in a L$_3$ in the dark-adapted compound eye. A: L$_3$ responses to saturating 2-ms light pulse recorded from the synaptic region. Top trace: in resting conditions. Middle trace: membrane potential has been lowered by steady-state current injection (-1.2 nA). Arrowheads point to the subthreshold depolarizations. Note that the 1st transient (arrow) was a reversed light-ON response, i.e., the membrane potential was lower than chloride equilibrium potential. The 2nd transient was equivalent to a light-OFF spike. Bottom trace: by further lowering of the membrane potential (-1.3 nA), the threshold of the voltage-gated channels responsible for the OFF spike was reached, spikes concomitantly with some subthreshold depolarizations (arrowheads) are seen. B, top trace: response of the same L$_3$ to a current step (+0.5 nA) applied in resting conditions recorded from the synaptic region. Middle and bottom traces: membrane potential has been lowered to the threshold of the OFF spike, i.e., to approximately -45 mV lower than the RP by steady-state current (-1.3 nA). Spikes and some subthreshold depolarizations (arrows) are seen during the current step. Note that the input resistance was ~30 MΩ when depolarizing step was used compared with the increase seen by negative current steps (see C). C, top trace: response of the same L$_3$ to a current step (-0.5 nA) in resting conditions recorded from synaptic region. Middle trace: membrane potential has been lowered with a steady-state current injection (-1.3 nA) to the threshold of the OFF spike. Subthreshold depolarizations (arrows) and spikes were elicited after the -0.5-nA current step. Bottom trace: similar stimulus as in middle trace, again some subthreshold depolarizations and spikes are seen. Note that the input resistance to -0.5-nA current step increases from ~30 MΩ in resting conditions to ~60 MΩ when the DC potential is lowered by 45 mV. This is not seen by positive current steps (see B); note also the scale difference between A and B. The LMC presented here had an RP of ~50 mV, an input resistance of 30 MΩ, and was identified as L$_3$ by electrophysiological criteria (see RESULTS).
of the blocker with the +2-nA current, the duration of the light-on response to a subthreshold light flash was increased threefold. In addition, the slow light-off depolarization either disappeared or was decreased remarkably (Fig. 6). This phenomenon was also seen in those neurons that did not originally generate spikes with the QX-314 in the electrode.

The findings thus revealed that the conductance responsible for the OFF spike is very likely to be a sodium conductance caused by the activation of the voltage-gated sodium channels. Further, the light-off response and the depolarization back to the resting potential from the hyperpolarization is at least partly caused by a similar sodium conductance (in addition to the reduced Cl conductance due to reduced transmitter release).

Properties of the OFF spike (and the ON spike) in the axon

Recordings were also made from the axons of the LMCs in the chiasma region (see METHODS) to investigate the OFF spike in the axons compared with those found in the synaptic region (lamina). Generally, the light-on responses recorded from the axons, as in the synaptic region, could be divided into two classes according to the appearance of the light-off spike. The threshold of the voltage-dependent depolarization was, as in lamina recordings, determined by hyperpolarizing the membrane potential with negative current. We found that the threshold of the OFF spikes was very near (-10 mV below, n = 16) or even at the RP producing now an ON spike (n = 4) with lowest stimulation intensity (Fig. 7A). The subsaturating light stimulus did not in the latter case cause any hyperpolarization but triggered the spike at exactly the same time with the hyperpolarizing light-on response observed with higher stimulation intensities. In neurons that showed spikes near RP, we observed also small depolarizations (as in lamina recordings, when the RP was hyperpolarized). In some of the high-quality recordings (n = 5), oscillations that could be interpreted as small spikes and/or subthreshold depolarizations were observed during the light-ON response.

Fig. 4. Current-clamp properties of the spikelike responses in a dark-adapted L3. A: inactivation removal experiment. The negative prepulse was varied from 0 to -1 nA while the positive test pulse was held constant (+0.3 nA). The amplitude of the spike (or initial depolarization) was increased from 7 to 21 mV (measured from RP). B: activation experiment, the negative prepulse was held constant (-1 nA) while the positive test pulse was increased from 0 to +3 nA. The amplitude of the elicited spike was increased from 3 to 54 mV (measured from RP). The cell tested here was identified as an L3 (RP approximately -55 mV) by electrophysiological criteria (see RESULTS).

Fig. 5. Inhibition of action potentials in LMCs by iontophoretic injection of QX-314, an intracellular Na+-channel blocker. First trace: normal light-on response to a 2-ms saturating light stimulus applied at RP. Second and third trace: membrane potential has been hyperpolarized by current (-2.5 nA) to the estimated threshold of the OFF spike (initially, in the second trace, near the reversal of the response). Note the decreased ON response and spikelike fast depolarizations. Fourth trace: same response after 1 min of QX-314 injection with positive current (+2 nA). Note that the spikes have disappeared. Fifth trace: control response after the experiment without hyperpolarization. The exact RP and the probable anatomical type of the cell cannot be given, because the QX-314 disturbs DC readings (electrode impedance is increased considerably).
FIG. 6. Effect of QX-314 injection on the graded flash response. Responses marked QX-314 were recorded after 5-min iontophoresis with +2 nA current. Top: saturating 10-μs light flash (~10,000 photons). Note that QX-314 injection decreased the light-off response and the duration of the light-on response was also increased considerably. Bottom: subsaturating light flash (~1,200 photons). Note again the almost disappeared light-off response with nearly 3-fold elongation of the light-on response.

response with long stimulation durations (>50 ms, Fig. 7B). These findings suggest that the threshold for these channels is indeed nearer to the RP in the axons than in the synaptic region (lamina).

The input resistance of the axons was found to be much larger (90–150 MΩ) than in the synaptic region (lo–30 MΩ), as reported earlier (Guy and Srinivasan 1988). Further, the A current was observed to be activated at ~10 mV below the RP (Fig. 8). In the synaptic region this A current is also seen but only ~50–90 mV below the RP (Hardie and Weckström 1990; Weckström, unpublished observations) in similar experiments. Thus the activation of the potassium conductance like the activation of the sodium conductance takes place nearer the RP in the axon than in the synaptic region.

FIG. 7. Recordings from LMCs in the axons in chiasma region. A: a subsaturating 2-ms light-on stimulus elicited a light-on spike. Bottom: response of the same cell now stimulated with a saturating 2-ms light-on pulse (~5·10⁵ photons/s). Note that the light-on spike (in top trace) appeared at exactly the same time as the peak light-on response at bottom. B: recording from another LMC with a subsaturating 50-ms light pulse (~5·10⁵ photons/s); note the oscillating and/or spiking activity. The input resistance in both of these LMCs (in A and B) was quite high, 120 MΩ in A and 110 MΩ in B; the RP was approximately ~40 mV in both of these cells. The actual LMC subtype was probably L₁/L₂ on the basis of the RP value.

possible function of the OFF spike

The propagation of the LMC light-on and -off responses to second optic neuropil medulla, is achieved by means of graded potentials (Autrum et al. 1970; Järvilehto and Zeitler 1971; Laughlin 1973; van Hateren and Laughlin 1990; Zeitler and Järvilehto 1971). The degree of the loss of visually acquired information during the passive propagation of the signal transfer through this centripetal pathway is very hard to determine. According to the cable model developed by van Hateren and Laughlin (1990), the signals transferred along the LMC cable are significantly low-pass filtered (Fig. 9B), when compared with the signals generated by the photoreceptor-LMC synapse (also Juusola et al. 1995).

The signal-to-noise ratio (SNR) of the photoreceptors and the LMCs were measured from the synaptic region to determine whether the high-frequency band is carrying information and not just noise. The results indicated that the SNR of the
FIG. 9. Information transfer properties in the LMCs. A: signal-to-noise ratio (SNR) of a photoreceptor and a LMC (a probable L1/L2 type, measured from the synaptic region). Note that the SNR in LMC exceeds to higher frequencies than in the photoreceptor. B: transfer function of an L1/L2 and L3, with the frequency transfer characteristics of the L0 axon (using a model presented by van Hateren and Laughlin 1990), assuming purely passive axon membrane, i.e., it does not contain the off-spike conductance or any voltage-activated K+ conductance. LMC subtypes were determined by electrophysiological criteria as stated in RESULTS.

Localization of the voltage-gated sodium conductance

The threshold of the sodium conductance was found to be considerably nearer to the RP in axon (chiasma) than in the synaptic region (lamina). The best explanation for this is that the actual location of the voltage-gated channels is in the axons. This in turn would mean that the DC voltage to which the membrane potential is current clamped in the synaptic region would attenuate as it propagates passively toward the axons and toward the location of the voltage-gated channels. Thus a bigger hyperpolarization would be required in the synaptic region compared with that of the axon region to reach the threshold (see, e.g., Guy and Srinivasan 1988; van Hateren and Laughlin 1990).

The variations found in the DC voltage of the threshold in the same region (≈10 mV) could also be explained in a similar manner. Recording sites vary from cell to cell, causing alterations in the distance between the recording site and the putative spike initiation zone. Thus the amount of attenuation and the observed threshold varies. The spikes and subthreshold depolarizations seen during the ON response in axon recordings indicate that the location of these voltage-gated channels is in the axonal portion. Alternatively, the spike initiation could, in principle, take place in the proximal lamina. The subthreshold spikes observed in the synaptic region recordings with current-induced hyperpolarization support this proposal. In patch-clamp studies it has been shown that the soma region of these cells show no voltage-gated sodium channels (Hardie 1988; R. C. Hardie, personal communication). Further, the finding that the A current (K+ channels) seems to be activated in the axon region with reasonable hyperpolarization from the RP (−10 mV) indicates that also these channels are operational in the axon in contrast to the soma of synaptic region.

The finding that L3 was the only interneuron generating the light-ON response caused by the increased chloride conductance (Hardie 1987, 1989; Zettler and Straka 1987) is sufficient to remove this inactivation inside the physiological voltage range of L3-type cells, at least in the axon region, creating conditions where the spike can be triggered with a depolarizing stimulus, i.e., during the cessation of the hyperpolarizing response.

The finding that L0 did not show any spiking properties was unexpected. Both L1 and L2 are closely related anatomically and differ mainly in the location and shape of their synaptic terminals in the medulla (Strausfeld 1976, and Fig. 2). In fact, previously L1 and L2 have been considered to have similar functions. The lack of spiking activity in L2 could be related to different anatomic connectivity of the neurons. This hypothesis is supported by the fact that the synaptic terminals of L1 and L2 are located in different layers in the second visual ganglion (medulla) and that in addition L2 synaptic terminal in the synaptic region forms a number of feedback synapses (Kral and Mcinertzhagen 1989).

DISCUSSION

Our results suggest that voltage-gated sodium channels are responsible for the off-spike generation in the first visual interneurons (L1 and L2) of the compound eye of the Calliphora vicina. The hyperpolarization of the membrane potential needed to initiate the action potentials points out that these channels are normally largely inactivated. Evidently, the hyperpolarizing light-ON response caused by the increased chloride conductance (Hardie 1987, 1989; Zettler and Straka 1987) is sufficient to remove this inactivation inside the physiological voltage range of L3-type cells, at least in the axon region, creating conditions where the spike can be triggered with a depolarizing stimulus, i.e., during the cessation of the hyperpolarizing response.

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The finding that L3 was the only interneuron generating the light-OFF spike under resting conditions, when measured from the synaptic region (lamina), is probably caused by the OFF spike propagating passively backward from its initiation point to the site of recording. This is the case in some ocellar second-order neurons (Ammermuller and Zettler 1986; Wil-
L3s higher input resistance (≈30 MΩ) is considered to be large enough to ensure bidirectional propagation of the spike-like transients (van Hateren and Laughlin 1990). In L1 and L2 the lower input resistance (10–20 MΩ) would cause strong attenuation of any axonal OFF spike. This hypothesis fits well to our data also in the regard that the OFF spike was not observed in all of the L3s recorded (Table 1). In these cases the recording site could have been in the very distal region of the cell.

Functional properties of the OFF spike

The actual triggering mechanism of the OFF spike could be related to a depolarizing synaptic input other than that of photoreceptor driven histamine release. Decamethonium greatly reduces or even totally eradicates the light-OFF response, and the application of acetylcholine depolarizes the RP, causing increased light-ON responses (Hardie 1988; Straka and Ammermüller 1991; Zettler and Straka 1987). These results together with our finding that QX-314 blocks the spiking activity and decreases the light-ON response could be explained by assuming that the LMCs would have another, probably a cholinergic synaptic input that would open voltage-gated sodium channels and trigger the light-OFF spike. Also the subthreshold depolarizations (Fig. 3) could be regarded as postsynaptic potentials (PSPs) and thus reflect the action of a depolarizing synaptic input. The neurons responsible could be amacrine cells that have been suggested to be cholinergic (Datum et al. 1989). In this case amacrine cells would interact via α-process and L4 neurons to LMCs (Shaw 1984; Strausfeld and Campos-Ortega 1973). It is plausible, however, that this kind of a mechanism would not be advantageous in systemic respect, because it is hard to determine what would be the role of such a horizontal signaling pathway in these centripetal neurons. In addition, the fact that the QX-314 application erased also the subthreshold depolarizations does not support this kind of mechanism.

The argumentation sometimes presented that these interneurons are in fact spiking and code light increments as trains of action potentials is also one to be considered (e.g., Arnett 1971, 1972). The fact that we could see ON spikes and subthreshold depolarizations during light-ON responses in axons (chiasma recordings, Fig. 8) could be interpreted in a way that this hypothesis is valid. The axon hillock would in this case probably be in the beginning of the axon. The cell damage when the cell membrane of these small neurons is penetrated with the microelectrode could be the reason why the spikes are not always present. It is also possible that some unknown modulator could shift these neurons between spiking and nonspiking states (see, e.g., Milde 1981, 1984). The above-formulated “spiking” hypothesis is supported by previous findings that extracellular spiking activity is seen in chiasma region ON-off and sustained units, the so-called “Arnett units” (Arnett 1971, 1972; Jansonius and van Hateren 1991, 1993). Thus the OFF spikes could, in principle, be a part of an OFF pathway in the compound eye.

Because the light-OFF spikes’ amplitude most probably is bigger in the synaptic terminal in the second neuropil than in the synaptic region (because of the higher input resistance of the axon terminal) (van Hateren and Laughlin 1990), the function of the OFF spike could also be involved in modulation of the response concerning the second interneuron. Interestingly, in the locust ocelli the second-order interneurons have been reported to be excited by depolarizing and to be inhibited by hyperpolarizing input from first-order interneurons (Simmons 1981). To resolve this kind of question, more information is needed on the function of the second-order interneurons (in 2nd optic neuropil).

The discussion above, although relevant, does not fit together with the main evidence presented in this paper. None of the LMCs, not even L3s, generate spikes when stimulated with small contrasts, or with white noise stimulation that corresponds more closely with the naturally occurring temporal distribution of the stimuli. Therefore the most probable function of the OFF spikes according to our results is involved in the restoration of the gain loss during the passive conduction of the signal toward the second neuropil, medulla. The SNR of the LMCs in the synaptic region is considerably better than that of the SNR in presynaptic photoreceptors at high frequencies (Fig. 9A) (also Juusola et al. 1995). This finding in turn tells us that this frequency band contains important information and it is meaningful for the system to restore it, instead of discarding it. The passive propagation of the light-induced responses attenuates the high-frequency signals considerably during the propagation toward the axon terminal (van Hateren and Laughlin 1990, see Fig. 9). We have shown that the OFF spike clearly affects the gain by shifting it toward, and increasing it in, the higher frequencies (Fig. 9). In this way these cells can compensate the attenuation of the high-frequency band and maintain the high SNR, analogously with photoreceptor axons of the bee (Coles and Schneider-Picard 1989; Vallet et al. 1992; Vallet and Coles 1993) and probably of the cockroach (Weckström et al. 1993). Moreover, it is probably not merely fortuitous that the L3s express delayed rectifier potassium channels (K channels) that are activated and deactivated continuously even when stimulated in resting conditions (Hardie and Weckström 1990). This same may apply also to the K channels in the L1/L2 axons (Fig. 8). These kind of mechanisms are according to our present results essential to antagonize the voltage gated sodium conductance.

As a conclusion, this visual system could have a mechanism where all the three interneurons are specialized in differentially amplifying slightly different bands from the visual signal, maybe used in different detection tasks in further processing. One of the function of the voltage-gated sodium channels in L3, and probably also in L1 (when sufficiently hyperpolarized), could be the compensation of the gain loss in the axon during the passive propagation of the signal toward the distal axon terminal. The exact function of the different spiking properties of L1 and L2 remain to be elucidated.

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