

Potential in the First Visual Synapse of the Fly Compound Eye

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Uusitalo, R. O. and M. Weckström. Potentiation in the first visual synapse of the fly compound eye. *J. Neurophysiol.* 83: 2103–2112, 2000. In the first visual synapse of the insect compound eye, both the presynaptic and postsynaptic signals are graded, nonspiking changes in membrane voltage. The synapse exhibits tonic transmitter release (even in dark) and strong adaptation to long-lasting light backgrounds, leading to changes also in the dynamics of signal transmission. We have studied these adaptational properties of the first visual synapse of the blowfly *Calliphora vicina*. Investigations were done in situ by intracellular recordings from the presynaptic photoreceptors, photoreceptor axon terminals, and the postsynaptic first order visual interneurons (LMCs). The dark recovery, the shifts in intensity dependence, and the underlying processes were studied by stimulating the visual system with various adapting stimuli while observing the recovery (i.e., dark adaptation). The findings show a transient potentiation in the postsynaptic responses after intense light adaptation, and the underlying mechanisms seem to be the changes in the equilibrium potential of the transmitter-gated conductance (chloride) of the postsynaptic neurons. The potentiation by itself serves as a mechanism that after light adaptation rapidly recovers the sensitivity loss of the visual system. However, this kind of mechanism, being an intrinsic property of graded potential transmission, may be quite widespread among graded synapses, and the phenomenon demonstrates that functional plasticity is also a property of graded synaptic transmission.

INTRODUCTION

The anatomy and electrophysiological properties of the neurons in the visual system of the fly make it an excellent model for studying the neural signal processing with graded potentials in the first visual synapse (Meinertzhagen and Frölich 1983; Shaw 1984; Strausfeld 1976, 1984). In the compound eye of the blowfly *Calliphora vicina*, six photoreceptors synapse with three first order visual interneurons (LMCs) according to the neural superposition principle (Kirschfeld 1967; Uusitalo et al. 1995b; van Hateren 1986) forming the first synaptic complex with multiple synapses (Shaw 1984). In this synapse the graded light-induced depolarization of the presynaptic photoreceptors is inverted, made more transient, and amplified (Autrum et al. 1970; Juusola et al. 1995a, 1996; Laughlin 1987). The photoreceptor transmitter histamine (Hardie 1987, 1989) opens ligand-gated Cl⁻ channels in postsynaptic LMCs (Hardie 1989; Zettler and Straka 1987) causing a hyperpolarization in response to light intensity increments (light-on response). The transmitter release has been proposed to be tonic even in dark (Laughlin et al. 1987; Uusitalo et al. 1995a). Similar tonically active graded potential synapses are also present in other visual

systems (vertebrate rods and cones, Baylor and Fettiplace 1971; Dowling and Ripps 1973).

In the fly compound eye, strong light adaptation causes the membrane potential of the photoreceptor soma to hyperpolarize by the activation of an electrogenic Na⁺/K⁺ ATPase (Hamdorf et al. 1988; Jansonius 1990). This hyperpolarization could conceivably reduce the tonic transmitter release causing an imbalance in the influx and efflux of the Cl⁻ ions into the postsynaptic LMCs. This would lead to increased light-on responses in the LMCs (Uusitalo et al. 1995a). The main hypothesis in this paper is that the presynaptic Na⁺/K⁺ transporter during light adaptation hyperpolarizes the axon terminals thereby decreasing the tonic transmitter release. During this hyperpolarization, the postsynaptic tonic Cl⁻ conductance is decreased whereas the Cl⁻-extrusion mechanism remains operational. This chain of events leads to decreased intracellular Cl⁻ concentration and to real postsynaptic potentiation.

We show that plasticity of the postsynaptic responses is also a property of a graded potential synapse. During the dark recovery the short-term plasticity resembles the potentiation seen in action potential synapses (Zucker 1989). However, the overall function of the potentiation in this visual system may be linked to dark recovery and could serve as a mechanism restoring the sensitivity loss after strong light adaptation and may also be linked to structural changes in the synapse during illumination (Meinertzhagen 1989; Pyza and Meinertzhagen 1995). We show that in the graded synapse the active regulation of the reversal potential of the transmitted mediated postsynaptic conductance may also contribute to the process of visual coding.

METHODS

Adult blowflies (*Calliphora vicina*) obtained from a frequently refreshed stock were used for the intracellular in situ recordings according to established procedures (e.g., Laughlin and Hardie 1978). All recordings were performed after an initial 30-min dark adaptation at room temperature (21 ± 1°C). A small hole filled with high vacuum grease was put into the back of the head capsule or to the marginal line of the cornea to penetrate the neuronal tissue. The animal was fixed to a holder and grounded with a Ag/AgCl wire via the hemolymph. The visual neurons were penetrated with sharp microelectrodes pulled from fiber-filled glass capillaries (Clark Electromedical, UK) with a microelectrode puller (Sutter Instruments). A piezoelectric micromanipulator (Burleigh PZ-550) connected to a cardan arm system was used to advance the microelectrode in tissue with 20-nm steps. The electrodes were filled with 2 M potassium acetate with 5 mM KCl (tip resistance 100–150 MΩ). A Xenon light source (Hamamatsu, Japan) connected to a shutter (Uniblitz 132, Germany) and a filter set (Schott, Germany) was used to light adapt the visual neurons. The test stimulus used was produced with a Xenon flash unit (Cathodeon, UK). The

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recorded signal was amplified with an intracellular amplifier (NPI-Electronics, Germany), filtered with an adjustable filter (dual channel elliptic filter, Kemo), and stored to a DAT-tape (Biologic, France). An IBM-compatible computer using an ASYST program (Keithley, Juusola 1993) was used to produce the stimulus and store the signals used. Two oscilloscopes (Tektronix 5A20A) were used to monitor the compensation of the electrode capacitance and to visualize the voltage responses of the neurons.

Power spectra of intracellularly recorded voltage samples were calculated via fast Fourier transform using standard methods (Bendat and Piersol 1971; Juusola et al. 1994) with Blackman-Harris 4-term window (Harris 1978). Current clamp experiments using discontinuous (switched) current injections were done as reported earlier (Finkel and Redman 1984; Juusola and Weckström 1993; Laughlin and Osorio 1989). The switching frequency was 3 kHz and the time constant of the electrode (after critical capacitance compensation) was $\sim 5 \mu\text{s}$ (Weckström et al. 1992b).

The ionophoretical injections were done by filling the electrode with 3 M potassium chloride (R_e 100–150 M Ω). The Cl^- ions were injected with negative DC current of different magnitudes from the recording electrode (Uusitalo and Weckström 1994). The injection was started well before (~ 1 min) the light adaptation and the recovery observed for ≥ 2 min. The ionophoretical experiments started with zero current and went up to -2.5 nA in 0.5-nA steps while the capacitance was critically monitored.

The best available method for studying the synapse between neurons is to try and make intracellular recordings from both pre- and postsynaptic neurons simultaneously. This, however, would be extremely difficult in the case of this synapse because the small diameter of the neurons involved ($\sim 1\text{--}2 \mu\text{m}$) makes simultaneous pre- and postsynaptic recordings extremely rare. At present the possible method is to make intracellular recordings from the pre- and postsynaptic neurons separately and to use a set of strict electrophysiological criteria to rule out recordings that are from injured or poorly penetrated cells. We mainly used the electrophysiological criteria we reported earlier (Uusitalo and Weckström 1994). The criteria for the good penetrations of the photoreceptor axon terminals were a low resting potential (r.p.) (from -50 to -60 mV), a large R_{in} (60–110 M Ω), and the presence of the fast-depolarizing transient (Weckström et al. 1992a). The criteria for the postsynaptic LMCs were as follows: resting potential (-40 to -60 mV), R_{in} (15–40 M Ω), and light-on response (greater than -35 mV; Hardie and Weckström 1990). To be qualified, the recorded neurons had to fulfill all the criteria above.

To study the process of dark recovery in the first visual synapse we stimulated the photoreceptors axon terminals with a long-lasting light-adapting pulse to which a 30- μs test flash was superimposed. The intensity of the adapting stimulus was varied from 2×10^5 to 2×10^8 effective photons/s and the duration was varied from 100 ms to 40 s. The test stimulus had a constant duration of 30 μs with a intensity from 73 to 1.5×10^5 effective photons/flash and an interstimulus period of 300 ms. The light output of the Xenon light source was calibrated in terms of effective light quanta by counting, after prolonged (>60 min) dark adaptation, the discrete small responses evoked by single photons (Lillywhite 1977) occurring in photoreceptors during dim illumination.

RESULTS

Dark recovery

To study the mechanisms behind dark recovery we recorded light-evoked responses from photoreceptor somata, axons terminals, and postsynaptic LMCs following different light-adaptational states (typical experiments shown in Fig. 1). Altogether 45 photoreceptors somata, 15 photoreceptor axon terminal recordings, and 40 LMCs were used for the results.

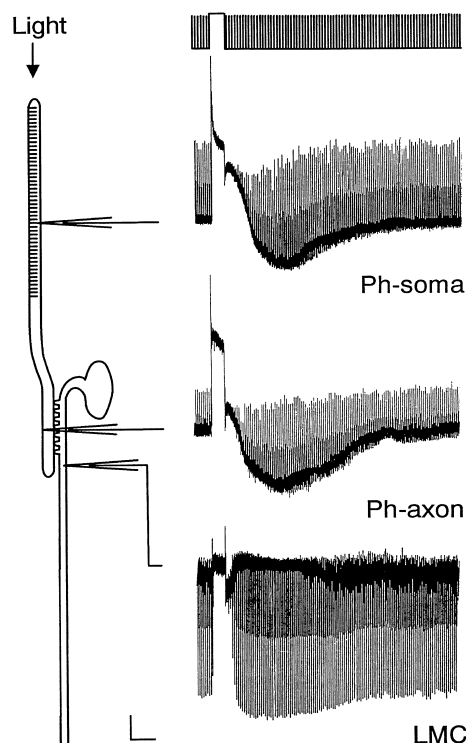


FIG. 1. Representative light-evoked responses of a photoreceptor soma (Ph-soma), an axon terminal (Ph-axon), and a postsynaptic postsynaptic 1st order visual interneuron (LMC) after 2 s light adaptation (2×10^8 photons/s). In all experiments there was a continuous stimulation by short (30 μs) test flashes and a longer light-adapting period (*top trace*: light stimulation) and thus recordings show responses to both. Hyperpolarization of resting potential of the soma and axon after the light-adapting pulse are caused by the Na^+/K^+ -pump (Hamdorf et al. 1988; Jansonius 1990). Depolarization, both in soma and axon just after the light-off, is likely caused by the Na^+/Ca^+ -exchanger (Minke and Kirschfeld 1984). During the presynaptic pump-potential the postsynaptic LMC shows a clear potentiation of the light-evoked responses. Note also the decreased level of noise and depolarized membrane potential of the LMC during this potentiation. The LMC also depolarizes slightly during adapting pulse. Bar in bottom right corresponds to 10 mV and 5 s calibration. Enlarged responses to test flashes are shown in Fig. 2.

After a strong light-adapting stimulus (2×10^8 effective photons/s) the photoreceptor soma recordings showed a marked hyperpolarization of the resting potential that reached a maximum of -21.7 ± 2.9 (SD) mV ($n = 10$). This hyperpolarization that was found in the photoreceptor axon recordings (Fig. 1; note that here the responses to test flashes are still depolarization transients) depended on the duration and intensity of the adapting light. Using exactly the same protocol, after the same stimulus, the postsynaptic LMCs depolarized by 4 ± 2 mV ($n = 10$) and showed a marked decrease in the conspicuous dark noise (Fig. 1 and Fig. 4, *inset*). The decrease in the dark noise was seen as a decrease in membrane fluctuation. Concomitantly with this, the LMC responses to a test stimulus showed a clear enhancement, or potentiation, that reached a maximum of 9 ± 0.2 mV ($n = 10$; Fig. 1), as shown before (Uusitalo et al. 1995a). The response enhancement lasted ~ 10 s after light adaptation. The amplitude and time course of the postsynaptic potentiation were found to depend on the intensity and duration of the adapting stimulus, in the same manner as the pump-induced afterhyperpolarization in the presynaptic axon recordings (see below, e.g., Fig. 6B). The presynaptic pump potential and the postsynaptic potentiation could also be

elicited by a high-frequency train (120 Hz) of short (30 μ s) saturating light-on stimuli to photoreceptors (data not shown).

Light-evoked responses in presynaptic photoreceptor somata, axons, and postsynaptic neurons after light adaptation

The amplitude of the test flash responses (duration 30 μ s) increased in the photoreceptor somata during the afterhyperpolarization when compared with responses to the same test flash during dark-adapted conditions. This was maximally 6 ± 1.2 mV ($n = 20$, typical single responses, enlargements from experiments of the type in Fig. 1 are shown in Fig. 2A). The increase can readily be interpreted to have been caused by the hyperpolarization of the resting potential by the Na^+ pump potential. This increased the driving force for the light-gated current and also reduced the effects of the voltage-gated potassium conductance (Juusola and Weckström 1993; Weckström and Laughlin 1995; Weckström et al. 1991). In the axons, the responses to the test stimulus increased and the transient characteristics were enhanced (Fig. 2B). The increase of the fast depolarizing transient was larger than the increase in the soma recordings (10 ± 2.5 mV; $n = 15$) and depended on

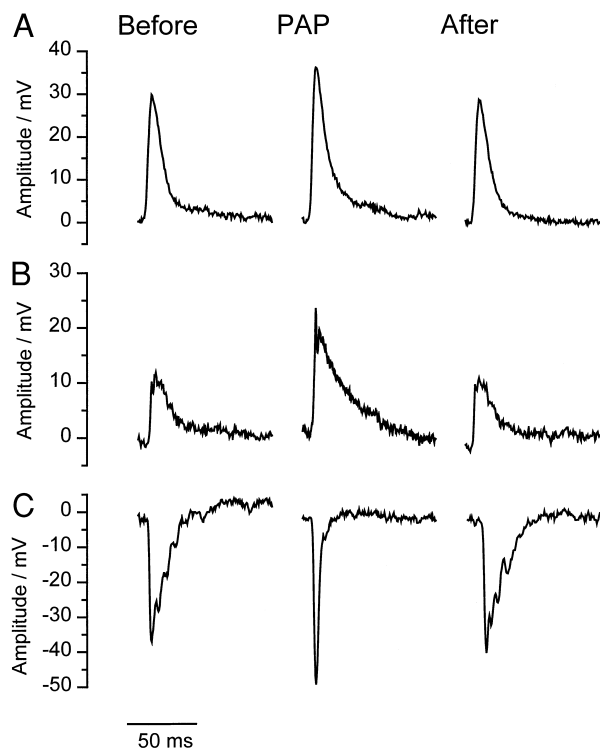


FIG. 2. Light-evoked responses to 30- μ s test stimuli (1.5×10^5 photons/flash) before, during (PAP), and after the postsynaptic potentiation that was elicited with a light-adapting pulse (2×10^8 effective photons/s) lasting for ~ 2 s. *A*: responses recorded from photoreceptor somata produced a 6 mV increase in amplitude during period of the presynaptic pump-potential (trace in PAP column). *B*: presynaptic axon terminal recordings showed at least a twofold increase in amplitude during the potentiation (PAP) with a considerable enlargement in amplitude of the fast depolarizing transient in rising phase of responses. Transient can also be seen before and after the potentiation but is much smaller. *C*: in postsynaptic LMCs the light-on responses during the potentiation were increased by ~ 10 mV during the PAP. Concomitantly the response became more transient and lost the normally present oscillations during the rising face of the light-induced hyperpolarizations. Shortened light-on responses in LMCs correlated well to increase in the presynaptic fast depolarizing transient amplitude (see *B*).

the hyperpolarization (i.e., the pump potential). Mimicking this, the increase in the fast depolarizing transient amplitude could be seen by hyperpolarizing the resting potential of the axon terminal by current clamp (see also Weckström et al. 1992a).

In the postsynaptic LMCs, both unsaturated and saturated responses to the test flash were potentiated, maximally by ~ 9 mV after light adaptation. The period of the potentiation after light adaptation coincided with the period of the presynaptic pump potential. During potentiation the hyperpolarizing responses were narrower than in dark-adapted conditions (Fig. 2C) and thus resembled mostly the transient component (the fast depolarizing transient) of the presynaptic axon responses during this same period (Fig. 2B). The more transient LMC responses were seen during the potentiation in every experiment. The oscillations often observed in the LMCs (e.g., Juusola et al. 1995a; van Hateren 1986) and the light-off depolarization were not present. The responses recovered when the potentiation effect also disappeared, ~ 10 s after its initiation.

The voltage versus light intensity functions ($V/\text{Log } I$ functions, see Laughlin et al. 1987) of photoreceptor somata in dark-adapted conditions and after light adaptation (Fig. 3A) showed that the responses increased from the test flash intensity of $\sim 10^3$ effective photons/flash upwards. In the presynaptic axons (Fig. 3B) this change was bigger and was present with lower stimulus intensities. The $V/\text{Log } I$ functions from the postsynaptic LMCs revealed that although both subsaturated and saturated LMC responses were potentiated, the near saturating responses were potentiated the most (Fig. 3C). Small (< 7 mV) LMC responses to stimuli with < 100 photons/s were actually smaller than in dark-adapted conditions. Thus only the LMC responses from ~ 7 mV upwards were enhanced. The slope of the $V/\text{Log } I$ function was steeper after light adaptation than under dark-adapted conditions and was shifted toward lower intensities during the potentiation. The slopes of the normalized functions were very nearly equal, suggesting that the sensitivity of transmitter release to the number of Cl^- channel openings did not change (for discussion of this see Laughlin et al. 1987).

Mechanism behind potentiation

Frequency domain analysis of the LMC responses under potentiation-inducing conditions, during the (now) depolarized resting potential, revealed that the power spectrum of the noise showed attenuation especially at the low-frequency band (Fig. 4, inset, shows a typical recording trace; note the absence of test flashes in this type of experiment). The input resistance R_{in} of the photoreceptor axons increased 8% from 62 ± 5 $\text{M}\Omega$ ($n = 3$) in dark-adapted conditions to 68 ± 4 $\text{M}\Omega$ ($n = 3$) with a hyperpolarization of ~ 20 mV. Concomitantly also the R_{in} of the postsynaptic LMCs increased by 7.4 ± 2.9 $\text{M}\Omega$ ($n = 5$; i.e., by 33% during the potentiation). This showed that during the attenuation of the membrane noise the total conductance of the cells as well as the LMCs is reduced.

In the postsynaptic LMCs, all of the changes (reduced noise levels, depolarized period of the resting potential, and period of the increased R_{in}) recovered to normal at a certain time point after the end of the adapting light pulse. The voltage of the presynaptic photoreceptor axons at this point was below the normal resting potential by 11 ± 1.5 mV ($n = 10$). We can

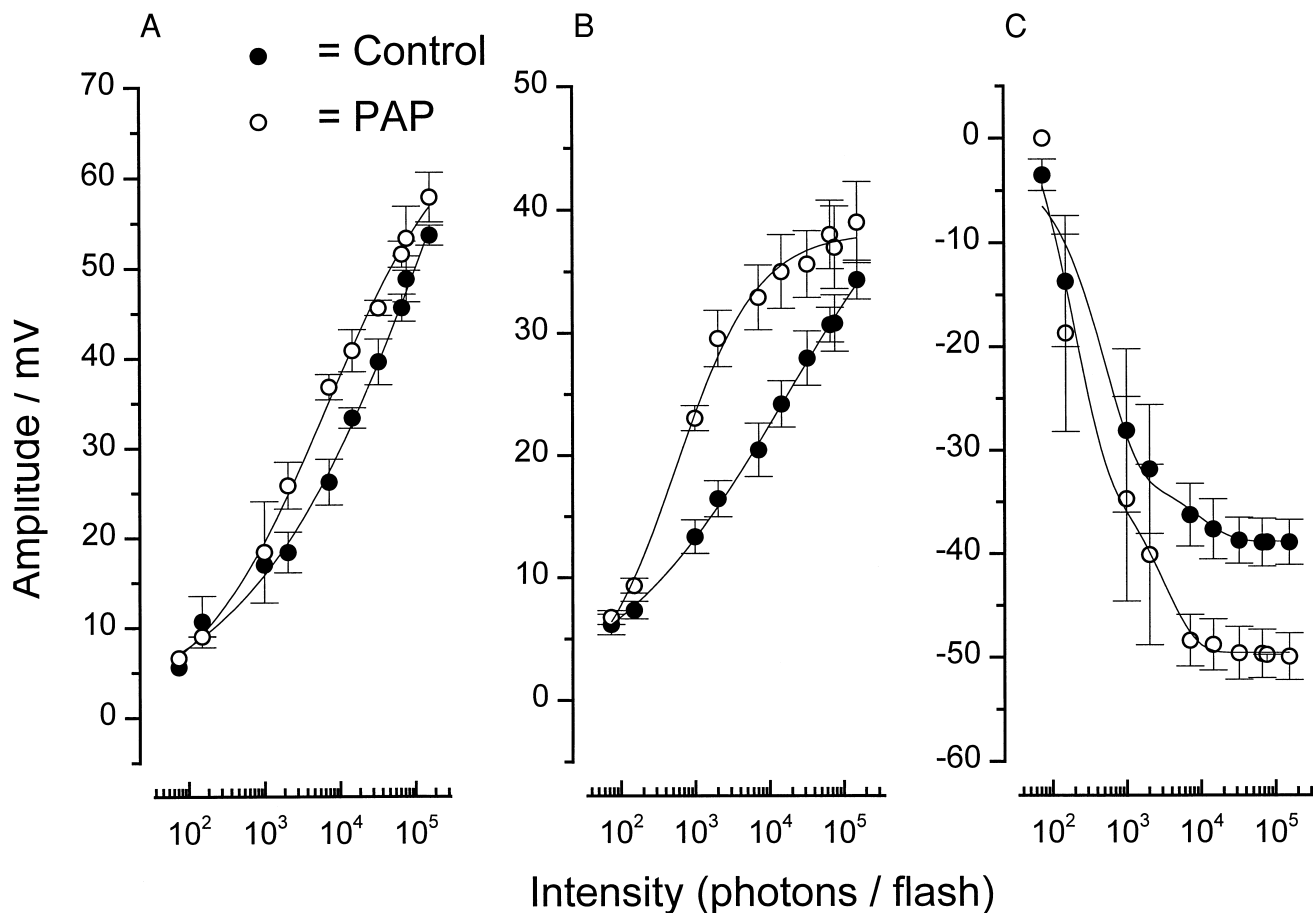


FIG. 3. $V/\log I$ -curves (maximum amplitude of response vs. light intensity of test pulse) from photoreceptor somata, presynaptic axon terminals, and postsynaptic LMCs in dark-adapted conditions (filled symbols) and during potentiation (open symbols). All curves were shifted leftwards during the period of potentiation just after light adaptation. Test flashes and the adapting light pulse were as before. A: $V/\log I$ curves of photoreceptor somata ($n = 5$). During the pump-potential (i.e., during the postsynaptic potentiation) responses were increased from the intensity of 10^3 photons onwards. Curves are best fitted with the Michaelis-type equation ($V = V_{\max}(I^n/(I^n + k^n)$, see Laughlin 1981) (in PAP: $V_{\max} = 72.7$, $n = 0.44$, $k = 19150$; in norm: $V_{\max} = 68.7$, $n = 0.49$, $k = 6430$). B: $V/\log I$ curves of the presynaptic axon terminals ($n = 4$) showed an even more significant increase in amplitude during potentiation. This was mostly mediated via the increase of fast depolarizing transient (see Fig. 2C). Fit is as in A (PAP: $V_{\max} = 38.2$, $n = 0.79$, $k = 558$; norm: $V_{\max} = 48.9$, $n = 0.36$, $k = 5569$). C: $V/\log I$ curves from LMCs ($n = 6$) demonstrate the postsynaptic potentiation (PAP) that was greatest with saturating responses (i.e., ~ 10 mV) but appeared with subsaturated responses as well. Curves show the best fit to a two-exponential dependence on intensity (PAP: $k_1 = 232$, $k_2 = 2809$, $y_{\max} = -49.6$ mV; norm: $k_1 = 260$, $k_2 = 4689$, $y_{\max} = -38.8$ mV).

argue that this result represents the value for the voltage in presynaptic axons below which the postsynaptic membrane noise was significantly decreased. The time that the presynaptic voltage spent below this voltage (during the pump potential) matched the period of depolarized r.p. and the decreased noise level in the postsynaptic LMCs.

Involvement of the postsynaptic Cl^- transport in the potentiation

Because the depolarization, noise reduction, and increased R_{in} of the LMCs during potentiation obviously point toward the possibility of reduced transmitter release, we tested if the postsynaptic Cl^- transport contributes to the overall potentiation of the postsynaptic responses. The Cl^- pump (extruding Cl^-) speeds up as the intracellular Cl^- concentration is increased (Uusitalo and Weckström 1994). Accordingly, the potentiation should be bigger if the intracellular Cl^- concentration is increased. We introduced a constant Cl^- load into the

LMCs prior to the potentiation-inducing light stimulus using ionophoretic injections while observing the dark recovery. Consistent with earlier reports, a large Cl^- load (-2.5 nA) was able to cause a reversal of the light-on response (the hyperpolarizing transient shown in Fig. 5A turned into a depolarizing one in Fig. 5B) by shifting the E_{Cl} to be more positive than the resting potential (Hardie 1987; Uusitalo and Weckström 1994; Zettler and Straka 1987). Under these conditions, when the response was reversed, the potentiation also occurred. The peak light response turned back (from a depolarizing transient) into a hyperpolarization (in Fig. 5C) for a period of time approximately as long as the potentiation lasted normally. The relative magnitude of the potentiation in terms of mV, when calculated relative to the on-response size, was 24 mV, which is larger than during the Cl^- load. The dark resting potential was also depolarized after the Cl^- load, apparently because of a large basal Cl^- conductance and the shift of the E_{Cl} to a more positive value (see also Uusitalo and Weckström 1994). After

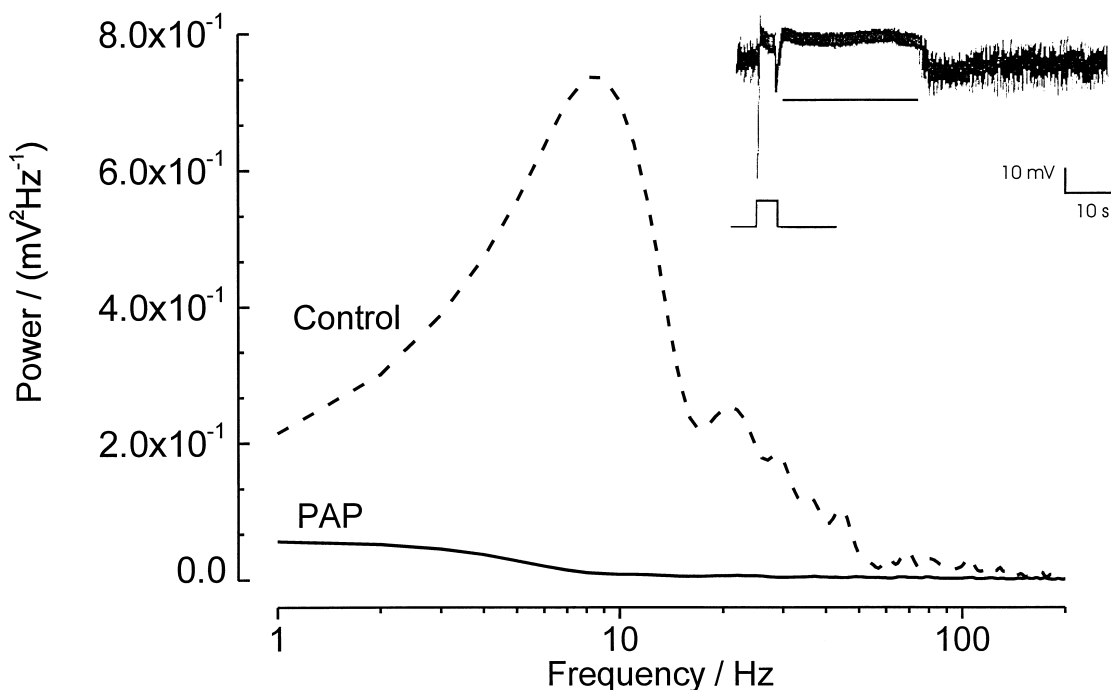


FIG. 4. Evidence for decreased transmitter release as a mechanism behind potentiation. Power spectra were calculated of postsynaptic dark-noise after the light-adapting stimuli when the membrane was virtually noiseless (PAP) and during normal conditions after the potentiation period (control). Note that during period of potentiation the power of the dark-noise in the low and middle frequency band was drastically attenuated, consistent with a reduction in the transmitter release. *Inset*: changes in membrane potential of a postsynaptic LMC after light adaptation (duration 2 s, intensity 2×10^8 photons/s). Note the period of decreased dark noise (underlined) and that here no test flashes were used.

the potentiation was over, the response returned back to the reversed state (Fig. 5D) These findings are consistent with the hypothesis that an increase (positive shift) in E_{Cl} was behind the potentiation.

Dependence of the potentiation on light-adapting stimulus

The magnitude of the pump-induced hyperpolarization in the somata of the presynaptic photoreceptors has been reported to depend on intracellular sodium concentration (Jansonius 1990). Because the potentiation is linked to presynaptic hyperpolarizing pump potential, it is evident that the amount of sodium entering the photoreceptors during light adaptation is of critical importance. We tested in the following changes that

take place in both the presynaptic pump potential and the postsynaptic potentiation (in terms of size and duration) by varying the intensity and duration of the light-adapting stimulus. Thus we also putatively varied the intracellular sodium concentration at the end of the adapting light pulse in the presynaptic photoreceptors.

When the duration of the light-adaptation (intensity 2×10^8 photons/s) was increased, both the pump-induced presynaptic hyperpolarization and the postsynaptic potentiation increased in size up to the duration of 2 s (Fig. 6). With longer light adaptation both the pump potential and potentiation started to decline. This seemed to be correlated with an additional depolarizing afterpotential that was manifested just after the end of

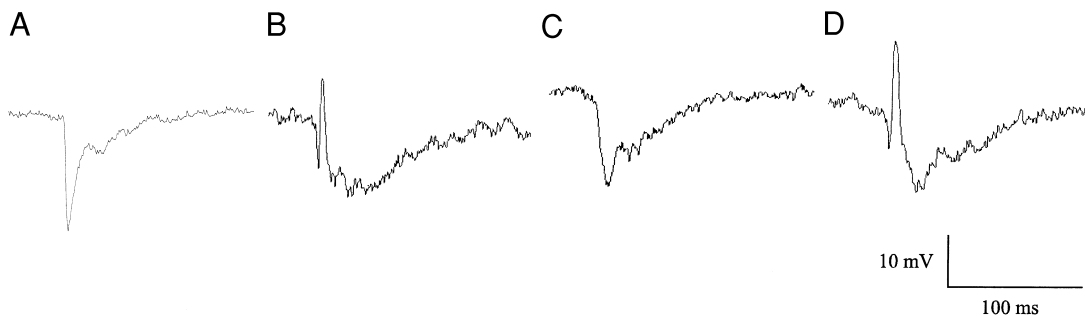


FIG. 5. Evidence for a shift in E_{Cl} during potentiation. The figure shows the LMC responses to the test flash ($30 \mu\text{s}$, 1.5×10^5 photons/flash) in a control situation (A) and with ionophoretic intracellular Cl^- -injection before (B), during (C), and after (D) the postadaptational potentiation (PAP). Steady-state ionophoresis with -2.5 nA current was started 1 min before light-adapting stimulus. Before the potentiation-inducing light-adaptation (2-s light adapting pulse 2×10^8 effective photons/s) the peak response was a depolarization above the resting potential ($E_{Cl} >$ resting potential). During the potentiation, response was transformed back into a hyperpolarization (C, $E_{Cl} <$ resting potential). After the potentiation the light-on responses recovered (D).

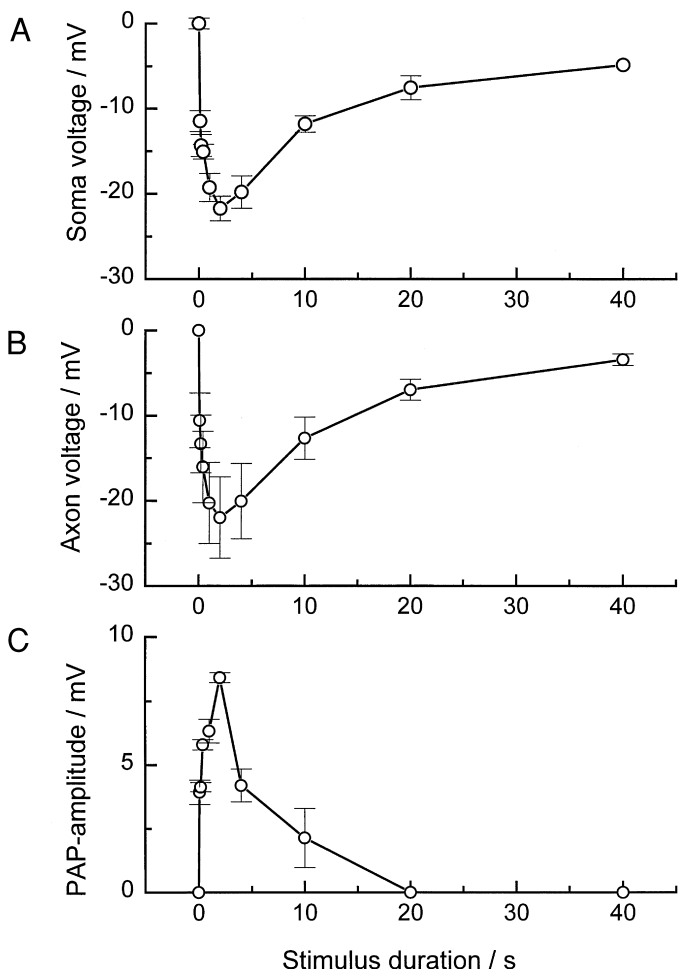


FIG. 6. Presynaptic pump-potential and postsynaptic potentiation (PAP) as a function of the duration of the light-adapting stimuli. Amplitude of the pump-induced hyperpolarization in (A) photoreceptor soma ($n = 5$) and (B) axon terminal ($n = 5$) increased from 100 ms to 2 s after which it started to decline. C: amplitude of the potentiation in LMCs ($n = 5$) increased similar to the pump-potential. After an adapting light pulse of 20 s, responses to test flashes in LMCs were not potentiated at all as the presynaptic voltage did not reach the critical voltage below which the transmitter release was found to be reduced.

the light-adapting stimuli, both in the photoreceptor somata and in the axon terminals. The second afterpotential started to mask the hyperpolarization caused by the Na^+ pump from a duration of 4 s onwards (data not shown). The afterpotential was also seen after preadaptation with green (555 nm) stimuli suggesting the noninvolvement of the prolonged depolarizing afterpotential (e.g., Hamdorf and Schwemer 1975). After >20 s of light adaptation the pump-induced presynaptic hyperpolarization did not reach the threshold for decreased transmitter release (~ 11 mV below the resting potential) and no postsynaptic potentiation was seen. The maximum amplitude of the potentiation corresponded well with the maximum hyperpolarization caused by the pump potential (Fig. 6).

Synaptic voltage transfer

We considered it important to characterize the function of the first visual synapse at voltages below the dark-adapted resting potential because the presynaptic resting potential is

relatively depolarized when compared with many other preparations, and in a tonic synapse these voltages could be used for signal coding (Laughlin 1987). Below the dark-adapted resting potential (i.e., during the pump potential) the gain of the synapse, as defined from the slope of the characteristic curve (the postsynaptic response in function of the presynaptic response, Fig. 7, e.g., Laughlin and Hardie 1978), was larger, especially just above the presynaptic threshold (-71 mV) for the transmitter release. During the potentiation the synaptic gain gradually decreased approaching values normally observed during dark-adapted conditions. This synaptic gain went even below the initial value from the presynaptic voltage of -55 mV onwards, when defined on the basis of presynaptic axon recordings. This was not surprising because the characteristic curve for the synapse below the dark-adapted resting potential was studied here immediately after light adaptation

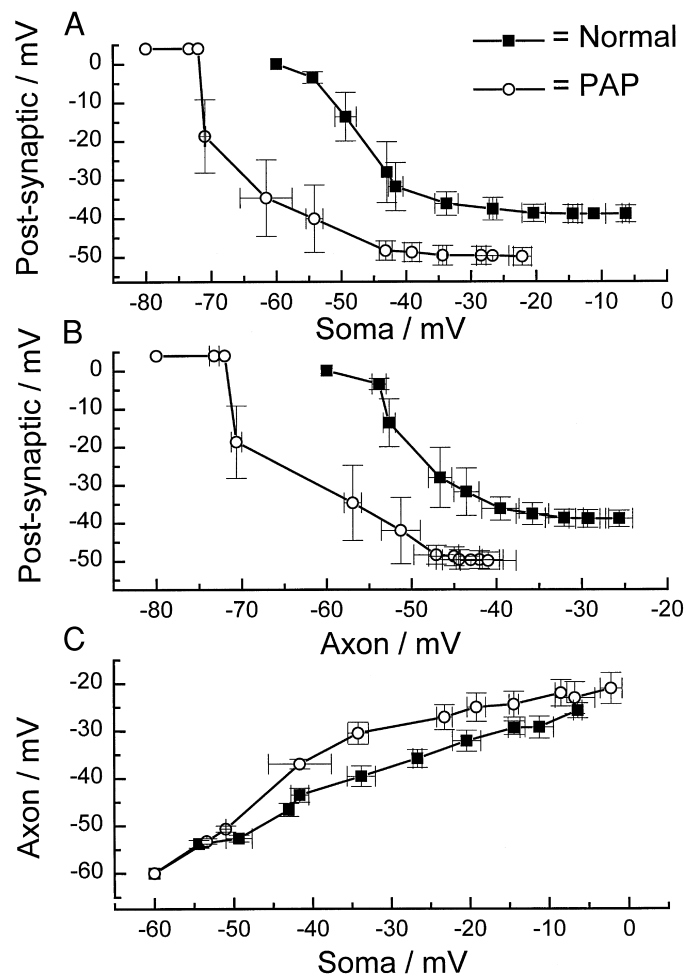


FIG. 7. Synaptic voltage transfer from the photoreceptors to LMCs. Characteristic curves were calculated in dark-adapted conditions (normal) and after light-adapting pulse of 2 s (PAP, $n = 5$). No functions are fitted to these equations because the one used earlier (Laughlin et al. 1987) was not applicable here and the true form is unknown. A: synaptic characteristic curve calculated from the photoreceptor somata to LMCs. Characteristic curve during the potentiation period (PAP) was very steep just near the "threshold" of transmitter release while decreasing steadily at higher presynaptic voltages. B: characteristic curve between axon terminals and LMCs. Slope was not as large, decreasing progressively with presynaptic voltage. C: characteristic curve between the photoreceptor somata and the photoreceptor axon terminals. This shows that activation of the fast depolarizing transient during the potentiation was partly behind the decreased gain of the synapse (see also Fig. 2).

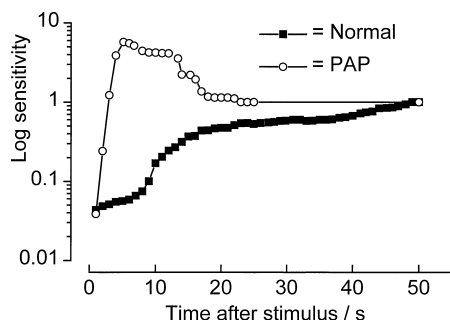


FIG. 8. Recovery of sensitivity in LMCs after light adaptation with 2×10^8 photons/s. Recovery is shown under conditions where the potentiation was manifested [i.e., after 2 s stimuli (PAP)] and when the potentiation was not seen [i.e., after the adaptation lasting 20 s (normal)]. During the potentiation the sensitivity after 3 s from the end of the light-adapting pulse was 2 log-units better than that without the potentiation. During a period of 4–18 s the sensitivity exceeded that of the initial dark-adapted sensitivity.

(i.e., during the potentiation). In contrast, the synaptic gain with small presynaptic voltages after the light adaptation was astonishingly high. The synaptic characteristic curve calculated from the axon terminals (Fig. 7B) showed a somewhat smaller synaptic gain increase with low voltages compared with those obtained on the basis of the soma recordings, probably related to the fast depolarizing transient in the axons.

Function of the potentiation

To study if the postsynaptic potentiation could have some contribution to the sensitivity recovery after light adaptation, we measured the recovery of subsaturated responses in the LMCs after saturating light adaptation (2×10^8 photons/s) with conditions where the potentiation was present and compared these results with those obtained in conditions without the potentiation. The amplitude of the flash responses was converted into intensities via the $V/\text{Log } I$ curve of the cells and from those on to sensitivities (in %). The sensitivity after the light adaptation under conditions without the potentiation (i.e., after strong light adaptation of 20 s) showed a more reduced sensitivity than expected. The sensitivity loss was more than tenfold and it recovered to normal after a period of ~ 50 s. Under normal conditions (i.e., without the potentiation) the recovery took place with two slow phases with a faster one in between (Fig. 8). During potentiation the sensitivity after adaptation was found to be recover to normal within 3 s. Thereafter it exceeded the normal sensitivity by almost eightfold. The sensitivity recovery after potentiation resembled the recovery of responses after mild light adaptation both in photoreceptors and LMCs (Laughlin and Hardie 1978). The difference in the sensitivity just after the light adaptation between these two conditions was 2 log-units in favor of the potentiation (Fig. 8). The sensitivity recovery should be faster with shorter light stimulation as demonstrated earlier (Laughlin and Hardie 1978), but normally it should not be enhanced beyond the dark-adapted level, which happens in this experiment. Accordingly, the potentiation could be an important mechanism in recovering the sensitivity loss after intense light adaptation.

DISCUSSION

The performance and adaptation of synapses where signals are transmitted with graded potentials are poorly understood.

We have utilized the first visual synapse in the fly compound eye (see reviews by Juusola et al. 1996; Laughlin et al. 1987) to investigate the processes after light adaptation (i.e., during dark recovery of the synaptic function). The dipteran visual system, like that of any other diurnal animal, is as frequently exposed to dark adaptation as it is to light adaptation. The efficiency of the processes underlying dark recovery can be argued to be of high significance to the synaptic function in general and to strongly contribute to the overall visual behavioral performance as well. As this study demonstrates, the light-on responses of LMCs are clearly enhanced after light adaptation. This enhancement, as judged from the time course, fulfills the criteria stated for the short-term potentiation (e.g., Zucker 1989) and thus can be called postsynaptic potentiation. The enhancement seems to be causally linked to tonic transmitter release in the photoreceptor–interneuron synapse (see Uusitalo et al. 1995a).

After light adaptation there seems to be two major processes operating simultaneously determining the DC voltage of the presynaptic photoreceptors. The first is the activation of the electrogenic Na^+/K^+ -ATPase (Fig. 9) that operates under stoichiometry of $\sim 3/2$ (Hamdorf et al. 1988; Jansonius 1990) and, when increasingly activated, hyperpolarizes the resting potential. The second is the process that depolarizes the resting potential after light adaptation with longer periods. This depolarization is likely to be related to the $\text{Na}^+/\text{Ca}^{2+}$ exchanger (Gerster et al. 1997; Hochstrate 1991; Minke and Kirschfeld 1984). It appears that light-adapting pulses lasting from 100 ms to 2 s cause an increase of the afterhyperpolarization (Fig. 6), which would be expected when the intracellular sodium concentration and thus the activation of the Na^+/K^+ pump increases (Jansonius 1990). When the stimulus duration is increased using the same light intensity, the hyperpolarizing pump-potential starts to decrease, thereby decreasing the potentiation amplitude in the postsynaptic LMCs as well (Fig. 6). The most obvious explanation for this is that the depolarizing afterpotential is increasingly masking the Na^+/K^+ pump potential, because the intracellular calcium rises to high levels (Oberwinkler and Stavenga 1998). Another process being activated as a result of the hyperpolarization of the resting po-

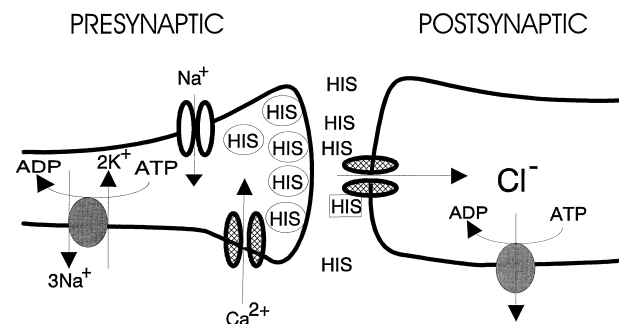


FIG. 9. Hypothesis for mechanism behind the postadaptational potentiation (PAP). The figure shows schematically the elements participating to generation of the afterpotentials and synaptic transmission in this histaminergic synapse. The presynaptic Na^+/K^+ -transporter is activated during light adaptation and hyperpolarizes the axon terminal, thereby inactivating the voltage-gated Ca^{2+} -channels responsible for the tonic transmitter release. During the decreased transmitter release the postsynaptic tonic Cl^- -conductance is decreased whereas the Cl^- -extrusion mechanism putatively a (Cl^- ATPase) remains operational. Activation of this complex chain of events leads to decreased intracellular Cl^- -concentration in the postsynaptic LMCs, a negative shift in the reversal potential of Cl^- , and thus to potentiation.

tential is the increasing amplitude of the fast depolarizing transient in the presynaptic axon terminals (Weckström and Laughlin 1995; Weckström et al. 1992a). When the resting potential is lowered, the fast depolarizing transient seems to be activated more (Fig. 2). Although the exact mechanism of this is beyond the scope of this paper, we may safely assume (see Weckström et al. 1992a) that it is caused by voltage-gated ion channels, analogously as in the bee photoreceptors (Vallet and Coles 1993; Vallet et al. 1992). The activation of the fast depolarizing transient is not directly linked to potentiation, however, because even the saturated LMC responses were clearly enhanced during the potentiation (see Figs. 1–3) and the increase of the presynaptic depolarization should not increase the postsynaptic response when the postsynaptic responses have reached saturation. On the other hand, the more transient nature of the LMC responses during potentiation may well be caused by the increased presynaptic fast depolarizing transient (Fig. 2).

Further exclusions can also be made. The mechanism behind potentiation cannot be increased sensitivity of the transmitter release mechanism in the photoreceptors. This increase and the resulting increased Cl^- conductance in the already saturated LMCs are not able to increase the response amplitude without a change in E_{Cl} . Another possibility would be an additional synaptic input to LMCs that would be activated after strong light (e.g., Hardie et al. 1989; Nässel 1987; Weckström et al. 1989). This would increase the conductance to some ions during the responses in the LMCs. Thus the activation of additional conductances would mean a drop in the R_{in} of the LMCs. Contrary to this hypothesis, we found that in the LMCs the R_{in} increased during the potentiation by 33%. Another possibility is that the ~ 4 mV steady-state depolarization of the LMC membrane potential could, by voltage-sensitivity of the histamine-gated channels, account for the potentiation. We have no evidence for this but it has been shown previously that the histamine-gated channels have only very weak voltage sensitivity that could not explain the potentiation (Hardie 1989).

As judged from the characteristics of the membrane noise (Fig. 4), the transmitter release seems to be decreased when the presynaptic axon terminals hyperpolarize. It is known that the release of the synaptic vesicles containing the transmitter is caused by activation of Ca^{2+} channels. Thus an increase in the release reflects the increase of the presynaptic intracellular free Ca^{2+} (Llinas 1982). The decrease in the transmitter release could be directly related to this if the probability of the Ca^{2+} channels being open is drastically decreased below the voltage of ~ 11 mV under the normal resting potential (-60 – -65 mV) of the photoreceptor axons. This means a relative threshold for the Ca^{2+} -channel activation of approximately -71 to -76 mV. This activation threshold does not agree with properties of the L-type channels likely to dominate the Ca^{2+} current in the presynaptic membrane (Juusola et al. 1996) but it agrees with findings in the axons of barnacle photoreceptors (Hayashi and Stuart 1993).

What is causing the enhancement of the postsynaptic LMC responses if the potentiation mechanism is substantially postsynaptic? The signal transfer in the photoreceptor-LMC synapse is relatively nonlinear (Juusola et al. 1995b). As a component of the nonlinearity, the LMCs have been reported to have an efficient Cl^- -extrusion mechanism to maintain a

stable ionic composition (Uusitalo and Weckström 1994). Apparently, during light adaptation the intracellular chloride accumulation exceeds the capability of the extrusion mechanism for the ion, resulting in depolarization of the resting potential and a decrease in the peak amplitude of the hyperpolarizing response (Uusitalo and Weckström 1994). It is conceivable that during the period of reduced transmitter release (the afterhyperpolarization, presynaptically) the chloride extrusion would still operate, at least for a relatively short period, as in normal conditions. This would cause a negative shift of the equilibrium potential of the histamine-gated conductance. During strong illumination the extracellular field potential is being depolarized by up to 25 mV (P. Kettunen, S. B. Laughlin, and M. Weckström, unpublished observations), rendering the transmembrane potential in the LMCs more hyperpolarized, which would still accelerate the Cl^- extrusion.

The ionophoresis of Cl^- into the LMCs (Fig. 5) clearly shifted the E_{Cl} to be more positive than the resting potential thus reversing the polarity of the responses. The potentiation after light adaptation appeared here with the usual time course but was clearly enhanced in size. This was a further indication that the Cl^- -extrusion mechanism was causing the potentiation. The extrusion process speeds up with increasing intracellular chloride (Uusitalo and Weckström 1994). The small 4 mV depolarization observed normally was not seen after Cl^- injection which was caused by the smaller Cl^- driving force when the E_{Cl} was near the resting potential and when the drop in the resting Cl^- influx did not change the membrane potential.

The recovery of sensitivity by the enhancement of light-on responses (Fig. 8) is not apparent under all conditions. This takes place in a "potentiation window" (determined by the preceding stimulation) in which the responses are enhanced and the sensitivity loss is recovered. This window extends in time from light-adapting durations from 100 ms to almost 20 s (Fig. 6). As we do not have enough behavioral evidence, we can only hypothesize about the conditions in which this recovery mechanism could be of functional significance. However, the blowfly is usually operating in daytime and normally moves constantly with considerable velocity, flying above and among (possibly moving) objects. For a fast moving animal, an adequate visual performance under all conditions is of crucial importance. This is true not only in dark- and light-adapted conditions but also during periods between them when shadows and sunshine are alternating. Our hypothesis is that the visual system is operating quite poorly when the fly moves from shadow to full sunshine, during which the light-adapting stimulus well resembles the one used here to elicit the potentiation. The loss of visual function is caused at least partially because the light-adapting process takes time whereas the gain in the first visual synapse remains high. It has been reported that the light-adapting process after intense light adaptation can take up to 2 min in the synapse (Juusola et al. 1995a). During this time it would be convenient for the fly to go back to shadow again to recover the adequate visual performance. The mechanism presented in this paper would allow the fly to spend a period of up to 20 s in light-adapted conditions and still recover the sensitivity very rapidly (Fig. 8) when reaching the shadow again, a clear behavioral advantage.

Regardless of the possible functional and behavioral significance of the potentiation, it has to be recognized that the

regulation of the Cl^- homeostasis in these interneurons is of great importance. As shown earlier, the Cl^- equilibrium potential is depolarized during light adaptation (Uusitalo and Weckström 1994). It was shown in this paper that the E_{Cl} is being shifted to a more negative value after light adaptation. Both of these changes require a role of the Cl^- -extrusion mechanism (Fig. 9) and suggest that modulation of the synaptic transmission via this Cl^- pump is of importance. The function of neurons is crucially dependent on different ion conductances and their modulation. The present evidence shows that the action of ion exchangers opposing the ionic conductances has to be given greater emphasis, at least in small graded potential neurons where the changes in intracellular ionic composition is relatively easily changed. These neurons have difficulties maintaining a constant ionic composition because they use very large ion fluxes compared with their size to generate the signals. Whereas this clearly is a problem, the fly LMCs have also developed a way of taking advantage of this property, which is to decrease the sensitivity loss after light adaptation.

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