

The Dark Adaptation in Single Visual Cells of the Compound Eye of *Aeschna cyanea*

Hansjochem Autrum* and Gertrud Kolb
Zoologisches Institut der Universität München

Received June 6, 1972

Summary. 1. Light and dark adaptation in single visual cells of *Aeschna cyanea* were examined by intracellular recording of the receptor potentials. A description of the characteristics of the receptor potential is given in Figs. 3 and 4.

2. A light stimulus of several seconds (light adaptation) is succeeded by an afterpotential of the receptor potential. In most cells, this afterpotential represents a decrease of the depolarization (Fig. 5) until the resting potential is recovered. Some cells, however, show temporary hyperpolarization during the afterpotential (Fig. 7 a).

3. The course of the dark adaptation was determined by test stimuli. This means that the sensitivity of the receptor was measured during the dark adaptation period. The afterpotential cannot be regarded as standard for the sensitivity of the receptor. The sensitivity will as well increase during the hyperpolarization period of an afterpotential. Even if after light adaptation the receptor potential has returned to the level of the resting potential, the sensitivity may still be lower than at maximum dark adaptation. Receptor potential and sensitivity for light stimuli do not correlate.

4. The maximum change of the sensitivity of a visual cell, caused by adaptation, is given by the factor 10^3 .

5. The dark adaptation is accelerated if the test stimuli during the dark adaptation are of higher intensity (Fig. 14). This is probably connected with reisomerization of the visual pigment by light, but limited, however, to stimuli of an intensity which is not high enough to cause light adaptation for its part (Figs. 9, 10).

6. The visual cells of the ventral eye region are slightly more sensitive than the dorsal ones (Fig. 14).

Introduction

Previous investigations concerning the adaptation of the compound eye used the ERG to prove the change of sensitivity (e.g. bees: Goldsmith, 1963; Seibt, 1967; *Dixippus*, *Tachycines*, *Calliphora*: Autrum, 1950; Hamdorf and Kaschef, 1965; *Drosophila*: Cosens, 1971). The ERG, however, yields only summated effects; the adaptation rate of single visual cells cannot be examined by means of the ERG. Nevertheless, the results show that the eyes of certain insects (*Apis*, *Calliphora*, *Drosophila*) adapt very quickly.

* Supported by a grant of the Deutsche Forschungsgemeinschaft.

In the following section the adaptation of single visual cells in the imaginal eye of *Aeschna cyanea* will be described. The spectral sensitivity of these visual cells is known (Autrum and Kolb, 1968; Eguchi, 1971). The most frequent maximal sensitivity of receptors in the ventral region of the eye is $\lambda_{\max} = 494/519$ nm, in the dorsal region $\lambda_{\max} = 445/458$ nm.

Methods

Animals. Most of the experiments were made on imagines of *Aeschna cyanea* hatched from their larvae the day before. Only occasionally 2 to 5 days old individuals were used. Up to their imaginal molting, the larvae were kept in aquaria.

Preparations were carried out according the method described in detail in Autrum and Kolb (1968); in case pulsations still occurred, one drop of nicotine salicylate dissolved in Ringer solution (0.002 g/100 ml) was dripped upon the cerebral ganglion.

Technical Equipment (Fig. 1): The light source is a Xenon high pressure lamp (900 W). The light is focussed by means of a quartz lens system *L* and conveyed to the eye through a flexible light guide *Ll* (Schott & Gen.). In the optical path are placed: 1. A cold mirror (Balzers) and a KG-glass (Schott & Gen.) for the absorption of heat radiation; 2. An interference filter (Schott & Gen.) generating monochromatic light and calibrated neutral filters (Balzers) regulating the intensity. 3. The adaptation unit (see below); 4. A diaphragm immediately in front of the eye in order to be able to work with a very narrow light beam.

The adaptation unit *G* (Fig. 1, bottom left) always works with the whole diameter of the light beam. Fig. 1 shows it in the position for light adaptation. The light enters through an opening next to the shutter lid *V*. During light adaptation the magazine *Mag* is situated in the upper position, whereas it is situated in the lower position (arrow) during the application of test stimuli. The magazine contains the holding inserts *E* for the neutral density filters. The intensity of the test stimuli is determined by the neutral filters in the upper opening of the insert. If stimuli are applied during light adaptation, no filter is positioned in the lower opening. — The duration of the test stimuli is 50 msec.

To examine the course of adaptation in a visual cell, above all the quick phases of dark adaptation must be registered immediately after the end of a light adaptation. The adaptation unit *G* allows the application of the first test stimulus just 40 msec after the completion of the light adaptation. The change from adapting light to dark adaptation as well as the duration and sequence of the test stimuli during the first 30 sec of the dark adaptation is electronically controlled by means of the control unit *StG* (Smola, 1965), via the solenoids *Sp*. The first 7 test stimuli are released automatically within the first 31 sec of dark adaptation in the following sequence: 0.5, 1, 2, 4, 8, 16, 31 sec after the end of light adaptation. When this automatic sequence is completed, further test stimuli are released manually every 30 sec. All these test stimuli are of equal intensity.

The eye is positioned in the center of a sphere (Fig. 2). A cap of this sphere, made of highly polished steel, lies in the corresponding recess of a magnet *M* in a gliding manner. This magnet retains the spherical cap in any position. By turning or tilting the cap, the head and the eye can be adjusted to the light beam in any direction required, without the distance between eye and light guide being changed.

Recording. Intracellular recording through capillary glass tubes (3 mol KCl), diameter of tip opening 0.5–0.1 μm ; resistance 35–70 megohm. Indifferent electrode connected to the thorax muscles.

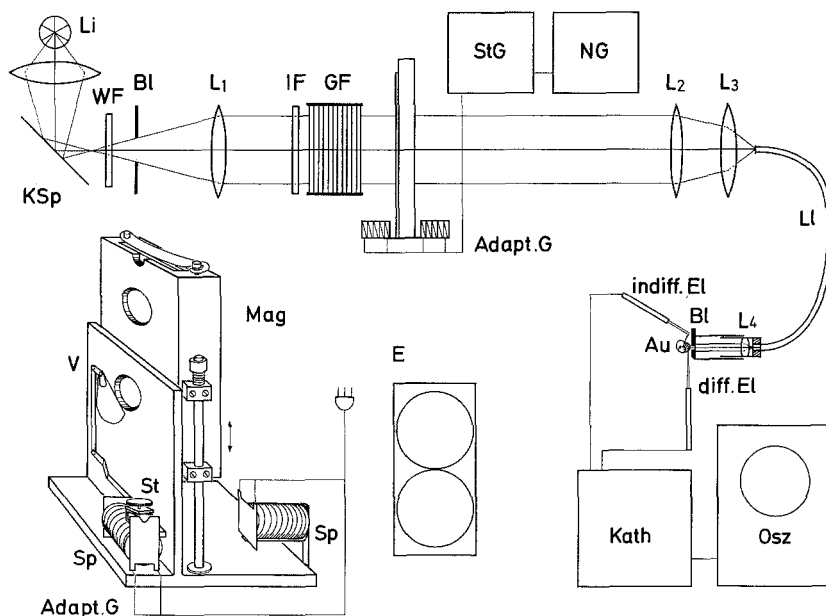


Fig. 1. Schematic diagram of the equipment. *Li* light source; *KSp* cold mirror; *WF* heat filter (*KG*-glass); *Bl* diaphragm; *L₁₋₄* lenses; *IF* interference filter; *GF* grey filter; *Adapt. G* adaptation equipment; *StG* control unit; *NG* power section; *Ll* light guide; *Au* eye; *indiff. El* indifferent electrode; *diff. El* different electrode; *Kath* cathode follower; *Osz* oscilloscope; *E* insert; *Mag* magazine; *V* shutter lid; *Sp* coil; *St* adjustment screw

The following criteria are regarded as evidence that the receptor potential of a visual cell is being recorded: 1. During the insertion of the microelectrode into the visual cell, the potential must drop immediately at least 25 mV (in most cases the potential decreases about 50–70 mV). 2. The receptor potential depolarizes monophasically.

For all visual cells we studied the maximum spectral sensitivity was measured (see Autrum and von Zwehl, 1964). Corresponding to each maximum, the light adaptation is made with $\lambda = 445$ nm (especially on receptors of the dorsal region), or with $\lambda = 495$ nm (especially on receptors of the ventral region, λ_{max} between 494 and 519 nm); the same wave-lengths are used to measure the course of the dark adaptation.

The light guide *Ll* is fixed to a rotation system (Fig. 2) so that the emerging light beam can rotate around the eye, remaining centered on it. The horizontal and vertical rotation axes intersect at the geometric center of the sphere. By means of roller bearings (Fig. 2), the rotation around the vertical axis can go on constantly and without vibrations. While a constant test stimulus is applied, the position of the light guide is adjusted so that the receptor potential reaches its maximum value. Then the light enters the ommatidium along its optical axis. — Checks, carried out during the experiments, show that the angle of the optical axis oc-

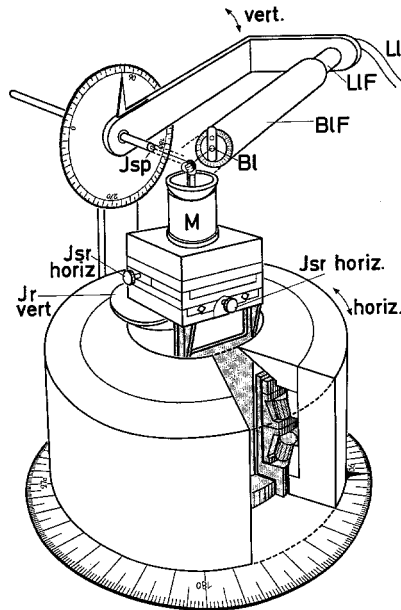


Fig. 2. Mount for eye light guide. *Bl* insert for diaphragm; *BlF* diaphragm holder; *Jsr* adjustment screw (horizontal movement); *Jr* adjustment disk (vertical movement); *Jsp* adjustment pin; *Ll* light guide; *LlF* light guide holder; *M* magnet holding spherical cap with eye

asionally shifts about 1–2° horizontally or vertically. This is probably due to a slow desiccation process at the surface of the retina cut. Only in rare cases a correction of the angle of incidence is necessary later on in the experiment.

Altogether 268 adaptation experiments on 29 receptors of the dorsal region, and 192 adaptation experiments on 11 receptors of the ventral region of the eye were analyzed. The temperature was between 22 and 25° C, occasionally 27° C.

Measurement of the Absolute Intensities. The absolute light intensity which strikes the eye ($\mu\text{Watt}/\text{cm}^2$) is determined by comparing it to a calibrated radiation receiver. The tolerances of this method are about $\pm 20\%$. In logarithmic scale this tolerance value (referring to the respective intensity = 1) corresponds to a deviation of -0.1 (-20%) and $+0.08$ ($+20\%$).

Duration of the Stimuli. Since the maximum amplitude of the receptor potential is already reached on test stimuli of 50 msec, only stimuli of such short duration are applied. The light adaptation time is 1, 5, 10 or 30 sec.

For tests made with absolute light intensities, the ratio of the intensity of the test stimulus to the intensity of the adapting light is determined by the transmission rate of the neutral filters applied during the release of the test stimulus. For the most part a test intensity of $1/10$, $1/100$, or $1/1000$ is chosen, only occasionally $1/2$ or $1/5$.

The intensity of an adapting light is fixed by means of neutral filters in front of the adaptation unit, the intensity of the test stimulus by additional neutral filters in the magazin of the adaptation unit.

Terminology and Analysis of the Results

For all measurements the maximum amplitude of the receptor potential is specified.

Efficiency. The efficiency of an equal test stimulus is given by the amplitude of the potential (in mV).

Sensitivity. Sensitivity curves during dark adaptation show the relative sensitivity of the receptor as function of the dark adaptation time. The sensitivity of a receptor is determined by the efficiency of the light stimulus and its dependence on intensity. Prior to each light adaptation, every 6 seconds (every 10 seconds for higher intensities) the dark adapted receptor is stimulated by a test stimulus of 50 msec. The amplitude of the receptor potential remains constant while these stimuli are applied. Thus the stimuli do not influence the adaptation level of the receptor. The amplitude of these constant potentials corresponds to the maximum sensitivity of the receptor in the dark adapted state. Between the different adaptation tests, the potential amplitude of each visual cell is measured as a function of the intensity. In this way misinterpretations due to base line fluctuations during longer experiments can be avoided. For calculation of the relative sensitivity after a light adaptation, the sensitivity curve of the same visual cell is used which has been established right before or after the respective adaptation tests.

Potential Type and Stimulus Intensity

The potential type of a dark adapted visual cell, dependent on the stimulus intensity of flickering light, is investigated on receptors of the dorsal and ventral eye region of imagines.

1. Dorsal Region of the Eye (Fig. 3)

Stimulus Intensity 0.01–0.1 $\mu W/cm^2$; λ_{\max} of the Visual Cells = 445 nm (Fig. 3A). In case of exposure to very low intensities, the receptor potentials will rise slowly to a plateau (oblique leading edge), and return to the resting level, or slightly below, after the light is switched off.

Stimulus Intensity 0.1–10 $\mu W/cm^2$; λ_{\max} of the Visual Cells = 445 nm (Fig. 3B). The depolarization rises faster within this intensity range, and, while exposure is still occurring, will decrease more slowly after having reached the maximum. When the stimulating light is switched off, the potential returns to the resting level, declining slowly in the beginning, then more abruptly (5 visual cells).

Stimulus Intensity 10–40 $\mu W/cm^2$; λ_{\max} of the Visual Cells = 445 nm (Fig. 3C). Within this intensity range the depolarization rises faster. After having reached the maximum depolarization, the potential will decrease stronger, exposure still occurring. The afterpotential, following the end of the stimulus, comprises two phases: a) Sometimes the afterpotential shows another smaller peak after the first one (Fig. 3C₂); in any case there will be a distinct hump (Fig. 3C_{1,3,4}). b) During the second phase, the afterpotential gradually returns to the resting level (Fig. 3C_{1,4}).

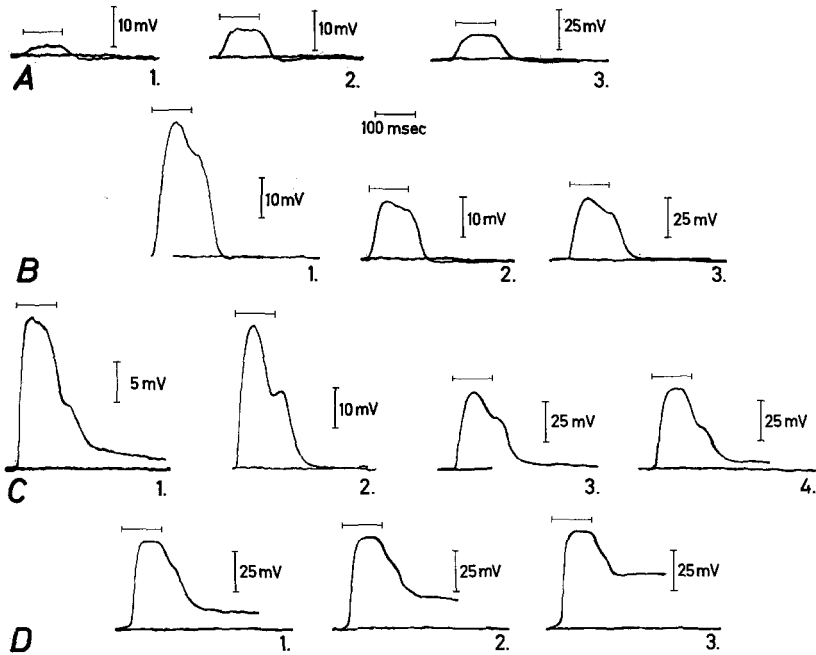


Fig. 3. Receptor potentials in dark adapted visual cells of the dorsal eye region with varying stimulus intensities. The time mark above the particular potentials gives the stimulus period of 100 msec each. This marking by means of a photo cell was not carried out synchronously to the registering of the receptor potential. It was done after each test, and was marked above the potential diagrams afterwards. A 1-3: λ_{\max} of the visual cells 445/458 nm. Intensities of the monochromatic light stimulus ($\lambda = 445$ nm): 1: $\sim 0.01 \mu\text{W}/\text{cm}^2$; 2: $\sim 0.05 \mu\text{W}/\text{cm}^2$; 3: $\sim 0.1 \mu\text{W}/\text{cm}^2$. Two different visual cells (1-2, 3). B 1-3: Potentials from three different visual cells; light stimulus: $\lambda = 445$ nm. 1: $\sim 10 \mu\text{W}/\text{cm}^2$; 2: $\sim 0.2 \mu\text{W}/\text{cm}^2$; 3: $\sim 1.7 \mu\text{W}/\text{cm}^2$. C 1-4: Potentials from three different visual cells. 1: $\sim 24 \mu\text{W}/\text{cm}^2$ (stimulating light $\lambda = 495$ nm); 2, 3: $\sim 10 \mu\text{W}/\text{cm}^2$ (stimulating light $\lambda = 445$ nm); 4: $\sim 35 \mu\text{W}/\text{cm}^2$ (stimulating light $\lambda = 445$ nm). D 1-3: Potentials from one visual cell. 1: $\sim 129 \mu\text{W}/\text{cm}^2$; 2: $\sim 260 \mu\text{W}/\text{cm}^2$; 3: $\sim 1287 \mu\text{W}/\text{cm}^2$ (stimulating light $\lambda = 445$ nm)

Stimulus Intensity 130-1300 $\mu\text{W}/\text{cm}^2$; λ_{\max} of the Visual Cells = 445 nm (Fig. 3D). The rising edge of the receptor potential is very steep. The afterpotential comprises two phases here as well: a) During the first phase of the afterpotential, the decreasing depolarization shows a small peak; b) During the second phase, the afterpotential only gradually approaches the resting level (Fig. 3D), and, the higher the intensity of the stimulus, the more slowly the afterpotential will decline. In case of high stimulus intensities ($> 10 \mu\text{W}/\text{cm}^2$; $\lambda = 445$ nm), the receptor

potential shows a peak after the end of the light stimulus (Fig. 3 B, C, D). The rise of the positive afterpotential in the ERG of *Calliphora*, following the end of a long stimulus, which was registered by Autrum (1950), may correlate with these results.

2. Ventral Region of the Eye

Within the ventral region of the eye, the types of potentials and their maximum depolarization vary from cell to cell (Fig. 4). Amplitude and duration of the afterpotentials relate to a high efficiency of the stimulating light on the cells: Amplitudes of the afterpotential, which occur in the dorsal eye region starting with $24 \mu\text{W}/\text{cm}^2$ or more, can be obtained in the ventral eye region even starting with $0.6 \mu\text{W}/\text{cm}^2$ (Fig. 4B₁). In the ventral region, the depolarization will already come close to the maximum with stimuli of $6 \mu\text{W}/\text{cm}^2$ (Fig. 4B). Unlike the amplitude of the afterpotential, this maximum remains constant with increasing intensity of the stimuli.

An increase of the stimulus intensity by the factors 10, 100, or 1000 causes a rise of the afterpotential. The duration of the depolarization maximum, however, remains unchanged in spite of increasing stimulus intensity. On 2 cells only, from 136, the maximum was prolonged by rising stimulus intensity (Fig. 4B_{2,3,4}).

The Course of the Repolarization after the End of a Stimulus

The resting potential represents the voltage across the membrane of a visual cell in a completely dark adapted state. The visual cell will respond to a light stimulus by a receptor potential; the repolarization process varies with different visual cells (Figs. 5 and 7).

The following types of repolarization, succeeding the end of a light adaptation, can be discerned:

Type I. After the end of a light adaptation, the depolarization will decline rapidly within the first 45 msec and then continuously approach the baseline during the following seconds. — Fig. 5 shows the response of a visual cell (Prot. Nr. 157/1) from the dorsal eye region after a light adaptation with monochromatic light of wavelength 445 nm, and the repolarization during dark adaptation. The time of light adaptation during the experiments, represented by Fig. 5a—c, takes 10 sec. The light intensity (J_A) during light adaptation is raised successively from $0.9 \mu\text{W}/\text{cm}^2$ (Fig. 5a) to $10 \mu\text{W}/\text{cm}^2$ (Fig. 5b), and to $260 \mu\text{W}/\text{cm}^2$ (Fig. 5c). The repolarization after $J_A = 0.9 \mu\text{W}/\text{cm}^2$ will just come close to the baseline within 50 msec; only in the course of the approximately next 6 sec the baseline will be reached (Fig. 5a). If J_A is raised to $10 \mu\text{W}/\text{cm}^2$, not only the depolarization will rise, but the abrupt dropping

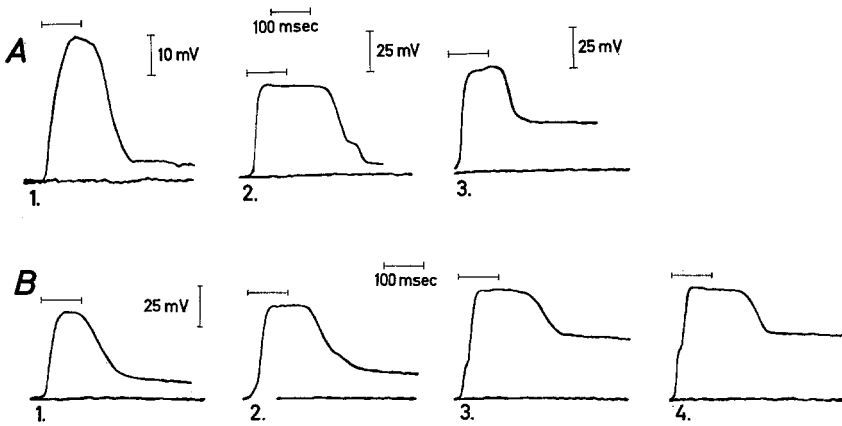


Fig. 4. Receptor potentials from visual cells ($\lambda_{\max} = 494/519$ nm) of the ventral eye region. A 1-3 from three different visual cells with equal intensity of stimulus ($142 \mu\text{W}/\text{cm}^2$). B 1-4 from one visual cell upon intensities of 1: $\sim 0.6 \mu\text{W}/\text{cm}^2$; 2: $\sim 6 \mu\text{W}/\text{cm}^2$; 3: $\sim 74 \mu\text{W}/\text{cm}^2$; 4: $\sim 760 \mu\text{W}/\text{cm}^2$. Stimulus period: 100 msec

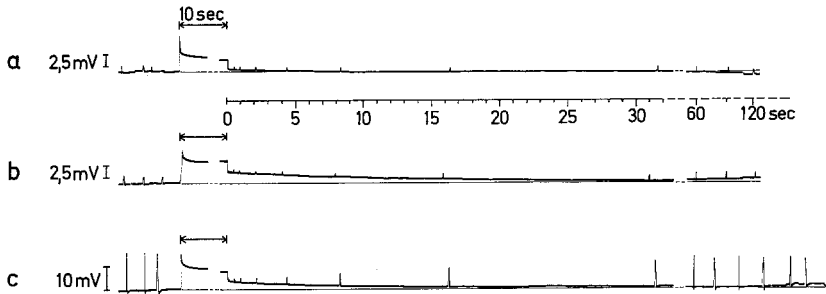


Fig. 5. An adaptation experiment on *one* visual cell in the dorsal region of the eye (Prot. Nr. 157/1) with monochromatic light ($\lambda = 445$ nm). Left: The level of the resting potential in dark adapted state and the depolarization during the light adaptation. Beginning and end of the light adaptation are marked by peaks of arrows; light adaptation time 10 sec. Light intensity J_A during the light adaptation in $\mu\text{W}/\text{cm}^2$ ($\lambda = 445$ nm) for a: 0.9; b: 10; c: 260; (ratio of the test stimulus intensity $J_T: J_A = 1/10$). Center and right hand: Course of the repolarization of the potential in the dark; the repolarization begins at the end of the light adaptation

of the depolarization, which starts at the end of the light adaptation, will not approach the base level up to 45 msec as closely as after low adapting light intensities ($J_A = 0.9 \mu\text{W}/\text{cm}^2$). Accordingly, in case the adapting light intensity is raised to $10 \mu\text{W}/\text{cm}^2$, the repolarization process will take about 16 sec. After a light adaptation to $260 \mu\text{W}/\text{cm}^2$, the repolarization will take up to 30 sec. An intensity increase of the adapting

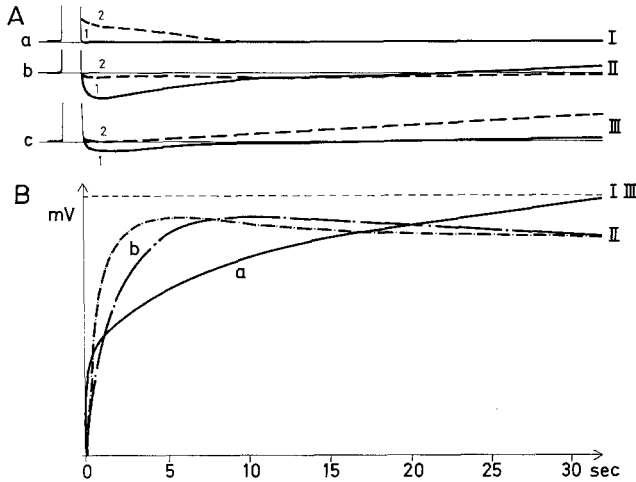


Fig. 6A and B. Schematic diagram of the repolarization (A) which follows the end of a light adaptation, and adaptation curves (B) during dark adaptation. Abscissa: Time of dark adaptation in seconds, beginning with 0 sec at the end of light adaptation. Ordinate: mV. Hyperpolarization downward, depolarization upward. A: Types of repolarization of the receptor potential during dark adaptation. a: Thin line: resting potential; fat line: repolarization after low intensity of adapting light, or short duration of light adaptation; interrupted line: repolarization after higher intensity of adapting light. Any intermediate stage between a_1 and a_2 will be possible, according to the intensity of the adapting light (J_A). b: Continued: hyperpolarization with succeeding slight depolarization; interrupted: hyperpolarization. Intermediate stages between b_1 and b_2 . c: The repolarization changes to a slight hyperpolarization, and the receptor potential returns to the resting level, followed by slight depolarization. Between c_1 and c_2 intermediate forms. B: Adaptation curves, established by determination of the sensitivity to a constant test stimulus. Function of the levels of the response potentials (in mV) to a constant test stimulus during dark adaptation (in sec). a and b: See text. I, II, and III: Types of dark adaptation curves

light, while the light adaptation period remains constant, will thus prolong the repolarization time. The repolarization of this visual cell (Prot. Nr. 157/1) always follows type I, as shown in Fig. 6Aa. In case of adaptation to equal light intensity (e.g. visual cell J 164/3 from the ventral eye region, $J_A = 0.6 \mu\text{W}/\text{cm}^2$, $\lambda = 495 \text{ nm}$) a longer light adaptation period will also prolong the succeeding repolarization time. After light adaptation periods of 5, 10, and 30 sec, the resting potential of this visual cell will be reached in the dark within 2, 6, and 8 sec respectively, after the end of the light adaptation.

Type II. After the end of a light adaptation, *hyperpolarization* will take place within the first seconds (Fig. 6Ab and 7a). This hyperpolarization returns to the resting potential within 30 sec or even later.

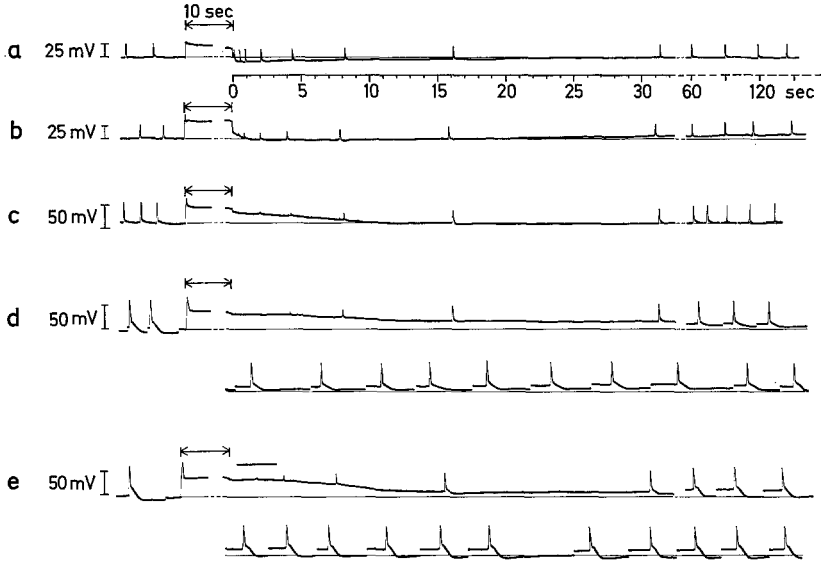


Fig. 7. Adaptation of *one* visual cell in the ventral region (Prot. Nr. I 162/1) to monochromatic light ($\lambda = 495$ nm) of different intensities (J_A); equal light adaptation time: 10 sec. The following was registered: 1. Level of resting potential in dark adapted state. 2. Response to the light adapting stimulus by depolarization, beginning and end of which is marked by peaks of arrows. — J_A : intensity during light adaptation; $J_T: J_A =$ intensity ratio between test and light adapting stimulus. a: $J_A = 0.07 \mu\text{W}/\text{cm}^2$, $J_T: J_A = 1/2$; b: $J_A = 0.6 \mu\text{W}/\text{cm}^2$, $J_T: J_A = 1/100$; c: $J_A = 6 \mu\text{W}/\text{cm}^2$, $J_T: J_A = 1/100$; d: $J_A = 74 \mu\text{W}/\text{cm}^2$, $J_T: J_A = 1/100$; e: $J_A = 760 \mu\text{W}/\text{cm}^2$, $J_T: J_A = 1/100$; 3. Repolarization in the dark after end of light adaptation. 4. The depolarizations caused by test stimuli, superimposed to the resting potential, respectively the repolarization potential

On some cells a slight depolarization may follow (Fig. 6A b, continued). On visual cells which in the dark, after the end of a light adaptation, i.e. during dark adaptation show hyperpolarization after light adaptations to rising intensity, the following types of repolarization may occur:

Type IIa. Light adaptations to rising intensity always lead to increased hyperpolarization.

Type IIb. On other cells, however, hyperpolarization will develop in a more complicated way (Fig. 7a—e). In case of low intensities of the adapting light (0.07 – $0.6 \mu\text{W}/\text{cm}^2$), hyperpolarization will increase with increasing intensity of the adapting light (Fig. 7a, b). If the adapting light intensity is raised to $6 \mu\text{W}/\text{cm}^2$, no more hyperpolarization will occur, and the potential will return after light adaptation slowly and continuously to the resting level (Fig. 7c). With higher intensities of adapting light (about $74 \mu\text{W}/\text{cm}^2$), the depolarization of the receptor potential

will decrease very slowly (Fig. 7d); there will be no hyperpolarization. In case of further increase of the adapting light intensity up to $760 \mu\text{W}/\text{cm}^2$, depolarization will decrease first, but after about 1 sec of dark adaptation, there will be a slight depolarisation rise of short duration (Fig. 7e, marked).

On type II as well, it will be possible to obtain an increase in hyperpolarization by extending light adaptation (5, 10, and 30 sec) to a constant light intensity. Repolarizations following type I, IIa, or IIb can be specific to a cell. Then a visual cell might show repolarization after light adaptation corresponding to only one of these types. In most cases, however, the type of repolarization will vary for the same visual cell. — Often intermediary repolarization types are found.

The Course of the Dark Adaptation

During dark adaptation, the sensitivity of the visual cells is examined using test stimuli of 50 msec and of varying intensity. The response to the test stimulus will always be another depolarization, superimposed on the repolarization of the light adaptation potential. The level of this response is determined with reference to the respective de- or hyperpolarization grade (only later when the repolarization towards the resting potential will be completed, will the reference be the resting potential). The function of the value of this response, dependent on the time of dark adaptation (adaptation curve), may differ for different visual cells (Fig. 6B):

1. The response level rises monotonically and will attain the maximum amplitude, which it showed before on complete dark adaptation, within about 30 sec—in accordance with the light adaptation conditions even later (Fig. 6Ba). This may as well be the case for visual cells, whose repolarization follows the pattern of Fig. 6Aa, as for cells following Fig. 6Ac.

2. The response level reaches a maximum within the first 5 to 10 sec; this maximum is not higher than the response to a test stimulus during complete dark adaptation. After this, the response level will decline within the subsequent 25–20 sec of dark adaptation and will regain the level of the test potential, which it holds during maximum dark adaptation, within the next 60 sec or longer (depending on the light adaptation conditions). This development pattern of potential levels during dark adaptation, will occur on visual cells which, after light adaptation, react with hyperpolarization (Fig. 6Bb).

Dark Adaptation after Light Adaptation to Different Intensities

In the following section the light intensity during light adaptation will be varied up to 5 logarithmic units; the light adaptation time remains

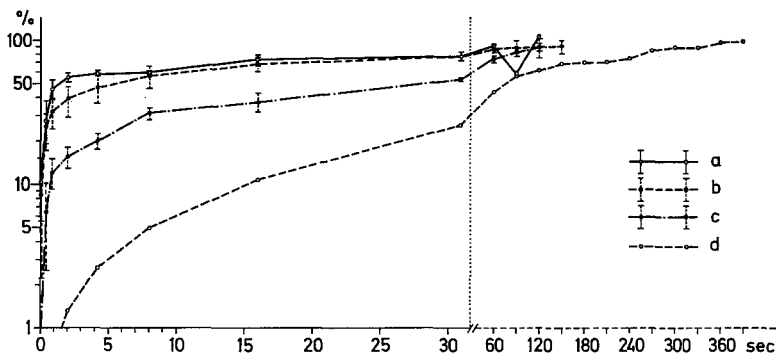


Fig. 8a—d. Adaptation curves of a visual cell ($\lambda_{\max} = 495$ nm) in the ventral eye region. $J_T = 0.1 J_A$; light adaptation time = 10 sec. a: $J_A = 0.07 \mu\text{W}/\text{cm}^2$; $J_T = 0.007 \mu\text{W}/\text{cm}^2$ (curve of average values from 2 experiments); vertical line shows maximum and minimum values. b: $J_A = 0.6 \mu\text{W}/\text{cm}^2$; $J_T = 0.06 \mu\text{W}/\text{cm}^2$ (curve of average values from 12 experiments); vertical line shows standard deviation. c: $J_A = 6 \mu\text{W}/\text{cm}^2$; $J_T = 0.6 \mu\text{W}/\text{cm}^2$ (curve of average values from 2 experiments); vertical line shows maximum and minimum values. d: $J_A = 74 \mu\text{W}/\text{cm}^2$; $J_T = 7.4 \mu\text{W}/\text{cm}^2$ (single curve). Ordinate: sensitivity in percent; Abscissa: time of dark adaptation in seconds

constant (10 sec). The ratio of the test stimulus intensity to the intensity of the adapting light also remains constant ($J_T : J_A = \text{const}$). Checks make sure that the same cell reacts with equal responses if a specific light adaptation is repeated.

$J_T = 0.1 J_A$; Duration of Light Adaptation 10 sec. Fig. 8 shows adaptation curves of a receptor from the ventral region with maximum spectral sensitivity 494/519 nm (stimulating light 495 nm). The constancy of the responses of this cell results from the deviation of the curve of average values (Fig. 8b; 12 tests). With the lowest intensity level ($0.07 \mu\text{W}/\text{cm}^2$; Fig. 8a) 55% of the sensitivity during dark adaptation will already be reached within 2 sec; the adaptation curve is situated above the other curves, which have been established for higher intensities. About 2 min after the beginning of dark adaptation, this visual cell will have recovered 100% of its sensitivity on maximum dark adaptation. An increase of the intensity of the adapting light and of the test stimuli by the factor 10 ($J_A = 0.6 \mu\text{W}/\text{cm}^2$; $J_T = 0.06 \mu\text{W}/\text{cm}^2$) will slow down the dark adaptation only negligibly within the first 8 sec (Fig. 8b). After 31 sec, the visual cell has attained the same sensitivity, no matter if it has been light-adapted to 0.07 or to $0.6 \mu\text{W}/\text{cm}^2$. In case of a further intensity increase ($J_A = 6 \mu\text{W}/\text{cm}^2$; $J_T = 0.6 \mu\text{W}/\text{cm}^2$), the adaptation curve will rise more slowly again (Fig. 8c).

With higher intensities (Fig. 8d), up to 1.7 sec after the beginning of the dark adaptation, the sensitivity will still be less than 1%. This

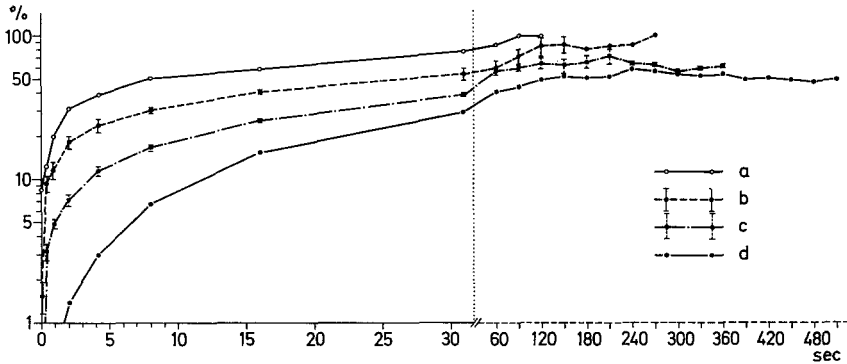


Fig. 9a—d. Adaptation curves of a visual cell ($\lambda_{\max} = 445$ nm) in the dorsal eye region for different intensities. $J_T = 0.1 J_A$; light adaptation time = 10 sec. a: $J_A = 0.1 \mu\text{W}/\text{cm}^2$; single curve. b: $J_A = 0.9 \mu\text{W}/\text{cm}^2$; curve of average values from 3 experiments (vertical line shows standard deviation). c: $J_A = 9.6 \mu\text{W}/\text{cm}^2$; curve of average values from 2 experiments (vertical line shows maximum and minimum values). d: $J_A = 258 \mu\text{W}/\text{cm}^2$. Ordinate: sensitivity in percent; Abscissa: time of dark adaptation in seconds

means that rising intensity of the adapting light will cause prolonged dark adaptation time.

The responses of this visual cell to test stimuli show a continuous rise during dark adaptation, with the afterpotential being slightly hyperpolarized in the beginning or with it being depolarized (Fig. 6 Aa, c). In other words: The initial hyperpolarization of the afterpotential during the first 10 sec does not influence the amplitude of the response to the test stimulus.

Adaptation experiments on a receptor from the dorsal eye region (Prot. Nr. I 152/1; Fig. 9) with maximum spectral sensitivity at 445 nm also show delayed dark adaptation in case of increased intensity of the adapting light and equal the test stimuli.

With low intensities ($0.1\text{--}0.9 \mu\text{W}/\text{cm}^2$), at the end of a stimulus visual cell Fig. 9 shows rapid decrease of the light adaptation potential towards the resting level (Fig. 6 Aa, continued), while a depolarizing afterpotential is correlated to higher intensities (e.g. $258 \mu\text{W}/\text{cm}^2$) (Fig. 6 Aa, interrupted). No matter which adapting light intensity is chosen, the amplitude of the responses to the test stimuli which this visual cell develops is rising steadily during dark adaptation (see diagram Fig. 6 Ba). The full initial sensitivity cannot be reestablished within 8 min (Fig. 9).

$J_T = 0.01 J_A$; Light Adaptation Time 10 sec. The investigation includes visual cells with maximum sensitivity at 445 nm, and at 494/519 nm.

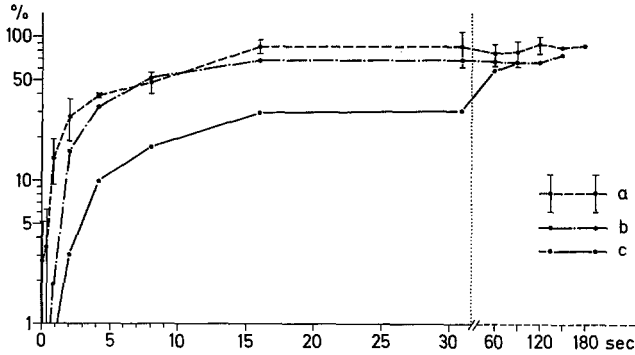


Fig. 10 a—c. Adaptation curves of a visual cell ($\lambda_{\max} = 445$ nm) in the dorsal eye region. $J_T = 0.01 J_A$; light adaptation time = 10 sec. a: $J_A = 0.9 \mu\text{W}/\text{cm}^2$; curves of average values from 3 experiments; vertical line shows standard deviation. b: $J_A = 9.6 \mu\text{W}/\text{cm}^2$; single curve. c: $J_A = 257 \mu\text{W}/\text{cm}^2$; single curve. Ordinate: sensitivity in percent; Abscissa: time of dark adaptation in seconds

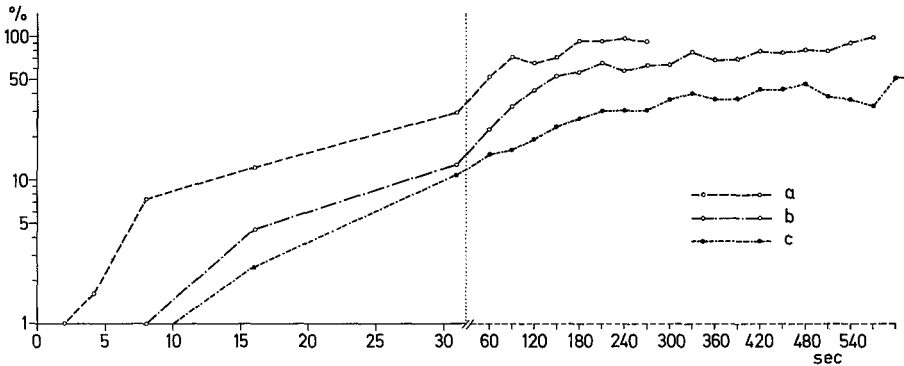


Fig. 11 a—c. Adaptation curves of a visual cell ($\lambda_{\max} = 494/519$ nm) in the ventral eye region. $J_T = 0.01 J_A$; light adaptation time = 10 sec. a: $J_A = 74 \mu\text{W}/\text{cm}^2$; b: $J_A = 380 \mu\text{W}/\text{cm}^2$; c: $J_A = 759 \mu\text{W}/\text{cm}^2$. Ordinate: sensitivity in percent; Abscissa: time of dark adaptation in seconds

Even if low test stimuli are applied, the dark adaptation following light adaptation to higher intensities is generally delayed (Fig. 10).

During the measurements, the visual cell (Prot. Nr. I 150/1; $\lambda_{\max} = 445$ nm; Fig. 10) shows an afterpotential which sometimes appears hyperpolarized, and sometimes depolarized (Fig. 6 A b, c). Independently the response to the test stimulus is rising continuously during dark adaptation.

$J_T = 0.001 J_A$; Light Adaptation Time 10 sec. In case of very high intensities of the adapting light, in experiments using very low test

stimuli (compared to the intensity of the adapting light), the following dark adaptation will develop very slowly—i.e. the more slowly, the higher the intensity of the adapting light is chosen (Fig. 11). After light adaptation to $74 \mu\text{W}/\text{cm}^2$ and test stimuli of $0.07 \mu\text{W}/\text{cm}^2$, the visual cell (Prot. Nr. I 164/3; ventral region; $\lambda_{\text{max}}=494/519 \text{ nm}$) recovers 99% of its maximum dark adaptation sensitivity after only 4 minutes of dark adaptation. After light adaptation up to $380 \mu\text{W}/\text{cm}^2$ (test stimuli $0.38 \mu\text{W}/\text{cm}^2$), it takes 9.5 minutes until 100% maximum sensitivity is regained (Fig. 11 b). After light adaptation up to $759 \mu\text{W}/\text{cm}^2$ (test stimulus $0.759 \mu\text{W}/\text{cm}^2$), only about 60% of the maximum sensitivity is reestablished within 34 minutes. Between the 24th and the 34th minute of dark adaptation, the sensitivity does not increase, only thereafter it gradually continues to rise.

After sufficiently high intensities of the adapting light, the test stimuli during the first seconds of dark adaptation will not be responded. With $J_A=759 \mu\text{W}/\text{cm}^2$ a succeeding test stimulus of $0.7 \mu\text{W}/\text{cm}^2$ remains without response for 10 sec (Fig. 11).

Also during this test series, the levels of the responses to the test stimuli during the afterpotential were neither influenced by hyperpolarization nor by depolarization.

Dark Adaptation after Different Durations of Light Adaptation

1. *Receptor with $\lambda_{\text{max}}=458 \text{ nm}$ (Dorsal Eye Region).* The intensity of the adapting light is $0.1 \mu\text{W}/\text{cm}^2$, the intensity of the test stimuli (J_T) is $0.01 \mu\text{W}/\text{cm}^2$. In case the light adaptation period is extended from 5 to 10 sec, respectively 30 sec, the dark adaptation will be increasingly delayed (Fig. 12). The retardation grade, however, varies only slightly, but not always significantly.

2. *Receptor with $\lambda_{\text{max}}=494/519 \text{ nm}$ (Ventral Eye Region).* J_A was $0.6 \mu\text{W}/\text{cm}^2$, $J_T=0.006 \mu\text{W}/\text{cm}^2$. With these low adapting light and test stimulus intensities, the dark adaptation delay after extended light adaptation periods (5, 10, and 30 sec) is significant (Fig. 13).

Adaptation to Constant J_A and Varied J_T

The light adaptation period is 10 sec. Light adaptation to $0.07 \mu\text{W}/\text{cm}^2$ is carried out on a receptor of $\lambda_{\text{max}}=494/519 \text{ nm}$; $J_T=0.007$ and $0.0007 \mu\text{W}/\text{cm}^2$. With the higher test stimulus ($0.007 \mu\text{W}/\text{cm}^2$) the dark adaptation is proceeding initially faster than with the lower one (Fig. 14a, b). After 5–9 sec, however, the sensitivity for the lower test stimulus exceeds the sensitivity for the higher one. With other visual cells the results are the same, only the point of intersection might be located on shorter or longer dark adaptation times. The sensitivity attained with 90 sec of dark adaptation is the same in both cases.

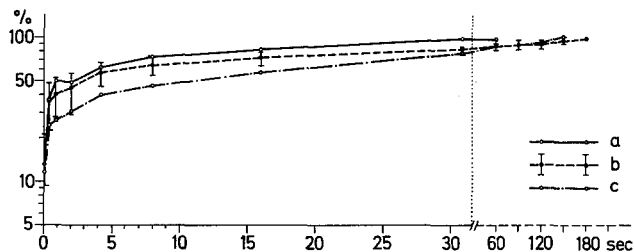


Fig. 12a—c. Adaptation curves of a visual cell ($\lambda_{\max}=458$ nm) in the dorsal eye region for different light adaptation times. Light adaptation to monochromatic light ($\lambda=445$ nm). $J_T=0.1 J_A$; $J_A=0.1 \mu\text{W}/\text{cm}^2$. a: light adaptation time=5 sec; single measurement. b: light adaptation time=10 sec; curve of average values from 7 measuring series (vertical line: standard deviation). c: light adaptation time=30 sec; single measurement. Ordinate: sensitivity in percent; Abscissa: time of dark adaptation in seconds

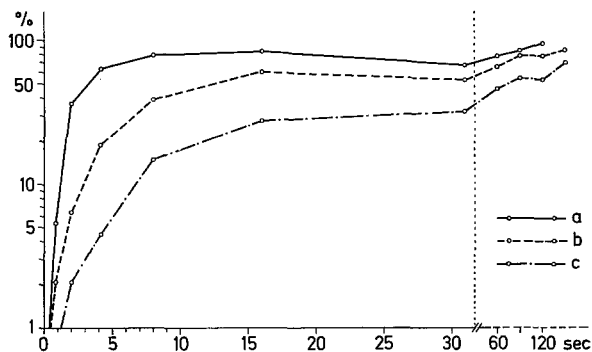


Fig. 13a—c. Adaptation curves of a green receptor ($\lambda_{\max}=494/519$ nm) in the ventral eye region for different light adaptation times. $J_T=0.01 J_A$; $J_A=0.6 \mu\text{W}/\text{cm}^2$; light adaptation times: a=5 sec; b=10 sec; c=30 sec. Ordinate: sensitivity in percent; Abscissa: time of dark adaptation in seconds

In case of very low test stimulus intensities ($J_T=1/100$, respectively $1/1000 J_A$; $J_A=10 \mu\text{W}/\text{cm}^2$; $J_T=0.1$ respectively $0.01 \mu\text{W}/\text{cm}^2$; Fig. 14b, c), the dark adaptation checked by the lower stimulus will proceed more slowly than the dark adaptation checked by the higher one. The progression of the dark adaptation is significantly differing during the first 20–30 sec. For all intensities of test stimuli, the state of maximum dark adaptation will be reached at about the same time: after about 60 sec of dark adaptation.

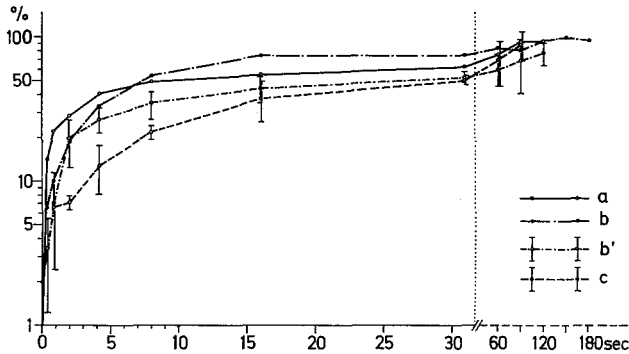


Fig. 14a—c. Adaptation curves of 2 visual cells for different ratios of $J_T : J_A$. a and b: Green receptor, $\lambda_{\max} = 494/519$ nm (ventral region) upon preceding light adaptation of 10 sec; $J_A = 0.07 \mu\text{W}/\text{cm}^2$ ($\lambda = 495$ nm). J_T on a = $0.1 J_A$, on b = $0.01 J_A$. b' and c: Average values of a visual cell in the dorsal region ($\lambda_{\max} = 445/458$ nm) upon preceding light adaptation of 10 sec. J_T on b' = $0.01 J_A$, on c = $0.001 J_A$; $J_A = 10 \mu\text{W}/\text{cm}^2$ ($\lambda = 445$ nm). Vertical lines: standard deviation for 3 tests; Ordinate: sensitivity in percent; Abscissa: time of dark adaptation in seconds

Discussion

1. A single visual cell in the eye of *Aeschna* will respond to a light stimulus by depolarization. At the end of the light stimulus the depolarization will fade away again. According to intensity and duration of the light stimulus, the resting potential will be reestablished sooner or later. If the visual cell has been adapted to high light intensities, it may take up to 60 sec or more until the resting level will be regained (Fig. 6Aa).

At the end of a light adaptation, some visual cells respond by a hyperpolarization (Figs. 6Ab, 7a), which gradually returns to the resting potential. This hyperpolarization may occur with all intensities of the adapting light; for some visual cells, however, it may first increase with rising intensity of the adapting light, but be completely absent after very high intensities.

After adaptation to low and medium intensities (drone eyes; relative intensity data) Hadjilazaro and Baumann (1968) found hyperpolarization during the afterpotential, but they did not observe hyperpolarization on higher intensities. We obtained similar results also for *Aeschna* after adapting light intensity of $760 \mu\text{W}/\text{cm}^2$ (Fig. 7c). It seems obvious that the receptor potentials of different visual cells develop in different ways, especially during the regeneration phase of the resting potential, which will follow after the end of an extended period of light adaptation. The change from one adaptation type to another cannot necessarily be

obtained on all visual cells by altering the adaptation conditions. This also suggests that there may be different types of visual cells.

2. One could presume that this varying behaviour of the receptor potential depends on the adaptation process. Adaptation is an alteration of sensitivity (Burkhardt, 1960). This alteration of sensitivity can only be deduced from the response to a test stimulus. The results of these investigations show: The afterpotentials which occur after a stimulus do not correlate to the sensitivity of the receptor towards a test stimulus. This is due to two completely different facts: a) During depolarization as well as during hyperpolarization after light adaptation, the sensitivity of a visual cell in relation to a test stimulus increases continuously during dark adaptation (diagram Fig. 6I and III). b) The response to a test stimulus may still be reduced during dark adaptation, even if the afterpotential has already returned to the resting level. Thus the sensitivity is longer reduced than the receptor potential (Figs. 5, 7). There is no interdependence between the course of the receptor potential after light adaptation, and the sensitivity.

The axons of the visual cells of *Aeschna* are relatively short; relatively i.e. as compared to e.g. the long axons of the visual cells of *Limulus*, or the stretch receptor of Crustacea. Nervous impulses do probably not occur in the axons of the visual cells of *Aeschna*. Thus there must be another way of excitation spread from the visual cells. In these visual cells, the receptor potential does not act as a generator potential for axonal impulses (as it is the case for many sensory cells; s. Fuortes, 1971).

The hyperpolarization which in the course of dark adaptation occurs in some (not all) sensory cells—and is specific for these—might (this is speculative) influence certain synapses in a way which is different from depolarization. Grundfest (1958, 1961) discusses the probability that certain synapses might release a transmitter substance with hyperpolarization. In this way it seems conceivable that hyperpolarization might control specifically different synapses between the axons of visual cells and second order neurons, as contrary to depolarization. The transfer of new light stimuli during dark adaptation could thus occur independently regarding the actual amplitude and sign of the receptor potential.

3. For *Aeschna* the extent of adaptation is low (measured as an alteration of sensitivity). Even upon light adaptation to high intensities, the sensitivity will change by only about the factor 100, at a maximum 1 000 (Figs. 8, 9, 10). Also with light adaptation to extremely high intensities (Fig. 11), the sensitivity will regain 1% of its maximum at complete dark adaptation: after $J_A = 74 \mu\text{W}/\text{cm}^2$ within 2 sec; after $J_A = 380 \mu\text{W}/\text{cm}^2$ within 8 sec; and after $J_A = 759 \mu\text{W}/\text{cm}^2$ within 10 sec. Even an extrapolation from the dark adaptation Fig. 11c ($J_A = 759 \mu\text{W}/\text{cm}^2$,

$J_T = 0.001 J_A$) will at the most show a sensitivity change by the factor 10^3 . Comparable sensitivity alterations were found (with different methods) by Goldsmith (1963) on *Apis*, Burt and Catton (1964) and Cosens (1966) on *Locusta*, Hamdorf and Kaschef (1965) on *Calliphora*, Seibt (1967) on *Apis*. In all these cases, apposition eyes are concerned. For the superposition eyes of moths, however, the threshold after light adaptation will increase to a value which is 10^5 to 10^6 times higher than the threshold value at complete dark adaptation (Bernhard, Höglund, and Ottoson, 1963).

Even after light adaptation to high intensities, the dark adaptation of imagines of *Aeschna* will develop very rapidly, especially within the first second, sometimes within the first few seconds. After adaptation to high light intensities, 10% of the maximum sensitivity will already be regained within 30 sec.

4. In case of low test stimulus intensities, the dark adaptation can be accelerated during the starting phase by raising the intensity of the test stimuli (Fig. 14). This peculiar effect may be explained by assuming that the stimulating light itself causes reisomerization of a second product of the visual pigment: With higher intensity of the test stimulus, the quantity of the reisomerized pigment will be larger and accordingly the sensitivity will increase faster. Likewise Hamdorf, Gogala and Schwemer (1971) found reisomerization of secondary products in *Ascalaphus* eyes. If the stimuli applied during dark adaptation are of high intensity, they will delay the dark adaptation (Fig. 9). In these cases the transformation rate of secondary products (isomerization) is higher than the reisomerization rate caused by light.

The authors would like to thank the Institute's Laboratory team, Mr. J. Weber, Mr. Th. Zschka, and Mr. H. Hein for designing and preparing the necessary equipment. They are particularly grateful to Mrs. M. Meinecke for her exact technical work and to Miss F. Althaus for the drawings.

References

- Autrum, H.: Die Belichtungspotentiale und das Sehen der Insekten (Untersuchungen an *Calliphora* und *Dixippus*). Z. vergl. Physiol. **32**, 176–227 (1950).
- Autrum, H., Kolb, G.: Spektrale Empfindlichkeit einzelner Sehzellen der Aeschniden. Z. vergl. Physiol. **60**, 450–477 (1968).
- Autrum, H., Zwehl, V. v.: Die spektrale Empfindlichkeit einzelner Sehzellen des Bienenauges. Z. vergl. Physiol. **48**, 357–384 (1964).
- Bernhard, C. G., Höglund, G., Ottoson, P.: On the relation between pigment position and light sensitivity of the compound eye in different nocturnal insects. J. Insect Physiol. **9**, 573–586 (1963).
- Burkhardt, D.: Die Eigenschaften und Funktionstypen der Sinnesorgane. Ergebn. Biol. **22**, 226–267 (1960).
- Burt, E. T., Catton, W. T.: Potential changes in the eye and optic lobe of certain insects during light- and dark-adaptation. J. Insect Physiol. **10**, 865–886 (1964).

- Cosens, D. J.: Visual sensitivity in the light- and dark-adapted compound eye of the desert locust. *J. Insect Physiol.* **12**, 871–890 (1966).
- Cosens, D. J.: Some factors affecting the rate of dark adaptation in certain insects. *J. Insect Physiol.* **17**, 955–968 (1971).
- Eguchi, E.: Fine structure and spectral sensitivities of reticular cells in the dorsal sector of compound eyes in the dragonfly *Aeschna*. *Z. vergl. Physiol.* **71**, 201–218 (1971).
- Fuortes, M. G. F.: Generation of responses in receptor. In: Handbook of sensory physiology (Autrum, H., Jung, R., Loewenstein, W. R., MacKay, D. M., Teuber, H. L., eds.), vol. I (Loewenstein, W. R., ed.), p. 243–268. Berlin-Heidelberg-New York: Springer 1971.
- Goldsmith, T. H.: The course of light and dark adaptation in the compound eye of the honey-bee. *Comp. Biochem. Physiol.* **10**, 227–237 (1963).
- Grundfest, H.: An electrophysiological basis for cone vision in fish. *Arch. ital. Biol.* **96**, 135–144 (1958).
- Grundfest, H.: Excitation by hyperpolarizing potentials. A general theory of receptor activities. In: Nervous inhibition (E. Florey, ed.). London: Pergamon Press 1961.
- Hadjilazaro, B., Baumann, F.: Afterpotentials of the visual cell of the honey-bee drone. *Helv. physiol. pharmacol. Acta* **26**, 351–352 (1968).
- Hamdorf, K.: Die Dauer der Dunkeladaptation beim Fliegenauge nach Belichtung mit heterochromatischen Blitzen. *Z. vergl. Physiol.* **75**, 200–206 (1971).
- Hamdorf, K., Gogala, M., Schwemer, J.: Beschleunigung der „Dunkeladaptation“ eines UV-Rezeptors durch sichtbare Strahlung. *Z. vergl. Physiol.* **75**, 189–199 (1971).
- Hamdorf, K., Kaschef, A. H.: Adaptation beim Fliegenauge. *Z. vergl. Physiol.* **51**, 67–95 (1965).
- Hartline, H. K., McDonald, P. R.: Light- and dark adaptation of single photoreceptor elements in the eye of *Limulus*. *J. cell. comp. Physiol.* **30**, 225–253 (1947).
- Seibt, U.: Der Einfluß der Temperatur auf die Dunkeladaptation von *Apis mellifica*. *Z. vergl. Physiol.* **57**, 77–102 (1967).
- Smola, U.: Entwurf und Aufbau eines Lichtreizgenerators zur Messung der selektiven Adaptation an einzelnen Sehzellen von Komplexaugen. Diplomarbeit, Inst. f. Nachrichtentechnik d. Techn. Hochschule München, 1965.

Prof. Dr. H. Autrum
Dr. Gertrud Kolb
Zoologisches Institut der Universität
D-8000 München 2
Luisenstraße 14
Germany