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Light Dependence of Oxidative Metabolism in Fly Compound Eyes Studied *in vivo* by Microspectrofluorometry

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Arthropod photoreceptors depend on oxidative metabolism for maintenance of the resting potential (horseshoe crab [1], bee [1], fruitfly [2]) and sensitivity to light (owlfly [3], locust [4]). In respiration measurements on isolated retinae (bee [5, 6], blowfly [5, 7, 8]) it was demonstrated that intense illumination distinctly elevates the rate of oxygen consumption by the photoreceptor cells. Here we report microspectrofluorometric measurements on light-induced oxidative metabolic processes in the compound eye of completely intact, living blowflies.

Fluorescence was measured from approximately 300 ommatidia of a compound eye of the blowfly *Calliphora erythrocephala* with a Leitz Orthoplan microscope equipped with a Leitz NPL 10 objective (aperture 0.20), a Ploemopak illuminator and a Compact photometer system. Completely intact and living animals could be investigated by selecting the white-eyed mutant chalky. This mutant lacks the retinal screening pigments, which in wild-type flies obstructs *in vivo* fluorometry, but otherwise the retinal properties of the mutant appear to be identical to those of the wild type [9–13].

The main visual pigment of blowflies is a rhodopsin absorbing maximally in the blue-green ($\lambda_{max}=495$ nm), which after light absorption converts into a thermostable metarhodopsin, absorbing maximally in the orange ($\lambda_{max}=580$ nm) [10–14]. Recently it was established that the metarhodopsin state upon orange excitation distinctly fluoresces in the far-red [13–15].

Applying blue excitation light and measuring the emission in the green, we failed to see any indication for rhodopsin fluorescence, but found that blue light, when delivered to the eye after a few seconds of darkness, induces a transient increase in green fluorescence. After longer dark times a biphasic process with a maximum at 1–2 s after light onset emerges (Fig. 1). Because the amplitude appears to be governed by a mechanism having a time constant in the order of 15–30 s we hypothesized that the system is related to recovery of the photoreceptors' light sensitivity in the dark. The main phase of this so-called dark-adaptation process occurs within that same span of time [11–16].

Fly photoreceptors respond to illumination by a depolarization of the cell membrane. Since the light sensitivity is reduced by a lowered oxygen tension [1–4] we applied a stream of nitrogen to a fly and measured its effect on the photoreceptors' fluorescence. Fig. 2 shows the effect of hypoxia during interrupted and continuous light, respectively. After a few seconds of depletion of oxygen the fluorescence signal falls and, furthermore, the light-induced dynamic process vanishes. On the other hand, recovery from hypoxia is almost instantaneous and appears to induce a strong increase in both the static and the dynamic fluorescence signal. (Measurements performed during application of pure oxygen yielded identical results to those obtained in air.) The dynamics of the fluorescence signal depends, furthermore, distinctly on temperature. At lower temperatures the process slows down, and, ultimately, no fluorescence change is observable anymore at $\lesssim 5$ °C.

A preliminary analysis of the eye's blue-induced emission spectrum shows a broad green peak around 520–530 nm (see also [15]), the height of which is lowered by anoxia.

These findings can be directly related to the studies of Chance et al. [17–19] on the flavins of the mitochondrial respiratory chain. They showed that anoxia of mitochondria results in a decrease in oxidized flavin compounds and thus in a fall in blue-induced green emission, and vice versa, that an increased rate of oxygen consumption is accompanied by an emission increase. Hence we suggest that the fluorescence changes induced by illumination of fly

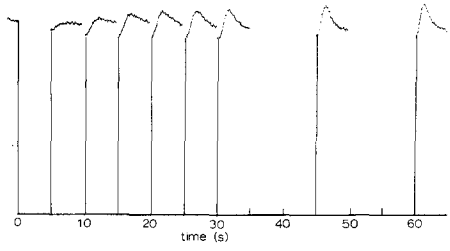

Fig. 1. Autofluorescence measured from the eye of a completely intact, living blowfly *Calliphora erythrocephala* mutant chalky. The excitation light, wavelength 456 nm and intensity 1.2×10^{17} quanta·cm^{-2}·s^{-1}, was applied during 5 s. Emission, measured in the wavelength range 510–600 nm, exhibits a biphasic time course, the shape of which depends on the dark time. The response is presented with time of onset at the preceding dark time (abscissa)

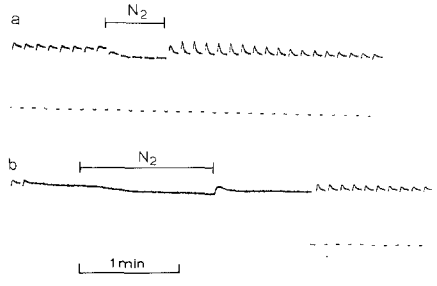

Fig. 2. Hypoxia severely affects the fly's eye autofluorescence. The conditions were the same as in Fig. 1, except that the 5-s flashes were interrupted by a 30-s dark time (during which time the recorder halted). Application of nitrogen rapidly lowers the fluorescence level, and the biphasic curve is flattened; after hypoxia an enhanced light-induced fluorescence change occurs which gradually settles down (a). Essentially the same effects are seen during continuous light (b)

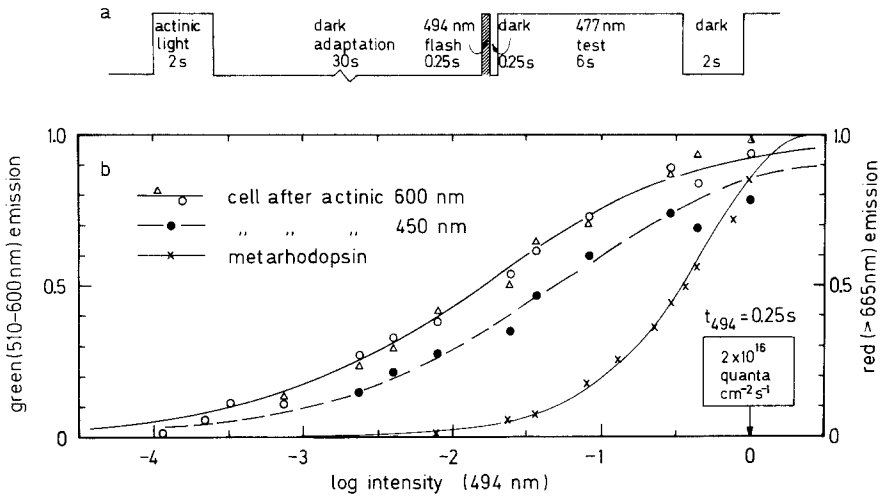

Fig. 3. Fluorescence changes in blowfly eyes caused by 494-nm flashes (a). Previous to the flash a 2-s actinic light of either ≈ 600 nm (Balzers K 60) or ≈ 450 nm (Balzers K 45) and a 30-s dark adaptation time was given. After the 0.25 s lasting flash a 477-nm test light (9.1×10^{15} quanta cm^{-2} s^{-1}) was applied 0.25 s later. The induced green emission (510–600 nm) was measured and in (b) the difference of the initial value from the value obtained after a low-intensity flash is drawn normalized to the maximum increment. △, ○: data from two runs of experiments performed from low to high intensities after actinic 600 nm. Hysteresis effects were negligible showing a complete reset by the actinic light and the following dark time to the same cell condition. The data are approximated by the hyperbolic function [22, 23] $(RI)^n/\{(RI)^n+1\}$, with $n=0.6$. I is the intensity of the 494-nm light; R^{-1}, the intensity where the fluorescence change is half-maximal, is 0.32×10^{15} quanta cm^{-2} s^{-1} and 10^{15} quanta cm^{-2} s^{-1} after 600-nm and 450-nm preadaptation, respectively. The range shift is explained from the difference in rhodopsin content being $\approx 100\%$ and $\approx 30\%$, respectively. The red fluorescence of metarhodopsin created by 494-nm flashes of various intensity, when starting from an initial 100% rhodopsin situation, is measured >665 nm (613 nm excitation). The resulting emission values are approximated by $1-\exp(-0.69\,IR)$ with $R^{-1}=7.3 \times 10^{15}$ quanta cm^{-2} s^{-1}

photoreceptors are caused by enhanced oxidative metabolic processes, also because the rapid dynamics of the green fluorescence, being in the order of a few seconds, accord with measurements of oxygen consumption induced by light flashes in isolated retinae [20, 21].

This view is supported by our measurements of ultraviolet-induced blue emission. The blowfly eye then exhibits a blue fluorescence peaking at about 460 nm [9, 15]. Upon illumination the ultraviolet-induced blue emission drops with the same dynamics as that of the green-emission increase seen in the blue-induced fluorescence experiments (the relative change is smaller, however).

Since oxidation of NADH results in a loss of blue fluorescence [18] we conclude that illumination of fly photoreceptors causes an increased oxidized state of the molecules in the mitochondrial respiratory chain.

The rapid metabolic processes manifest themselves at bright light intensities only. In the experiment of Fig. 3 the intensity dependence was investigated along the following procedure. In sequence, a 2 s lasting actinic light, a 30-s dark adaption time and a 0.25-s flash of 494 nm (at the wavelength of the rhodopsin absorption peak) was applied. The effect of the flash was tested by a blue (477 nm) light given 0.25 s later and the initial value of the blue-induced green (510–600 nm) emission was registered. We executed two series of experiments; in the first series the actinic light was orange (Balzers K 60) and in the second blue (Balzers K 45), in order to establish a high ($\approx 100\%$) and a low ($\approx 30\%$) rhodopsin concentration, respectively, in the photoreceptors [10–12, 20]. It appears that the initial green fluorescence depends on the intensity of the preceding 494-nm flash by a hyperbolic function [22, 23] (see legend of Fig. 3). A similar intensity dependence was previously found for the oxygen consumption by the isolated retina [7, 8].

Several lines of evidence confirm the assumption that rhodopsin conversion triggers the enhanced metabolic activity. Firstly, preliminary measurements of the action spectrum indicate a clear correspondence with the spectral dependence of oxygen consumption [8] and the spectral sensitivity of the main class of fly photoreceptors measured electrophysiologically [24]. Furthermore, the high- and low-rhodopsin curves (Fig. 3) are separated by 0.5 log unit, which can be explained from the difference in rhodopsin content. As the intensity-dependence curves in the two series are normalised we have to note that the saturating response is distinctly smaller in magnitude with low rhodopsin than with high rhodopsin, indicating a maintained demand for metabolic energy when metarhodopsin is abundant.

It is of obvious interest to relate light-induced mitochondrial activity with phototransduction processes in general. Because the principal step in phototransduction is rhodopsin conversion into metarhodopsin [11, 24], we estimated the degree of rhodopsin conversion caused by the 494-nm flashes by measuring the resulting metarhodopsin fluorescence (613 nm excitation, >665 nm emission; for procedures see [13, 15]). As indicated in Fig. 3 the brightest flash applied was just insufficient to establish within the 0.25-s flash time the photosteady state. When we consider that of the $1-2 \times 10^8$ visual pigment molecules of fly photoreceptors [11, 12, 25] $\approx 60\%$ exist in the metarhodopsin state after prolonged 494-nm light [10–12] we can read from Fig. 3 that mitochondrial activity is half-maximal when in the order of 10^6 rhodopsin molecules are converted. On the other hand, electrophysiological experiments indicate that the receptor potential from a dark-adapted cell is half-maximal when about 10^4 rhodopsin molecules are converted, i.e. at an intensity where activation of the mitochondrial process becomes slightly noticeable ($\log I \approx -4$ in Fig. 3).

Since rhodopsin conversion results in an increased membrane permeability and thus in ionic currents, ion pumps are activated [26]. The sodium pump is an ATP-ase and hence oxygen consumption is enhanced to recycle the depleted ATP [20–21, 26, 27]. In bee photoreceptors at least half, and probably more, of the energy produced by respi-

ration is needed for the sodium pump [27, 28]. However, several other processes following rhodopsin to metarhodopsin conversion but preceding the ionic changes draw on the supply of ATP as has been demonstrated recently for vertebrate rods (e.g. [29–31]) and *Limulus* ventral photoreceptors [32].
The recognition of the origin of the light-induced fluorescence changes opens the possibility for non-invasive measurements on photoreceptor metabolism, an essential part of the phototransduction machinery.

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Social Cues of a Hipposiderid Bat Inside a Cave Fail to Entrain the Circadian Rhythm of an Emballonurid Bat

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Insectivorous microchiropteran bats are abundant around our university complex (9°58′ N, 78°10′ E) and inhabit crevices, caverns, temples, ruins and cave environments. One of the caves is inhabited by a colony of about 500 individuals of the bat *Hipposideros speoris*. The deeper recesses show invariant temperature (27 °C) and humidity (95%) and the darkness is absolute (no light measurable over periods of 1000 s on the log scale of a UDT optometer). The bats entrain their flight activity by daily sampling of light just prior to flying out to forage at dusk and also through social cues. Thus caged individual members of *H. speoris* held 40 m deep in the cave entrained their activity/rest pattern to that of the free-flying members of the colony [1, 2].
We have now investigated whether an 'alien' bat confined in the deeper regions of the cave would also be able to entrain its circadian rhythm to the social cues emitted by the members of the colony of *H. speoris* during their nightly outflight and return before sunrise. Dim red light was used in conducting the experiments and the locomotor (flight) activity was recorded for four bats of an emballonurid species, *Taphozous nudiventris kachhensis*. The

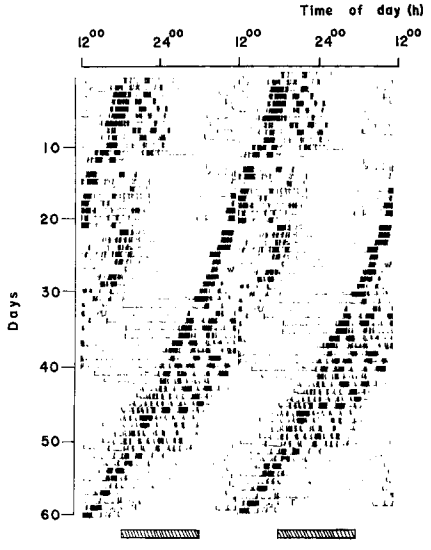

Fig. 1. A typical example of the *freerunning* of the flight activity pattern of an emballonurid bat confined in a hipposiderid cave for 60 days. Activity bouts are indicated by vertical patches and the horizontal lines indicate rest. The *original* felt pen tracings are double-plotted, the activity data for day 1 leading horizontally to data for day 2, data for day 2 to data for day 3 etc., to facilitate visual evaluation. The hatched area at the bottom of the figure indicates the time over which the members of the hipposiderid colony would be active, leaving the cave in a mass exodus early night and returning individually for the rest of the night

