Temperature dependence of ERG response attenuation due to the light adaptation in the owl-fly *Libelloides macaronius*

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Rationalle. Effective attenuation of owl-fly's dorsofrontal eye response due to light adaptation was evaluated by means of comparing whole ERG responses rather than just one parameter (i.e. peak amplitude).

Materials and Methods.

Preparation. The insect was immobilized with a mixture of bee's wax, colophony and contact paste onto a copper yoke. The temperature of the preparation was controlled with a water-cooled Peltier element, measured with a K-type thermocouple which was inserted into the unilluminated eye, and monitored on a digital multimeter.

Stimulation light was provided by two 150W Xenon Arc lamps. The adaptation light beam was selected with an IR-cutting Schott KG filter and a Schott SFK7 blue filter. The test beam was cooled with cold mirrors, the wavelength selected with an Oriel monochromator and intensity attenuated with Melles Griot fused silica neutral density filters with the smallest step of 0.1 log. Both beams were controlled with shutters, combined with a beam-splitting mirror and focused through a quartz lens onto the dorso-frontal part of the right eye of the insect.

Electrophysiology. A bevelled-tip extracellular glass micropippete, filled with Davenport saline, was inserted just underneath the cornea of the DF part of the right eye. Ag/AgCl wire, inserted into the unilluminated eye, served as a reference electrode. Signals were amplified with Axon AI-401 headstage, conditioned with Axon Instruments Cyberamp 320, digitized with National Instruments NI-DAQ 1200 Lab PC and stored in a Pentium PC running Strathclyde WinWCP software.

Experimental protocol & analysis. ERG responses elicited by test stimuli at constant intensity (1.3 log attenuation) were compared to ERG responses from the calibration sweep (25 non-saturating intensity steps ranging from -4 to 0 log intensity), performed at the beggining and at the end of the experiment. Test stimuli were applied each 3.1 seconds and consisted of two consecutive 50 milisecond monochromatic light pulses 1.5 seconds apart. (wavelenghts 385 nm and 400 nm). Adaptation was brought about by an adaptation stimulus (22 s of broad bandwitdh blue light passing through SFK7 filter). Data were processed using custom routines. Amplitudes of ERG responses from the calibration sweep at a given time after the onset of stimulus were interpolated with 9th grade polynomials in the log intensity dimension, producing a matrix of interpolated responses ranging over 4 log range with 0.01 log step. All twin test pulses were then compared to the calibration matrix. The interpolated responses, most similar to the test responses were picked with the least squares method, thus yielding a time course of effective stimulation light intensity. This method, whose only assumption is that the ERG shape at a given intensity does not change during the experiment, was developed and used because intensity-response relation of owl-fly's ERG does not fit well to a standard sigmoid curve used in intracellular experiments.

Results. Adaptation stimuli elicited attenuation of ERG with a biphasic time course; after cessation of adaptation light, sensitivity was first diminished, then the process reversed and sensitivity was slowly regained. Maximum effective attenuation ranged from 0.4 to 0.5 log units (figure 1). The whole process – rates of both phases and the time of reversal (figure 2) – shows a clear temperature dependence.

Discussion. Our results are very similar to those obtained by measuring eye glow (reflectance from the tapetum layer), showing that the ERG method, although very simple, can be used for monitoring light adaptation. The ERG method also has an important advantage over the eye-glow method, because **the adaptation is measured in absolute logarithmic units**, while the eye-glow method gives only arbitrary units. Moreover, this method measures whole adaptation of the eye, while the eye-glow method measures only optical (pupillary) adaptation.

Thanks to Gregor Zupančič & Gregor Belušič, they know why & they have know-how.

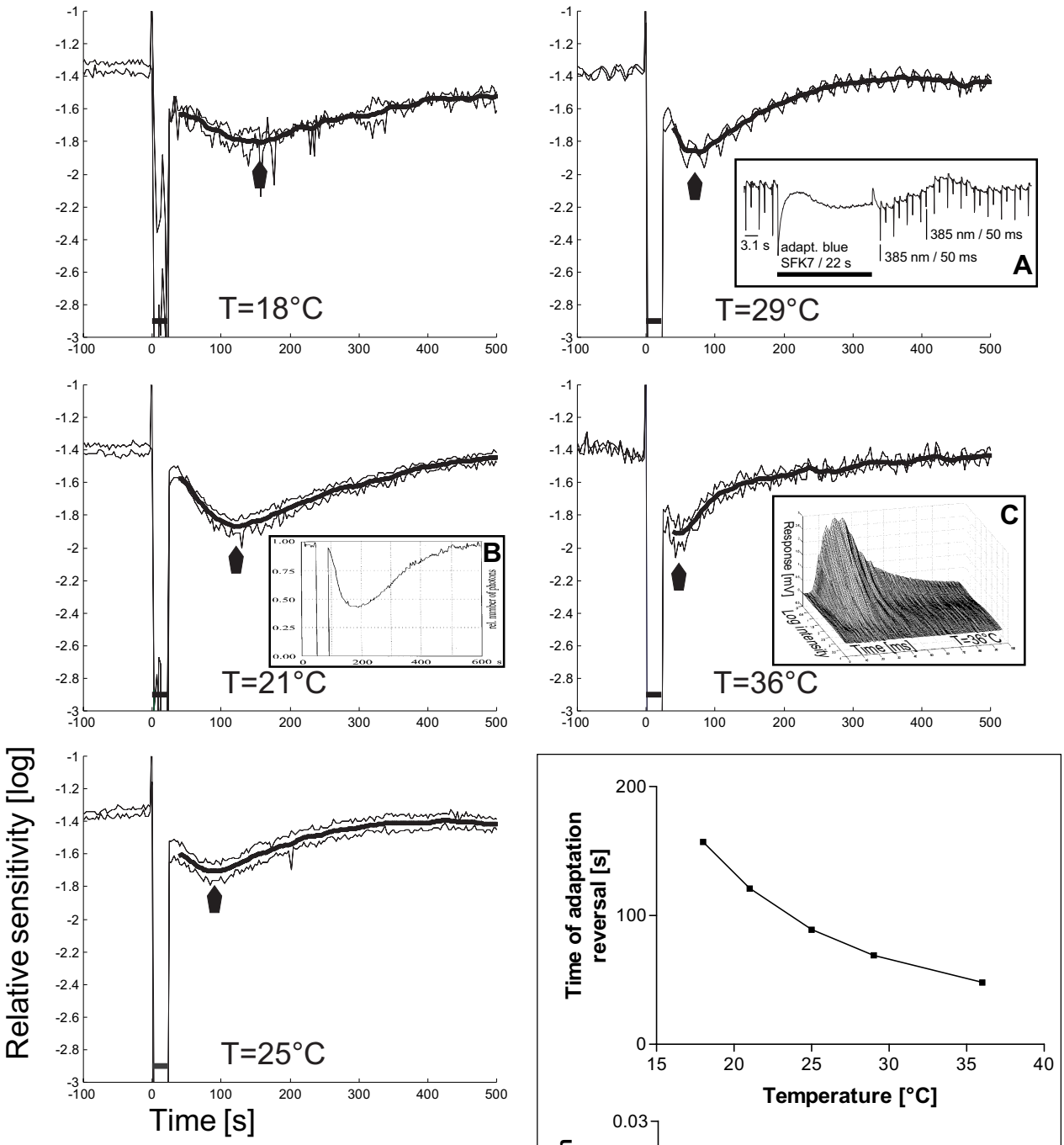

Figure 1: Whole adaptation measured with the ERG method at different temperatures.
The two thin traces represent time course of evaluated responses to two test stimuli (385 and 400 nm) at 1.3 log attenuation. Both traces were filtered with a low-pass digital filter, averaged and plotted with the thick trace. Adaptation was elicited with 22 seconds of broadband blue light passing through SFK7 filter. The adaptation stimulus is indicated with a bar at time 0. Inset **A** shows raw ERG data, the adaptation stimulus is again indicated with the bar. Arrows in the main graphs indicate reversals of adaptation processes. A measurement of eye-glow attenuation, measured at the room temperature, is shown in the inset **B** [adapted from Stušek, Hamdorf: J Comp Physiol A (1999), 184: 99-106]. Note that the method used therein measures relative counts of reflected and stray photons. Inset **C** shows the theoretical ERG matrix, calculated from calibration responses at 36°C.

Figure 2: Temperature dependence of the rate of adaptation processes.
Timestamps of adaptation reversal from Figure 1 are plotted against the temperature. The lower plot has the y-axis inversed, thus showing the rate of adaptation processes.