Ecophysiology through electroretinography: *Ascalaphus macaronius*, a case study

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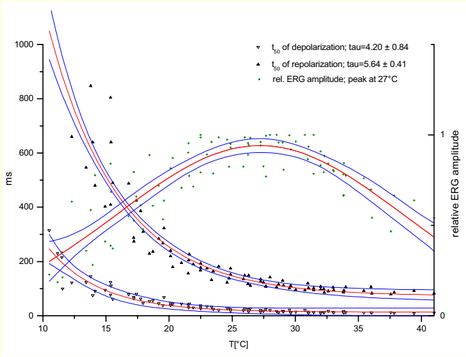
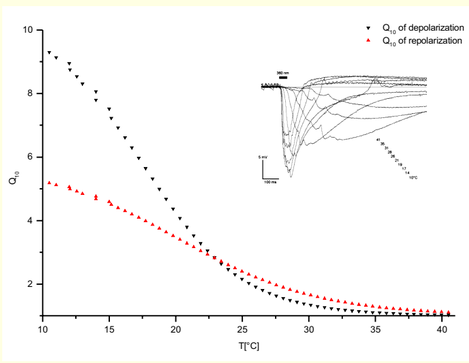
Introduction

The owlfly *Ascalaphus macaronius* (Insecta: Neuroptera) is a daytime predator famous due to the unique spectral sensitivity of its dorsofrontal (DF) eye that ranges only over the UV-part of the spectrum. It is a stenoecological animal inhabiting warm uncultivated meadows that lives as adult imago only for approximately two months in the summer. Even then, *Ascalaphus* is actively hunting only under unobscured skies. Field observations, measurements of its body temperature and spectral measurements of the sky have shown that its behavior is triggered by heat coming from direct insolation rather than by amount of UV light. Thus, electroretinography which had once offered an insight into *Ascalaphus*' unusual vision was this time used to evaluate the animal's thermical and aerobical demands.

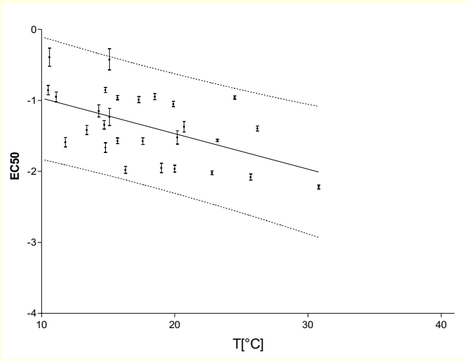
Temperature dependence of electroretinogram



a) Responses to 350 nm/50ms nonsaturating stimuli were measured in terms of amplitude, time to half maximal depolarization and repolarization, respectively. The speed of photoresponse decays exponentially with a "temperature constant" (analogue to time constant, tau) around 5°C. The asymptotes of the exponential fit give an estimate of fastest depolarization (t_{50}dep=14,6ms) and repolarization (t_{50}rep=73ms) times which do not coincide with the peak of ERG (T_{max}=27°C).



b) Temperature quotients for t_{50}dep and t_{50}rep were calculated from numerical matrices of exponential fits. The curves indicate that the photoresponse is approaching its optimum above 35°C and is dramatically retarded below 25°C. The bottleneck limiting the speed of photoresponse seems to be the repolarization since it exhibits a higher Q_{10} even at slightly suboptimal temperatures. Raw ERGs are presented in the inset.



c) The stimulus intensity that produces half maximal response (I_{50}) is increased at lower temperatures. This shows that a warm *Ascalaphus'* DF eye has an enhanced sensitivity. On the other side, the slope of Hill fit of log intensity/ response curves remains unaffected by lowering the temperature (not shown).

Experimental animals and methods

Ascalaphus adults were caught in the Karst region of Slovenia in July 2001. Animals were kept at a room temperature in individual vials and paper wraps, fed by liver and flies (*Calliphora*) and tested within two weeks after capture. *Calliphora erythrocephala* chalky was obtained from a laboratory culture maintained on a A vitamin rich diet.

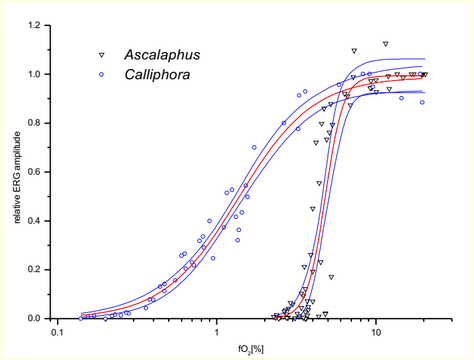
Experiments were performed on intact eyes with the exception of intensity/response measurements where pigment cells were removed with a razorblade and the eye was covered with a fragment of a coverslip.

For temperature dependence experiments, animals were immobilized with a mixture of bee's wax, colophony and contact paste onto a copper yoke. The temperature of the animal was controlled within a range between 12 and 42°C with a Peltier element lying underneath the preparation and monitored by a thermocouple inserted into an eye. Experiments with different oxygen fractions were performed at a room temperature (28°C). The animal was put into a chamber containing controlled mixture of N_2 and O_2 delivered by a Cole Parmer flow meter and the fO_2 was monitored by an O_2 sensor (ECHO, Slovenia).

Stimulation was provided by Xenon lamps (150 W for temperature dependence and 2000 W for fO_2 dependence experiments), IR was cut off with Schott KG filters and cold mirrors, wavelengths were selected with an Oriel monochromator, a Schott SFK blue monochromatic filter, a Schott OG cutoff filter and intensities were controlled with Melles Griot fused silica neutral density filters.

Extracellular potentials were recorded by glass pippetes filled with Davenport saline. Ag/AgCl wire inserted into thorax or into the unilluminated DF eye served as a reference. Signals were amplified with Axon 401 preamp and Axon Cyberamp 380, digitized with Cambridge electronics CED 1401plus or National Instruments NI-DAQ 1200 Lab PC, and stored in a Pentium PC running Strathclyde WinWCP software.

Oxygen dependence of ERG



Amplitude of ERG was determined at different fractions of oxygen in the experimental chamber. For comparison, the same experiment was done with *Calliphora erythrocephala* chalky. It is evident that *Ascalaphus'* oxygen demand is much higher than that of *Calliphora*.

Hypoxia halves the ERG amplitude at $fO_2$50=4,62% in *Ascalaphus* and $fO_2$50=1,37% in *Calliphora*.

Conclusions

Although a rough method, electroretinography reveals the high thermical and aerobical demands of *Ascalaphus*. The temperature optimum of the animal's vision reaches above 40°C which is in line with the measurement of its body temperature which reaches temperature over 45°C within a few minutes after exposure to direct sunlight.

The present experiments shed new light on previous data that had been obtained at laboratory room temperatures which had obviously posed serious limitations to *Ascalaphus'* visual performance. The high dependence of a temperature conformist animal is supplemented with data showing its relatively high oxygen demands.

Acknowledgement

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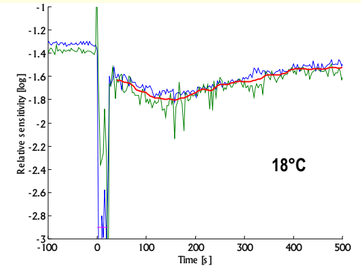
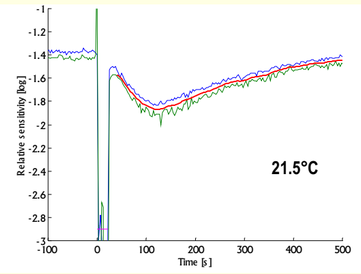
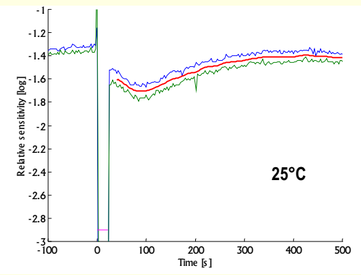
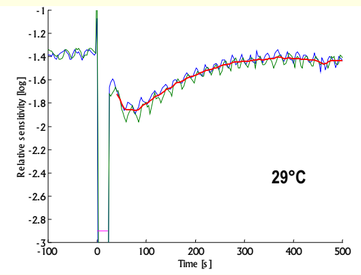
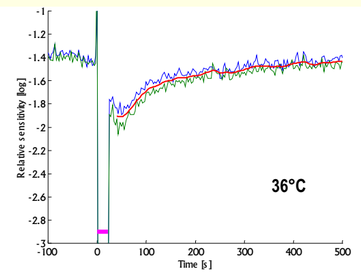
Temperature dependence of screening pigment migration











Effective attenuation caused by pigment migration was evaluated by means of comparing whole ERG responses rather than just one parameter (i.e. peak amplitude).

Method outline: *ERG responses elicited by test stimuli at constant intensity (-1.3 log attenuation) were compared to ERG responses from the calibration sweep (25 intensity steps in –3.9 to 0 log range, all under I_{50} intensity). Test stimuli, occuring 3.1 sec apart, consisted of two consecutive monochromatic light pulses (385 nm and 400 nm, 50 ms, 1500 ms apart). Pigment migration was triggered by an adaptation stimulus (22 s of broad bandwitdh blue light through SFK7 filter; a minor fraction of the transmitted light under 430 nm elicited ERG response approximately equal to that of test stimuli). Data were recorded with WinWCP software and processed using custom Matlab routines. Amplitudes of calibration ERG responses at a given time after stimulus onset were interpolated with 9th grade polynomials in the log intensity dimension, producing a matrix of theoretical responses in –4 to 0 log range with 0.01 log step. Each test pulse was then compared to the matrix, and the most alike theoretical response in the least squares sense was picked out, thus yielding a time course of effective attenuation of pigment.*

Adaptation stimuli elicited attenuation with biphasic time course; after cessation of stimulus, sensitivity was diminished, then the process reversed and sensitivity was slowly regained. Whole process – rates of both phases and the time of reversal – shows a clear temperature dependence. Maximum effective attenuation ranged from 0.3 to 0.5 log units, probably depending on the duration of previous dark adaptation.

Since the graphs represent data from a single animal, the processes were not quantified.

Our results are very similar to those obtained by measuring eye glow (reflectance from the tapetum layer), showing that ERG method, although very simple, can be used for monitoring pigment adaptation. ERG method also has an important advantage over the eye-glow method in giving out the information on effective attenuation of light reaching photoreceptors.