# Electrophysiological measurements of ERG response of owl-fly *Libelloides (=Ascalaphus) macaronius* in hipoxia

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**Introduction.** The insect visual system has a very high oxygen consumption. A pool of oxygen is stored in large air sacs in the head, mainly in the lateral and bottom part of the eye capsule. It is known that photoreceptor cells depend on continuous influx of oxygen and cease to function in anoxic conditions, where extracellularily measured response from the eye (ERG) gradually diminishes and finally dissappears (Hamdorf et al. 1988).

Oxygen is needed for production of metabolic energy by mitochondria. The electrochemical energy for ATP production is provided by a proton gradient that is built up across the inner mitochondrial membrane. The protons are pumped out from the matrix while electrons are transferred to oxygen through the electron transport chain consisting of a number of cytochromes and other carriers. Metabolic energy in photoreceptors is continously consumed at least by electrogenic pumps and protein phosphatases.

The present experiment was conducted in order to compare the oxygen dependence of owl-fly's vision to that of blowfly (*Calliphora erythrocephala*).

**Materials and methods.** The experiment was performed on intact dorso-frontal eye of female owl-flies, caught in Slovenian Karst region in July 2001. The preparation of animals was done under white light. Legs were cut off and the insect was fixed to a holder with wax-collophony. Finally, the wings were cut off and their bases immobilized. The insect was then placed into a small chamber with estimated volume of 10ml. The chamber was connected to nitrogen and oxygen supply through a flow meter/regulator. The chamber's atmospehere was monitored with a oxygen partial pressure ($pO_2$) sensor (ECHO d.o.o., Slovenia).

Gas mixture conditioning. Pure $N_2$ or regulated $O_2$/$N_2$ mixture was allowed to enter the chamber upon opening of the computer-controlled valve with a flow rate of approximately 60 ml/s. We estimated that the time needed for changing the chamber atmosphere into anoxic or hypoxic conditions and back to atmospheric conditions (21% $O_2$, 79% $N_2$) was 0.5 s

Light stimulation. Light stimulation was provided by a xenon arc lamp (900W). The light was attenuated with 25% neutral grey filters. The white light beam was directioned into the Leitz Orthoplan microscope and focused by microscope standard objective (4×) onto the insect's eye.

The shutter and gas valve were connected to the computer via CED1401 laboratory interface. The protocol was controlled with WinWCP software (Strathclyde Whole Cell Program, John Dempster, University of Strathclyde, Glasgow).

ERG measurements. Two electrodes where used to record ERG. Sharpened Ag/AgCl wire was used as a reference electrode. The measuring glass capillary electrode was bevelled under 45° angle to an aperture of about 20 µm. The electrode was filled with Davenport saline and inserted into a WPI electrode holder, held in a micromanipulator. The reference electrode was manually inserted between the eyes of the insect under binocular loupe.

The measuring electrode was positioned into the focus of the light beam and then retracted from the optical path. The chamber was then put onto the microscope platform and the central part of the dorsofrontal eye with ommatidia parallel with the light beam was focused. The measuring electrode was carefully inserted into the eye just underneath the cornea.

Extracellular potentials were amplified with Axon instruments Smart probe Al 401 (10× preamplifier) and conditioned with Axon CyberAmp 380. Amplified potentials were fed to CED1401plus laboratory interface and recorded at 160 Hz sampling rate into a PC computer running WinWCP Software.

**Results.** In the first experiment, the cessation of ERG response in anoxia was measured. Light stimuli (350 ms of white light, attenuated to elicit about half maximal response) were applied in 4 s intervals. Anoxic conditions were established 105 s after the beginning of the experiment and mantained for

195 s (figure 1). The amplitude of ERG responses remained unchanged for about 20 s of anoxia. Afterwards, ERG amplitudes gradually diminished and finally ceased after 95 s. After returning to normal atmosphere, ERG reappeared in 8 s (figure 2). ERG base line started to rise immediately after the aplication of $N_2$. The pre-anoxic baseline level was achieved approximately 4 min after the cessation of anoxia (not shown). In the control experiment without light stimuli (figure 3), we can also see the continuous rise of ERG baseline under anoxic conditions. The ERG baseline began to drop back towards the pre-anoxic level immediately upon changing to normal atmosphere.

The influence of low oxygen partial pressure on ERG amplitude was also measured. Oxygen fraction was gradually and slowly lowered from 20% towards 0%. The amplitudes were normalised to the amplitude of the ERG in normal conditions (figure 4). The ERG response amplitude is halved at around 5% $pO_2$ and ceases at about 2.5% $pO_2$, compared to 0.2% $pO_2$ in blow-fly (Perovšek, 2001, Graduation Thesis) or 0.6% $pO_2$ (R.P.Smits et al. 1995).

**Discussion.** It takes about 95s for the ERG to cease and 8s to reappear in comparison to blowfly's 60s/4s, so both ERG dissapearance and reappearance are slower in *Ascalaphus* than in *Calliphora*. This could be be due to larger air tanks, due to a higher concentration of cytochromes and therefore bigger capacitance of the electron transport chain, due to a larger stock of ATP or simply because the animal is larger. Reversibility of the process is cleary noticed. We can also notice the rise of base line under stimulation as in the dark, indicating yet unexplained hiperpolarization of the membrane.

The relatively high $pO_2$ at which *Ascalaphus'* photoreceptors stop responding to light stimuli could be explained with the predaceous life-style, its superior flying abilities or simply by the fact that this insect probably never copes with oxygen-depleted atmosphere, to which the blowfly is frequently exposed, especially in the larval stadium.

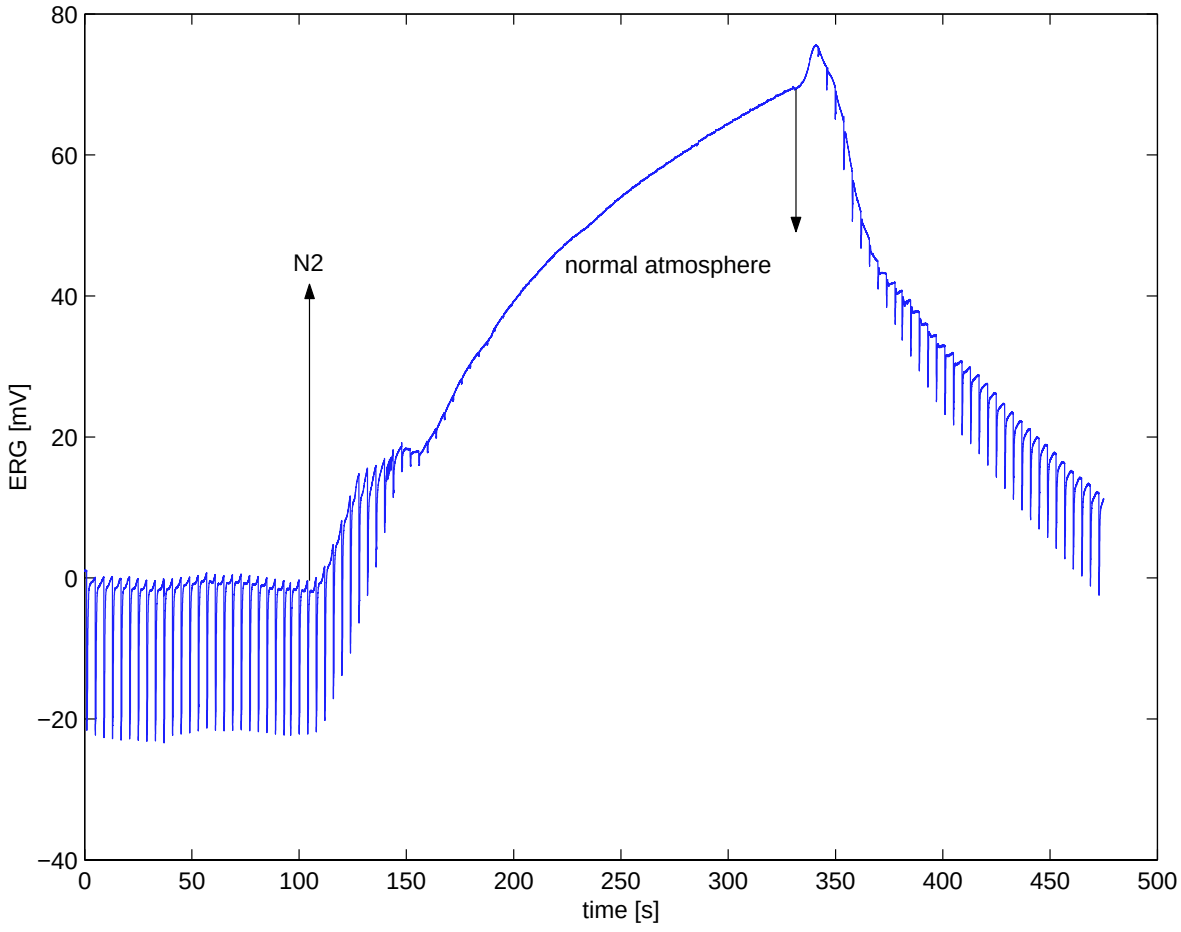


**Figure 1: Electrophysiological measurements of ERG in anoxic conditions under light stimulation**

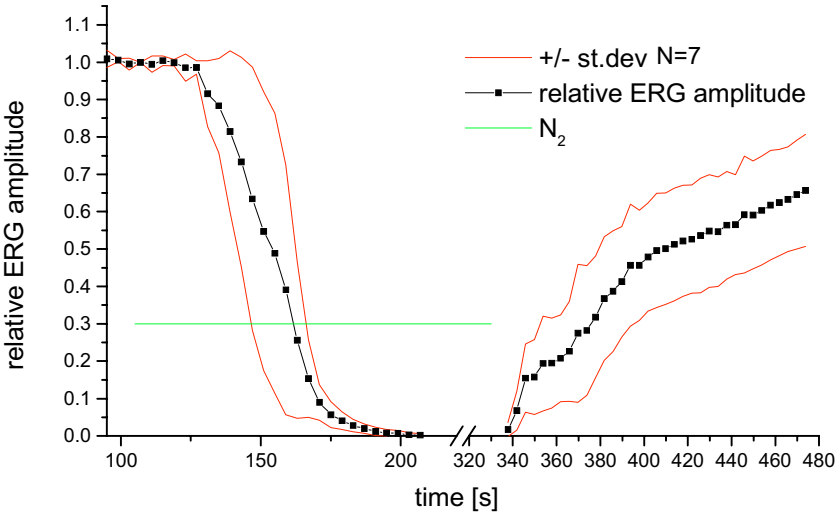


**Figure 2: Time course of relative ERG amplitude under anoxic conditions (its cessation and reappearance)**

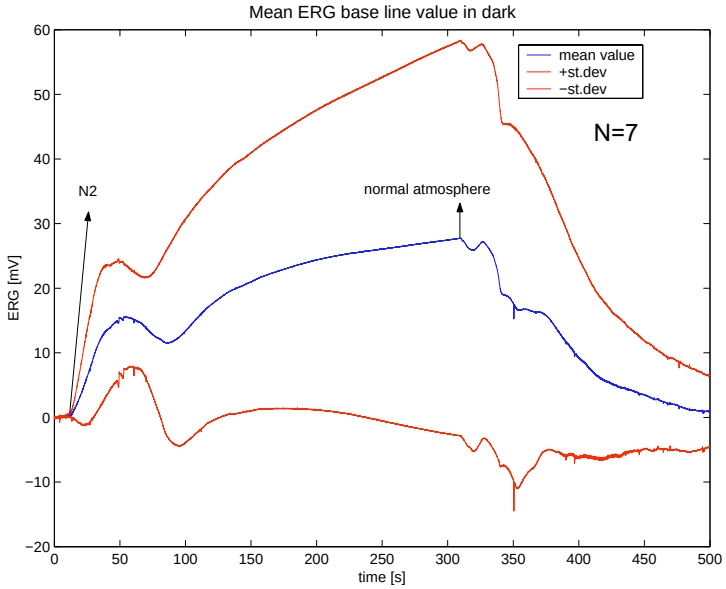


**Figure 3: Time course of ERG base line value under anoxic conditions without light stimuli**

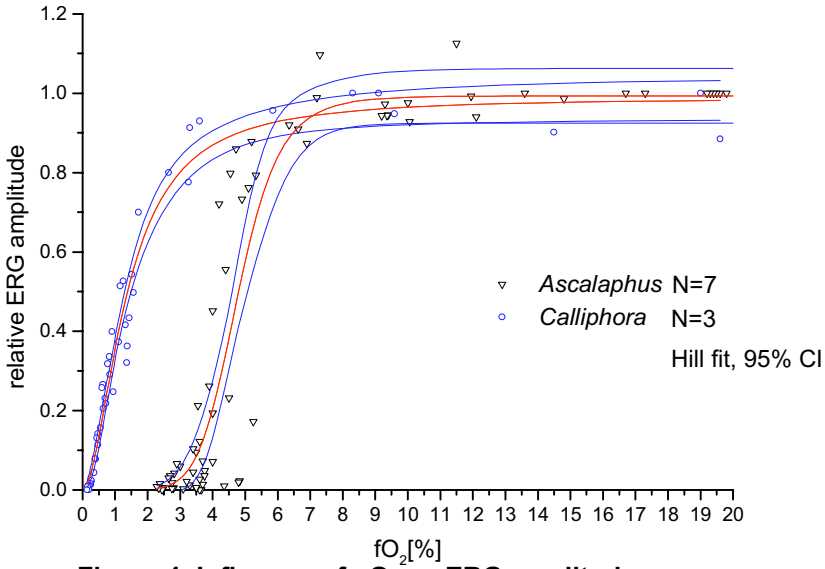


**Figure 4: Influence of $pO_2$ on ERG amplitude**