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Daily visual sensitivity pattern in the green lacewing *Chrysoperla carnea* (Neuroptera: Chrysopidae)

KARL KRAL and MICHAEL STELZL

Institut für Zoologie, Karl-Franzens-Universität Graz, A-8010 Graz, Austria;
e-mail: karl.kral@kfunigraz.ac.at

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Abstract. 24-hour experiments on dark-adapted compound eyes of the green lacewing, *Chrysoperla carnea* (Stephens) show significant daily changes in absolute sensitivity to stimuli of different colors, but no daily changes in the spectral sensitivity. The absolute sensitivity is highest for all wavelengths at midnight, and lowest at noon. The daily shift of sensitivity is, however, most pronounced in the working range of the eye in the bluegreen-green region of the spectrum. The electrophysiologically measured sensitivity of the compound eye to monochromatic stimuli correlates with the size change of its superposition aperture after illumination with white light. The daily sensitivity pattern is in good agreement with the daily flight activity pattern (see Duelli, 1986).

INTRODUCTION

As numerous field studies have shown, adult green lacewings are mainly nocturnal, but there are a few species that are also active during daytime (New, 1967; Duelli, 1980, 1986; Honěk & Kraus, 1981). Nonetheless they all have refractive superposition eyes which dominate the head in the form of golden-yellow to reddish shiny hemispheres (Ast, 1920; Horridge & Henderson, 1976). Owing to retina tiering and other optical and physiological properties, the compound eyes are optimally suited to all natural conditions of illumination (Horridge & Henderson, 1976). Duelli (1986) used light trapping to show that in the largely nocturnal lacewing *Chrysoperla carnea*, flight activity begins after sunset, as soon as it has become dark and continues until first light, i.e., during the time when its diurnal predators, such as birds and predatory insects, are not active. Our field studies further show that during the day, *C. carnea* tends to keep to the fresh green leaves on bushes where it finds food and at the same time enjoys camouflage from predators. Another lacewing species, *Chrysopa perla*, commence activity in the afternoon and is then active just after sunset, but it does not show much activity thereafter. According to Duelli (1986), in the mainly diurnal lacewing *Hypochrysa elegans*, flight activity begins after sunrise, is maximal between noon and early evening, and then decreases rapidly. He found in all lacewing species that the key factor associated with flight activity were darkness or light. The light intensity threshold for flight activity is about 10 Lux for *C. carnea*, thus lacewings sitting in dark places during daytime will get active earlier in the evening.

The aim of this project was to examine the extent to which the compound eyes of the common green lacewing, *Chrysoperla carnea*, are adapted to diurnal requirements of light. As a step toward answering this question, the spectral sensitivity and light sensitivity of the eyes at noon, evening, midnight and morning were analyzed electrophysiologically and optically.

MATERIAL AND METHODS

Animal

This study used adult green lacewings of both sexes of the species *Chrysoperla carnea* (Neuroptera: Chrysopidae). The insects were caught during the summer season in bushes at edges of fields sloping to the south on the outskirts of Graz. They were in full summer coloration of bright green. The insects so collected were kept in the laboratory for only a few days under natural ambient light and temperature. They were fed with a plant-juice extract.

Preparation and electrophysiological recordings

The experimental insect was attached by the thorax to a holder with a mixture of wax and resin. Tungsten electrodes were inserted with micromanipulators (HSE, Germany) into the right compound eye (recording electrode) or into the hemolymph space of the dorsal part of the head (indifferent electrode). Then the insect was dark adapted in a lightproof, electrically shielded chamber until the eyes were fully dark adapted (> 120 min). The extracellular potentials of the eye elicited with light stimulus were preamplified and displayed in DC mode on a dual-beam Tektronix 5103N storage oscilloscope and recorded with a Graphtec WR7800 thermal arraycorder.

Optical stimulation

For brief monochromatic stimulation, the light from a 150 W xenon arc lamp (Spindler & Hoyer, Osram XBO) was passed first through Schott interference filters with approximately 10 nm wide bands of monochromatic light, and than a heat-protecting filter, collimated with a set of quartz lenses and focused at the entrance to a high-grade fused silica fiber optic (UV-VIS; 3.2 mm diameter; numerical aperture 0.22; Oriel) that supplied the eye. At the output of the fiber optic at a distance of 10 mm from the eye surface, a calibrated BPW 34 photodiode measured a maximum stimulus intensity of 2.5×10^{12} photons $cm^{-2}s^{-1}$. The light intensity was varied with calibrated quartz neutral density filters (Schott). Stimulus duration was controlled with an electronic camera shutter (Oriel) and displayed on the screen of the oscilloscope using a silicon photocell located behind the shutter (see Mayer & Kral, 1993).

Electrophysiological protocol

In the 24-h experiment, the insects were kept in absolute darkness (background illumination would cause uncontrolled sensitivity deviations; see Discussion). Every 6 h the eye was stimulated with 12 selected stimulus wavelengths within a range of 366–633 nm and the light intensity measured that was required for near-threshold constant ERG responses and the criterion amplitude of the ERG. Therefore, light intensity was adjusted with neutral density filters at each wavelength until a criterion ERG-amplitude near threshold or of 0.5 mV had been met. A plot of log units of stimulus intensity as a function of wavelength gave the spectral sensitivity function (see e.g. Lall et al. 1980, 1985).

The monochromatic stimuli were offered to each experimental insect in a different random sequence at 3-min intervals and a reference stimulus of known wavelength and intensity was periodically given to control for reliability of the measured data. The duration of each stimulus was 1 sec. With such short stimuli, undesired light-induced motion of screening pigment could most likely be ruled out, which could have had an uncontrolled influence on the measurement results (see e.g. Nordtug, 1990). The beginning of the experiment was varied between noon, evening, midnight and morning. Six hours after the end of the 24-h experiment a control measurement was made to compare the final ERGs with the initial ERGs at the same time of day. Data were included in analysis only when results were comparable (less than 10% variation). This was the case in 60% of the studied insects.

Determination of effective acceptance angles of the ommatidium

A luminous (corneal) pseudopupil response elicited with orthodromic illumination using axially incident white light from a xenon arc was used to measure the effective acceptance angle of fully dark-adapted ommatidia every 6 h during a 24-h period. Control measurements were made over the next 24 h to determine the extent to which they agreed with the experimental measurements. Only data that were in agreement were evaluated (approx. 90%). The instrumentation used was a 3-D goniometer with electrical measuring apparatus in conjunction with a Dialux 20 microscope (Leitz) equipped with epi-illumination. For details on experimental procedures and methods see Eggenreich & Kral (1990). All experiments were carried out at room temperature of about 20 to 23°C.

Statistical procedure

Unnormalized data from each species were pooled and means and standard deviations plotted. Diurnal differences between treatments were tested for significance using two-sample analyses (t-test; $P < 0.05$).

RESULTS

ERG characteristics

The ERGs recorded from the dark-adapted compound eye of *C. carnea* with three different 1-sec monochromatic light stimuli (ultraviolet, 366 nm, blue, 450 nm and bright green, 546 nm) are shown in Fig. 1. Recordings under these stimulus conditions give a diphasic ERG with phasic on- and off-transients. Stimuli of these three different wavelengths elicit equivalent shapes of ERGs, in which relative intensities produce the same amplitudes (criterion of 0.5 mV; see Material and methods), i.e., there may not be a color-specific effect on ERG shape. This suggests that there is probably only one kind of spectral mechanism (in the large retinula cells R1-6) contributing to the response (see Kirschfeld & Lutz, 1977). This supposition is also supported by experiments with selective chromatic adaptation (unpublished observations). Regardless of whether adaptation was with UV, blue or green light, the sensitivity was decreased in the same way for all wavelengths. But the possibility cannot be excluded that there could be an adaptation effect with considerably higher stimulus intensities than those used here.

24-h experiments

The near-threshold spectral sensitivity curves and the spectral sensitivity curves of the dark-adapted compound eyes of *C. carnea* show a broad peak in the green at the times of day evaluated here, i.e., noon, evening, midnight and morning. Near threshold, the width of the maximum is always about the same (490–550 nm); it is greatest at midnight (490–550 nm), only slightly smaller in the morning and evening, and clearly smallest at noon (510–550 nm) (see Figs 2, 3). The maximum is generally in bright green (546 nm).

Near-threshold sensitivity and sensitivity measurements further show that in the bluegreen-green region of the spectrum, the dark-adapted compound eye reacts by about 1 log unit (i.e., ten times) more strongly to monochromatic stimuli at midnight than at noon.

In the evening and morning, the sensitivity is roughly between that of midnight and noon, or closer to midnight (near-threshold sensitivity). In the UV range, the difference for sensitivity between the times of day is at the limit; it is always significant for near-

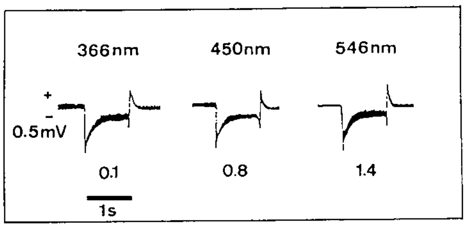

Fig. 1. This figure shows ERG waveforms for three colours (UV: 366 nm, blue: 450 nm and green: 546 nm) at different stimulus intensities (negative logarithm of I/I_{max}) recorded from the dark-adapted compound eyes of the lacewing *Chrysoperla carnea*. Criterion On-amplitude: 0.5 mV; duration of stimulus: 1 sec.

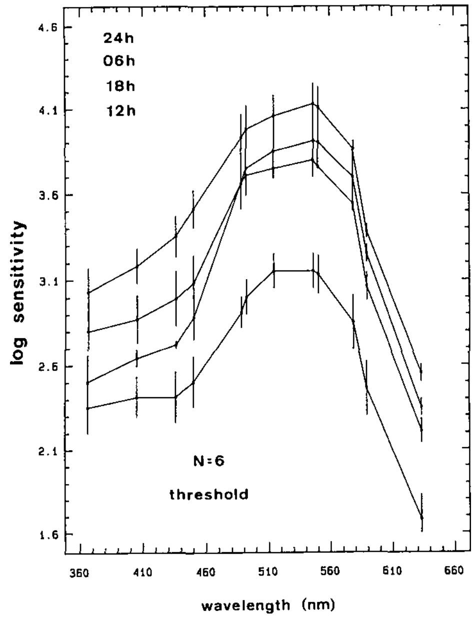

Fig. 2. Near-threshold spectral sensitivity curves of the dark-adapted compound eyes of the lacewing *Chrysoperla carnea* measured at noon, evening, midnight and morning. In this 24-h experiment, the animals were kept in absolute darkness. Values are mean ± S.D. N (number of studied animals) = 6.

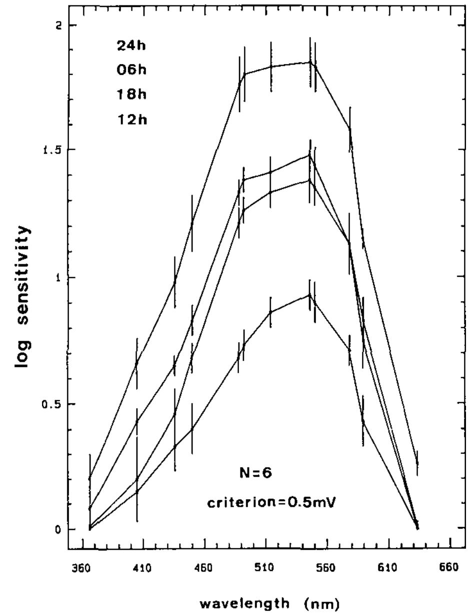

Fig. 3. Spectral sensitivity curves of the dark-adapted compound eyes of the lacewing *Chrysoperla carnea* for criterion amplitude of 0.5 mV measured at noon, evening, midnight and morning. In this 24-h experiment, the animals were kept in absolute darkness. Values are mean ± S.D. N (number of studied animals) = 6.

threshold responses except between noon and evening. Tables 1 and 2 summarize the calculated values.

TABLE 1. Difference in sensitivity to stimuli of different colours in log units between different day times in *Chrysoperla carnea*. N = 6 insects.

	366 nm	450 nm	546 nm
06–12 h	0.08 (P = 0.05)	0.43 (P < 0.00001)	0.55 (P < 0.00001)
12–18 h	–0.01 (P < 0.05)	–0.28 (P < 0.001)	–0.42 (P < 0.0001)
18–24 h	–0.19 (P < 0.05)	–0.53 (P < 0.00001)	–0.47 (P < 0.0001)
24–06 h	0.12 (P < 0.2)	0.38 (P < 0.001)	0.37 (P < 0.001)

TABLE 2. Difference in threshold sensitivity to stimuli of different colours in log units between different day times in *Chrysoperla carnea*. N = 6 insects.

	366 nm	450 nm	546 nm
06–12 h	0.45 (P < 0.01)	0.58 (P < 0.001)	0.76 (P < 0.00001)
12–18 h	-0.15 (P < 0.5)	-0.38 (P < 0.001)	-0.65 (P < 0.0001)
18–24 h	-0.53 (P < 0.01)	-0.63 (P < 0.00001)	-0.33 (P < 0.01)
24–06 h	0.23 (P < 0.05)	0.43 (P < 0.01)	0.22 (P < 0.000001)

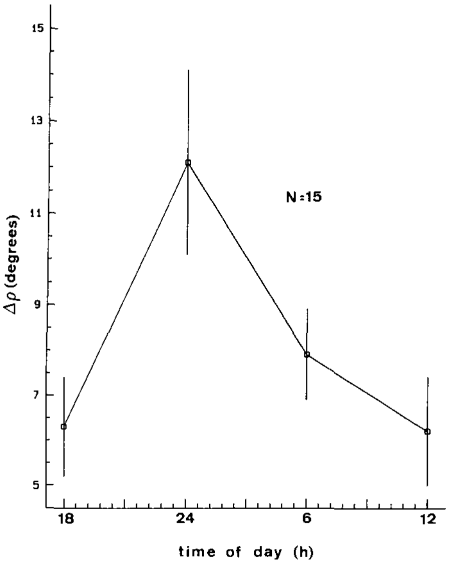

Fig. 4. Diagram shows relation of effective acceptance angles Δρ of dark-adapted ommatidia, which determine the size of the ommatidial visual field, to time of day in the lacewing *Chrysoperla carnea*. Δρ was estimated by measuring the angular span in which the pseudopupil remains in the ommatidium. In this 24-h experiment, the animals were kept in absolute darkness. Values are mean ± S.D. N (number of specimens) = 15.

Optical measurements

Pseudopupil measurements with white light from a xenon arc show in *C. carnea* that in the dark-adapted compound eye ("frontal equatorial region"), the average horizontal ommatidial acceptance angels Δρ are smallest between noon and evening (6.2° up to 6.3°), reach values averaging 12.1° at midnight and decrease toward morning to 7.9° (for more detail see Fig. 4). This means that the actual superposition aperture (due to the hemispherical shape of the eye, perhaps equivalent to the effective aperture) is about twice as large at midnight as it is at noon.

DISCUSSION

The presence of a dominant peak in the dark-adapted spectral sensitivity curves at 546 nm indicates the presence of a green receptor in the compound eyes of the lacewing *C. carnea* (however, the electrophysiologically-measured spectral sensitivity curves alone do not suffice to show whether the eyes can evaluate colors, or whether they only represent a light-dark system). The width of the curve plateau (490–550 nm) indicates that it must be a broad-band receptor (R1–6). The curve maximum seems to be in a range in which the fresh leaves on bushes reflect more light by percent than do stems, branches or dry grass and, seen absolutely, are especially bright (see e.g. Brown & Anderson, 1996). This brightness allows lacewings to recognize the fresh green leaves that they use to find honey dew produced by aphids, a site for egglaying and as a camouflaged resting place, as our field observations have shown.

Our findings from the 24-h experiments with the lacewing *C. carnea* show no significant daily changes in the spectral sensitivity of the dark-adapted compound eyes - with a broad peak in green – but do show significant daily changes in absolute sensitivity to stimuli of different colors. The sensitivity is highest for all wavelengths at midnight, and lowest at noon. Sensitivities are somewhere in between in the evening and morning. The daily shift of sensitivity to monochromatic stimuli is most pronounced (within the working range of the eye) in the bluegreen-green region of the spectrum. This shift is not remarkable in the UV range but near threshold of light sensitivity, in contrast, it is significant for all wavelengths.

The dial sensitivity pattern of the compound eyes of *C. carnea* is in quite good agreement with its flight activity pattern (Duelli, 1986): no activity during the day, commencement of activity at dusk, high activity during the night and an abrupt decrease in activity as soon as twilight at dawn reaches 10 Lux.

Because of the way the experiments were set up (start of experiment at different times of day, chromatic stimuli offert in different orders at absolute darkness, ERG amplitudes checked after every series of measurements as well as 6 h after the end of each 24-h period, constant temperature, see Materials and methods), we can probably rule out the possibility that in these experiments conditions of light or temperature in the experimental environment were responsible for the daily patterns of sensitivity. The fact that the light factor (the daily pattern of illumination) and temperature can be excluded indicates that internal circadian mechanisms must be responsible for the diurnal sensitivity to stimuli of different colors (see e.g. Tomioka et al., 1993). We know that endogenous rhythmicity of pigment migration (changes of superposition pupil) in superposition eyes, as impressively shown for nocturnal lepidopterans (Demoll, 1911; Bernhard et al., 1963), is nothing unusual.

Data of pseudopupil measurements presented on the diurnal dependence of the effective ommatidial acceptance angles $\Delta\rho$, i.e. of the superposition aperture, of the dark-adapted compound eye of lacewings also point in this direction. It is remarkable here that the size change of the superposition aperture generally correlates with the electrophysiologically measured sensitivity to stimuli of different colors (the evening values are an exception to this). Daily structural and physiological changes in visual cells (see e.g. Horridge et al., 1981; Blest et al., 1984) and/or movement of retinula cells (Horridge & Henderson, 1976) caused by internal mechanisms could also be responsible for the cyclical sensitivity pattern in lacewing eyes.

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