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Relative Spectral Sensitivity and Reverse Purkinje Shift in Identified L Neurons of the Ocellar Retina

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Summary. 1. The relative spectral sensitivities of identified large second order neurons (L neurons) of the dragonfly ocellus have been examined with dye filled intracellular microelectrodes placed in the brain.

2. Representative L neurons of the four geometric classes present in the median ocellar nerve show essentially similar waveforms in response to white, green and UV light.

3. Details of the retinal projections of these four neuron classes have been established.

4. L neurons exhibit a marked reverse Purkinje shift at low light intensities. Green sensitivity (485 nm) decreased relative to UV sensitivity (360 nm) as the intensity of illumination increased. At a mean intensity of 1.78×10^{12} photons cm^{-2} s^{-1} the L neurons are equally sensitive to UV and green light.

5. The reverse Purkinje shift is discussed in relation to data previously obtained for dragonfly receptor cell spectral sensitivity and in relation to its possible functional significance.

Introduction

Chappell and DeVoe (1975) have reported the spectral sensitivity of dragonfly ocellar receptor cells. At high intensities these cells were maximally sensitive in the UV with a secondary maximum in the green. In half of the receptor cells they examined there was a reverse Purkinje shift such that at low stimulus intensity the relative sensitivity reversed with the maximum sensitivity in the green and a lesser sensitivity in the UV. The crossover occurred at very small amplitudes of response, generally less than 15% of V_{max} (1.4–4.4 mV). Since the UV and green sensitivity response curves become parallel above about 25% of V_{max} (8 mV) it was not clear just how important the reverse Purkinje shift could be to the animal. Because of the high voltage gain reported at the first order synapse (Chappell and Dowling 1972; Patterson and Goodman 1974) it might be expected that this effect would be clearly reflected in second order cell response/intensity slopes. Wilson (1978), however, using wavelengths of 366 nm and 516 nm found no evidence of non parallel slopes of intensity response curves between 25% and 75% V_{max} in the large second order neurons (L neurons) of the locust ocellus.

It is of interest to examine the extent to which the spectral sensitivities of second order ocellar neurons correspond to those known for photoreceptor cells in the same insect. We have investigated the spectral responses of large second order median ocellar neurons of the dragonfly to UV and green wavelength stimulation corresponding to the two peaks of spectral sensitivity of its photoreceptor cells. The variety of geometries of individual median ocellar large second order cells in the dragonfly brain (Chappell et al. 1978; Patterson and Chappell 1980) suggests that there might be a difference in response dynamics and possibly spectral sensitivities of these second order cells. Here we examine the response characteristics and relative spectral sensitivities of all the large median ocellar neuron types by means of intracellular recording from their projections within the brain. These recordings have revealed a reverse Purkinje shift in dragonfly L cells which we report below.

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Materials and Methods

Three dragonfly species caught in the environs of Woods Hole were used for these experiments, *Aeschna tuberculifera, Sympetrum rubicundulum* and *Pachydiplax longipennis*.

Animals were maintained in the laboratory for up to three days prior to the experiments. For recording, dark adapted whole animals were held in a slotted tube with their heads waxed to the end. 'Plasticene' was used to keep the body still and to cover the compound eyes and the lateral ocelli. The frons and tissue overlying the brain were removed, leaving the ocellar lenses intact, and the electrode inserted. Sixty megohm electrodes were pulled from 1 mm capillary glass containing a small fused internal capillary to facilitate filling. These electrodes were filled with 3 M cobalt chloride solution. Signals were fed from the electrode via a head stage amplifier into a WPI 707 electrometer amplifier with current injection facilities. The resulting signals were displayed and photographed on a Tektronix 5111 storage oscilloscope. An indifferent electrode was filled with dragonfly Ringer (Duchâteau et al. 1953) and placed in contact with the brain.

Light stimuli were provided by a 100 W quartz halogen bulb focused, via glass optics, to a collimated beam (Dowling and Ripps 1971). The parallel beam was passed through a filter holder containing neutral density and spectral filters and deflected onto the preparation by a mirror. The three spectral filters used to obtain spectral sensitivity curves were a Corning CS 737 (λ_{max} 360 nm, 50% bandwidth 50 nm) and a Kodak no. 75 in combination with a Corning CS5-61 (λ_{max} 485 nm, 50% bandwidth 30 nm). The filters were chosen to approximate the two points of ocellar receptor peak sensitivity (Chappell and DeVoe 1975). A fourth filter (647 nm interference, 50% bandwidth 15 nm) was used to test the red sensitivity of four cells. The unattenuated white light intensity (log I=0) was 1.36×10^3 lux. Measurements of light intensity were made with a UDT 111A photometer equipped with a PIN 10DF diode calibrated from 350 nm, to 1,100 nm to the specification of the National Bureau of Standards (America). Corrections for changes in the optical density of glass components and neutral density filters were effected by direct measurement of the intensity of light incident upon the preparation with all the combinations of the filters used.

Cobalt was introduced to the neurons by applying a positive voltage at a constant current of 5×10^{-7} A to the electrode using 500 ms pulses (50% duty cycle) for 5 min. The heads were cut from the preparations into 5 ml of Ringer (Duchâteau et al. 1953) containing two drops of 10% ammonium sulphide. After 2 min the heads were washed in Ringer for 5 min before fixing for 3 h in alcoholic Bouin. Brains were removed from the head capsule, dehydrated through graded ethanols, cleared in xylene and coverslipped under balsam. Drawings were made with the aid of a camera lucida.

Results

Anatomy

Cobalt iontophoresis via the recording electrode resulted in the staining and definitive identification of 16 median ocellar L neurons. Central projections of dragonfly ocellar L neurons have been identified in the brain by Patterson and Chappell (1980) using whole nerve cobalt staining. Their nomenclature will be used in this paper. The dye injections of individual neurons made in the course of the experiments reported here permitted the examination of the retinal projections obscured in whole nerve fills. We found that all except a single unique midline neuron (MB) (Fig. 1c) have projections that are restricted to the left or right halves of the median ocellus. All of the cells apart from MB are paired neurons with an identical partner in the opposite half of the brain. Of the 16 cells stained 9 had projections from one half of the median ocellus to the contralateral half of the brain and 4 projected to the ipsilateral half of the brain. The remaining 3 cells were MB neurons with projections in both halves of, the median ocellus and, the brain. In one preparation slender collaterals from an MB cell could be followed into each of the two lateral ocellar nerves. Of the 9 contralateral median cells 3 were equivalent to the MC_1 neuron, recorded and identified by Patterson and Chappell, and two had rather different dendritic patterns in that they arborized slightly higher in the posterior brain and nearer the midline. These two cells match the pattern of the cell referred to as M_2 by Patterson and Chappell. In the remaining four cases sufficient detail was available only to identify the cell as unequivocally contralateral but not to resolve whether it was M_2 or MC_1. The four ipsilateral neurons were clearly identical in all respects and were one of the paired ipsilateral L neurons (M_3 and M_4). In one experiment, both M_3 and M_4 were stained in the same preparation demonstrating the cells to be an identical

Fig. 1. a The unique midline L neuron MB viewed in an unintensified whole-mounted preparation. The cell body is out of the plane of focus and is visible as a dark shadow (arrow). MB projects from both halves of the divided median ocellar plexus to the left and right hand protocerebral posterior slope neuropiles of the brain. Note dark pigment (*P*) in the brain sheath. Bar 50 μm. **b**, **c** and **d** are drawings made from *Pachydiplax, Sympetrum* und *Aeschna,* respectively. Note the conspicuous differences in brain size, the obvious bilateral division of the median ocellus in *Aeschna* and the different entry points of the lateral ocellar nerves. Bar 100 μm. **b** Median ipsilateral neuron. M_3 is one of two median ocellar neurons (M_3 and M_4) which project from one half of the median ocellus to the ipsilateral PSN. The two cells M_3 and M_4 are geometrically equivalent and are represented on both sides of the brain. **c** Median bifurcating neuron. MB is the median bilateral neuron illustrated in **a** above. MB has projections into both lateral nerves as well as in both halves of the median ocellar neuropile. **d** Median contralateral neuron. MC_1 is an L neuron projecting from one half of the median ocellus to the contralateral PSN of the brain. A similar cell (M_2), runs from one half of the median ocellus to the contralateral PSN but has a slightly different geometry to MC_1, arborizing nearer the brain's midline. The three inset traces show typical white light responses at log I = −2 for the three different fiber types. Calibration (lower trace): 5 mV, 500 ms (also shows light ON)

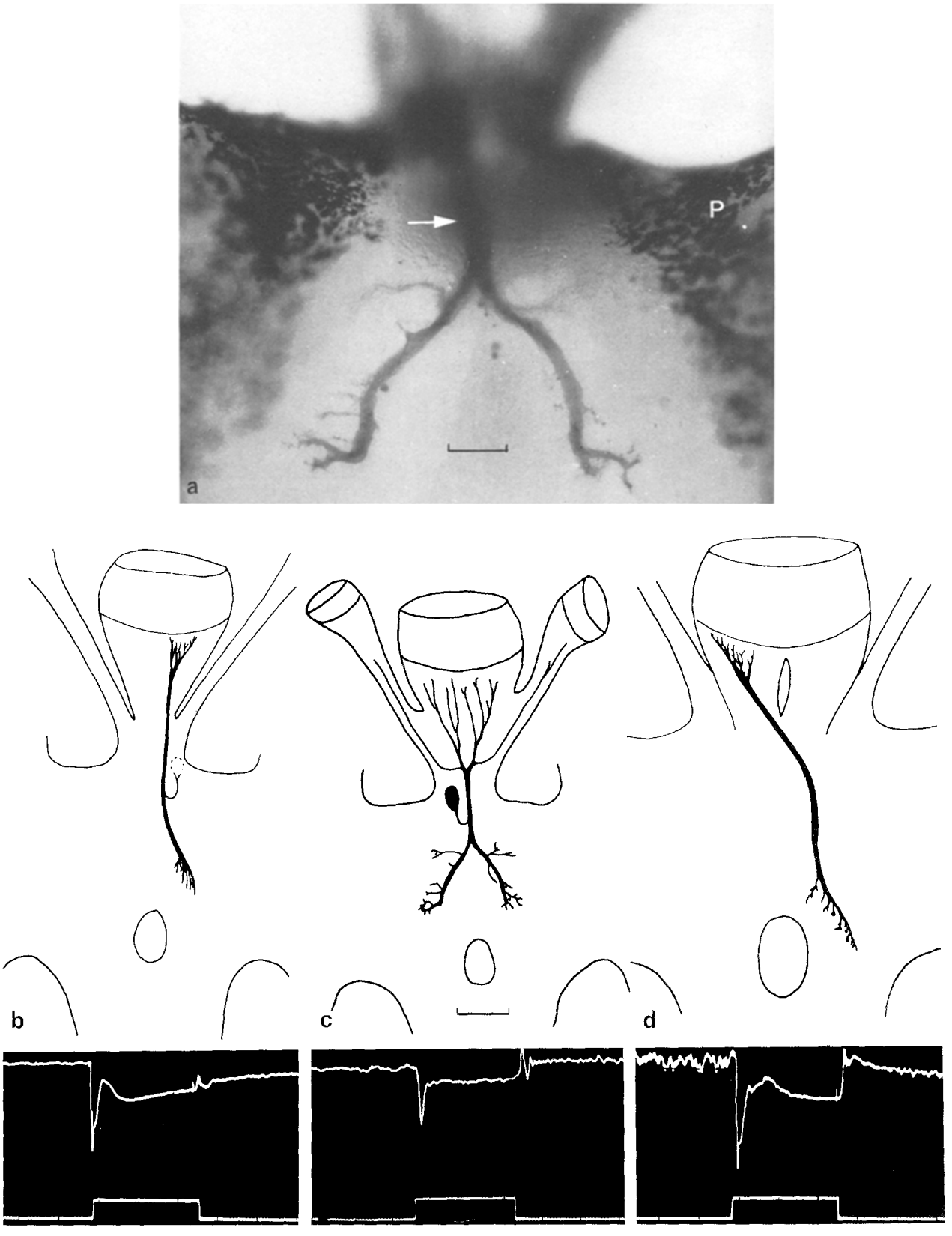

Fig. 1

pair of neurons. No significant differences in neuron geometries were found between the different species used in the experiments. The fibres M_3 and M_4 can now be more correctly referred to as MI fibres and M_2 as MC_2; the I and C indicating that their arborizations are, respectively, ipsilateral and contralateral to their dendritic branches in the ocellus.

Electrophysiological Responses and Spectral Sensitivity

All the neurons recorded and stained produced, on ocellar illumination, hyperpolarizing, non-spiking responses such as those shown in the insets of Fig. 1. These responses appeared very similar in neurons of different geometry. Several spiking ocellar neurons were encountered in the course of the recordings. Dye injection of one of these cells showed it to be a small cell of a diameter typical of ocellar S neurons as described by Goodman (1974). The waveforms recorded here from L neurons within the brain are essentially identical to those recorded in the retinal region by Chappell and Dowling (1972) and Patterson and Chappell (1980).

Relative spectral sensitivities were studied in 10 cells only one of which was not later identified by staining. The identified cells included four contralateral, three bifurcating and two ipsilateral L neurons. Both UV (360 nm) and green (485 nm) intensity-response curves were obtained since these correspond well with the peak sensitivities reported for dragonfly median ocellar photoreceptors (Chappell and DeVoe 1975). We did not find any evidence to suggest differences between cell types, or species, based on their spectral sensitivities. A typical intensity-response curve is shown in Fig. 2. This ipsilateral median ocellar L neuron (MI) was more than 1.5 log units more sensitive to green than UV light near threshold but became relatively more sensitive to UV as the intensity of illumination increased, reaching a point where equal photon fluxes elicited equal magnitude responses; the crossover point. At higher intensities, the cell became more sensitive to UV than green. This change from greater sensitivity in the green, toward greater sensitivity in the UV, represents a reverse Purkinje shift which was typical of all cells examined spectrally. For the ten cells examined, crossover occurred at a mean log flux of 12.3 ± 0.5 photons $cm^{-2}\ s^{-1}$. No obvious differences in waveform were noted between the response elicited by UV, green, or white light stimulation. Responses of comparable amplitude at green and UV wavelengths are shown in Fig. 2b. Changes in the response waveform were noted with different intensities, not different wavelengths. At higher intensities the responses became more phasic and looked like the white light responses at log $I = -2$ from the same cell (Fig. 1b). Several cells were tested with 647 nm light flashes. The responses elicited to red light were essentially similar in form to those obtained with UV and green light.

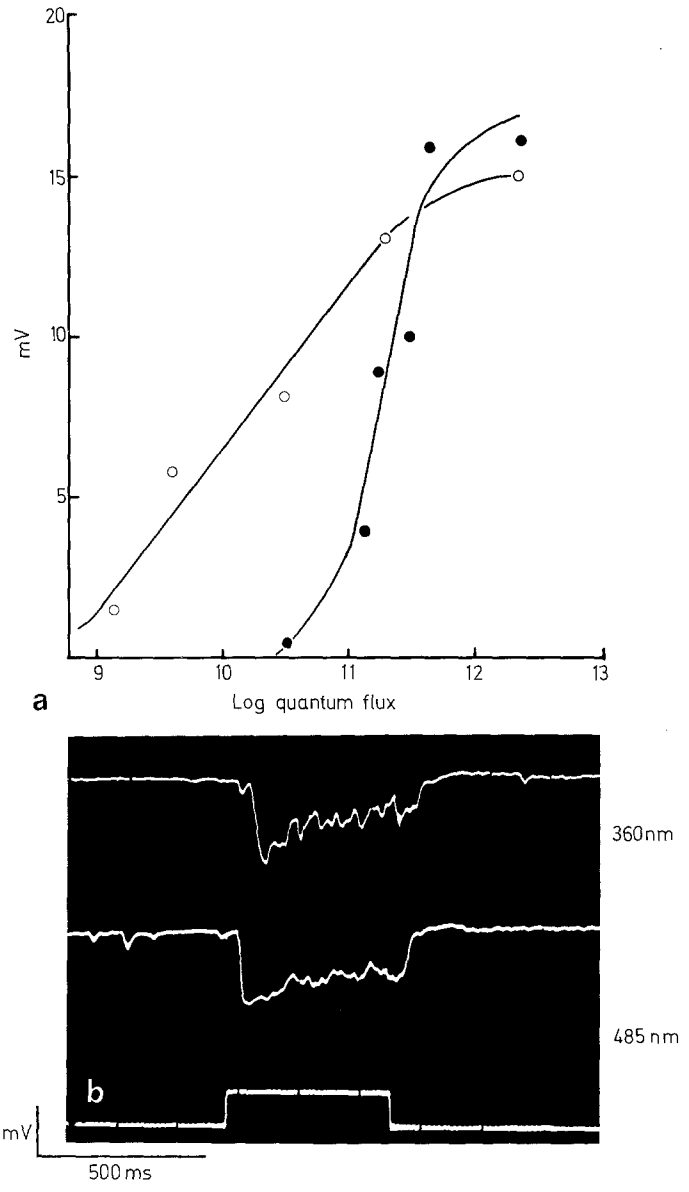

Fig. 2. a Median ipsilateral L neuron (*MI*) response versus intensity for UV and green stimulation. The amplitude of the hyperpolarising ON transient in mV (ordinate) is plotted as a function of the log quantum flux (log photons $cm^{-2}\ s^{-1}$) incident on the ocellar lens (abscissa). Green sensitivity (485 nm, open circles) decreased relative to UV sensitivity (360 nm, filled circles) as the intensity of illumination increased resulting in a reverse Purkinje shift with an equal sensitivity crossover point at 3.2×10^{11} photons $cm^{-2}\ s^{-1}$ (log flux -12.5). **b** Responses obtained near crossover are shown for each of the two wavelengths ($I = 2.2 \times 10^{11}$ photons $cm^{-2}\ s^{-1}$ for 360 nm response; $I = 3 \times 10^{10}$ for 485 nm. The cell's response to a white light flash is shown in Fig. 1b

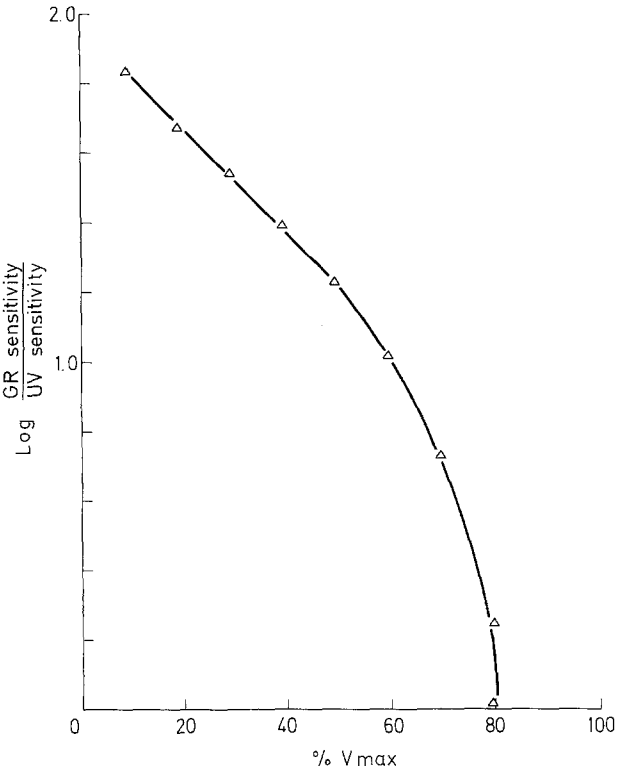

Fig. 3. Log ratios of green to UV sensitivity normalized as a function of percent of maximum response to white light (V_{max}). There was a decrease in the ratio of green to UV sensitivity as the median ocellar L neuron responses increased in amplitude with brighter flashes. The curve represents data averaged from 4 highly stable preparations (two contralateral and two ipsilateral L neurons) normalized as a percent of V_{max} for each cell. The rate of decrease in this ratio, which represents a reverse Purkinje shift, was quite linear between 10% and 40% of maximum response for most cells. The mean change in the log GR/UV sensitivity ratio was -0.25 ± 0.07 over this range when determined from the responses of 7 identified L neurons (4 contralateral, 2 ipsilateral, and 1 bifurcating)

The sensitivity of the cells to red light was approximately 3 log units less than to green light.

In comparing the Purkinje shift of the cells studied, it was helpful to normalize the results by plotting the log of the ratio of green sensitivity to UV sensitivity as a percentage of the cells' maximum response (V_{max}) to the brightest flash of white light (12.7 lux). Figure 3 shows such data averaged from four of the most stable cells. The green sensitivity decreased relative to UV sensitivity as the intensity of the flashes increased, reaching crossover at $80 \pm 8\%$ of V_{max}.

Discussion

Purkinje (1819) observed that for human observers the relative brightness of differently colored objects depended upon the level of illumination. A decrease in the relative sensitivity at the red (long wavelength) end of the spectrum as the level of illumination decreases is referred to as a Purkinje shift. This discussion considers a decrease in the relative sensitivity of the dragonfly ocellus in the UV (short wavelength) end of the spectrum as the level of illumination decreases, a reverse Purkinje shift. Determination of the anatomy of the cells by intracellular dye iontophoresis has shown that this reverse Purkinje shift is observed in all of the median ocellar L neuron types.

The anatomy of the ocellar system has now been well established in several different insects (see Goodman 1981 for review). The systems in all the insects so far examined are similar in several fundamental respects. All possess small numbers of large, (L), neurons connecting the ocelli to the posterior slope neuropile (PSN) of the protocerebrum as well as large numbers of smaller, (S), neurons that run to a variety of areas in the brain. Remarkable similarities exist in the distribution of the median ocellar L cells; the bilateral dragonfly neuron, MB, bearing a striking resemblance to the bilateral neuron M2 of the locust (Goodman 1974) and MOC 7/14 of the bee (Pan and Goodman 1977). The fact that all of the stained neurons, except MB, had arborisations restricted to just one half of the median ocellus emphasizes the importance of the internal bilateral division. Any functional significance of such a division is not yet apparent but while it is possible that it is simply a consequence of a bilateral origin it may be that the insect can derive some information from differences in the light flux on the median ocellar halves.

L cell responses of the whole, unanaesthetized, ventilating insect are comparable to those recorded in the isolated dragonfly head by Chappell and Dowling (1972). A slow, graded, hyperpolarizing potential in response to light, identical to the response recorded in the distal median ocellar retinal neuropile (Patterson and Chappell 1980), was recorded here well within the brain indicating that the graded slow potentials may be sufficient to drive the postsynaptic cells.

The presence of a varying ratio of UV to green sensitivity in different photoreceptors of the dragonfly median ocellar retina (Chappell and DeVoe 1975), suggests that with appropriate connectivity higher order cells might have significantly different spectral characteristics. Having studied spectral responses near the two peak sensitivities involved, we found that there were no apparent systematic differences between the spectral sensitivities of the dragonfly median ocellar L neurons with respect to the geometry of the L neuron identified by staining. Each exhibited greater sensitivity to green wavelength stimulation at lower intensities with the relative sensitivity to UV

light increasing at higher intensities. The reverse Purkinje shift is similar to that reported for the photoreceptor cells (Chappell and DeVoe 1975). Crossover for L neurons, however, occurred at response magnitudes near 80% of the maximum response of the cells as compared with less than 15% of the maximum response for the receptor cell reported by Chappell and DeVoe. The log quantum flux per square centimeter of 12.3 ± 0.5 for our L neurons falls well within the range of 10.9 to 13.3 for the two receptor cells they show. The magnitude of response at which crossover occurs for the L neuron is consistent with the observation by Chappell and Dowling (1972) that the L neuron ON transient shows greater sensitivity than that of the receptors. Based on their data it appears that the gain at this first synapse in the ocellar pathway is greater than four. Consequently, this shift in spectral sensitivity which occurs near the threshold end of the intensity-response curve for photoreceptors appears nearer the saturation end of the L neuron curves and may have considerable physiological significance. The functional importance of the shift is suggested by our observation of dragonflies, including *Aeschna* sp., hunting at dusk at low levels of illumination.

The data presented in Fig. 3 shows the considerable extent of the reverse Purkinje shift in dragonfly L neurons. A system such as this would be well suited for detecting the contrast between ground, and sky, or water. Wilson (1978) for example has suggested that the optics of the locust ocellus are ideally suited for the detection of the horizon by virtue of the contrast differences its motion through the ocellar visual cone will produce. In detecting contrast between sky and ground during the day, the high UV sensitivity of the dragonfly ocellus would be useful because the daytime sky is rich in UV which is absorbed by the ground. The green system, however, would detect significant amounts of green light reflected from foliage on the ground, thereby reducing the contrast. The relative sensitivity of the UV system may in fact be further enhanced by the presence of substantial green background illumination during the day since Chappell and DeVoe (1975) have reported selective adaptation of receptor cells in the green.

The available information on the daylight spectrum shows that as the solar altitude decreases the number of UV photons relative to green photons also decreases (Henderson 1977). The calculations of Deirmendjian and Sekera (1954) indicate that the ratio of the irradiance for UV light (360 nm) to green light (485 nm) will change from 0.87 with the sun overhead to 2.2 with the sun at a zenith angle of 84.3 °. At dusk an enhanced green sensitivity brought about by the reverse Purkinje shift would reduce the horizon image contrast because of the green light reflected by foliage. It may however be important to increase the probability of photon capture at low light intensities with a subsequent enhancement of the signal to noise ratio even at the expense of some contrast sensitivity.

It may be important not to consider the ocelli simply as horizon detectors as the ability to discriminate small changes in intensity within large visual angles may be used for several other purposes. The detection of the horizon, as what is, in effect, a shadow, predicts that the entry of any object into the visual field of an ocellus may be detected as a change in the light flux on the retina. This change in the light flux may be involved in roles as diverse as the detection of the edge of a pond surrounded by trees as an inverted horizon or mediating startle responses to objects entering the visual field nearer to the ocellus. The reverse Purkinje shift reported here for the dragonfly median ocellus will result in an improvement in sensitivity for both twilight and night sky and warrants further examination.

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Note Added in Proof

Since the submission of this manuscript G. Stange (J Comp Physiol 141:335–347 (1981)) has demonstrated a reverse Purkinje shift in the ocellar evoked head reflexes of the dragonfly *Hemicordulia tau*. His behavioural measurements of the point at which dragonflies are equally sensitive to green and UV light provides values between 1×10^{11} and 1×10^{12} photons cm^{-2} s^{-1} which compare favourably with the intracellularly determined mean figure of 1.78×10^{12} photons cm^{-2} s^{-1} given here.

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