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The orientation-dependent visual spatial cut-off frequency in a spider

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SUMMARY

Cupiennius salei **(Araneae, Ctenidae) has, like most spiders, eight camera-type eyes. The anterior median eyes are called principal eyes and have a movable retina; all of the other eyes are referred to as secondary eyes and are equipped with a reflecting tapetum. The photoreceptors in the secondary eyes are arranged in rows on the tapetum and the inter-receptor angle along such a row is smaller than normal to it. In this study, the vertical and horizontal spatial cut-off frequencies of moving gratings were measured for the posterior median (PM) eyes, and the data were then compared with the anatomical data reported in the literature. Detection of moving objects in the secondary eyes enhances the eye muscle potential frequency in the principal eyes. We thus recorded the eye muscle activity with a telemetric unit as a monitor for motion detection while moving stimuli – sinusoidally modulated bright and dark stripes – were presented to the PM eyes on a computer screen. A significant increase in the eye muscle activity was measured for gratings at an angular wavelength of 2.0 deg in the vertical orientation and of 2.7 deg in the horizontal direction. In the vertical orientation the critical wavelength is twice the inter-receptor angle; in the horizontal orientation the spiders responded to wavelengths that are smaller than twice the corresponding inter-receptor angle. The cut-off frequency seems thus to be limited by the visual field of the photoreceptors rather than the inter-receptor angle. The relative intensity modulations modelled for the two different grating orientations in single photoreceptor cells were in line with our data.**

Key words: spider eyes, electrophysiology, eye muscles, spatial resolution.

INTRODUCTION

Cupiennius salei (Keyserling, 1877) is a night-active hunting spider from Central America and it is most common in Mexico, Guatemala and Honduras. It prefers to live on monocotyledons, such as banana plants and bromeliads, where it remains sheltered during daytime (Barth et al., 1988b). At dusk the animals begin to hunt for prey and search for mates (Barth and Seyfarth, 1979). The spiders mainly rely on their excellent mechanosensory system in these behavioural contexts as has been shown in numerous studies (e.g. Melchers, 1967; Barth, 1986; Barth et al., 1988a; Baurecht and Barth, 1992; Barth, 1993) and vision seems to play only a minor role. Some manifestations of the behavioural significance of the visual system have been reported by Schmid (Schmid, 1997; Schmid, 1998) and Neuhofer et al. (Neuhofer et al., 2009). The anatomy of the eyes (Land and Barth, 1992; Kaps and Schmid, 1996) and the size and structure of the visual centres in the brain (Strausfeld et al., 1993; Strausfeld and Barth, 1993) suggest an even more important influence of the visual system in at least some behavioural contexts.

Cupiennius has, like most spiders, eight simple eyes with a cuticular cornea, a biconvex lens, a vitellar body and a retina. They can be divided into four different pairs according to their position on the carapace. The anterior median eyes (AM) are also called principal eyes, while the anterior lateral (AL), the posterior median (PM) and the posterior lateral (PL) eyes are referred to as secondary eyes.

The astonishingly low *F*-numbers (the ratio of the focal length to the lens diameter) of the spiders' eyes – ranging between 0.74 and 0.58 according to Land and Barth (Land and Barth, 1992) – indicate a high light sensitivity, and indeed the absolute corneal illuminance threshold was found to be well below 0.01 lx (Barth et al., 1993). The human eye has a maximal *F*-number of 2.1 [because the distance between the retina and the optical centre of the lens is 17.1 mm and the diameter of the fully open pupil is 8 mm (Hecht, 2002)] compared with the PM eyes of *C. salei* with an *F*-number of 0.71. This implies that the image of an extended surface at a given luminance on the retina in these eyes is roughly nine times brighter than the image in humans.

The structure of the principal and the secondary eyes differs considerably. All secondary eyes are inverted eyes with the photoreceptor cells turned away from the incident light. They are provided with a reflecting grid-shaped tapetum consisting of several layers of guanine crystals (Fig. 1). The tapetum strips in the PL and PM eyes are roughly orientated parallel to the spiders' longitudinal plane (Land and Barth, 1992). The principal eyes are everted eyes, with the rhabdomeres pointing towards the incident light. They lack a reflecting tapetum and their retina can be moved by two eye muscles each (Land and Barth, 1992; Kaps and Schmid, 1996).

The optics of the eyes, the quality of the image and the retinal resolution have been investigated by Land and Barth (Land and Barth, 1992). Neither diffraction at the aperture nor the optics of the lens but the fineness of its receptor mosaic limits spatial resolution in *C. salei*. The inter-receptor angles $\Delta\phi$ in rad are calculated as the separation of the receptor centres divided by the focal length. The posterior eyes (PM and PL) have the best resolution with inter-receptor angles of about 1 deg along the rows and 2–3 deg in the vertical direction. The poorest resolution was found in the AL eyes. The angular separation along the rows is 3–4 deg and between the rows is above 9 deg. For the AM eyes an inter-receptor angle of about 3 deg has been measured.

The inter-receptor angles determine the anatomical limit of spatial resolution. A grating can just be properly resolved if the image of one bar falls on a distinct receptor and the image of the next bar on the neighbouring receptor, i.e. the angular period λ of the grating is twice the inter-receptor angle ($\lambda=2\cdot\Delta\phi$) (Land and Nilsson, 2002).

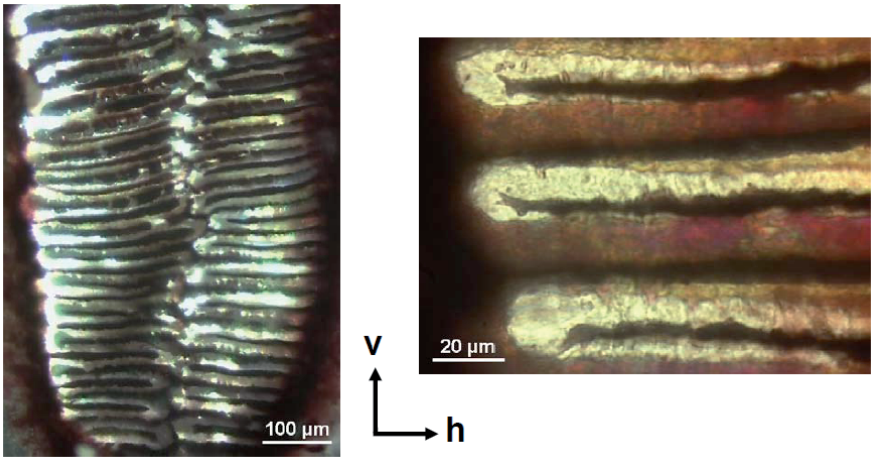

Fig. 1. Light microscope micrographs of a freshly removed posterior median (PM) eye retina. On the left side a large part of the grid-shaped tapetum can be seen (using both transmitted and reflected light). The greenish blue gloom is due to guanine crystals reflecting light through the photoreceptors. On the right side (dark field microscopy) only three tapetal strips are shown. One tapetal strip is equipped with two rows of photoreceptors and the axons of the photoreceptors leave the retina through the interjacent slits (Land and Barth, 1992). The arrows indicate the horizontal (h) and vertical (v) axis of the tapetum with respect to the body axis.

The temporal resolution of the photoreceptors was determined by Pirhofer-Walzl et al. using intracellular recordings (Pirhofer-Walzl et al., 2007). The integration time in the dark-adapted state was found to be 138±46 ms in the PM eyes and 86±23 ms in the AM eyes. For light-adapted eyes the authors measured an integration time of 79±17 ms in the PM eyes and of 44±19 ms in the AM eyes (Pirhofer-Walzl et al., 2007).

Kaps and Schmid investigated the structure and function of the eye muscles that move the AM retina (Kaps and Schmid, 1996). Each principal eye is provided with a dorsal muscle that is 600 µm long and consists of 15–18 striated muscle fibres and a ventral muscle that is 650 µm long and consists of 20–22 striated fibres. The ventral muscle inserts at the inner surface of the clypeus and the ventro-lateral surface of the eye cylinder. The dorsal muscle is attached to the exoskeleton in between the two PM eyes and runs to the dorso-lateral surface of the AM eye tube. The passive elasticity of the eye tubes and the eye muscles is assumed to be the counteracting force to the muscle contractions. Two different modes of eye movements have been observed: spontaneous microsaccades are generated by the dorsal muscle only. The muscle activity was shown to be around 12 Hz during the microsaccades, and the retina accomplishes recurring twitches of 2–4 deg in the dorso-median direction. This angle matches the inter-receptor angle in the AM eyes of 3 deg as reported by Land and Barth (Land and Barth, 1992), and it seems indeed reasonable to interpret the microsaccades as a mechanism to prevent the receptor cells from adapting when they are confronted with a static image. Induced saccades, however, are, as shown by Kaps and Schmid (Kaps and Schmid, 1996), generated by both the dorsal and the ventral eye muscles. The amplitude of these movements can go up to 15 deg and their direction can be varied depending on the activity of the two independent eye muscles. The resulting force is the vector sum of the forces produced by the dorsal eye muscle in the dorso-median direction and the ventral muscle in the ventro-median direction. The authors show the relationship between the muscle potentials frequency and the retinal displacement. Saccades are observed in walking animals (Kaps, 1998) and can be induced by mechanical or visual stimulation (Kaps and Schmid, 1996).

Schmid showed that the secondary eyes seem to be responsible for object detection whereas the principal eyes are used for object discrimination (Schmid, 1998). Neuhofer et al. have recently shown that a visual elicitation of the saccades in the eye muscles can only be induced if the secondary eyes are stimulated (Neuhofer et al., 2009). The authors showed that the retinae of the AM eyes move when objects are moving within the visual field of one or more secondary eyes, which suggests that the secondary eyes alone are responsible for motion perception. This specialisation has already been described for jumping spiders (Homann, 1928; Land, 1969), where the principal eyes that are responsible for pattern recognition show by far the best spatial resolution. Homann compared the principal eyes with the foveal parts and the secondary eyes with the peripheral parts of the human retina (Homann, 1928). Interestingly, in *Cupiennius*, the secondary eyes, the motion-detecting eyes, have the better spatial resolution.

The aim of this study was to determine to what extent these spiders exploit the optics of their eyes. In our experiments we make use of the fact that the perception of moving objects in the secondary eyes enhances the eye muscle activity in the AM eyes. The PM eyes were confronted with movable gratings of variable spatial frequency while the eye muscle activity was monitored *via* a small telemetric unit. The smallest spatial frequency that elicited a significant increase in the eye muscle potential frequency was then compared with the anatomical data reported by Land and Barth (Land and Barth, 1992). We also simulated the intensity changes produced by black and white bars moving relative to a single photoreceptor for the stimuli sizes and velocities used in our experiments by means of a model, which takes the geometry and the integration time of the photoreceptors into account. As the inter-receptor angles as well as the receptors themselves are smaller along the horizontal than the vertical axis, we expected to observe differences in the behavioural responses of the spiders to gratings in different orientations.

MATERIALS AND METHODS

Eye muscle potentials

Animals

Adult female *Cupiennius salei* were used in this study. The spiders were kept in a greenhouse in Vienna at a 12 h:12 h day:night cycle. Relative humidity (70–80%) and temperature (15–28°C) in the stock resemble natural conditions. The spiders were kept separately in glass jars and were fed on flies once a week. 19 spiders were used in the first experimental series, 14 in the second one.

In this study it was necessary to know the position of the PM eye investigated with respect to the stimulus and therefore the spiders had to be tethered. They were cooled down in a refrigerator (at approximately 3°C) and could then be fixed on a wooden hemisphere using parafilm. The small hairs on the upper side of the prosoma and between the eyes were removed. The telemetric unit was then

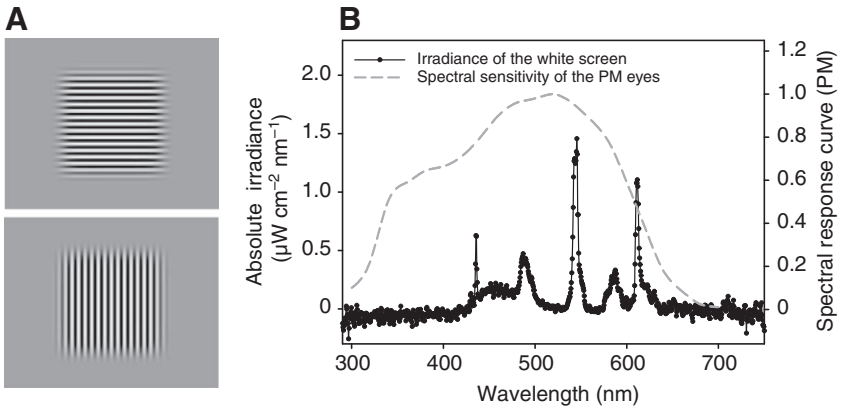

Fig. 2. (A) Screen shot of stimuli used in the experiments. This pattern corresponded to an angular wavelength of 3.3 deg for animals at a distance of 20 cm. (B) Spectrum of the light emitted by the LCD monitor used in the experiments together with the sensitivity spectrum of the posterior median (PM) eyes in the day state, modified from Barth et al. (Barth et al., 1993).

attached to the spiders' prosoma using beeswax. The reference electrode was inserted laterally into the prosoma, and the measuring electrode just below the PM eyes. The spiders were positioned with their body axis perpendicular to the screen at a distance of 20 cm. The spiders were then rotated 30 deg in the horizontal plane and 65 deg in the vertical plane with the PM eye of interest being the pivot point to ensure that the centre of the stimulus is approximately adjusted to the middle of the visual field of the eye. All but the PM eyes were covered with red acrylic paint.

Care and use of the animals comply with the Austrian animal welfare laws, guidelines and policies.

Telemetry

We used a telemetric device as proposed for the wireless transmission of the muscle potentials of a locust by Kutsch et al. (Kutsch et al., 1993), which was recently adapted for spiders (Neuhofer et al., 2009). Using a small, light and extremely sensitive emitter device it is possible to transmit the electric signals generated by the thin eye muscles in *Cupiennius*. The main component is an LC-oscillator circuit, which generates a carrier frequency of about 130 MHz and this carrier frequency is frequency- and amplitude-modulated by the action potentials of the eye muscles. A coil (4 to 5 turns) made of copper wire serves as an inductor. An insulated, very flexible and thin manganin wire is used as a recording electrode (alloy of copper, manganese and nickel; diameter d=30 μm; resistance per metre ρ_l=628.3 Ωm^{-1}, Isabellenhütte, Dillenburg, Germany). The reference electrode is made of silver wire (d=250 μm). The circuit is powered by a silver oxide battery (Renata or Maxell Watch Batteries; 1.55 V) weighing only 270 mg. The battery holder is made of hard PVC, and the mass of the transmitting device plus battery is about 660 mg. The signal could be received by a conventional world receiver (Conrad Voyager RY-630, Conrad Electronics, Hirschau, Germany). It was transmitted through an A/D converter (CED 1401, Science Park Cambridge, UK) to a PC using Spike2 (CED) for data analysis.

Stimulus

An LCD monitor (1280 × 1024 pixels, 60 Hz, Belinea, Wittmund, Germany) was used in this study. The stimuli were generated in Matlab (MathWorks, Inc., Natick, MA, USA), using the Psychophysics Toolbox extensions (Brainard, 1997; Pelli, 1997) and consisted in one-dimensional, sinusoidal, monochromatic gratings together with a transparency mask to prevent fringe effects (Fig. 2A). The spectrum of the screen is shown in Fig. 2B together with the spectral sensitivity of the PM eyes reported by Barth et al. (Barth et al., 1993). 40 lx (Pocket Light Meter, AZ Instrument, 8581, Taichung City, Taiwan) were measured at a distance of 20 cm from the screen showing the presented stimuli.

In the first series we used eight different spatial frequencies, ranging from 0.117 to 0.667 cycles per degree. The angular wavelength was 8.5 deg for the largest and 1.5 deg for the smallest stimulus. The gratings remained stationary for 50 s and then moved for 5 s at a constant speed (10 deg s^{-1}). Each spatial frequency was successively shown four times to the animals; the order in which the spatial frequencies were presented was different for each spider. Then the whole cycle was repeated so that each spider was confronted with each stimulus in all eight times. The movement onset was accompanied by a short trigger signal that could be registered directly with Spike2 together with the muscle potentials.

In the second series only the three gratings with the smallest wavelengths were shown to the animals. Thus, it was possible to confront each spider with the horizontal as well as the vertical orientation to allow a more direct comparison of the reactions. This was not possible in the first series because the presentation of eight different angular wavelengths in both orientations would have lasted too long and the experiments were limited by the lifetime of the battery. In the second series a constant temporal frequency of 2.5 cycles per second was chosen.

The stimuli sizes have an uncertainty of 0.1 deg mostly due to the positioning of the spiders in front of the screen.

Analysis

The eye muscle activity varied greatly during the experiments and we thus compared the mean muscle frequency in the three seconds prior to the movement onset with the frequency in the three seconds after the movement onset for each stimulus. These values were calculated by means of a Spike2 script file and the mean frequency change was determined for the eight stimuli presentations for each spider.

During the experiments the spiders occasionally moved their chelicerae and thus generated signals that were huge compared with the eye muscle potentials. This chelicerae movement was nearly always accompanied by an increase in the frequency of the eye muscle potentials. We therefore excluded stimulus presentations from the analysis whenever such a chelicerae signal was recorded within 25 s before stimulus onset or during the stimulus.

Spiders were excluded from the analysis if more than 10 responses (or more than three for a given stimulus size) were not valid due to chelicerae movements or because of a poor signal-to-noise ratio.

Differences between the mean muscle potential frequency for N spiders before and during stimulation were tested with the Wilcoxon signed-rank test using XLSTAT (Addinsoft, Paris, France). If the

frequency was higher during the movement of the gratings, the *P*-value for the one-tailed test was calculated.

Simulation of the intensity modulation

The temporal intensity modulation at a single model photoreceptor cell produced by a moving rectangular grating was simulated. We considered the size *s* of the image of a bar on the retina and the receptor size *r*. When the entire receptive area is filled with a white bar the intensity measured by the receptor is defined to be 1, when a dark bar covers the entire receptor the intensity is 0. When the grating moves relative to the retina the intensity varies over time depending on the ratio of the bar width to the receptor size, on the velocity of the grating and the integration time of the photoreceptor.

For an infinitesimally small integration time only the ratio r/s has to be considered. The maximum intensity differences ΔI are measured for bar widths *s* equal to or larger than the receptor, where at a given time the receptor field is entirely filled with the image of a bar or a fraction of it. For decreasing bar widths the maximal intensity differences fall as $\Delta I=2\bullet s/r-1$, and ΔI obviously equals zero when the stripe width is exactly half the receptor size. Intensity differences increase for further decreasing stripe widths following $\Delta I=-2\bullet s/r+1$, reaching a maximum at $r/s=3$. Considering only odd receptor-to-stripe ratios, where the intensity differences are maximal, the decrease follows $\Delta I=s/r$. If a finite integration time is considered, the maximal intensity differences begin to fall below 1 for bar widths bigger than the receptor size.

The values given above have been calculated assuming a rectangular photoreceptor cross section that is evenly filled with microvilli. In reality the microvilli are restricted to the two vertical boarders of the receptor, and the intensity for vertical bars was therefore calculated as the mean measured by the two microvilli stripes at a given time. The intensity variations for a full cycle were calculated for the wavelengths of the stimuli in our second experimental series. According to the micrographs given by Land and Barth (Land and Barth, 1992), the photoreceptor aspect ratio in the model was chosen to be 1:1.5, and one microvilli stripe was assumed to occupy one-fifth of the total receptor size. The integration time of the photoreceptors in the model was set to 79 ms as reported by Pirhofer-Walzl et al. (Pirhofer-Walzl et al., 2007).

RESULTS

Eye muscle potentials

An example of recorded eye muscle action potentials and the frequency increase induced by the movement onset of a grating are shown in Fig. 3. The results of the experiments are summarised in Tables 1 and 2. The mean responses of the spiders, i.e. the difference in the mean muscle potential frequency in the three seconds before and in the three seconds after the onset of the grating movement, are shown in Fig. 4A,B for the two experimental series.

In the first series gratings at angular wavelengths ranging between 8.5 deg and 1.5 deg were presented to the spiders. Nine spiders were shown the vertical gratings and 10 spiders the horizontal gratings. The movement onset of the gratings provoked an increase in the eye muscle potential frequency. The individual responses varied considerably, and the mean frequency increase diminished more or less steadily with decreasing wavelength. The highest mean frequency increase shown by a spider was in the order of 10 Hz and has been measured for the coarsest horizontal grating (8.5 deg). In the vertical subset as well as in the horizontal subset the frequency increases are significant for all stimuli sizes down to 2.7 deg ($0.003<P<0.033$) and not significant for 2.0 deg ($P=0.055$ for vertical gratings, $P=0.480$ for horizontal gratings). The change in

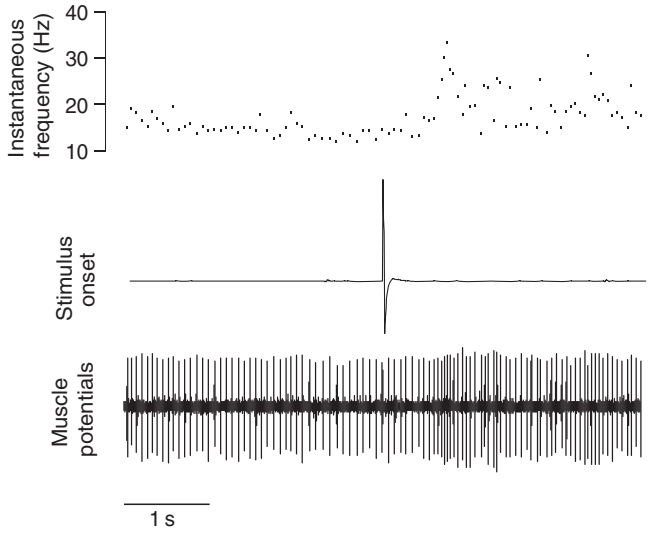

Fig. 3. An example for the electrophysiological recordings of the eye muscle action potentials can be seen at the bottom. The middle channel gives the time of the movement onset. Our method is not suited for the measurement of the absolute amplitude; Kaps and Schmid report amplitudes of 50–150 μV (Kaps and Schmid, 1996). At the top the instantaneous frequency of the eye muscle potentials is drawn. The frequency is typically in the order of 15–25 Hz before the grating movement and can go up to 100 Hz.

frequency is not significant for 1.5 deg ($P=0.953$ for vertical gratings and $P=0.386$ for horizontal gratings). If the two subsets are combined ($N=19$), the frequency increase is significant for all stimuli down to 2.7 deg ($P\leq0.002$); however, the increase for 2.0 deg is still not significant ($P=0.114$). Neither is the change in frequency for 1.5 deg ($P=0.443$). The linear regression (including all data points) has a coefficient of determination of $R=0.97$.

In the second series (Fig. 4B) we wanted to test if there were significant differences in the response to differently orientated gratings. The spiders ($N=14$) were shown only the stimuli with the smallest three spatial wavelengths of the first series (i.e. 2.7 deg, 2.0 deg and 1.5 deg). This time the gratings were presented at a constant temporal frequency (2.5 cycles per second) and each animal was presented with the horizontal as well as the vertical gratings. Therefore, the reaction of the spiders to gratings of the same spatial frequency but different orientations could directly be compared. The highest mean frequency increases measured for single spiders were in the order of 3 Hz. The response to the 2.7 deg grating was significant for the vertical ($P=0.005$) as well as the horizontal ($P=0.015$) orientation. However, the response to the 2.0 deg grating was significant only for the vertical orientation ($P=0.013$) and not for the horizontal one ($P=0.801$, two-tailed). The difference between the responses to the differently orientated gratings was also significant ($P=0.035$, two-tailed test). As in the previous series the 1.5 deg grating did not elicit a significant change in the eye muscle activity ($P=0.776$ for vertical gratings and $P=0.826$ for horizontal ones).

We thus could show significant responses to moving vertical gratings at angular wavelengths as small as twice the spiders' inter-receptor angle. Horizontal gratings provoked a frequency increase even for wavelengths smaller than twice the inter-receptor angle in this orientation – the inter-receptor angle between tapetum rows is

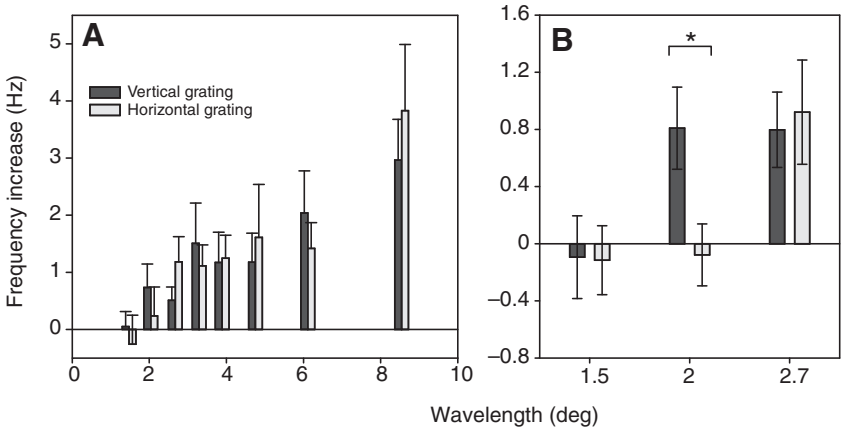

Fig. 4. (A) Eye muscle potential frequency increase (mean ± s.e.m.) for eight different angular wavelengths (see Table 1) moving at an angular velocity of 10 deg s^{-1}. To one group (N=10) the gratings were shown in their horizontal orientation, to the other group (N=9) in the vertical orientation. (B) Eye muscle potential frequency increase (mean ± s.e.m.) for angular wavelengths of 1.5 deg, 2.0 deg and 2.7 deg moving at a contrast frequency of 2.5 cycles per second. The increase in muscle potential frequency is significant for 2.7 deg in both orientations. A wavelength of 2.0 deg elicited a significant increase in the vertical but not in the horizontal orientation. The difference between the responses to the two orientations is also significant. The gratings with a wavelength of 1.5 deg did not significantly change the eye muscle frequency.

in the order of 2–3 deg (Land and Barth, 1992) whereas gratings at a wavelength of 2.7 deg still elicited a significant response.

Simulation of the intensity modulation

Fig. 5A–C shows the simulated light intensity variation measured by a single model PM eye photoreceptor for the angular wavelengths used in the second experimental series (2.7 deg, 2.0 deg and 1.5 deg) during a full cycle. The solid lines show the intensity variation for the horizontal gratings, the broken ones illustrate the intensity variation for the vertical gratings. In Fig. 5D the maximal intensity variations are shown for the three stimuli sizes considering the photoreceptor integration time of 79 ms as reported by Pirhofer-Walzl et al. (Pirhofer-Walzl et al., 2007). The grating velocity in the experiments was 2.5 cycles per second, which corresponds to roughly 0.2 cycles per integration time. Comparing the simulated values with the measured behavioural data we can indeed find a cut-off (indicated by the broken line in Fig. 5D). Above this limit the spiders showed a significant increase (2.7 deg vertical and horizontal gratings, 2.0 deg vertical gratings); the three stimuli for which we calculated lower intensity variations elicited no significant response in our experiments. This could explain why we found a difference between the responses to the different orientations that was less pronounced than expected from the inter-receptor angles. The temporal intensity variation is thus a possible explanation for the gathered data.

Table 1. Mean change of the eye muscle activity and the standard error of the mean (s.e.m.) for all spiders of the first series for the vertical (N=9) and horizontal subset (N=10) measured for the various angular wavelengths of the presented gratings

Wavelength (deg)	Vertical grating		Horizontal grating	
	Means±s.e.m. (Hz)	P	Means±s.e.m. (Hz)	P
8.5	3.0±0.7	0.005	3.8±1.2	0.003
6.1	2.0±0.7	0.014	1.4±0.5	0.014
4.8	1.2±0.5	0.014	1.6±0.9	0.023
3.9	1.2±0.5	0.019	1.2±0.4	0.014
3.3	1.5±0.7	0.019	1.1±0.4	0.010
2.7	0.5±0.2	0.033	1.2±0.4	0.008
2.0	0.7±0.4	0.055	0.2±0.5	0.480
1.5	0.1±0.3	0.953	–0.3±0.5	0.386

The P-values were calculated using the Wilcoxon signed-rank test. If the mean difference is positive the value for the one-tailed test is given, if it is negative the two-tailed value is shown.

DISCUSSION

In the secondary eyes of *C. salei* the photoreceptor size equals receptor spacing in the horizontal direction (i.e. along a tapetum strip) because the receptors are conjoined but the inter-receptor angle is larger than the angle subtended by the receptors in the vertical direction, i.e. normal to the strips. The finest grating that can be properly resolved by a given retinal mosaic has a spatial period equal to twice the receptor spacing (Land and Nilsson, 2002). In this case the image of one bright or dark bar of the grating falls on a given receptor and the image of the next bar falls on the neighbouring receptor.

In our experiments the finest vertical gratings that elicited a significant response matched exactly this so called Nyquist limit. This means that half the angular wavelength in the vertical orientation of the stimulus equals the inter-receptor angle (and the angle subtended by single receptors) of 1 deg in this orientation. For horizontal gratings we measured significant responses for wavelengths down to 2.7 deg, which is considerably smaller than twice the inter-receptor angle in this orientation. We found a significant difference between the responses to the two different orientations for the condition where the wavelength equals twice the inter-receptor angle in the vertical orientation; here only the vertical gratings provoked a significant activity increase.

There are several possible explanations for these findings. Firstly, the tapetum consists in reality, not only of strips but, as can be seen in Fig. 1, there are regions where the strips turn. These curves are present at the boarders and in the very middle part of the retina, and the inter-receptor angles in these regions are clearly smaller than the angle between the rows. The receptors in the curves could thus enhance acuity in the horizontal direction.

Table 2. Mean change of the eye muscle activity and the standard error of the mean (s.e.m.) for the second series (N=14) measured for the various angular wavelengths of the presented gratings

Wavelength (deg)	Vertical grating		Horizontal grating	
	Means±s.e.m. (Hz)	P	Means±s.e.m. (Hz)	P
2.7	0.8±0.3	0.005	0.9±0.4	0.015
2.0	0.8±0.3	0.013	–0.1±0.2	0.801
1.5	–0.1±0.3	0.776	–0.1±0.2	0.826

The P-values were calculated using the Wilcoxon signed-rank test. If the mean difference is positive the value for the one-tailed test is given, if it is negative the two-tailed value is shown.

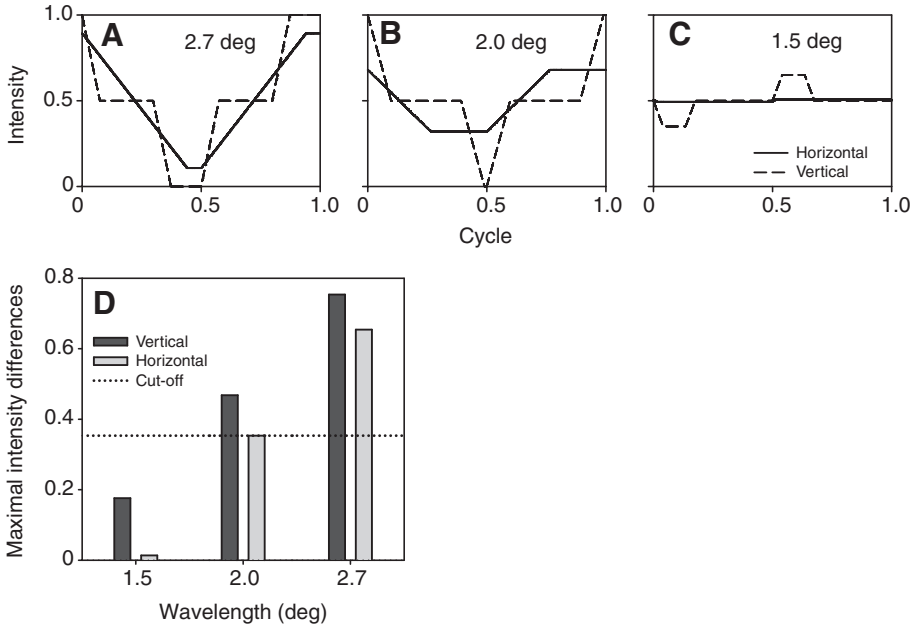

Fig. 5. (A–C) Simulated light intensity variation for the three wavelengths presented in the second experimental series (1.5 deg, 2.0 deg and 2.7 deg angular wavelength) measured by a single photoreceptor cell produced by a rectangular grating during a full cycle. The three subfigures show the values calculated for the *r*/*s* ratios in the experiments, taking the arrangement of the microvilli in the receptor cells into account. (D) Simulation of the maximum light intensity differences produced by rectangular gratings moving at a velocity of 0.2 cycles per integration time. Intensity changes above the indicated cut-off line (broken line) elicited a significant response in our experiments whereas intensity changes below this limit did not enhance the spiders' eye muscle activity.

Secondly, it is quite conceivable that the spiders respond to gratings with higher spatial frequencies than the Nyquist limit, because they could still perceive some sort of movement even if the grating is too fine to be properly resolved. The secondary eyes do not have a movable retina, and as *Cupiennius* is a typical sit and wait hunter the retinae of the secondary eyes are often completely stationary and the neural image in these eyes probably adapts (see also Land and Barth, 1992). It is thus thinkable that the spiders might not be able to perceive stationary objects with their secondary eyes but only objects moving in the visual field, producing intensity changes. The secondary eyes could then be imagined as a movement-detecting device using intensity changes over time. For the pure detection of intensity changes produced by small objects, telling the animal that there was something moving, the angle subtended by a receptor rather than the inter-receptor angle should be the critical factor. Ongoing experiments show that the spiders also respond very well to flicker stimuli (L.M.F. and A.S., unpublished) and this corroborates the assumption that temporal intensity changes trigger the muscular response.

However, even if we consider only the temporal intensity changes at a single receptor, a difference between the two orientations has to be expected because of the aspect ratio of the receptors, which are also larger in the vertical direction than in the horizontal one. We simulated the maximal intensity differences produced by gratings of a given spatial frequency at single model photoreceptors. It is possible to find a limiting intensity change above which the spiders responded to the stimuli and the intensity modulation can thus be considered as a possible explanation for the spiders' performance in our experiments; the difference in the measured spatial cut-off frequency is smaller than the difference between the inter-receptor angles but there is still a difference which can be explained by the geometry of the receptor cells and the resulting difference in the intensity modulations. The temporal intensity changes measured by the photoreceptor cells are the more pronounced, the better the image produced by the lens. This could be one of the reasons why animals would invest in lenses that provide much more detail than the receptor mosaic can resolve.

It might also be interesting to compare our data concerning an arthropod lens eye with the findings reported for insect eyes. Insect optomotor response is direction sensitive but we are unable to make any assumptions concerning the spiders' ability to perceive the direction of motion because there was no difference observed between the reactions to the two different motion directions of vertical gratings and so we can only compare the absolute value of the insect optomotor response with the muscle activity increase in the spider. Due to spatial aliasing the optomotor response of *Drosophila* to moving gratings disappears when the single stripes have a visual angle that exactly equals the inter-receptor angle, and for even smaller bars a response in the opposed direction is observed (Götz, 1964; Götz, 1965). Our data do not suggest spatial aliasing effects in *Cupiennius* similar to those observed in insects because the spiders showed a non-minimum significant response to gratings at wavelengths equal to twice the inter-receptor angle for both grating orientations, where a zero crossing would be predicted.

Our results suggest that spatial summation in subsequent neuronal processes seems not to impair acuity in, at least light-adapted, spiders. In a night-active animal one would then expect an important temporal summation to enhance the reliability of faint images; and indeed Pirhofer-Walzl et al. found integration times of 138 ms for dark-adapted and 79 ms for light-adapted PM eyes using intracellular recordings (Pirhofer-Walzl et al., 2007). Relatively large integration times and relatively good spatial acuity is what one would predict for sedentary animals interested in small, slowly moving objects (Warrant, 1999).

An inter-receptor angle of 1 deg (Land and Barth, 1992) is impressive for a night-active spider. *Dinopis*, a night-active visual superstar among arthropods, has inter-receptor angles of 1.48 deg in its enormous PM eyes (Blest and Land, 1977), the mean receptor angular sensitivity function having a half width of 2.3 deg (Laughlin et al., 1980). However, *Cupiennius* cannot challenge *Dinopis*' incredible sensitivity. The interommatidial angles of 15 species of bees (day and night-active ones) have been measured by Jander and Jander and were found to range between 1.2 deg and 4.7 deg (Jander and Jander, 2002). Somanathan et al. measured interommatidial angles in a night-active carpenter bee of 0.8 deg in the most acute

zone of its visual field (Somanathan et al., 2009). Spatial resolution in *Cupiennius* is thus comparable with the resolution reported for day and night-active bees.

It remains to be shown why this night-active spider invests in such a good eyesight and in what kind of behavioural contexts the visual sense plays an important role.

LIST OF ABBREVIATIONS

AL	anterior lateral
AM	anterior median
PL	posterior lateral
PM	posterior median
r	receptor size
s	size of the image bar
ΔI	intensity differences
λ	angular period

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