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"No evidence for the perception of polarised light in the ctenid spider *Cupiennius salei* "

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Table of contents

Introduction

The ability to perceive the polarisation of light is very common in the animal kingdom, especially in arthropods and cephalopods and may be even more widespread than colour vision. The reason lies in the arrangement of the photosensitive molecules organized in microvilli and their orientation to the incoming light. Polarisation sensitivity was found in a number of insect, crustacean and in two spider groups. Both these spider groups showed some morphological specialisation for enhancing the polarisation signal to use for true polarisation vision. However, on account of the structure of the retina, the basis for polarisation sensitivity might be present in more spiders. In this study *Cupiennius salei*, a nocturnal spider without any specialised structure for polarisation vision, was tested to be sensitive to polarised light. If that is the case, it might be a first hint, that polarisation sensitivity might be a widespread ability in spiders.

Cupiennius salei

General Biology

Cupiennius salei is a nocturnal wandering and hunting spider found in the neotropic rainforest of Central America. The genus contains 8 species and has been assigned to the Ctenidae (comb spider) family (Barth, 2002). It mainly uses its mechanosensory system for hunting and mating. However, in behavioural experiments attack behaviour could be elicited in *C. salei* by visual stimulus alone (Fenk et al., 2010). Spectral range and absolute sensitivity tests showed that C. *salei* has a well-developed visual system (Barth et al. 1993) and also uses it in behaviour to an extent (Orlando & Schmid, 2011; Zopf et al., 2013). They are able to select between two different targets due to shape, dimensions and orientations of the presented target (Schmid, 1998).

The Visual system of C. salei

Similar to most spiders, *Cupiennius salei* has eight eyes, arranged on the prosoma in two curved rows. They are named in accordance with their positons. The anterior-median (AM) eyes lie directly in front of the posterior-median (PM), same with anterior-lateral (AL) and posterior-lateral (PL). All the eyes are round and have a cuticular cornea and lens and a cellular vitreous boy. Both the front and the rear lens surfaces are mere hemispheres with different radii, but the same centre of curvature. The retina is formed by one layer of photoreceptor cells and several different support cell classes, like pigmented and non-pigmented glia cells (Grusch et al., 1997; Barth, 2002).

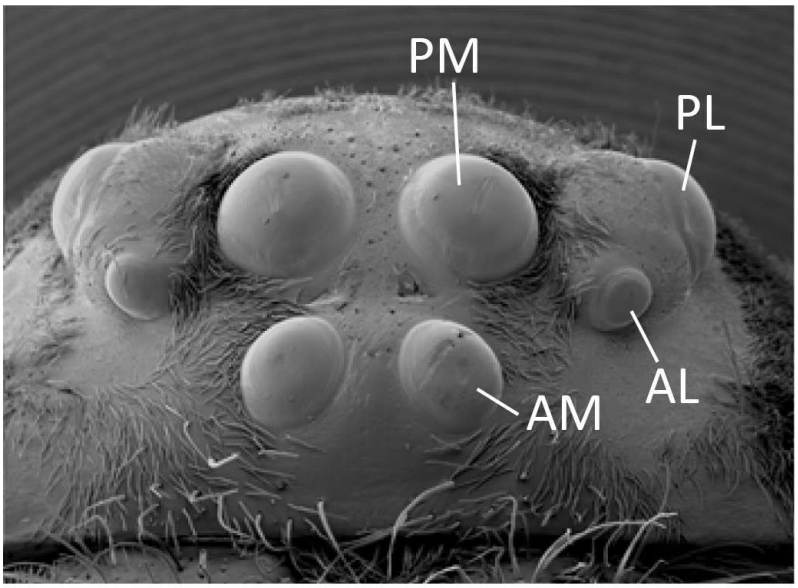

Figure 1: Eye position of adult Cupiennius salei under REM. AM = Anterior-median, AL = Anterior-lateral, PM = Posterior-median, PL = Posterior-lateral eyes (Zopf, 2010).

Eyes are divided by position, morphology and function into principal eyes (AM) and secondary eyes (AL, PL, PM). The structure and the function of the principal eyes is very different from the other three eye pairs. The AM eyes detect stationary objects (Schmid, 1998), whereas the PM, AL and PL eyes are responsible for detecting moving targets (Fenk et al., 2010).

In *C. salei,* the principal eyes are smaller than the PM and PL eyes and have everted photoreceptor cells. The retina can be moved by two pairs of eye muscles, but they lack a tapetum, an interference reflector, which sends light back from the back of the eye to the receptive segments (Barth, 2002). The secondary eyes, on the other hand, do have a tapetum. Their photoreceptors are inverted and they have no eye muscles. The three pairs of secondary eyes are similar in their morphology, but differ in size, lens diameter and number of photoreceptors. The PL eyes are the biggest and also have the most photoreceptors (Grusch et al., 1997).

The rhabdoms of the principal eyes are about 58 µm long with their rhabdomeric regions facing toward the vitreous cell and the incident light. The receptive segments of the photoreceptor cells have a rhabdomere on each of the four sides of the cross section. Rhabdomeres of neighbouring photoreceptors form a rhabdom. Inside the rhabdom the microvilli are tightly packed and regularly arranged, but the rhabdoms themselves are often strongly curved and form an irregular lattice. Non-pigmented glial processes can be found in the small columnar spaces, where four rhabdoms meet, but there are no glial processes between the rhabdomeres of one rhabdom. Only a few photoreceptors (about 5% of the retina surface) are not in the regular microvilli arrays, but instead form disorderly rhabdomeres consisting of irregular membrane loops (Grusch et al., 1997).

The secondary eyes are inverted, the photosensitive segments face away from the incoming light and the cell bodies are closest to the lens. The receptive segments are four-faced in cross-section and have two rhabdomeres on two opposite sides. Every rhabdomere is paired with the rhabdomere of the neighbouring photoreceptor, with the microvilli again in tightly packed and regular arrays. The rhabdomeres of the secondary eyes are shorter than in the principal eyes (about 50 µm in length).

The receptive segments are seated on the tapetum. The photoreceptors become narrower to pass through the gaps between the strips and then broaden again to form the intermediate segments. At

the end of each strip the row of receptive segments makes a 180° turn to from the next row. This way all receptive segments of one eye form one long meandering row. Pigmented glia cells separate the rows. The rows, but not the receptive segments within one row, are separated from each other by pigmented and non- pigmented glial cells (Grusch et al., 1997).

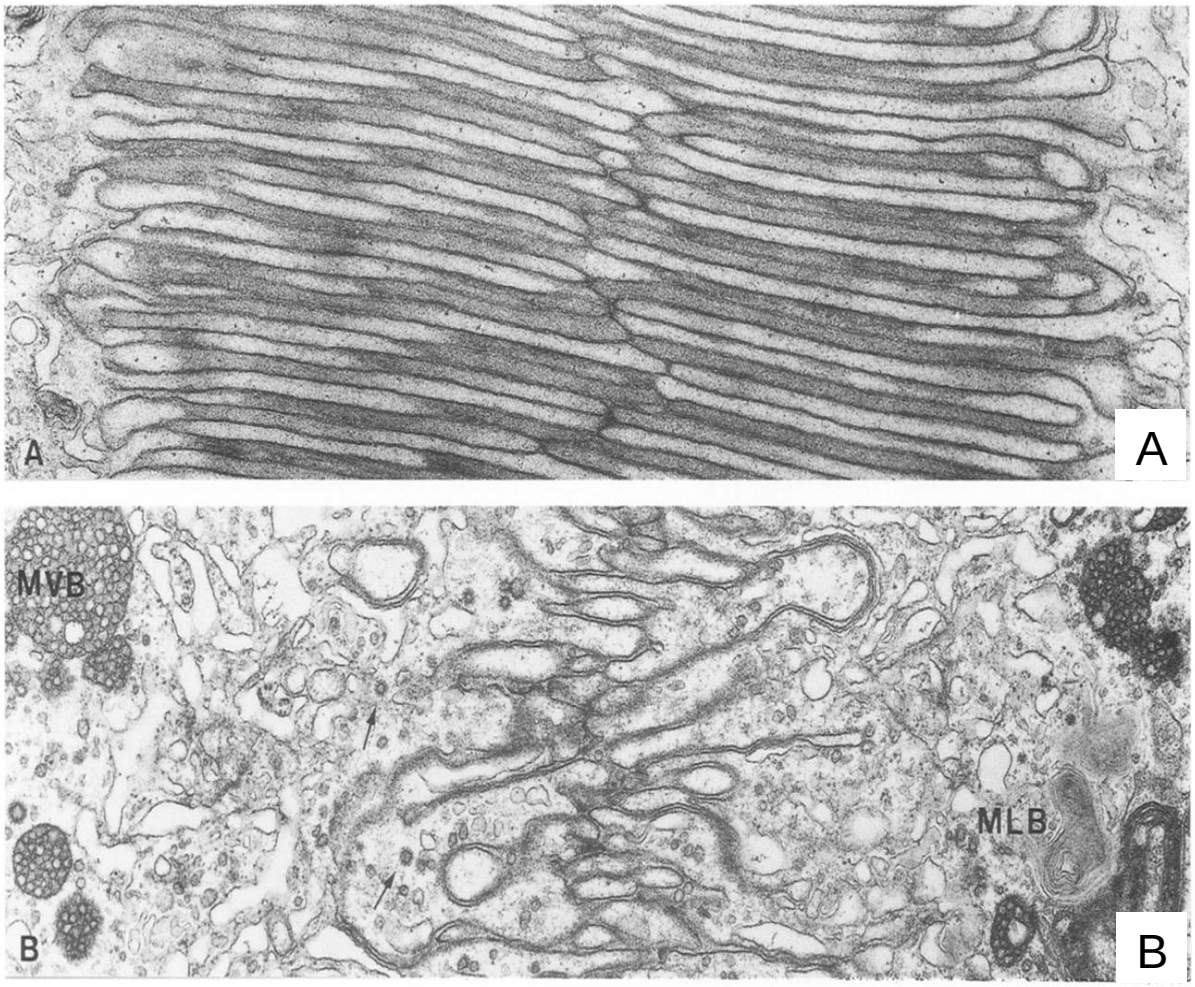

Figure 2: Microvilliar organisation comparison between night (A) and day (B) adapted eyes. The phototransductive membrane turnover in C. salei results in structural and sensitivity changes. During the night, the microvilli of two neighbouring rhabdomeres are ordered and tightly packed, whereas the structures are fragmented and degenerated during the day with numerous multivesicular bodies (MVB), multilamellar bodies (MLB) and coated vesicles (arrows) close to the rhabdoms (Grusch et al., 1997).

The tapetum acts as an interference reflector and reflects light from the back of the eye back to the photosensitive region, functionally doubling the length of the receptor. The exact shape of the tapetum is a taxonomic character and can be used to differentiate between various spider families. *Cupiennius*, like other hunting spiders has a grid-iron type. It consists of parallel horizontal strips of reflecting crystals. The strips are interconnected at the periphery and in the middle by a vertical middle strip (Grusch et al., 1997; Barth, 2002). All eyes show a phototransductive membrane turnover between day and night, resulting in structural and sensitivity changes (Barth et al., 1993). During the day, the rhabdoms become fragmented and about 80% of the microvilli are degenerated after two hours of light exposure and stay like this the whole day (Grusch et al., 1997).

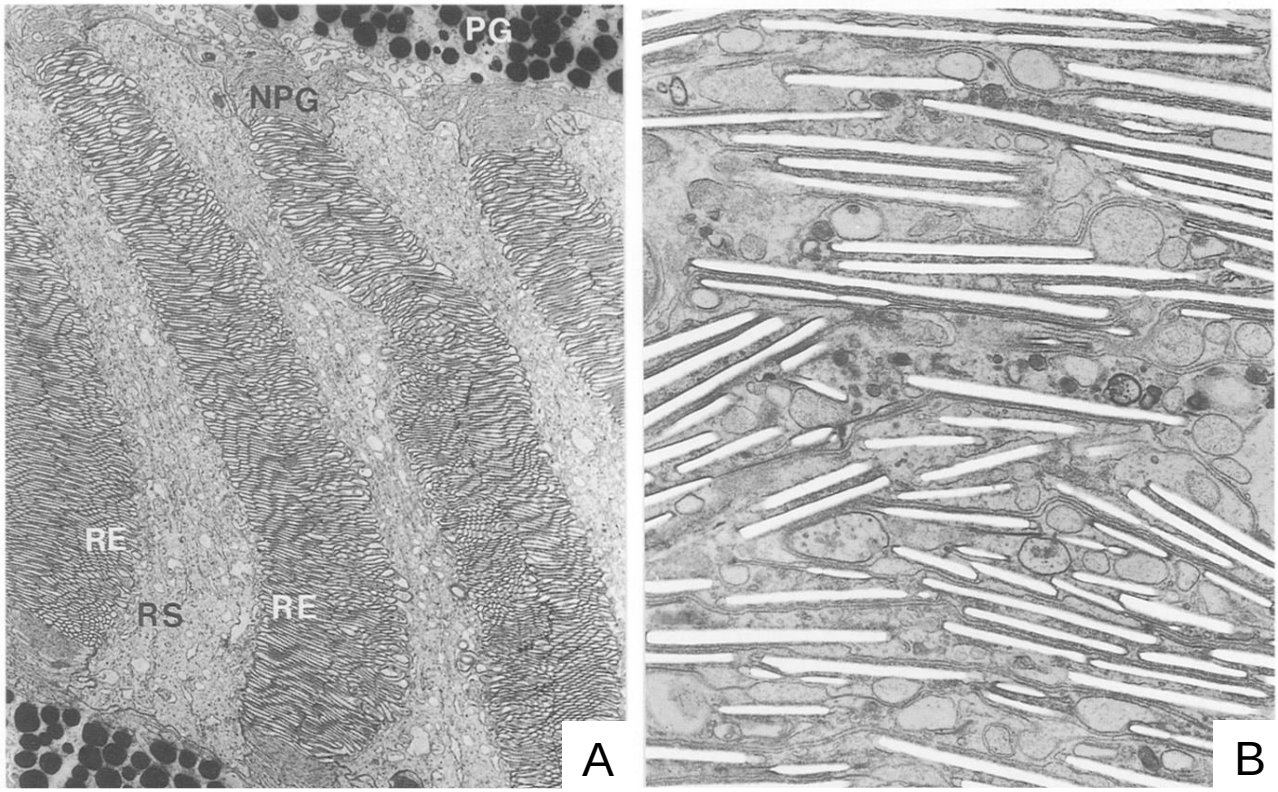

Figure 3: Organisation of the retina and the tapetum of secondary eyes in C. salei. A: Rhabdom of a night-adapted PL (Posterior lateral) eye (electron microscope, magnification 4250x). The rhabdomeres (RE) of each receptive segment (RS) are tightly packed and form orderly stripes of rhabdoms. The rows of the receptive segments are separated by pigmented glial (PG) and non-pigmented glial (NPG) processes. B: (x15 000) EM picture of tapetum cell from a PM eye. The guanine crystals are horizontally aligned and perpendicular to the incident light (Grusch et al., 1997).

Possible perception of polarised light in *C. salei*

Contrary to the day-adapted eyes, during the night the visual pigment molecules are organized in rhabdomeres and orderly aligned in the microvilliar membrane. As I will describe later in more detail, linear polarised light oscillates in one plane along its axis of progression. The angle of the plane, compared to its propagating axis, is described as the e-vector orientation of the polarised light. Eyes with their photosensitive molecules aligned in a more or less straight line, will have a preference to light oscillating along the same axis and therefore can be sensitive to polarisation. In the principal eyes, the irregular pattern of the rhabdoms makes it unlikely, that *C. salei* could discriminate different e-vector orientations. However, the microvilli of the night- adapted, secondary eyes are organised in a regular array. In the PM and PL eyes, the stripes of the tapetum are approximately horizontal, (parallel to the longitudinal plane of the spider or to the substrate on which the spider is sitting) and the guanine crystals, responsible for the light reflection abilities of the tapetum, are orderly aligned parallel to one another and perpendicular to the incident light.

Because of this level of regular pattern of the photoreceptive segments, the secondary eyes might have a preference for a specific e-vector orientation. Also, the rhabdoms in the secondary eyes are shorter than the ones in the principal eyes, but wider (Grusch et al., 1997), which reduces self-screening and increases sensitivity (Nilsson et al., 1987). This might be another hint, which favours polarisation vision in *C. salei.*

The visual fields of the PM and PL eyes cover almost the entire upper hemisphere. The AL eyes on the other hand look downward, just in front of the spider. If the PM and PL eyes can perceive polarised light, *C. salei* could use them to see the hemispheric polarisation pattern during moonlight. Or,

together with the AL eyes, perceive polarised light reflecting from wet surfaces in the canopy. However, the visual fields of the secondary eyes overlap only to a small degree, if we assume that the eyes have different preferences for e – vector orientations, the spider will need to change its point of view to compare between different eyes.

Why could it be advantageous for *C. salei* to perceive the polarisation of light?

C. salei lives in neotropic rainforest. It spends its days in retreat in plants, favouring ones with mechanically strong, large leaves, which provide shelter for the spider. Hunting, mating and molting also happens on the same type of plants (Barth, 2002). The light environment within forests consists of complex patterns of brightness and spectral distribution, the polarised light field adds to the complexity. In a study by Shashar et al., (1998) in the tropical rain forest of Guatopo National Park in Venezuela, they found that the celestial polarisation pattern remains visible underneath the forest canopy, but clouds and fog might reduce the polarisation signal. Using this signal for navigation therefore must be limited to the spaces exposed to several extended portion of clear sky (Shashar et al., 1998). However, sensing polarised light might be useful for *C. salei* in other ways. Different parts of or entire leaves will reflect polarised light according to their spatial position and structure. Other objects, that otherwise would be chromatically camouflaged, can be distinguished, when high variation of polarisation angle of the objects are compared to the homogenous reflection of leaves (Shashar et al., 1998). Even though *C. salei* does not see colour, but instead perceives differences in brightness (Orlando & Schmid, 2010), objects might seem brighter to the animal, if they reflect light at a specific polarisations angle. That is, if *C. salei* is more sensitive to specific e-vectors.

Another study by Bartha & Horváth, (2003) found that the degree of linear polarisations of cloudy skylight is highest in the UV, since in this spectral range the dilution of polarised light scattered in the air beneath the cloud by unpolarised light is the lowest. Similarly the degree of polarisation is also the strongest in UV, if the air under foliage is partly sunlit, because in the UV- range, unpolarised green light dilutes the polarised light the least. Therefore, the detection of polarised light under clouds or canopies is most advantageous in the UV-spectrum. Under clear skies, they found no favoured wavelength for the perception of the celestial polarisation, since the degree of polarisation is high enough. *C. salei* is most active during the night. Moonlight behaves like sunlight regarding polarisation, and can be used as a means of navigation (Dacke et al., 2003). The polarising pattern in rainforest should be the same during day and night.

Insects perceive skylight polarisation with UV, blue or green receptors. *Cupiennius salei* visual spectrum range also shows peaks in the blue (absorption maxima at 480nm), green (absorption maxima at 520), as well a shoulder in the ultraviolet (absorption maxima at 340) (Barth, 2002). The UV receptor cells were only found in the secondary eyes (Walla et al., 1996), as mentioned above the ones which have the highest probability to be used for polarisation vision in *C. salei.* However, only one UV-cell per eye was found. This could mean, that there are only very few UV- cells in the retina and therefore make them unlikely to be used for polarised vision, since this requires the ability to compare between many cells with different e-vector orientation preferences. Still, the UV-cells could be polarised light sensitive.

Another explanation for the few numbers of UV-cells found, is that the cells could be concentrated in a particular area, which was inaccessible to the electrode during recording. In wolf spiders, a family closely related to *Cupiennius*, an area in the periphery of the retina with rhabdomeres arranged perpendicular to one another has been identified and speculated to be linked to the wolf spider's

ability to orient themselves by polarised light (Barth, 2002). However, no comparable structure has been found in the retina of *C. salei* (Grusch et al., 1997). The discussion if UV-cells are used for polarised light detection might only be relevant in regard to the natural habitat of the spider. As mentioned before, the light environment in a forest under canopy shows the highest degree of polarisation in the UV- spectrum. It could be that the strength of the polarised light signal only surpasses the sensibility threshold of the spider in this spectrum. However, Grusch et al., (1997), found over 95% of all rhabdomeres in the retina of the secondary eyes of *C. salei* to be orderly arranged, regardless of their absorption maxima in the visual spectrum. Therefore, if the degree of polarisation is higher than the threshold of the receptor, all of these photoreceptors could be polarised light sensitive and potentially used for polarised light vision.

The requirements for the perception of polarised light

Photosensitive molecules in photoreceptors of animal eyes can only be activated if light, or rather the e-vector of the light wave, oscillates in the same plane as their absorptions axis (orientation of the 11-cis bond of the chromophore group) (Land & Nilsson, 2002). Additionally, the anatomy of the photoreceptor membrane containing visual pigments as well as the orientation of the photoreceptors themselves in relation to the outside world, are very important for polarisation sensitivity (Marshall & Cronin, 2011).

In vertebrates, the photosensitive molecule rhodopsin lies in discs, which form the rhabdoms of the rods and cones. These discs are oriented perpendicular to the incoming light and the rhodopsin molecules within are randomly distributed in all possible orientations, making it harder for the receptor cell to distinguish between different e-vector orientations of incoming light. A paper by Roberts et al. (2011) suggests that there may be more than just geometric mechanisms which could potentially increase photoreceptor sensitivity for polarised light, like protein-protein interaction and cytoskeletal coupling and might allow for some polarisation sensitivity in cone ciliary photoreceptors.

In invertebrates, on the other hand, the rhabdoms are organised in microvilli and have the chromophore molecules aligned to their long axis. Light oscillating in the same plane as the long axis of the microvilli, activates more photosensitive molecules than light oscillating in any other orientation. They therefore have a natural preference for a specific e-vector orientation (Land & Nilsson, 2002).

The degree to which a photoreceptor is polarisation sensitive depends on how its transmembrane rhodopsin molecules are aligned within the plane of the photoreceptor membrane, as well as how the microvilli themselves are aligned to the incident light (Wehner & Labhart, 2006; Nilsson et al. 1987). This makes a photoreceptor more sensitive to a specific e-vector orientation. But in order for true polarisation vision, (discriminating the visual signal due to the polarisation angle alone, independent of brightness or colour), at least 2 different e-vectors must be distinguishable in the same visual field by the visual system. Rhabdomeres in polarisation-sensitive ommatidia of insects and crustaceans as well as adjacent receptors in cephalopod retinas, generally show orthogonal microtubule arrangement, indicating sensitivity to e-vectors at 90° to each other. This orthogonal arrangement forms the basis of many polarisation- sensitive systems (Nilsson & Warrant, 1999; Labhart & Meyer, 1999; Marshall & Cronin, 2011).

In many insect species, the detection of polarised skylight has been shown both in behavioural experiments and by electrophysiological recording. All of them possess two sets of photoreceptors

with orthogonally oriented analyser, usually in specialised ommatidia situated at the dorsal rim of the compound eye (dorsal rim area).

In honey bees *Apis mellifera,* Rossel & Wehner, (1986) showed the use of a polarisation patterns in the sky for navigation, by rotating the field of view and transforming the polarisation information into modulation of perceived brightness. For this the bees use an array of polarisation- sensitive ultraviolet photoreceptors in the dorsal rim area (Labhard, 1980). Both electrophysiological recordings as well as behavioural studies show that the desert ant *Cataglyphis bicolor* uses polarisation pattern in the sky for path finding (Labhardt, 1986; Fent & Wehner, 1985). *Cataglyphis fortis,* relies predominantly on both the sky's polarised light pattern as a compass reference, as well as a simplified internal template to assess their walking direction (Lebhardt et al., 2012; Wehner & Müller, 2006). Polarisation vision was also discussed in controlling the flight course in flies *Calliphora* and *Musca*. *Musca domestica* was shown to respond to the presence of e-vector in both white and UV light by changing its turning tendency to changing e-vector orientations (Philipsborn & Labhart, 1990) and an electrophysiological study showed the high sensitivity to e-vector of polarised light in pure ultraviolet receptors in *Calliphora erythrocephala* and *Musca domestica* (Hardie, 1984).

The first behavioural evidence for the use of the polarisation pattern of the moonlit sky for orientation was found in the African dung beetle *Scarabaeus zambesianus*. *S. zambesianus* starts to forage at sunset and once it has located a food source, will use the polarisation pattern of the setting sun to find the most efficient path for escaping. After sunset, during moonlit nights, the dung beetle will continue to find the shortest path, but will choose random directions during moonless nights. By using both the sun's polarisation pattern as well as the moon's, the beetle can extend its foraging time (Dacke et al., 2003).

Apart from the arrangement of the photoreceptors, the rhabdoms in the specialised ommatidia also differ in shape. They are shorter, which favours polarisation sensitivity by reducing self-screening and have a larger cross-sectional area, which increases sensitivity (Nilsson et al., 1987; Labhart & Meyer, 1999). As mentioned above, for polarisation vision it must be possible to distinguish between different e-vector orientations. In the insect compound eye, each ommatidium has a specific preferred e-vector orientation sensitivity. In crustaceans, groups of ommatidia are specialised to differentiate not only different e-vector orientations, but also between linear and circular polarised light. The stomatopods, for example, have a mid-band of enlarged ommatidia in their eyes. 2- 6 rows of these ommatidia are specialised in circular polarisation vision and possess alternating layers of microvilli, which are highly ordered and in thinner layers than in the other parts of the retina. This increases these cells sensitivity to the stimulus. The other parts of the eye (ventral and dorsal hemisphere) are sensitive to linear polarised light. They are rotated 45° to each other and measure the degree of polarisation (Kleinlogel & White, 2008).

But also animals with single eyes has been shown to have polarisation vision. Both spiders and cephalopods possess microvilli, which are also orthogonally arranged within local retinal areas, or in the case of the spiders, are differently organized between individual eyes (Marshall & Cronin, 2011).

Polarised light detection in spiders

Spiders evolved simple eyes as their main visual system, usually having up to four pairs. These are highly developed in some species, with acuity that rivals that of primates. Eyes are characterized by position and morphology into one pair of principal eyes and three pairs of secondary eyes. The eyes

are named for their relative position on the head (anterior-median (AM) eyes, anterior-lateral (AL) eyes, posterior- median (PM), and posterior-lateral (PL) eyes). Apart from a few exceptions, the secondary eyes possess a reflecting tapetum, which is missing in the principal eyes in all species (Land, 1985). Having more than two eyes, makes it possible to devote different eyes to different tasks (Land & Barth, 1992; Schmid, 1998).

Although the retinal organization is very variable, all spider eyes possess microvilliar photoreceptors similar to those of other arthropods. Orthogonally arrangement of microvilli has been described in the eyes of spiders (Dacke et al., 1999; Dacke et al., 2001). Additionally, the tapetum can act as a polariser to enhance the polarisation sensitivity of the eye, due to the reflecting crystals which form the tapetum (Dacke et al., 1999). Behavioural evidence for polarisation vision was found in three families, the lycosid, agelenid and gnaphosid. In these families only the gnaphosid spider *Drassodes cupreus* uses its secondary eyes for polarisation detection, in the others the principle eyes are used (Dacke et al., 1999). The PM eyes of *Drassodes cupreus* are specialised as a compass, using the hemispheric polarised light pattern of the sky for navigation. The PM eyes are oriented upwards and their visual fields overlap to a great degree. The lenses are much reduced with no significant refracting power. This only provides a poorly focused image, but increases the receptive field of the photoreceptors. The canoe-shaped tapetum plays a very important part, polarising the reflected light and enhancing the polarisation sensitivity of the receptive segments of the photoreceptors. The rhabdoms in *Drassodes cupreus*, are oriented along the long-axis of the eyes, which are oriented approximately 90° to each other between the left and right PM eyes. Since they are oriented at different angles, each eye is most sensitive to a 90° different e-vector orientation. The gnaphosid spider therefore fulfils the morphological and electrophysiological requirements for true polarisation vision (Dacke et al. 1999; Dacke et al. 2001; Mueller & Labhart, 2010). Wolf spiders (lycosid) do not have specialised structures for polarised light detection in its principal eyes. The receptors are arranged orthogonal to each other in two layers. This arrangement not only leads again to co-axial analysers, but could potentially improve the polarisation sensitivity of the proximal layer by selective absorption of light orthogonal to its preferred direction by the other layer (Dacke et al., 2001). Both the gnaphosid as well as the lycosid spiders possess a rather well-developed system for the detection of polarised light and use this information for navigation and path finding (Dacke et al., 2001). But as previously mentioned, orthogonal arrangement of microvilli is found in the retina of many other spiders and the ability to at least polarisation sensitivity could be widespread in spiders.

The physics of polarised light

Light has three qualities, which are relevant for photosensitive structures: Luminous intensity, which describes the amount of photons that passes through, is emitted or reflected from a particular area (Heldmaier et al., 2013), the wavelength of the propagating wave, described as the spectrum of light and finally the polarisation of light (Marshall & Cronin, 2011). Light becomes polarised by reflection on shiny, non-metallic surfaces or by scattering on air or water molecules. Therefore polarised light can be found in abundance both in the atmosphere and hydrosphere and it provides another source of spatial information that can be perceived and used by animals (Wehner & Labhart, 2006).

There are two parameters, which describe polarised light, namely the angle and the degree of polarisation. These two aspects are corresponding to hue and saturation in colour vision (Nilsson & Warrant, 1999). A light wave consists of electric and magnetic fields, which oscillate with the same

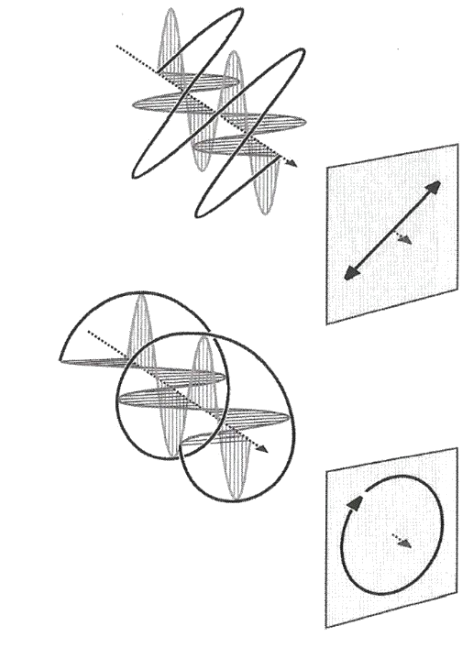

Figure 4: Physics of polarized light. The electric field vector of a propagating wave of light can be described by two orthogonal components (hatched horizontal and vertical wave traces). In the upper figure, the phase of these two components is 0°. In the lower figure, the phase relation is 90°. This results in linearly and circularly polarized light respectively (Wehner & Labhart, 2006).

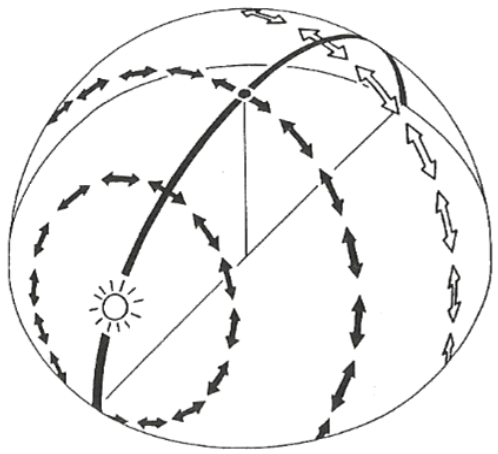

Figure 5: Celestial e-vector pattern. The white arrows indicate the region of the sky with the strongest polarisation at an angular distance of 90° to the sun. The pattern is visible even when the sun is obscured and can be used as a navigation aid (Land & Nilsson, 2002).

frequency, oriented perpendicularly to each other and to the direction of propagation. Only the electric field component can activate the photosensitive molecules in photoreceptors. Therefore only the orientation of the electric vector (or e-vector) can be perceived by animals.

Sunlight is usually unpolarised, meaning light oscillates in all direction without a preference in a specific orientation. However, by scattering or by reflection, light becomes linearly polarised.

The e- vector, which propagates along the z-axis, can be described by two orthogonal components, along the x- and y-axis. If the two wave components are of equal amplitude but different phase relations (0°- 180°), it will result in linearly, elliptically or circularly polarised light. In linear polarised light, the phases and the amplitudes of the x- and y- component of the e-vector are identical. The other extreme case occurs, if the phase relation of the two components is exactly 90°, then the light becomes circularly polarised (Wehner & Labhart, 2006).

The second parameter important in polarisation vision is the degree of polarisation. Within a theoretical (Rayleigh) atmosphere, light, which reaches an observer directly (0° scattering angle) is unpolarised (0%) and is maximally polarised (100%) at a 90° angle. At other scattering angles the light is only partially polarised, meaning apart from the dominant e-vector orientation, light oscillates in other orientation angles as well (Wehner, 2001). In nature, the degree of polarisation almost never surpasses 70% even while perfect conditions (Horvath & Wehner, 1999). Light which is reflected by shiny, non-metallic, dielectric surfaces is horizontally linear polarised with a maximum degree at a specific incident angle (Brewster's angle, 53° for the air/water interface) (Wehner, 2001).

As the sun light passes through the atmosphere and is scattered at air molecules, the light is maximally polarised at 90° angles from the lights pathway. This results in a pattern of polarisation across the sky, which is determined by the position of the sun. This celestial pattern is visible, even when the sun is obscured and can be used as navigation aid (Land & Nilsson, 2002). The same happens with moonlight with the moon in the zenith of the hemisphere, but at a lower luminance. This pattern is used by the African dung beetle to navigate after sunset (Dacke et al., 2003).

Goal of this study

As already discussed above in more detail, *Cupiennius salei* could be at least polarisation sensitive in its secondary eyes. Hints supporting this are the orderly arrangement of the microvilli in the rhabdomeres in the retina during the night, the perpendicularly aligned guanine crystals of the tapetum, the visual field, which, in the PM eyes, is oriented upwards and could allow *C. salei* to see the hemispheric polarisation pattern and the visual spectrum of the spider with its UV-peak, the spectrum with the highest degree of polarisation in the natural habitat of the spider.

In my study, I measure electroretinograms (ERG) of three of the secondary eyes of *Cupiennius salei*, when exposed to maximally linear polarised light. The left posterior-lateral eye (PL) and both the right and left posterior-median eyes (PM) are measured, to find out if different eyes have a different preferred e-vector orientation. I hope to answer the questions, if *Cupiennius salei* is polarised light sensitive or could even be capable of polarised light vision.

Materials and Methods

Animals

For this study adult females of *Cupiennius salei* were used, raised in the laboratory at the University of Vienna. The spiders were kept individually in glass jars (25cm high, 14.5cm diameter) and were fed once a week on flies (*Calliphora sp.*). They were held at an average temperature of 25°C and relative humidity above 60%. Females grow bigger than males and were therefore easier to handle and to study. There is no difference in the anatomy of the eyes between the sexes.

The animals were kept under an artificial day-night circle (12h: 12h, light: dark). For the experiments with night-adapted spiders, the circle was reversed. The spiders had at least three days to accommodate to the new night-day circle and the experiments started three hours after the begin of the night or day phase to make sure that the night and day microvilliar membrane turnover was completed before testing. All experiments were carried out in a room without another light source except the light stimulus.

In preparation for the experiments, each spider was cooled down for 1 hour at 4°C to make them easier to handle. It was then placed on a small wooden, hemispheric holder and fixated using parafilm.

Experimental setup

Polarisation of reflected light by the tapetum

In a first control experiment it was tested, if polarisation of light reflected by the tapetum would be sustained or not. For this linearly polarised light was directed at the tapetum, in an angle that the reflected light could be seen. A polarisation filter was then positioned between the reflecting light and the eye of the observer. By rotating the polarisation filter, the transmission of the reflected light decreases by absorption of different e-vector orientation. Maximally absorption occurs, when the pol-filter is rotated exactly 90° to the polarisation angle.

Recording

Electroretinograms (ERG) were recorded from the left and right posterior-median eyes (PM) and from the left posterior-lateral eye (PL) by placing the tip of a glass microelectrode filled with 1,5m KCL solution (resistance at room temperature 25°C: 120,8kΩ) on the lens of the eye. Electrical contact was made through the electrolyte itself or with electrode gel. The glass electrodes were made using a needle pipette puller (Kopf Needle Pipette Puller, Model 750, David Kopf Instruments, Tujunga, California, USA). Resistance was measured with a multimeter (MT-51 Multitester, Voltcraft)

A chlorinated silver wire slightly inserted into the opisthosoma of the spider served as the reference electrode. The received signal was amplified, measured and recorded with conventional equipment. (Preamplifier, amplifier, oscilloscope, PC). The experimental setup was positioned inside a Faraday cage on an air-suspended table to reduce vibration. The software Spike2 was used to record the measurements as well as the start and duration of each stimulus on a PC.

Stimulation

During the experiments, light impulses were emitted using white light, ranging in wavelength from 403 to 730 nm (see appendix), transported through an optical fibre. The light source was positioned directly in front of the eye between in 5 – 10 mm distance. A polarisation filter (pol-filter) was attached at the end of the fibre, which polarised the light linearly. The pol-filter could be turned 360° in 10° steps. Stimulus duration was 60ms. The interval between stimuli was 1s. 7 different e-vector orientations were tested, starting with polarised light oriented horizontally at 0° or 180° and then continuing in 30° steps until the pol-filter was turned 180°. This allowed to test a whole circle (0°, 30°, 60°, 90°, 120°, 150°, 180°). The testing order of the pol-filter orientations was different each measurement. Furthermore, the light impulses were emitted using two different luminances, the higher luminance at 104,5 cd/m^2, the lower at 15,16 cd/m^2. This was done, to make sure, that less activated photoreceptors would lead to a smaller ERG and therefore test the ERG. The luminance measurements, as well as spectral measurements were done with the polarisation filter positioned in front of the light source.

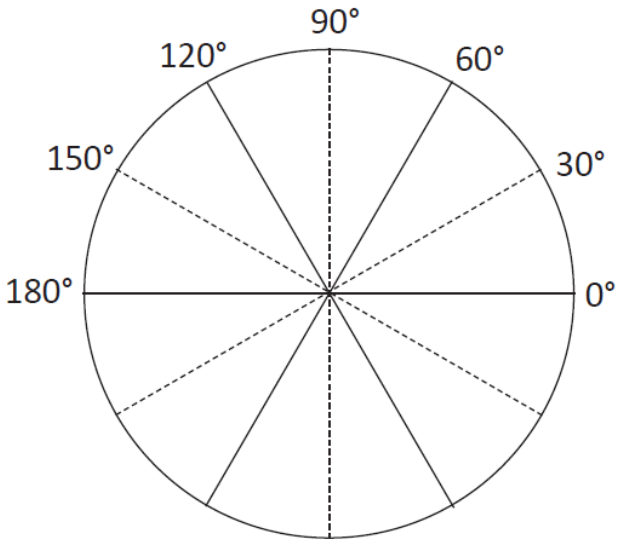

Figure 6: Tested e-vector orientations. The pol-filter was turned in 30°-steps, starting at 0° and continuing until the whole circle was tested. 0° and 180° both describe the same e-vector orientation (horizontally polarised light), therefore the data of these two test-runs were combined.

During the recording, each pol-filter position was tested with 11 light impulses, the first one was not included in the analysis. At the higher luminance the stimulation protocol was carried out two times and later compared to assure that the signal was correct during the whole measurement cycle. Which led to 20 light impulses per e-vector position at the higher luminance in total and 10 at the lower luminance. Since *Cupiennius salei* has a sensitivity peak in the UV-spectrum between 340 to 380nm, (Walla et al. 1996) and the UV-cells are suspected to be used for polarised light detection (Barth, 2002), the same experiment was carried out using a UV-light diode. The experiments with UV-light were done with the same stimulation protocol. The UV-light ranged in wavelength between (365-400nm) (see appendix). Again the same 7 e-vector directions were tested. The luminance of the UV-Diode was 1,641 cd/m^2. The pol-filter used for the other experiments had to be changed, because it shifted the emitted light spectrum. A glaspolfilter was used instead (for spectral measurements see appendix).

In a study by Barth et al. (1993), the absolute sensitivity of the visual system of *C. salei* was measured to be maximally 900lx and minimally 0.01lx and they concluded, that the sensitivity threshold was clearly below that. The stimulation signals in these experiments, both the white light as well as the UV-light, were clearly above the sensitivity threshold of the spider.

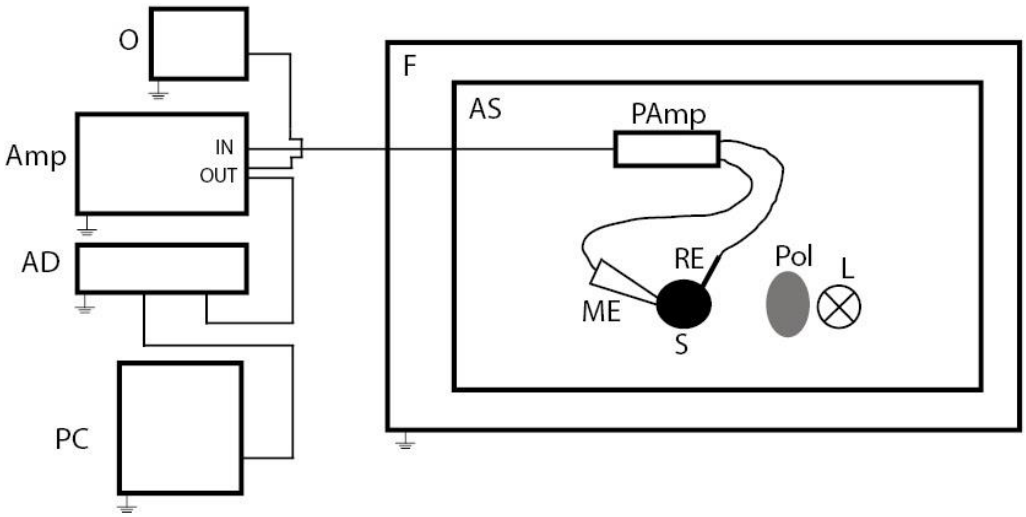

Figure 7: Experimental setup. ERGs were measured of secondary eyes of C. salei while exposed to linearly polarized light. AD = analog-to-digital converter, Amp = amplifier, AS = air-suspended table, F = Faraday cage, L = light source, ME = measurement electrode, O =oscilloscope, PAmp = preamplifier, PC = computer, Pol = Pol-filter, RE = reference electrode, S = spider

Statistical Analysis

During the recording, each pol filter position was tested with 11 light impulses, the first one was not included in the analysis. At the higher luminance each pol filter position was tested two times in two repeated experiments (overall 22 light impulses per polarisation filter orientation), the lower luminance with 11 light impulses. The 22 light impulses at the higher luminance were separated in two different repetitions cycles (11 impulses each) and later compared to make sure the measurement was correct during the whole measurement period. When there was no difference between the first and second repetition, the data were combined.

The arithmetic average was calculated for each e-vector direction for each individual spider and each eye. It was not possible to exactly replicate the amplification factor between different animals or eyes, therefor the absolute values could not be compared. Instead the data were normalised, calculating the difference in percent between the signal amplitudes received by different pol filter position using the amplitude size of the ERG at 0°/180° as a reference. After that, the mean difference of amplitude size between the different polarisation angles was compared using ANOVA. The statistical analysis was done with Microsoft excel and IBM SPSS Statistic 21.

Results

Polarisation of reflected light by the tapetum

The tapetum maintains polarisation of incident light to some degree. However, the reflected light shows an irregular pattern. Even when the second polarising filter was rotated full circle, the polarised light reflected from the tapetum is never fully absorbed by the second filter.

Also, the polarised light is not equally well reflected from the whole area of the tapetum, forming an irregular pattern of higher and darker areas, when presented with the same e-vector orientation.

Electroretinogram results

UV– light

For the experiment with the UV – diode, no ERG could be measured, since the signal-to-noise ratio was insufficient and could not be kept apart. The position of the electrode was changed multiple times, but no clear signal could be found. The data were not further analysed.

White light

Recordings of 3 spiders with day adapted eyes and 10 with night adapted eyes could be completed.

Since there was no significant differences in amplitude size between the first and second cycle at the higher luminance, the data were combined (t-test with Levene-test F (2,314) = 0, 823, p =0,059). This lead to a sample size of 200 measurements per orientation and per eye for night adapted eyes at the higher luminance and 100 measurements at lower luminance. For the day adapted eyes, this means 60 measurements per orientation and per eye at higher luminance and 30 at lower luminance.

In the night-adapted eyes, the mean peak size of the higher luminance was 101, 01 % with 0,718 STD. At the lower luminance the mean peak size was 75,27% ± 0, 738 STD the size of the peak at 0° with higher luminance. In day-adapted eyes, the mean peak size was 99,202 % with 1,10 STD (higher luminance) and 78,988 % with 1,49 STD (lower luminance).

There was a difference in amplitude size between the higher and lower luminance in night-adapted eyes of about -24,74 ± 7,45 % overall (-25,55±6,38 at 0°, -25,49±7,71 at 30°, -24,87±8,01 at 60°, -23,58 ±8,97 at 90°, -24,52± 6,88 at 120°, -24,45± 6,74 at 150°). The difference is significant (t – test with

Levene test F(2,10) = 0,012, p= 0,000). This shows, that a difference in luminance leads to a difference in peak size in night- adapted eyes.

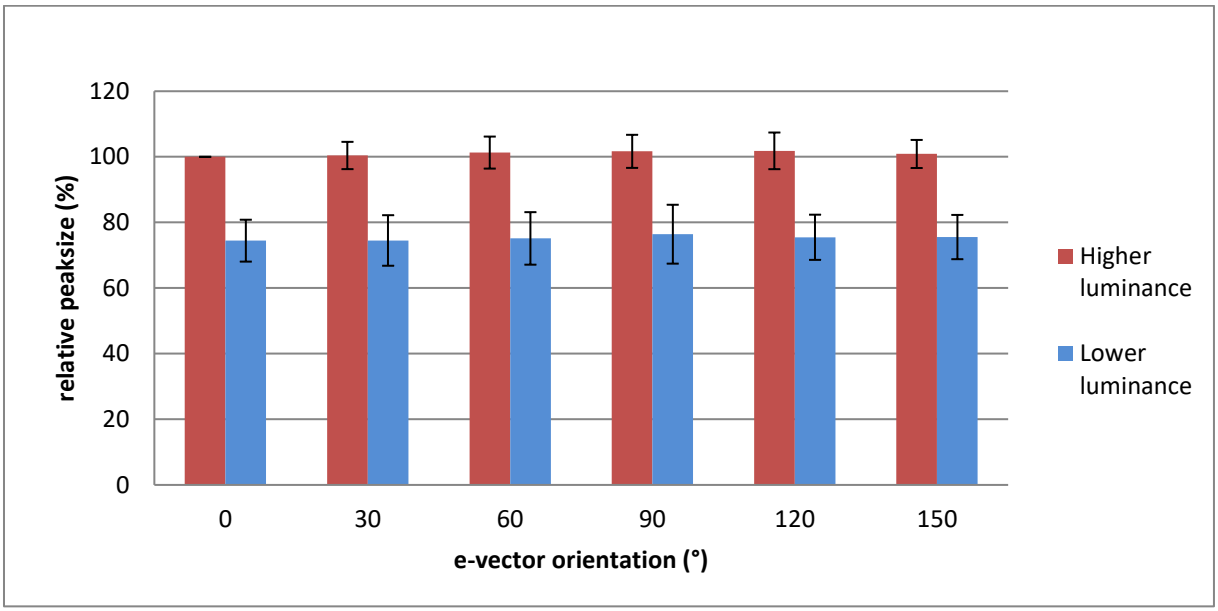

Figure 8: Normalised peak sizes at higher and lower luminance at different e-vector orientations in the night-adapted eye of Cupiennius salei. The difference in amplitude size was 24, 74 % and 0,745 STD overall. Data were normalised using the average peak size at 0° at the higher luminance as reference. n= 10

Homogeneity of variance between the higher and lower luminance setting in the day and night adapted eyes was tested using Levene- test, but was not tenable, F(2,320)= 34,821, p = 0,000. Therefore the data of the two different luminance groups was not combined and instead tested individually.

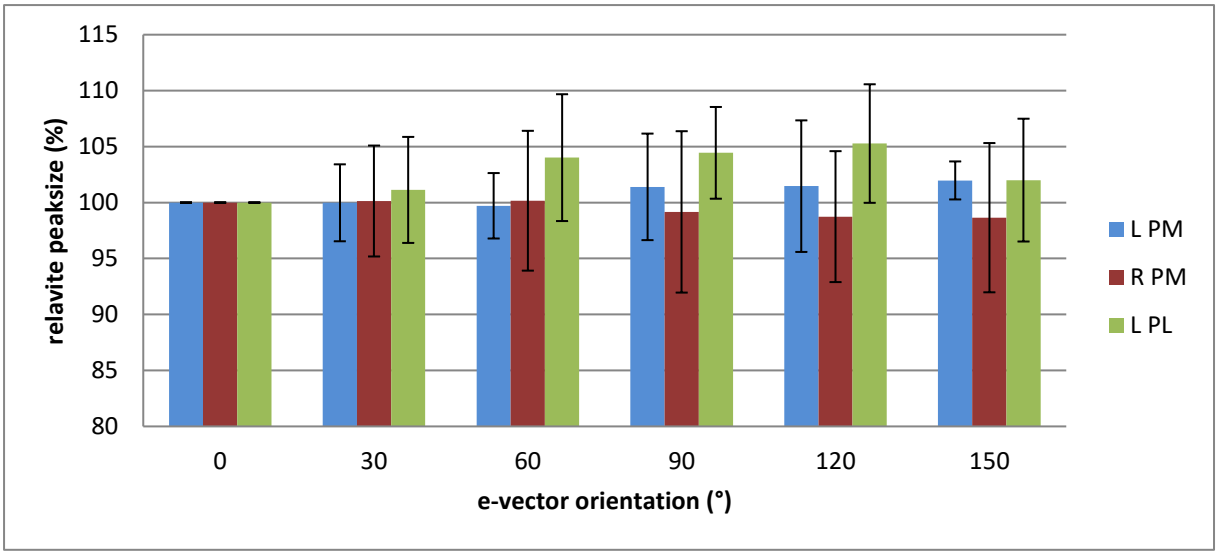

Figure 9: Normalised Peak sizes at the higher luminance adjustments in the night adapted eye of Cupiennius salei, sorted by measured eye. The data was normalised with the mean peak size at 0° being used as reference. None of the eyes showed any significant preference for any tested e-vector orientation. (L PM = Left Posterior Median eye, L PL = Left Posterior Lateral eye, R PM = Right Posterior Median eye). n= 10

The total mean peak size at the higher luminance setting was 101,415 % with 4,226 SD (total n = 150). Since mean peak size at 0° was used as a reference to normalised the data, the amplitude at 0° is exactly 100% and not included in the ANOVA. Using again the mean peaks size at 0° as a reference, the mean peak size at the lower luminance setting was 101,567% with 8,012 SD (total n = 148).

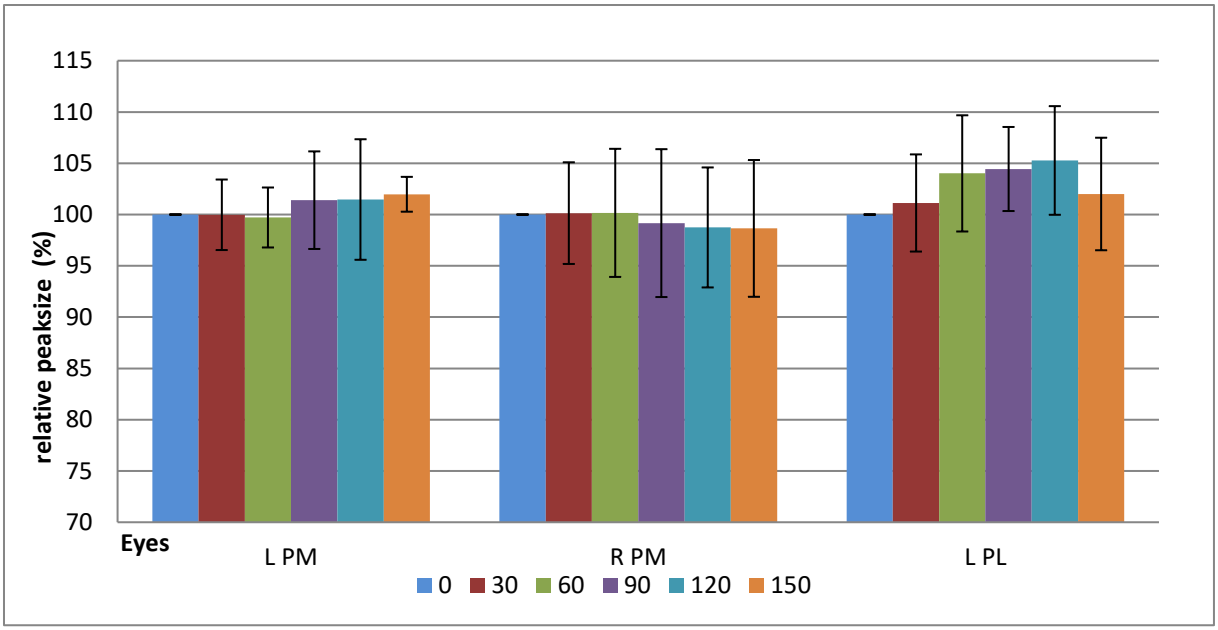

Figure 10: Normalised Peak sizes at the higher luminance adjustments in the night adapted eye of Cupiennius salei. The peak size was measured at 0°, 30°, 60°, 90°, 120° and 150° e-vector orientation. The data were normalised with the mean peak size at 0° being used as reference. No e- vector was preferred by any of the three tested eyes. (L PM = Left Posterior-Median eye, L PL = Left Posterior-Lateral eye, R PM = Right Posterior-Median eye), n = 10

The differences in amplitude size was tested using ANOVA both for the higher and lower luminance with all eyes combined. Homogeneity of variance across all eyes was tested using Levene- test and confirmed ($F(4,145)= 0,197$, $p = 1,530$). As expected, in the day adapted eye, there are no significant differences between the tested e-vector orientation in all eyes in no luminance setting (high luminance: $F (4, 44) = 0,34$, $p = 0,849$; low luminance: $F (4, 44) = 0,467$, $p= 0,76$). However, also in the night- adapted eyes, the differences between the different e-vector angles at the higher luminance setting is not significant ($F(4,149)= 0,420$, $p = 0,794$) (see table 1). Neither were there any differences at the lower luminance ($F (4, 147) = 0,353$, $p= 0,841$) (see table 2).

Table 1: ANOVA of peak sizes at different e-vector angles of all measured eyes (night adapted) of C. salei at the higher luminance setting. The differences between the e-vector angles are not significant. (F(4,149)= 0,420, p = 0,794).

e-vector angle		mean difference	Standard error	significance	95%-confidence interval	
					lower limit	upper limit
30	60	-0,882	1,265	0,957	-4,375	2,612
	90	-1,255	1,265	0,858	-4,749	2,239
	120	-1,412	1,265	0,797	-4,906	2,081
	150	-0,462	1,265	0,996	-3,956	3,032
60	30	0,882	1,265	0,957	-2,612	4,375
	90	-0,373	1,265	0,998	-3,867	3,120
	120	-0,531	1,265	0,993	-4,025	2,963
	150	0,419	1,265	0,997	-3,074	3,913
90	30	1,255	1,265	0,858	-2,239	4,749
	60	0,373	1,265	0,998	-3,120	3,867
	120	-0,157	1,265	1,000	-3,651	3,336
	150	0,793	1,265	0,971	-2,701	4,287
120	30	1,412	1,265	0,797	-2,081	4,906
	60	0,531	1,265	0,993	-2,963	4,025
	90	0,157	1,265	1,000	-3,336	3,651
	150	0,950	1,265	0,944	-2,544	4,444
150	30	0,462	1,265	0,996	-3,032	3,956
	60	-0,419	1,265	0,997	-3,913	3,074
	90	-0,793	1,265	0,971	-4,287	2,701
	120	-0,950	1,265	0,944	-4,444	2,544

The data of the night-adapted eyes were separated for each eye to test differences in amplitude size to e-vector orientation in each eye individually.

Again, in all tested eyes there was no significant difference between any e-vector orientation in both luminance settings (see table 2).

Table 2: ANOVA of peak sizes of different eyes (night adapted) of C. salei at both luminance settings and all e-vector orientations. There was no significant difference between the amplitude sizes at different e-vector angles in none of the tested eyes and at no luminance setting (high = white background, low = grey background, df = degrees of freedom).

Eye	sum of squares	df	df total	mean of squares	F -value	significance
LPM	81,824	5	53	16,365	0,464	0,801
RPM	26,506	5	53	5,301	0,273	0,926
LPL	222,996	5	53	44,599	0,892	0,494
LPM	115,017	4	49	28,754	0,479	0,751
RPM	266,654	5	52	53,331	0,566	0,725
LPL	160,195	5	54	32,039	0,46	0,804

Discussion

Despite the hints described in the introduction, which made a polarised light sensitivity in *C. salei* very likely, no rise in the activity level could be measured for any of the tested e-vector orientations in none of the tested eyes with white light. In the UV-spectrum, no valid measurements could be made, due to insufficient signal-to-noise ratio. However, as described above, all photoreceptors were assumed of being sensitive to polarised light, due to their morphological attributes. This was not the case. Therefore, I try to find some possible explanations for these findings.

Polarised light sensitivity – White light

The most straight forward explanation for these results is that the degree of organisation of the rhabdoms is insufficient to discriminate between different e-vector orientations. In the day-adapted eyes, the microvilli and rhabdoms are degenerated and no longer form a regular array. Therefore, the experiments with day-adapted spiders were expected to show no preference for a specific e-vector. This was the case. The measurements showed no preference with a high standard deviation. The night-adapted eyes, showed lower deviations, but still no preference for a specific e-vector. Due to the higher degree of organisation of rhabdoms, a higher consistence in signal output was expected.

Apart from the photoreceptors themselves, other structures could also have a negative impact on the spider's capability to detect polarised light. The receptors of *Cupiennius salei* "sit" on the rows of the tapetum, which is oriented horizontal to the substrate on which the spider is sitting.

In the first control experiment, it was shown that the tapetum maintains polarisation of incident light to some degree. However, the reflected light showed an irregular pattern. This means, that even if a nearly fully polarised light source is directed at the tapetum, the reflected light is less polarised. This works directly against polarised light sensitivity by reducing contrast for the perception of e-vector orientation.

Also, the polarised light was not equally well reflected from the whole area of the tapetum. The variability in the degree and angle of polarisation could be caused by a variability in the orientation of the guanine crystals in the tapetum. This was already discussed by Dacke and colleagues (2001) when they studied polarised light detecting optics in canoe-shaped tapeti.

Apart from the form of the tapetum, the presence of a well-developed lens, which might lessen the polarisation signal, in the secondary eyes is another finding that is different between *C. salei* and *Drassodes cupreus* and other spiders that are able to see polarised light.

Aside from morphological features, it might be possible that the stimulation signal in the experimental design was too bright in the higher luminance setting. This would lead to an undiscriminating activation of all photoreceptors, no matter the preference of the rhabdoms. Subtle differences might have been overshadowed. However, also at the lower luminance setting, the ERG showed no preference for any e- vector orientation.

Polarised light sensitivity – UV light

In this setup no measurements of UV-cells could be analysed, as the signal to noise ratio was insufficient to distinguish a clear signal even after changing the electrodes multiple times and positioning the recording electrode on different areas of the eye. As mentioned before, UV–cells are believed to be either very rare or to be located in areas inaccessible in some previous ERG recordings. So it is possible that the electrode never recorded UV cells or that the recording setup might not be sensitive enough to detect a single cell signal from a UV-cell, due to the fact, that the ERG was a multi-cell recording through the cuticula and the intact lens of the eye.

Polarisation sensitivity in UV – cells might have had a big role in a natural environment. As previously described, in nature, the degree of polarisation is highest in the UV –spectrum, especially under the rainforest canopy. On the basis that the microvilli arrangement in the receptors are sufficient to enable polarisation sensitivity, UV – cells should be as sensitive as other receptor cells, since no previous study found a completely different microvilli organisation in UV-cells and also might be the ones actually most useful in nature. If other photoreceptors would have shown polarisation-sensitivity, recordings focusing on UV- cells would have been interesting in future studies.

However, since no preference for e-vector orientation, even with maximally polarised light, could be found in any receptor cells, it seems unlikely that UV-cells should be polarisation sensitive. Even though no measurement could be completed in this study, it is improbable that UV – cells alone should be enough to enable *Cupiennius salei* to detect polarised light, especially if their morphological structure does not differ significantly from the other photosensitive cells.

Concluding remarks

Contrary to the spider *Drassodes cupreus*, *Cupiennius salei* does not seem to be able to detect the polarisation of light. This does seem surprising, considering the apparently high degree of organisation of the rhabdoms in the retina of its secondary eyes.

There are several possible explanations for this. First, the amount of sufficiently aligned microvilli could not be enough for the necessary sensibility for a specific e-vector orientation. Additionally, other structures like the tapetum or the lens might even weaken the incoming polarisation signal or reduce visual field of individual photoreceptors.

Lycosid and gnaphosid spiders, who detect polarised light, both have specialised structures. In lycosid, there are two layers of orthogonal receptors. In gnaphosid, parallel arranged microvilli cover the whole retina and the lens power is drastically reduced, enhancing the visual field of individual photoreceptors. Furthermore, the middle axis of the two opposing PM eyes are orthogonal to each other, allowing true polarisation vision by comparing the signal from both eyes. In both groups the tapetum also plays a big role in polarisation perception and show a high degree of specialisation to allow the spatial information of polarised patterns to be used for navigation and path finding.

Cupiennius salei mainly depends on its mechanosensory system and even though its visual system is much more developed than previously thought, the same morphological structure can be found in many other spider families and shows no morphological specialisation for polarisation sensitivity.

A positive result in the ERG when exposed to polarised light might have been a hint, that polarisation sensitivity is a uniform sensory capacity for eyes with similar microvilliar arrangement. Whether or not the information can actually be processed and used would depend on higher functions.

If *C. salei* had the neural network to actually perceive polarised light and if and for what purpose it might use this information, would be the topic for future research.

However, it seems that, a seemingly high degree of organisation and parallel arranged rhabdoms alone, are not enough to guarantee polarised light sensitivity, even on the very first level of sensory input.

Even though *C. salei* fulfils some of the morphological requirements for polarised light perception, the degree of microvillar organisation alone seems to be insufficient and additional structures or a higher degree of organisation are needed. The role of the UV-cells in polarised light vision remains very doubtful and their usefulness for *Cupiennius salei* unclear.

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Appendix

Spectral measurements

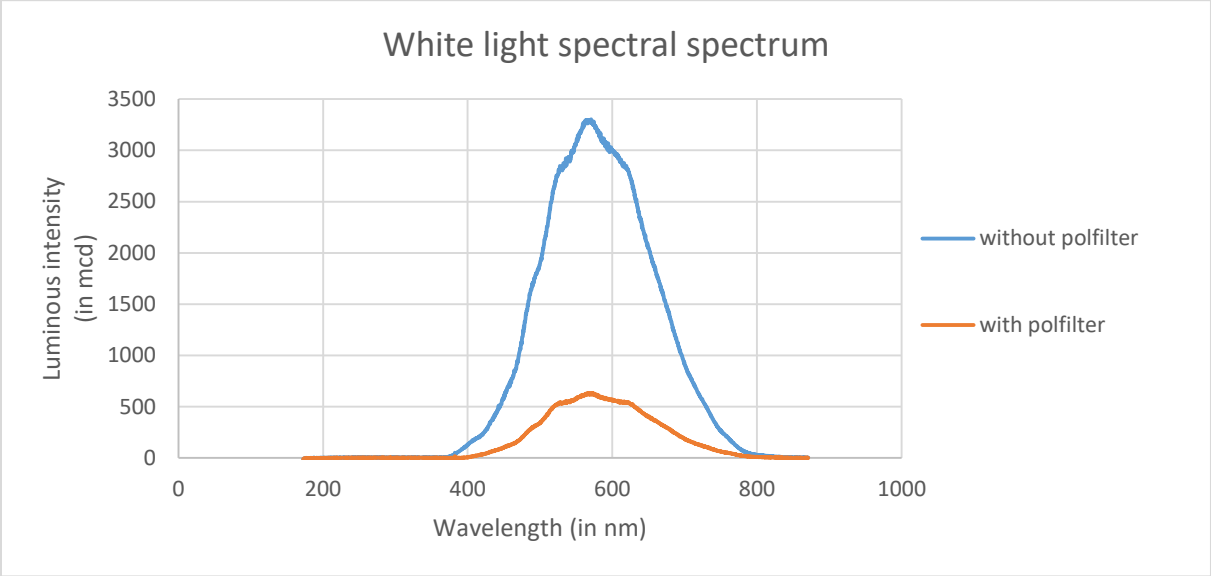

Figure 11: Spectral measurement of the white light source with and without attached polarisation filter. Peak with polarisation filter at 577 nm, without at 576nm.

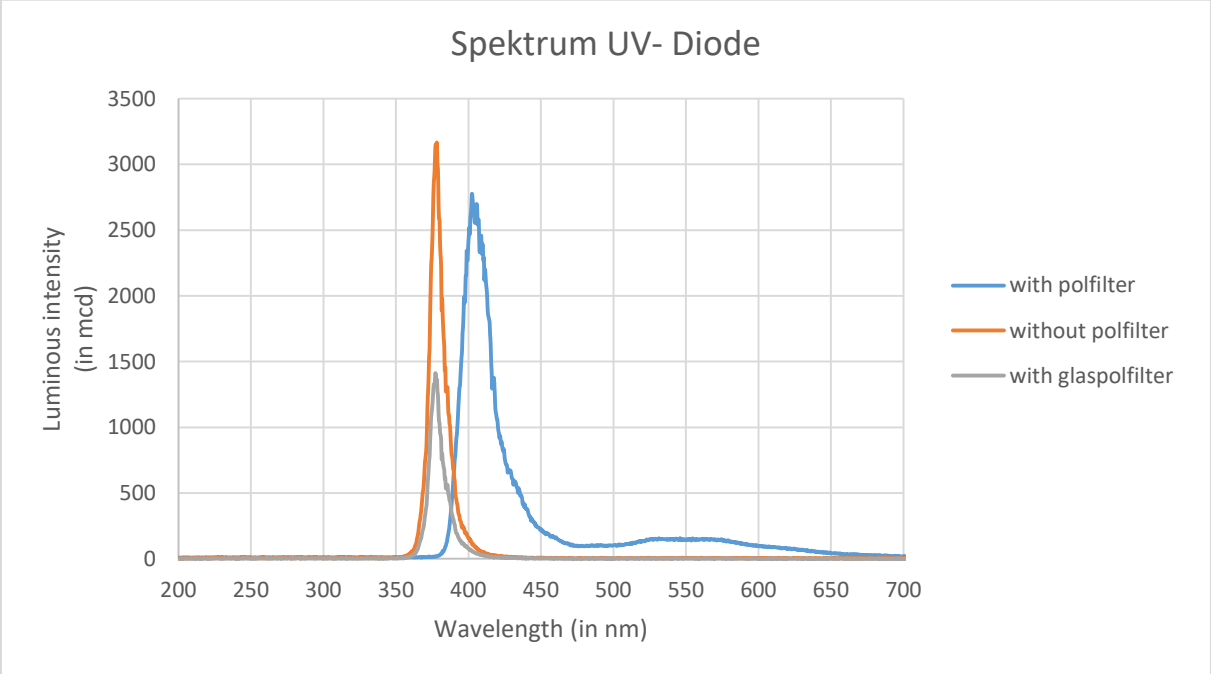

Figure 12: Spectral measurement of the UV light source with and without attached pol-filter and with glasfilter. Peak with pol-filter at 402 nm, without at 378nm. Peak with glaspolfilter at 377nm. The polarisation filter caused a changed in the UV-spectrum and was replaced by a polarisation filter made out of glass.

Summary

The ability to perceive polarised light is a widespread ability in arthropods and cephalods. The basis for this is the organisation of photosensitive molecules in microvilli and their orientation towards incoming light. Linear polarised light oscillates in one plane along its axis of progression. Photoreceptors will naturally have a preference to light oscillating along the same long axis as their microvilli. Polarisation sensitivity was found in a number of insects, crusatacean and in two spider groups: gnaphosid and wolf spiders (lycosid). Both the gnaphosid as well as the lycosid spiders possess a rather well-developed system for the detection of polarised light, based on orthogonal arrangement of microvilli and additional morphological structures and use this information for navigation and path finding. However, similar arrangements are found in the retina of many other spiders and the ability to at least polarisation sensitivity could also be found in other spiders without any morphological specialisation. *Cupiennius salei*, a nocturnal spider without any specialised structure for polarisation vision, was tested to be sensitive to polarised light. In the night-adapted secondary eyes of *C. salei*, the visual pigment is organized in rhabdomeres and orderly aligned in the microvillar membrane. In the secondary eyes, the receptive segments only have 2 rhadomeres on opposing sides and two neighbouring rhadomeres for a rhabdoms with the microvilli tightly packed and in regular arrays. Additionally, the tapetum of *C. salei* consists of parallel, horizontal strips with reflecting guanine crystals. Polarisation sensitivity was therefore tested in the night- adapted secondary eyes. Electroretinograms were measured in three of the secondary eyes when exposed to maximally linear polarised light. A first control experiment tested if light reflected by the tapetum would sustain its polarisation. The recordings were made for the left and right posterior-median eyes and from the left posterior-lateral eye with conventional equipment. The light impulses where emitted using white light, ranging from 403 to 730 nm in wavelength and UV – light between 365-400, and maximally linearly polarised light using pol-filter. 7 different e-vector orientations were tested with 2 different luminance settings at 104,5 cd/m^2 and at 15,16 cd/m^2. The data of the night-adapted eyes were separated for each eye to test differences in amplitude size to e-vector orientation in each eye individually using ANOVA. Homogeneity of variance across all eyes was tested using Levene- test and confirmed ($F(4,145) = 0{,}197$, $p = 1{,}530$). However, there were no significant differences in amplitude size between the different e-vector angles at the higher luminance setting ($F(4,149) = 0{,}420$, $p = 0{,}794$) or at the lower luminance ($F(4, 147) = 0{,}353$, $p= 0{,}841$). No measurement of UV-cells could be analysed due to insufficient signal-to-noise ratio and the polarised light was not equally well reflected from the whole area of the tapetum, which could be caused by a variability in the orientation of the guanine crystals. Contrary to the gnaphosid or lycosid spiders, *C. salei* does not seem to be able to detect the polarisation of light. A possible explanation could be insufficiently aligned microvilli, which do not allow for a preference to a specific e-vector. Additionally the tapetum or lens might weaken the incoming polarisation signal or reduce the visual field of individual photoreceptors. A positive result in the ERG when exposed to polarised light might have been a hint, that polarisation sensitivity is a widespread ability in spiders even without specialised structures. Whether or not the information could be processed or even used in behaviour would depend on higher cognitive function. However, it seems that a seemingly high degree of organisation and parallel arrangement of photosensitive segments alone are not enough to guarantee polarised light sensitivity even on the first level of sensory input and a higher degree of organisation or additional structures are needed. The role of the UV-cells in polarised light vision remains very doubtful for *C. salei* and their usefulness for *Cupiennius salei* unclear.

Zusammenfassung

Die Wahrnehmung der Polarisation von Licht ist eine weitverbreitete Fähigkeit bei Arthopoda und Cephalopoda. Die Grundlage dafür ist die Organisation der photosenstivien Moleküle in Microvilli und deren Orientierung zu einfallendem Licht. Linear polarisiertes Licht schwingt überwiegend in einer Ebene in seiner Fortbewegungsachse. Microvilli welche in derselben Achse orientiert sind wie das ankommende Licht, werden stärker stimuliert als von Licht welches in einer anderen Ebene schwingt und besitzen so eine natürliche Präferenz für eine bestimmte Orientierung des linear polarisierten Lichts (Orientierung des e-Vektors). Diese Sensibilität für die Polarisierung von Licht konnte in vielen Insekten, Krustentiere und 2 Spinnengruppen gezeigt werden. Sowohl die Gnaphosid wie auch die Wolfspinnen (Lycosid) besitzen ein gut entwickeltes System für die Detektion von polarisiertem Licht, basierend auf einer orthogonalen Orientierung der Microvilli zueinander und zu einfallendem Licht. Zusätzlich haben beide Spinnen spezialisierte morphologische Strukturen, welche polarisierte Signale verstärken. Sie nutzen diese Information zur Navigation und Wegfindung. Das grundlegende System, die orthogonale Orientierung von Microvilli, ist allerdings eine Anordnung die auch in vielen anderen Spinnen zu finden ist. Es ist daher möglich, dass die Sensibilität für polarisiertes Licht eine weitverbreite Fähigkeit im Spinnenreich ist. Es wurde daher *Cupiennius salei*, eine nachtaktive Jagdspinne ohne zusätzliche morphologische Spezialisierung zur Wahrnehmung von polarisierten Licht, auf ihre Sensibilität für verschiedene e-Vektoren getestet. In *C. salei* ist, während der Nacht, das visuelle Pigment in Rhabdomeren angehäuft und ausgerichtet in der Microvilli Membran. Die Rezeptor Segmente der Nebenaugen besitzen nur 2 Rhabdomere an gegenüberliegenden Seiten und formen zusammen mit den benachbarten Rhabdomeren Rhabdome mit regelmäßiger Anordnung und eng gepackten Microvilli. Zusätzlich besteht das Tapetum von *C. salei* aus parallel angeordneten, horizontalen Reihen mit reflektierenden Guanin Kristallen. Es wurden Elektroretinogramme von drei der Nebenaugen gemessen, mit einem maximal polarisierten Lichtstimulus als Signal. Die Messung erfolgte am linken und rechten Posterior-median und dem linken Posterior-lateral Auge, um zu testen, ob verschiedene Augen unterschiedliche e-Vektoren präferieren. In einem ersten Kontrollexperiment wurde ebenfalls getestet, ob Licht, welches vom Tapetum reflektiert wird, seine Polarisation beibehält. Als Lichtimpulse wurde weißes Licht benutzt mit einer Wellenlänge zwischen 403 und 730nm und UV-Licht mit 365 bis 400nm Wellenlänge. Die Polarisierung des Lichts erfolgte durch Polfilter. Insgesamt wurden 7 verschiedene e-Vektor Orientierungen mit zwei unterschiedlichen Helligkeitsstufen (104,5 cd/m^2 und 15,16 cd/m^2) getestet. Die erhobenen Daten wurden für jedes Auge einzeln mittels ANOVA analysiert, um einen Unterschied in der Amplitude der entstehenden Potentiale bei beschiedenen e-Vektor Orientierungen zu ermitteln. Varianzhomogenität über alle Augen konnte mittels Levene-Test bestätigt werden ($F(4,145) = 0{,}197$, $p = 1{,}530$). Ein signifikanter Unterschied in der Amplitudengröße für verschiedene e-Vektor Orientierungen konnte in keinem Auge gefunden werden, weder in der höheren Helligkeitsstufe ($F(4,149) = 0{,}420$, $p = 0{,}794$), noch in der niedrigeren ($F(4, 147) = 0{,}353$, $p = 0{,}841$). Aufgrund eines unzureichenden Signal-Rausch-Verhältnisses, konnten die Ergebnisse der UV-Licht Messung nicht weiter analysiert werden. Aufgrund ihrer Seltenheit in der Retina von *C. salei*, bleibt aber die Rolle der UV-Rezeptoren für die Wahrnehmung von polarisierten Licht fragwürdig. Bei dem Kontrollexperiment zeigte sich eine ungleiche Reflektion des polarisieren Lichts vom Tapetum, möglicherweise aufgrund der Variabilität der Guanin Kristalle. Anders als bei Gnaphosiden oder Lycosiden, ist es *C. salei* nicht möglich die Polarisation von Licht wahrzunehmen. Eine mögliche Erklärung könnte sein, dass die Microvilli nicht ausreichend ausgerichtet sind, um eine Präferenz für einen bestimmten e-Vektor zu haben. Zusätzlich könnte sowohl das Tapetum, als auch die Linse selbst das einfallende Polarisationssignal schwächen und so das visuelle Feld individueller Photorezeptoren

einschränken. Ein positives Resultat des ERG hätte ein erster Hinweis darauf sein können, dass die Wahrnehmung von polarisiertem Licht, oder die Sensibilität dafür eine weit verbreitete Fähigkeit sein könnte, selbst bei Spinnen ohne zusätzliche morphologische Spezialisierung. Ob und wie diese Information dann verarbeitet und im Verhalten genutzt worden wäre, wäre abhängig von höheren kognitiven Prozessen gewesen. Allerdings scheint ein hoher Grad an Organisation und eine parallele Ausrichtung von photosensitiven Segmenten allein keine Sensibilität für polarisiertes Licht zu garantieren und ein höherer Grad an Organisation oder zusätzliche Strukturen sind bereits in der ersten Ebene der Signalübertragung nötig.