

RESEARCH ARTICLE

Brightness discrimination in the day- and night-adapted wandering spider *Cupiennius salei*

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ABSTRACT

Cupiennius salei is a nocturnal spider with eight eyes, which undergo a remarkable circadian cycle: the rhabdomeric membrane of the photoreceptor cells is dismantled during the day and rebuilt at the beginning of the night. Such drastic changes might influence the brightness discrimination ability. We tested this hypothesis by presenting square-shaped flickering stimuli with certain luminances on stationary backgrounds with other luminances to spiders with day- or night-adapted eyes. When the spider, through its three pairs of so-called secondary eyes, perceives a visible contrast between the stimulus and the background, its principal eye muscle activity should increase. We therefore recorded this activity *in vivo* to assess the brightness discrimination ability of *Cupiennius salei*. Our results show that this spider has good brightness discrimination ability, which is significantly better with dark-adapted eyes. A Michelson contrast of 0.1 to 0.2 at night, and of 0.2 to 0.3 for day-adapted eyes, is sufficient to elicit a significant response, except below a critical value of luminance ($\sim 16 \text{ cd m}^{-2}$), where the minimal perceivable contrast needs to be higher. In the Discussion we compare these performances with those of other animals, in particular with jumping spiders.

KEY WORDS: Circadian cycle, Spider eyes, Telemetry, Photoreceptor cycle

INTRODUCTION

Cupiennius salei Keyserling 1877 (Ctenidae) is a wandering spider from Central America. At night, it becomes active and adopts a ‘sit and wait’ predation behavior in which the spider waits for prey to pass by and then catches it rapidly (Barth and Seyfarth, 1979). This hunting method, without any webs or traps, implies good sensory systems. *Cupiennius salei* can use its extremely sensitive mechano-sensory system alone to catch its various crawling or flying prey (Hergenröder and Barth, 1983). The visual system was therefore initially neglected by earlier research, and the focus was on investigating the mechano-sensory system (Albert et al., 2001; Barth, 2002; French et al., 2002; Hergenröder and Barth, 1983). The visual system of this spider has been studied more recently and is apparently also very efficient (Fenk and Schmid, 2010; Fenk and Schmid, 2011; Zopf et al., 2013). Interestingly, vision alone can also elicit attack behavior in *C. salei* (Fenk et al., 2010).

As in nearly all spiders, *Cupiennius salei* has eight eyes, arranged in four pairs. The anterior-medial (AM) eyes are called ‘principal eyes’: their retina can be moved by muscles (a dorsal and a ventral muscle for each eye) and these eyes are involved in discriminating targets (Schmid, 1998). The three other pairs, termed ‘secondary

eyes’, are the posterior-medial (PM), the anterior-lateral (AL) and the posterior-lateral (PL) eyes. They differ from the principal eyes morphologically and functionally: they lack muscles and are involved in detecting targets and motion (Neuhofer et al., 2009; Schmid, 1998). Behavioral experiments show that the secondary eyes are color blind (Orlando and Schmid, 2011). Moreover, the eyes of *C. salei* are extremely sensitive: vision is possible up to a threshold of 0.0001 to 0.001 lx (Barth et al., 1993; Fenk and Schmid, 2011), and the spider’s spectral sensitivity ranges from 365 to 695 nm as a result of three different types of photoreceptors (Walla et al., 1996; Zopf et al., 2013).

All eyes of *C. salei* exhibit an interesting phenomenon: histological observations reveal a circadian cycle of degradation and rebuilding of the photoreceptor cell membrane (Grusch et al., 1997). The photoreceptor cells possess rhabdomeres, i.e. microvilli where phototransduction occurs. In spiders exposed to a 12 h light:12 h dark cycle, after 2 h of dark adaptation, the rhabdomeres are abundant and measure approximately 2.5 μm in the principal eyes and 2.5–3.5 μm in the secondary eyes. After 2 h of light adaptation, however, the rhabdomeres become fragmented: the diameter of the microvilli is irregular, the density of the microvilli is reduced to less than half and microvilli length decreases ($\sim 0.75 \mu\text{m}$). Moreover, numerous coated vesicles and huge multivesicular bodies are observed close to these degraded rhabdomeres. About 80% of the rhabdomeric membrane is thus removed.

A similar membrane turnover of the photoreceptor cells has been described for other nocturnal arthropods: Insecta [the mosquito *Aedes aegypti* (White and Lord, 1975)], Chelicerata [Deinopidae *Deinopis subrufa* (Blest, 1978), Pisauridae *Dolomedes* (Blest and White, 1978)] and Crustacea [the crayfish *Cherax destructor* (Stowe et al., 1990), the rock crab *Grapsus* (Nässel and Waterman, 1979), and the ghost crab *Ocyropsis* (Nässel and Waterman, 1979)]. Among diurnal species, some Salticidae (jumping spiders) have been investigated: *Phanias harfordi* and *Phidippus johnsoni* showed no modification of the rhabdomere architecture (Eakin and Brandenburger, 1971), but in *Servaea vestita* an exocytotic shedding of the photoreceptor membrane was observed (Blest and Maples, 1979). In the horseshoe crab *Limulus polyphemus* (Chelicerata) (Chamberlain and Barlow, 1984), a circadian cycle also affects the eyes: the rhabdomeric membrane is only briefly dismantled; furthermore, the aperture of each ommatidium (visual unit of a compound eye) is reduced and the responsiveness of the photoreceptor cells and optic nerves is decreased (Barlow et al., 1980). Neither of these last two mechanisms occurs in *C. salei*.

The ecological relevance of such a costly structural conversion remains unclear. One interpretation is that the phototransduction machinery is repaired or ‘regenerated’ in the multivesicular bodies during the day, in particular through fusion with non-coated vesicles of the endoplasmic reticulum (Blest and White, 1978). It has also been proposed that this phenomenon might primarily be used to adjust the sensitivity of the eye to the ambient light (Blest, 1978). Another interesting hypothesis proposes that it would be

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‘uneconomical’ to preserve such a quantity of membrane in the photoreceptor cells (Blest, 1978).

Examination of *Limulus polyphemus* revealed that the circadian anatomical and physiological changes in the eyes modulate their behavior (Powers and Barlow, 1985; Powers et al., 1991). However, no behavioral studies have been carried out to assess the impact of a rhabdomeric membrane cycle, such as the one found in *C. salei*, on visual performance. Therefore, we investigated the brightness discrimination ability, a visual performance not yet studied in this spider, with day- and night-adapted eyes.

Brightness is the perception of the luminance of an object. It can be linked to the perception of contrast (C), which quantifies the difference between light and dark, and is defined by $C=(L_{\max}-L_{\min})/(L_{\max}+L_{\min})$, where L_{\max} and L_{\min} are the luminances for two compared areas (in cd m^{-2}) (Michelson, 1927). The better an animal’s perception of contrasts, the better its brightness discrimination ability and the closer the two discriminated values of luminance. The few studies examining the brightness discrimination ability in animals are mainly restricted to diurnal or arrhythmic vertebrates (Cornsweet and Pinsker, 1965; Geisbauer et al., 2004; Griebel and Schmid, 1997; Huang et al., 2002; Lind et al., 2013; Scholtyssek et al., 2008). The diurnal jumping spiders (Salticidae) *Menemerus bivittatus* (Tiedemann, 1993) and *S. vestita* (Zurek et al., 2010) have also been investigated. To compare the brightness discrimination ability among different species, Weber’s fraction (k) is used. Weber’s law states that the size of the noticeable difference threshold is related to the mean of the two levels of brightness, except at very low and very high stimulus intensities, and is thus defined as $k=\Delta L/L$, where L is the mean luminance and ΔL is the just-noticeable difference between two luminance values (Geisbauer et al., 2004; Huang et al., 2002). Thus, the lower the Weber’s fraction for an animal, the better its brightness discrimination ability.

It is possible to assess *in vivo* the brightness discrimination ability of *C. salei* using a telemetric device and the following protocol. The dorsal muscles of the principal eyes of this spider are spontaneously active (frequency of 12 ± 1 Hz) (Kaps and Schmid, 1996). This frequency increases after stimulus detection, which can either be mechanical (Kaps and Schmid, 1996) or visual (Fenk and Schmid, 2010; Fenk and Schmid, 2011; Neuhofer et al., 2009; Orlando and Schmid, 2011), in order to move the gaze of the principal eyes towards the stimulus. A flickering stimulus whose luminance is perceived as different from that of the background can be detected by the secondary eyes of *C. salei*. This increases the muscle activity of the principal eyes. The activity of a dorsal principal eye muscle can be recorded extracellularly through a single-channel telemetric transmitter device (Fenk and Schmid, 2010; Fenk and Schmid, 2011; Kaps and Schmid, 1996; Neuhofer et al., 2009; Orlando and Schmid, 2011). This approach enables investigation of the brightness discrimination ability of *C. salei*. Moreover, the performance of the spider can be compared between day- and night-adapted eyes, and with other species.

RESULTS

Secondary eyes perceive flickering stimuli

The assumption is that the secondary eyes of *C. salei* are the only ones that perceive flickering stimuli because they are involved in the detection of targets rather than their discrimination.

Therefore, a perceivable contrast displayed to spiders with covered secondary eyes should elicit no increase in the principal eye muscle activity. These control contrasts consisted of the brightest green flickering square (green 255) on the darkest green background (green 0) (see also supplementary material Table S1), and vice versa;

these contrasts are equal to 1 according to Michelson’s law (Michelson, 1927).

For three tested spiders with only the principal eyes uncovered, no statistically significant increase was recorded: 1.08 ± 0.64 Hz (results always given as means \pm s.e.m.) for the control with a brighter stimulus and -0.01 ± 0.37 Hz for the control with a darker stimulus; each control was shown nine times to each spider. This demonstrated that the principal eyes do not detect the flickering stimuli that we used, and consequently that the secondary eyes are required for this task, as suggested by our subsequent experiments with all eyes uncovered.

The minimal perceivable contrasts

During the search for the minimal contrasts seen by *C. salei*, the previous controls with maximal Michelson contrasts were displayed regularly to the spiders. For the studied spiders with day-adapted eyes, the means of the increase in muscle frequency were 5.16 ± 0.33 Hz ($N=91$) for the control with the lightest green flickering on the darkest background and 3.31 ± 0.24 Hz ($N=73$), for the control with the darkest green flickering on the lightest background. For the spiders with the night-adapted eyes, a mean increase of 5.79 ± 0.47 Hz ($N=27$) was measured for the control with the lightest green flickering square and a mean of 4.29 ± 0.61 Hz ($N=26$) for the control with the darkest green flickering square. These statistically significant increases confirm that the tested spiders could see contrasts and that the telemetric device recorded the principal eye muscle activity.

Next, the spiders were confronted with different combinations of green, with different shades for the backgrounds and different shades for the stimuli, thus eliciting different contrasts. The results are given in Table 1, for spiders with either day- or night-adapted eyes. We determined the minimal contrasts perceived by the spider by assessing the significance (Wilcoxon signed-rank test) of the increase in the principal eye dorsal muscle activity. Fig. 1 presents the minimal contrast perceived by *C. salei* as a function of the luminance of the background and the state of the eyes. All four curves show a characteristic shape with clearly discriminable slopes. Above a luminance of 16 cd m^{-2} (corresponding to green 127; see supplementary material Table S1) for the background, either with darker or brighter stimuli, the necessary minimal contrast that elicits a behavioral response is constant: between 0.2 and 0.3 for spiders with day-adapted eyes and between 0.1 and 0.2 for spiders with night-adapted eyes. Below 16 cd m^{-2} down to the minimal brightness level (close to 0 cd m^{-2}), another slope is observed. When the background becomes darker, the minimal contrast perceived by *C. salei* is higher. Thus, the highest value of a perceivable contrast is reached with brighter stimuli on the darkest background, with day-adapted eyes (contrast $C=0.87$).

For all the tested combinations, the minimal contrast perceived by the spider is lower when its eyes are night-adapted, meaning that the brightness discrimination ability is better with night-adapted eyes.

Interestingly, below 16 cd m^{-2} for the luminance of the background, the minimal perceived contrasts are lower with brighter stimuli than with darker stimuli for the same backgrounds. Thus, the brightness discrimination ability is better with brighter than with darker stimuli. This is not evident for backgrounds brighter than 16 cd m^{-2} .

The differences between darker and brighter stimuli or between day- or night-adapted eyes are statistically significant. However, these differences may be finer as Fig. 1 shows, because the minimal perceived contrasts may be lower. We determined the ‘minimal’ perceived contrast in a relative manner by using different background/stimuli combinations. Applying even more combinations would enable more precise determination of the minimal contrasts detected by *C. salei*.

Table 1. Increase in muscle frequency for different displayed contrasts, for spiders with either day- or night-adapted eyes

Background	Stimulus	Contrast	Increase in muscle frequency (mean \pm s.e.m., Hz)	
			Day-adapted eyes	Night-adapted eyes
0	30	0.67	0.35 \pm 0.57	0.33 \pm 0.79
		0.81	1.91 \pm 0.95	0.99 \pm 0.34*
		0.87	2.00 \pm 0.76*	2.59 \pm 0.69**
31	1	0.69	1.15 \pm 0.42*	2.40 \pm 0.88*
		0.62	0.44 \pm 0.30	1.48 \pm 0.48*
		0.35	0.82 \pm 0.58	0.17 \pm 0.70
31	51	0.47	1.06 \pm 0.32**	–
63	33	0.59	0.71 \pm 0.50*	–
		0.39	0.56 \pm 0.40	–
63	83	0.28	0.13 \pm 0.26	–
		0.39	1.08 \pm 0.45*	–
95	55	0.52	2.84 \pm 0.62**	2.36 \pm 0.52**
		0.37	0.32 \pm 0.45	1.76 \pm 0.58*
		0.25	0.72 \pm 0.40	0.11 \pm 0.54
95	115	0.21	0.26 \pm 0.34	0.35 \pm 0.57
		0.29	0.48 \pm 0.38	1.91 \pm 0.95*
		0.37	1.18 \pm 0.46*	2.00 \pm 0.76*
127	102	0.24	1.53 \pm 0.52*	3.30 \pm 0.75**
		0.19	1.24 \pm 0.85	1.34 \pm 0.43*
		0.14	0.65 \pm 0.60	–0.99 \pm 0.66
127	137	0.08	–0.63 \pm 0.54	–0.15 \pm 0.66
		0.12	0.66 \pm 0.61	0.99 \pm 0.57*
		0.16	0.75 \pm 0.34*	2.68 \pm 0.81**
191	151	0.26	–	–
		0.19	–0.13 \pm 0.37	–
191	221	0.22	0.82 \pm 0.37	–
		0.29	1.33 \pm 0.42*	–
215	245	0.20	1.87 \pm 0.79	1.02 \pm 0.75
		0.22	2.03 \pm 0.66*	1.76 \pm 0.74*
255	205	0.30	2.21 \pm 0.70**	2.90 \pm 0.90**
		0.22	0.73 \pm 0.61	2.72 \pm 0.69**
		0.15	1.49 \pm 0.69	1.38 \pm 0.41**
		0.08	–0.04 \pm 0.58	–

Michelson contrasts were calculated using luminance values listed in supplementary material Table S1. The significance of an increase is assessed with the Wilcoxon signed-rank test ($N \geq 11$); significant results are printed in bold; * $P < 0.05$, ** $P < 0.01$; –, no data. The minimal perceived contrast by *Cupiennius salei* depends on the state of the eyes, on the background, and whether the stimulus is brighter or darker. Not all the combinations of background and stimuli are presented here, i.e. only the relevant ones helping visualize the minimal contrasts perceived by the spider. Backgrounds and stimuli values correspond to the value of the green channel in the RGB color model (see also supplementary material Table S1).

Calculation of Weber fractions

We used the Weber fraction k to compare species (see Introduction). Because of the difference between the brightness discrimination ability with darker or brighter stimuli, and with day- or night-adapted secondary eyes, four different fractions should be distinguished. We calculated $k_{\text{day,d}}=0.87$ from the results with day-adapted eyes and darker stimuli, $k_{\text{day,b}}=0.63$ for day-adapted eyes and brighter stimuli, $k_{\text{night,d}}=0.66$ for night-adapted eyes and darker stimuli, and finally $k_{\text{night,b}}=0.42$ for night-adapted eyes and brighter stimuli. The dependence between the noticeable difference threshold (ΔL , cd m^{-2}) and the mean of the two luminances (L , cd m^{-2}) was checked graphically (data not shown): the trend curves fit well with our data (at minimum $R^2 > 0.94$). This validates Weber's law.

DISCUSSION

Brightness discrimination ability differs between day- and night-adapted eyes

Our data show that the state of the secondary eyes of *C. salei*, i.e. those perceiving contrast, affects the brightness discrimination ability. Night-adapted eyes enable better brightness discrimination ability than day-adapted eyes, for brighter or darker stimuli. The Weber fractions for the day-adapted eyes are about 1.4 times higher than for night-

adapted eyes. This also indicates that the night-adapted eyes improve the contrast sensitivity whatever the background luminance and regardless of whether the stimulus is darker or brighter.

During the day, the multi-vesicular bodies derived from the dismantled rhabdomeres probably contain proteins for phototransduction, particularly opsins (photoreceptor proteins). Thus, the conversion of light to electrical signals may be lowered during the day.

Our results, however, disagree with an earlier study in which the sensitivity of the PM eyes, measured using electroretinograms, remains the same both day and night (Barth et al., 1993). On the contrary, the sensitivity of the principal eyes, also showing a turnover in their photoreceptor cells, increased about 10-fold at night in *C. salei*. In the spider *D. subrufa*, in which a circadian rhabdomeric membrane cycle has also been described (Blest, 1978), sensitivity is constant for day- and night-adapted PM eyes (Laughlin et al., 1980). In that study, the authors suggested a possible experimental bias because they observed that the light they used interfered with the rebuilding of the rhabdomeric membrane. We cannot offer an explanation for the findings in *C. salei*. As the sensitivity depends on the pupil diameter D , which remains constant, and the acceptance angle $\Delta\rho$, which varies according to the size of

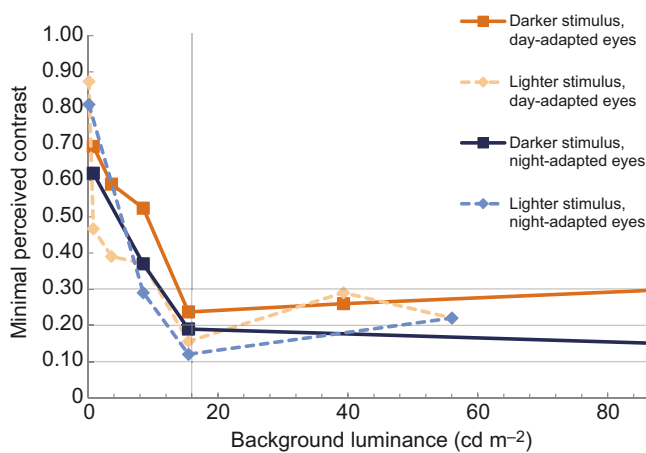


Fig. 1. Minimal contrast perceived by *Cupiennius salei* as a function of the luminance of the background and the state of the eyes. Beyond a certain luminance of the background (~16 cd m⁻²), the brightness discrimination ability is constant for the spider, with either darker or brighter stimuli, and day- or night-adapted eyes. Under this luminance value, the brightness discrimination ability declines: the darker the background, the higher the minimal perceived contrast. The brightness discrimination ability apparently improves with brighter stimuli. The brightness discrimination ability is better with night-adapted eyes throughout the tested luminance range.

the rhabdomeres, it is expected that eye sensitivity also increases at night when the rhabdomeres are larger (Land, 1981).

The brightness discrimination ability improves more for brighter (than darker) stimuli, with a 42% decrease in the minimum perceivable contrast from day- to night-adapted eyes versus a 19% decrease for darker stimuli. The calculated average diminution percentages stem from four values and are not significant. One potential explanation for this difference is that interneurons from more central parts of the nervous system may include a 'behavioral filter'. Accordingly, the neural circuits could be improved to see fine contrasts with brighter versus darker stimuli. From an ecological point a view, this speculation is less convincing because the prey of *C. salei* is extremely variable and can be either darker or brighter than the environment (Barth and Seyfarth, 1979). This difference could be confirmed in an electrophysiology study (Pirhofer-Walzl et al., 2007) using intracellular recordings of photoreceptor cells.

Brightness discrimination ability depends on background luminance

The brightness discrimination ability of *C. salei* depends on the brightness range of the background. Above a luminance of about 16 cd m⁻² for the background, the minimal contrast perceived by the spider is between 0.2 and 0.3 with day-adapted eyes, and between 0.1 and 0.2 for night-adapted eyes. Below this luminance value, the perceived minimal contrast increases with decreasing background luminance.

The relationship between the recorded behavioral response and the physiology of the photoreceptor cells is unknown. Nonetheless, there is evidently a physiological threshold around 16 cd m⁻², below which the perception of contrasts decreases rapidly. An eye needs a certain amount of photons to activate sufficient photoreceptor cells, which then permit the neural circuits of the brain to 'calculate' a brightness difference and thus perceive a contrast. This also applies for humans: at threshold vision, a human may see light but not enough to discriminate a dark circular patch on a bright field; intensities between 100 and 1000 times the threshold are required to reliably detect the circle (Land and Nilsson, 2002). Although single

photoreceptor cells can detect single photons, enough light is needed for the downstream neural circuits to reliably detect the circle.

At night, the natural environment of the nocturnal spider *C. salei* is clearly below 16 cd m⁻² (luminance during the night begins below approximately 1 cd m⁻²). In fact, under natural conditions, this spider uses other sensory modalities, above all its mechano-sensory ones (Barth, 2002). It can, however, use vision to a lesser extent, e.g. for 'obvious' visual cues, because brightness discrimination is not totally impaired. Fenk et al. showed that visual cues are sufficient to elicit attack behavior in this spider (Fenk et al., 2010). Moreover, Lindner has shown that *C. salei* exhibits attack behavior more often towards a moving dot with a contrast of 1 (58% of positive reactions to the test), whereas this value drops to 13% if the contrast is reduced to 0.7 (Lindner, 2013).

Brightness discrimination ability differs between brighter and darker stimuli

With both day- and night-adapted eyes, the brightness discrimination ability of *C. salei* is better with brighter versus darker stimuli compared with the background. The Weber fractions underline this: values obtained with darker stimuli are about 1.5 times higher than those with brighter stimuli.

This observation is initially surprising because a previous study (Fenk et al., 2010) indicated that the spider 'responded' better with a dark stimulus on a light background than vice versa. That study considered the attack behavior and both the tested stimuli had a maximal contrast. Our results, however, are not in contradiction with the above. We can speculate that the spider has a better brightness discrimination ability with brighter stimuli because it receives more photons and thus more photoreceptor cells are activated. This, in turn, increases the AM eye muscle activity. At the level of the attack behavior, however, darker stimuli may be more relevant for *C. salei*.

Comparison between *C. salei* and jumping spiders

In behavioral tests, Tiedemann investigated the brightness discrimination ability in the jumping spider *M. bivittatus* (Tiedemann, 1993). The method he applied was different, and only three backgrounds, for which the relative reflection is given, were tested. By using percentages, the results of *M. bivittatus* and *C. salei* can be compared on the same graph (Fig. 2). For *C. salei*, the data are given for the night-adapted secondary eyes. The data for *M. bivittatus* may also pertain to the secondary eyes, thought to be the contrast detectors in jumping spiders too (Zurek et al., 2010); however, the existence of a circadian cycle in the eyes of this spider has not been studied. The pattern of brightness discrimination ability in *C. salei* and that in *M. bivittatus* are similar in the sense that, below a certain luminance of the background, they need a higher contrast to perceive a stimulus, and above this value, the brightness discrimination ability is constant.

More recently, Zurek et al. studied the performances of another jumping spider, *S. vestita* (Zurek et al., 2010). Again, another method was used; in particular, only darker stimuli were displayed to the secondary eyes. For that jumping spider, the lowest perceived Weber contrast (defined as the difference between the stimulus and background luminance, divided by the background luminance) that was statistically significantly perceived was 0.01. For *C. salei*, that value with darker stimuli was 0.26.

On the one hand, the brightness discrimination ability of *C. salei* is slightly better than that of *M. bivittatus* (Fig. 2). On the other hand, the performance of *S. vestita* is better than that of *C. salei*. The difference between *M. bivittatus* and *C. salei* can be attributed to differences in spider lifestyle and morphology. Jumping spiders are diurnal and have huge principal eyes, enabling a high spatial

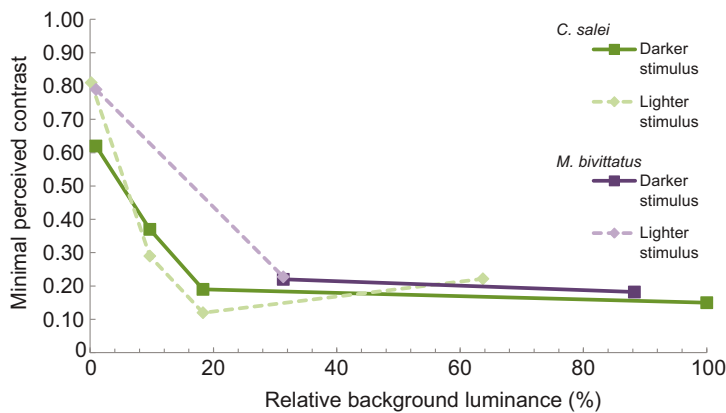


Fig. 2. Minimal contrast perceived by *C. salei* (with night-adapted eyes) and *Menemerus bivittatus*, with darker or brighter stimuli, as a function of the brightness level of the background. Above a certain luminance value, the brightness discrimination ability is constant for the two spiders, with either darker or brighter stimuli. Below this critical value, this ability declines. Indeed, the darker the background, the higher the minimal perceived contrast, with either darker or brighter stimuli. *Cupiennius salei* detects finer contrasts than *M. bivittatus*. The x-axis gives the relative luminance of the background, from 0% (darkest) to 100% (lightest). Data for *M. bivittatus* are recalculated from Tiedemann (Tiedemann, 1993).

resolution through large lenses. This is necessary given that they stalk and hunt prey down. Their secondary eyes, which are the contrast detectors (Zurek et al., 2010), are smaller. In contrast, nocturnal spiders have huge secondary eyes with great light-gathering power, thus increasing the brightness discrimination ability (Land and Nilsson, 2002). Consequently, they can detect and immediately catch moving prey in their dim habitat (without tracking). This resembles the behavior of *C. salei*, whose PM and PL eyes are larger than its AM eyes. Nevertheless, the better brightness discrimination ability of *S. vestita* remains extraordinarily impressive.

Comparison between *C. salei* and some vertebrates

Weber fractions of some vertebrates have been calculated (for review, see Scholtyssek et al., 2008). Briefly, the values range from 0.11 for humans (*Homo sapiens*) (Cornsweet and Pinsker, 1965; Griebel and Schmid, 1997) and rhesus macaques (*Macaca mulatta*) (Huang et al., 2002) to 0.42 or 0.45 for horses (*Equus caballus*) (Geisbauer et al., 2004), with birds such as budgerigars (*Melopsittacus undulatus*) (Lind et al., 2013) having intermediate values of 0.18. Fenk and Schmid suggested that a comparison with the toad would be interesting (Fenk and Schmid, 2011). This vertebrate has a lifestyle similar to that of *C. salei*: it is nocturnal and also has a ‘sit and wait’ hunting behavior. Moreover, toads’ eyes remain immobile and are thus thought to adapt to the stationary surroundings. In this manner, only moving targets are perceived. This is comparable to the secondary eyes of *C. salei*. Vertebrates have morphologically different eyes from spiders. The eyes of *C. salei* are smaller, which probably explains its poorer brightness discrimination ability. Consequently, fewer photons are available for the spider, lowering its brightness discrimination ability (Land and Nilsson, 2002). Nonetheless, Weber fractions of *C. salei* are not very high for a small arthropod: its $k_{\text{night,b}}$ value is the same as that of a horse! We can therefore claim that *C. salei*’s brightness discrimination ability is quite good, no doubt thanks to relatively big lenses, three different types of photoreceptors and therefore a wide spectral sensitivity range.

MATERIALS AND METHODS

Animals

The experiments were conducted with adult female *C. salei* from our in-house long-term breeding population. Their body sizes were about 3.3 cm, with total body length (including legs) up to 10 cm. The spiders were kept individually in glass jars under a cycle of 12 h light:12 h dark, at $\sim 23^{\circ}\text{C}$, and at a relative humidity of 70–80%, in order to recreate natural conditions. The spiders were fed once a week with flies (*Calliphora* sp.). For control experiments, the secondary eyes of some spiders were covered with matt acrylic paint (blue and black) and a piece of Parafilm band, permitting them to see solely using their principal eyes. The paint was removed after the experiment.

Telemetry

A single-channel telemetric transmitter device was used to record the activity of a principal eye dorsal muscle of *C. salei*. This device, adapted for the spider (Fenk and Schmid, 2010; Fenk and Schmid, 2011; Neuhofer et al., 2009; Orlando and Schmid, 2011) from one used previously with locusts (Kutsch et al., 1993), comprises an amplifier, an oscillator, a transmitter and a small battery. The recording electrode (a coated Manganin wire, diameter $30\ \mu\text{m}$) was inserted into the muscle; the reference electrode (a silver wire, diameter $250\ \mu\text{m}$) was inserted laterally into the prosoma. The mass of the device with a battery, carried on the prosoma of the spider, was about 750 mg. The signal emitted by the telemetric device was received by a wide-band receiver (Conrad Voyager RY-630, Conrad Electronics, Hirschau, Germany), digitized by an A/D converter (CED 1401, Cambridge, UK), and finally sent to a computer for further analysis.

Stimuli

The stimuli were created with Microsoft PowerPoint and were presented to the spider on an LCD screen (Samsung SyncMaster 226BW, Samsung Electronics, Daegu, South Korea). The colors were generated on a computer with three color channels (red, blue, green). The ‘color’ white is a mixture of these three primary colors. In our tests we used only the color green, of different brightness, for two reasons. First, by using only green (i.e. only one channel), the intensity depends minimally on the viewing direction. Second, the spectral composition of the green channel matches best with the spectral sensitivity of *C. salei* eyes (Barth et al., 1993), which is not the case for the blue (partial matching) or the red channel (no matching).

In this study, 51 shades of green with different luminances were used (see supplementary material Table S1). The luminances were measured with a luminance meter (Luminance Meter LS-100, Konica Minolta, Osaka, Japan). Using a spectroradiometer (International Light IL 1700, Research Radiometer-Photometer, Newburyport, UK), we confirmed that all shades of green used in our experiments were approximately the same wavelength (544 nm); only their relative intensities change, according to their luminance. The lightest green is green 255, the darkest one is green 0. Several combinations of green (the green of the stimulus and that of the background) were used. For each background, at least three different brighter or darker stimuli were used to determine the minimal perceived contrast. During training, each stimulus was presented five to six times to each spider. Each spider saw three to four ‘sets’ of stimuli. A set is defined as a background and all the tested darker or brighter stimuli. Table 1 presents results pinpointing the minimal contrasts from all the different sets used. The sets were displayed in random order for each animal. Within a set, the stimuli were also displayed randomly.

Examination of the temporal cut-off frequency in *C. salei* (Fenk and Schmid, 2011) showed that a flickering stimulus elicits a strong response. Consequently, flickering stimuli were chosen: a green square ($\sim 24 \times 15$ cm, luminance L_1) flickered (frequency 0.5 Hz) on a background ($\sim 48 \times 30$ cm, luminance L_2). If the spider perceived two different levels of brightness, a response should be recorded. The flickering stimulus lasted 11 s. It was preceded and followed by pauses (solely the background) lasting 30 s each.

Experimental setup

For ease of handling, each spider was first cooled down for 1 h at 4°C. Then it placed on a holder in the shape of a wooden hemisphere using Parafilm bands. The small hairs on the dorsal part of the prosoma and on a small lateral area were removed with round-ended tweezers in order to install the telemetric device, which was fixed on the spider with beeswax. Before the recording electrode was inserted into the dorsal muscle of a principal eye, some hairs between the eyes were also carefully removed. The spiders were always manipulated with care and in accordance with Austrian animal welfare laws, guidelines and policies.

During stimulus display, the spider was positioned in front of the LCD screen, at a distance of 34 cm. The body axis of the spider was orthogonal to the screen. Thus, the screen covered 70 deg of the visual field of the spider, i.e. it covered the visual field of the two PM eyes (Land and Barth, 1992). The experimental room was kept dark during the tests, except for the light from the LCD screen presenting the stimuli to be discriminated. The spider and the LCD screen displaying the stimuli were placed within a Faraday cage positioned on an anti-vibration table to minimize the risk of spiders responding to mechanical stimuli.

The tests began about 3 h after the onset of the dark or light cycle for the spider, i.e. at a time when the degradation, or, respectively, the rebuild of the membrane, is completed (Grusch et al., 1997). Moreover, this period corresponds to the spiders' peak locomotor activity during their subjective night (Seyfarth, 1980).

Data analysis

The AM eye dorsal muscle activity was recorded on a computer using the software Spike2 (CED). A screenshot is provided in supplementary material Fig. S1. Using the software Spike2, we calculated the mean muscle frequency for the 3 s before and the 3 s after the stimulus onset. Longer recording durations were not chosen because spiders can move their chelicerae and the generated noise can interfere with recordings. Recordings invalidated because of such artifacts or insufficient signal-to-noise ratio were excluded from the analysis. Statistical differences in the muscle activity frequency before and after stimulus presentation were tested using the Wilcoxon signed-rank test.

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Competing interests

The authors declare no competing financial interests.

Author contributions

E.C. and A.S. conceived and designed the experiments. E.C. performed the experiments and analysed the data. E.C. drafted the article. E.C. and A.S. revised the manuscript.

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Supplementary material

Supplementary material available online at <http://jeb.biologists.org/lookup/suppl/doi:10.1242/jeb.103366/-DC1>

References

- Albert, J. T., Friedrich, O. C., Dechant, H. E. and Barth, F. G. (2001). Arthropod touch reception: spider hair sensilla as rapid touch detectors. *J. Comp. Physiol. A* **187**, 303-312.
- Barlow, R. B., Jr, Chamberlain, S. C. and Levinson, J. Z. (1980). Limulus brain modulates the structure and function of the lateral eyes. *Science* **210**, 1037-1039.
- Barth, F. G. (2002). *A Spider's World: Senses and Behavior*. Berlin: Springer.
- Barth, F. G. and Seyfarth, E.-A. (1979). *Cupiennius salei* Keys (Araneae) in the highlands of central Guatemala. *J. Arachnology* **7**, 255-263.
- Barth, F. G., Nakagawa, T. and Eguchi, E. (1993). Vision in the ctenid spider *Cupiennius salei*: spectral range and absolute sensitivity. *J. Exp. Biol.* **181**, 63-80.
- Blest, A. D. (1978). The rapid synthesis and destruction of photoreceptor membrane by a dipterid spider: a daily cycle. *Proc. R. Soc. B* **200**, 463-483.
- Blest, A. D. and Maples, J. (1979). Exocytotic shedding and glial uptake of photoreceptor membrane by a salticid spider. *Proc. R. Soc. B* **204**, 105-112.
- Blest, A. D. and White, W. A. (1978). The rhabdomere organization of some nocturnal pisaurid spiders in light and darkness. *Philos. Trans. R. Soc. London B* **283**, 1-23.
- Chamberlain, S. C. and Barlow, R. B., Jr (1984). Transient membrane shedding in *Limulus* photoreceptors: control mechanisms under natural lighting. *J. Neurosci.* **4**, 2792-2810.
- Cornsweet, T. N. and Pinsker, H. M. (1965). Luminance discrimination of brief flashes under various conditions of adaptation. *J. Physiol.* **176**, 294-310.
- Eakin, R. M. and Brandenburger, J. L. (1971). Fine structure of the eyes of jumping spiders. *J. Ultrastruct. Res.* **37**, 618-663.
- Fenk, L. M. and Schmid, A. (2010). The orientation-dependent visual spatial cut-off frequency in a spider. *J. Exp. Biol.* **213**, 3111-3117.
- Fenk, L. M. and Schmid, A. (2011). Flicker-induced eye movements and the behavioural temporal cut-off frequency in a nocturnal spider. *J. Exp. Biol.* **214**, 3658-3663.
- Fenk, L. M., Hoinkes, T. and Schmid, A. (2010). Vision as a third sensory modality to elicit attack behavior in a nocturnal spider. *J. Comp. Physiol. A* **196**, 957-961.
- French, A. S., Torkkeli, P. H. and Seyfarth, E.-A. (2002). From stress and strain to spikes: mechanotransduction in spider slit sensilla. *J. Comp. Physiol. A* **188**, 739-752.
- Geisbauer, G., Griebel, U., Schmid, A. and Timney, B. (2004). Brightness discrimination and neutral point testing in the horse. *Can. J. Zool.* **82**, 660-670.
- Griebel, U. and Schmid, A. (1997). Brightness discrimination ability in the West Indian manatee (*Trichechus manatus*). *J. Exp. Biol.* **200**, 1587-1592.
- Grusch, M., Barth, F. G. and Eguchi, E. (1997). Fine structural correlates of sensitivity in the eyes of the ctenid spider, *Cupiennius salei* Keys. *Tissue Cell* **29**, 421-430.
- Hergenröder, R. and Barth, F. G. (1983). The release of attack and escape behavior by vibratory stimuli in a wandering spider (*Cupiennius salei* Keys). *J. Comp. Physiol.* **A 152**, 347-359.
- Huang, X., MacEvoy, S. P. and Paradiso, M. A. (2002). Perception of brightness and brightness illusions in the macaque monkey. *J. Neurosci.* **22**, 9618-9625.
- Kaps, F. and Schmid, A. (1996). Mechanism and possible behavioural relevance of retinal movements in the ctenid spider *Cupiennius salei*. *J. Exp. Biol.* **199**, 2451-2458.
- Kutsch, W., Schwarz, G., Fischer, H. and Kautz, H. (1993). Wireless transmission of muscle potentials during free flight of a locust. *J. Exp. Biol.* **185**, 367-373.
- Land, M. F. (1981). Optics and vision in invertebrates. In *Handbook of Sensory Physiology*, Vol. VII/6B (ed. H. Autrum), pp. 471-592. Berlin: Springer.
- Land, M. F. and Barth, F. G. (1992). The quality of vision in the ctenid spider *Cupiennius salei*. *J. Exp. Biol.* **164**, 227-242.
- Land, M. F. and Nilsson, D.-E. (2002). *Animal Eyes*. New York, NY: Oxford University Press Inc.
- Laughlin, S., Blest, A. D. and Stowe, S. (1980). The sensitivity of receptors in the posterior median eye of the nocturnal spider, *Dinopis*. *J. Comp. Physiol. A* **141**, 53-65.
- Lind, O., Karlsson, S. and Kelber, A. (2013). Brightness discrimination in budgerigars (*Melopsittacus undulatus*). *PLoS ONE* **8**, e54650.
- Lindner, P. (2013). Visually elicited prey capture behavior in *Cupiennius salei*. Master's thesis, University of Vienna, Vienna, Austria.
- Michelson, A. A. (1927). *Studies in Optics*. Chicago, IL: University of Chicago Press.
- Nässel, D. R. and Waterman, T. H. (1979). Massive diurnally modulated photoreceptor membrane turnover in crab light and dark adaptation. *J. Comp. Physiol. A* **131**, 205-216.
- Neuhof, D., Machan, R. and Schmid, A. (2009). Visual perception of motion in a hunting spider. *J. Exp. Biol.* **212**, 2819-2823.
- Orlando, E. and Schmid, A. (2011). Colour blindness of the movement-detecting system of the spider *Cupiennius salei*. *J. Exp. Biol.* **214**, 546-550.
- Pirhofer-Walzl, K., Warrant, E. and Barth, F. G. (2007). Adaptations for vision in dim light: impulse responses and bumps in nocturnal spider photoreceptor cells (*Cupiennius salei* Keys). *J. Comp. Physiol. A* **193**, 1081-1087.
- Powers, M. K. and Barlow, R. B., Jr (1985). Behavioral correlates of circadian rhythms in the *Limulus* visual system. *Biol. Bull.* **169**, 578-591.
- Powers, M. K., Barlow, R. B., Jr and Kass, L. (1991). Visual performance of horseshoe crabs day and night. *Vis. Neurosci.* **7**, 179-189.
- Schmid, A. (1998). Different functions of different eye types in the spider *Cupiennius salei*. *J. Exp. Biol.* **201**, 221-225.
- Scholtyssek, C., Kelber, A. and Dehnhardt, G. (2008). Brightness discrimination in the harbor seal (*Phoca vitulina*). *Vision Res.* **48**, 96-103.
- Seyfarth, E.-A. (1980). Daily patterns of locomotor activity in a wandering spider. *Physiol. Entomol.* **5**, 199-206.
- Stowe, S., De Couet, H.-G. and Davis, D. (1990). Photoreceptor membrane turnover in the crayfish *Cherax destructor*: electron microscopy and anti-rhodopsin electron-microscopic immunocytochemistry. *Cell Tissue Res.* **262**, 483-499.
- Tiedemann, K. (1993). Visual brightness discrimination of the jumping spider *Menemerus bivittatus* (Araneae, Salticidae). *J. Arachnology* **21**, 1-5.
- Walla, P., Barth, F. G. and Eguchi, E. (1996). Spectral sensitivity of single photoreceptor cells in the eyes of the ctenid spider *Cupiennius salei* Keys. *Zoolog. Sci.* **13**, 199-202.
- White, R. H. and Lord, E. (1975). Diminution and enlargement of the mosquito rhabdom in light and darkness. *J. Gen. Physiol.* **65**, 583-598.
- Zopf, L. M., Schmid, A., Fredman, D. and Eriksson, B. J. (2013). Spectral sensitivity of the ctenid spider *Cupiennius salei*. *J. Exp. Biol.* **216**, 4103-4108.
- Zurek D. B., Taylor A. J., Evans C. S. and Nelson X. J. (2010). The role of the anterior lateral eyes in the vision-based behaviour of jumping spiders. *J. Exp. Biol.* **213**, 2372-2378.

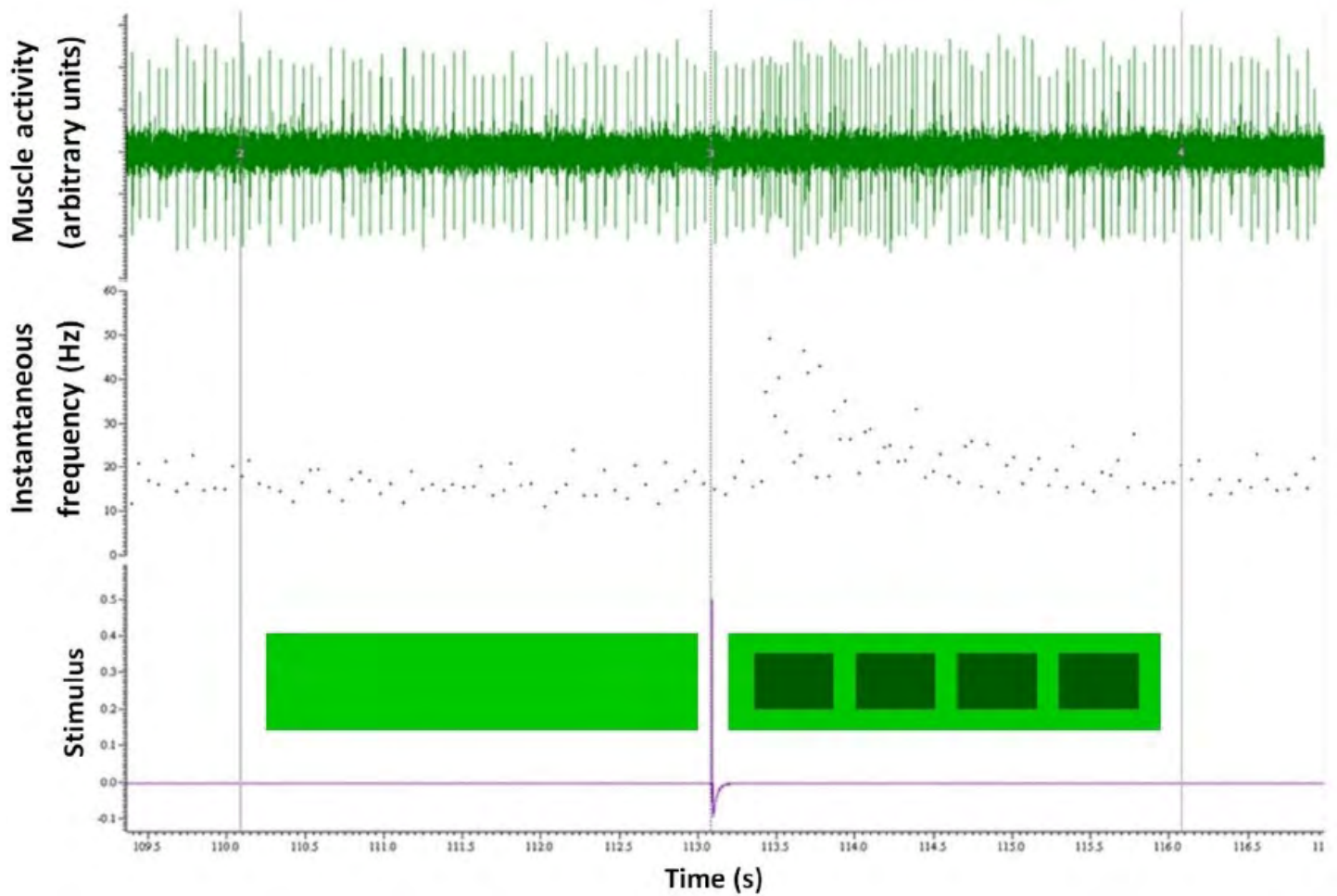





















































Fig. S1. Screenshot of a recording. The vertical cursor in the middle indicates stimulus onset, also indicated by the spike in the bottom trace. In this example, the stimulus displayed to a *Cupiennius salei* with night-adapted eyes consists in a dark flickering square (green 11) on a brighter background (green 31) (graphics above the bottom trace are presented in heightened contrast to simulate the stimulus for the reader). The AM eye muscle activity is presented by spikes (upper trace). From it, the instantaneous frequency can be calculated (black dots, middle trace), and, subsequently, the mean frequency during the 3 s before the stimulus (between the two first cursors) and after the stimulus (between the two last cursors). Here, the frequency increases, which indicates that the spider perceives the contrast through its secondary eyes and reacts with its principal eyes.

Table S1. 51 different shades of green were created using Microsoft PowerPoint

Green value	Color	Luminance (cd/m ²)	Green value	Color	Luminance (cd/m ²)	Green value	Color	Luminance (cd/m ²)
0		0.146	65		3.900	137		18.93
1		0.147	71		4.399	142		20.42
10		0.186	75		5.080	147		22.02
11		0.191	83		6.415	151		23.27
20		0.352	93		8.165	152		23.63
21		0.391	95		8.504	161		26.78
23		0.455	102		9.921	171		30.54
30		0.751	103		10.14	191		39.33
31		0.814	107		11.01	205		47.59
33		0.918	112		12.17	211		52.39
40		1.356	115		12.94	215		56.02
43		1.579	117		13.31	221		61.67
50		2.161	122		14.54	225		65.33
51		2.238	125		15.47	231		71.16
55		2.660	127		16.09	235		74.92
61		3.283	132		17.55	245		83.45
63		3.611	135		18.54	255		87.95

Their luminance, coming from the LCD-screen seen by the spiders, was measured. The “green value” corresponds to the value of the green component in the RGB (red, blue, green) color model; this model, using the three additive primary colors, codes all the colors in a computer. (The values for the blue and the red components are here equal to 0.)