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Visual perception of motion in a hunting spider

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SUMMARY

Like most other spiders *Cupiennius salei* (Keyserling 1877) has two different eye types, one pair of principal eyes and three pairs of secondary eyes. The principal eyes have two eye muscles each, which allow movement of the retina and are mainly used for the discrimination of stationary objects. The secondary eyes without such eye muscles are supposed to detect moving objects. Masking experiments were used to analyse the role of these two eye types in motion detection. In a white arena the animals were stimulated with short sequences of moving black bars. The principal eyes move involuntarily when objects are moving within the visual field of an ipsilateral secondary eye. The eye muscle activity of the principal eyes was recorded using single channel telemetry, and activity changes were taken as an indicator for the perception of motion. Masking the principal eyes with black paint and presenting a moving visual stimulus did not modify the induced muscle activity, whereas masking the secondary eyes eliminated the increase in eye muscle activity. This suggests that the secondary eyes are responsible for movement detection. We conclude that the animals are able to detect moving targets visually only with the secondary eyes. The principal eyes, by contrast, do not seem to be involved in the detection of moving targets.

Key words: *Cupiennius salei,* motion vision, eye movement, telemetry, electromyogram.

INTRODUCTION

In most arthropods the investigation of vision is a difficult task because it is often hard to find behaviours which can be used to clearly demonstrate distinct aspects of visual perception. *Cupiennius salei* (Keyserling 1877), a Central American hunting spider, lives on bromelias and banana plants. These plants are important as substrata for hunting and courtship behaviour (Barth, 1993). The animals find these plants mainly by vision, and this behaviour was used to investigate the visual perception of stationary objects (Schmid, 1998).

Cupiennius salei has, like most other spiders, four pairs of eyes. Morphologically they are of two types, one pair of principal eyes (AM or anterior median eyes) and three pairs of secondary eyes (AL or anterior lateral, PM or posterior median, PL or posterior lateral eyes). The principal eyes have photoreceptor cells with rhabdomeres oriented towards the light, whereas in the secondary eyes the rhabdomeres point towards a reflecting tapetum (Blest, 1985). Among many other differences the eyes also differ in their phylogeny. The principal eyes developed from an ancestral pair of lens eyes, whereas the secondary eyes derived from decomposed compound eyes (Paulus, 1979). The overlap of the visual fields of the anterior median (AM) and the posterior median (PM) eyes is thought to indicate a difference in function between these eyes (Land 1985; Land and Nilsson, 2002). Previous behavioural experiments suggest that the principal eyes are responsible for the processing of shapes (Schmid, 1998). The secondary eyes are instead believed to detect moving objects.

The loss of movement perception after covering the secondary eyes was demonstrated in salticids in behavioural experiments nearly 50 years ago (Dzimirski, 1959). Salticids are visually guided hunters and visual perception can be investigated easily using clear behavioural responses. According to Forster (Forster, 1985) and Rovner (Rovner, 1993) orientation and approach were the first behavioural responses of jumping spiders and wolf spiders towards moving targets when either prey or potential mates were presented. But all of the species investigated in these studies are active predators.

Cupiennius salei follows an alternative hunting strategy. Our model species is a sit and wait predator, and vision does not play a major role in courtship behaviour either (Barth, 1993).

The principal eyes of *Cupiennius salei* possess two muscles each, which are used to move the retinae (Kaps, 1998). There are two types of such movements. (1) Spontaneous microsaccades, which occur as a result of the contraction of the dorsal eye muscle at a frequency of about 12 Hz. This prevents an adaptation of the photoreceptors by slightly shifting (1–2 deg.) the visual field. (2) Induced eye movements or saccades are caused by the contraction of both the dorsal and the ventral eye muscles; they deflect the visual field laterally (up to 15–20 deg.). The induced muscle activity increases in response to mechanical stimulation before locomotion (Kaps, 1994; Trischler, 2003), or in response to visual stimulation (Kaps and Schmid, 1996). The visually stimulated change of the eye muscle activity can therefore be used as an alternative and reliable indicator of visual perception.

Masking experiments were used to investigate the role of principal and secondary eyes of *Cupiennius salei* in motion detection. Moving visual stimuli were presented to the animals, with either the principal or the secondary eyes covered with black paint, and the changes in eye muscle activity were measured.

MATERIALS AND METHODS

The spider

The experiments were carried out with 16 adult females of *Cupiennius salei* Keys (Ctenidae) from our own breeding stock in Vienna. The temperature, light and humidity regime resembled natural conditions (Barth et al., 1988). The animals were kept

individually in glass jars, and fed with flies (*Calliphora* sp.) once a week. In these experiments the animals had to be correctly positioned in the arena and should not move while the visual targets were presented. Therefore only females were used, because they naturally move around much less than males (Schmitt et al., 1990). This is justifiable as there is no sex specific difference in the visual system.

Electrophysiology

After immobilization by chilling at 7°C for approximately 30 min the animals were tethered with Parafilm onto a wooden holder, dorsal side up. To localize the eye muscles, an electrolytically tapered tungsten electrode was inserted into the frontal part of the prosoma. Extracellulary recorded action potentials were amplified using standard electrophysiological equipment (Kaps and Schmid, 1996). If the signal-to-noise ratio was at least 4:1 (referred to spontaneous activity) or better, both the tungsten electrode and the reference electrode were removed, and the telemetry-transmitter device (see below) was mounted dorsally onto the prosoma with a mixture of beeswax and rosin. A silver wire, inserted laterally into the prosoma, functioned as the reference electrode. The recording electrode, a manganine wire, was implanted through the hole in the cuticle previously pierced with the tungsten electrode. Both electrodes were then fixed with beeswax (Fig. 1A).

Telemetry

A single channel telemetry device was used in all experiments to monitor the eye muscle activity. It consisted of amplifier, modulator, oscillator and battery as described by Kutsch et al. (Kutsch et al., 1993), and had one additional amplifier stage to obtain a final amplification of about 400 fold.

Flexible insulated manganine wire (30 µm in diameter; 628.3 Ωm^{-1}; Isabellenhütte, Dillenburg, Germany) served as the recording electrode, which allowed a local recording of the eye muscle activity. In free moving animals this is necessary in order to reduce the interference from other nearby muscles such as the cheliceral muscles.

The modulated signal generated and sent by the transmitter device was received by a conventional world receiver (Conrad Voyager RY-630, Conrad Electronics, Hirschau, Germany) and fed into an A/D converter (CED 1401, Cambridge, UK) connected to a PC for further data analysis.

Visual stimulation

All experiments were carried out in a white cardboard arena (56 cm×70 cm base and 60 cm high). The stimulus sequences were generated on a computer and projected at 30 frames per second with a video beamer (1024×768 pixels; 2000 ANSI-Lumen). The target image was a vertically oriented rectangle (15×45 cm) moving over the back wall of the arena from right to left within 5 s (0.112 $m s^{-1}$). The presentation of the moving rectangle was repeated up to 16 times with a 30 s break between each trial. Nevertheless not every reaction to stimulation could be analysed because of disturbances such as movements of the animal or increasing electrical noise.

Experiment 1: masking of the principal eyes

The principal eyes were masked with black water colour paint and the video sequence was presented to the animals at a distance of 50 cm. The target moved from 30 deg. right to 30 deg. left of the animal's midline, and thus fell within the visual fields of both AM and PM eyes (Fig. 1B). Six animals were used for this experiment.

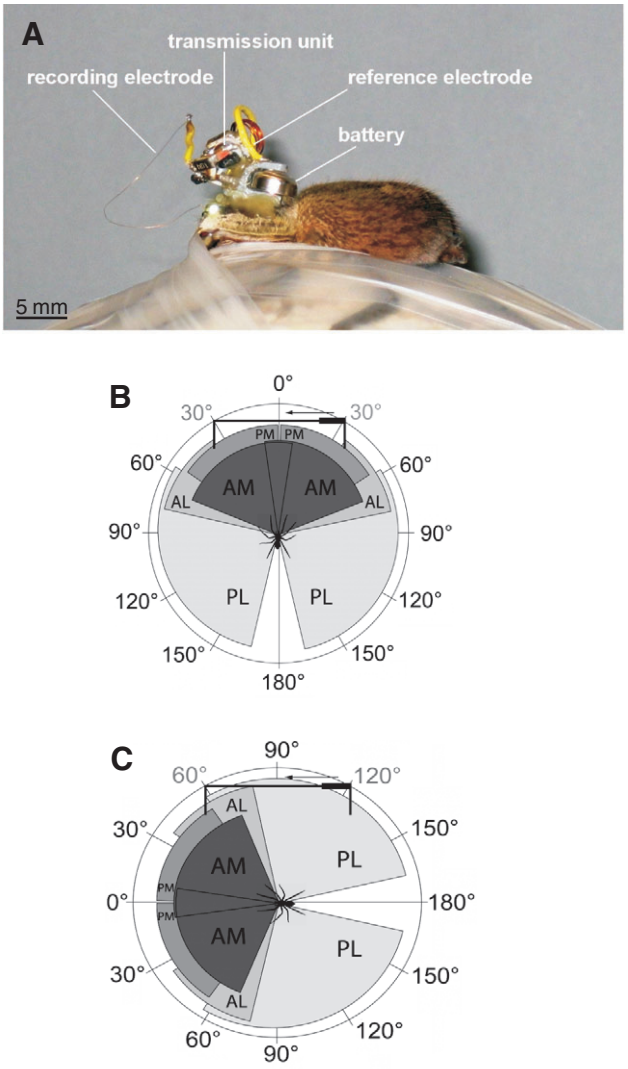

Fig. 1. (A) Female *Cupiennius salei* prepared for experimentation. The transmission unit including the battery was mounted with beeswax on the dorsal side of the prosoma. A manganine wire was used as the recording electrode; the reference electrode, consisting of silver wire, was inserted laterally into the prosoma. (B) Top view of the arena during an anterior median (AM) or posterior median (PM) masking experiment. The spider was positioned 50 cm in front of the projection area (back wall). Visual fields of the eyes (AM; PM; AL, anterior lateral; PL, posterior lateral) are plotted according to Land and Barth (Land and Barth, 1992). A black bar (15 cm×45 cm) moving from 30 deg. right to 30 deg. left (arrow), stimulated only AM and PM eyes. (C) Top view of the arena for testing the AL and PL eyes. The spider was oriented with the body long axis parallel to the projection area. The distance between the prosoma and the projection area was 50 cm. In this case, the black bar was moved within 5 s from 120 deg. right to 60 deg. right (arrow). Only AL and PL eyes were stimulated in this preparation.

Experiment 2: masking of the secondary eyes

In this experiment the secondary eyes were masked with water colour paint. The experimental procedures were the same as in the previous experiment. Again six animals were used for this experiment.

Experiment 3: testing AL and PL eyes

To test the relevance of AL and PL eyes for motion detection, the spider was aligned with its body axis parallel to the back wall. Facing the left side of the arena, the right AL and PL eyes then could see

the stimulus (Fig. 1C). The experimental procedure was the same as in experiment 1. The target moved from 60 deg. to 120 deg. and stimulated both the AL and PL eyes. Four animals were used for this experiment.

Recovery control experiments

With water and soft tissue the masking was thoroughly removed from the eyes, and after a break of 2 h, to calm down the animals, the experiment was repeated.

Data processing

The muscle potentials were recorded in Spike 2 (CED). In animals free to move, as was the case in these experiments, the amount of spontaneous eye muscle activity varied considerably. Therefore the increase of muscle activity due to visual stimulation was calculated by subtracting the mean spontaneous frequency measured in a time window of 5 s preceding stimulation from the mean stimulus activity within the 5 s time window during visual stimulation. Differences between the spontaneous and stimulus-induced eye muscle activity and differences between masking experiments and recovery control experiments were tested with the Wilcoxon matched-pairs signed rank test (Sachs, 1988).

Differences in the eye muscle activity for stimulation of different eye types were tested with the Kruskal–Wallis test.

RESULTS

Different animals showed different degrees of spontaneous eye muscle activity. Consistently, visual stimulation led to retinal movements as long as the secondary eyes were not covered. Fig. 2 shows traces of electromyograms of four experiments. With all secondary eyes covered, the animals did not react to the moving targets, and there was no change in spontaneous muscle activity correlated to visual stimulation (Fig. 2A). If subsequently the paint was removed from the secondary eyes (recovery control) the animal again detected the moving target, as seen from the increase in the eye muscle activity during visual stimulation (Fig. 2B). Masking the principal eyes had no influence on the ability of the secondary eyes to detect movement (Fig. 2C) and therefore elicited an increased eye muscle activity in the principal eyes in the same way as during the control experiment with uncovered AM eyes (Fig. 2D).

The mean muscle activity during experiments (N=6 animals) with covered secondary eyes was compared with the mean muscle activity of the animals after recovery (recovery control experiment). The stimulus-induced eye muscle activity is shown as the increase in activity relative to the prestimulus activity. Masking the secondary eyes resulted in no response of the AM muscle to visual stimulation (Fig. 3). The mean activity increase was almost zero (0.2 Hz) and there was no significant difference between spontaneous activity and stimulus-induced activity (P=0.345; Wilcoxon test). In the control experiments, with all eyes recovered, the eye muscle activity showed a mean increase of 5.1 Hz ,which was a significant increase compared with spontaneous activity (P=0.028; Wilcoxon test). Furthermore the Wilcoxon test showed a significant difference in eye muscle activity between the masking experiment and recovery control experiment (P=0.028).

In order to understand the role played by the AM eyes in movement detection and to demonstrate a possible neuronal connection between these eyes and all of the secondary eyes, the AM eyes were covered with black paint. The mean increase of eye muscle activity due to visual stimulation was 3.3 Hz for the masking experiment and 2.6 Hz for the recovery control experiment (Fig. 3);

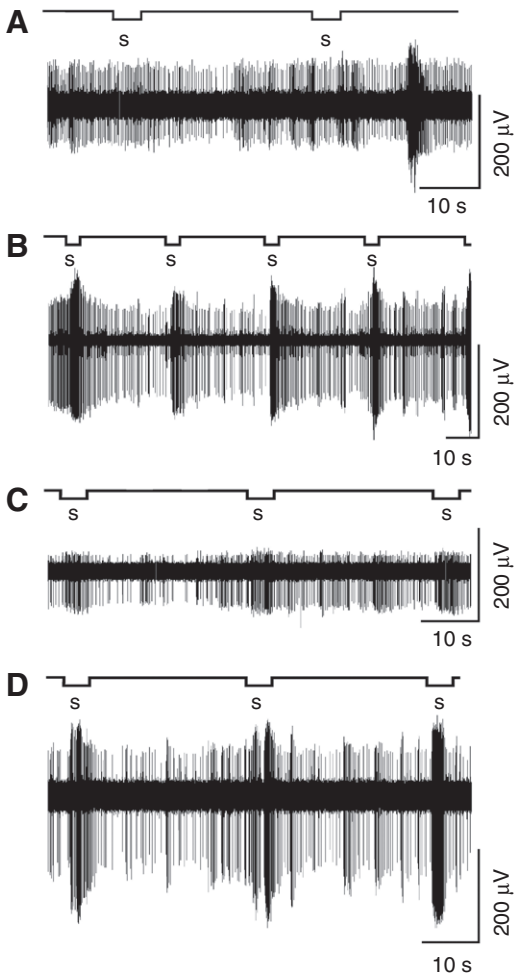

Fig. 2. Electromyograms from two different spiders. The upper trace indicates the presence of the stimulus S, the lower trace shows the activity of the dorsal eye muscle. During PM masking (A) there was no stimulus-related activity compared with the control experiment (B). In the AM masking experiment (C) there was increased eye muscle activity due to visual stimulation which did not differ from the AM control experiment (D). The asterisk in A indicates an artefact caused by movement of the animal.

there was no significant difference between these firing rate distributions (P=0.753, Wilcoxon test). For both experiments the stimulus-induced eye muscle activity was significantly different from spontaneous activity (masking experiment; P=0.028; recovery control experiment: P=0.046; Wilcoxon test).

Owing to the limited extension of the visual fields (Fig. 1B) it was not possible to investigate the potential influence of the AL and PL eyes for movement detection. However, to quantify the influence of these eye types in movement detection, we aligned the animals in such a way that the moving target fell into the visual fields of the AL and PL eyes (see Fig. 1C). The results of this experiment were compared with the recovery control experiments of the AM masking and secondary eye masking experiments (Fig. 4). The mean reaction of the AM eye muscles to the stimulation of AL and PL eyes was in the order of 7.1 Hz and therefore slightly higher than the activity in response to stimulation after recovery of AM (2.6 Hz) or PM (5.1 Hz) eyes. Nevertheless, the Kruskal–Wallis test just failed to indicate significant differences (P=0.058).

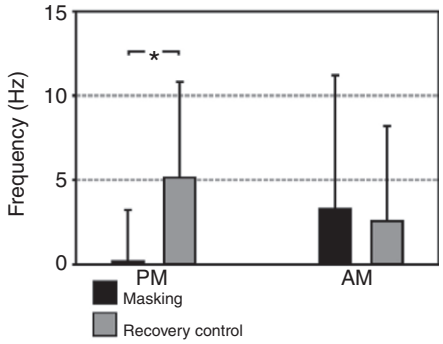

Fig. 3. Responses to moving targets found in the AM and PM masking experiments. The first bar shows the mean eye muscle activity with the PM eyes masked [number of animals (*N*)=6; number of trials (*n*)=57], the second one indicates the activity with unmasked eyes (*n*=54). Vertical lines represent standard deviation. The asterisk indicates a significant difference between the results of the PM masking experiment and the corresponding recovery control experiment (Wilcoxon matched pair test *P*=0.028). The third and fourth bars show the activity with the AM eyes masked (*N*=6, *n*=57), unmasked (*n*=58), respectively. Wilcoxon matched pair test showed no significant difference between the AM masking and the corresponding control groups.

DISCUSSION

Motion detection

Owing to the lack of easily observable locomotory movements in response to moving stimuli in *Cupiennius salei*, it is not possible to investigate the different functions of the eyes by means of conventional behavioural experiments. The present study therefore used eye muscle activity as an indicator of the visual perception of motion. When presenting a moving visual stimulus, the eye muscle activity increased whereas the spider itself did not move. After covering the secondary eyes the animals showed no response, i.e. increased eye muscle activity, to the presentation of moving targets. This result clearly indicates the relevance of the secondary eyes in movement detection.

Schmid (Schmid, 1998) investigated the functional role of the AM and the PM eyes with respect to target discrimination and target detection. It could be shown that the principal eyes serve as a target discriminating system. Our experiments showed that covering the principal eyes had no influence on movement detection, whereas covering the secondary eyes eliminated stimulus induced eye muscle activity.

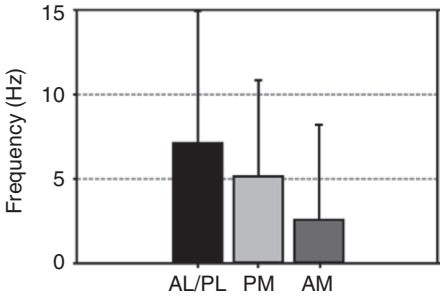

Fig. 4. The mean reaction of the different eye types to moving targets. The first bar shows the reaction of the eye muscles to a target moving within the visual field of the AL and PL eyes (*N*=4, *n*=36), the second one the reaction to targets in the visual field of AM and PM after recovery of the PM eyes (*N*=6, *n*=58), and the third one indicates the reaction to motion in the visual field of the AM and PM after recovery of the AM eyes (*N*=6, *n*=54). The Kruskal–Wallis test showed no significant difference.

The muscle activity of the principal eyes increases in response to moving visual stimuli presented in the visual field of the secondary eyes only, inducing a lateral shift of the visual field. A reason for the stronger influence of the AL and PL eyes might be that their visual fields are more posterior compared with those of the PM eyes. In this case the shift of the visual field of a principal eye should be more pronounced in order to detect lateral objects.

Our results show that there is a connection between the secondary and the principal eyes in the visual system. The presented data support the idea that the principal and the secondary eyes represent two different visual systems which are responsible for the perception and computation of different visual qualities.

The principal eyes are responsible for object discrimination (Schmid, 1998) and the secondary eyes are responsible for motion detection. This is in accordance with results of neuroanatomical investigations of the two different visual pathways of *Cupiennius salei* (Strausfeld and Barth, 1993; Strausfeld et al., 1993).

Object size and locomotion

The orientation towards moving objects does not seem to play an important role in the behavioural repertoire of *Cupiennius salei* and therefore could not be detected in this study. The targets used in the present study might have been too large for visual induced behaviour. The stimulus used (150×450 mm) exceeds the size of any potential mate or prey. The presented targets may have indicated a predator, therefore staying immobile in these situations may be an adaptive strategy.

Pros and cons of telemetry

Using telemetry a serious constraint is battery life time, determined by size and tolerable weight. In our present study it was limited to between 5 and 6 h.

According to a comparison of data received from animals that were tethered to different extents, the fixation of the animals greatly influences the results (Kutsch and Stevenson, 1981). In *Cupiennius salei*, it seems that the degree of tethering of the animal strongly influences eye muscle activity. For our early investigations of eye muscle activity in *Cupiennius salei*, the spiders were firmly fixed onto an animal holder and extracellular recordings were made with standard electrophysiological equipment. Under these conditions a continuous spontaneous activity was recorded in all experiments (Kaps and Schmid, 1996).

The next step towards less restricted recording conditions was to fix the animal's prosoma to a flexible holder and to have the animal walk on an air-suspended styrofoam sphere. Long and flexible manganine wires were used for recording. Under these conditions, the occurrence of spontaneous activity alternated with phases of complete quiescence of the eye muscles (Kaps, 1998). The application of telemetry combined with video analysis of the locomotor behaviour on the air-suspended sphere showed for the first time that there is a slight increase of eye muscle activity before the onset of locomotion (Trischler, 2003).

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REFERENCES

Barth, F. G. (1993). Sensory guidance in spider precopulatory behaviour. *Comp. Biochem. Physiol.* **104**, 717-733.

Barth, F. G., Seyfarth, E. A., Bleckmann, H. and Schüch, W. (1988). Spiders of the genus Cupiennius Simon 1891 (Araneae, Ctenidae). I. Range distribution, dwelling plants, and climatic characteristics of the habitats. *Oecologia* **77**, 187-193.
Blest, A. D. (1985). The fine structure of spider photoreceptors in relation to function. In *Neurobiology of Arachnids* (ed. F. G. Barth), pp. 79-102. New York: Springer.
Dzimirski, I. (1959). Untersuchungen über Bewegungssehen und Optomotorik bei Springspinnen (*Salticidae*). *Z. Tierpsychol.* **16**, 385-402.
Forster, L. (1985). Target discrimination in jumping spiders (*Araneae: Salticidae*) In *Neurobiology of Arachnids* (ed. F. G. Barth), pp. 249-274. New York: Springer.
Kaps, F. (1994). Retinabewegungen bei *Cupiennius salei* (Araneae, Ctenidae). Diplomarbeit, Universität Wien.
Kaps, F. (1998). Anatomische und physiologische Untersuchungen zur Funktion der Retinabewegungen bei *Cupiennius salei* (*Araneae, Ctenidae*). Dissertation, Universität Wien.
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.
Keyserling, E. (1877). Über amerikanische Spinnenarten der Unterordnung *Citigradae*. *Verh. Zool. Bot. Ges. Wien* **26**, 609-708.
Kutsch, W. and Stevenson, P. (1981). Time-correlated flights of juvenile and mature locusts: a comparison between free and tethered animals. *J. Insect. Physiol.* **27**, 455-459.
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. (1985). The morphology and optics of spider eyes. In *Neurobiology of Arachnids* (ed. F. G. Barth), pp. 53-78. New York: 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*. Oxford: Oxford University Press.
Paulus, H. F. (1979). Eye structure and the monophyly of the Arthropoda. In *Arthropod Phylogeny* (ed. A. P. Gupta), pp. 299-383. New York: Van Nostrand Reinhold.
Rovner, J. S. (1993). Visually mediated responses in the lycosid spider *Rabidosa rabida*: the roles of different pairs of eyes. *Mem. Queensl. Mus.* **33**, 635-638.
Sachs, L. (1988). Allgemeines und Vergleichendes zum statistischen Test. In *Statistische Methoden: Planung und Auswertung*, pp. 171-192. Berlin: Springer.
Schmid, A. (1998). Different functions of different eye types in the spider *Cupiennius salei*. *J. Exp. Biol.* **201**, 221-225.
Schmitt, A., Schuster, M. and Barth, F. G. (1990). Daily locomotor activity patterns in three species of *Cupiennius* (Aranea: Ctenidae): the males are the wandering spiders. *J. Arachnol.* **18**, 249-255.
Strausfeld, N. J. and Barth, F. G. (1993). Two visual systems in one brain: II. Neuropils serving the secondary eyes of the spider *Cupiennius salei*. *J. Comp. Neurol.* **328**, 43-62.
Strausfeld, N. J., Weltzien, P. and Barth, F. G. (1993). Two visual systems in one brain: I. Neuropils serving the principal eyes of the spider *Cupiennius salei*. *J. Comp. Neurol.* **328**, 63-75.
Trischler, C. (2003). Telemetrische Untersuchungen zur Augenmuskelaktivität bei *Cupiennius salei*. Diplomarbeit, Universität Wien.