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Brightness discrimination in a nocturnal hunting spider:
a telemetric study

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Magistra der Naturwissenschaften (Mag. rer.nat.)

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Für meine Mutter

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1. Introduction

1.1 *Cupiennius salei*

1.1.1 Taxonomy

The hunting spider *Cupiennius salei* belongs to the family of wandering spiders (Ctendiae). As many other spiders *Cupiennius salei* has eight eyes. The arrangement of the eyes is also found in Thalassiinae. The genus *Cupiennius* can be identified by the position and the circular shape of all eight eyes. Up to now, nine species could be described (Lachmuth et al. 1984; Revision in Barth and Cordes 1998).

1.1.2 Habitat and activity patterns of *Cupiennius salei*

The spatial distribution includes Central America and regions of northern South-America. *Cupiennius salei* is a nocturnal hunting spider, which uses different plants like Amaryllidaceae, Araceae, Bromeliaceae, Liliaceae and Musaceae as a dwelling during the day. At night these plants are also used as an area for mating, prey catching and moulting.

The active phase starts about an hour after sunset at an illumination level of 15 lx. Prey catching starts after nightfall at an illumination of 0.01 lx (Seyfarth 1980; Schmitt et al. 1990).

1.2 The eyes of *Cupiennius salei*

1.2.1 Morphology

There are four pairs of lens eyes in *Cupiennius salei*, which are arranged median respectively lateral in two rows on the prosoma. Therefore the different eyes are called AM-eyes (antero-median), AL-eyes (antero-lateral), PM-eyes (postero-median) and PL-eyes (postero-lateral) (Fig.1). The AM-eyes are called the principal eyes, while the other three pairs are known as secondary eyes (Foelix and Choms 1992). All eyes have a similar shape, while the size of the eyes differs noticeable: The PM eyes are the largest, the PLs are slightly smaller, followed by the AM eyes and finally the ALs. The arrangement of the eyes is shown in Fig.1 (Land and Barth 1992). The secondary eyes are specialized for viewing movement of objects, whereas the principal eyes are especially suitable for the detection of shape and texture (Schmid 1998; Neuhofer et al. 2009).

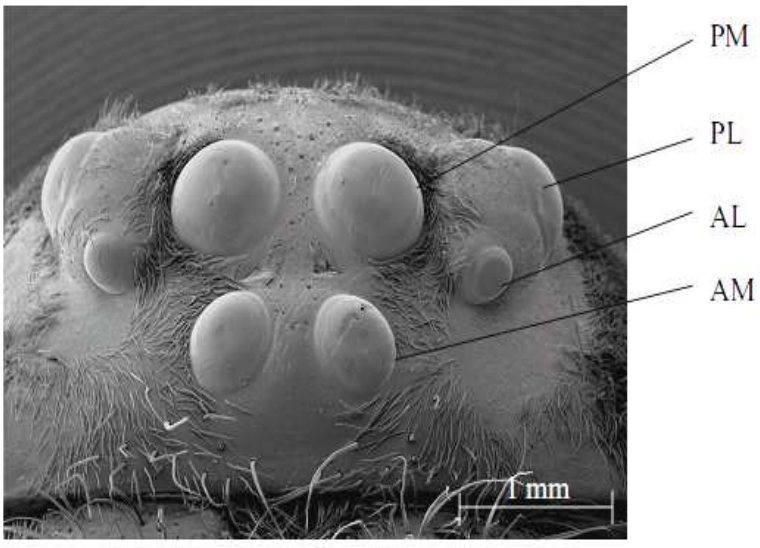

Fig. 1. REM – Picture of the eyes of an adult *Cupiennius salei*. The eyes are arranged in two strongly curved rows, the AM and AL eyes in front of the PM and PL eyes. AL – antero-lateral, AM – antero-median, PL – postero-lateral, PM – postero-median (after Zopf 2010).

The origin of the AM eyes is found in a previously existing pair of simple eyes (Paulus 1979). The principal eyes have everse photoreceptor cells, the rhabdoms are faced towards light incidence (Fig.2). The retina of the AM eyes is the only one which is movable because of the dorsal and ventral eye muscles. Hence, a deflection of the visual field of 15° is possible. (Barth 2001). The principal eyes lack a tapetum, a reflecting layer behind the receptors which is present in the secondary eyes (Fig.2) (Land 1985). The secondary eyes descend from splitting up of the ancestral compound eyes. These eyes have inverse photoreceptor cells, the rhabdoms are averted from light incidence. To maximize the light efficiency these eyes have a tapetum, which consists of several layers of guanine crystals (Fig.2) (Paulus 1979). The retinae of the secondary eyes are immobile (Land 1985).

All eyes have a cuticle cornea and lens, and moreover a cellular glass body. The retina consists of a single layer of photoreceptor cells. The axons of these cells merge and form the visual nerves that leave the eye cup and proceed to the visual ganglia (Fig.2) (Grusch et al. 1997).

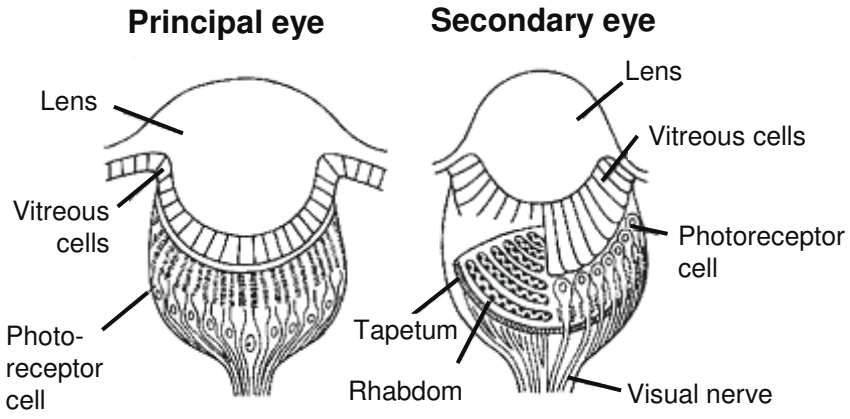

Fig. 2. The different eye types of *Cupiennius salei*. A schematic organization of the principal eyes (AM) and the secondary eyes (PM, PL, AL) is shown. The rhabdoms of the secondary eyes are averted, those from the principal eyes are inverted. The secondary eyes have a tapetum, which reflects the incoming light. Both types have a lens and vitreous cells. Axons of the photoreceptor cells form the visual nerves (after Grusch et al. 1997).

1.2.2 Retinal resolution and Neuroanatomy

The resolution of the eye is determined by the inter-receptor angle as well as the lens diameter. The bigger the diameter of the lens the smaller the diameter of the airy disk (Land 1985).
The image is of good quality in all eyes. The principal eyes (AM eyes) have an inter-receptor angle of 2.9°. The three secondary eyes (AL, PM and PL eyes) all have gridiron tapeta with the receptors arranged in rows. Here the inter-receptor angle is between 0.9° (PM) and 3.6° (AL) between the rows and 2.3° (PM) and 9.2° along the rows, respectively (Land and Barth 1992).

The structure of the AM retina is different from that of the other three eyes. The receptor cells are about 90 µm long and 14 µm wide, each receptor has rhabdomeres on three or four sides (Land and Barth 1992), whereas the receptor cells of the secondary eyes form only two rhabdomeres (Barth 2001).
All receptor cells of one eye form the optic nerve, which runs to the first optic neuropile (Land and Barth 1992).

The two types of eyes each have their own visual pathway, with two separate sets of neuropil regions (Barth 2001).
The optic nerves of the **secondary eyes** each end in the first optic neuropile, the lamina, which is comparable to the lamina of insects. This lamina is connected through interneurons with the second optic neuropile, the medulla. All secondary eyes converge to a third optic neuropile, the so called ‘mushroom body’ (Strausfeld and Barth 1993).
In the **principal eyes** the visual pathways process a similar way as those in the secondary eyes. The so called ‘central body’ describes the third common optic neuropile of the principal eyes (Strausfeld et al. 1993).

1.2.3 Visual fields of *Cupiennius salei*

Fig. 3 shows the visual fields of *Cupiennius salei*. The visual fields of AM and PM eyes overlap nearly completely. The visual fields of the PM and PL eyes cover almost the whole upper hemisphere, and down to 40° below the horizontal plane.

Barth and Land (1992) tested two spiders and found a gap of 5-20° between the visual fields of the PM and PL eyes in both animals. They presumed that this is not an artefact of the method, because no such gap was found between the two PM fields at the frontal section. A second small gap was found at the rear of the animal where the abdomen is situated.

The PM eyes seem to have an elongated field while the field of the PL eyes is rather orbital. The AL eyes field is small and downward-pointing, looking at the region just in front of the spiders chelicerae. It overlaps the lower areas of the fields of view of both the PM and PL eyes (Land and Barth 1992).

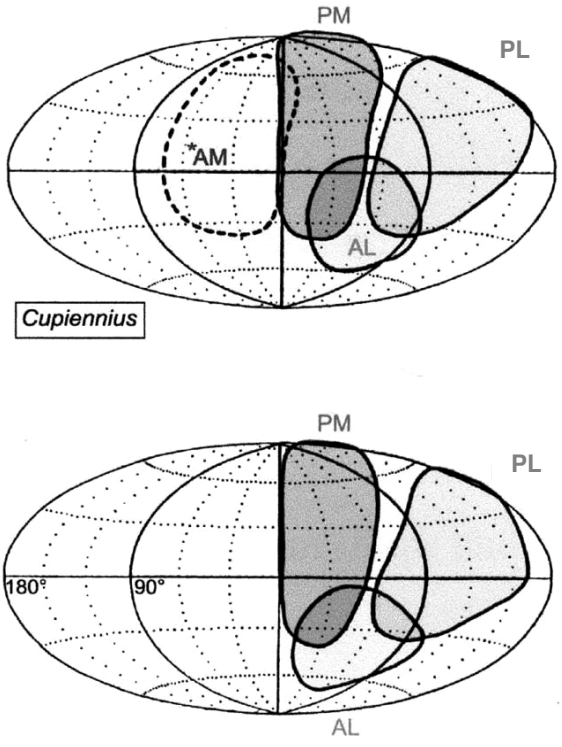

Fig. 3. Visual fields of the principal (AM) and secondary (PM, PL, AL) eyes of *Cupiennius salei*. The fields are plotted onto a globe with the spider at the centre, and the projection used depicts the whole of that globe, marked off at 90°, 30° and 5° intervals. The visual fields of AM and PM eyes almost overlap completely. Fields of PM and PL allow vision almost over the whole upper hemisphere. The small field of the AL eyes points downwards to the spiders chelicerae (after Land and Barth 1992).

1.2.4 Eye musculature

The AM eyes of *Cupiennius salei* possess two eye muscles each, a dorsal and a ventral one, which are used to move the retina (Kaps and Schmid 1996). A scheme of the arrangement of the eye muscles in the prosoma is shown in Fig. 4.

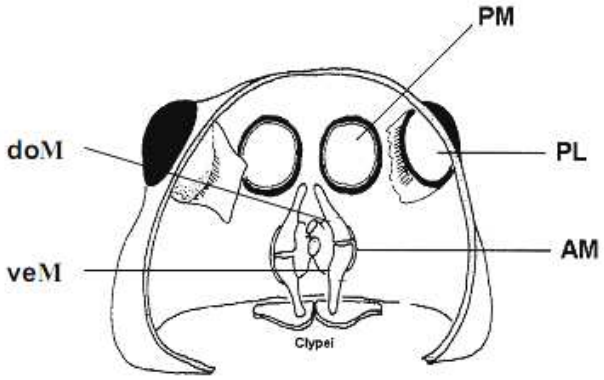

Fig. 4. Muscles of the AM eyes (principal eyes) of *Cupiennius salei*. Inside view of anterior region of prosoma; dorsal and ventral eye muscles attach on the AM eyes. AM - antero-median eyes, doM – dorsal muscle, PL - postero-lateral eyes, PM - postero-median eyes, veM - ventral muscle (Kaps and Schmid 1996).

The **dorsal eye muscle** arises dorso-laterally on the AM eye tube and attaches at the dorso-median carapace between the PM eyes. It is 600 µm long and consists of 15-18 striated fibres. It varies in breath from 50 µm at its dorsal insertion point to 300 µm in the ventral region.

The **ventral eye muscle** consists of 20-22 striated fibres and is 650 µm long. It is attached to the ventro-lateral surface of the eye tube and inserts at the carapace on the ventral internal surface of the clypei. It is 75 µm wide at its ventral insertion point and widens to 300 µm at the insertion area in the eye tube (Kaps and Schmid 1996).

The muscles of the two AM eyes are not active synchronously, neither the occurrence nor the direction of the movements of both eyes are correlated.

At simultaneous activity of both muscles in one eye, the eye tube can be deflected between dorso-median and ventro-median directions, the visual field therefore can only be shifted laterally (Fig. 5). The retina is shifted in a direction determined by the vector sum of the forces generated by them. The binocular visual fields cannot be enlarged (Kaps and Schmid 1996).

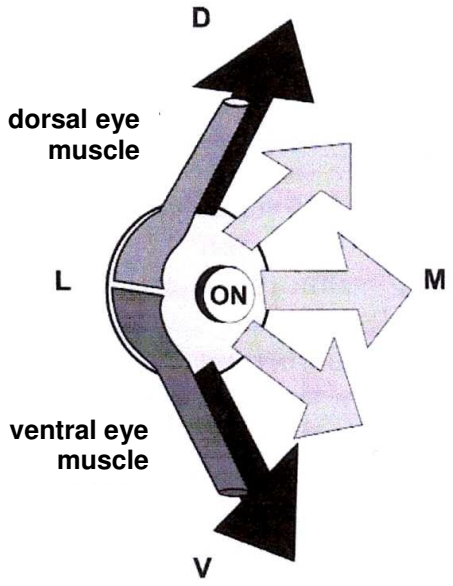

Fig. 5. Deflection of the eye tube caused by activity of the eye muscles. Black arrows mark deflection course of activity either of dorsal or ventral eye muscle. Grey arrows are examples of possible moving directions if both eye muscles contract simultaneously. ON – optic nerve, D – dorsal, M – medial, V – ventral, L – lateral (Kaps and Schmid 1996).

Two kinds of retinal movements can be distinguished:

Spontaneous microsaccades continuously 'vibrate' the retinae of unstimulated spiders. These short retinal movements are produced by the dorsal eye muscle only and might avoid visual adaptation.

Induced movements of the retina can be performed by both eye muscles, either dorsal or ventral. This causes a deflection of the visual field of the AM eye. Hence, moving objects, which are detected by the secondary eyes, can come in the spiders' visual field of the AM eyes and be identified. Perception of moving stimuli is therefore correlated with eye muscle activity (Kaps and Schmid 1996).

1.2.5. Motion sensitivity

To verify previous findings in jumping spiders (Land 1971; Duelli 1977) that the secondary eyes are responsible for motion detection, Neuhofer (2009) did several electrophysiological masking experiments with *Cupiennius salei*. When the principal eyes of the spider were masked with black colour, the animal still reacted to moving targets with an increase of eye muscle activity, while masking the secondary eyes eliminated the increase in muscle activity. It is shown that there might be a neuronal crosstalk between the secondary and the principal eyes in the visual system.
This clearly indicates the relevance of the secondary eyes in movement detection, whereas the principal eyes are not motion sensitive (Neuhofer et al. 2009) but responsible for target discrimination (Schmid 1998).

1.3 Brightness discrimination

In *Cupiennius salei*, a nocturnal hunting spider, vision is a highly developed sensory system. *Cupiennius* can see in extremely dim light at an illumination level between 15 lx after sunset and 0.01 lx during the night (Seyfarth 1980; Schmitt et al.; 1990Barth 2001).
As Orlando (2005) has shown, *Cupiennius salei* seems not to be able to see colors although there are three types of photoreceptor-cells with maximum sensitivities at 520 nm, 480 nm and 340 nm (Walla et al. 1996).
To date, neither there are investigations about the brightness discrimination ability in *Cupiennius salei*, nor is much known about brightness discrimination in animals in general.

Studies on brightness discrimination involve only a handful of species, predominantly mammals: humans (Cornsweet and Pinsker 1965; Griebel and Schmid 1997, Dain and Ling 2009), the harbour seal (*Phoca vitulina*) (Scholtyssek et al. 2008), the West Indian manatee (*Trichechus manatus*) (Griebel and Schmid 1997), two species of the South African fur seal (*Arctocephalus pusillus* and *Arctocephalus australis),* dogs (Pretterer et al. 2004), the macaque monkey (Huang et al. 2002) and horses (Geisbauer et al. 2004).

Investigations concerning brightness discrimination in invertebrates have been performed by Tiedemann (1993) in the jumping spider *Menemerus bivittatus*.

From such a small sample, there is no basis to draw conclusions about differences between diurnal, arrhythmic and nocturnal species (Pretterer et al. 2004). By calculating the Weber fraction it is roughly possible to compare the different species.

1.3.1 Weber's law

Weber's law states that the difference between two stimuli that is just noticeable depends on the magnitude of the starting stimulus. It is found that the greater the magnitude of the starting stimulus, the greater is the just noticeable difference (Griebel and Schmid 1997)

$$\Delta I/I=k$$

where I is the intensity, ΔI is the absolute intensity difference threshold and k is the relative difference threshold, i.e. the Weber fraction. Weber's law does not apply to very low and very high stimulus intensities.

1.3.2 Brightness discrimination in humans and monkeys

Hendley (1948) demonstrated that the visual acuity of humans depends on the contrast between object and background. He showed that increasing the contrast above the threshold improves the identification of details to some extend.
Psychophysical studies on humans adapted to the respective light level showed that their brightness discrimination threshold decreases in increasing light intensity. At high luminance values this decline becomes smaller until it reaches a relatively steady value (Craik 1938, Hendley 1948). Dain and Ling (2009) showed that children aged 5-12 are able to order series of 15 different shades of grey from lightest to darkest in the correct order. This ability increases in humans in precision as they grow older.

Different investigations about brightness discrimination in humans have been made with calculated Weber fractions of 0.11 (Griebel and Schmid 1997) and 0.14 (Cornsweet and Pinsker 1965). Huang et al. (2002) found, that the macaque monkey (*Macaca mulatta)* is quite similar to humans both in its visual physiology and in perception.

The calculated Weber fractions were 0.11 and 0.18 for two macaque monkeys. Other researchers found values of ~0.1 in the Rhesus monkey (Crawford 1935) and Brooks (1966) computed ~0.2 in the squirrel monkey. So the brightness discrimination ability of humans and monkeys seems to be quite similar.

1.3.3 Other species and their ability in brightness discrimination

Harbor seal (*Phoca vitulina*) and South African fur seal (*Arctocephalus pusillus* and *Arctocephalus australis*)

In experiments with the harbor seal (*Phoca vitulina*) Scholtyssek et al. (2008) determined a mean Weber fraction of 0.14, which indicates a comparable brightness discrimination ability to that of humans.
The brightness discrimination ability of the South African fur seal *Arctocephalus pusillus* and *Arctocephalus australis* were investigated by Busch and Dücker (1987). Griebel and Schmid (1997) calculated a Weber fraction of 0.3 for both species, which means that the brightness discrimination ability of the fur seal is approximately half as good as that of the harbor seal.

West Indian Manatee (*Trichechus manatus*)

The results of a twofold single-choice test showed that manatees are able to discriminate a 2.8% difference in relative reflection in a very dark range of grey stimuli, with a calculated Weber fraction of 0.35 (Griebel and Schmid 1997).

Haflinger Horse (Equidae)

Even though horse eyes are among the largest in the vertebrates, their visual capabilities are considered to be poor, based on a low ganglion cell density and a low count of cones in the retina (Geisbauer et al. 2004).
Geisbauer et al. (2004) tested two horses, which had to choose the lighter of two grey panels. The experiment showed that brightness discrimination is rather moderate in horses in comparison with other mammals, with calculated Weber fractions of 0.42 and 0.45.

German Shepherd, Belgian shepherd, Fox Terrier (Canidae)

Dogs are arrhythmic animals, active during both day and night. It is suggested that all canids might have a very similar dichromatic color vision system (Pretterer et al. 2004). A twofold simultaneous-choice test has been constructed and with the results Weber fractions of 0.22 (German Shepherd) and 0.27 (Belgian Shepherd) were calculated. The brightness discrimination ability seems to be about 2 times better in humans than in dogs (Pretterer et al. 2004). An earlier investigation of Stone (1921) on the brightness discrimination ability in two young fox terriers revealed a lower difference threshold. Only one standard intensity was tested, but the results he obtained were consistent for the two subjects with Weber fractions of 0.12 and 0.10, respectively.
The relatively high brightness discrimination threshold found by Pretterer et al. (2004) appears to be a consequence of the experimental methods. Therefore the brightness discrimination ability tested by Pretterer et al. (2004) may have been underestimated while the lower values obtained by Stone (1921) are more realistic (Scholtyssek et al. 2008).

It could therefore be suggested, that the brightness discrimination ability of dogs is as good as of humans.

Jumping Spider (*Menemerus bivittatus*, Salticidae)

The visual system of jumping spiders (Salticidae) is highly developed when compared to other families of spiders. The most specialized eyes are the AM eyes, which are capable of color vision. The secondary eyes' function is primarily to detect movement and to elicit orientation towards a target (Land 1971). Tiedemann (1993) showed in a behaviour experiment that the jumping spider *Menemerus bivittatus* has a high contrast discrimination ability.

The spider showed a rapid increase in response as the stimulus gets darker compared to the background. This rapid change in respond was not shown when the stimulus was lighter than the background (Tiedemann 1993). Unfortunately, only behavioural responses were registered and there where no Weber fractions calculated.

1.4 Aim

Cupiennius has a highly developed visual system. Three types of photoreceptors have been identified, however, colour vision seems to be impossible for the spiders (Orlando 2005), therefore the brightness discrimination ability should be investigated to indicate an alternative use of this three receptor types.

In this research an experimental set up to test the ability of brightness discrimination of *Cupiennius salei* was developed. Moveable stimuli in 24 different shades of grey - from white to black - were presented in front of five different backgrounds, which also varied from white to black.

By extracellular recording of the eye muscle activities with a single-channel telemetric transmitter a significant change in frequency should show when the spider is able to discriminate between stimulus and background. If there is no change in frequency, the stimulus is not visible for the spider and should therefore not be discriminated.
Thereby conclusions on the brightness discrimination ability of *Cupiennius salei* should be allowed.

2. Material and Methods

2.1. Experimental animal

Adult females of the Central American hunting spider *Cupiennius salei* Keys (Ctenidae) were used. They were bred at the Department of Neurobiology, Vienna, Austria, and kept under a 12/12 hour circadian rhythm. Once per week they were fed on flies (*Calliphora erythrocephala*). The temperature (22-28° C) and relative humidity (70-80 %) were similar to those of their natural habitat, the Central American forest. Each animal was kept individually in a glass jar.

2.2. Single-channel telemetric transmitter device

A single-channel telemetric transmitter was used in the experiments and was developed by Dipl.Ing. R. Machan at the electronic laboratory at the Department of Neurobiology, Vienna, Austria. A circuit diagram is shown in Fig. 6.

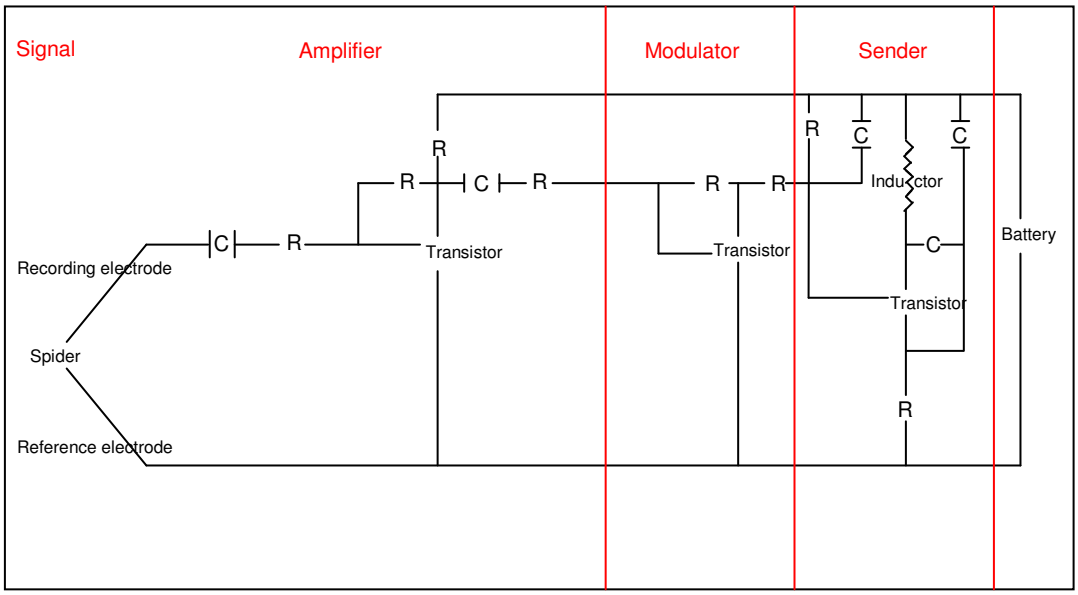

Fig. 6. Circuit diagram of the telemetric single-channel transmitter. There are eight resistors (R), five capacitors (C), an inductor and three transistors. The signal is recorded and then amplified. After amplitude modulation the signal is sent to a wide band receiver. A battery acts as a voltage source (after Orlando 2005, modified).

The three subunits – amplifier, modulator and sender - consisted of eight resistors (R), five capacitors (C), three transistors and one inductor.
A recording electrode and a reference electrode were attached to the transmitter. As a voltage source acted a battery (Maxell, 319 Silver 1.55V), which delivered electricity approximately for three hours.

The recording electrode was made of isolated manganin-wire with a diameter of 30 µm (628.3 Ω/m; Isabellenhütte, Dillenburg, Germany), the reference electrode consisted of silver-wire with 250 µm diameter.
The signal was enhanced by the factor of about 120-fold, amplitude-modulated and sent through the inductor, which was made of isolated copper-wire, to a wide band receiver (CONRAD Voyager RY-630, Conrad Electronics, Hirschau, Germany). The amplitude-modulation enabled a transfer of the eye-muscle-potentials over the carrier frequency, which was about 135 MHz and generated by the inductor. The weight of the transmitter - battery included – was 650 mg.

2.3. Visual Stimulation

To detect the ability of brightness discrimination in *Cupiennius salei*, 24 paperstripes in different shades of grey (from black to white) with a size of 41 cm length and 5 cm width were used as stimuli. These stimuli could be moved in front of a background, which was replaceable and available in nine different shades of grey.
The 24 grey-steps from white to black were printed on „matt coated paper“ (180 g/m²) in a professional print office. The backgrounds were printed on papers in size A1, the foregrounds on size A2. The papers were cut into their final size, then the 24 stripes were stuck with an aerosol fixative (3M Display Mount) on 1 mm stiff cardboard.
The relative reflectance of the single papers, compared to a white-standard (white paper, same series), was measured by using a radiometer (IL 1700 Research Radiometer/Photometer, Newburyport, England) for a wavelength range of 530 – 730 nm. For the required illumination for the measurements a daylight lamp (Radium Parabol R95, 75 Watt, matt) was used. The data for the 24 stimuli is shown in Tab.1.

Tab. 1. Relative reflectance in percent (R [%]) of the 24 stimuli. Stripe number 1 = white, stripe number 24 = black. Also shown is the course of the greyshading from white to black.

Number of paperstripe	R [%]	Greyshading
1	99,98	
2	93,39	
3	90,86	
4	84,85	
5	82,79	
6	75,51	
7	71,75	
8	64,36	
9	58,82	
10	55,29	
11	52,05	
12	49,64	
13	44,61	
14	41,67	
15	36,57	
16	34,98	
17	31,46	
18	27,08	
19	24,92	
20	22,72	
21	20,73	
22	19,37	
23	18,68	
24	15,22	

2.3.1. Illumination Level

The ambient light level in the arena was measured with a Multimeter (MT-51 Multi-Tester, Voltcraft, Hirschau, Germany) and varied from 1186 lx at background 1 to 486 lx at background 9 (Tab. 2).

Tab. 2. Measured illumination level in the experimental arena in Lux [lx] for each used background.

No. of Background	Measured Illumination Level in Lux [lx]
1	1186
4	732
5	600
7	523
9	486

2.4. Stimuli – Paperstripes

The 24 stripes had a length of 41cm and a width of 5 cm and were stuck on 1 mm stiff cardboard to guarantee stability. To fix them on a movable bar in the setup, two stripes of magnetic adhesive tape (Magnetoplan, 19 mm x 5 m) were stuck on the backside of each stripe. Therefore also on the movable bar two magnetic stripes were applied. Then the stimulus could be attached without any difficulty in the experimental setup and could also be changed quickly.

2.5. Backgrounds

The grey papers which acted as backgrounds had a size of 50cm width and 59,1cm height. They were printed in nine different shades of grey from white to black (Tab. 3). The backgrounds could be fixed with two clips on the frontal part of the setup, hence the background-papers could be replaced easily (Fig. 9). Five of the nine backgrounds - 1, 4, 5, 7 and 9 - were used in the experiment (Tab. 3). At these five backgrounds the relative reflectance showed percentaged distinctions, which seemed to be most suitable for the investigation. The differences in relative reflectance are approximately 50 % between background 1 and 4, nearly 20 % between 4 and 5, 10 % between 5 and 7 and about 5 % between background 7 and 9.

Tab. 3. Relative reflectance in percent (R [%]) of the nine backgrounds. Background number 1 = white, background number 9 = black. The second numbers point to the according stimuli in Tab. 1. The used backgrounds for the experiment are marked bold. Also shown is the course of greyshading from white to black.

Number of background and according stripe	R [%]	Greyshading
1 ≙ 1	**99,98**	
2 ≙ 5	80,83	
3 ≙ 8	64,95	
4 ≙ 12	**48,28**	
5 ≙ 17	**30,96**	
6 ≙ 19	23,60	
7 ≙ 21	**20,26**	
8 ≙ 23	18,31	
9 ≙ 24	**15,12**	

2.6. Preparation of the experimental animal

To immobilize the animal and to arrange it for preparation, the spider was cooled down in a refrigerator for 45-60 minutes at 4° C. After that the animal could be placed on a specimen holder and be fixed with Parafilm (Fig. 7).

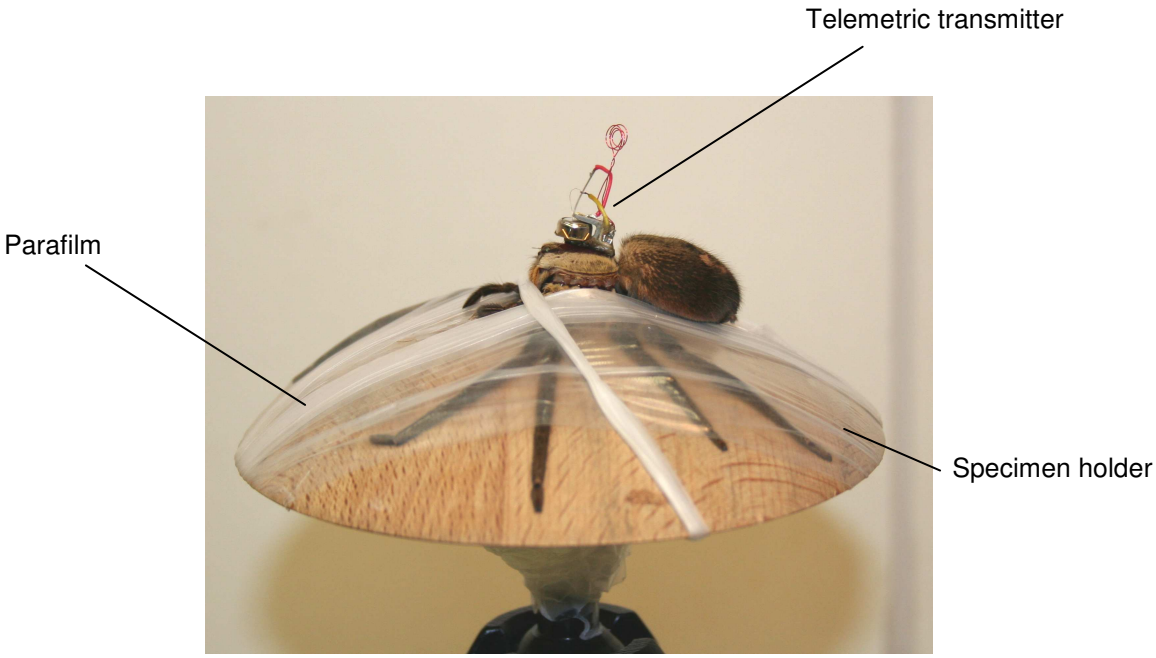

Fig. 7. Lateral view of a prepared *Cupiennius salei* with the telemetric transmitter. The animal was fixed on a specimen holder with parafilm. The transmitter was placed on the spiders' prosoma with heated bees wax.

For an easier implantation of the electrodes, the hair between AM and PM eyes and on a small area on the lateral prosoma was removed. A battery was inserted into the transmitter which now could be attached on the prosoma by using heated bees wax. The reference electrode was implanted in the lateral prosoma subsequently (Fig. 8). Then the insertion of the recording electrode followed, either in the muscle of the left or the right AM eye. The cuticle at the injection site was perforated with an electrolytically tapered tungsten-electrode first, then the recording electrode could be implanted (Fig. 9). By moving the electrode carefully it was possible to localize the eye-muscle. The signal was received by the antenna of the wide band receiver and was viewable on the oscilloscope.

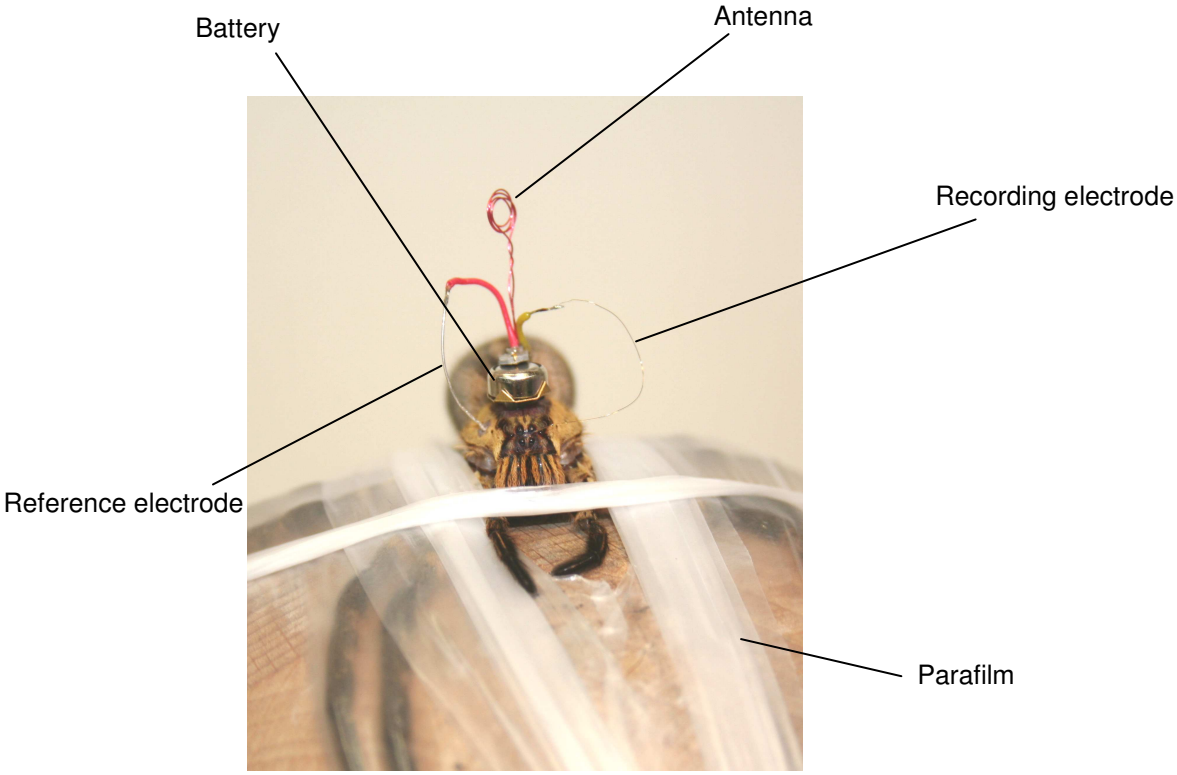

Fig. 8. Frontal view of a prepared *Cupiennius salei* with the telemetric transmitter device. The reference electrode is implanted in the lateral prosoma, the recording electrode is inserted in the muscle of the left AM eye.

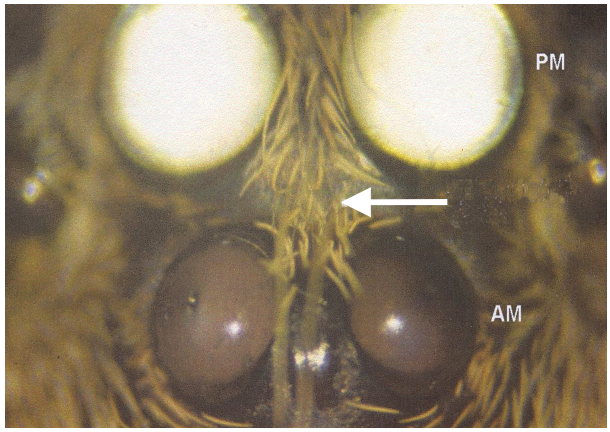

Fig. 9. Position of the recording electrode in the left dorsal eye muscle (arrow) between the PM and AM eyes of *Cupiennius salei* (after Orlando 2005).

It was important that the signal-to-noise-ratio was at least 4:1, otherwise it was hard to distinguish between spikes and noise in the analysis.

As soon as the signal remained constant on the oscilloscope the preparation was finished and the animal could be placed in the experimental setup.

2.7. Experimental setup

The setup was located in a faraday cage on a vibration-isolated table (TMC micro-g, Technical Manufacturing Inc., Peabody, USA). Figure 10 shows a schematic description.

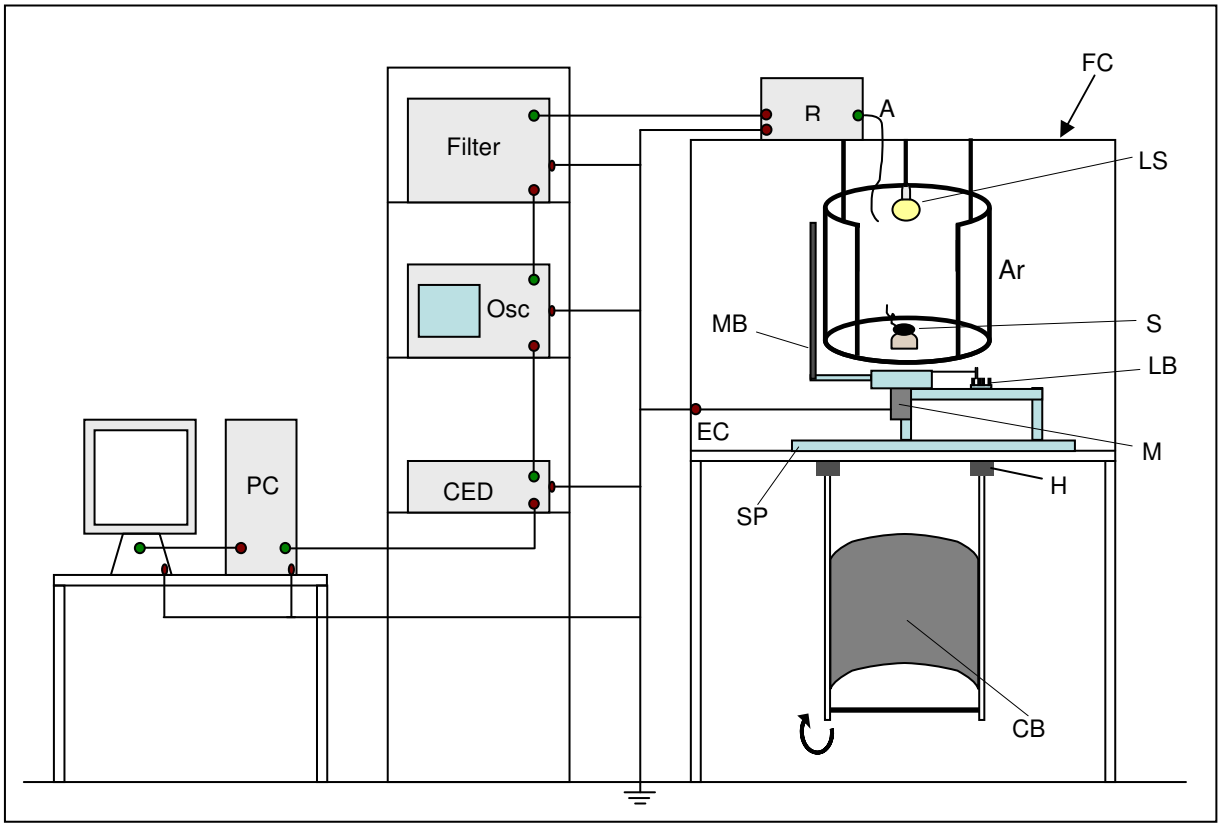

Fig. 10. Scheme of the experimental setup. An arena (Ar) is hanging in the Faraday cage (FC). In the middle the prepared spider (S) is placed. A daylight lamp acts as a light source (LS). Trough an open window in the arena the spider can see the movable bar (MB) rotating around the arena. This bar is driven by a motor (M) beneath the arena. Also two lightbarriers (LB) are installed, to register the stimulus on-and-off-set. The components which are independent of the arena stand on a steel plate (SP) to reduce vibration. On the front side of the cage the changeable background (CB) is fixed with hinges (H) and can be moved in front of the window (arrow). A receiver (R) with an antenna (A) is placed on the cage. The signal is transferred to a Filter, an oscilloscope (Osc) and an analog-digital converter (CED). At last it is transmitted to a PC, where the signal can be recorded. All components are earthed by an edge-connector (EC).

The animal was placed in the middle of the arena, which was suspended with thread rods on the ceiling of the faraday cage. The spider was placed in a way in the arena, that the visual fields of the prepared AM-eye and the associated secondary eyes were orientated to the middle of the background. The distance of the spider to the background was always 25 cm.

The arena had a diameter of 50 cm, a height of 34 cm and an open window with a width of 43 cm at the front-side. The inside of the arena was covered with light grey paper and not changeable.
A bar made of plastic, acted as a holder for the stimuli-stripes and could be moved around the arena clockwise and was powered by a motor beneath it (Fig. 10). The bar had a length of 43 cm, the width was 3 cm and the thickness 5 mm.

Two light barriers indicated the moments of stimulus onset (bar became visible) and stimulus offset (bar disappeared), this time was determined as '**stimulus-time**'. The rest of the time was described as '**interstimulus-time**'. The signal of the light barrier was shown on the oscilloscope and on the monitor of the PC. One rotation lasts about 11.5 s. This corresponds to an angular velocity of 31.3 °/s or a velocity of 0.145 m/s. The stimulus time was 4.3 s, whereas the interstimulus time was 7.2 s (Fig. 11).

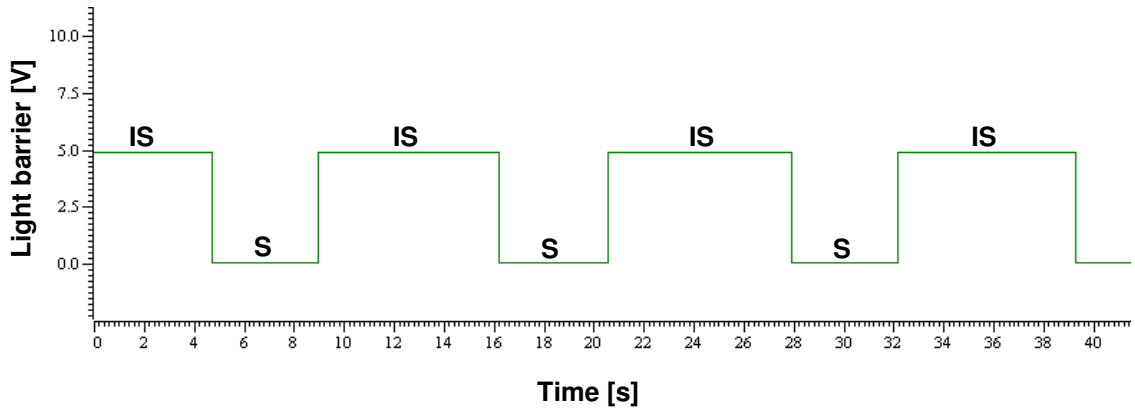

Fig. 11. Visibility of the movable bar is determined as ‚stimulus time' S and takes 4.3 s. Rest of rotation-time is called 'interstimulus time' IS and takes 7.2 s. Voltage signal (5V) of the light barriers is shown on the ordinate.

When these adjustments had been completed, one of the grey-paper backgrounds was fixed on the semicircular holder, which could be shoved on a guardrail to the experimental arena to almost close it. The bar with the stimulus now directly moved clockwise in front of the grey background through the visual field of the spider. A lamp (Radium, Parabol R95, 75 Watt, matt) was placed in the upper background of the arena and acted as a light source in the setup (Fig. 10). By using this kind of wide field lamp, shadows between background and stimulus should be reduced. Illumination values are shown in Tab. 2.

2.7.1. Signal processing

The signal from the transmitter was received by a wide band receiver (CONRAD Voyager RY-630, Conrad Electronics, Hirschau, Germany) and relayed to a filter to reduce noise and to amplify the signal 10 times. To make the signal visible it was conducted to an oscilloscope. To analyse the analog signal it was A/D converted by an analog-digital converter (CED micro1401 mkII, Science Park, Cambridge, England). Now it could be recorded with the program Spike 2 version 6.10 (Cambridge Electronic Design, Cambridge, England) on the PC. The whole equipment was earthed by an edge connector (Fig. 10).

2.8. Experimental procedure

When the animal was placed in the experimental setup, a background was fixed on the semicircular holder and then the arena was closed. The stimulus was fixed on the movable bar and the recording started. The stimuli were presented from 1 to 24 and each stimulus rotated at least 8 times around the spider.
Therefore, for each stimulus eight ‘stimulus-‘ and ‘interstimulus-times’ could be recorded at least. When all 24 stimuli were presented to one spider, the experimental procedure was finished and the animal was released. To evaluate the data statistically, 6 animals were tested for each background minimum.

Although it was tried to avoid shadows in the arena, a control before each experiment is necessary. Therefore the white and the black stimulus were tested with the white background. With the white stimulus there should be no changes in frequency, with the black stimulus a reaction of the spider should be shown. Unfortunately it was not possible to analyse the results without doing the statistics because change in frequency was not discernable during the ongoing experiment with the naked eye.

2.9. Analysis

2.9.1. Eye muscle activity

The activity of the eye muscles of the several animals was not always correlated with the presentation of the stimulus. Different activity-situations are shown in Fig. 12, 13 and 14.

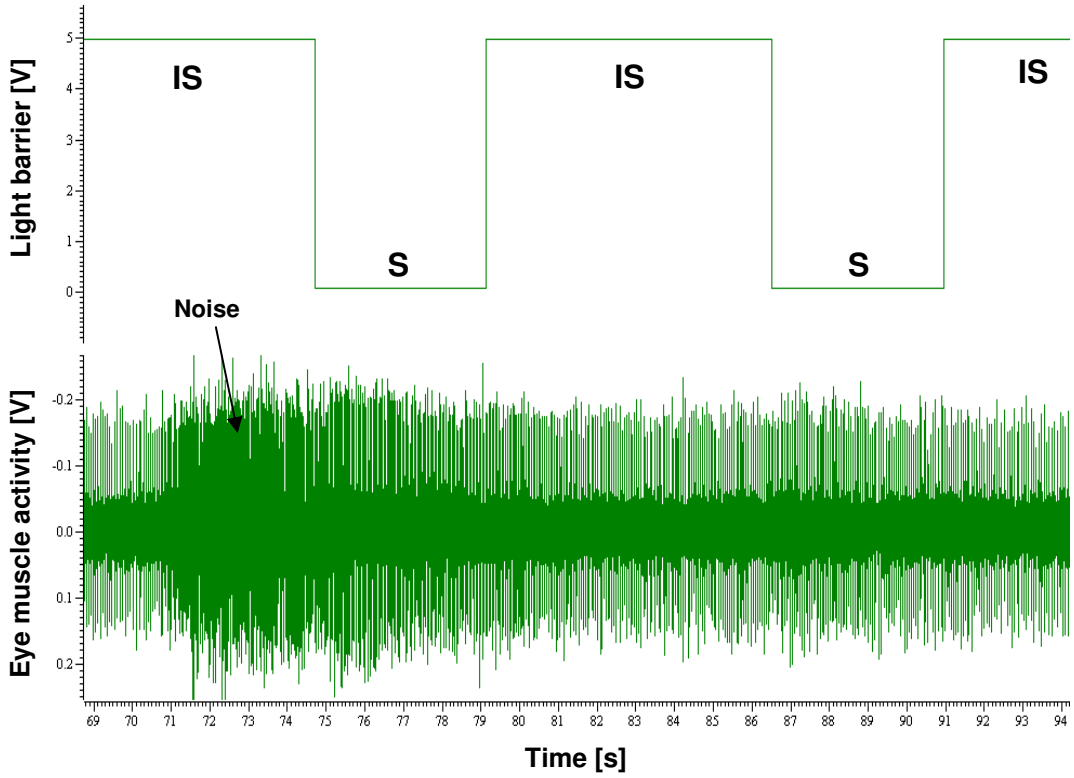

Fig. 12. Part of a record. Continuous eye muscle activity and a disturbance in the recording sequence are shown. The disturbance is recorded as a noise and is caused by movements of the spider or reactions on stimuli from outside. Stimulus time – S, Interstimulus Time – IS.

If there was continuous eye muscle activity, the test-session started. Sometimes the record was disturbed by a stimulus from outside (wind, vibrations) on which the spider reacted with increased eye muscle activity or a movement of the whole body. This was visible as an activity increase in the record (Fig. 12). Then the record was stopped and was started again, when the spider once more showed a normal activity. If such noisy parts were recorded, they were later excluded from the analysis.

A record, which could be used for the analysis is shown in Fig. 13. The spider had a continuous eye muscle activity and showed an increase of eye muscle activity when the stimulus was presented.

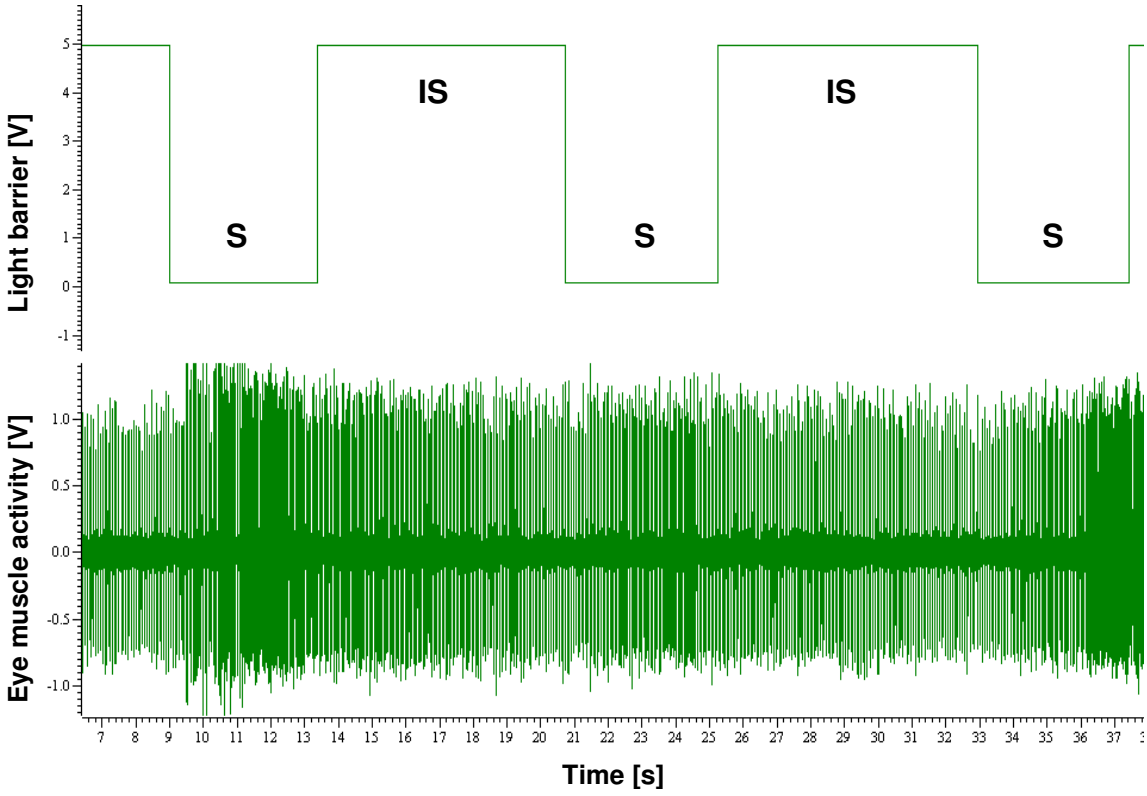

Fig. 13. Part of a record. An increase of the eye muscle activity is shown only during the stimulus time (S). Interstimulus time – IS.

Only records of continuous eye muscle activity and an evaluable signal-to-noise ratio were taken for the analysis.

2.9.2. Frequency analysis

As written above, each spider was shown all 24 stimuli in front of one background. For one background, 6 or 7 animals were tested. Each stimulus was presented at least 8 times.

Since the durations of stimulus time and interstimulus time were not equal, an analysis-area was determined for both. This area amounts to a duration of 2.2 s both for stimulus- and interstimulus time (Fig. 14).

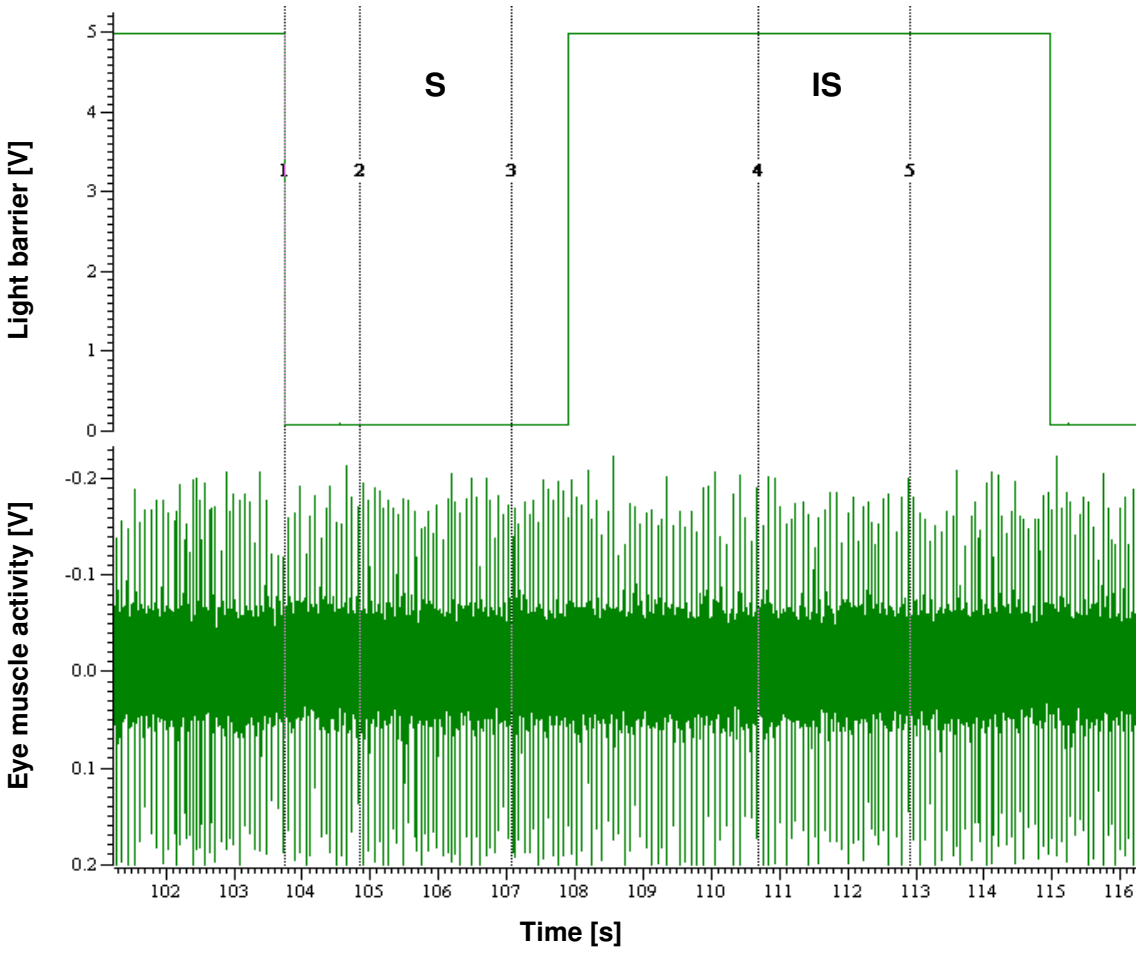

Fig. 14. Part of a record of continuous eye muscle activity during the stimulus- (S) and the interstimulus time (IS) without a reaction. The analysis-area between cursor 2 and 3 for the stimulus time and between cursor 4 and 5 for the interstimulus time is shown.

Each usable value of the rotations was implicated in the statistical analysis.

Therefore, the difference between stimulus and interstimulus frequency of *each* rotation was calculated. This happened for *all* animals and *all* stimuli at *each* background. Hence, the frequency increase is regarded.

When the tests for one background were done, the 6 or 7 difference-values for each of the 24 stimuli were averaged. The program 'MatLab R2006a' (The MathWorks, Inc., Natick, Massachusetts, USA) was used for the analysis of the statistical significance.
The difference-values for all stimuli were imported in the program, which analysed the statistical significance with the ‚Wilcoxon signed rank test for zero median'. The level of significance was 5 % ($p<0.05$).

This process was repeated for each background.

3. Results

3.1. Eye muscle potentials

As the position of reference and recording electrode always varies, there are distinct kinds of signal forms with different durations. Also dielectric characteristics of muscle and connective tissue can be responsible for variations in the signals. The figures 15 – 17 show such examples.

Figure 15 shows a tri-phasic muscle potential which lasts 1.7 ms. Noise of the transmitter is bordered by horizontal cursors.

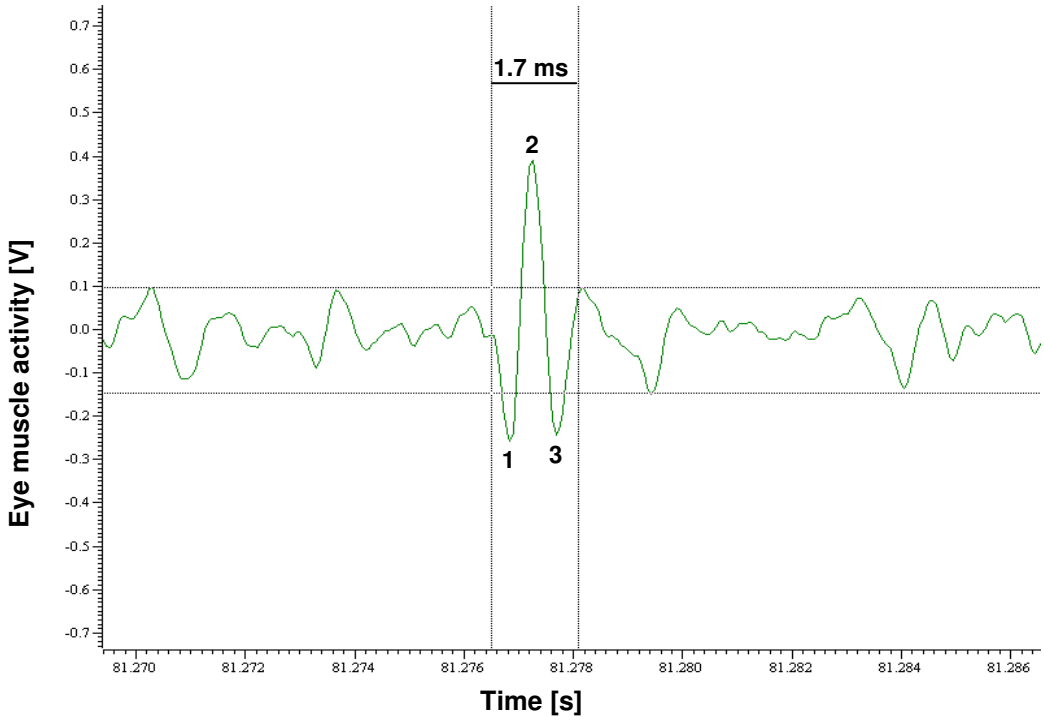

Fig. 15. Single tri-phasic eye muscle potential. Between the horizontal cursors the course of noise is shown. The different phases are marked with numbers. Duration of the potential is 1.7 ms.

Figure 16 shows a tetra-phasic eye muscle potential, which lasts 2.7 ms. Noise of the transmitter is bordered by horizontal cursors.

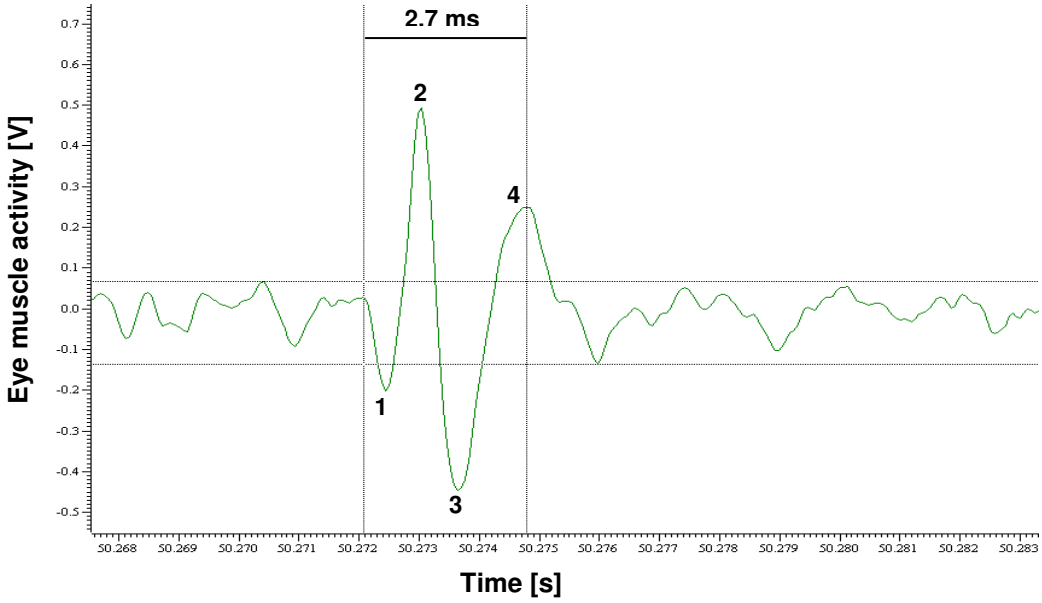

Fig. 16. Tetra-phasic eye muscle potential. Between the horizontal cursors the course of noise is shown. The different phases are marked with numbers. Duration of the potential is 2.7ms.

Figure 17 shows a penta-phasic eye muscle potential, which lasts 4.3 ms. Noise of the transmitter is bordered by horizontal cursors.

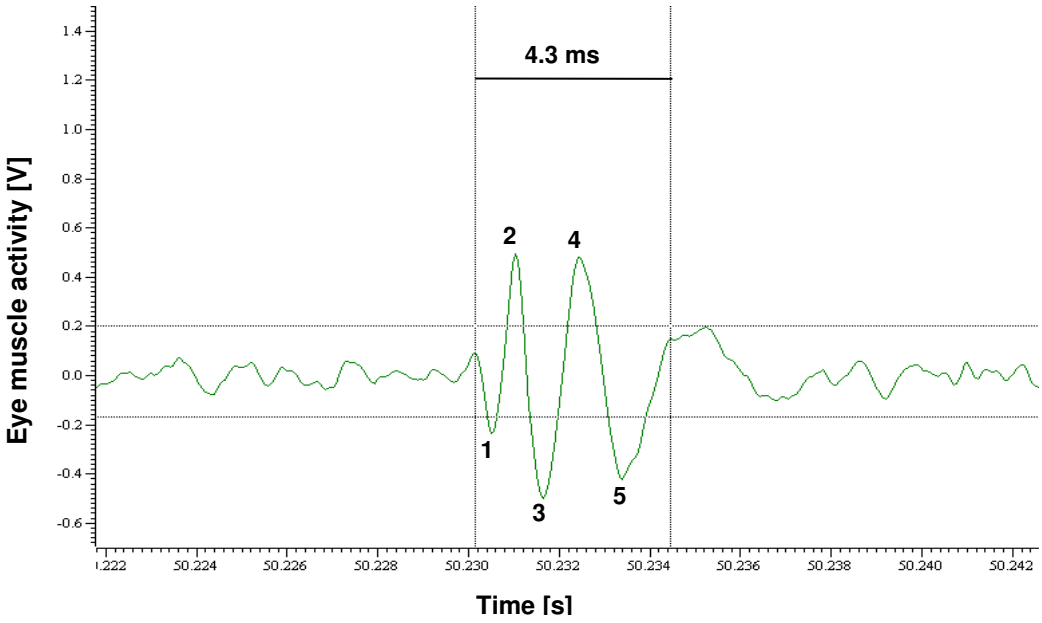

Fig.17. Penta-phasic eye muscle potential. Between the horizontal cursors the course of noise is shown. The different phases are marked with numbers. Duration of the potential is 4.3 ms.

If the position of the recording electrode did not change, the signal remains the same during one recording session. The duration of a signal was not necessarily correlated with the number of phases.

All these different types of signals were used for the analysis. For this investigation, the form of a signal was not the determining factor, but the frequency. Nevertheless it is also important to know how variable muscle potentials can be for a better understanding of the method.

3.2. Main Experiments

As performing a control experiment was not possible (see above), the experiments were started with the white background (1).

3.2.1. Background 1

Six spiders were tested here, each animal was shown every stimulus 8 times minimum. All recordings were analysed and then combined for interpretation. Fig. 18 shows the frequency modulation of all 24 stimuli (mean values with standard deviation) for background 1.

For more clearness, also the median values for all stimuli at background 1 are shown in Fig. 19. By the median values a clearer tendency is displayed.
Stimulus 1 has the same relative reflectance as background 1, namely 99.98 %.

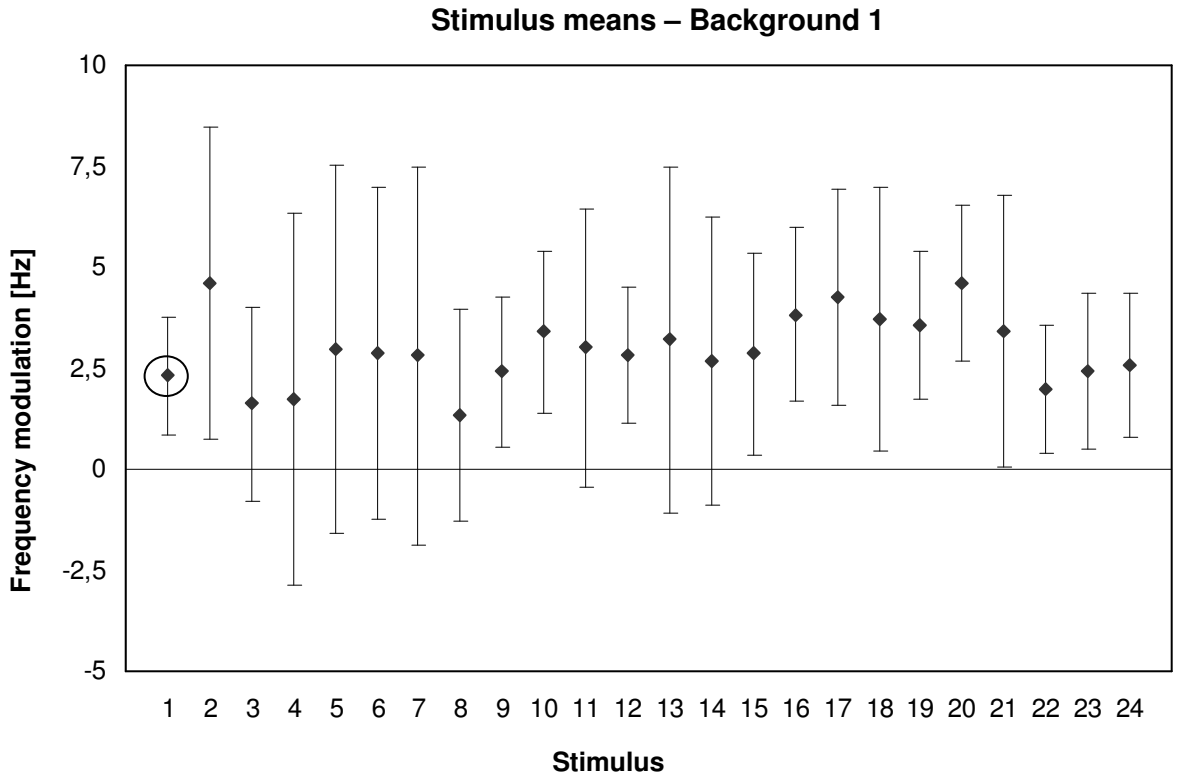

Fig. 18. Mean values with standard deviations of the frequency modulation of the 24 stimuli presented to 6 animals in front of background 1. An increase of frequency indicates discrimination between stimulus and background. Stimulus 1 (encircled) has the same relative reflectance as the background.

At stimuli, which are not discriminated, a frequency modulation of about 0 should be shown. If there is an increase in frequency, a discrimination of the stimulus is to be assumed. Both figures (18 and 19) show an increased frequency for the lighter stimuli and especially stimulus 2, which shows a very high increase.

When the stimuli become darker (at about stimulus 10), brightness discrimination ability increases (stimulus 10 and 11). A small decrease of frequency can be registered at stimulus 12 – 15 and again at 21 – 24. Remaining stimuli show a frequency increase of 4-5 Hz (Fig. 19).

Therefore, a tendency for brightness discrimination ability cannot be registered for this background.

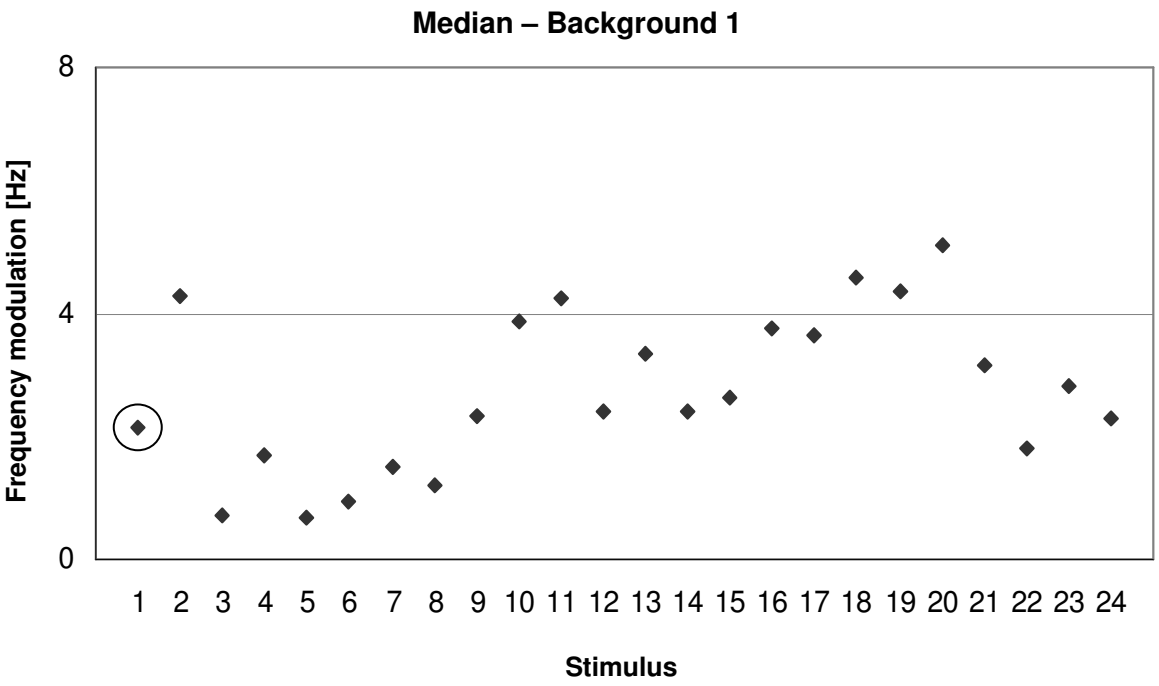

Fig. 19. Median values of frequency modulation of the 24 stimuli presented to 6 spiders in front of background 1. An increase of frequency indicates discrimination between stimulus and background. Stimulus 1 (encircled) has the same relative reflectance as the background.

For a better demonstration of brightness discrimination ability, the range of significance of all spiders (N = 6) and all 24 stimuli at background 1 is shown in Fig. 20. If p-value is < 0.05 the stimulus is discriminated significantly from the background.

As Fig. 20 shows, the both lightest stimuli (1 and 2) are discriminated significantly. Further significantly discriminated stimuli are: 9, 10, 12, 15, 16, 17, 19, 20 and 24. The remaining stimuli are not discriminated significantly from background 1. The stimulus, which has the same relative reflectance as the background, stimulus number 1, is significantly discriminated.

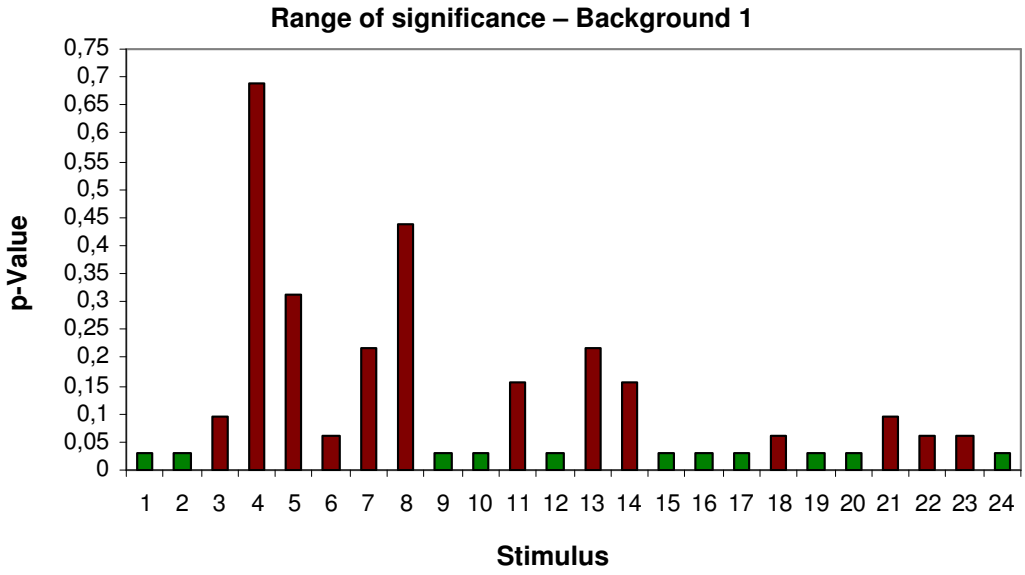

Fig. 20. Range of significance for background 1. On the x-axis the 24 stimuli are plotted, N = 6. The ordinate shows the p-values. Every value below 0.05 is significantly discriminated and marked green. Red values are not significant and the stimulus cannot be distinguished. Stimulus 1 has the identical relative reflectance as the background.

3.2.2. Background 4

Again six spiders were tested for this background. Every stimulus was shown at least 8 times. All recordings were analysed and then combined for interpretation. Fig. 21 shows the frequency modulation of all 24 stimuli (mean values with standard deviation) for background 4.

As for background 1 the median values for all stimuli at background 4 are shown in Fig. 22 for better clarity. By the median values a clearer tendency is displayed.

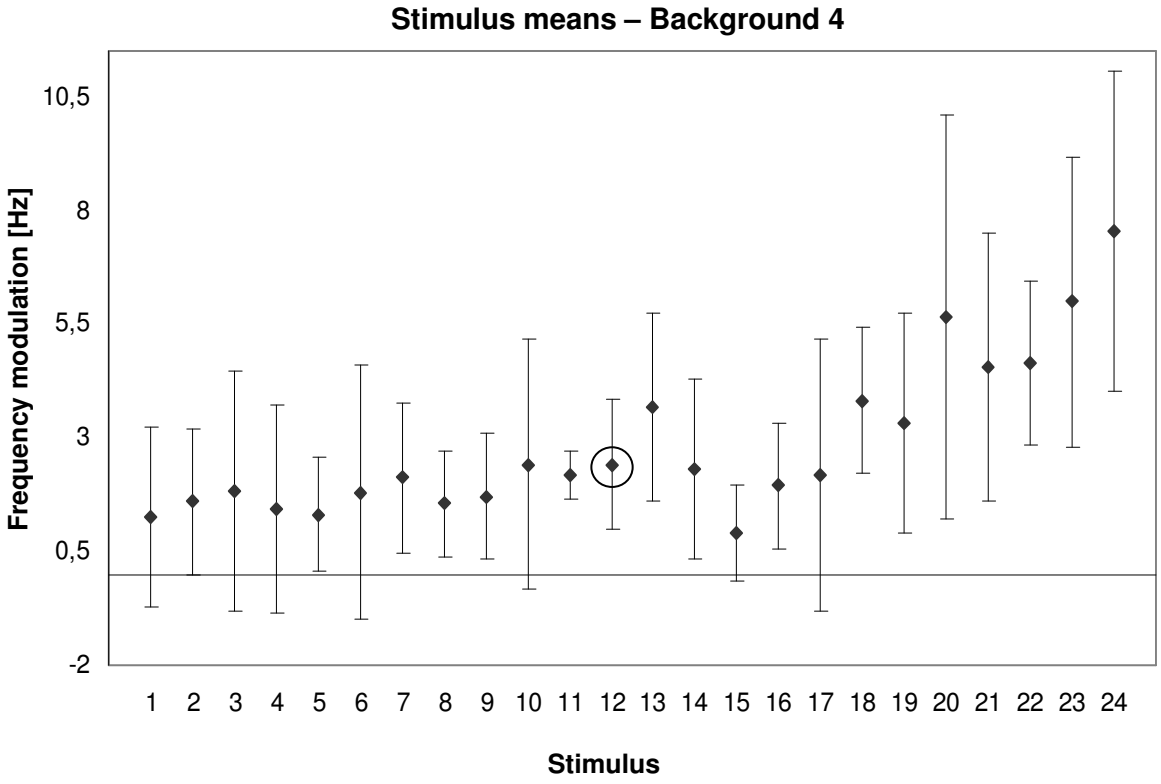

Fig. 21. Mean values with standard deviation of frequency modulation of the 24 stimuli presented to 6 spiders in front of background 4. An increase of frequency indicates discrimination between stimulus and background. Stimulus 12 (encircled) has the same relative reflectance as background 4.

For the lighter stimuli (1 – 12) no high frequency modulation is registered. Stimulus 12 has the same relative reflectance as the background (49.64 %) and should therefore not be discriminated. As Fig. 21 and 22 indicates an increase in frequency about 3 Hz is recorded for this stimulus. Fig. 23 points out, that this stimulus is in fact significantly distinguished.

From stimulus 15 on, a steady increase in frequency from about 2 to 7 Hz and thus brightness discrimination is shown. A frequency modulation about 4 Hz can be recorded mostly. A straight ascent in brightness discrimination just can be registered for the darker stimuli (Fig. 21, 22).

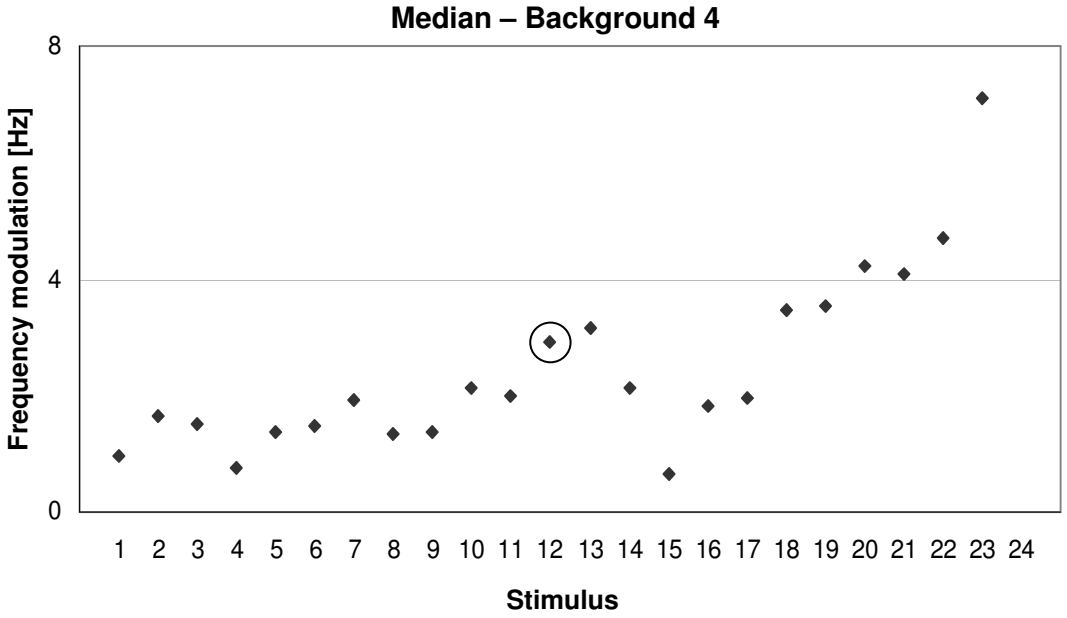

Fig. 22. Median values of frequency modulation of the 24 stimuli presented to 6 spiders in front of background 4. An increase of frequency indicates discrimination between stimulus and background. Stimulus 12 (encircled) and background 4 have the same relative reflectance.

For a better demonstration of brightness discrimination ability, the range of significance of all spiders (N = 6) and all 24 stimuli at background 4 is shown in Fig. 23. If p-value is < 0.05 the stimulus is discriminated significantly from the background.

As Fig. 23 shows, the lighter stimuli are not significantly discriminated till stimulus 8. Further significantly discriminated stimuli are: 9, 11, 12, 13, 14, 16, 18, 20, 21, 22, 23 and 24. The remaining stimuli are not significantly discriminated from background 4.

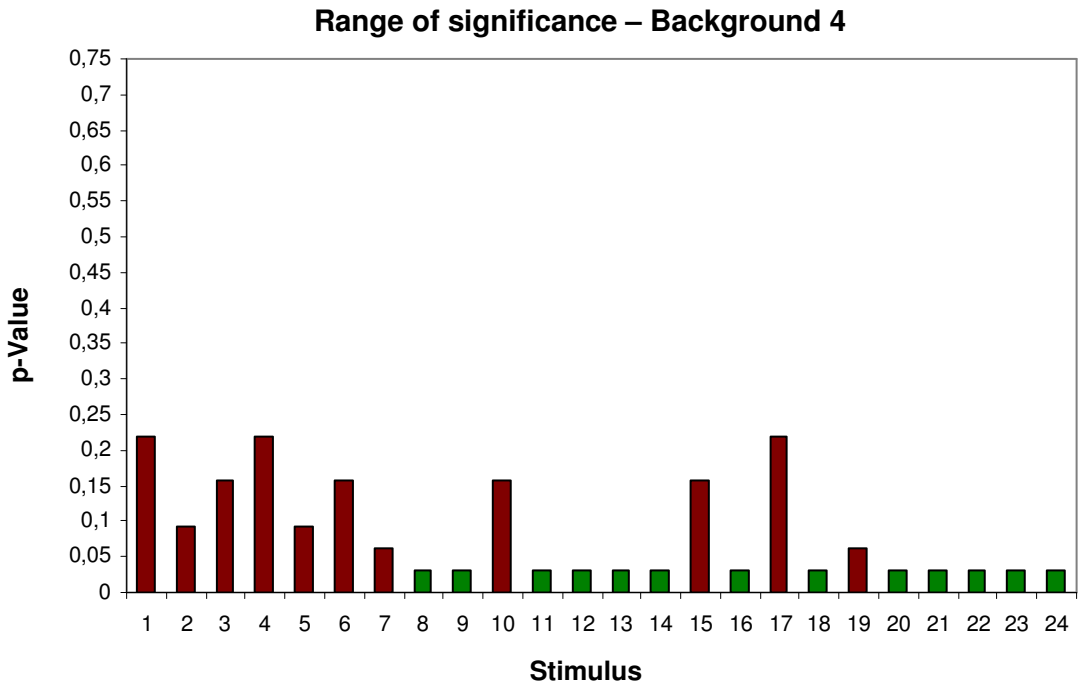

Fig. 23. Range of significance for background 4. On the x-axis the 24 stimuli are plotted, N = 6. The ordinate shows the p-values. Every value below 0.05 is significantly discriminated and marked green. Red values are not significant and the stimulus cannot be distinguished. Stimulus 12 has the identical relative reflectance as the background.

The stimulus, which has the same relative reflectance as background 4, stimulus number 12, is significantly discriminated.

Again, a meaningful result cannot be given for this background. A tendency of increased brightness discrimination ability is shown at the darker range of stimuli (20 – 24).

3.2.3. Background 5

Seven spiders were tested for this background. Again every stimulus was shown at least 8 times. All recordings were analysed and then combined for interpretation. Fig. 24 shows the frequency modulation of all 24 stimuli (mean values with standard deviation) for background 5. The identical stimulus to background 5, stimulus 17, has a relative reflectance of 31.46 %.

As for the other backgrounds the median values for all stimuli at background 5 are shown in Fig. 25 for better clarity.

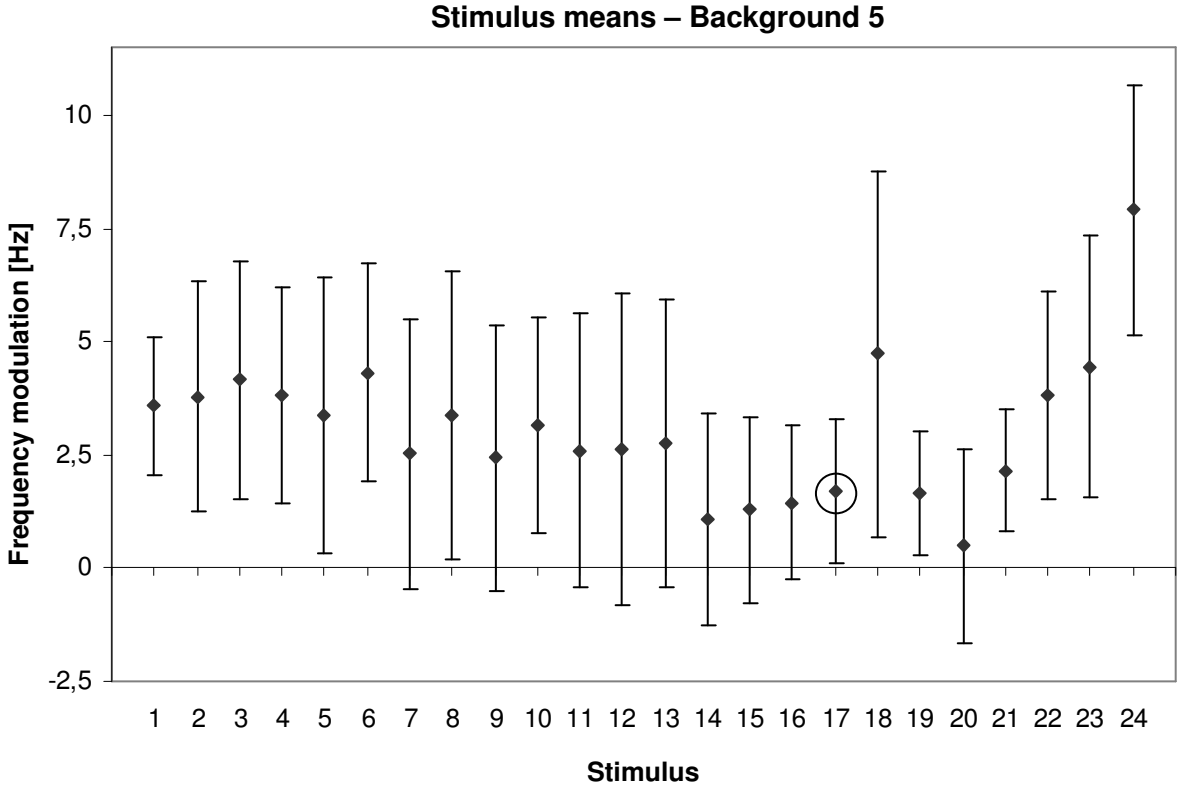

Fig. 24. Mean values with standard deviation of frequency modulation of the 24 stimuli presented in front of background 5. An increase of frequency indicates discrimination between stimulus and background. Stimulus 17 (encircled) has the same relative reflectance as background 5. Seven animals were tested.

Till stimulus 13 an increased frequency modulation of approximately 3 Hz can be registered. A sharp decline is shown at stimuli 14 and 15. As Fig. 25 shows, the frequency modulation at stimulus 14 drops nearly to zero. Stimulus 17 has the same relative reflectance as the background and should therefore not be discriminated. But as Fig. 24 and 25 show, again a frequency increase is recorded for this stimulus. Fig. 26 points out, that this stimulus is in fact significantly distinguished.

From stimulus 16 to 19, a steady increase, especially at stimulus 18 (5 Hz), in frequency and thus brightness discrimination is shown. With stimulus 20 an outlier with a frequency modulation about zero is found in all three figures (Fig. 24, 25 and 26). However, stimuli 21-24 are well discriminated.

A straight ascent in brightness discrimination can be registered for the lighter stimuli till stimulus 13 and for stimuli 21 to 24 (Fig. 24, 25). This fact is also shown in Fig. 26, where the significances for all stimuli are applied.

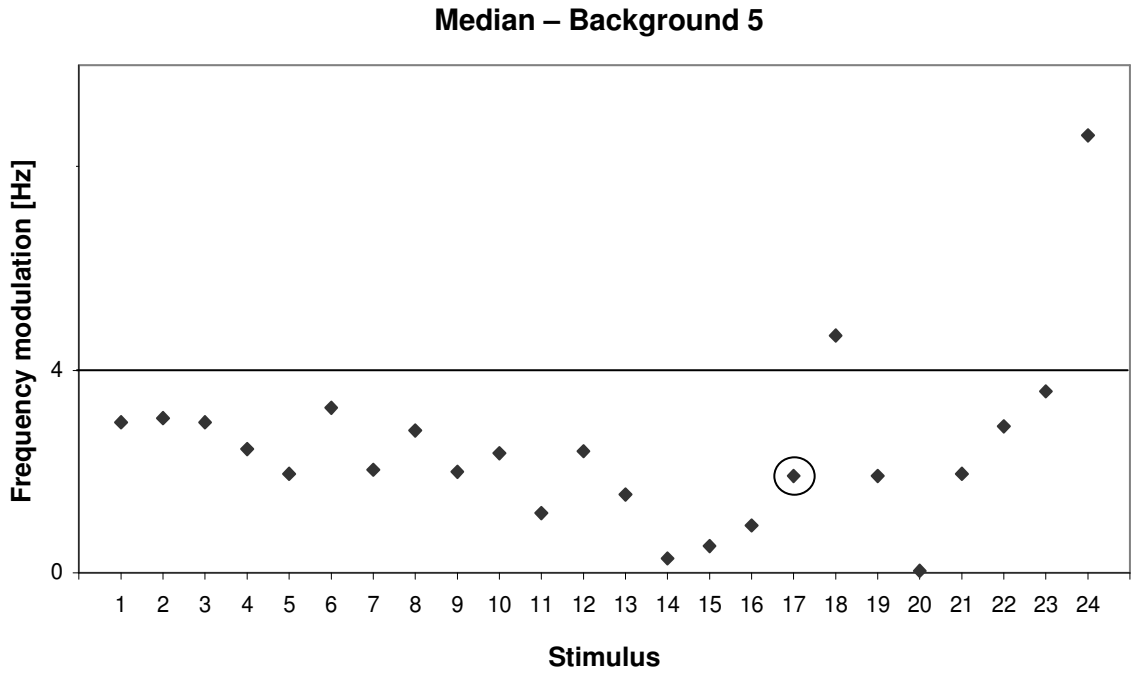

Fig. 25. Median values of frequency modulation of the 24 stimuli presented to 7 spiders in front of background 5. An increase of frequency indicates discrimination between stimulus and background. Stimulus 17 (encircled) and background 5 have the same relative reflectance.

For clarification of brightness discrimination ability at background 5, the range of significance of all spiders (N = 7) and all 24 stimuli at background 5 is shown in Fig. 26. If p-value is < 0.05 the stimulus is significantly discriminated from the background. As Fig. 26 shows, all stimuli are significantly discriminated except stimuli 14, 15 and 20. Those stimuli were not significantly discriminated from background 5.

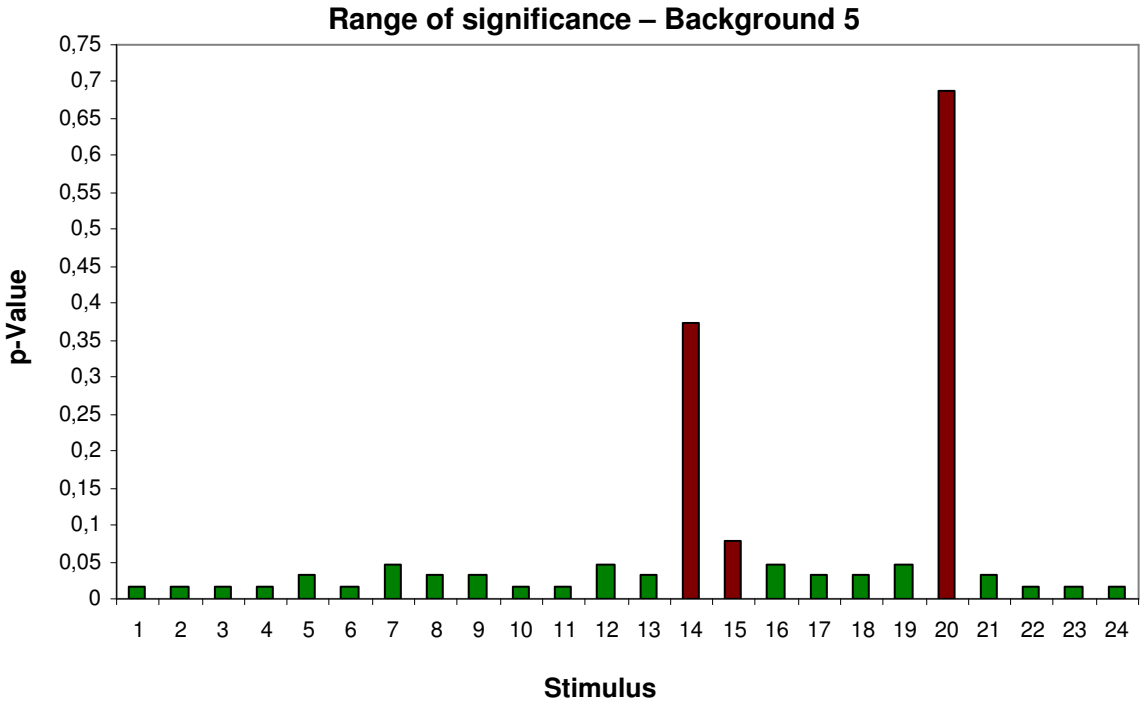

Fig. 26. Range of significance for background 5. On the x-axis the 24 stimuli are plotted, N = 7. The ordinate shows the p-values. Every value below 0.05 is significantly discriminated and marked green. Red values are not significant and the stimulus cannot be distinguished. Stimulus 17 has the same relative reflectance as the background.

Despite the better results for background 5, the stimulus with the same relative reflectance as background 5 (number 17) is again significantly discriminated, just as the stimuli around the same relative reflectance of the background (16 and 18).
Given these results no clear conclusion about the brightness discrimination ability at background 5 can be made.

3.2.4. Background 7

Six spiders were tested for this background. As before, every stimulus was shown at least 8 times. All recordings were analysed and then combined for interpretation. Fig. 27 shows the frequency modulation of all 24 stimuli (mean values with standard deviation) for background 7. The identical stimulus to background 7 was stimulus 21, with a relative reflectance of 20.73 %. As for the other backgrounds, also the median values for all stimuli at background 7 are shown in Fig. 28 for better clarity.

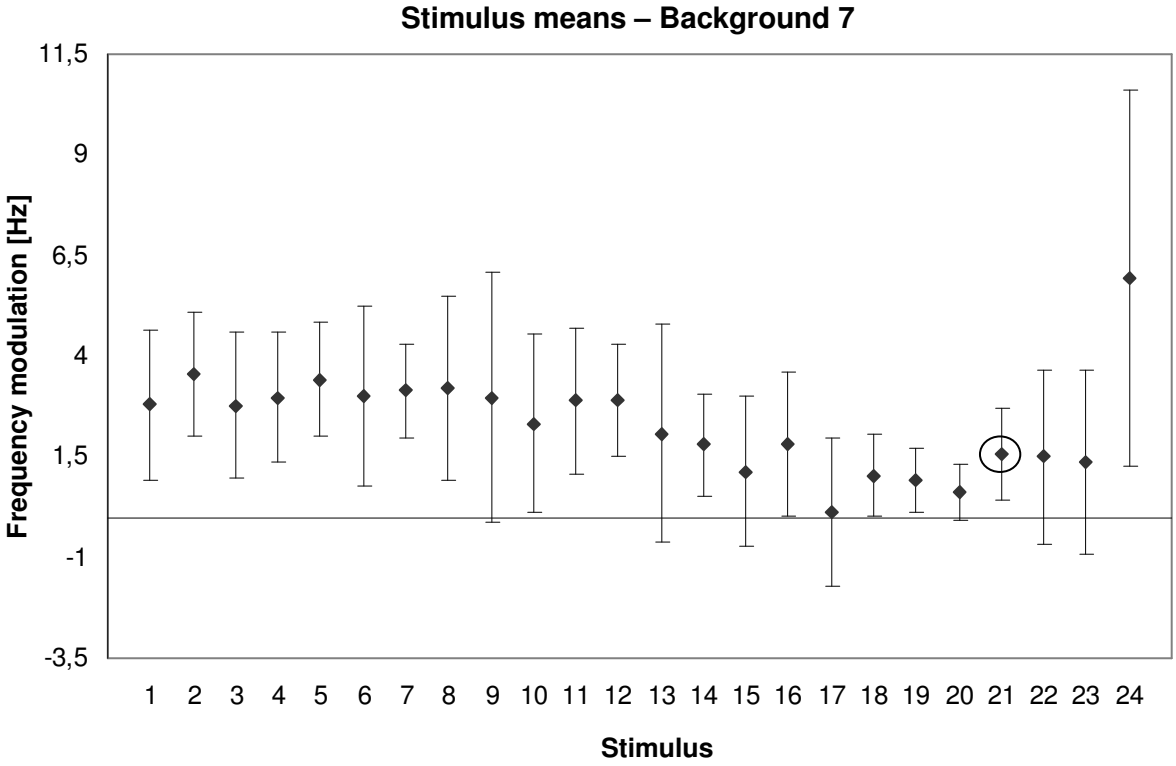

Fig. 27. Mean values with standard deviation of frequency modulation of the 24 stimuli presented in front of background 7. An increase of frequency indicates discrimination between stimulus and background. Stimulus 21 (encircled) has about the same relative reflectance as background 7. Six animals were tested.

Till stimulus 14 a heightened frequency modulation between 2 and 4 Hz can be registered, except for stimulus 15. A sharp decline is shown from stimuli 17, which is converged near zero (Fig. 27). A mean ascent is shown at stimulus 21. Stimulus 24, however, increases highly with frequency, namely at about 7 Hz (Fig. 27 and 28).

Stimulus 21 has the same relative reflectance as the background and should therefore not be discriminated. But as Fig. 27 and 28 show, a frequency increase is recorded for this stimulus. Fig. 29 points out, that this stimulus is in fact significantly distinguished.

A relative straight ascent in brightness discrimination can be registered for the lighter stimuli till stimulus 14 and again for stimuli 24 (Fig. 27 and 28). The more meaningful significances for all stimuli at background 7 are applied in Fig. 29.

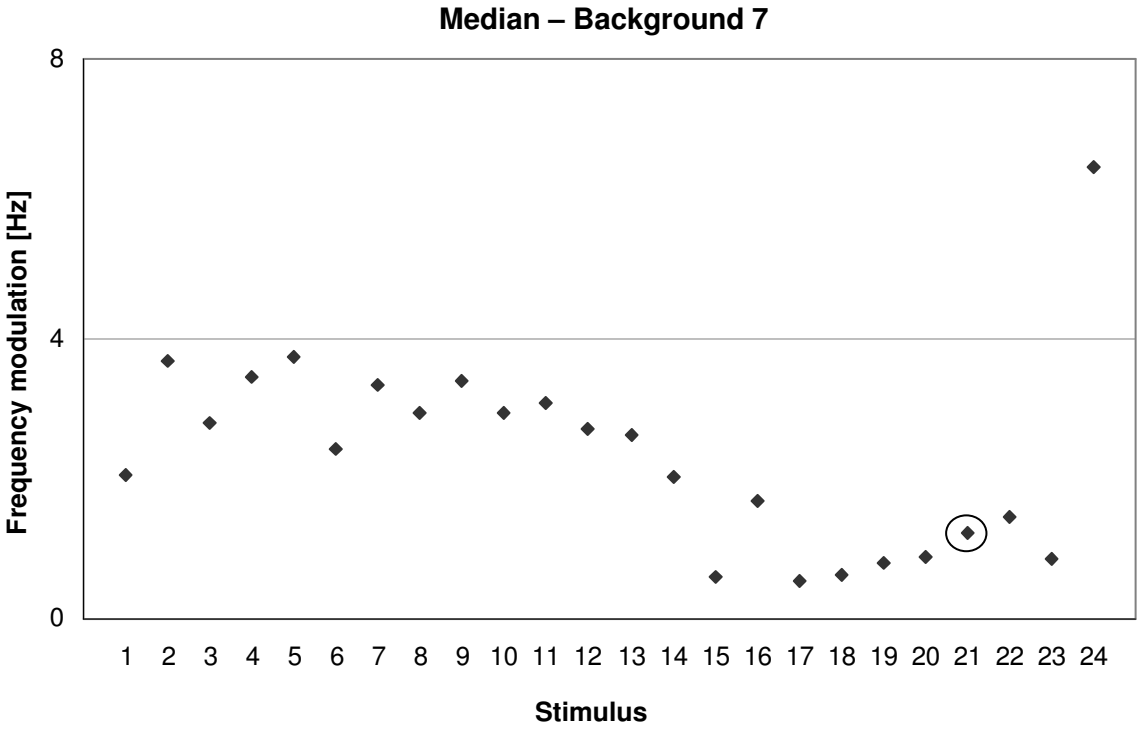

Fig. 28. Median values of frequency modulation of the 24 stimuli presented to 6 spiders in front of background 7. An increase of frequency indicates discrimination between stimulus and background. Stimulus 21 (encircled) and background 7 have the same relative reflectance.

For clarification of brightness discrimination ability at background 7, the range of significance of all spiders (N = 6) and all 24 stimuli at background 7 is shown in Fig. 29. If p-value is < 0.05 the stimulus is significantly discriminated from the background. As Fig. 29 shows, all stimuli from 1 to 8 are significantly discriminated. Further significant discriminated stimuli are 11, 12, 18 and 21.

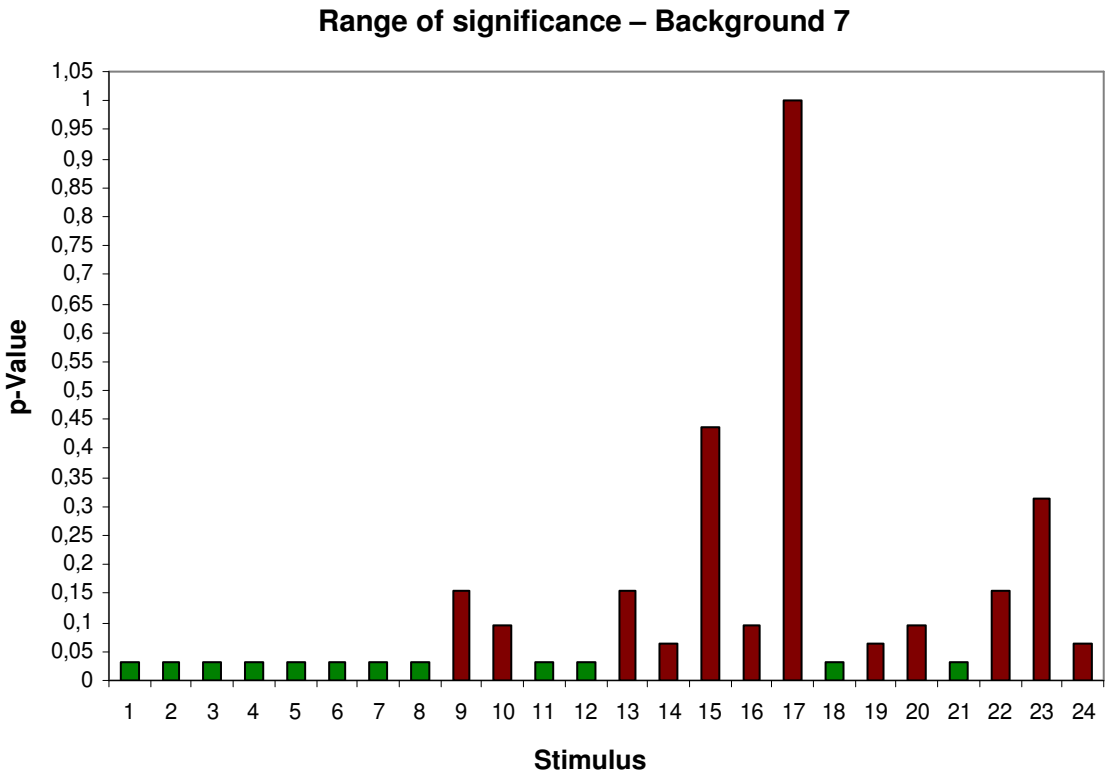

Fig. 29. Range of significance for background 7. On the x-axis the 24 stimuli are plotted, N = 6. The ordinate shows the p-values. Every value below 0.05 is significantly discriminated and marked green. Red values are not significant and the stimulus cannot be distinguished. Stimulus 21 has the identical relative reflectance as the background.

Again the stimulus (stimulus 21) with the same relative reflectance as the background is significantly discriminated, but the stimuli around are not (except stimulus 18). For the first time, also the darkest stimulus, 24, is not significantly discriminated from the background.

A tendency of brightness discrimination ability for the lighter stimuli is obvious, but again, no clear conclusion about the brightness discrimination ability at background 7 can be made based on these results.

3.2.5. Background 9

Seven spiders were tested for the last background, number 9 (black). As before, every stimulus was shown 8 times at least. All recordings were analysed and then combined for interpretation. Fig. 30 shows the frequency modulation of all 24 stimuli (mean values with standard deviation) for background 9. The identical stimulus to background 9 was stimulus 24, with a relative reflectance of 15.22 %.

As for the other backgrounds, also the median values for all stimuli at background 9 are shown in Fig. 31 for better clarity.

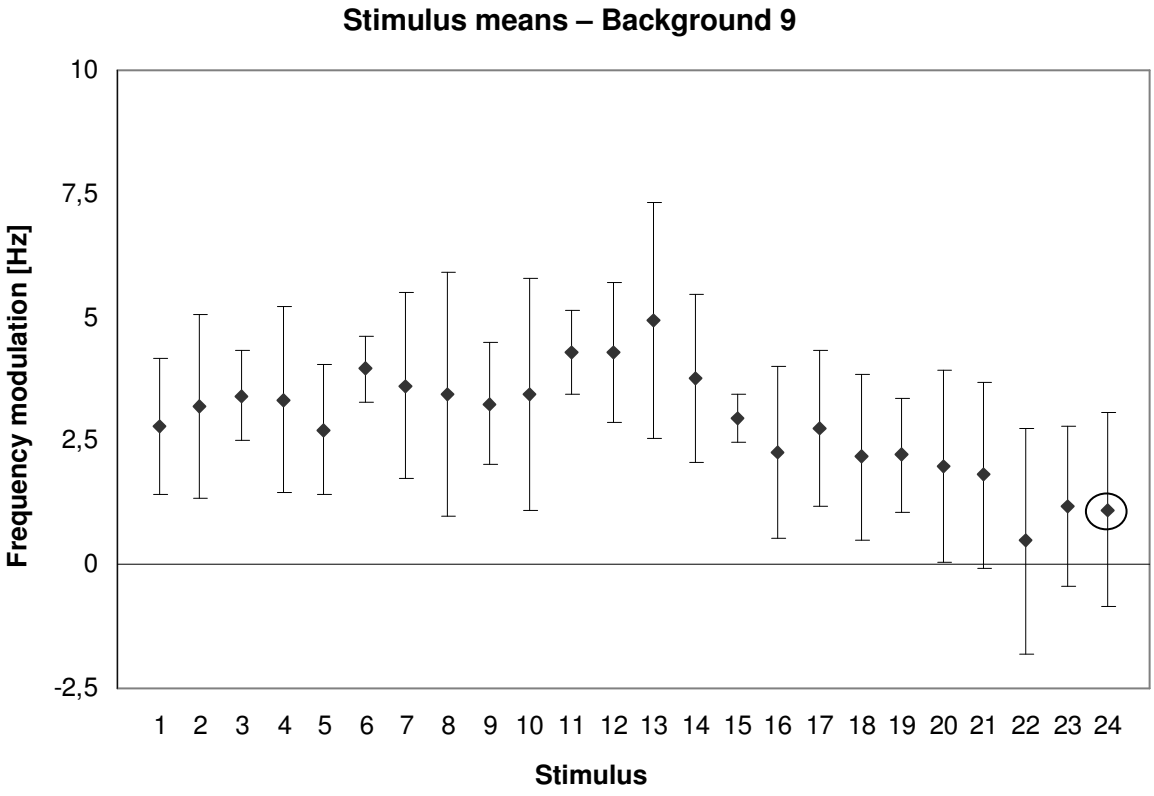

Fig. 30. Mean values with standard deviation of frequency modulation of the 24 stimuli presented in front of background 9. An increase of frequency indicates discrimination between stimulus and background. Stimulus 24 (encircled) has the same relative reflectance as background 9. Seven animals were tested.

There is a continuous frequency modulation between 2.5 and 5 Hz registered from stimulus 1 to stimulus 15. From stimulus 16 to 21 the frequency varies only between 2 and 2.5 Hz. At stimulus 22 a very low modulation in frequency is recorded (~ 1 Hz). At stimuli 23 and 24 it increases about 1.5 Hz (Fig. 30 and 31).

Stimulus 24 has the same relative reflectance as the background and should therefore not be discriminated. Fig. 32 indicates that this stimulus is not significantly distinguished. The statistical significances for all stimuli at background 9 are applied in Fig. 32.

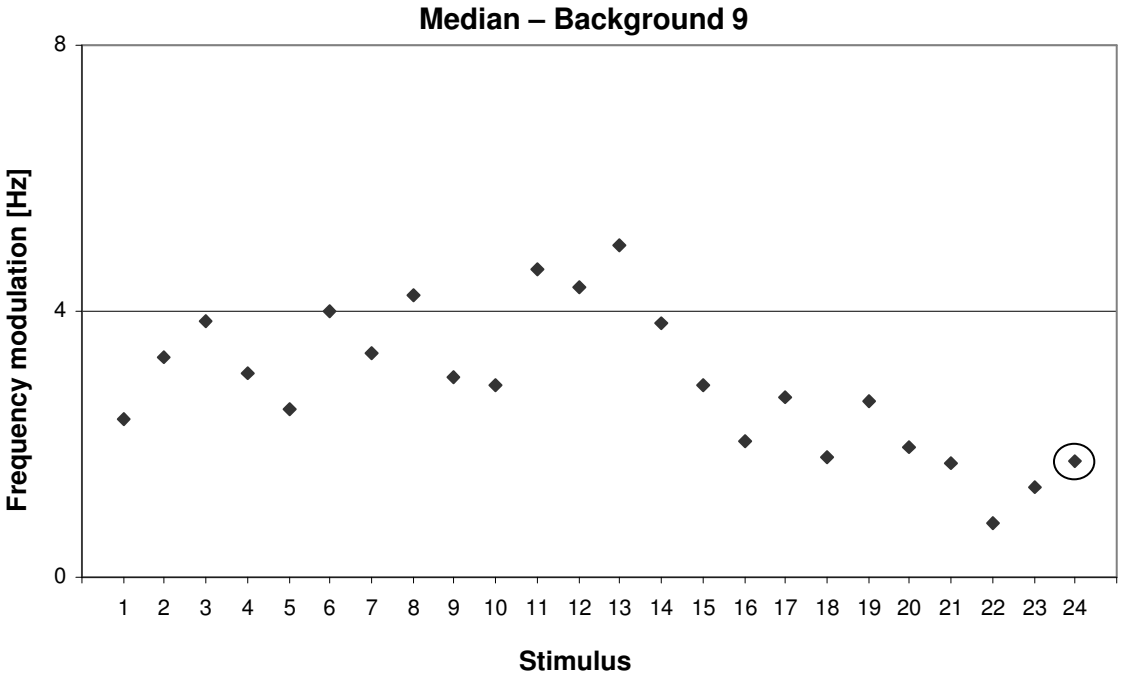

Fig. 31. Median values of frequency modulation of the 24 stimuli presented 7 spiders in front of background 9. An increase of frequency indicates discrimination between stimulus and background. Stimulus 24 (encircled) and background 9 have the same relative reflectance.

For clarification of brightness discrimination ability at background 9, the range of significance of all spiders (N = 7) and all 24 stimuli at background 9 is shown in Fig. 32. If p-value is < 0.05 the stimulus is significantly discriminated from the background. As Fig. 32 shows a significant discrimination of stimuli 1 to 19 is given. A further discriminated stimulus is 21, which is, with a value of 0.04688, barely under significance level. The stimuli 20, 22, 23 and 24 are not distinguished from the background.

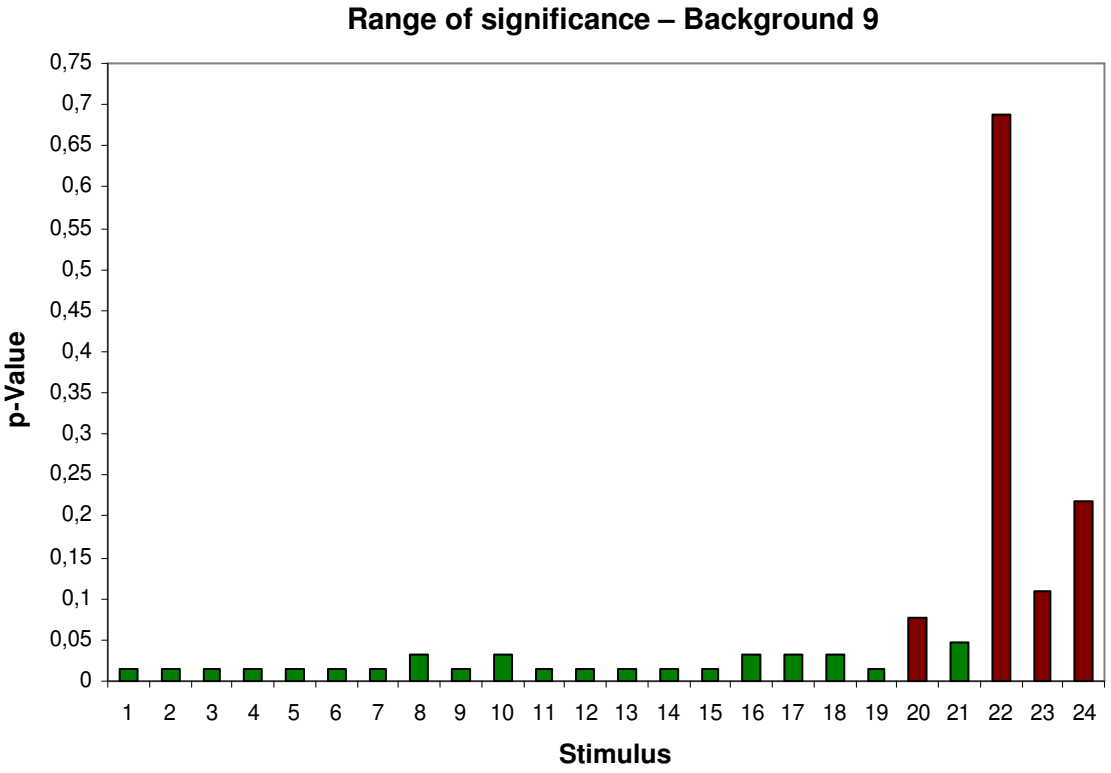

Fig. 32. Range of significance for background 9. On the x-axis the 24 stimuli are plotted, N = 7. The ordinate shows the p-values. Every value below 0.05 is significantly discriminated and marked green. Red values are not significant and the stimulus cannot be distinguished. Stimulus 24 has the same relative reflectance as the background.

For the first time, the stimulus with the same relative reflectance as the background cannot be significantly discriminated.

A tendency of brightness discrimination ability for the lighter stimuli can be clearly seen, but with the significant discrimination of stimulus 21, a clear conclusion about the brightness discrimination ability is again hard to draw.

To sum up, the results of all five backgrounds do not point to a clear conclusion relating to brightness discrimination ability in *Cupiennius salei*. Furthermore, it makes little sense to calculate the Weber fractions for these results.

4. Discussion

In this thesis the brightness discrimination ability of *Cupiennius salei* should be investigated. First, the different assets and drawbacks of telemetry will be discussed. Second part of the discussion will be the consideration of the results of brightness discrimination ability. A future prospect on possible continuative investigations regarding brightness discrimination ability in *Cupiennius salei* will be proposed.

4.1. Telemetry

By using single-channel telemetry it is possible to investigate animal behaviour under naturalistic conditions. With this method it was shown in *Cupiennius salei* that there exists a dependence between state of eye muscle activity and experimental conditions.
Kaps and Schmid (1996) found, that the state of activity depends on the experimental terms. If the animal is fixated, there is a continuous spontaneous activity of the dorsal eye muscle of about 12 Hz. Kaps (1998) showed, when the spiders' motility was limited by running on a styrofoam globe, that there was variability in frequency modulation from a high level decreasing to a temporarily total absence of the action potential-frequency to zero.
In freely moving spiders, the eye muscle activity is correlated with the running direction and depends on the state of activity (Trischler 2003).

For this investigation the spiders were fixed on a specimen holder. Nevertheless, there occurred various problems with the transmission of the signal. The movable part of the experimental setup, where the backgrounds can be fixated, was made of metal and although the movable bar for the stimuli was made of plastic, electromagnetic interferences appeared.

Combined with the impossibility of placing the recording electrode always at the same position to the antenna of the wide band receiver and other interference factors like the electronic equipment (CED, PC, light source), the signal transmission was disturbed and a signal recording was not possible.

To work against these problems, the prepared spider was shifted marginally in the arena till the signal was good enough to record. If thereby no bettering could be registered, the spider was released and the experimental session was stopped. Not only the electronic equipment caused problems in recording. Some spiders did not show continuous eye muscle activity or even totally stopped their muscle activity. These recordings were not useable for the analysis. Such spiders were released and a new preparation-run with another spider was started.

4.2. Brightness discrimination ability

4.2.1. The idea behind this work

There are only a few studies on brightness discrimination ability in animals and most of them have dealt with mammals (Cornsweet and Pinsker 1965; Griebel and Schmid 1997; Huang et al. 2002; Geisbauer et al. 2004; Pretterer et al. 2004; Scholtyssek et al. 2008; Dain and Ling 2009).
In invertebrates, the jumping spider *Menemerus bivittatus* was tested for its ability in brightness discrimination (Tiedemann 1993). This work was a behavioural experiment, without an electrophysiological component. Tiedemann (1993) found, that these jumping spiders are able to discriminate sperical shaped stimuli in different shades of grey from a background (black, white or grey). In summary, the spiders react the better the more attractive the stimulus is. But there is no indication from which point on the spiders can actually discriminate the stimulus from the background.

This was the aim of this thesis. Using the electrophysiological method with single channel telemetry it was examined how good a nocturnal hunting spider, *Cupiennius salei*, can discriminate different grey stimuli from a variable background in different shades of grey.

However, the main outcome of this work does not indicate a clear conclusion about the brightness discrimination ability in *Cupiennius salei*.

4.2.2. The brightness discrimination ability of *Cupiennius salei*

Because the receptor-cell mosaic of *Cupiennius salei* is relatively coarse-grained, the resolution is not nearly as good as in humans. The calculated minimal distance of an object at which the spider eye still forms a sharp image is about 4 mm for the principal eyes (AM eyes) and 7-19 mm for the PM eyes (Barth 2002).
In the experimental setup of this thesis, the spider was placed 25 cm away from the moving stimulus. According to that fact, the spiders should have not resolved edges of the stimuli. But because of the high illumination level, it would be obvious that the animals detected either the edges of the stimuli or even shadows, which the experimenter did not discover. As written above, the high illumination level was selected because of prevention of shadows. Before starting the experiments, several checks had been carried out with other lamps and the best solution was found with a 75 W lamp.

Reactions on mechanical stimuli like switching the movable bar on or off could be recorded, but only at the beginning of a recording session. This part of the record was not analysed and can therefore be excluded as an influencing factor. Also ‘click’ sounds of the light barriers did not irritate the spiders since increases in frequency could not be detected. Eventual external disturbances like door slams etc. were filtered out and were not analysed. Summing up, aforesaid stimuli could not have effects on the present results.

Results of the five tested backgrounds do not allow to draw a clear conclusion. The least meaningfull results were shown at the lightest background (number 1). There are no indications for concrete brightness discrimination ability or a tendency for it. Especially the significant discrimination of stimulus 1 and 2 support this fact (Fig. 20) and lead to the idea of edge-recognition. Here, the illumination level was highest with 1186 lx and a relative reflectance of 99.98 % was measured at this background.

The other light background (number 4) displays a slightly different result. Here, when looking at Fig. 21 to 23, the tendency for brightness discrimination ability increases, but still the stimulus with the same relative reflectance is discriminated with statistical significance (Fig. 23). This finding again supports the theory of edge-recognition, although the relative reflectance is decreased about a half from 99.98 to 48.28 % and illumination level was diminished from 1186 lx to 732 lx.

By decreasing the illumination level to 600 lx (30.96 % relative reflectance) at background 5, a tendency in brightness discrimination ability for the lighter stimuli (Fig. 25) can be demonstrated. Here the illumination is not as bright as for the previous backgrounds but there is repeatedly the problem of significant discrimination between the related stimulus (number 17) and the background (Fig. 26). Although the results are quite good for the lighter stimuli, a concrete conclusion cannot be made. Presumably there is again the problem of edge-recognition.

With background 7, which had a relative reflectance of 20.26 % and an illumination level of 523 lx, the results were significant for the lighter stimuli (1 – 8) but not meaningful for the darker ones (Fig. 28 and 29). Though the tested stimulus was identified significantly, this finding again suggests that edge-recognition has an influence even at this slightly lower illumination level.

For the black background (number 9) a constant significant discrimination range at the lighter stimuli till stimulus 19 (Fig. 32) is shown. Here, the first non-significant value for the identical stimulus (number 24) can be displayed. Coevally, the best results regarding to brightness discrimination ability could be achieved for this background. This could be explained by the low relative reflectance (15.12 %) and the illumination level (486 lx). Nevertheless, the darkest stimuli results in a significant value that cannot be explained. This fact could again point to edge-recognition and does not allow a concrete conclusion. However, with decreasing illumination level the results became clearly better.

Diverse outliers (i.e. in Fig. 19 stimulus 2; stimulus 18 in Fig. 24 and 25; stimulus 24 in Fig. 28) cannot be explained and could potentially be avoided by raising the number of experimental animals.

In the work of Orlando (2005) a very similar experimental setup was used. Only illumination level discerned from the existent setup. Orlando used three lamps to avoid shadows and created an ambient illumination level of 350 lx. It is suggested that this constant illumination led to more conclusive findings than were found in the present study.

Besides edge-recognition the daily patterns of *Cupiennius salei* potentially had an influence on the results. *Cupiennius* is only active during the night. Activity phase starts at about 15 lx, hunting even happens at 0.01 lx (Seyfarth 1980). In this study, spiders were tested only during their day-phase at much higher illumination levels. Therefore, it is possible that the experimental animals were irritated by the high illumination.

Given these inconclusive results, further studies are necessary to examine brightness discrimination ability in *Cupiennius salei.* Furthermore, a new concept of the experimental setup would be required. First of all, the high illumination level has to be decreased. If the illumination level is low enough, i.e. about 10 lx, the remaining setup could be used for further recording sessions.

A next step would be the total modification of the experimental setup. A screen could replace the paper stimuli and also the backgrounds and there would then be no more motor sounds or other mechanical disturbances. Additionally, more spiders should be tested for a more significant result.

To sum up, it would be worthwhile to further investigate the interesting field of brightness discrimination ability in *Cupiennius salei*.

5. Summary

The Central American ctenid spider *Cupiennius salei*, a nocturnal hunter, has four pairs of eyes, which are arranged in two rows. Because of their morphology, they are classified in principal eyes and secondary eyes. Due to their location on the prosoma the principal eyes are called AM-eyes (anterior-median), whereas the secondary eyes are divided in PM-eyes (posterior-median), PL-eyes (posterior-lateral) and AL-eyes (anterior-lateral). The AM-eyes are discerned from the secondary eyes also because of their functionality. The principal eyes are especially suitable for the detection of shape and texture, whereas the secondary eyes are specialized for detecting the movement of objects.

Only the retina of the AM-eyes is movable. These eyes possess a dorsal and a lateral eye muscle each, which allow a lateral deflection of the visual field.

The retinal resolution is limited by the retina mosaic, with inter-receptor angles between 0.9° and 9.2°. All eyes have about the same light sensitivity; with the absolute sensitivity being about 0.01 lx. In *Cupiennius salei* three types of photoreceptor-cells with maximum sensitivities at 520 nm, 480 nm and 340 nm are found.

As previous studies showed, colour vision in association with moving targets is not possible for *Cupiennius salei*. This thesis investigated the brightness discrimination ability of *Cupiennius*.

24 stimuli, grey-shaded from white to black, were presented in front of five replaceable backgrounds, which also ranged from white to black. The activity of the eye muscles, which change when a moving stimulus crosses the visual field of the secondary eyes, was registered by extracellular recordings through a telemetric transmitter. If the spider is able to discriminate a stimulus from the background, the frequency of eye-muscle activity should increase. Stimuli which have the same relative reflectance as the background should not be distinguished by the subjects. Paperstripes with a length of 41 cm and a width of 5 cm were used as stimuli. They moved clockwise in front of one of the backgrounds through the visual field of the spider.

It was the aim to find out which difference of brightness between stimulus and background is sufficient to elicit a significant discrimination.

The present results do not allow to draw a clear conclusion in terms of the brightness discrimination ability in *Cupiennius salei*. A significant discrimination between background and related stimulus was found in four out of five backgrounds. When using the black background the spiders could not significantly discriminate between the background and the related stimulus.

Especially the lighter backgrounds do not allow a clear conclusion. But there seems to be at least a tendency for brightness discrimination ability at the darker backgrounds.
Due to the high illumination level in the experimental arena it is likely that the spiders detected either the edges of the stimuli or shadows which the experimenter did not discover. Particularly the result of the lighter backgrounds would support this theory.

Given these poor results, a clear conclusion cannot be made and further studies will be necessary to evaluate brightness discrimination ability in *Cupiennius salei.*

6. Zusammenfassung

Die zentralamerikanische Kammspinne *Cupiennius salei*, ein nachtaktiver Jäger, besitzt vier Paar Augen, die in zwei Reihen angeordnet sind. Aufgrund ihrer Morphologie werden sie in Haupt- und Nebenaugen unterteilt. Wegen ihrer Lage am Prosoma spricht man bei den Hauptaugen von AM-Augen (anterior-median), während sich die Nebenaugen in PM-Augen (posterior-median), AL-Augen (anterior-lateral) und PL-Augen (posterior-lateral) unterteilen. Auch aufgrund ihrer Funktionalität werden die Haupt- von den Nebenaugen unterschieden. Während die AM-Augen der statischen Wahrnehmung und der Objektdetektion, sowie ihrer Unterscheidung dienen, sind die Nebenaugen für die Bewegungsdetektion verantwortlich. Nur die Retinae der Hauptaugen sind beweglich, und verfügen über je einen dorsalen und einen lateralen Augenmuskel, wodurch das Gesichtsfeld in lateraler Richtung verschoben werden kann. Das optische Auflösungsvermögen wird durch das Retina-Mosaik begrenzt, die Interrezeptorwinkel liegen zwischen 0,9° und 9,2°. Alle Augen sind in etwa gleich lichtempfindlich, die absolute Empfindlichkeit liegt bei 0,01 lx.

Bei *Cupiennius salei* sind drei Typen von Photorezeptorzellen bekannt. Diese zeigen Empfindlichkeitsmaxima bei 520 nm, 480 nm und 340 nm.

Wie aber in vorangegangenen Studien gezeigt werden konnte, ist Farbwahrnehmung über den Bewegungskanal bei *Cupiennius salei* nicht möglich. Die vorliegende Arbeit untersuchte deshalb das Graustufenunterscheidungsvermögen von *Cupiennius*.

Dazu wurden 24 Stimuli, abgestuft von weiß bis schwarz, vor fünf Hintergründen unterschiedlicher Graustufen, ebenfalls von weiß bis schwarz, präsentiert. Die Aktivität der Augenmuskeln, die reagieren, sobald ein bewegtes Objekt das Gesichtsfeld der Nebenaugen durchquert, wurde mittels extrazellulärer Ableitung erfasst und telemetrisch aufgezeichnet. Kann die Spinne einen Stimulus vom Hintergrund unterscheiden, sollte sich die Frequenz der Augenmuskelaktivität erhöhen. Bei einem Stimulus, der die gleiche relative Reflektanz wie der Hintergrund aufweist, sollte keine Reaktion gezeigt werden. Papierstreifen von 4 cm Breite und 50 cm Länge stellten die Teststimuli dar.

Diese bewegten sich vor einem der fünf Hintergründe durch das Gesichtsfeld der Spinne. Ziel war es herauszufinden, wie groß die Helligkeitsunterschiede zwischen Stimulus und Hintergrund sein müssen, damit *Cupiennius* sie noch signifikant voneinander unterscheiden kann.

Die vorliegenden Ergebnisse lassen jedoch keine klare Schlussfolgerung über die Graustufenunterscheidungsfähigkeit von *Cupiennius salei* zu. Bei vier von fünf Hintergründen kam es zu einer signifikanten Unterscheidung zwischen Hintergrund und jeweiligem zugehörigen Stimulus. Nur bei dem dunkelsten Hintergrund (schwarz) konnten die Spinnen nicht signifikant zwischen Hintergrund und zugehörigem Stimulus unterscheiden.

Vor allem bei den hellen Hintergründen ist keine klare Aussage möglich. Erst bei den dunkleren Hintergründen lässt sich eine Tendenz in Richtung Graustufenunterscheidung erkennen. Die hohe Beleuchtungsstärke in der Versuchsarena legt daher die Vermutung nahe, dass trotz intensiver Versuche Schattenbildung zu vermeiden, die Versuchstiere dennoch Schatten oder noch wahrscheinlicher, die Kanten der Stimuli wahrgenommen haben. Vor allem die Ergebnisse der hellen Hintergründe unterstützen diese Theorie.

Die nicht signifikanten Ergebnisse lassen keine klare Aussage zur Graustufenunterscheidungsfähigkeit von *Cupiennius salei* zu, weshalb weitere Untersuchungen auf diesem Gebiet notwendig wären.

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