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„The role of the different eye-types in visual distance discrimination in *Cupiennius salei*“

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1. Introduction

Cupiennius salei (Keyserling 1877) from the family Ctenidae lives in Central America and is a night-active hunting spider. It is most widely spread in Mexico, Guatemala and Honduras and lives on monocotyledons, such as bromeliads, the lily family and banana plants, where it stays hidden during daytime (Barth et al. 1988). At dusk the spider begins to hunt for prey as a sit-and-wait hunter and also searches for mates (Barth and Seyfarth 1979). The mechanosensory system of the spider is well developed and it was shown that the spider is able to catch its flying prey without any visual input (Melchers 1967; Barth and Seyfarth 1979). Not only in its prey-catching behaviour, but also in its pre-copulatory behaviour, where chemical and vibrational communication plays a major role, *Cupiennius salei* mostly relies on its mechanosensory system (Barth 1986; Baurecht and Barth 1992; Barth 1993). The visual system seems to play a minor role in this context (Barth 1993).

However, there also has been evidence, that the visual system, which is well developed despite the spider's nocturnal activity, is significant in at least some behavioural contexts. It was shown that the spiders approach visual targets and distinct between them (Thill 1998). The role of the two sets of eyes was also investigated in this context (Schmid 1998). The visual perception of motion has been in the centre of research (Neuhofer et al. 2009) and was shown to be color-blind (Orlando and Schmid 2011). Although the spiders are able to catch prey without any visual input (Melchers 1967; Barth and Seyfarth 1979), the visual stimulus alone can also elicit attack behaviour (Fenk et al. 2010). Recently the visual spatial and temporal cut-off frequency was investigated and compared to anatomical data (Fenk and Schmid 2010; Fenk and Schmid 2011). Therefore, the visual system seems to be far more of importance than primarily expected.

1.1. The visual system of Cupiennius salei

Cupiennius salei has, like most spiders, eight simple camera-type eyes, which are arranged in rows and can be distinguished according to their position (Fig. 1). In the front row the anterior median eyes (AME), which are also referred to as principal eyes, and the anterior lateral eyes (ALE) are located. The posterior median eyes (PME) and the posterior lateral eyes (PLE) are situated in the back row. Anterior lateral (ALE), posterior median (PME) and posterior lateral (PLE) eyes are also called secondary eyes. All eyes consist of a cellular vitreous body and a lens, but the two eye-types, principal and secondary eyes, differ primarily in their structure (Land and Barth 1992) (Fig. 2).

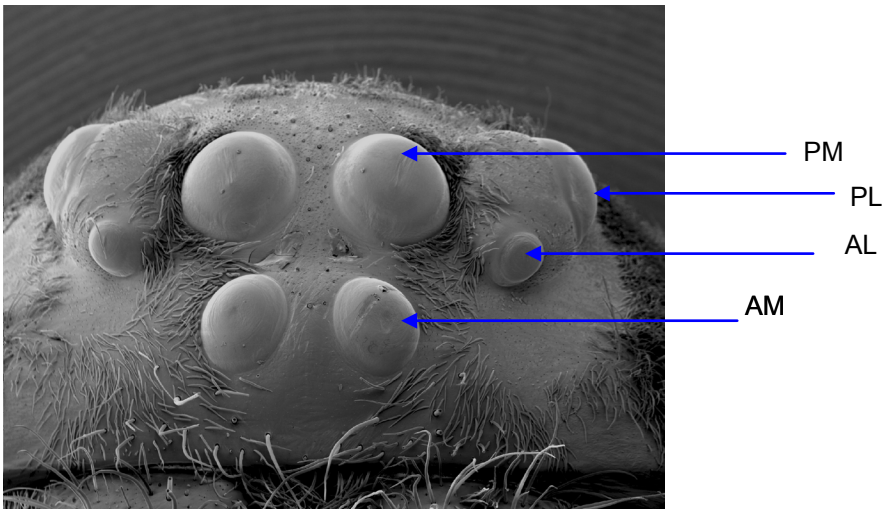


Fig.1: REM-picture of the eyes of an adult *Cupiennius salei*. AM: anterior median eyes; AL: anterior lateral eyes; PM: posterior median eyes; PL: posterior lateral eyes (Zopf 2010).

The two different eye-types do not only differ in their morphological organisation as shown in Fig. 2, but also in their visual pathways. Each of the two eye-types has its own visual pathway and two separate sets of neuropile regions (Strausfeld and Barth 1993; Strausfeld et al. 1993). This indicates that there is parallel processing of visual information from principal and secondary eyes and that the different eye types might be specialized for perceiving different visual information.

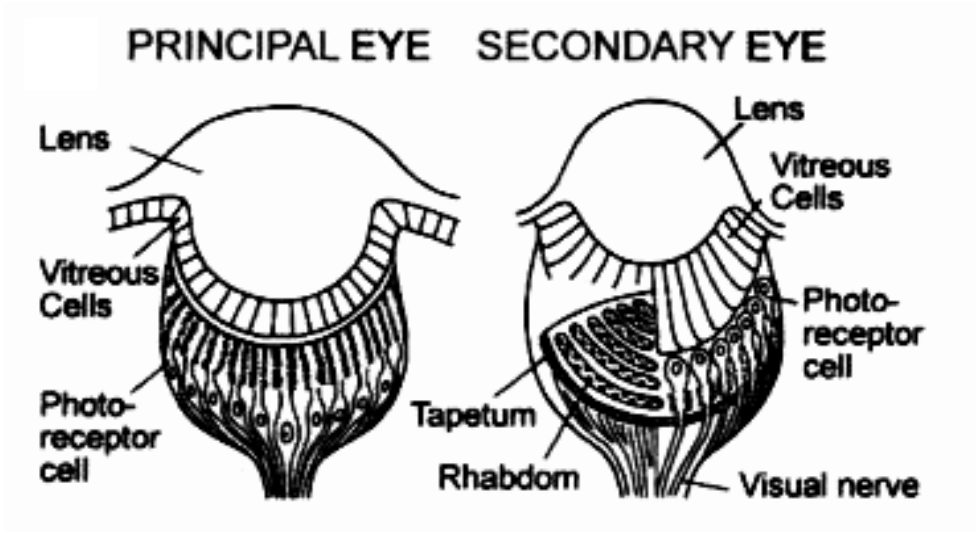
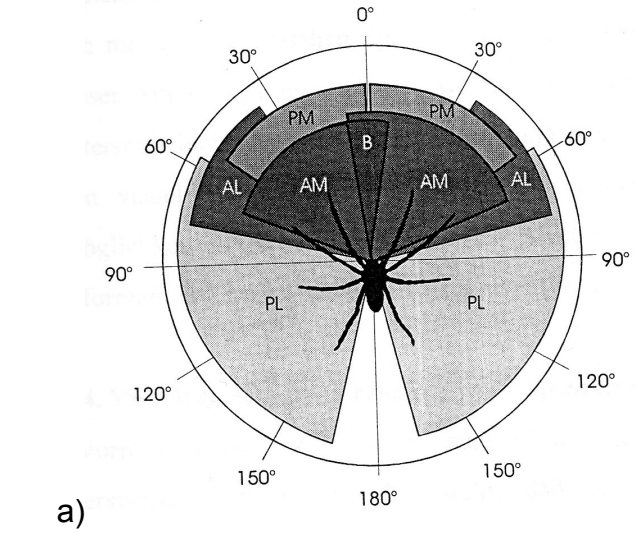
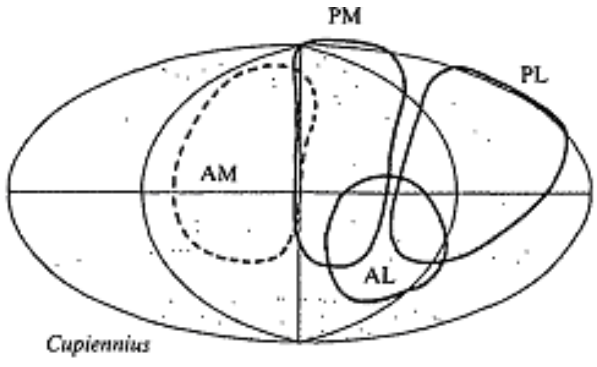


Fig. 2: Organisation of principal (AM) and secondary eyes (AL, PM, PL): The principal eyes consist of a lens, vitreous cells and photoreceptor cells with their cell nuclei proximal to the rhabdomers, which are pointing towards the incident light (everted eyes). The secondary eyes also consist of a lens and a cellular vitreous body. The cell nuclei is situated distal and the rhabdomers are turned away from the incident light (inverted eyes) (Grusch et al. 1997).

The visual fields of the eyes of *Cupiennius salei* allow a nearly all-around view, except a posterior gap of about 10-15° (Land and Barth 1992) (Fig. 3). The fields of view of the PM and PL eyes cover almost the entire upper hemisphere and about 40° of the lower hemisphere. There is a small gap between the visual fields of PM and PL eyes. The AL eyes cover the field directly in front of the chelicerae. The visual fields of the two AM eyes are the only one, which overlap a very small extent and therefore allow binocular vision only directly in front of the spider. The visual fields of the principal eyes also correspond approximately with the ones of the PM eyes, which again led to the assumption that they have different functions (Land and Barth 1992; Kaps 1998) (Fig. 3).



a)



b)

Fig. 3: Visual fields of *Cupiennius salei*. The fields of view of AM and PM eyes overlap to a large extent, whereas the AM eyes show a small area of overlapping visual fields. PM and PL eyes cover a large area in front of and beside the spider with a small gap between them. The AL eyes have the smallest visual fields in front of the chelicerae. **a)** Scheme from Kaps 1998. **b)** Drawing by Land and Barth 1992.

Principal eyes

The principal eyes are everted eyes, which means that their rhabdomeres point towards the incident light and the cell nucleus is situated proximal (Land 1985) (Fig. 2). Additionally, the retina of the principal eyes can be moved by two eye-muscles per eye, which allow a movement of the visual field of up to 15° (Land and Barth 1992; Kaps and Schmid 1996) (Fig. 4). The dorsal muscle is attached to the dorso-lateral part of the eye and is attached to the carapax between the PM eyes, whereas the ventral muscle reaches from the ventro-lateral side of the eye to the clypei. The retina can be displaced by the dorsal muscle alone or by both, the dorsal and ventral muscle, resulting in the full deflection of 15° (Kaps and Schmid 1996).

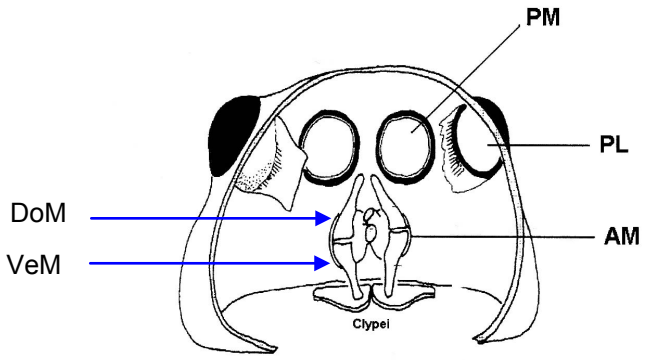


Fig. 3: View into the frontal aspect of the prosoma. Dorsal and ventral eye-muscles are attached to the AM eyes. AM: anterior median eyes; PM: posterior median eyes; PL: posterios lateral eyes; DoM: dorsal eye-muscle; VeM: ventral eye-muscle (Kaps and Schmid 1996).

Secondary eyes

All secondary eyes, on the contrary, are inverted eyes with their rhabdomeres turned away from the incident light and distal cell nuclei. The secondary eyes are equipped with an interference reflector, the tapetum (Land 1985) (Fig. 2). This reflective section of the eye consists of several layers of guanine crystals, which are aligned in stripes parallel to each other and in the case of the PME and the PLE parallel to the spider's longitudinal axis. Each of the tapetum stripes carries two receptor cells. The tapetum reflects the incident light, which increases the sensibility of the secondary eyes (Land and Barth 1992).

1.2. The role of principal and secondary eyes

Because of the differences in morphology (Land 1985; Land and Barth 1992) and visual pathways (Strausfeld and Barth 1993; Strausfeld et al. 1993), it is suggested that principal and secondary eyes are accountable for perceiving different visual information. This was first investigated by Schmid (1998), who could show that the spiders are able to detect visual targets using either their principal or their secondary eyes. However, to discriminate between different visual targets, the principal eyes are required (Fig. 5). Thus, it is suggested that the principal eyes are necessary to distinct visual targets, whereas the secondary eyes are suitable for detecting if a

visual stimulus is present (Schmid 1998). In addition it should be mentioned, that in all behavioural experiments it could be constantly shown, that the spiders approached the targets with a zig-zag-movement (Fig. 5). Schmid also suggested that this behaviour could be shown to induce motion parallax (Schmid 1998).

Later on it was shown, through measuring the eye muscle potential, that the eye muscles in the AM eyes only respond to the movement of a visual stimulus presented in the visual field of the secondary eyes. So, it seems that the secondary eyes are responsible for movement detection, whereas the principal eyes are suitable for the detection of form and texture (Neuhofer et al. 2009). Very recently it was found that the motion-detecting system is color-blind (Orlando and Schmid 2011) and that the spiders look in the subsequent walking direction before turning, indicated by an increasing eye muscle potential in the ipsilateral eye (Schmid and Trischler 2011). On the contrary to Melchers (1967) and Barth and Seyfarth (1979), who demonstrated the prey catching behaviour without any visual stimulus (Melchers 1967; Barth and Seyfarth 1979), it could be attested very recently, that visual stimuli alone can release attack behaviour and hence it is very likely that visual cues are also used in the context of hunting behaviour (Fenk et al. 2010).

Different functions of principal and secondary eyes were not only shown in *Cupiennius salei,* but also in salticids and lycosids, which are closely related to ctenid spiders (Land 1971; Duelli 1978; Forster 1985).

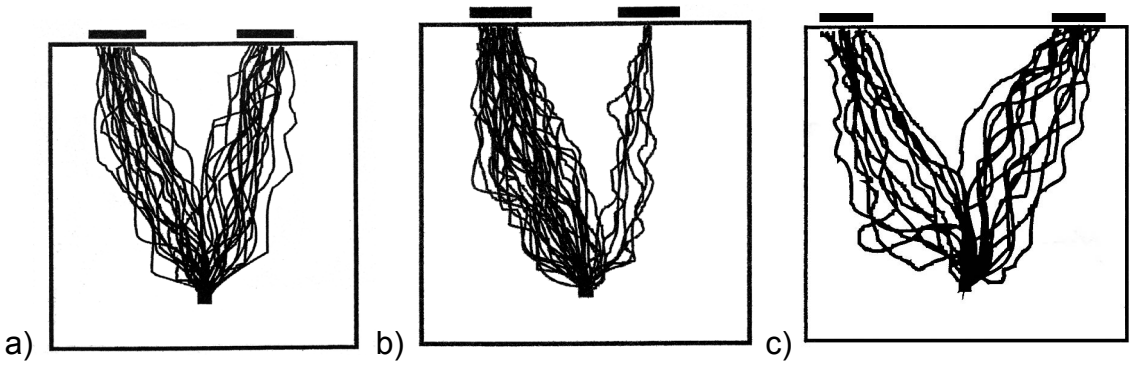


Fig. 5: Walking paths of spiders in the experiments of Schmid (1998). **a)** Two identical vertical objects were offered. 21 spiders ran towards the left target, 19 chose the right target. **b)** A vertical target on the left side and a sloping target on the right side were presented. Spiders could discriminate between the two different objects and preferred the vertical target 33 times, the sloping target 7 times (p=<0.001). **c)** When confronted with the same set-up as in **b)** and covered AM eyes, the spiders were not able to discriminate between the different objects and performed 14 runs towards the vertical object and 14 runs towards the sloping object. It could be recognized that the animals often approach the objects in zig-zag-movements (Schmid 1998).

1.3. Distance discrimination

Although, the picture on the retina is two-dimensional, the perception of the three-dimensional environment is possible for many animals and obligatory for the orientation in space. There are different depth cues that allow the perception of depth and distance, which were summarised by Goldstein (1997) as oculomotorical, monocular, motion-induced and binocular depth cues. Oculomotorical depth cues, such as convergence and accommodation, imply that the eyes can be moved and the position of the eye and the eye-muscle contraction can be processed. Monocular cues, like linear perspective and texture gradient, provide depth information when viewing at an unmoving picture and are also possible with only one eye. Motion-induced cues, such as motion parallax, are due to the movement of the observer or the object and binocular depth cues produce depth perception by comparing the two slightly different images on the retinas of the two eyes (Goldstein 1997).
Because of the morphology of the eyes, the lack of binocular overlap and the zig-zag-movement when approaching a target, it can be suggested that motion parallax might play a significant role in the depth perception of *Cupiennius salei.*

Motion parallax

The term motion parallax is referred to as the phenomenon that objects that are close to the observer display a greater apparent displacement when the observer moves, than objects at a greater distance. It can be used to estimate the absolute distance between observer and objects and the relative distance between two or more objects. Especially in animals that lack binocular vision, such as insects, self-induced motion parallax seems to play a major role in distance detection.
Mantids and locusts are able to estimate the absolute distance of an object through translational side-to-side movements of the head, also known as peering movements (Wallace 1959; Sobel 1990; Walcher and Kral 1994; Poteser and Kral 1995; Kral and Poteser 1997). Wallace (1959) was the first to show, that desert locusts (*Schistocerca gregaria*) estimate the absolute distance of an object incorrectly when the landing target was moved synchronously but counter to the peering movement of the animals (Wallace 1959). In the locust *Schistocerca americana* and in juvenile

praying mantis (*Tenodora sinensis, Polyspilota sp.*) it was also possible to quantify the over- and underestimation of the distance in relation to the movement of the landing target (Sobel 1990; Poteser and Kral 1995). However, in preying mantis larvae, distance estimation and therefore well-directed jumps were only possible, when both eyes were intact (Walcher and Kral 1994). Locusts overestimated the distance with one eye blinded (Sobel 1990).

Motion parallax as a distance cue has also been demonstrated in crickets (Goulet et al. 1981), bees (Srinivasan et al. 1990), amphibians like toads (Burghagen and Ewert 1983), but also in mammals such as gerbils (Goodale et al. 1990) and hooded rats (Legg and Lambert 1990).

1.4. Questions

It was already shown that *Cupiennius salei* uses its two different eye-types, namely principal and secondary eyes, for perceiving different visual information. While principal and secondary eyes alone can detect if an object is present, only principal eyes are able to distinct visual targets (Schmid 1998). Furthermore, a moving stimulus in the visual field of the secondary eyes is sufficient to induce movement of the eye-muscles of the principal eyes (Neuhofer et al. 2009). In this study it should be investigated if *Cupiennius salei* is able to discriminate the relative distance of two objects and which eye-type is sufficient for this task.

To detect distance, motion depth cues may play an important role. Retinal displacement is referred to as the phenomenon that the image on the retina moves, when the observer or the object is moving. The fact that the image of nearer objects grows faster on the retina than farther objects when approaching the objects is called retinal expansion and may also be involved in the spider's depth perception. The relative movement between an object and the background can also be taken into account when perceiving depth. Another aim of this study was to determine which of these mechanisms plays the major role in distance discrimination.

2. Material and Methods

2.1. Experimental animals

As experimental animals 20 adult males of the Central American hunting spider *Cupiennius salei* were used (Fig. 6). The animals were bred at the Department of Neurobiology, Vienna. During the time span of the experiments, the animals were kept under an artificial photoperiod of 12 hours day and 12 hours night with a temperature between 22°C and 25°C and a relative humidity around 60%. The spiders were kept individually in glass jars. Once a week, they were fed on flies (*Calliphora* sp.) and fresh water was regularly provided.

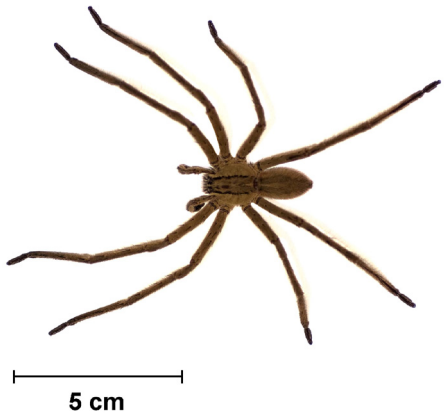


Fig. 6: Picture of a male *Cupiennius salei* (Zopf 2011).

2.2. Experimental set-up and procedure

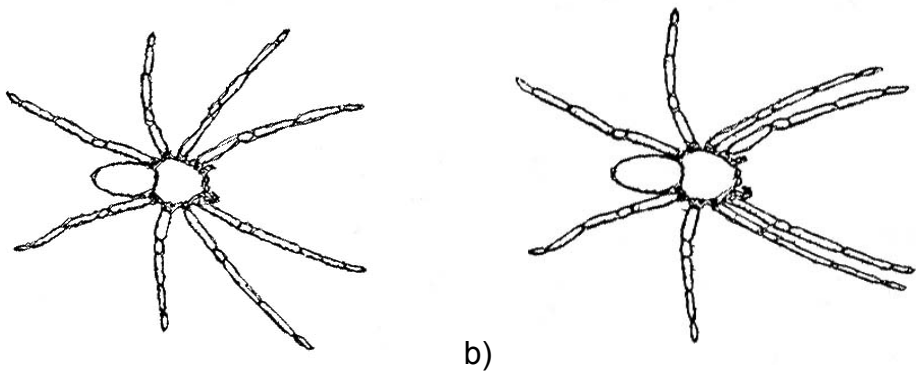
Although *Cupiennius salei* is a nocturnal hunter, the experiments took place under the artificial daylight condition, because in previous experiments the spiders did not show any difference in target discrimination either in darkness or under brighter illumination.

The experimental arena was located in the same room where the spiders were kept. The size of the arena was the largest possible in this room and was 2.36 m wide and 2 m long. The arena was enclosed with three white walls and the floor was covered

with white paper. The front wall was 60 cm high allowing to handle the spiders and to walk into the arena, whereas on the back wall two target objects made of black cardboard were presented. Above the arena there were two diagonal arranged fluorescent tubes installed to light the arena equally.

To exclude side preference, the two rectangle targets were presented in a twofold simultaneous-choice alternately at both positions. The animals were released in 1.80 m distance from the back wall from their glass jars, facing the objects. The run was recorded as “successful”, if the spider touched one of the two objects with Object 1, which will be referred to as the physically nearer object, or Object 2, which will be referred to as the physically distant object placed directly on the background. However, a “false” run was recorded, if the animal did not touch any object and ran up the plane wall or moved around in the arena without touching any wall. After a maximum time span of 10 minutes without moving, the spiders were touched with a stick with cotton wool on its end to motivate the spiders to run. After another two minutes without moving, the run was finished and also recorded as “false” run. If the spider displayed panic posture with its typical leg position (Fig. 7) for the time span of 10 minutes, the run was recorded as “panic posture”.

The running traces, number of the spider, date and time were recorded by hand. For the statistical analysis the sign test from Dixon and Mood was used.



a) b)

Fig. 7: a) Leg position in normal readiness. The spider’s body is raised in position. **b)** Leg position during panic posture. The body is pressed flat on the ground (Kaps 1998).

2.2.1. Preliminary studies

Set-up and procedure

To investigate if the spiders can discriminate relative distance, two rectangle objects made of black cardboard were arranged in five different distances in front of a structured background (Fig. 8). An artificial decision line at a distance of 30 cm in front of every object was defined, as in Kosenburger (2005). When viewed from this decision line, the two objects appeared to have the same (Fig. 9).

The structured background consists of a pattern of black and white stripes running diagonal crossing each other. Each stripe was 6 cm thick. This structured background is necessary to enable relative motion between object and background.

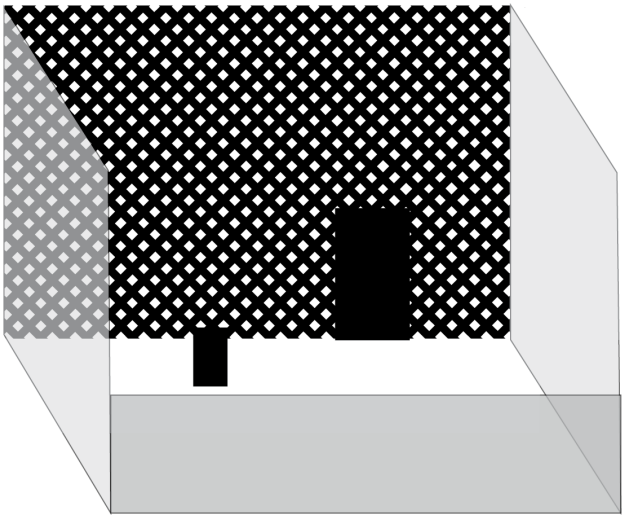
Five consecutive series of target combinations were tested in this experiment. The more distant object (Object 2) was always placed directly on the structured background with a height of 70 cm and a width of 39 cm. It was placed 39 cm apart from the middle line. The nearer object (Object 1) was presented either in 70 cm, 50 cm, 30 cm, 13 cm or 0 cm distance from the background. When regarded from the artificial decision line, which was place 30 cm in front of the respective Object 1, Object 1 appeared to have the same size as Object 2 on the back wall. The respective size of each object was calculated with the tangent of the angle α (angle α in degrees) (Fig. 9).

The experiment started with two same objects on the back wall and a strong side preference could be observed. After the arrangement of the lightning was changed, no more side preference occurred and four more consecutive series in different distances took place.

In the first series Object 1 was removed 70 cm from the back wall and had the size of 21 cm x 11.7 cm. When Object 1 was placed 50 cm from the back wall it was sized 26.3 cm x 14.6 cm. In 30 cm distance from the wall, it was 35 cm x 19.5 cm and in 13 cm distance from the wall, Object 1 had the size of 48.8 cm x 27.2 cm.

For every distance 40 runs (n=40) with 20 animals (N=20) were conducted and the two objects changed side every 10 runs.

a)



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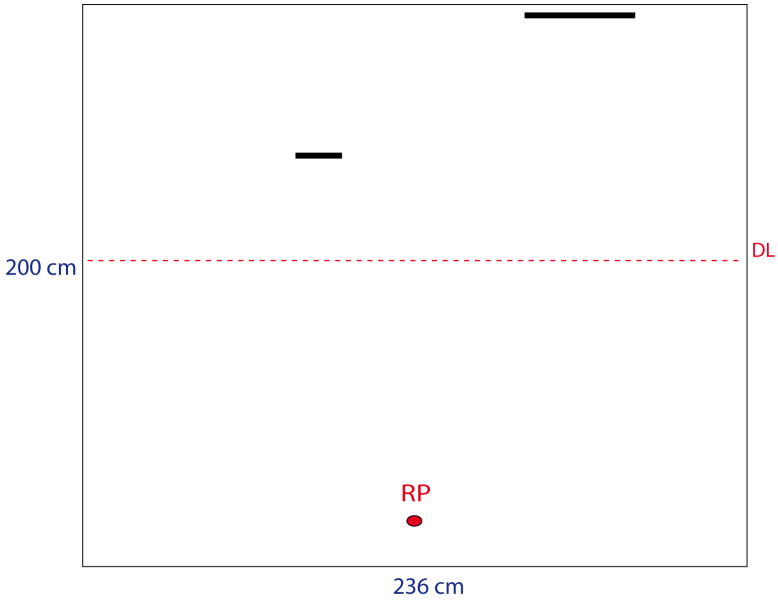


Fig. 8: a) Experimental arena with the structured background, the nearer Object 1 and Object 2 on the back wall. **b)** Dimensions of the experimental arena in the preliminary studies with Object 1 at a distance of 50 cm from the back wall. The decision line (DL) is located 30 cm in front of Object 1. The release point (RP) is also marked.

Calculation

In this experiment the angle α had to be calculated for every distance, because the decision line was 30 cm in front of the respective object. In the first series with Object 1 in a distance of 70 cm, the height of Object 2 (70 cm) was h and d was the distance from Object 2 to the decision line (100 cm). The angle α was calculated with the formula tan α = h/d. This results in an angle α of 35°. With this angle the height of Object 1 (h_1) in the distance of 70 cm could be calculated with the formula $h_1 = d_1$ x tan α, whereas d_1 is the distance between decision line and the respective Object 1 and is always 30 cm (Fig 9).

The width of Object 2 (39 cm) was w, and again d was the distance from Object 2 to the decision line (100 cm). Angle β was calculated with the formula tan β = (2 x w)/d. So, angle β was 38° for Object 1 in a distance of 70 cm and the width of Object 1 (w_1) could be calculated with the formula 2 x $w_1 = d_1$ x tan β, whereat d_1 = 30 cm (distance between Object 1 and decision line) (Fig. 9).

For Object 1 in 50 cm distance, d = 80 cm and therefore α = 41,35° and β = 44.3°, for Object 1 in 30 cm distance, d = 60 cm and α = 49.4° and β = 52.4° and for Object 1 in 13 cm distance, d = 43 cm and α = 58.4° and β = 67°. In all cases, the distance between Object 1 and the middle line was the same as the width of the respective object.

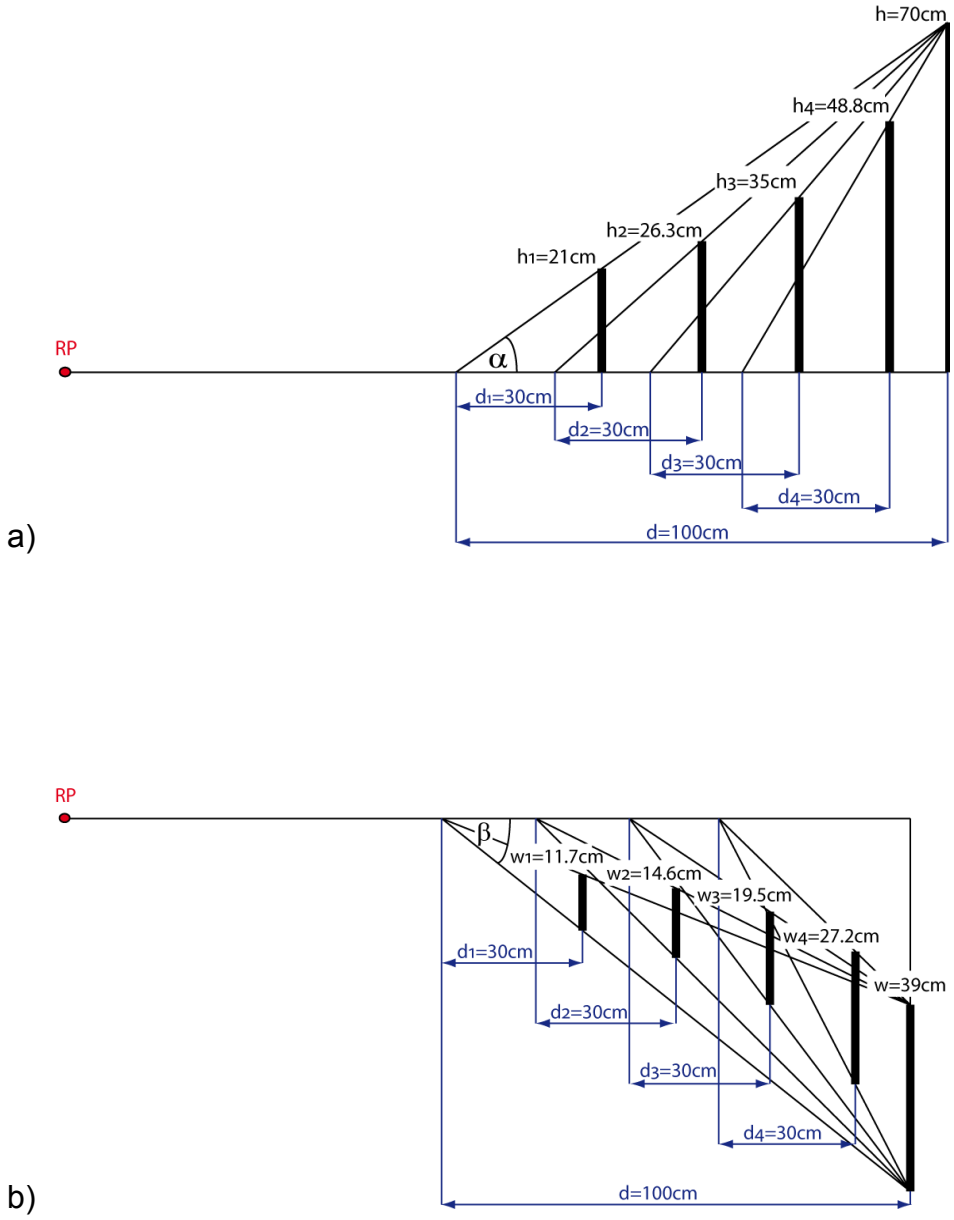


Fig. 9: Schematic view of the calculation of the set-up. **a)** Calculation of the height $h_{1...4}$ of Object 1. RP: release point; α: angle of view from decision line for Object 1 in 70 cm distance from the wall (35°); $h_{1...4}$: height of Object 1 in 70 cm, 50 cm, 30 cm and 13 cm distance from the wall; h: height of Object 2; $d_{1...4}$: distance between decision line and respective Object 1 (30 cm); d: distance between decision line and Object 2 on the wall. **b)** Calculation of the width $w_{1...4}$ of Object 1. RP: release point; β: angle of view from decision line for Object 1 in 70 cm distance from the wall (38°); $w_{1...4}$: width of Object 1 in 70 cm, 50 cm, 30 cm and 13 cm distance from the wall; w: width of Object 2; $d_{1...4}$: distance between decision line and respective Object 1 (30 cm); d: distance between decision line and Object 2 on the wall.

2.2.2. Distance discrimination with a virtual object

Set-up and procedure

This experiment was conducted to test, if the spiders are able to discriminate distances only by motion parallax. Therefore a new experimental set-up had to be established. The structured background was replaced by a plane white wall. Object 2, the object placed directly at the wall, had the size of 70 cm x 39 cm and consisted of the same pattern as the structured background in the previous experiments, namely black and white stripes running diagonal crossing each other with each stripe being 6 cm wide.

Object 1 was established by a box, 1 m high, made of Styrofoam (Fig. 10). In the back of this box, there was the structured background arranged in a semicircle, with stripes 6 cm wide. The front wall had a rectangular opening, through which the spider could go. The edges of the opening were cut sharply. From the spider's view the background pattern was visible through the opening and appeared to be an object (Fig. 10b). If the spider approached the two objects through zig-zag-movement, the edges of the opening in front of the structured background induced a motion-parallax, whereas there was no motion parallax at Object 2 at the wall. So, Object 1 was similar in size but located at the position of the opening and therefore closer to the animal.

The front walls of the boxes were built in 5 different distances (70, 50, 30, 13 and 0 cm) from the back wall. The seperation wall was 30 cm longer than the box and therefore ended where the artificial decision line was located. The decision line was defined to be 30 cm in front of the respective object and therefore changed for every distance, as described by Kosenburger (2005). The respective size of each opening was calculated with the tangent of the angle α, as in 2.2.1. (Preliminary studies) (Fig. 9).

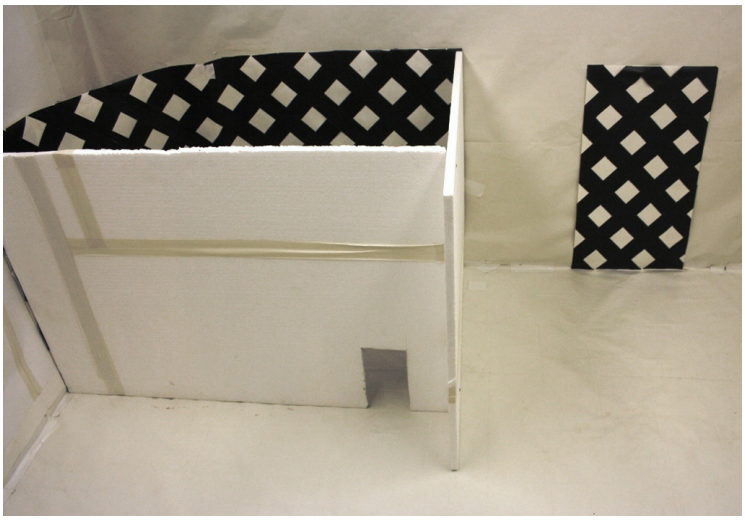
In the first series the front wall of the box was placed 70 cm from the back wall, the opening had the size of 21 cm x 11.7 cm. In the next series, the front wall of the box was placed 50 cm from the back wall and the opening was sized 26.3 cm x 14.6 cm. In 30 cm distance from the wall, the opening was 35 cm x 19.5 cm, in 13 cm distance from the wall, the opening had the size of 48.8 cm x 27.2 cm. In the last series, the front wall of the box was placed directly on the back wall with the structured

background visible through its opening, which was 70 cm x 39 cm like Object 2. This last series took place as a control experiment.

Additionally a control experiment was conducted, with the front wall of the box in a distance of 50 cm from the back wall (opening 26.3 cm x 14.6 cm) and a black background instead of the structured background. Object 2 in this experiment also had no pattern and was plane black.

For every distance 40 runs (n=40) with 20 animals (N=20) were conducted.

a)



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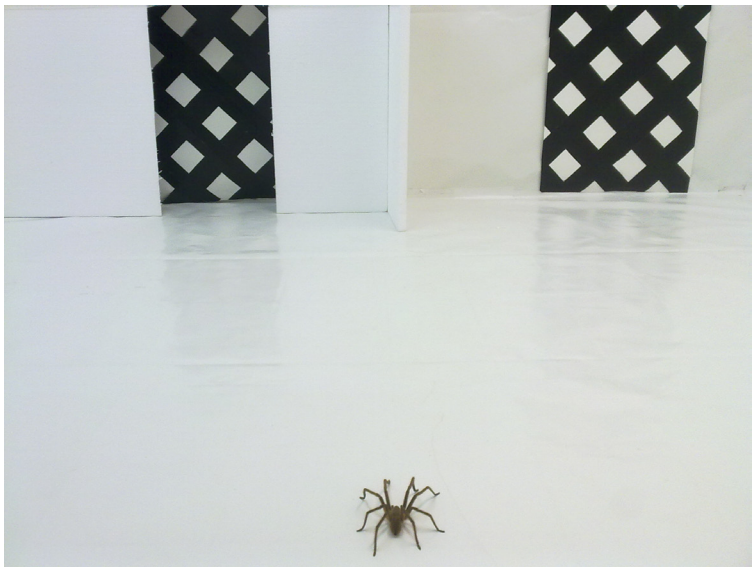


Fig 10: a) Example picture with the front wall of the box in a distance of 50 cm. **b)** Example picture from the spider's view with the front wall of the box in a distance of 30 cm.

2.2.3. Distance discrimination with structured background

Set-up and procedure

Again it should be investigated, if the spiders can discriminate relative distance. The artificial decision line was placed backwards at a distance of 1.30 m from the back wall, as described by Thill (1998). When viewed from this decision line, the two objects appeared to have the same size. Two rectangle objects made of black cardboard were arranged in different distances in front of a structured background.
A wall made of Styrofoam separated the arena in the middle (Fig. 11 and 12). This wall was 70 cm high and 1.30 m long. So, the separation wall reaches until the defined decision line. When walking towards the separation wall, the spider can decide whether to approach the nearer Object 1 or the farther Object 2. When reaching the separation wall and therefore the decision line, both objects appear to have the same size (Fig. 13). After crossing the decision line, the nearer Object 1 is larger than Object 2 on the back wall and the spider can not change its decision due to the separation wall.
The structured background consists of a pattern of black and white stripes running diagonal crossing each other. Each stripe was 6 cm thick, which is equivalent to a visual angle of 2.14° from the decision line. This structured background is necessary to enable motion parallax between object and background.

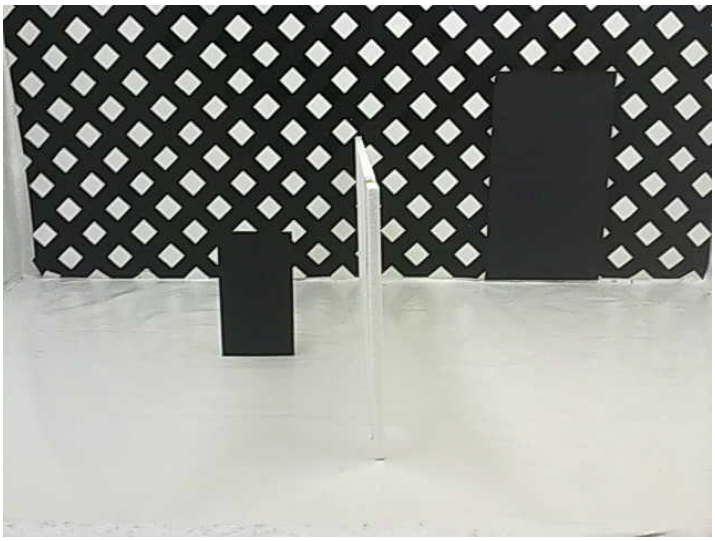


Fig. 11: Example picture of the experimental arena with Object 1 on the left side in a distance of 50 cm from the back wall and Object 2 on the right side.

Four consecutive series of target combinations were tested in this experiment. The more distant object (Object 2) was always placed directly on the structured background with a height of 70 cm and a width of 39 cm. It was placed 39 cm apart from the middle line. The nearer object (Object 1) was presented either in 70 cm, 50 cm, 30 cm or 0 cm distance from the background. The size of this object was calculated to have the same size as the other object, when regarded from the decision line. The respective size of each object was calculated with the tangent of the angle α.

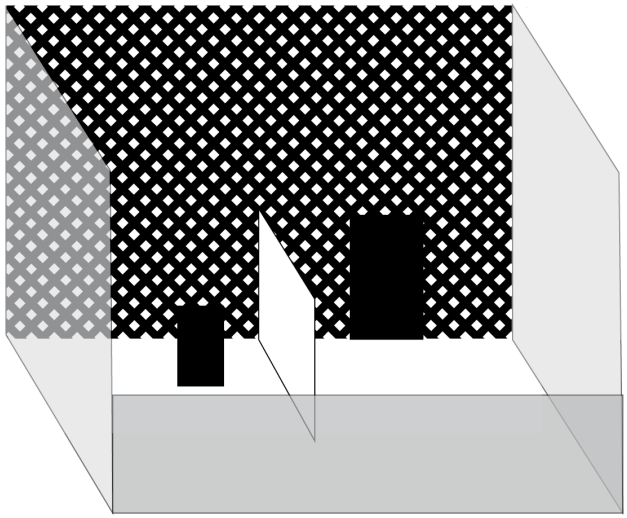
In the first series Object 1 was removed 70 cm from the background, sized 32.3 cm x 18 cm. Secondly, Object 1 was placed 50 cm from the back wall and had the size of 43 cm x 24 cm. In 30 cm distance from the wall, the third stage, the object was 53.8 cm x 30 cm and in the last series Object 2 was placed at the background and has the same size as Object 2 (70 cm x 39 cm). This last series took place as a control experiment. For every distance 40 runs (n=40) with 20 animals (N=20) were conducted and the two objects changed side every 10 runs.

Calculation

The height of Object 2 (70 cm) was h, d was the distance from Object 2 to the decision line (130 cm). The angle α was calculated with the formula $\tan \alpha = h/d$. This results in an angle α of 28.3°. With this angle the height of Object 1 (h_1) in the different distances can be calculated with the formula $h_1 = d_1 \times \tan \alpha$, in which d_1 is the distance between decision line and the respective Object 1 (Fig. 13).

The width of Object 2 (39 cm) was w, and again d was the distance from Object 2 to the decision line. The width w and the distance between the middle wall and Object 2 was the same. So, angle β was calculated with the formula $\tan \beta = (2 \times w) /d$. So, angle β was 31° and the width of Object 1 (w_1) could be calculated with the formula $2 \times w_1 = d_1 \times \tan \beta$ (Fig. 10). In all cases, the distance between Object 1 and the middle line was the same as the width of the respective object.

a)



b)

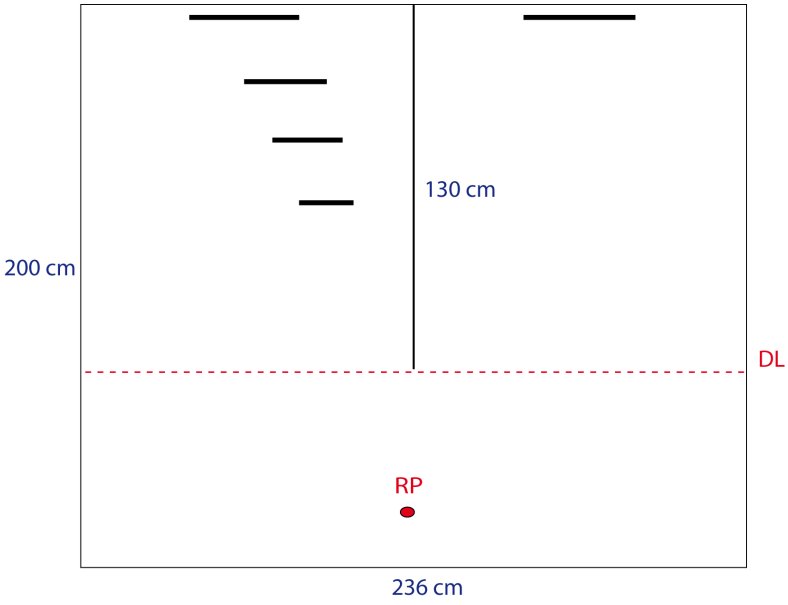
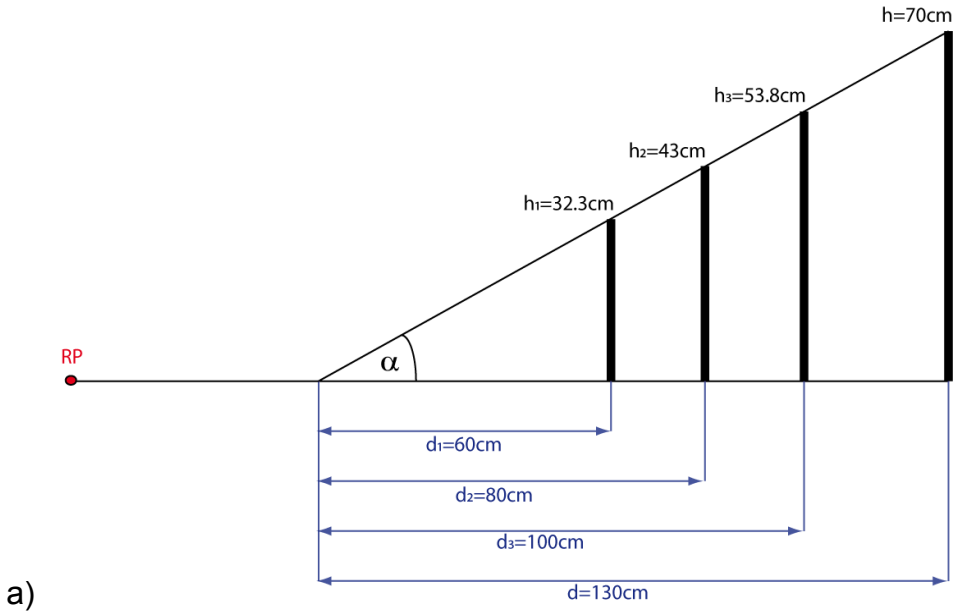


Fig. 12: a) Experimental arena with the structured background, the separation wall in the middle, the nearer Object 1 and Object 2 on the back wall. **b)** Dimensions of the experimental arena and the dividing wall. The release point (RP) and the decision line are also marked.



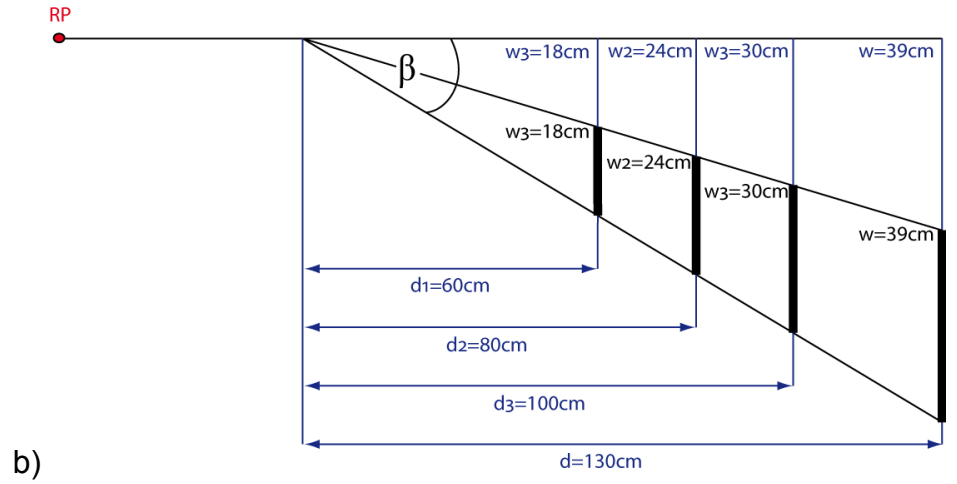


Fig. 13: Schematic view of the calculation of the set-up. **a)** Calculation of the height $h_{1...3}$ of Object 1. RP: release point; α: angle of view from decision line (28.3°); $h_{1...3}$: height of Object 1 in 70 cm, 50 cm and 30 cm distance from the wall; h: height of Object 2; $d_{1...3}$: distance between decision line and respective Object 1; d: distance between decision line and Object 2 on the wall. **b)** Calculation of the width $w_{1...3}$ of Object 1. RP: release point; β: angle of view from decision line (31°); $w_{1...3}$: width of Object 1 in 70 cm, 50 cm and 30 cm distance from the wall; w: width of Object 2; $d_{1...3}$: distance between decision line and respective Object 1; d: distance between decision line and Object 2 on the wall.

2.2.4. Distance discrimination with covered eyes

Covering of the eyes

To cover the eyes of the spiders, the animal's eyes were colored with black paint for model building (Revell, Acryl Paint) (Fig. 14). Therefore the spiders were put into the refrigerator and kept there for about 30 minutes at a temperature of 7°C. The cooled down spiders were then attached to a mushroom-shaped holder and put under a binocular. Either the six secondary or the two principal eyes were covered with two layers of paint. The spiders were illuminated from below to see, if the layers of paint let pass any light. After 30 minutes of drying the spiders were put back into their glass jars. The upper layer of paint was light-blue to be able to observe without a binocular if the paint was tight or scraped off.

a)  b) 

Fig. 14: a) Male spider with colored secondary eyes. **b)** Male spider with colored principal eyes.

Distance discrimination with covered secondary eyes

To test whether the principal eyes are responsible for distance discrimination, the experiment was performed with animals which had all secondary eyes covered. Afresh four consecutive series of target combinations were tested. For every of the four series 40 runs (n=40) were carried out with 8 spiders (N=8) whose secondary eyes were covered. The two objects changed side every 10 runs to control for side preference.

Distance discrimination with covered principal eyes

This experiment was planned to show if the secondary eyes are necessary for the spiders to perceive distance. Again the same experiment with four consecutive series of objects in different distances was carried out with 40 runs (n=40) per distance. This time 8 spiders (N=8) with covered principal eyes were used for the experiment and again the objects changed side every 10 runs.

2.2.5. Distance discrimination without structured background

Set-up and procedure

To investigate whether the spiders are able to detect distance without structured background, the pattern used previously was removed. The background in this experiment consisted of a plane, white wall. As in the prior experiments, a Styrofoam-wall (1.30 m x 0.70 m) separated the arena in the middle and two objects were arranged in different distances. From the artificial decision line at a distance of 1.30 m from the rear wall at the end of the Styrofoam-wall, the two objects appear to have the same size.

As before four consecutive series of target combinations were tested. The nearer object (Object 1) was presented either in 70 cm, 50 cm, 30 cm or 0 cm distance from the background. The more distant object (Object 2) was always placed directly on the back wall with a height of 70 cm and a width of 39 cm. It was placed 39 cm apart from the Styrofoam-wall. This object appeared to have the same size as the other object, seen from the decision line in front of the Styrofoam-wall. The respective size of each object was calculated as described in 2.2.3. (Fig. 13).

In this experiment 40 runs (n=40) with 20 animals (N=20) were made for every object-combination and the objects again changed side every 10 runs.

2.2.6. Luminous intensity

Procedure

In order to investigate if different areas in the arena, especially the inside of the boxes in experiment 2.2.2., differ in their luminance, a luminance meter (LS-100/LS-110) was used. The apparatus was held in a right angle at a distance of 1 m from the measured area. Three measurements of the same area were made and the mean value was calculated.

For every box the luminance of the white and black squares on the background was measured. It was compared to the luminance of the white and black squares of the structured Object 2. Additionally, the luminance of the white squares of the structured background of the arena without any objects was measured on different points, namely in the middle of the background, left, middle-left, middle-right and right. White squares in the low area near the floor were used for this measurement.

3. Results

3.1. Preliminary studies

The first experiment conducted for this work consisted of two same objects both positioned on the structured background. The animals showed a strong side preference ($p<0.01$) (Fig. 15a). The number of runs towards Object 1 was counted to be 3, whereas the number of runs towards Object 2 was 21. There were 9 false runs and 7 panic postures.

After changing the light conditions and Object 1 in a distance of 70 cm from the wall, no difference could be observed (Fig. 15b). Object 1 was chosen 12 times and Object 2 was chosen 14 times. The spiders performed 7 false runs and 7 panic postures. When Object 1 was placed 50 cm afar from the background, it was preferred 14 times and Object 2 on the back wall was chosen 8 times (Fig. 15c). In this series 12 false runs and 6 panic postured were counted. In the next series, with one target in 30 cm distance from the back wall, this object was chosen 9 times by the spiders and Object 2 on the wall was preferred 14 times (Fig. 15d). The spiders showed 8 false runs and 9 runs ended with a panic posture. With Object 1 in 13 cm afar from the back wall, the animals ran towards this object 13 times and preferred Object 2 also 13 times (Fig. 15e). The number of false runs was counted to be 7 and panic posture also was performed 7 times.

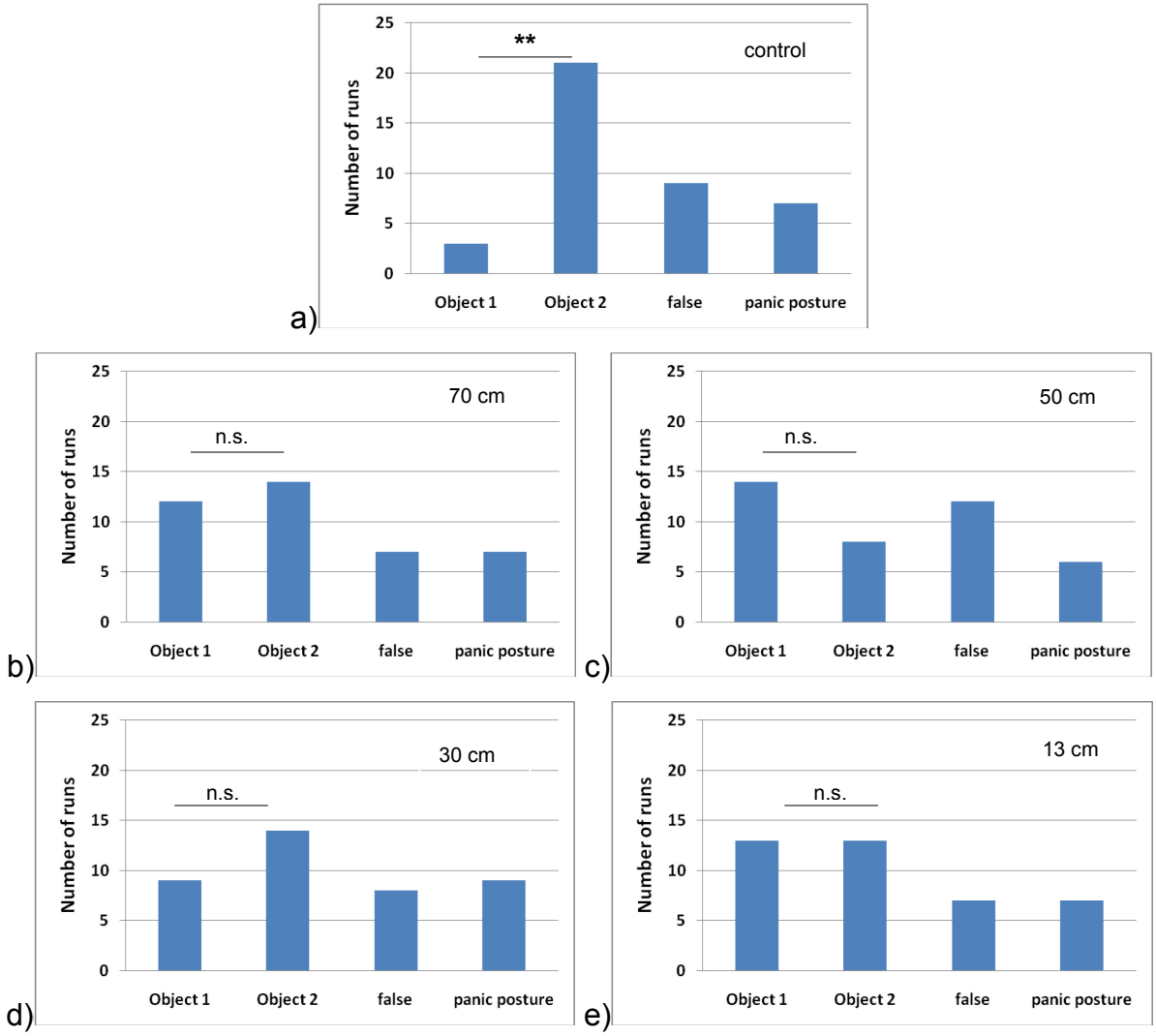


Fig. 15: Numbers of runs towards Object 1, Object 2, false runs and panic postures in the preliminary studies. n=40; N=20; **: $p \leq 0.01$; n.s.: not significant. **a)** Control set-up with Object 1 placed directly on the wall **b)** Object 1 in 70 cm distance from the wall; **c)** Object 1 in 50 cm distance from the wall; **d)** Object 1 in 30 cm distance from the wall; **e)** Object 1 in 13 cm distance from the wall.

3.2. Distance discrimination with a virtual object

When confronted with a virtual object just produced by motion parallax by means of an opening in front of a structured background, the spiders showed a significant difference in the number of runs towards the two targets in most cases. When the opening of the box was arranged to be in 70 cm from back wall, the spiders preferred running towards the opening 16 times, whereas they ran towards Object 2 only 3 times ($p<0.01$) (Fig. 16a). There also were 11 false runs and 10 panic postures. In the second series, with the opening of the box in a distance of 50 cm from the wall, the spiders again did show a significant preference for the box ($p=0.05$). Object 1 was chosen 15 times, Object 2 was chosen 6 times, 10 false runs were performed and panic posture was shown 9 times (Fig. 16b). There was no significant difference in the third series in 30 cm distance, but a trend can be seen. In this series, Object 1 was chosen 15 times, Object 2 was chosen 8 times (Fig. 16c). There were 5 false runs and 12 panic postures. The next situation with the box-opening 13 cm afar from the back wall, again resulted in a significant difference between the number of runs, with a p-value of 0.05. The number of runs towards the box-opening was counted to be 17, the spiders performed 8 runs towards Object 2 on the back wall, 6 false runs and 9 panic postures (Fig. 16d). In the first control experiment, where the front wall of the box was put directly on the back wall of the arena, no significant difference could be shown. The spiders showed 14 runs towards Object 1, 11 runs towards Object 2, showed 8 false runs and 5 runs ended with a panic posture (Fig. 16e). In the second control experiment the structured background of the box and the structured Object 2 were replaced by a black background and a black object and again no significant difference could be found, but a strong trend could be seen. The spiders preferred to run towards Object 1 10 times, whereas the chose Object 2 19 times. They showed 5 false runs and 4 panic postures (Fig. 16f).

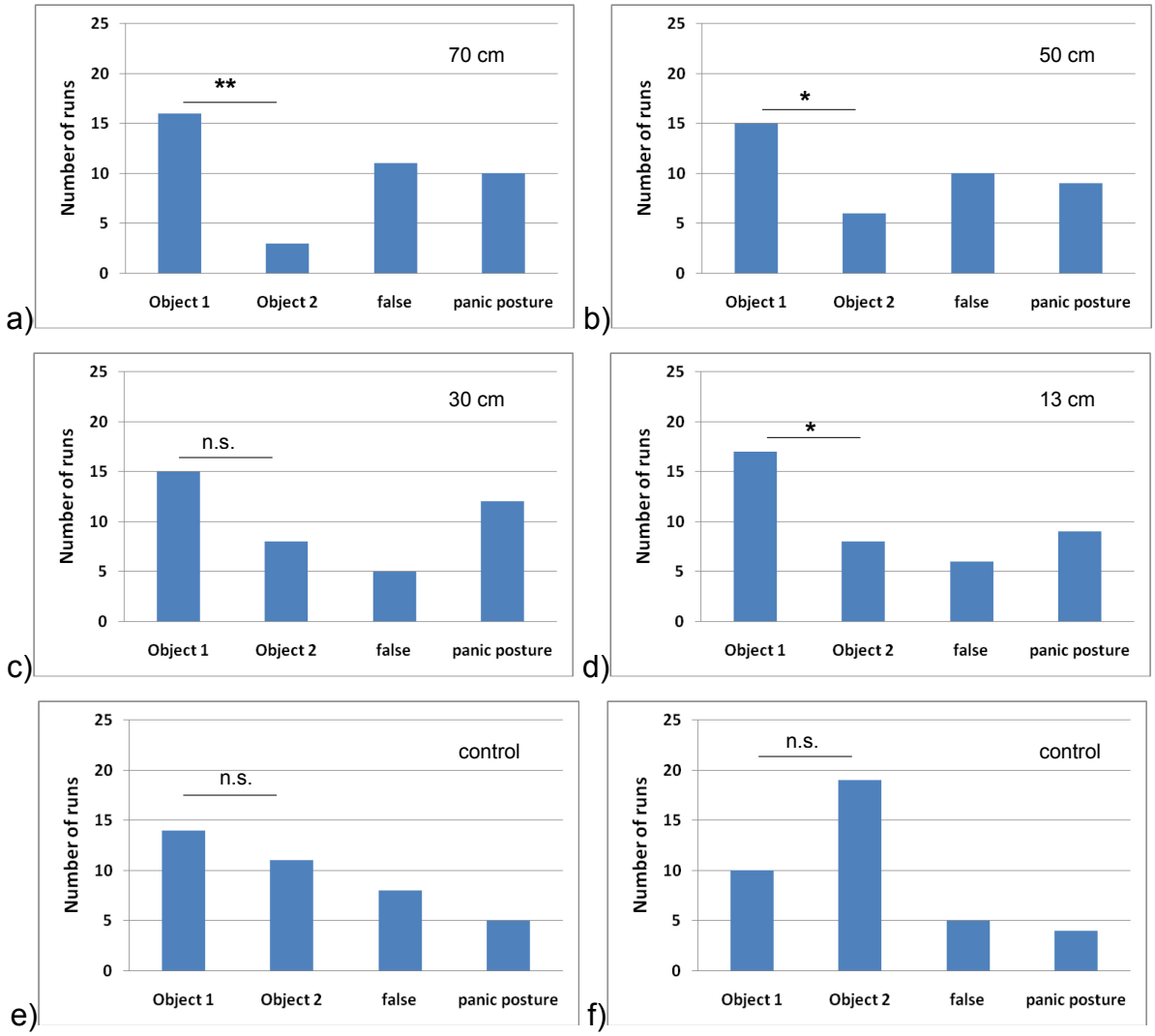


Fig. 16: Numbers of runs towards Object 1, Object 2, false runs and panic postures in the experiment "distance discrimination by motion parallax". n=40; N=20; *: $p \leq 0.05$; **: $p \leq 0.01$; n.s.: not significant. **a)** Object 1 in 70 cm distance from the wall; **b)** Object 1 in 50 cm distance from the wall; **c)** Object 1 in 30 cm distance from the wall; **d)** Object 1 in 13 cm distance from the wall; **e)** Control set-up with Object 1 placed directly on the wall; **f)** Control set-up with Object 1 in 50 cm from the wall and a black background.

3.3. Distance discrimination with structured background

In this experiment the spiders approached Object 1 significantly more often than Object 2 in all three distance combinations (Fig. 17). When Object 1 was placed at a distance of 70 cm from the back wall, it was chosen 22 times and Object 2 was chosen 8 times (Fig. 17a). This is a significant difference with a p-value of 0.01. In these experiments there were 7 false runs and 3 runs ended with a panic posture. With Object 1 at a distance of 50 cm from the back wall, Object 1 was approached 21

times and Object 2 was chosen 10 times (Fig. 17b), the difference remained significant with p=0.05. This time 6 false runs and 3 panic postures were performed. In the third series with Object 1 at a distance of 30 cm from the back wall, the p-value also was 0.05, with 20 runs to Object 1 and 9 runs to Object 2 (Fig. 17c). The spiders showed 8 false runs and 3 panic postures in this set-up. In the control experiments, where both objects were arranged directly at the wall, the spiders showed no difference in their preference (Fig. 17d). They were attracted 13 times by Object 1 and 15 times by Object 2. There were 8 false runs and 3 panic postures.

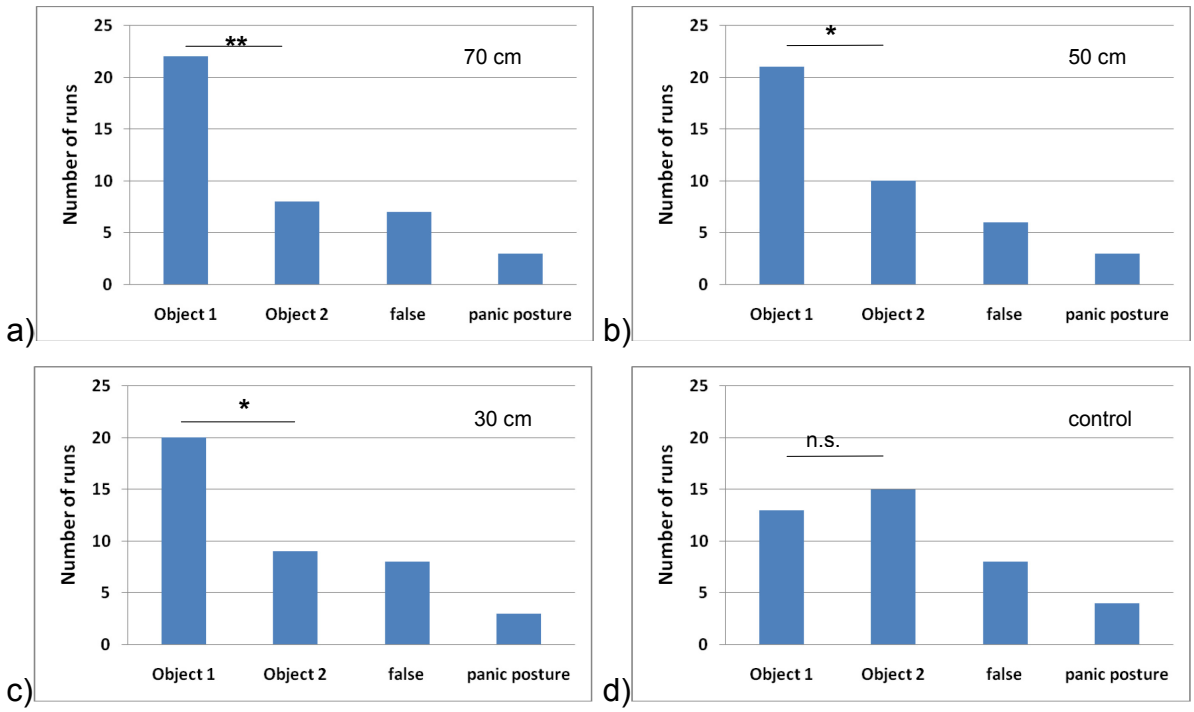


Fig. 17: Numbers of runs towards Object 1, Object 2, false runs and panic postures in the experiment "distance discrimination with structured background". n=40; N=20; *: p ≤ 0.05; **: p ≤ 0.01; n.s.: not significant. **a)** Object 1 in 70 cm distance from the wall; **b)** Object 1 in 50 cm distance from the wall; **c)** Object 1 in 30 cm distance from the wall; **d)** Control set-up with Object 1 placed directly on the wall.

3.4. Distance discrimination with covered eyes

Distance discrimination with covered secondary eyes

With all three pairs of secondary eyes covered and only perceiving visual information with their principal eyes, the spiders again showed a significant preference for Object 1, the nearer one, compared to Object 2, which was again in all experiments located directly on the back wall. The animals favored Object 1 in 19 runs and Object 2 in only 7 runs in the first series, when Object 1 was arranged in 70 cm distance from the background (Fig. 18a). The p-value in this case was 0.02. In this set-up, there were 9 false runs and 5 runs ended with a panic posture. In the next series with Object 1 at a distance of 50 cm from the wall, the p-value was 0.05, with 17 runs to Object 1 and 8 runs to Object 2 (Fig. 18b). There were 9 false runs and 6 panic postures. When Object 1 was placed at a distance of 30 cm from the back wall, Object 1 was chosen 17 times and Object 2 was chosen 7 times (Fig. 18c), which means that the p-value is 0.05. They showed 9 false runs and 7 panic postures. In this experiment, again a control set-up was conducted, where both Objects are placed directly at the wall. Here, the spiders did not show any difference and preferred Object 1 in 11 runs, Object 2 in 14 runs, performed 8 false runs and 7 panic postures (Fig.18d).

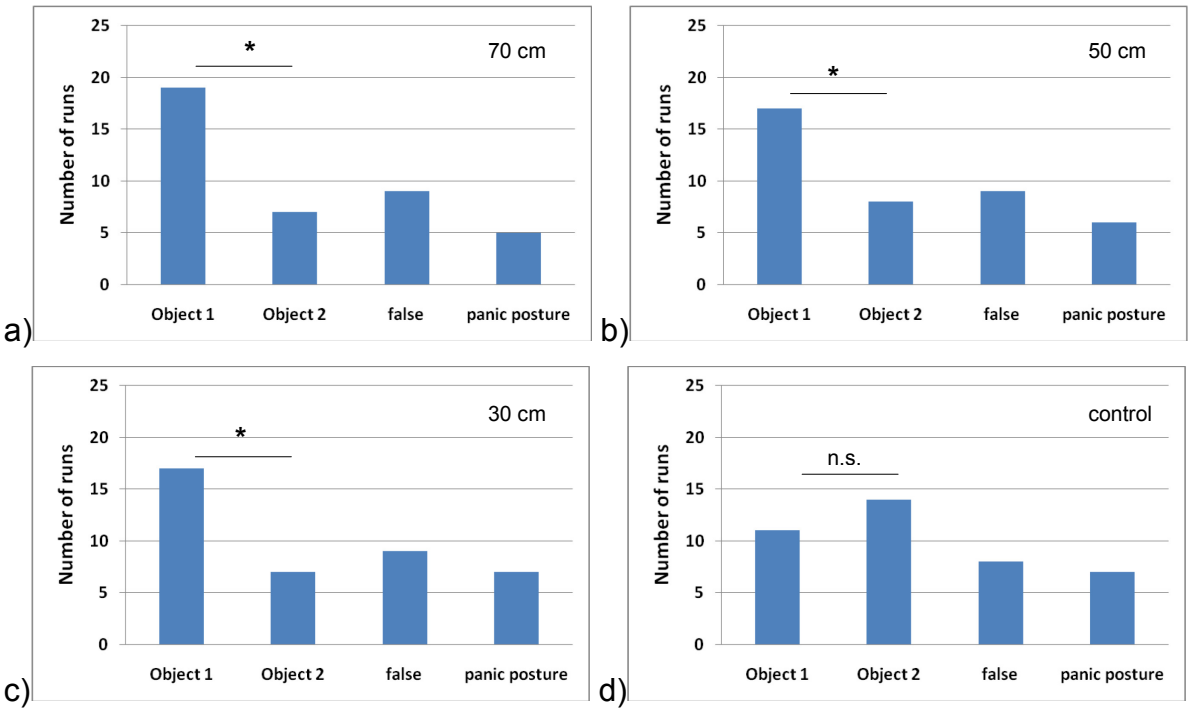


Fig. 18: Numbers of runs towards Object 1, Object 2, false runs and panic postures with covered secondary eyes. n=40; N=8; *: $p \leq 0.05$; n.s.: not significant. **a)** Object 1 in 70 cm distance from the wall; **b)** Object 1 in 50 cm distance from the wall; **c)** Object 1 in 30 cm distance from the wall; **d)** Control set-up with Object 1 placed directly on the wall.

Distance discrimination with covered principal eyes

The spiders did not show a significant preference towards either the closer or the other object, when their principal eyes were covered and they were able to perceive their environment with their secondary eyes only. In the first series, when Object 1 was arranged at a distance of 70 cm, the number of runs towards Object 1 was counted to be 14, and 10 runs towards Object 2 (Fig. 19a). Besides, there were 9 false runs and 7 panic postures. When Object 1 was arranged in 50 cm distance from the back wall, the spiders ran towards Object 1 in 15 cases and towards Object 2 in 10 cases (Fig.19b). Also 10 times they performed a false run and 5 times they showed a panic posture. In the third series with Object 1 in a position of 30 cm distance from the wall, Object 1 was chosen 14 times and Object 2 was chosen 12 times (Fig. 19c). The spiders performed 10 false runs and showed the panic posture

in 4 cases. In the control experiment Object 1 was preferred 13 times and Object 2 was chosen 10 times (Fig. 19d). In this experiment there were 9 false runs and 8 runs ended with a panic posture.

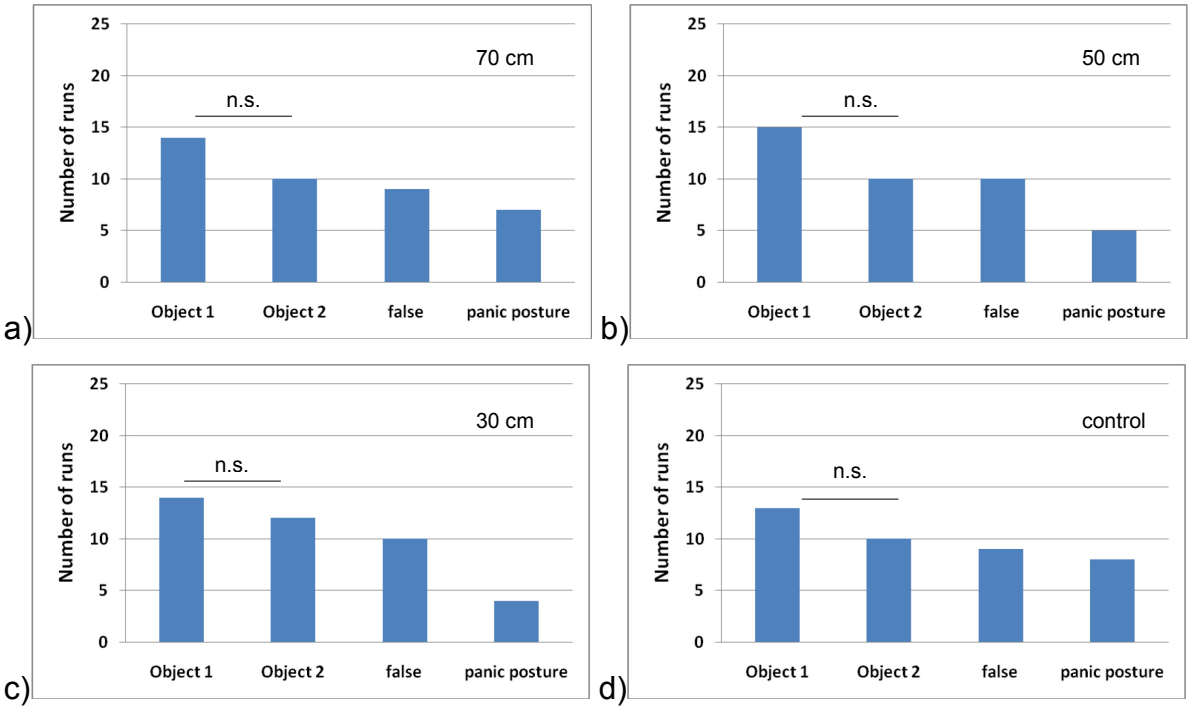


Fig. 19: Numbers of runs towards Object 1, Object 2, false runs and panic postures with covered principal eyes. n=40; N=8; n.s.: not significant. **a)** Object 1 in 70 cm distance from the wall; **b)** Object 1 in 50 cm distance from the wall; **c)** Object 1 in 30 cm distance from the wall; **d)** Control set-up with Object 1 placed directly on the wall. In all four experiments no significant difference could be found.

3.5. Distance discrimination without structured background

In this experiment, where the structured background was removed, the spiders did not distinguish between the two targets. In Figure 20a, one can see that in the first series with Object 1 being in 70 cm distance from the plane back wall, Object 1 was preferred 18 times, Object 2 was preferred 14 times, 4 false runs and 4 panic postures were shown. When the nearer target was placed 50 cm from the wall, the spider ran towards Object 1 in 14 runs and towards Object 2 in 16 runs (Fig. 20b).

There were 6 false runs and 4 panic postures. In 30 cm distance, the number of runs towards Object 1 was counted to be 16 and towards Object 2 there were 15 runs (Fig. 20c), with 6 false runs and 3 panic postures. In the control experiment the situation was similar with 15 runs towards Object 1, 14 runs towards Object 2, 8 false runs and 3 panic postures (Fig. 20d).

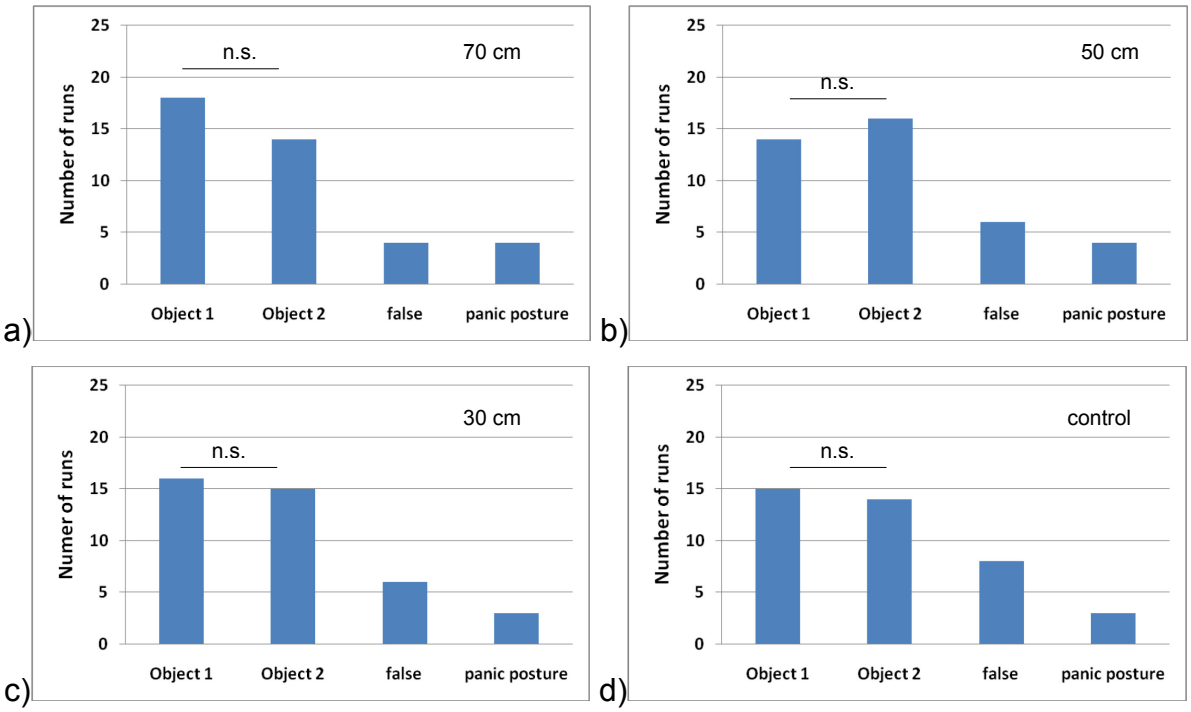


Fig. 20: Numbers of runs towards Object 1, Object 2, false runs and panic postures in the experiment "distance discrimination without structured background". n=40; N=20; n.s.: not significant. **a)** Object 1 in 70 cm distance from the wall; **b)** Object 1 in 50 cm distance from the wall; **c)** Object 1 in 30 cm distance from the wall; **d)** Control set-up with Object 1 placed directly on the wall. In all four experiments no significant difference could be found.

3.6. Luminous intensity

The box made of Styrofoam in experiment 2.2.2. (Distance discrimination with a virtual object) had a structured background, which could be observed through the opening of the box. The luminous intensity of white and black squares of this background and of white and black squares of the structured Object 2 on the back wall was measured.

The measurements show that a white square of the structured background in the box is lower than of a white square of the structured Object 2 on the back wall (Tab.1). When the opening of the box is arranged at a distance of 70 cm, the luminous intensity of a white square of the structured background in the box is 84.1 cd/m^2. When arranged at a distance of 50, 30 and 13 cm, the luminous intensity of the white squares inside the box only slightly varies between 65.8 and 67.7 cd/m^2. When the box is put directly on the wall, the white square of the structured background of the box has a luminous intensity of 101.8 cd/m^2. The white square of Object 2 on the back wall slightly varies between 110.3 and 114.9 cd/m^2.

A black square of the structured background inside the box, otherwise, does not vary in its luminous intensity as much throughout the experiment. It amounts between 2.2 and 5.4 cd/m^2 and is only slightly lower than a black square of the structured Object 2, which was between 5.2 and 7.2 cd/m^2.

Additionally the luminous intensity of white squares of the structured background of the arena without any objects was measured. The measurements show that the left and the right side do not vary much in their luminous intensity (Tab.2). Near the corners they are slightly dimmer than towards the middle of the background.

Tab. 1: Luminous intensity in cd/m^2 of white and black squares in the box in 70 cm, 50 cm, 30 cm, 13 cm and 0 cm distance from the back wall and of white and black squares of Object 2 on the back wall.

Distance box	White square in box	White square of object	Black square in box	Black square of object
70 cm	84.1	114.9	5.2	6.7
50 cm	65.8	113.7	3.9	5.2
30 cm	67.7	114.7	3.6	6.9
13 cm	63.6	113.8	2.2	7.2
0 cm	101.8	110.3	5.4	6.1

Tab. 2: Luminous intensity in cd/m^2 of white squares of the structured background on the left side, middle-left, middle, middle-right and right side of the arena.

	left	middle-left	middle	middle-right	right
Structured background	77.2	96.3	89.1	97.6	87.6

4. Discussion

The data gathered in these experiments lead to the conclusion that the principal eyes are in charge of distance detection in *Cupiennius* salei. In the experiment with structured background it was investigated, if the spiders are able to discriminate between two objects at two different distances. Object 1 was closer to the spider but appeared to have the same size as Object 2 at a distance of 130 cm from the back wall. At the release point Object 1 is much smaller than Object 2. When approaching the targets, the size of Object 1 grows faster than the size of Object 2. Additionally the spiders perform a zig-zag-movement when walking towards the objects. It was suggested, that the animals produce a motion parallax to estimate the absolute and relative distances, as it was shown in mantids and locusts (Wallace 1959; Sobel 1990; Walcher and Kral 1994; Proteser and Kral 1995; Kral and Proteser 1997). It could be shown that the spiders are able to distinguish between two objects in different distances, as Nina Thill did in 1998 (Thill 1998). The p-value was best ($p \leq 0.01$), when the closer object was in 70 cm distance from the wall, but the p-value did not decrease with the smaller distance between Object 1 and the back wall. This is on the contrary to Thill (1998). There, the spiders also perform better, when the nearer object is in 70 cm from the back wall, but their performance constantly decreases with the decreasing distance between the nearer object and the back wall. (Thill 1998). In this study the p-value was the same in 50 and 30 cm distance ($p \leq 0.05$) (Fig. 17). Without the structured background, there were no significant differences between the number of runs towards Object 1 and Object 2 (Fig. 20). Therefore, it can be assumed that the spidera relative motion between object and a structured background to detect the relative distance.

With covered secondary eyes the spiders were still able to discriminate between the two objects in front of a structured background (Fig. 18). Object 1 was chosen slightly more often with all eyes uncovered than with covered secondary eyes. The total number of runs from all three distances towards Object 1 was 63 with all eyes uncovered and 53 with covered secondary eyes (Tab. 3). When the principal eyes were covered, there was no significant difference. Although there were no significant results, they chose the closer Object 1 slightly more often than Object 2 on the wall (Fig. 19).

It could be possible that the spiders normally use all eyes to discriminate distance and therefore show a slightly better performance with all eyes uncovered. As shown by Neuhofer et al. (2009) the AM eye-muscles only respond to movement of a stimulus, if it is presented within the visual field of the secondary eyes (Neuhofer et al. 2009). If motion parallax is a major cue for depth perception in *Cupiennius salei*, also the secondary eyes should be involved in distance detection.

Tab. 3: Number of total runs towards Object 1, towards Object 2, false runs and panic postures in all experiments. The total number was calculated from the three results of Object 1 in 70 cm, 50 cm and 30 cm from the back wall. n=120; N=20;**: $p \leq 0.01$;

Experiment	Object 1	Object 2	false run	panic posture
preliminary studies	39	35	26	20
virtual object	46 **	17	26	31
structured background	63 **	27	21	9
covered secondary eyes	53 **	22	27	18
covered principal eyes	45	32	29	16
without structured background	48	45	16	11

False runs and panic posture

In the experiments with and without structured background, the total number of false runs was 21 with structured background and 16 without structured background (Tab. 3). A possible explanation for this could be that the spiders also accepted the stripes at the background as a appropriate target at small distances and therefore touched the black stripes of the back wall instead of the black object. This would lead to more false runs than with a plane white back wall. The total number of panic postures was 9 with structured background and 11 without structured background (Tab. 3).

The covering of the eyes increased the number of false runs and panic postures. There was a total of 27 false runs among the spiders with covered secondary eyes and a total of 29 with covered principal eyes (Tab. 3). The total number of panic postures nearly doubled. There were 18 panic postures in total when the secondary eyes were covered and 16 panic postures when the principal eyes were covered (Tab. 3). This could also be a possible explanation for the fact that the spiders chose Object 1 a little more often in the experiment with structured background and all eyes

uncovered. Because the spiders were distracted by their covered eyes, they showed more false runs and panic postures and hence less positive runs towards Object 1.
Both false runs and panic postures were very high in the experiment with the virtual object and in the preliminary studies. This could be, because these experiments were the first ones for the spiders and therefore the spiders had to get used to being outside the glass jar and walking on the floor. However, panic postures are extremely high in the virtual object experiment, which is in line with previous studies (Kosenburger 2005). Therefore one can conclude that the less attractive an object appears to the spider the more false runs and panic postures occur.

Luminous intensity

The first experiment of the preliminary studies explicitly shows that the illumination of the experimental arena has to be fairly equal for the spiders in order to run towards the presented targets, because they seem to prefer the darker area of the arena (Fig. 15a). The measurements of the luminous intensity of the structured back wall show that the intensity slightly varies (Tab. 2). The luminous intensities on the left and on the right corner differ a little, but it seems that this small extent does not influence the spiders, because after the first experiment no side preference was found. The background between the middle and the corners (middle-left and middle-right) is brighter than in the middle and the corners. This is due to the two fluorescent tubes diagonally installed above the arena.
Table 1 illustrates that especially the white square of the background inside the box appeared to be dimmer than the white squares of the structured Object 2 on the back wall. Because it is known that the spiders are sensitive to luminous intensity, this could be the reason for the spiders to prefer the virtual object (Fig. 16). This theory is underlined by the fact that they did not prefer Object 1 in the preliminary studies (Fig. 15), which was calculated like the virtual object and therefore had the same size.
The fact that the spiders preferred the box because of the dimmer illumination was excluded by Kosenburger in her studies, because in her experiments the animal did not prefer any target in the control experiment with the black box. However, the luminous intensity was not measured throughout these experiments (Kosenburger 2005).

Virtual object with black background

The spiders seem to prefer, if not significantly, Object 2 on the back wall in the virtual object experiment when it is black and also the background of the box is black (Fig. 16f). As Table 1 shows, the black parts of the virtual object and Object 2 on the back wall do not differ much in their luminous intensity. It can be suggested that therefore the spiders were not prone to prefer by illumination in this situation. The virtual object with the black background was arranged at 50 cm distance from the back wall.
This experiment should be comparable to the preliminary studies, but in this same distance (50 cm from the back wall), the spiders did not prefer any object (Fig. 15c). The spiders prefer Object 2 on the back wall only when there is no structured background, like in the virtual object experiment. It seems that the spiders decide only by size and not by distance in this experiment, because there is no background, and therefore prefer the much bigger Object 2 on the back wall.
This is not true for the preliminary studies. Because there is a structured background, the spiders can also include distance (Fig. 15). Anyhow, the animals do not prefer the nearer object in the preliminary studies, probably because it was calculated to be too small. This was due to the decision line, which was located 30 cm in front of the nearer object.

Decision line

In the preliminary studies and in the virtual object experiments, Object 1 was calculated like in Kosenburger's experiments (Kosenburger 2005). In these experiments Object 2 is bigger than Object 1 until the animals are at a distance of 30 cm in front of Object 1 (decision line). Until this point most of the spiders had already made their decision. When observed from the release point, Object 2 was much bigger than Object 1. However, the animals do not seem to decide by size, because in the preliminary studies, the spiders did not prefer Object 2 on the back wall (Fig. 15b-f). If they had only chosen by distance, they would have preferred the closer Object 1. With the decision line rather close to Object 1, the spiders seem to be confused and alternate their strategies.

The decision line was placed backwards and stayed the same throughout the remaining experiments at a fixed distance of 130 cm from the back wall and therefore 60 cm in front of Object 1, with this object positioned at a distance of 70 cm from the back wall. The closer Object 1 was calculated and relatively bigger in size than in the previous experiments. This, additionally to the dividing wall in the middle, changed the animal's behavior. The size seemed to play a minor role and the spiders decided to prefer the closer target (Fig. 17). Thus, the position of the artificial decision line has to be considered during the whole experiments.

Outlook

The aim of this study was to investigate, whether the principal or the secondary eyes are in charge of distance discrimination in *Cupiennius salei*. It could also be shown, that a structured background is necessary for the spiders to detect distance. The structured background used here consists of diagonal stripes and therefore the zig-zag movement of the spiders creates an up and down movement of the stripes at the edge between object and background. The next step would be to investigate which features a background has to provide to ensure distance discrimination. In following experiments a checkerboard structure will be used to produce only coverage and no up and down movement.

Furthermore, it has to be mentioned that this experiments only dealt with walking spiders. In their natural environment the spiders spend most of their time sitting and waiting. It is not clear if and how they are able to detect the distance of prey only by visual stimuli. They are able to detect prey and jump towards it only by visual input (Fenk et al. 2010), but it is not clear if they also detect the absolute distance to the prey, as mantids and locusts do through their peering movements (Wallace 1959; Sobel 1990; Walcher and Kral 1994; Proteser and Kral 1995; Kral and Proteser 1997).

5. Summary

The nocturnal hunting spider *Cupiennius salei* has four pairs of simple camera-type eyes, which can be classified due to their morphology in principal and secondary eyes. The principal eyes, or anterior median eyes (AME), are everted eyes, which means that their rhabdomeres point towards the incident light (Land 1985). Their retina can be moved by two eye-muscles per eye (Kaps and Schmid 1996). The anterior lateral eyes (ALE), the posterior median eyes (PME) and the posterior lateral eyes (PLE) are referred to as secondary eyes, which are inverted eyes with their rhabdomeres turned away from the incident light and are equipped with an interference reflector, the tapetum (Land 1985). The two different eye types are known to differ not only in their morphology, but also in their function. While the principal eyes are necessary to distinct visual targets, the secondary eyes are suitable for detecting if a visual stimulus is present and are sufficient to motion detection (Schmid 1998; Neuhofer 2009). It the present study it could be investigated which eyes *Cupiennius salei* uses to detect distance. Therefore a behavioural test was conducted, in which the spiders had to discriminate between two targets in different distances. One object was placed on the back wall of an experimental arena and the other object was positioned in different distances in front of the wall. At a virtual decision line in front of the objects, both appeared to have the same size. The experiments showed that the animals need a structured background to detect distance. Without this pattern of black and white stripes running diagonal crossing each other, the spiders did not show a preference for the closer object. This leads to the suggestion that *Cupiennius salei* uses a self-induced relative motion between background and object, produced by their zig-zag-movement, to obtain distance information. The spiders were able to discriminate between the two objects, if the structured background was present. By covering either the principal or the secondary eyes, it could be shown that the principal eyes are in charge of distance detection.

6. Zusammenfassung

Die nachtaktive Jagdspinne *Cupiennius salei* besitzt vier Augenpaare, die aufgrund ihrer Morphologie in Haupt- und Nebenaugen eingeteilt werden können. Die Hauptaugen, oder AM-Augen (anterior-median), sind everse Augen, das heißt, ihre Rhabdomere sind dem Lichteinfall zugewandt (Land 1985). Ihre Retina kann aufgrund zweier Augenmuskeln bewegt werden (Kaps and Schmid 1996). Die AL-Augen (anterior-lateral), PM-Augen (posterior-median) und PL-Augen (posterior-lateral) werden als Nebenaugen bezeichnet. Sie weisen einen inversen Bauplan auf, mit Rhabdomeren, die dem Lichteinfall abgewandt sind, und besitzen einen Interferenz-Reflektor, das Tapetum (Land 1985). Die beiden Augentypen unterscheiden sich nicht nur in ihrer Morphologie, sondern auch in ihrer Funktion. Während die Hauptaugen notwendig sind, um visuelle Ziele zu unterscheiden, eignen sich die Nebenaugen, um einen visuellen Stimulus zu erkennen und sind in der Lage, Bewegungen wahrzunehmen (Schmid 1998; Neuhofer 2009). In der vorliegenden Studie konnte gezeigt werden, welchen Augentypus *Cupiennius salei* für die Distanzwahrnehmung benutzt. Dafür wurde ein Verhaltenstest ausgeführt, bei dem die Spinnen zwei Objekte in unterschiedlichen Distanzen unterscheiden mussten. Eines der Objekte wurde an die hintere Wand der Versuchsarena platziert, das andere in unterschiedlichen Distanzen vor der Wand. An einer virtuellen Entscheidungslinie vor den Objekten, scheinen sie die gleiche Größe zu haben. Die Experimente zeigten, dass die Tiere einen strukturierten Hintergrund brauchen, um die Distanz detektieren zu können. Ohne diesen Hintergrund, der aus schwarzen und weißen Streifen bestand, die sich diagonal kreuzten, zeigten die Spinnen keine Präferenz für das nähere Objekt. Dies führt zur Annahme, dass *Cupiennius salei* die selbst-induzierte Relativbewegung von Hintergrund und Objekt, die sie durch ihre Zick-Zack-Bewegung hervorruft, benötigt, um Informationen über Distanz zu beziehen. Die Spinnen waren in der Lage, die beiden Objekte zu unterscheiden, wenn der strukturierte Hintergrund vorhanden war. Durch Abdecken der Haupt- oder Nebenaugen konnte gezeigt werden, dass die Hauptaugen für die Distanzwahrnehmung verantwortlich sind.

7. Literature

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