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„Visually elicited attack behaviour

in *Cupiennius salei*“

verfasst von

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1. Introduction

The habitat of *Cupiennius salei* from the family of Ctenidae spans parts of Central America from Mexico, Guatemala, Costa Rica down to Panama. Observations undertaken in their natural habitat have shown that they prefer a temperature between 15°C to 20°C with a relative humidity of 90%. *Cupiennius salei* lives on plants, which have mechanically strong leaves and also provide shelter at its base for instance banana plants or bromeliads (Barth and Seyfarth, 1979).

Those spiders are nocturnal sit and wait predators with their highest activity at night, approximately 30 minutes after sunset when the light intensity is below 0.1lx (Barth and Seyfarth, 1979; Schmitt *et al.* 1990). Due to their active period in the dark it is well documented that they use their mechano-sensory system for prey-catching and courtship behaviour. Stimuli such as airflow, substrate vibrations provide information about the surrounding environment (Barth, 2001).

Concerning the hunting behaviour, spiders sit and wait on leaves, which transfer the vibrations caused by prey and allow them to locate its position. When the prey is close enough *Cupiennius salei* leaps for it (Barth and Seyfarth, 1979).

It is also well-established that just an airflow caused by a flying insect can elicit attack behaviour, even with blinded eyes (Barth *et al.* 1995).

The visual system of this spider species has been underestimated because of its nocturnal activity but recent research has provided evidence that it is well developed and seems to play an important role in different behavioural contexts.

The visual system of *Cupiennius salei* consists of four pairs of eyes, placed in two curved rows and are named after their position. This arrangement is also important for the taxonomic identification of spiders. They are differentiated into two types (Fig.1):

Principal or anterior-median eyes (AM) and secondary eyes, which consist of posterior-median eyes (PM), anterior-lateral eyes (AL) and posterior-lateral eyes (PL).

The PM eyes are the largest ones followed by the PL eyes, both positioned in the posterior row and the AM and AL eyes are located in the anterior row (Land and Barth, 1992).
The two eye types differ in their structure, function, processing of visual information and also in their evolutionary development. Principal eyes originate from lens eyes whereas the secondary ones descend from decomposed compound eyes (Paulus, 1979).

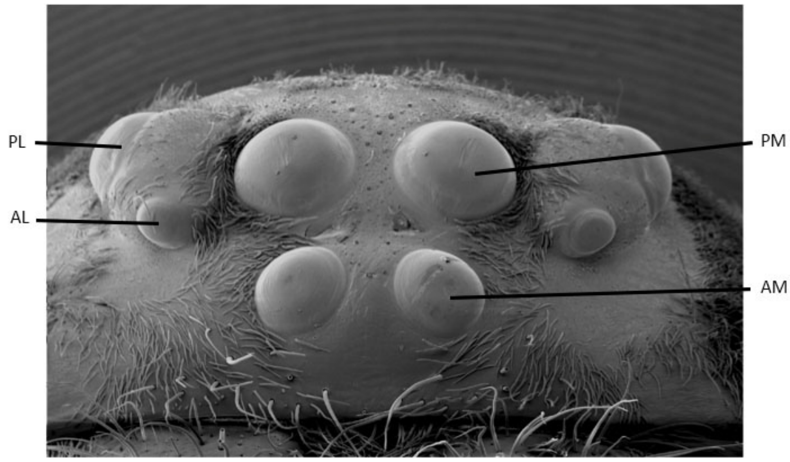

Fig.1: REM-image of the arrangement of the eyes from an adult *Cupiennius salei*. AM…anterior-median, AL…anterior-lateral, PM…posterior-lateral**,** PL…posterior-lateral (Zopf, 2010).

The principal eyes are everted which means the rhabdomeres (=photosensitive part of the eye) are positioned towards the incident light and the cell nuclei close to the rhabdomeres (Grusch *et al.* 1997). The anterior-median eyes are lacking a light reflecting structure named tapetum. They have two eye muscles each which makes it possible to move the retina, the dorsal and ventral eye muscle. The ventral eye muscle is attached at the clypei and leads to the ventro-lateral side of the eye tube, it is activated by mechanical stimulation. The dorsal eye muscle attaches dorso-lateral to the eye tube and travels to the dorso-median carapace and is spontaneously active. If both muscles contract the retina shifts laterally to 15° (Kaps and Schmid, 1996).

Secondary eyes on the other hand are inverted, they lack the ability of a moveable retina and have a tapetum which is a reflecting structure and consists of several layers of guanine crystals. The rhabodmeres are pointing to the tapetum and the major part of the rhabdomeric region is supported by the reflecting structure. As a result light that does not get absorbed gets reflected by the tapetum and passes through the rhabdomeres a second time. By doing so the light available to the photoreceptors is elevated and therefore vision under low-light conditions increases (Grusch *et al.* 1997; Land and Barth, 1992).

Not only do the eyes differ in morphology but also in their visual pathway with two separate sets of optical neuropils which depicts parallel processing of visual information.

As shown in Figure 2 the retina of each secondary eye projects into its own first optic neuropil (ON1). The size of each neuropil corresponds with the size of the eye (PM eyes have the largest first optic neuropil). The first one connects through interneurons with a separate secondary optic neuropil. Finally, all secondary neuropils converge in a third one, the mushroom body.

The visual pathway in the principal eyes is similar, for there are separate first and second order visual neuropils, and a third common optic neuropil which is the central body (Strausfeld and Barth, 1993; Strausfeld *et al*. 1993*)* (Fig.2).

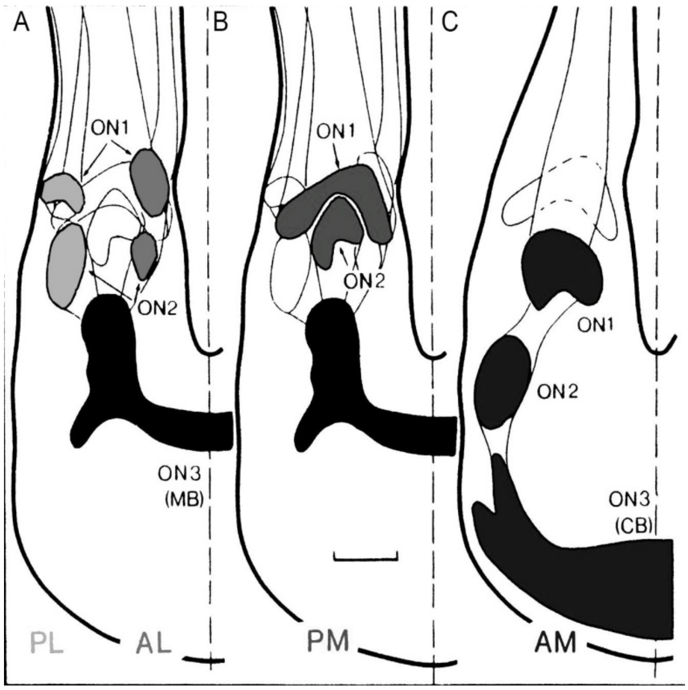

Fig.2: A, B: Dorsal view of the visual system of the secondary eyes (ON1=lamina, ON2=medulla, ON3=mushroom body);
C: Visual system of the principal eyes (ON3=central body);
(Strausfeld and Barth, 1993).

The arrangement of the eight eyes makes it possible to get an overall view of the surrounding area except for a small gap (10-15°) at the rear part of the spider. The field of the AM eyes overlaps with that of the PM eyes to a large extend and both largely cover the upper hemisphere making it possible to see what is happening in the front and partially in the back of them. The AL field is smaller, overlaps with the lower part of PM and PL field and is oriented downwards the front of the chelicerae. There is a small gap between the PL and the PM eyes, the PL eyes provide a view to the side of the animal (Land and Barth, 1992; Kaps, 1998) (Fig.3).

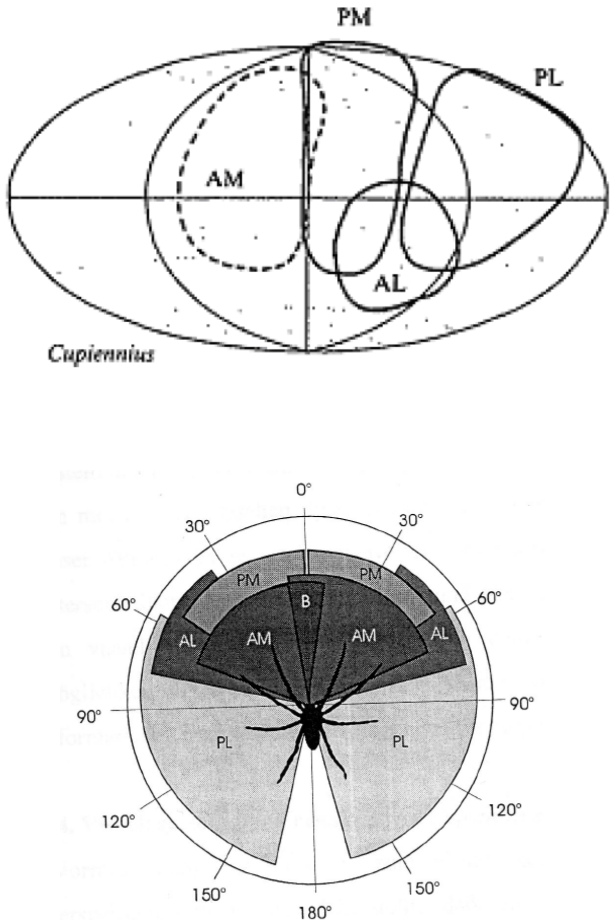

Fig.3: Visual field of *Cupiennius salei*: Overlapping of the AM and PM field to a large part and AM eyes have a small part of overlapping as well; PL eyes are covering a great part sideways of the spider and have a small gap between its fields; AL field overlaps with the lower part of the PM and PL eyes. (Upper image (Land and Barth, 1992), lower image (Kaps, 1998)).

All the differences in morphology and visual pathway and especially the overlapping visual fields of AM and PM eyes, indicate that principal and secondary eyes have different functions. In a study by Schmid in 1998 it was shown that the AM eyes are necessary to discriminate targets. Through covering the principal eyes and only being able to use the PM eyes *Cupiennius salei* was capable to detect targets but could not discriminate between different targets. An electrophysiological study provided data showing that presenting a moving stimulus causes an increase in the eye muscle activity, however, when covering the secondary eyes no response to the stimulus could be observed. This indicates that the secondary eyes are specialized in movement detection and principal eyes in target discrimination (Neuhofer *et al*. 2009). Moving the retina in the AM eyes prevents the neural image from adapting and enables them to detect stationary objects whereas the secondary eyes do not have this ability. The lack of a moveable retina results in an adaption of the secondary eyes. The background dissolves and only moving objects can be perceived (Land and Barth, 1992). Another study indicates that the movement-detecting system in *Cupiennius salei* seems to be colour blind. Experiments have shown that the animals could not discriminate coloured stimuli (green, red, blue) from a range of 29 grey backgrounds (ranging from white to black) (Orlando and Schmid, 2011).

Although the visual system is very well developed and the knowledge about it expands, there are still open hypotheses. For example, if it is possible to elicit a prey catch behaviour by only using visual cues without also stimulating the mechano-sensory system.

Studies focusing on this subject have provided results which give evidence that solely a moving visual stimulus can elicit such behaviour.

Fenk *et al.* in 2010 have tested two different stimuli. For the first experiment a black disc moved along jerky curves on a green background. The appearance of the second stimulus was identical to the previous one but the colours of the disc and background were switched, a green disc was presented on a black background. The data showed a significantly higher response to the black disc on a green background.

In the pursuing study the same stimulus path (Fenk *et al.* 2010) was used but the size and speed of the disc was altered. The stimulus was presented with 3cm, 4cm, 6cm and 8cm in diameter, and three different velocities (slow, medium and fast) were chosen. The increase

in size resulted in higher responses. The preference in speed decreased the slower the disc moved along the path (Lindner, 2013).
A second experiment was conducted in Lindner's study in 2013 and its aim was to find out if there is a bias for the initial appearance of the disc during the presentations. For this purpose four vertical paths with different start and end positions of the disc (centre, bottom or top on the presenting screen) were analysed.

It was already shown that solely using a visual cue could elicit prey-catching behaviour in *Cupiennius salei*. Previous studies proved that presenting a black dot on a green background results in reactions towards it (Fenk *et al.* 2010). Also varying the features of the stimulus like size and speed causes different responses (Lindner, 2013) but there are still more possibilities to modify the stimulus.
In this study the main focus is to gain more information on what kind of characteristics a visual stimulus has to have to elicit a high rate of responses. A specific focus will be put on the slope of the stimulus path in order to find out if there is a preference for a dot moving along horizontal or sloped paths.

2. Material and Methods

2.1. Experimental animals

The test objects of these experiments were 45 sub-adult females of the nocturnal hunting spider *Cupiennius salei*. Those spiders were provided by the Department of Neurobiology`s own breeding. Each individual was kept in a glass jar with the size of 13cm in diameter and 25cm in height. The bottom was filled with peat and a plastic cup filled with water was added to obtain a higher humidity within the jar. They were fed once a week on *Calliphora sp.*.

The surrounding conditions in the experimental room were at a relative humidity of 55% and the temperature varied by 2°C between 23°C and 25°C.The artificial light and dark cycle was set to 12 hours each (12:12).

2.2. Experimental setup

In order to examine if visual cues could elicit attack behaviour an experimental setup (Fig.4) was constructed to exclude behavioural influences through mechanical stimuli.

The setup consisted of two screens (SamsungSyncMaster T220, Samsung Electronics, Seoul, South Korea) separated into four equally sized areas, two Webcams (Logitech Pro 9000, Logitech, Apples, Switzerland) and two Laptops (Hp 6730b, Hewlett-Packard, Paolo Alto, California).

Four jars were positioned in front of the screens at a distance of 24 cm. They were visually separated by polystyrene walls to eliminate interference from individuals tested next to each other at the same time. Additionally, the jars were placed on top of styrofoam blocks to reduce influences in behavioural response through vibrations caused by the research scientist or other external disturbances.

Behind the jars two Webcams were placed to record the behaviour shown by the animals during the experimental trials. The data was saved on two laptops, which in addition provided the presentation of the moving stimuli on the screens.

A visual cover between the area of the research scientist and the rest of the set up prevented possible visual disturbances caused by the movement of the researcher, which could have provoked a reaction from the spiders (Fig.4).

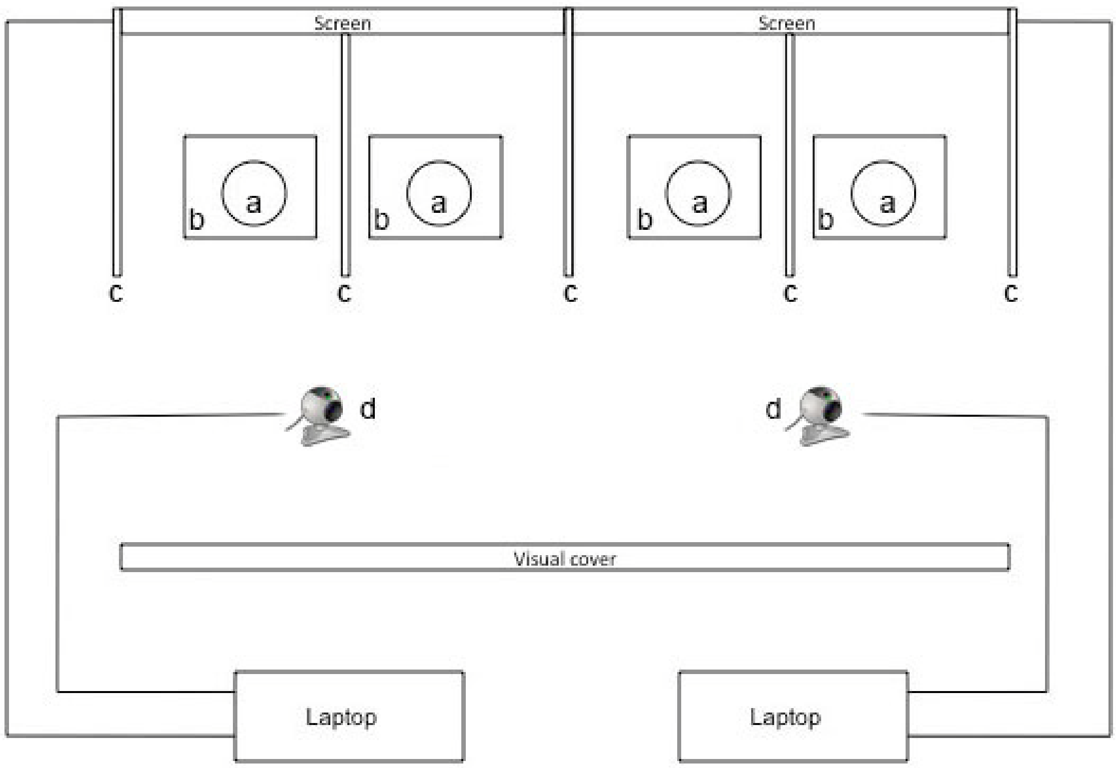

Fig.4: Experimental set up from above: Two screens are separated by polystyrene walls (c) into four equally sized areas. The jars (a) are positioned on top of styrofoam blocks (b). Two Webcams (d) record the behaviour, which gets saved on the laptop. The laptop in addition delivers the stimuli presented on the two screens.
The visual cover separates the rest of the set up from the area of the research scientist.

2.3. Testing procedure

The testing was carried out the same way in all the experiments undertaken in this study: Four jars were placed in front of the screens at the same time. All spiders were positioned in an identical way, their prosoma pointing downwards, eyes facing the screen and their ventral side to the webcam (Fig.5).
Between placing the jars and starting the presentation a delay of 10 minutes was given to

allow the individuals to get used to the screen and calm down. Most spiders got tested no more than twice a day with a resting period of two to three hours in between. The break between the trials was necessary because increasing the number of tests per day meant that fewer reactions could be observed.
The light intensity could be regulated and according to a previous study (Lindner, 2013) the brighter the light the lower the response rate to the stimulus, therefore the light was dimmed to achieve an ambient illumination of 35.2lx.

Not every individual out of the 45 got tested in each experiment because they need to be in a certain state of ecdysis to show any behaviour towards a visual cue. When they are too young *Cupiennius salei* does not react to such a stimulus and when they get closer to their adult stage there is a decrease towards visual cues observable. Data provided by spiders which moulted during the testing phase had to be excluded three days prior and three days after their ecdysis because they would not react to any stimulus during this time.

Fig.5: Position of *Cupiennius salei* before starting the presentation.
The prosoma is pointing downwards and the eyes are facing the screen.

2.4. Stimulus

According to the study by Fenk et al. (2010) *Cupiennius salei* reacts to a visual stimulus consisting of a black dot. Therefore, a stimulus of the same shape was chosen to be used in these experiments.

The stimuli for all the experiments were generated with Microsoft Powerpoint. They consisted of a black dot varying in size and moved along an animated path on a green background. Green was chosen because it is beneficial using one of the three colour channels (green, blue, red) of the screen to decrease the colour variation by facing it from different angles. Especially green was selected because *Cupiennius salei* has a peak in its spectral sensitivity at 520nm which lies within the green spectrum (Barth et al., 1993; Walla *et al.* 1996).

Each presentation lasted 40s in total, during which the stimulus moved along the animated path back and forth 4 times without a break in between. It took the stimulus 5s from the appearance in the left corner to reach the end of its path.

2.4.1. Horizontal stimulus path (size, velocity, position)

The preliminary study (Lindner, 2013) observed that *Cupiennius salei* shows attack behaviour by presenting a black dot on a green screen moving along a vertical path. Therefore, the first experiment was conducted to investigate if the animals also react to a stimulus moving horizontally.

For this first experiment 531 trials were conducted by testing 15 individuals between six and seven months old.

To assess what characteristics might be important preliminary studies were performed. For the assessment 3 dots were used with diameters of 2, 4 and 6cm (Fig.6) and were presented at a distance of 24cm, which corresponds to an angular width of 4.8°, 9.5° and 14.3°.

The three dots were presented moving along horizontal paths and were combined with three different velocities (slow (15.8°/s), medium (23.8°/s) and fast (47.65°/s) speed) and varying also in the positions on screen (bottom, centre and top). The stimulus appeared at the left side and going back and forth four times (Fig.6).

One size of the stimulus with each speed and just one position was tested per day. For example the size of 4cm in diameter at the centre of the screen combined with the three different velocities. Every individual was tested with each speed setting once a day.

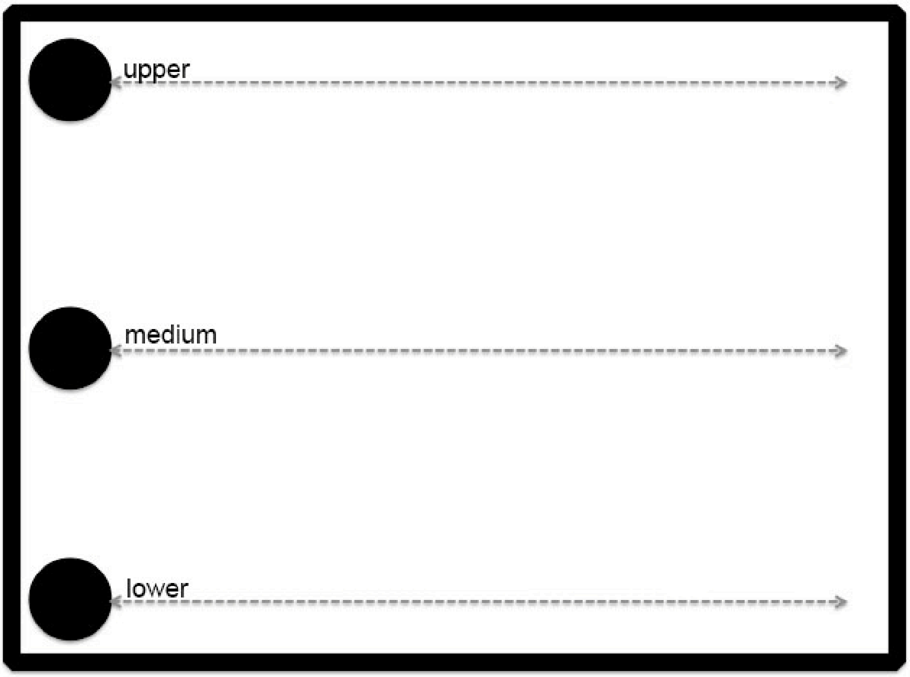

Fig.6: The rectangle including the dots symbolizes one of the four presentation areas. The description lower, medium and upper indicate the three different positions the stimulus was presented.

2.4.2. Sloped stimulus path

The difference between the first experiment and the three subsequent experiments is a change in the orientation of the stimulus path.

Prior to the main experiments preliminary tests were performed to find out if there was a preference in size and velocity for a stimulus moving along a sloped path. Therefore the settings of the first experiment were replicated, but sloped paths were used.

26 individuals (age between six and seven months old) were tested in 584 presentations.

After analysing the preliminary data the main experiments were carried out with a stimulus of 6cm (corresponds to an angular width of 14,3°) and the slowest speed setting.
In the following presentations the dot always appeared at the lower left corner and moved to the end of the sloped path (going back and forth four times).

To determine if the slope of the stimulus path is a crucial characteristic to elicit attack behaviour the following experiments were conducted:
In the first one the dot moved along a sloped path at an angle of 55° (Fig. 7b). 305 trials with 26 individuals were carried out.
Two more experiments were conducted using the same 21 spiders. One path had a nearly horizontal path with a slope of 25° (Fig. 7a), 293 trials were performed in this test.
The other experiment included 292 trials using a path close to vertical angle of 75° (Fig. 7c).

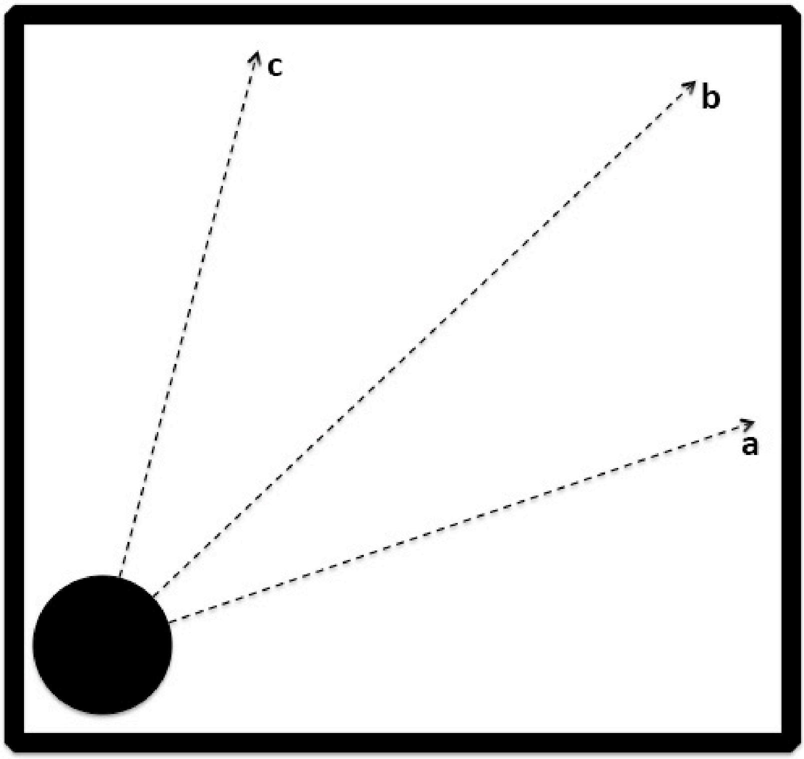

Fig.7: The rectangle symbolizes the presentation area where the stimulus is moving along a sloped path of 25° (**a**), 55° (**b**) and 25° (**c**).

2.5. Latency

The time between the appearance of the dot and the initial response was measured using a stopwatch. Additionally, the distance the stimulus moved along the path until the first response was calculated.

3. Results

3.1. Observed behavioural responses

Throughout this study three major behavioural responses triggered by a visual stimulus could be observed. Those could easily be distinguished because of their specialized sequence of movements.

1. orientate: this one shows the least movement of all. The spider is not actually moving around but it stays in the same place with only its prosoma bending in the direction of the stimulus, it orientates towards the moving dot.
2. following: the animal is running around in its jar, along the glass wall or the ground trying to get close to the stimulus /or tries to catch it.
3. attacking: the spider is jumping (no leg is touching the wall or ground) towards the dot. Also classified as attacking is when the animal has shown a previous behavioural pattern and their ventral side is now facing the screen and they try to "grab" the stimulus by rapidly pulling their first and second pair of legs toward their body.

A fourth behavioural response was detected which could be seen as the "threatening" that has been described in another study (Lindner, 2013) where the spider is lifting its first pair of legs. This type of activity occurred very rarely and it could not be clearly classified. It was not possible to differentiate between threatening behaviour or an aborted following activity.

Before presenting the stimulus the spiders were sitting motionless in their jars. When the dot appeared on the screen and the animals started to present one of the described behaviours, no particular order could be observed. Sometimes only one movement was performed and on other trials a combination of up to 6 separate behavioural responses were shown during one presentation.

For example 2.3 seconds after the appearance of the stimulus the spider performed the following combination of behavioural responses: orientation twice, following, attacking, following and another attack were shown.

3.2. Horizontal stimulus path

In this preliminary experiment the spiders were confronted with a stimulus following a horizontal path where the combination of three different positions, velocities and sizes were presented to see whether there is a preference in any of these characteristics.
The results showed that there is no bias for any of the attributes. In only 6 out of 531 trials a spider responded towards the stimulus. The six positive reactions were performed by the same individual but the stimulus has never had the same combination of characteristics.
After analysing the preliminary results it seemed that *Cupiennius salei* does not consider this kind of stimulus as a possible prey.

3.3. Sloped stimulus path

3.3.1. Preference in size

In the second experiment the features of the stimulus stayed the same except for a change in angle of the slope on the path. The preliminary studies again were conducted with three different sizes and velocities, which in combination followed an animated path with an angle of 55°.

Focusing on the stimulus size presenting the dot with the smallest size (2cm) in diameter there was no behavioural response recorded. With regards to the two bigger ones the reaction rate increased. Confronting the spiders with the medium-sized (4cm) dot 33.46% out of 260 presentations elicited an attack behaviour. As for the largest stimulus (6cm) an even higher response rate could be recorded, 37.9% of the runs showed behavioural response (Tab.1).

The comparison between the number of positive reactions towards the medium-sized dot and the largest one do not show a significant difference but they indicate that a bigger stimulus could elicit a higher response rate (Tab.1).
As a result further experiments were conducted only with a stimulus of 6cm in diameter.

The statistical analysis using a Chi-square test showed that the difference in positive reactions between the two stimuli of 4cm and 6cm were not statistically significant (χ^2=1,080, p≤0,299).

Tab.1: Number of presentations for each of the dot sizes and the response rate to each of the stimuli in number and percentage.

Size of stimulus (cm)	Number of trials	Number of trials with reactions	Percentage of trials with reactions
2	84	0	0
4	260	87	33.4
6	240	91	37.9

3.3.2. Preference in stimulus speed

Looking at the response rate as a function of speed and comparing the medium-sized and largest stimulus in 37 cases out of the 91 positive trials the spiders showed a reaction towards the slowest speed setting and largest dot. Furthermore a slight decrease in reactions was recorded for the other two velocities namely 32 for the intermediate speed and 22 attack behaviours at the fastest speed setting (Fig.8).

Regarding the medium-sized dot in 36 trials out of the 87 positive trials the spiders showed movement at the slowest speed in 27 runs at the intermediate speed and in 24 runs at the fastest velocity (Fig.8).
To sum up for both stimulus sizes the test objects showed an increase in positive runs when presented with the stimulus at a decreased velocity.

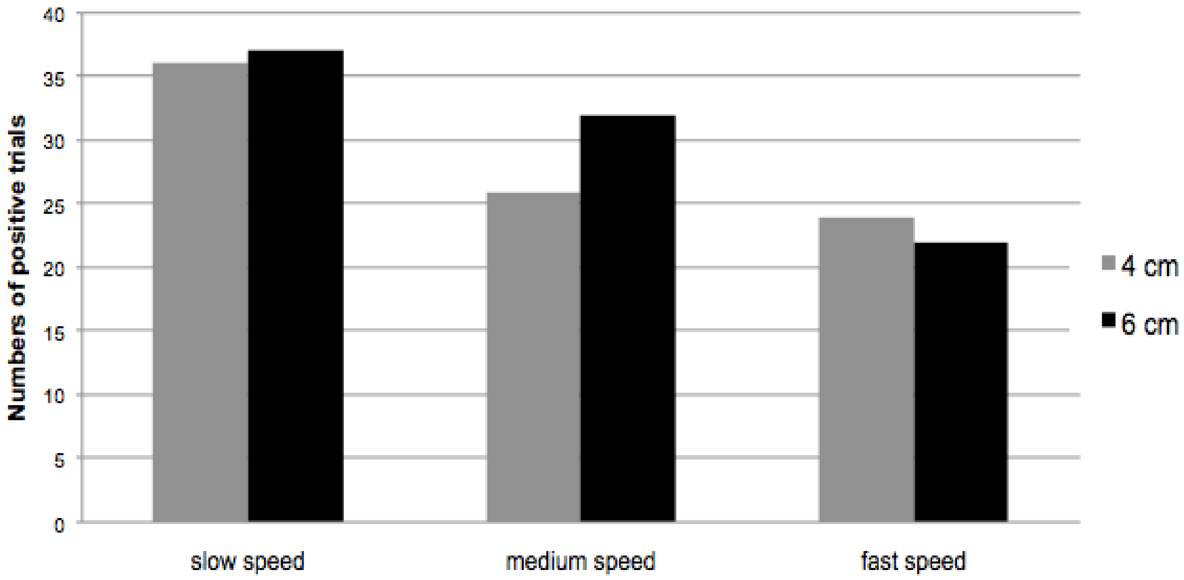

Fig.8: Comparison between the medium-sized and the largest stimulus focusing on the response rate as function of speed. A decrease in positive runs in both dot sizes is noticeable the faster the speed setting.

3.3.3. Slope of stimulus path

To determine if the slope of the stimulus path is a crucial characteristic to elicit an attack behaviour two more experiments have been conducted. The stimulus follows a line with an angle of 25° which is closer to the horizontal axis than in the previous test (55°) and in the second experiment the stimulus travels along a slope of 75°.

Nearly 300 runs have been performed for each of these set-ups which resulted in a response rate of 32.9% for the steeper angle and 65 trials with reactions (22.2%) for the path closer to the horizontal axis. The highest elicited behaviour namely in 44.3% of the presentations resulted by testing the dot along the sloped path of 55° (Tab.2).

Tab.2: The number of trials performed for each slope and the positive response rate in number and percentage.

Slope	Number of trials	Number of reactions	Percentage of reactions
25°	293	65	22.2
55°	305	135	44.3
75°	292	96	32.9

A statistical analysis using a Chi-square test was performed to see if the distinctions in the positive response rates are significant. The results confirm that the differences in the number of elicited movements are significantly different (Tab.3). Comparing the outcomes of 75° to 25° and 75° to 55° lead to a p-value of 0.004 and checking the results of 55° against 25° showed a p-value of 0.001. The outcomes of the statistical analysis confirm that the diversity in the response rate of the experiments with different slopes are statistically significant (Tab.3).

Tab.3: A comparison of each slope with each other to see if the difference in their response rate is significantly different by using the chi-square-test.

Slope	Chi-square	p-value
75° - 25°	8.38	0.004
75° - 55°	8.15	0.004
55° - 25°	32.73	0.001

3.4. Frequency of behavioural types

The amount of recorded behavioural types is distributed differently between the three experiments (Fig.9).

The highest number of positive presentations occurred at a slope of 55° furthermore the highest total number of shown behaviour throughout those 135 trials, namely 362 single reactions have been shown at this gradient. The smallest amount of reactions was recorded at the stimulus path closest to the horizontal axis (25°), which also had the lowest number of positive presentations, only 65 trials recorded response behaviour. In comparison to the intermediate sloped path the steepest one (75°) showed a decrease in positive trials, in 96 presentations 247 single reactions were observed (Tab.4).

The number of behavioural responses corresponds to the quantity of positive presentations (Tab.4).

As for the frequency of behavioural types, the most dominant behaviour throughout the three experiments was attacking. The other two types of behaviour were distributed differently. Following was elicited twice as often as orienting at the slope of 25°. In comparison at 55° orienting was performed more often than following. The stimulus along the sloped path of 75° caused approximately equal numbers of following and orienting (Fig.9A).

Because of the different quantity of spiders tested in each experiment an additional analysis of the frequency of behavioural responses expressed as percentage of total number of reactions is shown in figure 9B. The distribution is the same like mentioned above. Attacking was carried out the most, around 50% in all three experiments, following and orienting occurred differently throughout the trials.

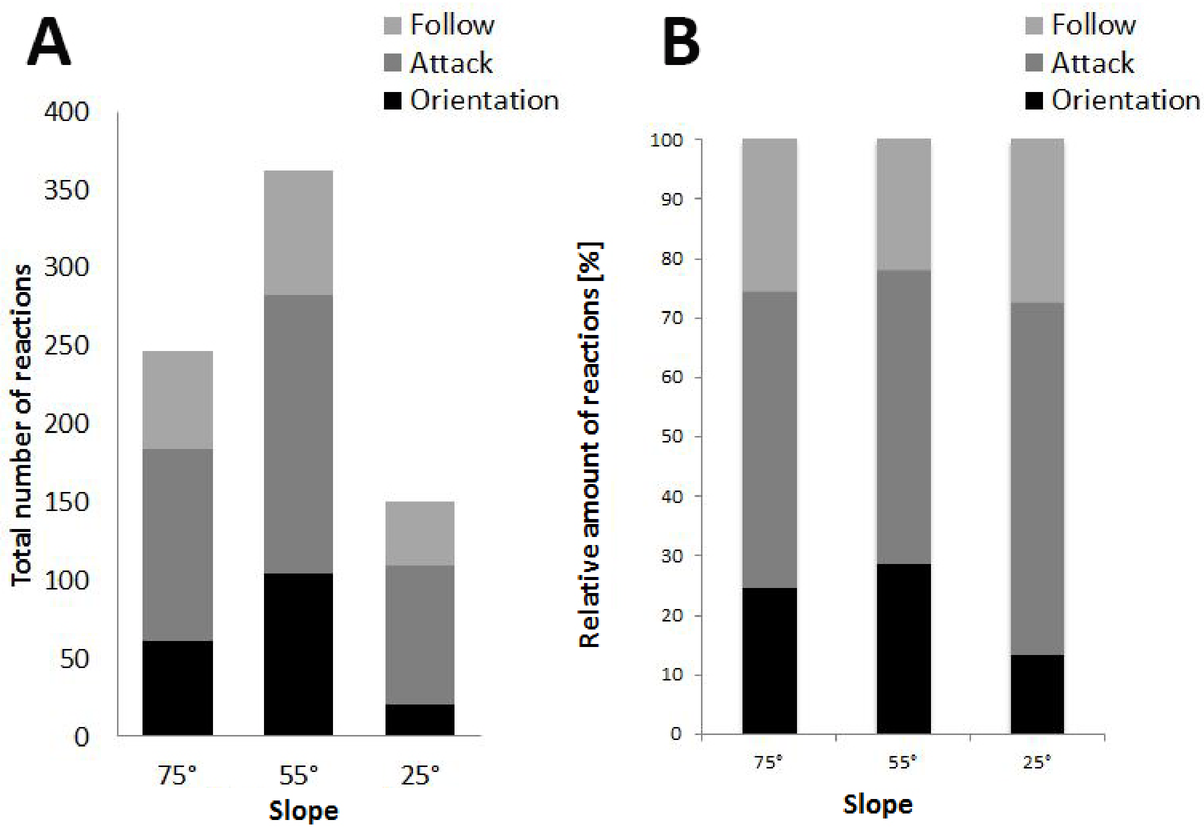

Fig.9: Analysis of the distribution of the three types of behaviours for each slope (25°, 55°, 75°) (**A**); Comparison of the occurrence of the behavioural responses between the three slopes (25°, 55°, 75°) expressed as percentage of total number of reactions (**B**).

Tab.4: Number of behavioural types in the positive trials split into the amount of recorded behavioural types per experimental set up. (O=orientation, A=attack, F=following)

Slope	O	A	F	Total number of behaviour	Trials with reactions
75°	61	123	63	247	96
55°	104	178	80	362	135
25°	20	89	41	150	65

3.5. Latency

The medium latency of each slope corresponds with the total number of reactions. The highest amount of responses was shown by presenting the dot at a slope of 55°, which also had the shortest latency with a minimum of 3.4s. Whereby the lowest number of positive reactions was at the 25° angle with an average value of 5.2s and in between is the medium latency of 4.1s for the third experiment with 75°.

Figure 10 presents the time it took the spiders to show an initial response at each positive presentation after the appearance of the dot comparing the different slopes.

All three show a major peak in their elapsed time at 3s. Presenting the path closest to the horizontal axis no reaction before 2.5s has been observed but it is the only one, which exhibits a small peak after 5s namely at 7s. The other experiments show a constant decrease in reactions after 4s, except at the slope of 75° a slight increase at 5s occurred and one statistical outlier at 22s (not shown in Fig.10).

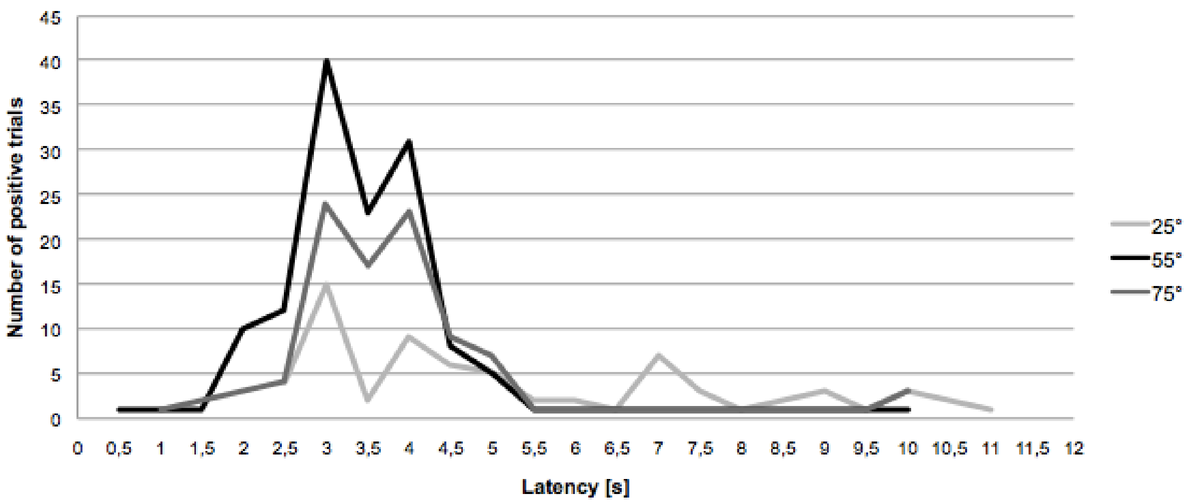

Fig.10: Latency of the initial responses to a stimulus over the course of time for each slope. The latency is defined as the time span between the appearance of the dot and the first reaction towards the stimulus.

During the experiments it could be observed that many responses were elicited when the stimulus passed the position of the spider and before reaching the end of the path. The jar was placed at a level that the animal was located in the centre of the screen.

The major peaks of the latency are the same in all three experiments at a range of 3-4s after the appearance of the stimulus. Table 5 shows the sloped path in centimetres and where the dot is located at these particular elapsed times.
In all three trials the majority of responses are in the upper third of the path before reaching the end for the first time when the dot is still moving upwards.

Tab.5: The three different experiments have their maxima in response at three major points of time. The table shows where the dot is located along the path at these certain response times.

Slope	path [cm]	3 seconds	3,5 seconds	4 seconds
25°	25	15cm	x	20cm
55°	37	22.2cm	25.9cm	29.6cm
75°	34	20.4cm	23.8cm	27.2cm

Looking at Figure 11 the x-axis represents the actual length of the stimulus path, which is different for all three experiments. To be able to make a comparison between them the x-axis were set to a fixed length with respective scaling.
These diagrams show which location along the stimulus path represents the peaks in latency within the first 5s (the time it takes the dot from the appearance in the lower left corner to the end of the path).
For the experiment with the slope of 25° the distance is 25cm which has its maxima in responses at 15cm and 20cm (Fig.11A).
For the 55° and 75° angles they are also in the upper part of the path. The intermediate angle has its maxima at around 22, 25 and 29cm and the latter at around 20, 24 and 27cm (Fig.11B; Fig.11C).
Due to the normalization of the three diagrams one can see that the highest response rate for each experiment, in relation to each other, is in the same area in the upper part of the stimulus path.

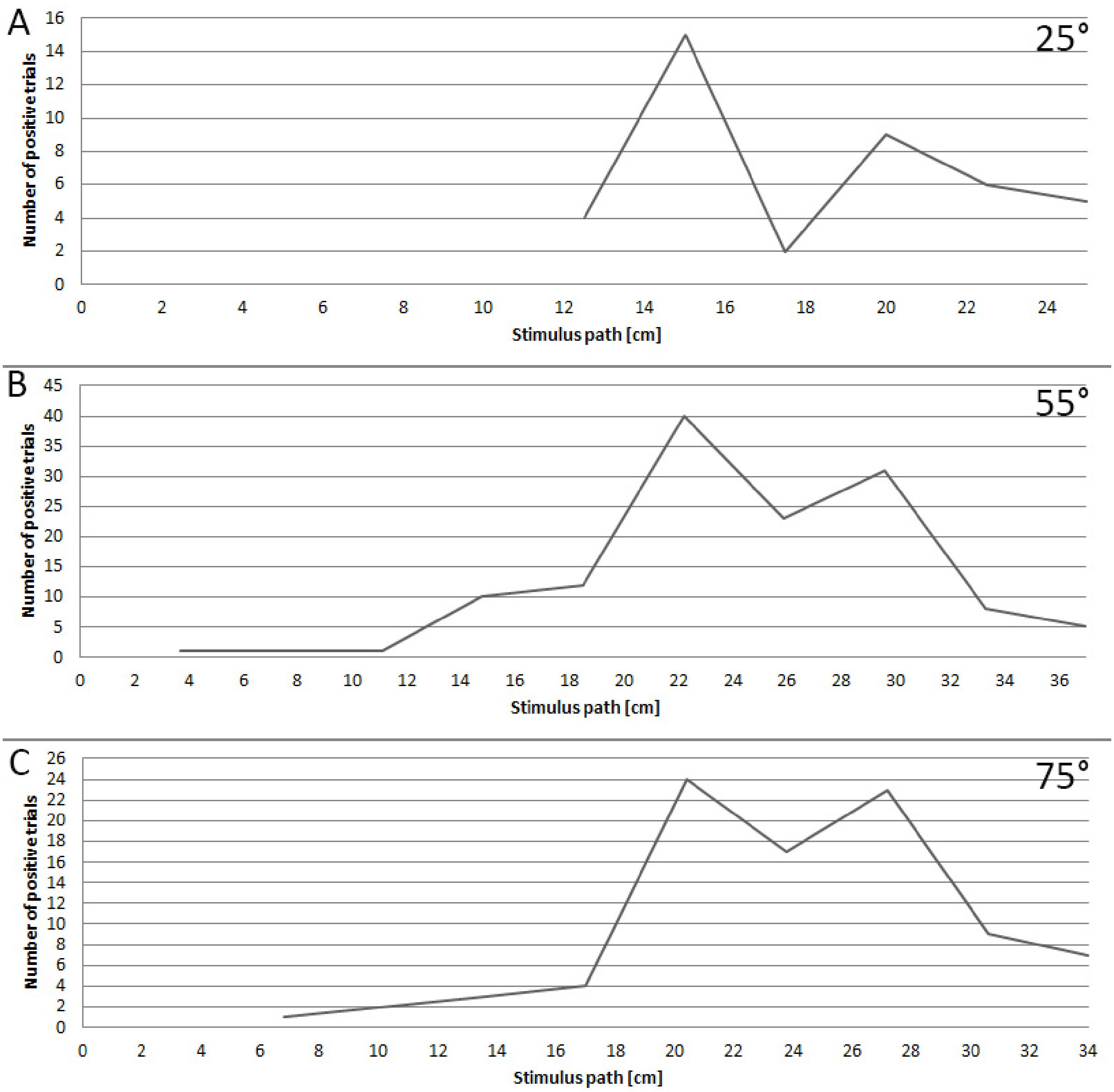

Fig.11: Graphical representation of table 6 shows the location along the stimulus path representing the maxima of reaction in latency. It can be observed that the highest response rate for each experiment, in relation to each other, is in the same area the upper part of each stimulus path.

4. Discussion

The results of the previous study (Lindner, 2013) have established that *Cupiennius salei* reacts towards a visual stimulus by showing prey catching behaviour. Varying the parameter of the visual cue led to differences in response rates. The aim of the recently undertaken research was to gain more information on what attributes a perfect visual cue must have to elicit the most frequent attack behaviour. The main focus was on altering the slope of the stimulus path and presenting the dot along different sloped paths.

The collected data from these experiments led to the conclusion that the angle of the stimulus path is one of the attributes, which may cause an increase in attack behaviour by providing solely visual information.

4.1. Stimulus size and speed

The purpose of the preliminary tests was to find out if there is a preference in speed and size of the stimulus. To assess this idea, combinations of those two attributes were evaluated. A 2cm, 4cm and 6cm sized dot (which corresponds to an angle of 4.8°, 9.5° and 14.3° of the spiders visual field at a distance of 24cm to the screen) combined with slow (22.8deg/s), medium (57.03deg/s) and fast (114.06deg/s) speed were tested.

The highest results could be achieved by combining the size of 14.3° and the slowest velocity (22.8deg/s). The fastest and smallest dot could only elicit reactions in 1% of the trials. The differences between medium-sized and largest stimulus are not significant just a slight tendency towards the bigger one could be observed (Tab.1). The same is true for preference in speed the slowest movement elicited the highest number in responses (Fig.8). Comparing the findings regarding stimulus size to the previous experiment (Lindner, 2013) it complies the findings namely the bigger the dot the higher the response rate. On the other hand the results concerning reactions to speed differ.

The preference in visual cues of a larger size could be explained with the efficiency of the food source. The smallest dots would not elicit any reactions in 99% of all experiments. This could be traced back to the fact that trying to catch a prey this size would cost the spider too

much energy for too little reward. In line with the previous argument an increased size of the stimulus makes it more attractive as a food source. Catching a prey like this would result in an acceptable energy source.
A further explanation could be that a bigger stimulus may represent rather an enemy than prey and attacking it prevents the spider from becoming the victim in this scenario.
Another reason could be that smaller sized stimuli appear to be too far away to even try to capture it and bigger dots seem to be much closer and a hunting success is more likely.
In the study by Lehnert (2011) the spiders could choose between two stationary objects. They offered a bigger one further away and a smaller one closer to the animal. This study showed that the spider’s choice seemed not to depend on the size but the distance of the stimulus as the closer objects were preferred. *Cupiennius salei* needs a structured background (Lehnert, 2011) to detect distance. They perform a zig-zag movement which generates an up and down movement of the stripes which are used as the structured background. Such a background is not given in this recent study when a black dot is moving on a green background. In this case distance discrimination could not be performed.

4.2. Behavioural responses and frequency of occurrence

Throughout the tests the same behavioural responses as in the previous study by Lindner (2013) could be observed except the one described as threatening behaviour. A rare appearance of the latter might have occurred but it was very difficult to discriminate it as an individual movement. It happened mostly after an attack when the spider was on the bottom of the jar on the transfer to performing “following” behaviour.

Analysing the occurrence of the three types of behaviour in all experiments during this study showed that the act of attacking was always the most dominant reaction (Fig.9). Its distribution was around 50% of all responses in the different tests.
No chronological order of appearance of the three types of behaviour could be observed. Each type could occur as an initial response, followed by any of the others. Sometimes just one reaction towards the stimulus was observed; in other trials up to 7 individual behavioural responses could be recorded.

If as a first response towards the stimulus an attack behaviour was elicited, it mostly happened shortly after the appearance on the screen. It seemed that the spiders would not want to risk the so-called prey getting out of reach. Regarding their natural habitat, a further reason for this immediate attack might be that showing of orientation or following as an initial behaviour could warn/frighten the prey. Another possible explanation could be that they attack the stimulus because they perceived it as a threat and tried to eliminate it.
During different trials it was observed that the same visual cue could cause less aggressive behaviours and no actual attacking. When the dot passed by, the spiders just orientated towards the direction the stimulus moved or followed it but never tried to grab it.
It could be the case that *Cupiennius salei* was assessing the stimulus because during the time the dot was moving along the sloped path the distance between the spider and the stimulus changed. Upon appearance in the lower left corner the stimulus seemed to be far away. When it moved up on the diagonal it got closer to the point where spider sat and therefore seemed bigger. As the stimulus moved forward the distance to the test subject increased and the dot diminished in size. Orienting and following would bring them closer to their prey after it passed them and sometimes this “assessing” was followed by an attack. The spiders probably perceived the stimulus as a worthy prey or they would not show any more responses towards it.

Different responses from same individuals to same stimulus

During the experiments different responses from the same individuals were recorded while presenting the same stimulus. In one trial a spider would react towards it very aggressively and the next time absolute no interest towards the stimulus was shown.
One reason might have been their ecdysis state. When the test subjects were chosen from the department’s breeding only their age in months is known but not how often they have moulted so far. Having moulting information would be important because a certain amount of moults seemed to be necessary before *Cupiennius salei* is reacting towards a visual stimulus. Prior to this specific moulting stage no visual information elicits any behavioural response.
Unfortunately with an increase in age a decrease in activity was noticed, fewer reactions

towards the same stimulus were recorded. Therefore it is important to select individuals of the appropriate age/moulting state. Two spiders can have the same age but might not have performed the same number of moults. This depends on food availability. Another reason for variation in activity towards the stimulus was if the spider moulted during the experimental phase. If this happened the collected data of three days prior and after had to be excluded. During this time no visually elicited behaviour occurred.

Another reason for differences in responses by the same spider might be the surrounding conditions which were not perfect. The light intensity could only be dimmed to 32lx whereas in their natural habitat the activity increases after sunset and is at its highest at an illumination level below 0.1lx (Barth, 1993). The location of the experimental room was not isolated from the daily life of the university. Hence disturbances like slamming doors, repair works and lectures caused vibrations during the trials which could have had an influence on the behaviour. Finally there also exists a difference in locomotor activity between the sexes, which is in males 12.7 times larger than in females (Schmitt et al. 1990).

4.3. Horizontal stimulus path

When conducting the first experiment a stimulus was presented along a horizontal path (0°) and three different positions were tested (bottom, centre and top of the screen (Fig.6)). Nearly no response could be elicited neither when the presentation was set to the centre screen (spider and stimulus at approximately the same height), nor when the dot was moving along the bottom of the monitor. Testing the latter a higher number of reactions was expected as the study by Lindner (2013) provided results that there was a preference when the dot appeared at the bottom of the screen. The reason being that the position of the spider, sitting above the stimulus, made it easier to attack by just jumping down.

4.4. Sloped stimulus path

The experiments along the horizontal path elicited a reaction in only 1% of the trials.

Compared to the Lindner`s findings this outcome appeared strange and for the next testing phase a change in the slope of the stimulus path from 0° to 55° (which is the diagonal of the testing area for one spider, because two could get tested at the same time) was undertaken which resulted in a remarkable increase of the reaction rate.

The fact that a vertical path and a slope of 55° elicits attack behaviour but a horizontal line results in nearly no response led to the assumption that the slope of the pathway could be one of the characteristics necessary to visually elicit an attack behaviour.
Therefore another two experiments were conducted, using a slope closer to the horizontal axis (25°) and one nearer to the vertical axis (75°).
Table 6 shows the increase in reaction rate the steeper the slope of the stimulus path. In contrast to what was expected the highest percentage of trials with reactions was observed at the sloped path of 55° and not at the slope closest to the vertical axis. The unexpected decrease of responses at the slope of 75° might be an indication that the angle at which the stimulus moves through the visual field of *Cupiennius salei* is a necessary characteristic for them to attack, whereas a horizontal movement does not fulfil such criteria.
The arrangement of their eyes makes it possible to get a nearly overall view of their surroundings, which means they should notice the stimuli moving along the other sloped paths but they showed less interest in those.

4.5. Latency

While analysing the recorded videos it came to the attention that a high number of first responses appeared when the dot was moving from the lower left corner upwards. Especially when the stimulus had passed the position of the spider, where they were at a same height and therefore was in the upper third of the length of the path, which is within the first 5s (Tab.5). Of course there were some presentations where the principal responses took place after five seconds when the dot was moving back down again, coming closer to the spider`s position. Nevertheless the highest number of initial reactions took place within the first five seconds.

Dot velocity

While analysing the data an anomaly was detected. When creating the power point presentations it is possible to set different velocities but it has to be mentioned that slow does not define an actual speed but is dependent on the length of the path. This means the dot always travelled for five seconds whether the path was 25cm or 37cm long.

In the presentations at a slope of 55° the length of the path was 37cm and it took the stimulus 5s from one end to the other. In the other two presentations the velocity was set to slow as well which means that the stimulus also travelled for 5sec along the path although they had different lengths.

Table 7 shows the difference in time the stimulus actually would have needed to pass the length of the path if the movement of the dot would not have changed because of the different lengths of the sloped paths. The difference between 55° and 75° is just 2.5deg/s. It is not known if such a small variation can be detected by the spider, also the chi-square test confirmed that the results of the response rate differentiated very significantly. Therefore the obtained results should still be valid.

Along the 25° path it should only have taken 3.4s (27.1deg/s) for the same speed setting. This means that the stimulus actually moved at a slower velocity along the 25cm than in the experiment with a slope of 55°, which resulted in the highest number of positive reactions.

The outcomes of the preliminary tests on speed resulted in the conclusion that the slower the stimulus moves the higher the response rate therefore this error should have caused more interest of the spiders in this presented stimulus, which did not happen. Consequently it appears the slope is still the factor determining the lower response compared to the other slopes (Tab.7).

The preliminary studies on velocity and size are not affected by this error because this was tested with the same distance.

Tab.7: The detected anomaly of the speed setting in PowerPoint: The three experiments have different path lengths but the stimulus always takes 5s (=actual time). Time needed describes the time (°/s) the stimulus would have needed if the velocities were not dependently on the length of the path.

Slope	Diagonal	Actual time	Time need
25°	25cm	5s (18.47°/s)	3.4s (27.1°/s)
55°	37cm	5s (22.8°/s)	5s (22.8°/s)
75°	34cm	5s (21.9°/s)	4.6s (24.3°/s)

4.6. Location of highest responses rate

Disregarding the time needed for the stimulus to reach the end of its path but concentrating on the length of the sloped path showed that the location of the dot seemed to be essential for an increase in eliciting behaviour.

Although the angle and the length of the path differ, the peaks of initial responses were observed in the upper part of the sloped path. More specifically after passing in front of the spider and moving upwards before reaching the end.

This is in contrast to Lindner`s findings where the largest number of reactions were recorded as the dot reached the end of the screen, when it had to slow down to turn around and was on the way down again. The second preferred position was when the stimulus appeared from the bottom where an immediate reaction occurred.

Those results again correspond with the idea that a visual stimulus has to move through the visual field at a certain angle to elicit a behavioural response

4.7. Conclusion and Outlook

The aim of this study was to gain more information on what attributes are necessary to elicit attack behaviour by solely using visual input. The results provided evidence that size and velocity of the stimulus and the slope of a stimulus path seemed to be an important characteristic to increase behavioural responses.

The results suggested a bigger stimulus moving with a slow velocity leads to a higher response rate, whereas a combination of fast speed and small size causes no attacks.

Cupiennius salei also performed a significantly higher number of responses at the sloped path of 55°. Compared to these results a decrease in the experiments with a slope of 25° and 75° occurred. The lowest amount of positive trials was observed towards the stimulus of 25°. The least interesting stimulus seemed to be the horizontal path (0°) only 1% of the presentation caused any response.

The next steps would be to examine if there is a very specific slope, which would increase the reaction rate above the results of the sloped path of 55°.

This study could show that eliciting attack behaviour in *Cupiennius salei* by solely using a visual cue is possible and presenting a larger stimulus with a slow velocity along a sloped path of 55° caused the highest behavioural responses.

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6. Summary

The visual system of *Cupiennius salei* consists of four pairs of eyes separated into two types. The principal eyes are specialized to discriminate targets whereas secondary eyes are necessary to detect if a visual stimulus is moving (Schmid, 1998; Neuhofer *et al.* 2009).

The aim of this research was to find out if an attack behaviour can be elicited by solely using a visual stimulus, furthermore it was investigated what characteristics would be needed to cause such a reaction. A specific focus was on the slope of the stimulus path to find out if there is a preference for a dot moving along a sloped path or a horizontal one.

As a prerequisite the experimental setup had to exclude any other mechano-sensory inputs like substrate vibrations or airflow. In order to provide this environment the visual stimulus was produced on a computer screen using Microsoft Powerpoint, spiders were separated through a visual cover and the jars containing the spider were put onto styrofoam blocks.

The preliminary tests focused on size and speed of the stimulus. The results suggested an increase in responses towards the bigger sized and slow moving stimuli. The following experiments were performed with a stimulus of 6cm in diameter and a slow velocity.

The main focus was on the orientation of the stimulus path, four different possibilities were tested: the stimulus was presented along a horizontal path and on a sloped path with three different angles of elevation (25°, 55°, 75°).

The stimulus along the horizontal path elicited hardly any attacks, increasing the angle of the path resulted in an increase of reactions. The highest number of responses was recorded at the angle of 55°. Bringing the stimulus closer to the vertical axis by using the path of 75° showed a reduction in attacks.

The preference in larger stimuli could be explained by efficiency of the food source or also defensive behaviour. A possible explanation for the spiders preferably reacting towards certain sloped paths may be that a visual stimulus has to move through their visual field at a certain angle.

7. Zusammenfassung

Das visuelle System von *Cupiennius salei* besteht aus vier Augenpaaren, die in zwei Augentypen unterschieden werden. Die Hauptaugen sind spezialisiert um Ziele zu unterscheiden, während die Nebenaugen notwendig sind um die Anwesenheit eines bewegten visuellen Stimulus wahrzunehmen (Schmid, 1998; Neuhofer *et al.* 2009).
Diese Studie beschäftigt sich mit der Frage, ob ein Angriffsverhalten alleine durch einen visuellen Stimulus ausgelöst werden kann und welche Eigenschaften dieser haben muss, um solch eine Reaktion hervorzurufen. Der Fokus lag auf dem Steigungswinkel des Stimuluspfades, um zu überprüfen ob es eine Präferenz für einen Reiz entlang eines horizontalen oder schrägen Pfades gibt.
Der Versuchsaufbau musste so gestaltet werden, dass alle mechano-sensorischen Informationsquellen, wie Vibrationen und Luftströme ausgeschlossen werden konnten. Aus diesem Grund wurde der Stimulus auf einem Computerbildschirm gezeigt, die Spinnen durch Trennwände separiert und zusätzlich ihre Aufbewahrungsbehälter auf Styroporblöcke gestellt.Die Vorversuche konzentrierten sich auf die Größe und Geschwindigkeit des Reizes. Die Ergebnisse zeigten einen Anstieg in Reaktionen gegenüber größerer Stimuli, welche sich mit langsamer Geschwindigkeit fortbewegten. Die weiteren Experimente wurden mit einem Stimulus von 6cm im Durchmesser und einer langsamen Geschwindigkeit durchgeführt.
Das Hauptinteresse in dieser Studie galt der Ausrichtung des Pfades dem der Stimulus folgt. Vier verschiedene Möglichkeiten wurden getestet: ein Reiz entlang einer horizontalen Linie und entlang eines Pfades mit drei verschiedenen Steigungswinkeln (25°, 55°, 75°).
Präsentierte man den Reiz entlang der Horizontalen konnten nur wenige Reaktionen ausgelöst werden. Die Erhöhung des Steigungswinkels führte zu einem Anstieg an Reaktionen. Die stärkste Antwortsrate wurde bei einem Winkel von 55° erreicht. Testete man entlang des 75° Winkels wurde ein Rückgang an Reaktionen beobachtet.
Die starke Reaktion gegen große Stimuli könnte durch höheren Nahrungsgehalt oder Abwehrverhalten erklärt werden. Eine Erklärung für die Präferenz von gewissen Steigungswinkeln wäre, dass ein Reiz in einem bestimmten Winkel das visuelle Feld von *Cupiennius salei* durchqueren muss.

8. Acknowledgement

First and foremost special thanks to Ao. Univ.-Prof. Dr. Axel Schmid, for the opportunity to work on this project, his guidance and his patience.
I also want to thank Miroslav Dragasev for his help choosing the right test subjects and teaching me how to handle them and Elisabeth Fritz-Palank for the assistance with the supplies.

9.Appendix

9.1 Curriculum Vitae

Persönliche Daten	**Sarah SCHÜTZINGER**
Geburtsdatum und Ort	25.04.1987, Spittal/Drau (Kärnten)
Nationalität	Österreich

Ausbildung

1993-1997	Volksschule Möllbrücke
1997-2005	Bundesrealgymnasium Spittal/Drau
Mai 2005	Abschluss mit Matura
Oktober 2006	Beginn des Biologistudiums an der Universität Wien
Sommersemester 2010	Abschluss des Bakkalaureatsstudium Zoologie
2010-2014	Masterstudium Verhaltens-, Neuro- und Kognitionsbiologie and der Universität Wien
April 2014	Abschluss des Masterstudiums

Praktika während des Bakkalaureats- und Masterstudiums

Sommersemester 2009	Aktivitätsmuster des sibirischen Tigers im Tiergarten Schönbrunn, Wien
Wintersemester 2009	Bioakustische Studie an der Nacktmullkolonie im Tiergarten Schönbrunn, Wien
Wintersemester 2010	Tagesaktivität bei stachellosen Bienen (*Meliponini*) in La Gamba, Costa Rica
Sommersemester 2011	Stressbelastung bei freilebenden Feldhamstern (*Cricetus cricetus*) in der Stadt Wien
Sommersemester 2011	Ethologisches Praktikum mit handaufgezogenen Graugänsen (*Anser anser*) an der Konrad Lorenz Forschungsstation, Grünau
Wintersemester 2013	Visuell gesteuertes Angriffsverhalten von *Cupiennius salei*

Tutorien

Wintersemester 2012	Tutorin bei Organ- und Kommunikationsysteme
Sommersemester 2013	Tutorin bei den Tierphysiologischen Übungen 2

9.2 Image of the Powerpoint presentations

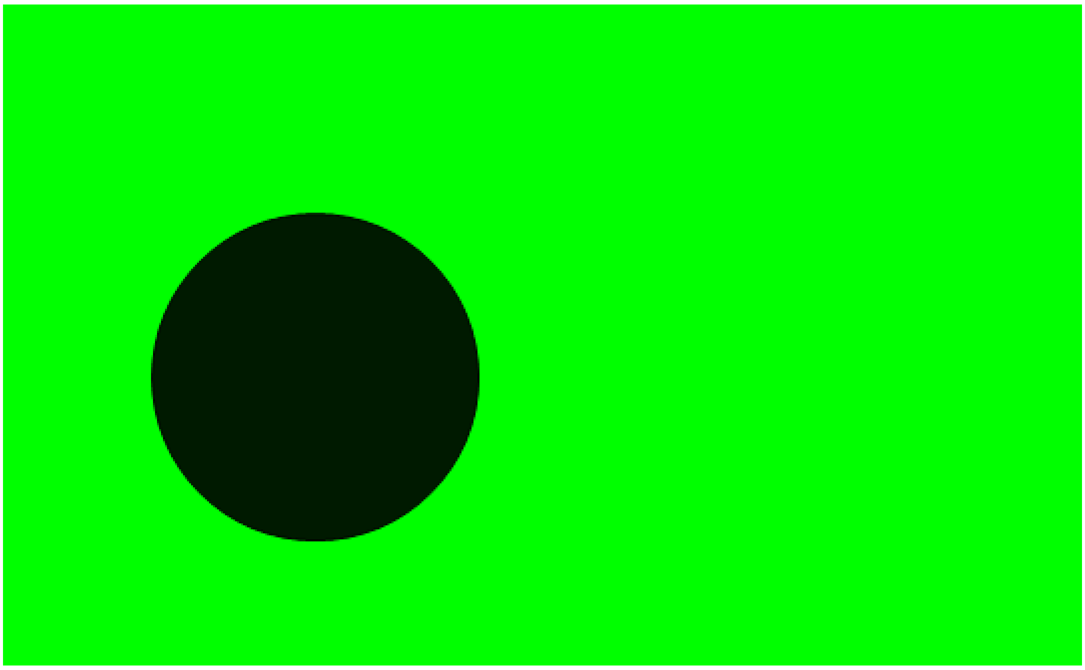

Fig.12: Shows the contrast between the background and the stimulus which was used in the presentations.