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"Visually elicited prey capture behavior in *Cupiennius salei*"

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Content

1 Abstract

The main interest of this study is to find out whether visual stimuli alone can elicit attack behavior in *Cupiennius salei,* a nocturnal sit-and-wait hunting spider. After behavioral studies involving target discrimination, this study is interested in the spiders' ability to detect moving rather than stationary objects. For this reason, all other cues which could interfere when an object is presented, like vibrations of the substrate or sound which could occur are excluded by presenting a moving dot - stimulus on a computer screen. Different settings with different stimuli are used, to gain insight how a stimulus has to be presented in order to elicit the strongest response. Furthermore, an experiment is conducted, masking the different types of eyes to check for their respective influence in detecting moving objects on the screen. The results indicate that vision alone is sufficient to elicit attack behavior and the strength of response is dependent of different factors, such as the size of the stimulus. Furthermore, it is shown, that one pair of eyes is specifically useful in detecting moving objects in the front of the spider.

2 Introduction

The geographic distribution of *Cupiennius salei* ranges from the southern USA, over Mexico, the Antilles and Central America to Brazil (Melchers 1963). Without question, there are differences in climate between the different regions, so it is hard to give certain values for the habitat of *Cupiennius salei.* Barth and Seyfarth (1979) conducted a study concerning the habitat of the spider in the highlands of central Guatemala, which acts as a representative for the whole range of distribution. There, the average temperature ranges from 15°C to 20°C and the relative humidity is about 90% year round. In this region, *Cupiennius salei* lives on banana plants (*Musa sapientium),* which is used to provide shadow for young coffee plants on the plantations (Barth and Seyfarth 1979). *Cupiennius salei* prefers plants with relatively stiff leaves as host plants, because of their property to transmit vibrations very well. This is important for the spider, because mechanosensation is used in many circumstances such as hunting or courting. Also, the base of the leaves provides shelter during the day. After dusk, the spider leaves this shelter and after some time it moves up a leaf in order to wait for prey. This takes place in the dark, at light

intensities as low as 0.1 lux and below, which is even ten times above the sensitivity measured in electroretinogram recordings, which lies below 0.001 lux, using white light stimuli (Barth et al. 1993). The prey the spider is waiting for can be any small insect which passes by, as *Cupiennius salei* is extremely polyphaguous and can cope even with strongly poisonous or distasteful insects, much better compared to other spiders (Nentwig 1986).

Regarding *Cupiennius salei,* there is a variety of studies investigating the mechanosensory system, most of them focused on the detection of substrate vibrations or airflow. It could be shown which kinds of vibrations *Cupiennius* salei has to deal with (Barth et al. 1988) and how the different frequencies of substrate vibrations are able to elicit attack or withdrawal behavior (Hergenröder and Barth 1983). Besides chemosensory cues to detect a possible partner, the mechanosensory system plays a big role in courtship behavior, i.e. during vibratory courting and tactile interactions thereafter (Barth and Schmitt 1991). During courtship, the male spider raises and lowers its opisthosoma in quick succession, producing vibratory cues which are transmitted along the leaves of the bromeliads where the female spiders are sitting most of the time. These signals consist of extremely specific syllables and carrier frequencies (Schüch and Barth 1990) and also differ greatly from vibrations produced by potential prey, predators or the wind.

As other spiders, *Cupiennius salei* has 4 pairs of eyes. These are the anterior median (AM), the posterior median (PM), the anterior lateral (AL) and the posterior lateral (PL) eyes. The AM eyes are referred to as principal eyes, whereas the others are the secondary eyes. The reason for this differentiation is the difference in structure between the eyes, and a difference in use as well. The principal eyes are the only ones which possess muscles in order to move their retinae (Land 1969). This has two effects on the use of the eyes. First, with a moveable retina, the spider can follow objects with its gaze, without moving itself and thereby giving away its own position. The second advantage is that by inducing micro saccades, the eyes do not adapt to their surrounding and stationary objects can be observed better. In the secondary eyes, this is not possible, and therefore the eyes saturate and the background fades away if the animal is not moving. When this happens, the spider becomes blind, except for moving objects which enter its visual field. These are then detected easily. This phenomenon has also been shown in *Bufo bufo* L., where on top of that, a mechanism has evolved to cope with blinking, where the image

would be refreshed. During a blink, retinal on-off and off ganglion cells are inhibited to remain in a state of purposeful blindness (Ewert and Borchers 1974). Once an object is detected, the spider can turn in its direction to better observe it with its principal eyes. As Duelli has shown, a moving object in front of the spider is more probable to elicit a response than an object positioned more laterally (Duelli 1978).

This differentiation in use of the eyes might be part of the reason why the visual fields of the AM and PM eyes overlap to a great extent. In general, the AM eyes cover roughly a fourth of the visual field and the PM eyes about a third. Both are covering what is happening in front of the spider and partly in its back, until the view is blocked by the opisthosoma anyway. The PL eyes expand the visual field to the sides for about another fourth of the visual field, whereas the AL eyes provide a view of directly in front of the chelicera, covering about a fifth of the visual field and overlapping a little with the visual fields of the AM and PM eyes. For each pair of eyes, there is no distinct binocular overlap (Land and Barth 1992).

Another structural difference between principal and secondary eyes is the presence of a tapetum in the latter. The tapetum is a reflecting structure built from guanine crystals behind the photoreceptors, which enables the photoreceptors to detect photons which were not registered upon entering the eye. This structure enables the spiders to see at low light intensities. The possibility of using incoming light twice because of the reflecting tapetum lowers the light intensity threshold down below 0.001lx (Barth et al. 1993). To compliment this, the rhabdomers of the photoreceptors in the secondary eyes are oriented away from the light, whereas in the principal eyes, they are everse. These structural differences can be deducted from a different evolutionary background. While the principal eyes are derived from lens eyes, the secondary eyes are derived from compound eyes (Weygoldt and Paulus 1979).

With respect to the visual system, there were some behavioral studies performed with animals walking towards stationary objects. Mainly, the setup in these studies is an arena, in which the spiders were put, with two different stimuli located at the back wall and the spiders had to discriminate between them. This discrimination was indicated by the spiders choosing one of the stimuli by walking towards it. In these experiments mainly distance discrimination (Lehnert 2011) or shape discrimination have been tested (Schmid 1998).

Another aspect of the visual system was explored by Orlando and Schmid (2010). Using colored stripes, moving on a grey background, it could be found that the movement – detecting system in *Cupiennius salei* is in fact colorblind.

The main point of this study is to determine the role of the visual system in regards to detecting moving objects. This is conducted altering different parameters of a dot stimulus, like its size or direction, and eventually gain a setting, at which most of the spiders react towards the stimulus. With a setting where the spiders showed attack behavior on a regular basis, the two different eye types were then tested separately, masking the other with black paint, in order to determine which set of eyes plays the bigger role in detecting moving objects.

3 Material and Methods

3.1 Animals

All *Cupiennius salei* used in this study were subadults around 5 to 7 months of age after hatching. They derived from the department's breed, and were kept at 25 °C and a relative humidity of 75 % with a 12/12 hours circle of light. Each individual was kept in a jar made of glass, 13 cm diameter and 25 cm height, the bottom fifth filled with peat. The only object in this environment was a little plastic cup filled with water in order to maintain humidity and provide the spider with water. The spiders were fed once a week with 6-8 *Calliphora sp.*

Altogether, 56 spiders were used in the various experiments. It was this many, because on one hand, it was not possible to test an individual more than five times a day, because its probability to react to a stimulus decreased dramatically, so that nearly no spider reacted in later tests, whether they did before or not. On the other hand, spiders that molted during the time they were tested had to be excluded, because they would not react to a stimulus some days before and after the molt. Unfortunately it is not possible to determine when a spider will be molting beforehand, so they had to be excluded and their results discarded when it happened.

3.2 Operational setup

The spiders in their jars were placed in front of two computer screens (Samsung SyncMaster T220), at a distance of 20 centimeters. They were oriented in a way that they were sitting with their prosoma pointing downwards on the sector furthest away from the screens, so that the stimulus moved through a majority of the spider's visual field, at least of the AM and PM eyes. In front of each screen, two spiders could be placed at the same time, visually separated from each other by a Styrofoam barrier. This barrier was necessary to ensure that a spider is not influencing the behavior of its neighbor, for example by giving it another visual cue when jumping and distracting it from the stimulus it should focus on, or by its mere presence, because the territorial *Cupiennius salei* females would not tolerate another individual this close to them. Underneath the jars, there were

polystyrene platforms on top of foam to reduce vibrations made by the other spiders, the experimentator or other sources. On the other side, where the spider's ventral side was facing, two webcams (Logitech Pro 9000) recorded its behavior using the program Logitech Webcam Software. The data were saved on two computers.

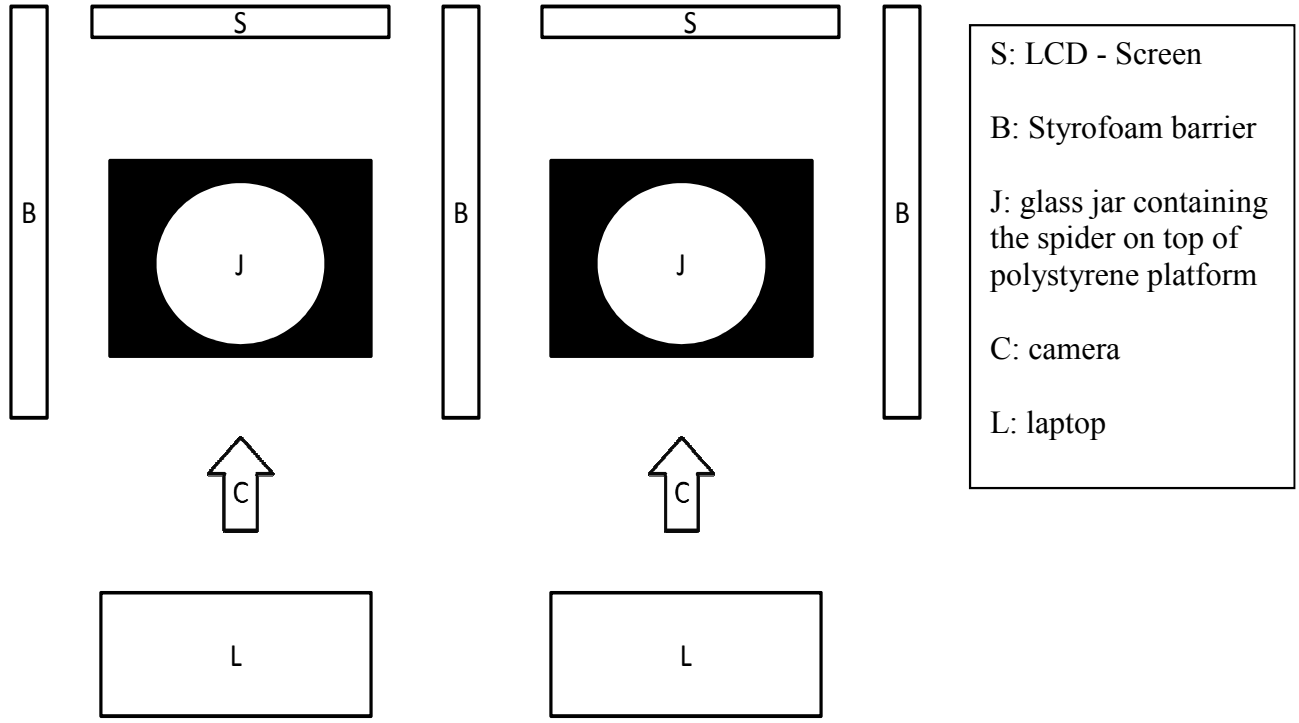


Figure 1: Top-view of the operational setup.

The ambient light was regulated by a commercial dimmer and held stable at about 35lx. The temperature was always 28 to 30 °C.

3.3 Stimulus

3.3.1 Stimulus appearance

According to Land (1969), a variety of shapes, namely dots, squares, crosses, triangles, etc. elicit attack behavior, whereas spider-shaped stimuli tend to elicit courting. Therefore, a dot – shaped stimulus was chosen to elicit attack behavior in this study. An advantage of using a stimulus this simple is the ease to replicate it.

The stimulus was generated using Microsoft PowerPoint. In all experiments conducted, it consisted of a black dot moving on a green background. Green was used as background, because it is easier to reproduce one of the three true colors of the computer system (which uses red, green and blue) than a shade of gray. Any color except those three would be harder to define exactly, because it would be dependent of the screen's settings and, because flat screens were used, also of the angle of view. Moreover, green was selected, because *Cupiennius* has a peak in its spectral sensitivity at 520 nm, which perfectly meets the spectral composition of the screen (Walla et al. 1996).

3.3.2 Stimulus path

In most of the experiments, the path the dot stimulus followed was the one Fenk and colleagues (2010) used in their study, where they determined whether the spiders reacted to stimuli generated by computer screens at all and is shown in figure 2. Essentially it is a custom animation of a path drawn manually in Microsoft PowerPoint, with the dot appearing at the start, then following this path and disappearing at the end, at the same position it appeared.

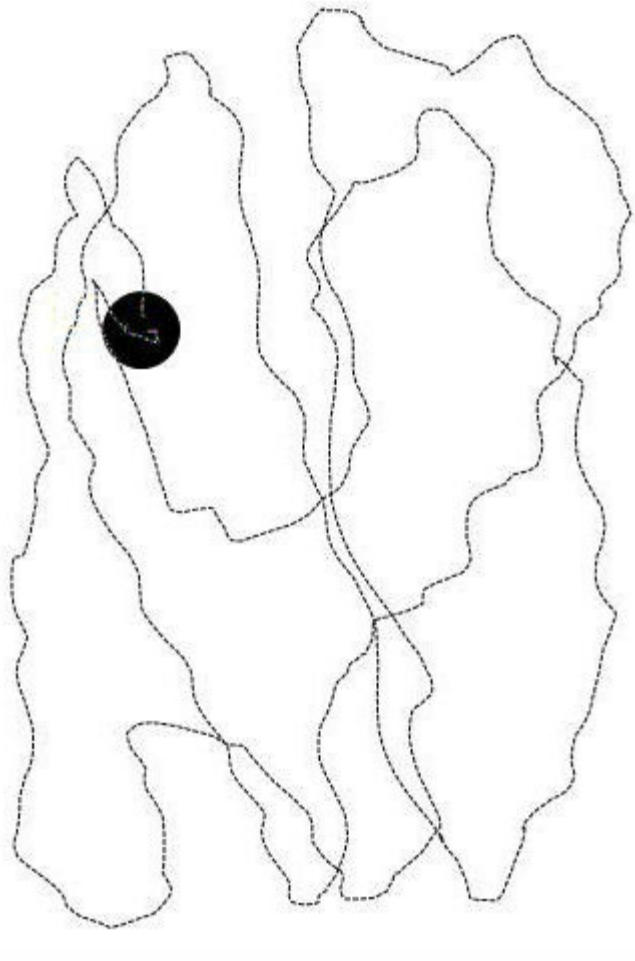


Figure 2: Path of the moving dot stimulus used in most of the experiments of this study. It is a hand-drawn animation-path created in Microsoft PowerPoint. The dot starts at the location shown here, moving upwards, following the dotted line, until it reaches its starting position once again and disappears there.

Only in one experiment the path differed from this one. For this, four different paths were used, where the dot moved only vertically, with the difference being the start and end position of the dot's presentation. In the first, the dot appeared in the center of the screen, then moved upwards until the topmost part, where it returned, moving all the way to the bottom. There again, it changed direction and moved upwards again, until it reached the starting position in the middle of the screen, where it disappeared. The second path was essentially the same as the first, only mirrored, so the dot moved down first, then up to the top, then back to the starting position. In the third path, the dot entered from the top, moved all the way to the bottom, where it changed direction and moved back up, leaving the screen at the top. The last presentation again was the same path as before, only mirrored, so the dot entered from the bottom, moved up, then down again, where it left the screen. These four paths are shown in figure 3.

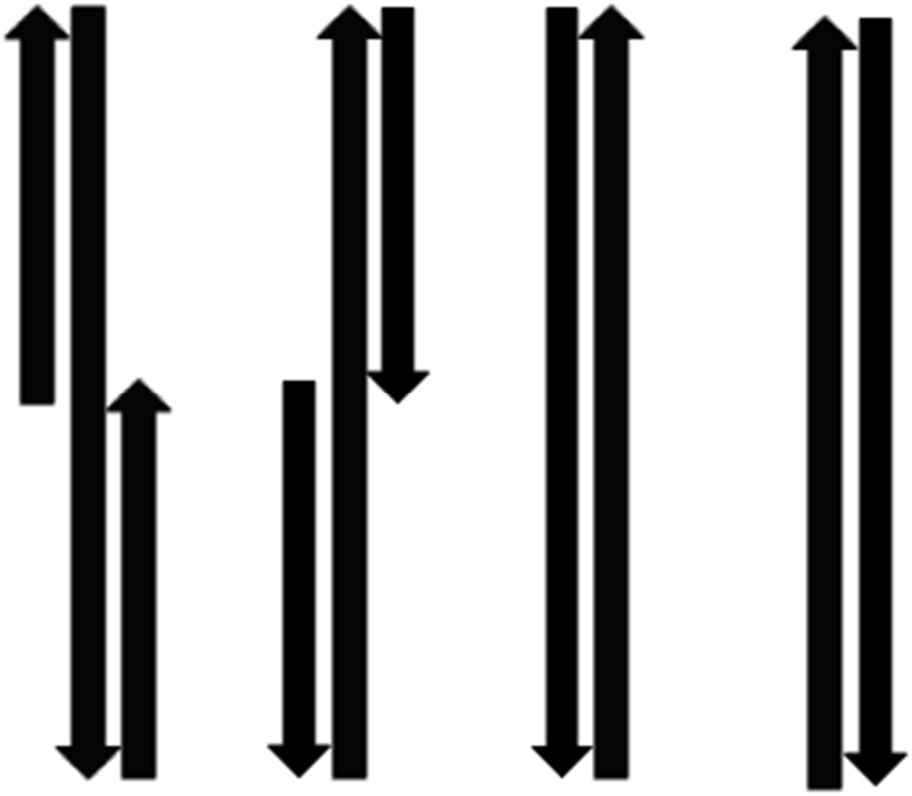


Figure 3: The four different paths used in the experiment concerning latency of the reaction and position of the stimulus at the time of the reaction.

3.4 Different behavioral responses

Until now, it was not tested, if the spider *C. salei* would react to a visually presented stimulus. Thus, a series of preliminary tests was performed to gain insight into the different behavioral responses. For this series, the spiders weren't prepared for the presentation in a way they were in the actual study, i.e. placed on the opposite outline of the jar facing the screen, sitting in a downward oriented position. Rather, they were allowed to be wherever they were when the presentation started, and furthermore, many sets of presentation were performed after one another with various delays. This was also to determine, if it was necessary to wait for a certain amount of time after a presentation before the spiders were responsive for the next. For this preliminary study, a total of 1404 presentations was recorded, using 12 spiders.

3.5 Stimulus size

Five different sizes of stimuli were used during the experiment, namely dots of 2, 3, 4, 6 and 8 cm diameter. This translates into objects which, for a spider 20 cm away from the screen, cover an angle of about 5°, 8°, 11°, 17° and 22° respectively. Each of the five different sizes of stimuli was tested 120 times in total, using 24 spiders.

3.6 Stimulus speed

For this experiment the speed of the moving dot was altered, using three different speed settings. The one used in the other experiments, with a speed of about 56 deg/s and one faster option with about 84 deg/s and one slower, with about 43 deg/s. In total, 24 runs were made for each setting, using eight spiders.

3.7 Ambient light intensity

With the aid of a commercial dimmer the ambient light of the testing room was set to four different intensities, namely 1lx (with the lights completely turned off and therefore the only source of light were the computer screens), 60lx, 330lx and 2800lx (which was the maximum intensity possible. These intensities were measured directly behind the spider, which was sitting on the side of its glass jar, using a multimeter (MT-51, Voltcraft). Additionally, the luminance of the stimulus and the background was measured, using a Konica Minolta LS-100 luminance meter. Then, the contrast was calculated by dividing the difference in luminance by the luminance of the background. For this part of the study, 24 runs were made for each light setting, using eight spiders in total.

3.8 Latency and location

It was not clear, whether time passed between the first appearance of the stimulus and the reaction of the spider towards it or the location of the stimulus within the visual field of the spider had an impact on eliciting a reaction. To determine the influence of these factors, an additional experiment was performed, using four different paths of the moving dot. In all experiments, the dot only moved vertically, the difference between the paths was the position where the dot started and ended (see 3.3.2).

In the experiment, all 24 possible combinations in which order the presentations could be shown were performed, to exclude any effect a certain sequence of showing the four presentations could have. For example if the spiders react only to the first two presentations would provide a false picture of how the spiders would react to the remaining stimuli. Each set was presented four times, because of the four test-subjects per run, equaling a total of 96 runs.

3.9 Covered eyes

The spiders were anesthetized using carbon dioxide, and placed on a holder. It consists of a wooden calotte, where the spider is placed and strapped down with parafilm. The holder was mounted on a magnetic socket, so it can be fixed on a metallic plate to exclude moving it by accident and a spherical joint connecting these two parts facilitates the manipulation of the spider under a stereomicroscope. This ensures that the spider can be placed in the optimal position with easy access to the eyes. When the spider was finally tethered, a hole was ripped into the parafilm using forceps to gain access to the eyes.

Under the stereomicroscope, the spiders' eyes were covered with a paint used in model making. First a black layer was applied, to guarantee that no light could pass into the spider's eye. Then on top of that, a layer of blue ink was applied, so it could be checked with the naked eye, if the paint was still there, covering the eyes. This was necessary, in order to reduce the number of times the spiders had to be anesthetized and tethered, because the spiders could only be used for the experiments after some time had passed and they were used to having some of their eyes painted.

To ensure that the cover was lightproof, the spiders were placed on another holder, quite similar to the one described above, but instead of the wooden calotte it has a round platform with a excentric hole. The spiders were placed in a manner that their prosoma was fixated above the hole. Then, using a lamp with a flexible light guide (Schott KL 1500-Z) the spider was illuminated from below and it could be checked if light passed through the covered eyes, because one could easily discriminate between the illuminated cuticle and the dark stained eyes.

The first experimental group consisted of animals that had all their principal eyes covered, and in the second group, the animals had their secondary eyes covered. In a follow-up, for the former group, only the PM eyes were left open.

In the first part, 40 runs were made with all spiders used in the experiment with all their eyes open, to have a control. Then, they were divided randomly into two groups of four spiders each, one to have the AM eyes covered, and one with the three pairs of secondary eyes covered. For each group, another set of 40 runs was performed. The stimulus used had a diameter of 4 cm and was following the arbitrary path at 56 deg/s.

After the first set of runs, the group with only the AM eyes covered got their AL and PL eyes covered too and was tested again, for 20 runs. The other group still with only their AM eyes open, was tested again as well, but with a stimulus twice the size.

4 Results

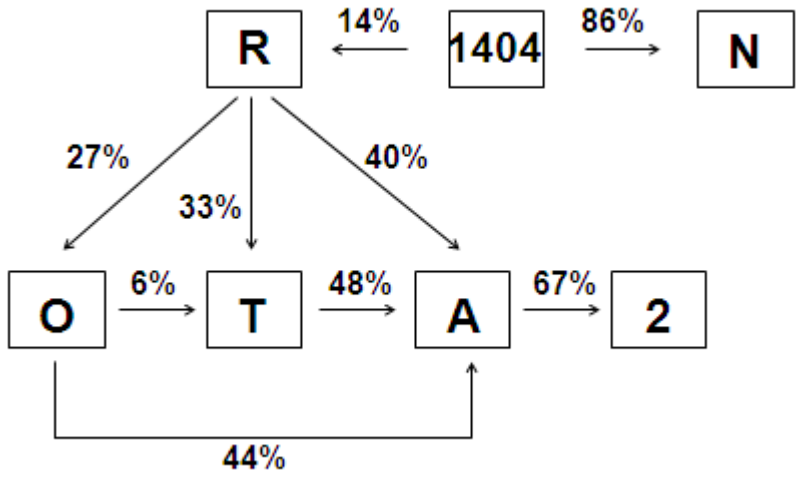
4.1 Different behavioral responses

The spiders are sitting motionless in their jars, so it is very easy to detect non-spontaneous behavior, which is elicited by an external stimulus. Mainly four different kinds of directed behaviors were observed during this study.

First, the slightest reaction is solely a turn of the body axis resulting in an orientation towards the stimulus, so that the spider is sitting in a position that seems to be optimal for attack, with her prosoma facing downwards. In the second behavior shown the spider is lifting its first pair of legs a few times. This is referred to as threatening behavior. Then, even more aggressive, the spider can leap towards the moving dot in an attempt to catch it. This is referred to as attack behavior. Lastly, some spiders, after failing their first attempt, tried to attack the dot once again when it moved past them. This is referred to as secondary attack and occurred only after a first leap.

All these different forms of behavior (except for the secondary attack) could occur separately from one another, so during one presentation the animal responded either in one way, or in several combinations of the different responses. If the latter occurred, it was always a strict order of behavior shown. The only variation was that one or more steps could be skipped, meaning that none of the different kinds of behavior could precede another which had to occur later in this particular order. For example, no spider showed threatening behavior after attacking the dot, but the step of threatening could be skipped and the spider attacked right after orienting towards the dot.

Figure 4 shows an ethogramm depicting the various reactions and their respective chance of occurrence during this preliminary study, using the data for all the different stimulus sizes and the manually drawn path. The numbers shown in percentages indicate the probability of different follow-up behavior and add up to 100% for the amount of spiders the previous box includes. The missing percentages are the spiders showing no further behavior after a certain step and for example remain in a position oriented towards the screen, or don't attack after showing threatening behavior.



N… the spider showed no reaction at all and remained still.

R… a reaction was shown.

O… the spider oriented towards the stimulus.

T… the spider showed threatening behavior.

A… the spider attacked the stimulus.

2… the spider showed a secondary response after attacking the stimulus.

Figure 4: Ethogram depicting the different responses a spider shows when confronted with a visual stimulus on a computer screen. The numbers in percentages indicate the rates at which any kind of behavior is shown, the arrows leaving a box sum up to 100% with the remaining percentages covered by the box itself, indicating that the spiders showed no further reaction after that step. In this figure, all different stimulus sizes are pooled.

Regarding the different sizes of the dot stimulus separately, it could be observed, that in the smaller sizes, less spiders reacted towards the stimulus. In the test with dots of the smallest size (2 cm), only 2 % of the spiders showed a reaction, and all of them instantly attacked the stimulus (figure not shown). Concerning the next bigger size (3 cm), only a bit more spiders (5 %) showed a reaction, but only about half of them attacked immediately (Fig. 5). A small portion (11 %) showed threatening behavior. None of those spiders which threatened followed up with an attack. And lastly, a third of the spiders showed orientation towards the stimulus, after which the majority proceeded to attack, many of them also a second time.

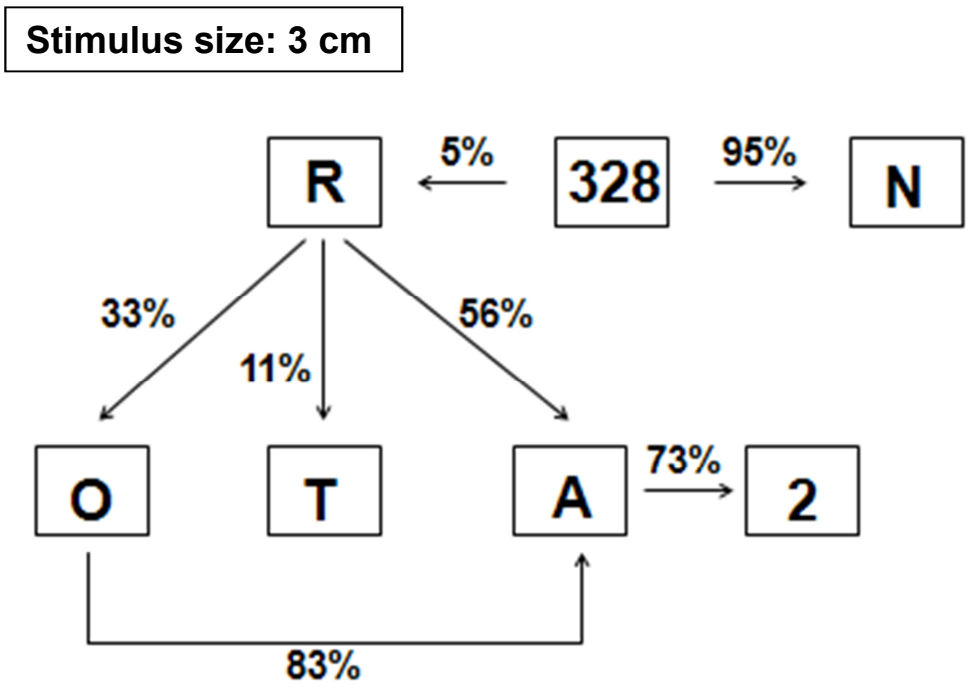


Figure 5: Ethogram depicting the responses the spiders showed towards a dot stimulus of 3 cm diameter. More than half of the spiders which showed a reaction attacked the stimulus immediately, about a third only after orienting towards it. (For abbreviations see figure 4)

When confronted with a stimulus of 4 cm diameter, 12 % of the spiders showed a reaction towards the dot (Fig. 6). All three possible categories of reactions (orientation, threatening, and attack) occurred in the range of 28 – 36 %. If the spiders oriented themselves towards the dot, three quarters followed up with an attack. If the spiders showed threatening behavior, the majority also tried to attack the stimulus. Finally, most of those who attacked tried a second time when the stimulus was passing by again.

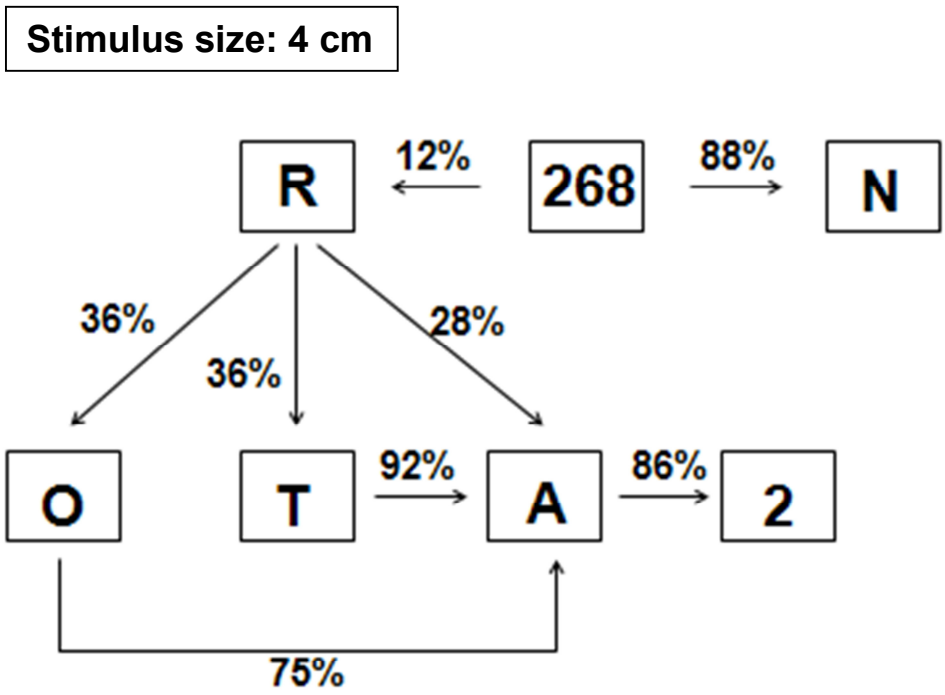


Figure 6: Ethogram depicting the responses the spiders showed towards a dot stimulus of 4 cm diameter. The three main behaviors (orienting, threatening and attack) were shown about equally often. Most of the spiders which oriented themselves towards the dot or threatened it tried to attack it afterwards. (For abbreviations see figure 4)

When confronted with a stimulus of 6 cm diameter, even more spiders showed a reaction, namely 27 % (Fig. 7). Like for the stimulus of 4 cm diameter, all kinds of reaction were shown, only in this case, threatening became a bit more frequent. Attacking was not shown as often, half of the spiders did not do so after orienting or threatening, likewise. One sequence of behavior that was not present in the former dot – sizes could be observed, namely threatening the stimulus after orienting towards it.

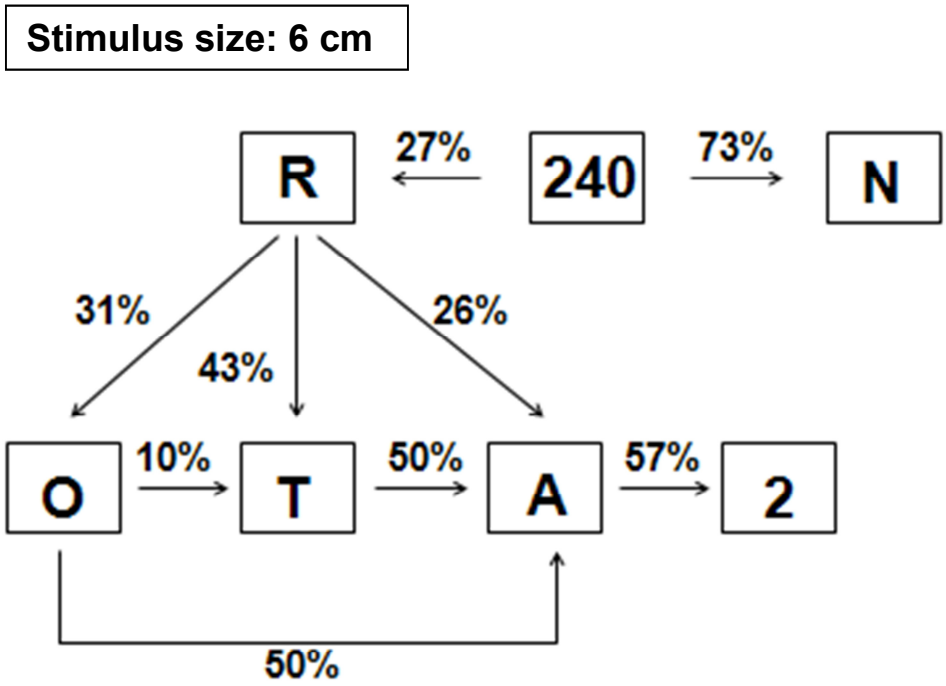


Figure 7: Ethogram depicting the responses the spiders showed towards a dot stimulus of 6 cm diameter. Compared with the smaller sizes, more threatening behavior could be observed. Also a transition from orienting to threatening behavior in 10 % of the spiders which oriented themselves towards the dot could be observed, a behavior not shown in smaller stimulus – sizes. (For abbreviations see figure 4)

Confronted with the biggest stimulus (8 cm), 26 % of the spiders showed a reaction and nearly half of them attacked it. In general, the spiders tried to attack this stimulus – size the most. Only after threatening, the majority remained where they were (Fig. 8).

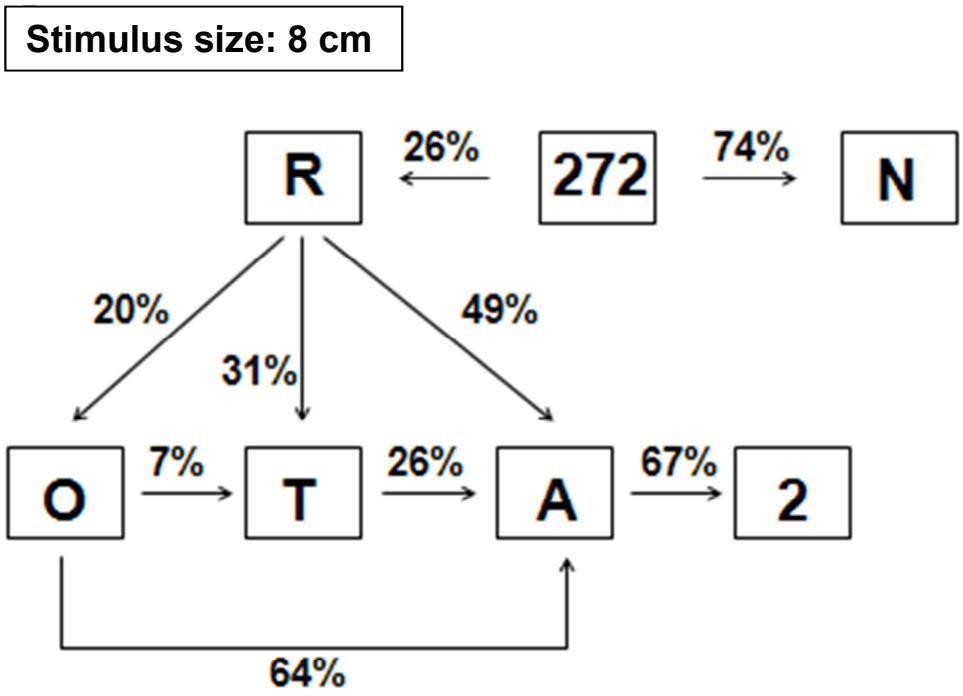


Figure 8: Ethogram depicting the responses the spiders showed towards a dot stimulus of 8 cm diameter. About half of the spiders which showed a reaction tried to attack the stimulus immediately. On the other hand, the percentage of spiders which attacked after showing threatening behavior was smaller than at a stimulus of 6 cm diameter. (For abbreviations see figure 4)

4.2 Stimulus size

The results of the experiments where five different dot-sizes were used are shown in Table 1 and contain the data of a total of 536 runs, with runs in which the spiders showed a reaction towards the stimulus, and runs where they did not, as well as the percentage of positive runs. For the smallest dot-size, only 3% of runs were positive, i.e. in only 3% of the runs the spider reacted towards the stimulus. For the dots with a diameter of 3 cm, 21% of the runs were positive, for 4 cm diameter 35% and for 6 and 8 cm diameter, 54 and 65%, respectively. These results indicate a direct relation between size of the stimulus and probability to elicit a response in the spiders.

Table 1: Total number and percentage of reactions towards dot stimuli of different size, ranging from 2 to 8 centimeters in diameter.

dot-size (cm)	Total runs	no reaction	pos. reaction	% pos
2	106	103	3	3
3	107	84	23	21
4	105	68	37	35
6	107	49	58	54
8	111	39	72	65

To check whether these differences were random or significant, a step-wise comparison of two consecutive stimulus sizes was performed, using Pearson's chi-square test. The results of this test for significance supports this point well for the sizes 2 – 3 cm and 6 – 8 cm, where the difference between these categories was very significant ($p < 0,01$). When comparing the sizes 3 – 4 cm and 4 – 6 cm, no significant difference could be observed, but a trend in this direction ($p \sim 0,15$) is obvious. When skipping the stimulus of 4 cm diameter and comparing 3 – 6 cm, the result was highly significant ($p < 0,001$). The exact values are shown in Table 2.

Table 2: A step-wise comparison of two differently sized stimuli concerning their chance of eliciting a positive reaction in the spiders using Pearson's chi – square test.

dot – sizes (cm)	chi - square	p - value
2 – 3	10,6	0,001
3 – 4	2,04	0,153
4 – 6	2,14	0,143
6 – 8	7,16	0,007
3 – 6	12,66	0,0004

4.3 Stimulus speed

Out of the 24 runs per speed setting (43, 56 and 84 deg/s), in only 6 runs the spiders showed a reaction towards the stimulus during the fastest setting and in 4 runs during the medium setting. Not a single spider showed any reaction during the slowest setting. For the human eye, the movement of the dot in the highest speed setting does not appear fluid. It can only be guessed how it is perceived by the spiders. Since the stimulus at the intermediate setting appeared more fluid and only a few spiders less reacted towards it, all other experiments were done with the intermediate speed.

4.4 Ambient light intensity

In the experiment where the ambient light intensity was altered, a gradient of responsiveness could be observed. The brighter the room was, the less frequently the spiders showed a reaction towards the stimulus. Table 3 shows the percentages of positive runs for all eight spiders during which the animals showed directed behavior towards the stimulus (i.e. orienting towards it, showing threatening behavior or attempting to attack it) for each of the four light intensities. Additionally, the contrast between the stimulus and the background is shown.

Table 3: Percentages of positive reactions towards a moving dot during four different ambient light intensities.

light intensity (lx)	% pos. reaction	contrast
1	58	0,99
60	54	0,97
330	29	0,89
2800	13	0,71

A test for significance of the differences between the four categories was performed, using Pearson's chi – square test. By step - wise comparing the different light settings, it can be shown that the categories differ significantly from one another (see Table 4).

Table 4: Step – wise comparison of different light settings concerning their chance of eliciting a positive reaction in the spiders using Pearson's chi – square test.

Light intensities	chi - square	p – value
1 – 60	13,47	0,00024
60 – 330	3,96	0,04655
330 – 2800	8,33	0,00391

4.5 Latency and location

During the tests considering the stimulus size (4.2), it was observed that most of the spiders showed a reaction after a more or less distinct time. Therefore the latency between stimulus onset and the first reaction of the animal was measured for the five different stimulus sizes used in 4.2.

4.5.1 Stimulus size of 2 cm diameter

During the tests, only four spiders showed any reaction towards the smallest dot-size, after about 5, 6 and two at 9 seconds after stimulus onset.

4.5.2 Stimulus size of 3 cm diameter

Using the stimulus with 3 cm diameter, a few more responses than with using the smallest stimulus could be elicited. The distribution of responses at any given time shows no acute peak, but rather is distributed over a range of 3 to 19 seconds after stimulus onset.

4.5.3 Stimulus size of 4 cm diameter

The stimulus with a diameter of 4 cm was the smallest where distinct peaks of activity could be encountered. There were two, one at 7 and one at 11 seconds after stimulus onset.

4.5.4 Stimulus size of 6 cm diameter

For this size, there was one major peak at 11 seconds after stimulus onset and two minor peaks at 8 and 16 seconds.

4.5.5 Stimulus size of 8 cm diameter

Using the largest stimulus of 8 cm and the arbitrary path (figure 2), most spiders showed a reaction after 12 seconds after stimulus onset.

Figure 9 shows the distribution of positive reactions over time for all the different stimulus sizes. The most reactions were observed between 11 and 13 seconds. This was the time at which the stimulus reached the bottom of the screen.

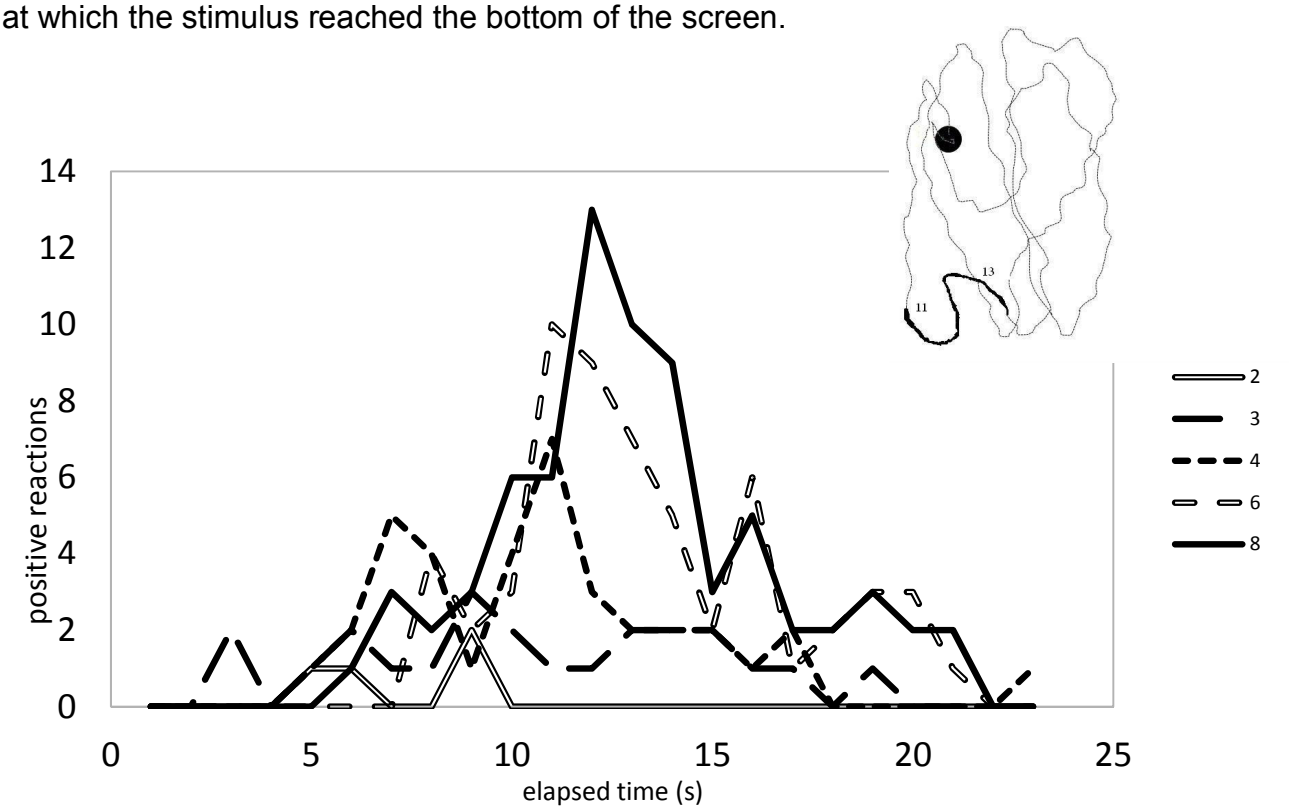


Figure 9: Positive reactions to the presentation of a moving dot of different size. For the two smallest sizes, no definite peak of behavioral response is shown, whereas for the three bigger stimuli, a peak is shown between 11 and 13 seconds. For all sizes, no reactions were shown before 3 seconds and after 22 seconds after stimulus onset.

To check whether this reflects the spiders' latency or if a certain position of the dot is preferred by the spiders, another series of tests was performed, using four different paths. The most apparent difference between these paths was that in two of them the stimulus appeared in the center of the screen, whereas in the other two it entered from outside the screen. Also, due to the design of the paths, the stimulus had to reverse directions twice in the former, and only once in the latter two paths. This difference is also reflected in the distribution of positive responses. In the two paths where the stimulus appeared in the middle of the screen and then moved upwards or downwards, a big portion of the spiders showed directed behavior when the dot came to stop before returning back down, or up, respectively. Then, five seconds later, on the other edge of the screen, the same happened and another big fraction of test-subjects reacted.

For the other two paths, the distribution pattern looks quite different. For both of them, there is a prominent peak after only one or two seconds after stimulus onset. The fastest responses were observed in the path, where the stimulus entered the screen from below, with the spiders reacting almost immediately when the dot was visible to them. For the other path, most of the spiders reacted after two to three seconds, when the dot has passed the middle of the screen, reaching the bottom. The distribution is shown for all paths in Figure 10.

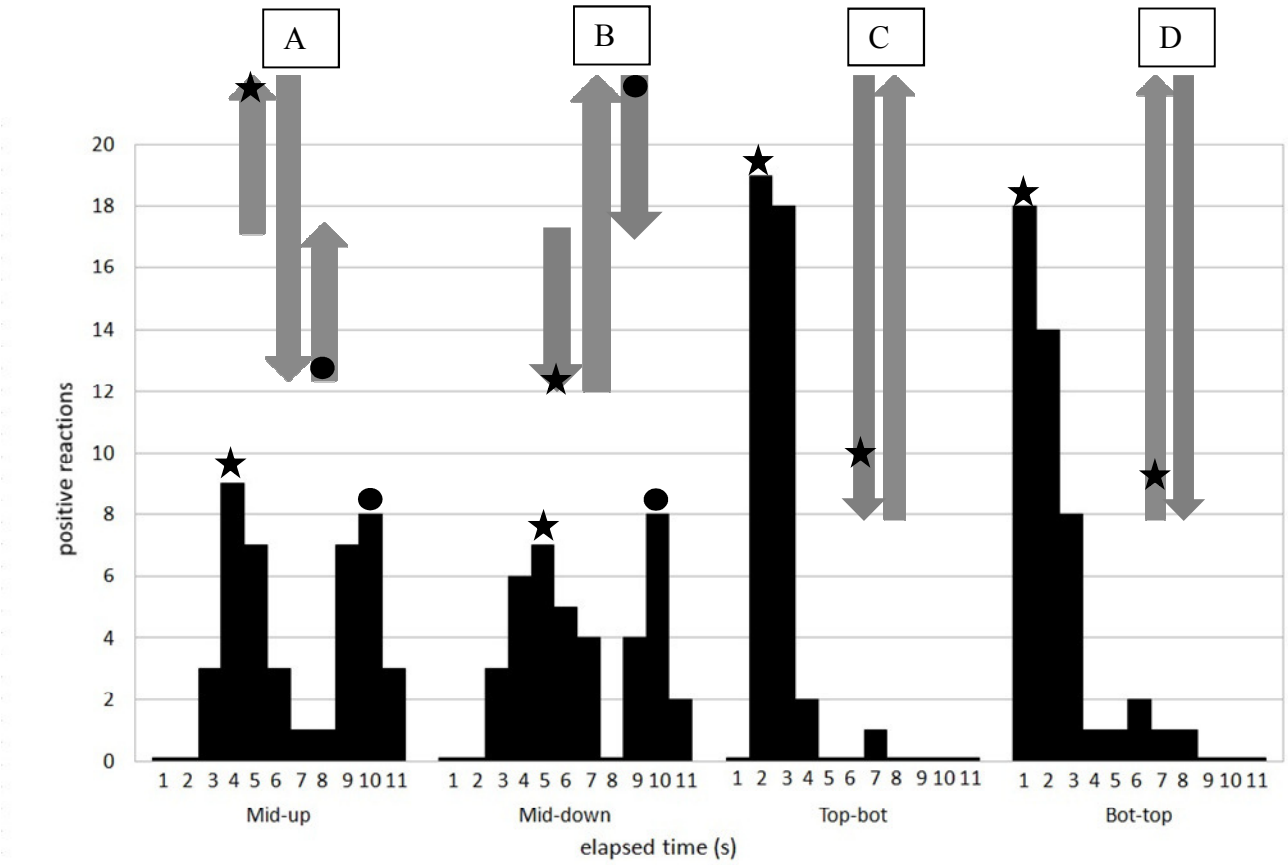


Figure 10: Positive reactions for the four different presentations of vertically moving dots. The stars and dots indicate the corresponding peaks of responses in reaction time (in the histogram) and location (on the arrows indicating the path) during the presentation. For the first path, (A), 2 peaks of activity are formed, one 4 seconds, the other 10 seconds after stimulus onset. For (B), there are 2 peaks, also at 4 and 10 seconds. For (C), there is only one peak, after one second of the presentation, and for (D) there is a singular peak, at less than 1 second after the beginning of the presentation.

4.6 Covered eyes

In the experiment where the spiders had their eyes covered with black and blue paint, a great difference in responsiveness between the two groups could be observed. The group of spiders with only the AM eyes covered showed nearly as many positive reactions as the control group of unimpaired spiders. The group of spiders with their AM eyes open and their secondary eyes covered showed no reaction at all throughout the experiment. Even

when doubling the size of the stimulus, the animals in this group did not show any reactions.

When the group with their AM eyes covered got the rest of the eyes except the PM eyes covered as well, the percentage of directed response did not decrease, but remained the same.

An overview of the various runs and their percentage of reactions is given in Table 5.

Table 5: Responses shown towards dot stimuli when only a part of the eyes could be used to perceive it. For the AM eyes only, no reactions whatsoever were recorded, whereas if the secondary eyes or even only the PM eyes were left open, no impairment in means of response frequency could be detected.

eyes open	stimulus size (mm)	runs	pos. reaction	no reaction	%pos	latency (s)
all	40	40	17	23	43	9,41
AM	40	38	0	38	0	0
AM	80	20	0	20	0	0
secondary	40	40	18	22	45	10,06
PM	40	20	9	11	45	11,33

5 Discussion

The goal of this study was to find out whether *Cupiennius salei* spiders react towards visual stimuli and if so, how the stimuli have to be shaped in order to elicit behavioral responses at a higher probability. The first part could indeed be shown, for in many cases, attack behavior could be elicited. The parameters which were tested to create the optimal stimulus were stimulus size, ambient illumination and stimulus speed. The first two had a relatively large impact on the chance of eliciting a response. For the latter it could not be shown explicitly, because of a too low number of responses.

5.1 Different behavioral responses

The different ways a spider could react towards a dot stimulus presented on the computer screen were orienting behavior, where the spiders would turn themselves to face the stimulus, threatening behavior, where the spiders waved their first pair of legs towards the stimulus and attack behavior, where the spiders leaped towards the stimulus.

Overall, the biggest amount of behavior shown was aggressive behavior, where the spiders leaped towards the screen, supposedly in order to catch the moving stimulus. The second most often occurring behavior was the one referred to as "threatening" behavior. These two made up about 70% of the behavioral responses. A large number of the spiders showed aggressive behavior after orienting towards the stimulus. This occurred in about half the cases, whereas orienting followed by threatening occurred rather rarely. This could possibly be, because for the spider it is better not to be revealed before repulsing a potential threat. In the time spent orienting, it could have been eaten before showing its predator the potential threat it poses. Related to this it seems logical, that the percentage of spiders that initially showed threatening behavior and then attacked the stimulus gets smaller the bigger the stimulus was. Opposed to that, the most aggressive behavior, i.e. leaping immediately, without assessing the situation beforehand, was shown when confronted with the biggest stimulus size. A possible reason could be that for the spider a stimulus this size meant that the spider had to react quickly and repulsing a potential opponent or escaping was not an option, but capturing a prey this size would outweigh the risk. This could also explain why secondary attacks were more common when confronted

with smaller stimuli. After an attack, the spider was generally about ten centimeters closer to the screen, and therefore the stimulus was perceived as bigger and represented a more attractive prey. In the case of stimuli of 6 or 8 cm in diameter, the stimulus then appeared maybe too big to elicit attack behavior. It could be that a stimulus this size would more likely represent a threat to the spider and therefore, lesser spiders reacted to it aggressively.

5.2 Stimulus size

While the probability to which the spiders reacted towards the different - sized stimuli differed a little, the overall increase in probability was directly dependent on the size. That means, the bigger the stimulus was, the more spiders showed directed behavior towards it. This correlation is supported by the chi – square test for differences between the sizes which indicates at least a strong tendency towards dissimilarity between the different stimuli in respect to chances of eliciting a response.

It could be that the spiders compare the benefit of catching its prey to the risk of missing it and in doing so avoid wasting precious energy. Therefore they would risk an attack more often on bigger prey, because it yields more benefit and smaller prey just is not as attractive.

On the other hand, the spiders had no possibility to measure the distance to the stimulus. Firstly, because in general they lack depth perception if sitting motionless, and secondly, there is no depth to perceive on 2D monitors. Thus, the size of the stimulus cannot be interpreted as size alone, but also as information about the distance to the stimulus, meaning that bigger stimuli might be objects closer to the spider. Bearing this in mind, it seems natural that more spiders react to the bigger stimuli, because it should be easier to catch a prey that is not too far away.

It was often observed, that some spiders jumped in the direction of humans, walking by their jars. Obviously, the spiders won't catch humans as prey, but as they were at a certain distance, they might appear to the spiders as potentially small enough to be caught.

When comparing the size of the stimulus to the spatial resolution of the eyes, it is also unsurprising that almost none of the spiders reacted to the smallest stimulus, which covered only 5,72° of their visual field. This is only about twice the spherical distance between two receptor rows which is 2,6° for the PM eyes, and they have the best resolution compared to the other eyes (Land and Barth 1992, Fenk and Schmid 2010).

5.3 Stimulus speed

A possible reason why this experiment yielded no results at the lowest speed of the dots might be that they were not perceived well by the spiders. In general, for the spiders, the stimuli might not be resolved properly, due to their lower temporal resolution. So they may need faster movements, and slow movements might be seen by them not as smooth movements but rather little jumps. On the other hand, if exposed to a flickering stimulus, the spiders did not show any reaction, in this case activity of the eye muscles, if the frequency of the flicker is too high (Fenk and Schmid 2011). It is hard to tell how exactly the spiders perceived the stimuli, but this problem would occur in a non-experimental setup as well, when confronted with real moving animals. It could be that the slow stimulus was not attractive for the spiders, because it does not appear to them as possible prey, which might move at faster speed. It has been shown, that some animals mask their movement by travelling at very slow speed (Barth et al. 1988). Maybe the same is possible using the visual system.

5.4 Ambient light intensity

Cupiennius salei is a nocturnal hunter and adapted to catch its prey under light intensities where it is hard for humans to see anything. When the light was turned off completely and the laptop screen was the only source of light in the room, the contrast between stimulus and background was very high (0,99). On the other hand, having the room extremely bright results in a decreasing contrast of the flat screens. In the brightest setting the contrast was lowered by about a third (0,71). The rate of behavioral response decreased when the room was illuminated more strongly. It could very well be that the spiders would hunt their prey under these circumstances, but were not able to see the stimulus that well. After all, the percentage of directed behavior dropped from over 50% at the high contrast

to only about 13% at the low contrast. Therefore, the contrast has a remarkable influence on the rate at which *Cupiennius salei* spiders show attack behavior against the moving dot stimuli.

5.5 Latency and location

During the experiments described above, most of the spiders reacted after a certain amount of time, at least when the larger stimuli were presented. In the path used, this was after about 11 to 12 seconds, when the stimulus first reached the bottom of the screen. The question that therefore emerged was whether this time was the latency after which the spiders normally react to moving objects, or the position of the dot was perfect for an attack. The logical consequence was to alter the path and therefore the four different paths described above were created.

For each pair of the four alternative paths, the distribution of reactions over time was very similar. The only case in which an internal latency could explain the time passed until the reaction was the path, where the stimulus enters the screen from below. There, most spiders reacted very fast, almost immediately after the dot appeared, when it was still in the lowermost part of the screen. For the spider, the position of the dot and its direction of movement, up the screen, towards the spider, which is sitting approximately in the level of the middle of the screen, the situation is optimal for an attack. For all other paths, the most responses were shown when the stimulus reached the edge of the screen and began to turn around.

The reason for this distribution could be that it provides an advantage for the spiders when the stimulus is reversing its direction of movement, because in order to do so, it has to slow down and come to a stop, even if only for a very short amount of time. The spider leaps for the target which would then be easier to catch, because of standing still. Either this or the spider attacks, because the dot would soon reach the edge of the screen and then disappear.

Interestingly, the attack itself was not always directed exactly towards the stimulus. This was only true when it was in the bottom half of the screen, below the spider's vantage

point. If the stimulus was above the spider when it attacked, it also jumped downwards, without any chance to actually catch the dot. Maybe this has something to do with the spider not knowing how far away the dot is positioned, whether it is inside the jar or 20 cm away on the outside, so jumping downwards is merely a result of finishing the jump, which the spider intended, while trying to catch its prey in midair.

Though it cannot be determined if the change in velocity or direction or even the position itself is the adequate stimulus to elicit the attack behavior, it is certain, that the delay in response is not caused by a latency between the spider detecting the stimulus and reacting towards it.

5.6 Covered eyes

This part of the study provided the strongest results of all, because one group, the one with only the AM eyes open never reacted to the stimulus at all. This complements the findings of Neuhofer et a. (2009), who performed experiments with moving stimuli and masked the different eye types as well. In their study, eye muscle activity was measured in the AM eyes in response to black bar stimuli. The activity increased in response to stimuli when the AM eyes were covered, but not when the secondary eyes where.

That the difference in attack rate was no artifact produced by the impairing act of covering the eyes and leaving the spiders unable to do anything at all, because the other group, where the secondary eyes were left open, still showed directed behavior towards the stimulus. In addition they reacted at a very similar rate, so one could say that the act of covering the AM eyes did not affect the spider's ability to detect the moving dot. Even when the AL and PL eyes were covered, this fact remained unchanged. Probably because they are not needed in detecting objects directly in front of the spider, with only the visual field of the AL eyes barely reaching into the middle, and only in the lower half, so it makes no difference if they are left open or are covered.

This indicates that for detecting a moving object, not only the secondary eyes suffice, but the principal eyes play no role whatsoever. This was predicted a priori, because of the structure of the eyes. The AM eyes, possessing a moveable retina (Land 1969), are used in observing non-moving objects and help in orienting by observing the background. The

secondary eyes, lacking the retinal muscles, are used in detecting moving objects, because after sitting still for a while, the image of the world saturates the retina of the secondary eyes and they become as good as blind. The only thing that could be detected is a change in the environment, i.e. something passing by the spider. Therefore it is detected much more easily, because it is the only thing seen by the secondary eyes (Ewert and Borchers 1974).

These findings complement those of Schmid (1997), who performed a study covering the eyes in a similar way, but presenting stationary objects which the spiders had to approach. When the AM eyes were covered, the spiders could still detect the stimuli and approach them, albeit unable to discriminate between them. With the AM eyes left open, they discriminated between the stimuli. Bearing this in mind, a question that remains is why the spiders did not react at all when they were left with only the AM eyes open. Ignoring that the detection of the dot is not optimal, the spiders still should be able to see it. Still, there is a difference between moving and stationary objects and the way they are perceived by the spider might as well be different.

In conclusion, it can be said, that a behavioral response in *Cupiennius salei* can be elicited by only stimulating the visual system. The best way for doing so is choosing as big a stimulus as possible under the darkest possible ambient illumination. Moreover, it can be said, that the functionality of the secondary eyes is necessary for the attack behavior to be expressed, whereas the functionality of the principal eyes is no prerequisite.

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8 Appendix

8.1 Summary

This study is concerned with the question, how attack behavior can be elicited in the nocturnal hunting spider *Cupiennius salei*, using only visual stimuli. For this reason, all other sensory inputs had to be excluded, meaning that a stimulus should not elicit a response using another channel. Therefore, a presentation of visual stimuli using computer screens was chosen, because then the stimulus did not have a physical appearance, but was only an image which could not produce vibrations for example. Furthermore, the different spiders were isolated from each other, so that they won't influence each other as well. When using stimuli of different sizes, the spiders responses increased in likeliness, the bigger the stimulus was. Also, a darker ambient illumination was a better condition to test the spiders, because much fewer spiders reacted during light intensities resembling daylight, than when it was darker. Under brighter ambient illumination, the contrast was not so strong, which made it harder for the spiders to see the stimulus.
During the first experiments using always the same animation path for the stimulus, many spiders reacted after a certain amount of time passed. To check whether this was influenced by the path chosen itself, another set of presentations was made, consisting of four different paths. It could be shown that the way the dot moved influenced the time when the spider would react towards it. Most responses were recorded when the dot was at the edge of the screen, especially at the bottom.

A last experiment was performed, covering the different pairs of eyes with paint and confronting the spiders again with the dot – stimulus. It could be shown that the PM eyes play the biggest role in detecting moving stimuli, whereas the AM eyes seem to not contribute at all.

8.2 Zusammenfassung

Diese Arbeit beschäftigt sich mit der Frage, wie Angriffsverhalten bei *Cupiennius salei,* einer nachtaktiven Jagdspinne, ausgelöst werden kann, wenn nur der visuelle Kanal zugelassen wird. Zu diesem Zweck mussten alle anderen Informationsquellen für die

Spinne ausgeschaltet werden, das heißt, dass ein Stimulus, der auf den optischen Kanal abzielt, nicht auch noch auf einem anderen Weg eine Antwort auslöst. Aus diesem Grund wurde eine Präsentation von Stimuli auf Computerbildschirmen gewählt, da diese nur virtuell vorhandene Bilder darstellen und keine wirklichen Körper sind, die zum Beispiel Vibrationen auslösen, wenn sie bewegt werden. Außerdem wurden die Spinnen selbst voneinander isoliert, sodaß sie sich nicht gegenseitig beeinflussen konnten. Bei Konfrontation mit verschieden großen Stimuli, mehr Spinnen reagierten, je größer der Stimulus war. Auch eine dunklere Umgebung hatte positiven Einfluß auf die Antwortwahrscheinlichkeit. Um einiges weniger Spinnen reagierten bei Lichtverhältnissen entsprechend denen bei Tageslicht, als wenn die Umgebung dunkler gehalten war. Außerdem war der Kontrast bei helleren Lichtverhältnissen schlechter, was den Spinnen auch erschwerte, den Stimulus zu sehen.

Während der ersten Experimente wurde immer derselbe Pfad für den Stimulus gewählt und es konnte beobachtet werden, dass die meisten Spinnen nach etwa der gleichen Zeit reagierten. Um zu überprüfen, ob das der Einfluß des Pfades selbst war, wurde ein Set aus vier verschiedenen Pfaden erstellt. Es stellte sich heraus, dass der Pfad wirklich Einfluß auf die Zeit, zu der die Spinnen reagierten hatte. Die meisten reagierten, wenn sich der Stimulus am Rand des Bildschirms befand, vor allem am unteren.

Als letztes Experiment wurde die Rolle der unterschiedlichen Augentypen in der Erkennung von sich bewegenden Objekten untersucht. Zu diesem Zweck wurden abwechselnd die verschiedenen Augentypen mit Modellack übermalt und die Spinnen wieder mit dem wandernden Punkt konfrontiert. Es stellte sich heraus, dass die PM – Augen die größte Rolle im Erkennen des sich bewegenden Punktes spielen, während die AM – Augen anscheinend hierbei nicht mitwirken.

8.3 Curriculum vitae

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Ausbildung

1993-1997 Volksschule St. Ursula
1997-2001 Unterstufe Gymnasium St. Ursula
2001-2005 Oberstufe Gymnasium St. Ursula (sprachlicher Zweig)
Juni 2005 Matura mit gutem Erfolg abgeschlossen
Okt. 2005 – Juni 2006 Grundwehrdienst
seit Oktober 2006 Studium an Universität Wien: Biologie
2008: Abschluss erster Studienabschnitt
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August 2003: Ferialpraktikum im Dorotheum Wien
Mitarbeit bei Neuerfassung und Wertschätzung diverser Schmuckstücke
seit Juli 2005: Nachhilfe in Mathematik für alle Schulstufen
März bis Mai 2006: Door to door Kampagnen bei XPR
Verkauf von „Frühstücksservice“
Juni 2006 bis September 2011: geringfügig Angestellter bei Impacts
Eventbetreuung: Catering, Aufbau, Transport, Service. Verkauf (inkl. Teamleitung)
z.B. Juli 2008: Stadionverkauf bei EM 2008 für SellAct
Mai 2007 bis September 2008: Bienenzucht
Ankauf von drei Bienenkolonien, Beitritt Imkerverein, Honigproduktion, Verkauf
Juli 2009: Forschungsstation Wilhelminenberg: unentgeltliche Pflege der Keas
September 2010 bis Juni 2011: HR – Controlling bei UPC Austria
September 2010 bis Juli 2013: Tutor an der Universität Wien
Im WS: Organ- und Kommunikationssysteme
Im SS: Physiologie – Nerv, Muskel, Sinne
Oktober 2011 bis September 2012: Freelancer bei Kulinarik (Stadionverkauf)
seit September 2012: Lehrer am BG 13 Fichtnergasse (Unterrichtsfach BIUK)

Sprachen

Deutsch: Muttersprache
Englisch: ausgezeichnete Sprachkenntnisse in Wort und Schrift,
laufende Anwendung im Rahmen des Studiums, 2 Wochen Sprachaufenthalt 2002,
Teilnahme European Youth Parliament 2004
Spanisch: sehr gute Sprachkenntnisse in Wort und Schrift,
mehrmalige Sprachaufenthalte (2 Wochen 2004, 1 Monat 2005 und 1 Monat 2008)
Französisch: gutes Textverstehen, Grundkenntnisse im Dialog,
2 Wochen Sprachaufenthalt 2004