

DIFFERENT FUNCTIONS OF DIFFERENT EYE TYPES IN THE SPIDER *CUPIENNIUS SALEI*

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Summary

The Central American hunting spider *Cupiennius salei* relies mainly on its mechanosensory systems during prey-catching and mating behaviour. The behavioural relevance of its eight eyes has not been studied before, although their optics and sensitivity suggest highly developed visual capabilities. The visual system was examined in a twofold simultaneous-choice experiment. Two targets were presented at a distance of 2 m from the animals, and their walking paths towards the targets were monitored. Spiders showed no preference when choosing between two identical targets, but when choosing between two different targets they strongly preferred a vertical bar to a sloping bar or a V-shaped target. By covering all eyes except the anterior median or posterior median eyes, it

could be shown that the spiders were able to detect the targets using any of the eyes. Discrimination between different targets was only possible with the anterior median eyes uncovered, although the visual fields of the anterior median and posterior median eyes overlap completely. It seems most likely that the animals separate visual information in the periphery and therefore that the eyes have different functions. The posterior median eyes support a target-detecting mechanism and the anterior median eyes a target-discrimination mechanism.

Key words: eye, spider, *Cupiennius salei*, target discrimination, vision.

Introduction

One possible reason why some animals have more than two eyes could be to increase the size of the overall visual field. Another advantage could be to allow the world to be perceived in different ways; that is, to capture different features from the visual world using different eyes. If the visual fields of two eyes on one side of the body overlap almost completely, a difference in function is highly likely. This functional separation could be achieved in the peripheral parts of the visual system within the eyes themselves, by differences in the optical systems of the eyes, including the extent of enhancement by a tapetum, by differences in the absolute and spectral sensitivities of photoreceptors, or in the way that these are distributed within the retinae. Alternatively, differences in the type of information extracted could be achieved in the more proximal parts of the visual system, the visual pathways, where the visual world can be split into different information channels by neuronal mechanisms.

Experiments with jumping spiders (Salticidae) and lycosid spiders (Lycosidae) have revealed that these animals, in contrast to the ctenid spider *C. salei*, rely mainly on their visual system during courtship and prey-catching behaviour (Duelli, 1978; Forster, 1985; Rovner, 1993). The behavioural roles of the different pairs of eyes in salticids and lycosids have been studied previously. When a small object moves in the visual

field of the lateral eyes of a jumping spider, the animal turns rapidly with an angular extent almost equal to the angle between the object and the body axis, and then fixes the object with its anterior median eyes (Land, 1971). The probability that the animal will walk towards a target depends on its size and shape (Forster, 1982, 1985).

In lycosid spiders, the anterior median and the posterior median eyes also have different roles (Rovner, 1993), and vision, olfaction and the mechanosensory systems are all involved in conspecific interactions (Rovner, 1996).

In the spider *Cupiennius salei*, the visual fields of the anterior median (AM) or principal eyes and of the posterior median (PM) eyes overlap almost completely (Land and Barth, 1992). The PM eyes together with the anterior lateral (AL) and the posterior lateral (PL) eyes are called the secondary eyes. The electroretinogram (Barth *et al.* 1993) and the spectral sensitivities of three photoreceptor types (green, blue-green and ultraviolet) have been described previously (Walla *et al.* 1996). The distribution of specific photoreceptor types within the retina has not been investigated, but there is some evidence that it differs between the different eye types. The PM eyes have a tapetum and photoreceptors with cell bodies located distal to the rhabdomeres. The AM eyes have photoreceptors with cell bodies located proximal to the rhabdomeres and no

tapetum. The visual pathways of the AM and PM eyes are also completely different. The two AM eyes have separate first- and second-order visual neuropiles and a common third-order neuropile. The PM eyes, like the other secondary eyes (AL, PL), have separate first- and second-order neuropiles. All six pathways of the secondary eyes then merge into a common third-order neuropile (Strausfeld and Barth, 1993; Strausfeld *et al.* 1993). Thus, completely separated visual information processing is possible with these two systems, up to at least the fourth-order processing stage.

The influence of the visual system on the walking mode of the animal was shown by Schmid (1997). Each of the AM eyes has two clearly separated eye muscles which can move the retina. The visual fields can be moved in any direction between dorso-lateral and ventro-lateral because of the arrangement of these muscles (Kaps and Schmid, 1996). All these differences, together with the overlapping visual fields, indicate separate functions for the AM and PM eyes.

In order to show conclusively the existence of functional separation between the principal and the secondary eyes in the visual system of *C. salei*, it is necessary to demonstrate such a separation in behavioural experiments. The structural differences described above clearly suggest such a functional difference but do not in themselves provide sufficient proof. The use of the different eye types in a behavioural context can be studied using different visual stimuli in combination with experimentally covering the AM or PM eyes. *Cupiennius salei* probably uses vision to detect prey and predators, but this has not yet been demonstrated. Furthermore, *C. salei* is likely to use its visual system to search for the plants in which it lives, because none of its other sensory systems is appropriate for the performance of this task.

In the present study, the different functions of the AM and PM eyes in detecting and discriminating visual targets are described. It is shown that *C. salei* can detect visual targets using either the AM or the PM eyes. However, the animal uses its AM eyes exclusively to discriminate among different visual targets. In addition, some types of visual features that the animal is able to distinguish are identified.

Materials and methods

Experimental animals

Adult males ($N=42$) of the Central American hunting spider *Cupiennius salei* Keys were used. The animals had a body length of 3–3.5 cm and a leg span of 10–12 cm. They were bred at the Institute of Zoology, Vienna, Austria, under natural daylight and were fed once per week on flies (*Calliphora erythrocephala*) or house crickets (*Acheta domestica*). The temperature (22–28 °C) and relative humidity (80–95 %) were similar to those of the Central American forest. Each animal was kept individually in a glass jar.

Experimental apparatus

The animals used in experiments were kept under an artificial photoperiod (12 h:12 h L:D) for at least 3 days prior

to the experiments. Experiments were performed in a room without natural light or air-conditioning in order to avoid vibrations transmitted through the floor. The size of the experimental arena was 2.5 m×2.5 m. The floor and three of the walls around the arena were homogeneously bright up to 2.5 m high. The fourth wall was lit up to a height of 80 cm. The animals were put into the arena. In some experiments, the animals were filmed using a video camera mounted above the arena (Fig. 1A). The video-recorded walking paths were reconstructed using frame-by-frame analysis, digitized and pooled for each target combination.

Experiments were carried out at two different illuminations, during subjective day (bright, 200 lx) and night (dark, 1 lx). The low intensity in our experiments is far above the threshold of the spider, which is approximately 0.01 lx (Barth *et al.* 1993). The animals were released at a distance of 2 m from the targets, which were constructed from black cardboard. The twofold choice experiments were carried out with the targets presented alternately at both positions (left or right) to exclude any effects of side preferences of the animals. The distance between the targets was 1.5 m. The glass jar containing the animal was placed at the release site and the cover was removed. The animal was then very slowly and carefully coaxed to leave the jar at the side facing the targets.

In all experiments, the number of animals is given and also the number of trials. *P* is the significance (tested using χ^2 or paired sign tests) of possible position or illumination effects and of any differences in the attractiveness of the two targets.

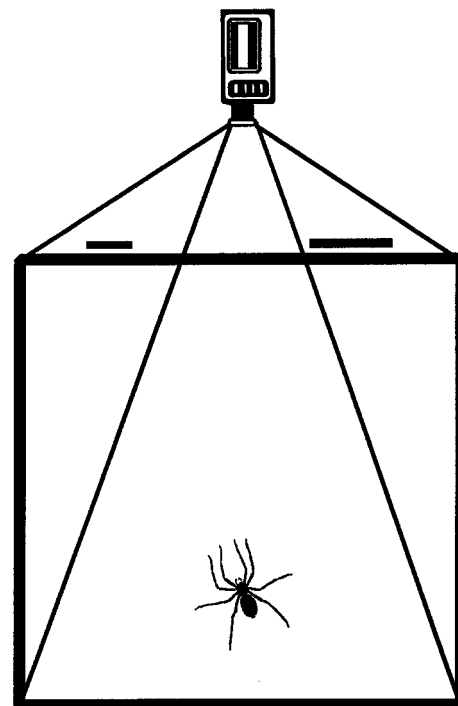


Fig. 1. (A) The experimental arena (2.5 m×2.5 m) with a spider (not to scale) facing two different targets (bold lines at the top). A video camera mounted above the arena recorded the walking paths of the animals.

Table 1. Target combinations type 1–5 with shape, dimensions and orientations used in the behavioural tests

Target combinations	Shape of targets		Width/height (mm)	Orientation (degrees)
	A	B		
Type 1	I	I	240/500 versus 240/500	0
Type 2	I	/	240/500 versus 240/500	22
Type 3	I	^	240/500 versus 2×120/500	22
Type 4	I	V	240/500 versus 2×120/500	22
Type 5	V	^	2×120/500 versus 2×120/500	22

The types and shapes of the targets used are shown in Table 1. The targets had the same overall size (area, height, width, orientation) unless stated otherwise. Where a target was split into two parts, as was the case for the V-shaped targets (types 3, 4 and 5 in Table 1), each part was tested individually as a target to ensure that it could be detected by the animals.

Coating of the eyes

In some experiments, the eyes were covered with beeswax mixed with charcoal powder. This provided an opaque covering that the animals were not able to remove (unlike the lacquer-coating used in preliminary experiments). Different combinations of eyes were covered. Untreated animals and animals with all eyes covered were used as controls. Animals with both AM eyes or both PM eyes untreated and all other eyes covered were used, as were animals with only one AM eye or one PM eye left untreated. In the latter group, animals with one left or one right eye untreated were used equally to eliminate any effects of side preferences. If the animals walked towards a target and reached it with at least the tip of one leg, the path was regarded as a ‘directed’ walk. If the animals did not reach a target and touched the wall of the arena but not the target, the path was regarded as an ‘undirected’ walk and the experiment was repeated.

Results and discussion

Untreated animals

Walking paths were recorded in two experiments (target combination types 1 and 2 with control animals). Spiders followed a characteristic zig-zag approach towards the targets, in which the body axis was turned alternately left and right at an angle to the overall walking direction (Fig. 2). This zig-zag movement could be a mechanism to help the animals distinguish between the object and the background by using motion parallax.

Animals with all their eyes untreated walked towards the different sets of target combinations shown in Table 1. They were able to distinguish significantly ($P<0.001$) between the target combination types 2, 3 and 5 (Table 2). In the control experiment ($N=10$), in which two identical targets were presented (type 1), the animals walked towards each target the same number of times (Table 2). There was no significant difference in the number of choices for target type 4, although there was a trend towards detectable discrimination in control animals. In an additional experiment, we used a bromeliad and a black target shaped like a bromeliad in combination with a rectangular target of equal area. In both tests, the animals could not discriminate between the rectangular target and the real or cardboard bromeliad.

Table 2. Numbers of choices made by spiders with the five types of target combinations presented shown in Table 1

Type	Control		Two AMs		Two PMs		One AM		One PM	
	A	B	A	B	A	B	A	B	A	B
1	21	19	15	17	18	14	13	19	16	16
2	33	7***	21	7**	14	14	22	10*	17	15
3	33	7***	24	4**	14	14	20	12	18	14
4	25	15	20	12	15	17	16	16	18	14
5	35	5***	23	9**	17	15	23	9**	19	13

Control animals had all their eyes untreated; in the other experiments, the untreated eyes are indicated, the other eyes were covered with a beeswax/charcoal mixture.

* $P<0.05$; ** $P<0.01$; *** $P<0.001$.

Seven to eight animals were used in each category, the number of runs was 28–32 (four runs for each animal). In the control experiments, ten animals were used, with four runs each.

AM, anterior median eye; PM, posterior median eye.

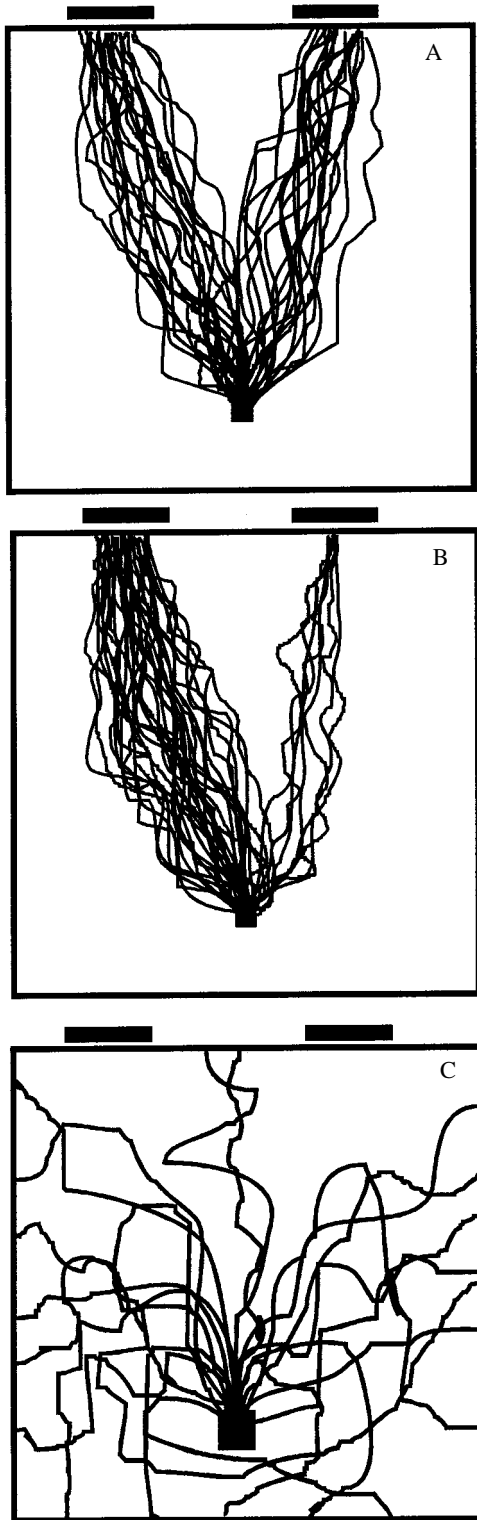


Fig. 2. Recorded paths of spiders walking towards two different target combinations: type 1 (A) and type 2 (B) (see Table 1). The animals were released at the starting point (filled squares). Note that the animals often approached the targets in a zig-zag fashion. In A, a total of 40 runs is shown (10 animals, four runs each), with 21 runs to the left and 19 runs to the right side. In B, the same animals made 33 runs to the vertical target (left) and seven to the oblique target (right). (C) Undirected walking paths of animals with covered eyes.

Treated animals

Four different combinations of eyes were covered with beeswax in order to investigate possible functional differences between the AM and the PM eyes.

AM eyes untreated; all other eyes covered

In this case, as in the control experiments, type 1 targets were chosen at similar frequencies (15 versus 17, $N=7-8$), whereas the targets in combinations 2, 3 and 5 were clearly distinguished ($P<0.01$). Again there was a tendency towards discrimination for type 4, but the difference was not statistically significant. The results for animals with only the AM eyes untreated were therefore the same as those for control animals.

In summary, *C. salei* is clearly able to discriminate vertical from sloping targets (type 2 combination). Furthermore, they seem to be able to discriminate a vertical bar from an upside-down V-shaped target (type 3); the animals prefer the vertical bar. The results with targets in combination 5 show that the spiders are able not only to distinguish between vertical and sloping bars but also between targets composed from bars sloping in different orientations.

PM eyes untreated; all other eyes covered

With only the PM eyes untreated, the spiders ($N=8$) were still able to detect the targets and walk towards them. However, for all five target combinations, there were no significant differences between the frequencies with which targets were chosen (Table 2).

One AM eye untreated; all other eyes covered

With only one AM eye untreated, the animals were still able to detect the targets and walk towards them ($N=8$). They were also able to distinguish between targets in types 2 and 5, but their performance was slightly poorer than in the previous tested groups ($P<0.05$ for type 2; $P<0.01$ for type 5). Discrimination for target type 3 dropped below the significance level. These results show clearly that one active AM eye is better than two PM eyes for discriminating between targets.

One PM eye untreated; all other eyes covered

As for the spiders with both PM eyes untreated, these animals could not distinguish between the different targets in any combination and walked towards all targets with almost equal frequencies. However, the animals were still able to detect the targets and walk directly towards them.

Effects of covering the eyes

The proportion of undirected walks increased drastically when the eyes were covered, although when the AM eyes were untreated, the number of undirected walks (no target chosen) equalled that obtained with all eyes untreated. In control experiments with all eyes functional, 19% of the walks were undirected compared with 21% in experiments with the AM eyes untreated and all other eyes covered. However, 42% of

the walks were undirected with one AM eye untreated, whereas 48% and 52% were undirected for one and two PM eyes untreated, respectively.

The proportion of failed runs shows that the animals are impaired in their ability to walk towards a target if they are using one or two PM eyes only. Their relatively intact ability to distinguish between different targets using only one AM eye indicates that binocular interaction is not necessary for this ability.

Animals with all their eyes covered were not able to detect a real bromeliad as a target in a further control experiment. Therefore, we conclude not only that the covering of the eyes was light-proof but also that the animals were not using other sensory systems such as olfaction, for example, to detect a target.

Side and light intensity preferences

χ^2 -tests were used to determine whether covering the left or right AM/PM eyes elicited walking towards the left or right target. This was not the case. The same test was used to compare the results under light and dark conditions to make sure that the possible difference in sensitivity of the eye types had no influence on the detectability of the targets.

Conclusions

The animals were able to detect targets using either the AM or the PM eyes. Differences in attractivity between two targets found using animals with all their eyes intact remained in animals that could use only their AM eyes. Using only the PM eyes, the animals could still detect targets but were not able to discriminate between them. This indicates that the AM eyes are used for target discrimination, whereas the PM eyes are involved in target detection only. The AM eyes are presumably used to detect distinct features of importance in the animals' environment, such as dwelling plants or retreats. The reason for the greater attractiveness of a vertical target relative to a sloping target is not clear at present, but it may be related to indicators used by the spider to choose dwelling plants or trees with retreats.

The ability of *C. salei* to detect and discriminate different targets therefore consists of two mechanisms: a target-detecting mechanism present in both the AM and PM eyes, and a target-discrimination mechanism which requires the AM eyes to be intact. Thus, *C. salei* does see differently with its different eyes.

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