

RESEARCH ARTICLE

Role of the different eyes in the visual odometry in the wolf spider *Lycosa tarantula* (Araneae, Lycosidae)

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ABSTRACT

The wolf spider *Lycosa tarantula* returns home by means of path integration. Previous studies demonstrated: (i) that the angular component of the outbound run is measured using a polarized-light compass associated with the anterior median eyes; (ii) changes in direction of the substratum are detected by the anterior lateral eyes (ALEs); and (iii) in relation to the linear component of the outbound run, an increase of optic flow, in either the lateral or ventral fields of view, caused spiders to search for the burrow at a point nearer to the goal. However, the role of the secondary eyes [ALEs, posterior lateral eyes (PLEs) and posterior median eyes (PMEs)] in the perception of this optic flow and the importance of them for gauging the distance walked is still unknown. In this study, lateral or ventral gratings of wavelength $\lambda=1$ cm were used, with two groups of spiders in each setup: (1) PLEs+PMEs covered and (2) ALEs covered. The largest reduction in the distance walked to return to the burrow was observed with the ventral grating/ALEs covered. These results show the importance of the previously neglected ALEs for the visual behavior of these spiders. The possibility of gathering information for locomotion from the three pairs of secondary eyes in the mushroom bodies is discussed.

KEY WORDS: Optic flow, ALEs, PLEs, PMEs**INTRODUCTION**

Animals that do not navigate following a particular route can do so by path integration. For this, the animal must be kept informed about the distance and direction to a goal. To calculate the distance walked, the animal must possess an odometer to record how far it is from its home to a goal location, and it must possess a compass to record the direction of travel.

Odometry – the measurement of the distance walked or flown – has been studied in depth mainly in walking (desert ants of the genus *Cataglyphis* and *Melophorus*; Wolf, 2011) and flying insects (e.g. honeybees *Apis mellifera*; Srinivasan, 2014). In these insects, two parameters have been proposed to be used for odometry: optic flow and stride integration.

In a previous study (Ronacher et al., 2000), the possible contribution of optic flow in desert ants (*Cataglyphis fortis*) was studied in channels with different width lined with a grating of black-and-white stripes which had a wavelength (λ) according to the channel width; the smaller λ was 2 cm. When the grating was placed in the lateral visual fields, there was no influence on the distance walked by the ants before searching for the nest began (Ronacher


et al., 2000). When the grating was placed in the ventral visual field, Ronacher and Wehner (1995) found a very small effect of optic flow on the distance walked when the visual patterns they used (e.g. gratings of black-and-white stripes) were moved in the direction of insect walking or in the opposite direction. However, when the pattern was stationary and the ventral halves of the eyes were covered, the mean traveled distance was not statistically different from the distance walked by ants without eye covers (Ronacher and Wehner, 1995). Wittlinger and Wolf (2013) investigated the possible interactions of the two mechanisms by which deserts ants estimate distance: stride integration and ventral optic flow. When they covered the ventral part of the ant's eye, the absence of ventral optic flow had no influence on homing distance gauged.

The optic flow hypothesis in honeybees was addressed in experiments by Esch and Burns (1995, 1996) to explain the results of their balloon and high building experiments. Kirchner and Srinivasan (1989) began studies of honeybee visual odometry by using channels with two lateral walls, in each of which there was a vertical black-and-white grating. By using this kind of channel, honeybee researchers subsequently introduced variables such as one-wall movement (Kirchner and Srinivasan, 1989), stripe period and direction (Srinivasan et al., 1997) or channel width (Baird et al., 2005; Hrnčir et al., 2004; Srinivasan et al., 1996).

The wolf spider *Lycosa tarantula* (Linnaeus 1758) is an ambush predator that lives in a burrow of ~20 cm depth with an external part made of twigs, leaves and small stones fastened together with silk. From this turret, the spiders ambush their prey and can walk to chase them, returning afterwards to the burrow from distances of 30–40 cm (personal observations). The mechanism by which *L. tarantula* returns to the burrow is path integration. The angular aspects of path integration in *L. tarantula* have been more studied than the linear aspects. Under natural conditions, *L. tarantula* uses a polarized-light compass associated with the functioning of the anterior median eyes (AMEs), as shown by Ortega-Escobar and Muñoz-Cuevas (1999). In that study, under a clear sky, with sun but with no UV light available, spiders either exhibited a systematic search or headed in a random direction. The inbound runs of spiders in which the AMEs were blinded or all eyes except the AMEs were blinded showed that the AMEs are the only ones able to detect sky polarized light. Under indoor conditions, *L. tarantula* needs visual input for the integration of the angular component (Ortega-Escobar, 2002) and this input is only obtained through its anterior lateral eyes (ALEs) (Ortega-Escobar, 2006). But besides being necessary to measure the angular aspect of the outbound run, ALEs are able to perceive a 90 deg change of a black-and-white grating ($\lambda=0.6$ cm) and, accordingly, the spiders showed a significant change in bearing dispersion in relation to the burrow position. When ALEs were covered, this bearing dispersion disappeared, while when ALEs were the only uncovered eyes, the directional bearing dispersion persisted. This

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result suggests that only ALEs perceive the visual change of the substratum (Ortega-Escobar, 2011).

In order to return to the burrow, the spiders must measure the distance walked during their outbound runs. How do they do this? In our previous study (Ortega-Escobar and Ruiz, 2014), female *L. tarantula* were placed in an experimental channel in an indoor setup and were made to walk from their burrow to a point 30 cm from the superior part of it. They were then transferred to an experimental channel (from which all silk cues had been removed) at the same distance from the burrow. We were interested in the possible use of the self-generated optic flow by *L. tarantula* to gauge the distance walked. In control experiments, spiders were made to walk through channels with plain white walls and substratum and with all eyes uncovered or all eyes covered. When all eyes were covered and the spiders could use only proprioceptive information, there was more variation in the distance walked before they began searching for the burrow or making Turner's loops (Ortega-Escobar and Ruiz, 2014; Wehner, 2016), also called 'search loops' (Wehner and Srinivasan, 1981). The optic flow induced by a grating ($\lambda=2$ cm) perpendicular to the direction of walking had a great influence on the distance walked by *L. tarantula* but the effect was more apparent when the grating was lateral than when it was over the substratum. With a half-period lateral cross-stripe grating, the spiders looked for the burrow at an even shorter distance.

In the present study, we made spiders walk from their burrow through a channel which had a grating of black-and-white stripes ($\lambda=1$ cm) either on its walls or on its substratum and analyzed the effect of covering different eyes [ALEs, posterior lateral eyes (PLEs) and posterior median eyes (PMEs)] according to the position of a grating either in the lateral or ventral fields of view.

MATERIALS AND METHODS

In all experiments, adult virgin females of *L. tarantula* were used. These animals were captured from a wild population in Madrid (central Spain; 40°32'N, 3°42'W) and had undergone their final two to three molts in the laboratory; the age of all the animals was very similar and all the trials were conducted after maturation. The spiders were maintained in individual containers measuring 17×13×8 cm with sufficient substratum (earth) to move around

and dig burrows. They were fed blow flies (*Calliphora vomitoria*) and given water twice a week.

The required sample size was determined from previous data gathered in comparable conditions (Ortega-Escobar and Ruiz, 2014). Assuming a nominal significance level $\alpha=0.05$ and a power level of $(1-\beta)=0.80$, the need to detect differences corresponding to an effect size of 0.348 with a correlation between repeated measures of 0.56, and using repeated measures ANOVA with two groups and 10 repeated measurements, the total number of measurements needed would be $n=8$. The sample size was increased to $n=9$ spiders in order to guard against missing information. Animals were assigned at random to each experiment according to availability at the time of the experiment.

General procedure

The general procedure was similar to that described in Ortega-Escobar and Ruiz (2014) but with some changes described below. The spiders were placed in a terrarium that was divided into three channels (Fig. 1A); the lateral channels (A and B in Fig. 1A) were occupied by spiders. The channels were 52 cm long and 9.5 cm wide, with 10 cm high walls. The spiders were placed in the terrarium 3 days before the beginning of the study and they lived in these channels during the experiment. Light was provided by four 36 W fluorescent tubes positioned 160 cm above the terrarium. These tubes supplied a light intensity of 1193–1155 lx at the test channel (the first measurement is for the point of release of the spider; the second measurement is for a point 30 cm from the point of release). These intensities are lower than those measured in the field. At 12 cm from one end of the lateral channels, an artificial burrow measuring 17 cm deep and 2.5 cm in diameter was built.

The spiders were gently pushed 30 cm away from the burrow in their channel; when they reached this point, they were captured in a transparent glass cup and transferred to a similar point in the test channel (Fig. 1C). In contrast to our previous study (Ortega-Escobar and Ruiz, 2014), the length of the test channel was 90 cm, allowing the spider a more unrestricted search of the burrow. If the spider did not move after 20 min, it was taken back near to its burrow via the glass cup. The spiders were filmed as they searched for the burrow, using a Panasonic SDR-H80 video camera. As the spiders moved in

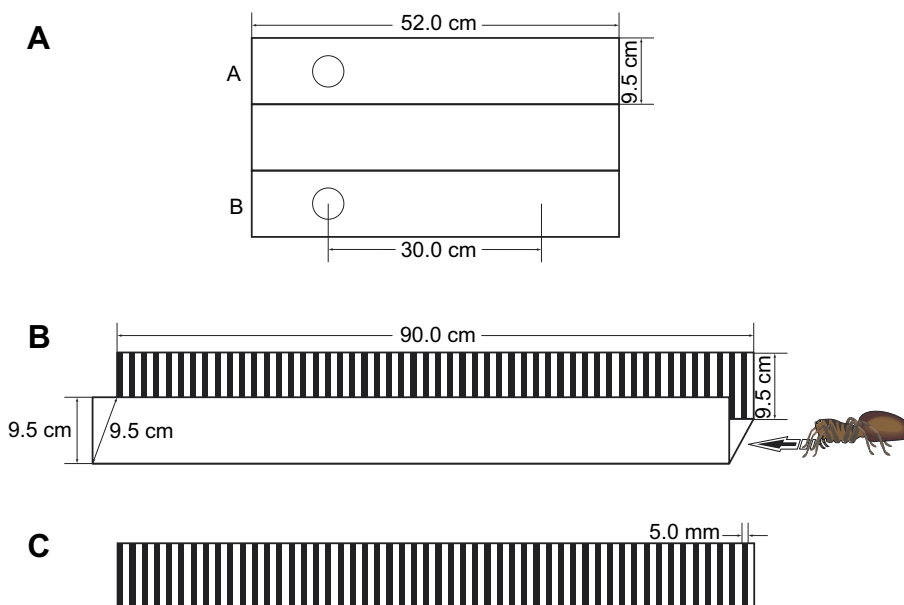


Fig. 1. Setup used to study odometry by *Lycosa tarantula*. (A) Top view of the terrarium divided into two channels (A and B) and an intermediate region. Channels A and B were the 'spider channels', each containing one spider. Spiders walked along their channel to a point positioned 30 cm from the burrow and were afterwards transferred to the 'test channel'. (B) Lateral view of the test channel for an experiment in which the grating was placed on the walls of both training and test channels. Although the spider is represented out of the test channel, it was actually placed at a point equivalent to that at which it was removed from channel A or B. (C) Top view of the test channel for an experiment in which the grating was placed on the substratum of both training and test channels.

contact with one wall of the channel (thigmotaxis), a paper ruler was placed in the center of the substratum to measure the distance walked. All the runs were carried out between 10:00 h and 18:00 h.

The spiders could walk either following the direction they had taken before being captured in the glass cup (this was considered a non-valid run) or turn 180 deg and walk in search of the virtual burrow. It was considered that a spider had walked the correct distance to its burrow if it made a complete (180 deg) change in direction (Turner's loop). The number of fresh female spiders is indicated in each experiment. Each spider was used in 10 training trials and 10 experimental trials. For each trial, the distance walked by the spider before searching began was measured, as well as the latency to walk. The eye-covering procedure is described in a previous paper (Ortega-Escobar, 2006). We covered only three of the four pairs of eyes (Fig. 2; ALEs with ventral field of view, PLEs with lateral field of view from 60–80 deg to near 180 deg, and PME with ventral and lateral fields of view; Kovoov et al., 1992; Land, 1985). AMEs were not covered given that their optic axis is orientated 20 deg upward and is placed 15 deg lateral to the sagittal plane (Kovoov et al., 1992). Two different experiments were performed: (1) with lateral placed grating and (2) with ventral placed grating.

Data analysis and statistics

We measured two parameters in each inbound run, the distance (cm) walked and the latency (s) to begin walking – that is, the time elapsed between the moment the spider was placed on the test channel substratum and the moment it began to walk. This latency period is considered to be a measure of the motivation to search for the burrow although other explanations may be also valid.

All data were analyzed using the statistical software IBM SPSS Statistics 20.0 (IBM, 2011). All nominal levels of significance were set to $\alpha < 0.05$. The distances walked are reported as means \pm s.d. and distribution was also illustrated using box and whiskers plots, where the center is the median, the spread is the interquartile range (25th and 75th percentiles) and the whiskers are the 10th and 90th percentiles, depicting the dispersion of the data. One-sample *t*-tests were performed to compare the inbound distance against the burrow distance from the point of release (established as a fixed distance of 30 cm for all animals). In all experiments, a mixed effects longitudinal linear model (similar to a repeated measures ANOVA) was carried out to study the effect of changing any visual condition in the animals. In the proposed model, the two experimental conditions (training versus test) are treated as repeated measures. Within each experimental condition, 10 trials were measured for each animal, giving a total of 20 measurements. Hence, repeated measurements for each animal should be nested within the experimental condition factor. In the model, the experimental condition factor was considered as a fixed effect, repeated measurements within condition were considered a random effect, and the different animals measured were also considered a random effect. Only differences between the two experimental conditions were interpreted and Bonferroni-adjusted *post hoc* tests were used when necessary (more than two experimental conditions compared, i.e. uncovered versus PLEs covered versus PLEs+PMEs covered).

RESULTS

In all the experiments, in both the training and test conditions, the spiders made their inbound path in a multiple-step trajectory, generally in contact with the walls of the channel, and this pattern was intermixed with walks from one wall to the other. On some

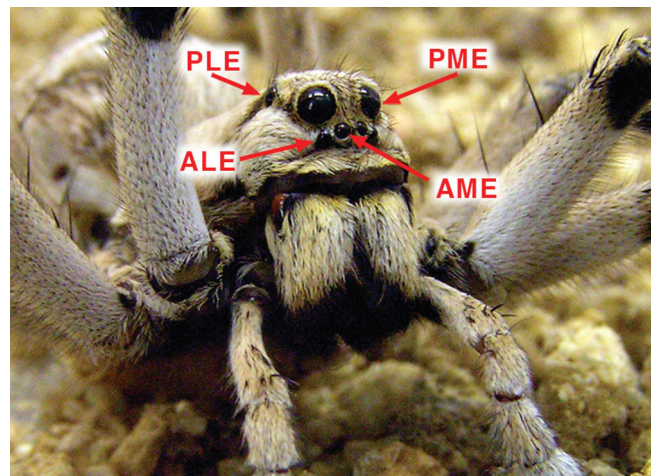


Fig. 2. Eye disposition in the *L. tarantula* cephalothorax. ALE, anterior lateral eye; AME, anterior median eye; PLE, posterior lateral eye; PME, posterior median eye.

occasions, it was observed that the spider walked through the central part of the channel for a long distance but this type of walking was not frequent.

In test conditions of both experiment 1 and 2 (see below), some animals showed a movement of the palps upwards to the frontal superior part of the carapace, touching it in the region of the PMEs, from outside to inside. This behavior has been observed when PMEs and ALEs are covered.

As in our previous study (Ortega-Escobar and Ruiz, 2014), spiders that had some eyes covered did not make exploratory leg movements during walking.

Experiment 1: lateral grating

Stimuli in the lateral field of view are perceived mainly by the PLEs and the PMEs (see Materials and methods, 'General procedure', above). The first eyes, PLEs, have a visual field from 60–80 to 180 deg while the PMEs have a visual field from near 0 to 60–80 deg. So, in this experiment, it would be expected that the more important eyes for detecting the lateral grating would be the PLEs and PMEs.

The spiders in this experiment were moved in the channels as described in Materials and methods (see 'General procedure', above). The walls of the spider channel and the test channel were always lined with a grating of black-and-white stripes (stripe width 0.5 cm, $\lambda = 1$ cm) orientated perpendicular to the long axis of the channel. The substratum of the spider channel and the test channel were made of earth with very fine particles.

This experiment was divided into two complementary setups: (1) PLEs-PMEs covered: in this setup, PLEs and PMEs were covered successively after spiders had been trained with all eyes uncovered; (2) ALEs covered: in this setup, ALEs were covered after spiders had been trained with all eyes uncovered. In each setup (PLEs-PMEs covered and ALEs covered), nine fresh spiders were used.

Lateral grating and PLEs-PMEs covered

In the first part of this experiment, when the animals had only their PLEs covered, movement of the palps was not observed. In the second part of this experiment, when the animals had covered PLEs and PMEs, movement of the palps was observed in two animals, in one of them in the 2nd and 3rd displacements and in the other in the four initial displacements.

The mean (\pm s.d.) distance walked during training was 35.3 ± 8 cm ($t_{13} = 22.09$, $P < 0.001$), further than the target point. When the PLEs were covered, the mean distance walked changed to 31.7 ± 7.4 cm ($t_{261} = -5.26$, $P < 0.001$), nearer to the position of the virtual burrow. All the animals with covered PLEs searched for the burrow at a shorter distance than that during training. When PME were additionally covered, the mean distance walked changed to 30 ± 7.4 cm ($t_{261} = 1.68$, $P < 0.001$) (Fig. 3).

The fixed effect 'PLEs-PMEs covered' had a significant effect on the distance walked (mixed effects model analysis, $F_{2,261} = 14.399$, $P < 0.001$). However, this effect was due only to the covering of the PLEs. When PMEs were additionally covered, the mean distance walked was not statistically different. This result shows that only the PLEs have an influence on the distance walked to the burrow under these conditions. Although the AMEs and ALEs were uncovered, spiders did not walk the same distance as they did with all eyes uncovered.

The shortest distance spiders walked with their PLEs covered was 20 cm and the longest distance was 53 cm. When PMEs were covered in addition to PLEs, the shortest distance spiders walked was 20 cm and the longest distance was 51 cm.

The mean (\pm s.d.) latency period during training was 223.4 ± 154 s. When the PLEs were covered, it was 210.3 ± 133.6 s; when the PMEs were covered in addition to the PLEs, it was 224.8 ± 192.7 s.

The fixed effect 'PLEs-PMEs covered' had no significant effect on the latency to walk ($F_{2,261} = 0.394$, $P = 0.675$).

Lateral grating and ALEs covered

In the experiment with the ALEs covered, the same movement of the palps was observed in three animals: in one of them, in the 3rd, 6th and 7th displacements; in another, in the 2nd and 4th displacements; in the last, in the 8th and 10th displacements.

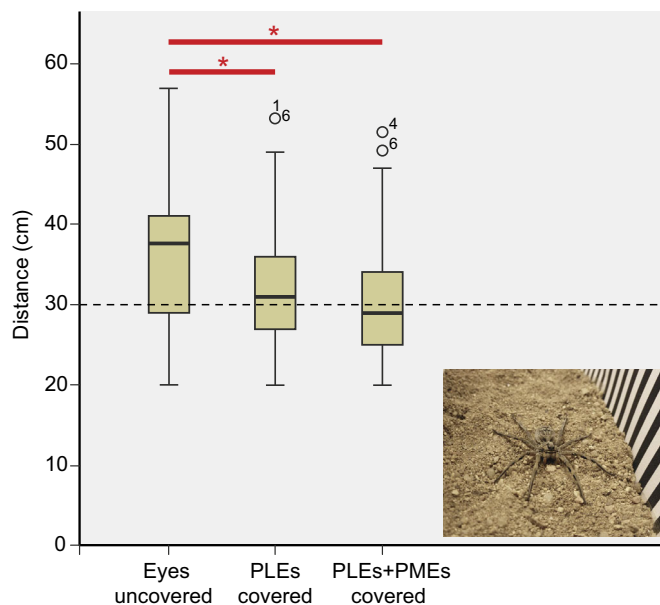


Fig. 3. Mean distance walked by spiders in the lateral grating experiment with the posterior lateral eyes and posterior median eyes (PLEs-PMEs) covered. Data are shown in the control (eyes uncovered; $n=9$ spiders and $N=90$ trials) and test conditions (PLEs covered and PLEs+PMEs covered; $n=9$ spiders and $N=90$ trials). Boxes show medians and interquartile ranges, whiskers correspond to extreme values and circles represent outliers (numbered outliers correspond to different spiders). *Significant difference ($P < 0.001$); repeated measures ANOVA. The dashed line at 30 cm indicates the position of the virtual burrow.

In this experiment, the mean (\pm s.d.) distance walked during training was 34.5 ± 6.4 cm ($t_{27} = 41.034$, $P < 0.001$). When the ALEs were covered, the mean distance walked changed to 32.1 ± 7.5 cm ($t_{171} = 2.37$, $P < 0.05$) (Fig. 4).

The fixed effect 'ALEs covered' had a significant effect on the distance walked (mixed effects model analysis, $F_{1,171} = 5.618$, $P = 0.019$). This effect was due mainly to 3 animals walking a 10.2, 5.3 and 2.6 cm shorter distance than in the training condition. If we do not consider the data from these 3 animals, the mean distance walked during training was 34.5 ± 6.3 cm. When the ALEs were covered, the mean distance walked changed to 33.9 ± 7.2 cm. In this case, there was no significant difference in the distance walked due to covering of the ALEs (mixed effects model analysis, $F_{1,120} = 0.256$, $P = 0.614$). The other six animals showed either small increases or decreases in the distance walked.

The shortest distance walked by the spiders under this visual condition was 11 cm and the longest distance was 51 cm.

The mean (\pm s.d.) latency period during training was 289.6 ± 242.1 s and when the ALEs were covered it was 246.7 ± 226.9 s.

The fixed effect 'ALEs covered' had a significant effect on the latency to walk (mixed effects model analysis, $F_{1,171} = 4.438$, $P = 0.037$).

In both setups (PLEs+PMEs covered and ALEs covered), in the control displacements the animals walked further in the test channel than the distance required to find the virtual burrow (30 cm).

Experiment 2: ventral grating

Stimuli in the ventral field of view can be perceived through the ALEs and the PMEs (see Materials and methods, 'General procedure', above). In this experiment, the walls of the spider channel and test channel were plain white. The substratum of both channels was a grating of black-and-white stripes (stripe width 0.5 cm, $\lambda = 1$ cm) orientated perpendicular to the long axis of the channel.

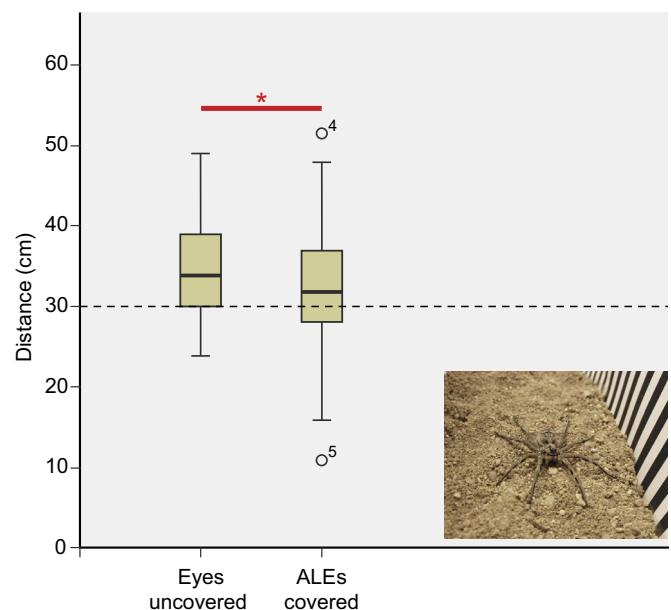


Fig. 4. Mean distance walked by spiders in the lateral grating experiment with the anterior lateral eyes (ALEs) covered. Data are shown in the control (eyes uncovered; $n=9$ spiders and $N=90$ trials) and test condition (ALEs covered; $n=9$ spiders and $N=90$ trials). *Significant difference ($P < 0.001$); repeated measures ANOVA. See legend of Fig. 3 for further details.

As in experiment 1, this experiment was divided into two complementary setups: (1) PLEs-PMEs covered: in this setup, PLEs and PMEs were covered at the same time after spiders had been trained with all eyes uncovered; (2) ALEs covered: in this setup, ALEs were covered after spiders had been trained with all eyes uncovered. In the first setup (PLEs-PMEs covered), 9 spiders were used and in the second setup (ALEs covered), 10 spiders were used.

Ventral grating and PLEs+PMEs covered

In this experiment, three animals showed palp movements: one of them in the 1st, 2nd and 10th displacements; the second one in the 1st and 3rd displacements; and the third one in the 4th displacement.

The mean (\pm s.d.) distance walked during training was 39.0 ± 4.9 cm ($t_{16}=51.591$, $P<0.001$). When the PLEs+PMEs were covered, the mean distance walked changed to 34.4 ± 5.8 cm ($t_{171}=-6.1$, $P<0.001$) (Fig. 5). All animals, with one exception, walked a distance between 3 and 9.7 cm shorter than in the training condition to find the virtual burrow.

The fixed effect 'PLEs+PMEs covered' had a significant effect on the distance walked (mixed effects model analysis, $F_{1,171}=37.185$, $P<0.001$). Given that the PLEs cannot perceive the grating in their visual field, the effect of searching for the burrow at a closer range than in the control condition is due only to the covered PMEs.

The shortest distance walked by the spiders in this condition was 22 cm and the longest distance walked was 48 cm.

As in experiment 1, the distance walked by the spiders searching for the burrow was greater than that in the outbound run.

The mean (\pm s.d.) latency to walk during training was 416.4 ± 263.3 s and when the PLEs+PMEs were covered it was 434.7 ± 314.9 s. The fixed effect 'PLEs+PMEs covered' had no significant effect on the latency to walk (mixed effects model analysis, $F_{1,171}=0.641$, $P=0.425$). That is, without functioning PLEs and PMEs, the spiders had a latency to walk that was not different from that measured with all eyes functioning.

Ventral grating and ALEs covered

In this experiment, two animals exhibited palp movements; one of them showed it in the 2nd displacement, and the other in the 1st and 2nd displacements.

The mean (\pm s.d.) distance walked during training was 34.5 ± 8.4 cm ($t_{18}=21.660$, $P<0.001$). When the ALEs were covered, the mean distance walked changed to 26.1 ± 9.7 cm ($t_{190}=6.89$, $P<0.001$) (Fig. 6). All animals, with one exception, walked a distance between 8 and 12.5 cm shorter than in the training condition to find the virtual burrow. The tenth animal walked the same distance in the training and the test conditions.

In this experiment, the highest variance in the distance walked was observed. The shortest distance walked was 5 cm and the longest distance walked was 60 cm (see Fig. 5).

The fixed effect 'ALEs covered' had a significant effect on the distance walked (mixed effects model analysis, $F_{1,190}=47.527$, $P<0.001$). Although all the other eyes (PMEs, PLEs and AMEs) were uncovered, the animals did not walk the distances gauged during training.

The mean (\pm s.d.) latency period during training was 248.4 ± 152.2 s and when the ALEs were covered it changed to 195.6 ± 121.3 s. The fixed effect 'ALEs covered' had a significant effect on the latency to walk (mixed effects model analysis, $F_{1,190}=9.943$, $P<0.05$). That is, with the ALEs not functioning, the spiders had a latency to walk smaller to that with all eyes functioning.

DISCUSSION

We studied the role of three eye pairs (ALEs, PLEs and PMEs) in gauging the distance walked during outbound runs. The inbound distance walked during training was always larger than 30 cm, the distance between the external part of the burrow and the point where we took the spider to transfer it to the test channel. As spiders began to walk from either the bottom or middle of the burrow, the overestimation of the distance to walk could be due to the fact that in the outbound run they walked 30 cm plus the distance inside the

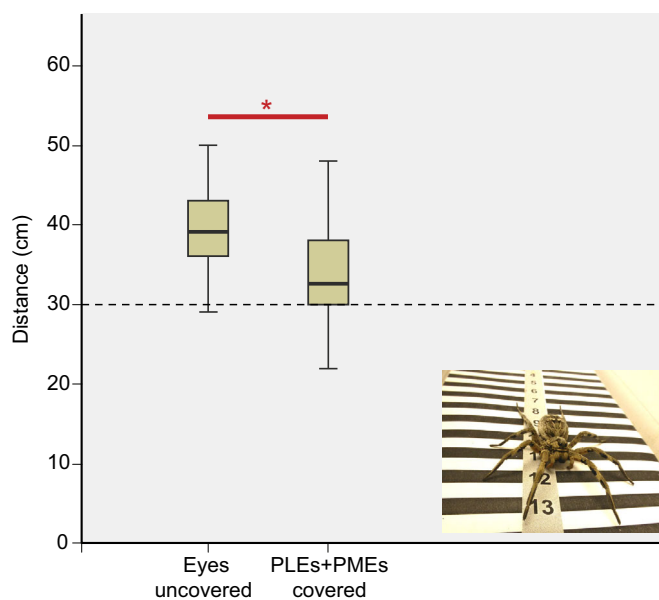


Fig. 5. Mean distance walked by spiders in the ventral grating experiment with the PLEs-PMEs covered. Data are shown in the control (eyes uncovered; $n=9$ spiders and $N=90$ trials) and test condition (PLEs+PMEs covered; $n=9$ spiders and $N=90$ trials). *Significant difference ($P<0.001$); repeated measures ANOVA. See Fig. 3 for further details.

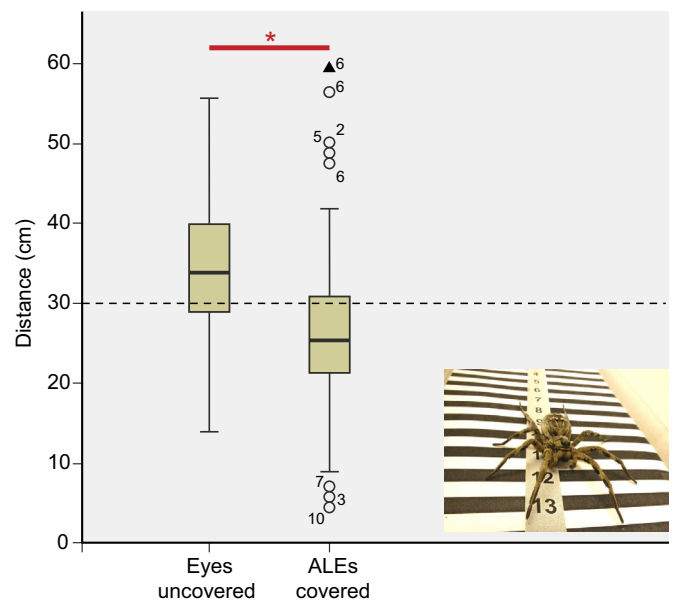


Fig. 6. Mean distance walked by spiders in the ventral grating experiment with the ALEs covered. Data are shown in the control (eyes uncovered; $n=10$ spiders and $N=100$ trials) and test condition (ALEs covered; $n=10$ spiders and $N=100$ trials). *Significant difference ($P<0.001$); repeated measures ANOVA. See Fig. 3 for further details.

burrow. Additionally, self-generated optic flow should be taken into consideration, because in our previous study (Ortega-Escobar and Ruiz, 2014), in which animals walked the same distance but without any lateral or ventral grating, the mean distance walked in inbound runs was 26.5 ± 11 cm; however, when there was some lateral or ventral grating, the inbound distance walked was always longer than 30 cm (Ortega-Escobar and Ruiz, 2014). Regardless, with our experiments we cannot disentangle the effect of walking inside the burrow from that of the presence of a grating placed perpendicular to the walking direction.

When the grating was placed on the lateral walls of the channel, the most important effect on the inbound distance gauged was due to the absence of performance of the PLEs, with a reduction near 10.2%. The posterior covering of the PME had no significant effect on the distance gauged. This means that, although lateral grating can be perceived through the PMEs, mainly at a certain frontal distance, this is not taken into consideration when gauging the distance walked in the outbound run. In relation to the ALEs, the visual fields of which could perceive the inferior part of the grating, only 3 of the 9 animals showed a reduction in the distance gauged to return to the burrow when the ALEs were covered. When these 3 animals were not considered in the statistical analysis, the difference between the distance walked during the training and test runs was not significant. However, we cannot know whether the shorter distance walked by these 3 animals was due to the absence of perception of the inferior part of the grating or to the absence of perception of the substratum structure. Their behavior was not different from that of the other animals.

The largest reduction in the distance gauged was observed when the grating was placed ventrally over the substratum and the ALEs were covered. In this case, the reduction in the distance walked was 24.3% on average. There was also a reduction in the distance walked when the PMEs were covered (11.8%). Therefore, *L. tarantula* probably integrate the information gathered through the ALEs and PMEs to get an image of the changes observed in the substratum, which can be used for orientation when returning to the burrow after looking for prey, for example.

Is there a possibility of integration of the information from the three pair of eyes that detect changes in both lateral and ventral visual fields during the outbound runs? The visual centers of *L. tarantula* females have been the object of a detailed histological study. The PLEs have 15% of their axons present in the PME nerves and PMEs have a 6% of their axons in PLE nerves; these data suggest the possibility of functional integration between these two posterior eyes at least at the level of the first optic neuropil (lamina) (Kovoor et al., 1992). In contrast, the laminae of the ALEs do not receive information from the PME or PLE retinas. ALEs show less convergence over the cells of their lamina; the ratio of retina cells to lamina cells is approximately 1:1, which means that near and different photoreceptors will have a physiological effect (excitatory or inhibitory) on different lamina cells. In comparison, the rate of convergence of retina cells to lamina cells for PLEs and PMEs is 3.9 and 3.6, respectively (Kovoor et al., 1992). The second optic neuropils (medullae) of ALEs, PLEs and PMEs make contact with a third neuropil called mushroom bodies (MBs) (Kovoor et al., 2005). In another spider species, *Cupiennius salei*, the MBs receive visual information from the three secondary eyes (PMEs, PLEs and ALEs) (Strausfeld and Barth, 1993). In particular, Strausfeld and Barth (1993) propose a specific role for the spider MBs in processing visual motion. They do not specify whether this visual motion comes from the prey or is self-generated by the spider when

walking. This hypothesis has not been proved in *C. salei*. However, a previous study showed that this spider is able to come back to a place that it had been chased away from by using proprioceptive information alone, as all its eyes had been made non-functional (Seyfarth et al., 1982).

MBs have been relatively well studied in insects and in relation to spatial navigation. Mizunami et al. (1998) showed that cockroaches (*Periplaneta americana*) are capable of place memory through using distant visual cues and that lesions of the medial lobes and pedunculi of the MBs abolish the capacity to relate distant visual cues to a target. The MBs have also been hypothesized to be involved in spatial navigation in honeybees and ants, in spite of the absence of direct evidence (Wolff and Strausfeld, 2016). There is some indirect evidence of the involvement of the MBs in bee and ant navigation, as visual centers connect with them (ants: Gronenberg, 2001; bees: Paulk and Gronenberg, 2008). Additionally, the region of the MBs that receives visual information – the collar – is selectively developed when bees (Durst et al., 1994) or ants (Kühn-Bühlmann and Wehner, 2006; Stieb et al., 2010) begin to forage. The expression of an activity-dependent gene has been used to identify the MBs as a region that is active during navigation (Lutz and Robinson, 2013); this gene showed upregulated expression exclusively in the MBs after a single orientation flight.

The results of this study in relation to the ALEs and those obtained by Ortega-Escobar (2011) on the involvement of the ALEs in detecting changes in the orientation of the ventral substratum show that these eyes are very important for homing orientation. We think that the suggestion made by Land (1985; p. 59) that ‘their function may well have been usurped by the PM eyes’ is not correct and nor is the suggestion by Lehmann et al. (2016; p. 459) that ‘in Lycosidae (wolf spiders) and Deinopidae (net-casting spiders) the posterior median eyes, i.e. one of the secondary eye pairs, are the main visual organs’. It is true that the studies carried out by Rovner (1993) on the lycosid spider *Rabidosia rabida* (both females and males) showed that the most important eyes for perceiving courtship displays were PLEs and PMEs. But this does not take into consideration two aspects of the vision of a lycosid spider such as *L. tarantula*: orientation by polarized light carried out by the AMEs (Ortega-Escobar and Muñoz-Cuevas, 1999) and changes in the substratum orientation (Ortega-Escobar, 2011).

In another species of spider, the nocturnal wandering spider *Leucorchestris arenicola*, the role of different eyes in homing has been studied in a natural context (Nørgaard et al., 2008). In *L. arenicola*, covering AMEs and ALEs causes a reduction in homing success.

In relation to the latency to walk, we observed that there was a significant difference between training and testing only when the ALEs were covered, either with lateral or ventral grating. When the PLEs and PMEs were covered, there was no significant difference in latency. This means that covering the eyes does not reduce the motivation to return to the burrow. On the contrary, covering of the ALEs caused the spiders to being to walk sooner than when all their eyes were uncovered. We think that one possible explanation for the larger reduction in latency times when ALEs were covered is that the animal starts moving earlier in order to start generating the optic flow needed to guide displacement.

Finally, we observed a characteristic movement of palps only when the PMEs or ALEs were covered and not when PLEs were covered. This movement was the same with either PMEs or ALEs covered. We did not observe an ‘antennation’ movement of the first

pair of legs such as that described in *Cupiennius salei* when it walked in complete darkness (Schmid, 1997). The palp movements we observed occurred only at the beginning of the first step of the inbound run or at the beginning of one of the posterior steps of the trajectory (see fig. 3 of Ortega-Escobar and Ruiz, 2014); they did not occur during walking. This behavior probably indicates that the visual information obtained through the PMEs and ALEs is processed in the same visual center, although we do not have any physiological data to support this.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

J.O.-E. conceived the study, designed and carried out the experiments, collected the data, carried out interim data analysis, and guided the interpretation of findings and the development of the manuscript, both in drafting and in review. M.A.R. contributed to the design of the experiments, carried out statistical data analysis, participated in the drafting and development of the manuscript, participated in the interpretation of data and study results, and critically reviewed the manuscript. Both authors read and approved the final manuscript.

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