Circadian Rhythms of Locomotor Activity in *Lycosa tarentula* (Araneae, Lycosidae) and the Pathways of Ocular Entrainment

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Abstract

*Lycosa tarentula* is a ground-living spider that inhabits a burrow where it awaits the appearance of prey or conspecifics. In this study, circadian rhythms of locomotor activity were examined as well as the ocular pathway of entrainment. Thirty-three adult virgin females were examined under constant darkness (DD); all of them exhibited robust circadian rhythms of locomotor activity with period averaging 24.1 h. Fourteen of these spiders were studied afterwards under an LD 12:12 cycle; they usually entrained to in the first or second days, even when light intensity was as low as 1 lx. During the LD cycle, locomotor activity was generally restrained to the darkness phase although several animals showed a small amount of diurnal activity. Ten males were also examined under LD; they also were nocturnal but were much more active than the females. Seven females were examined under constant light (LL); under this they became arrhythmic. Except for the anterior median eyes (AMEs), all the eyes were capable of entraining the locomotor activity to an LD cycle. These results demonstrate that under laboratory conditions and low light intensities locomotor activity of *Lycosa tarentula* is circadian and in accordance with Aschoff’s ‘rule’. Only AMEs are unable to entrain the rhythm; the possible localization of circadian clock is therefore discussed.

Keywords: Circadian rhythm, locomotor activity, ocular pathway, entrainment, spiders, *Lycosa tarentula*

Introduction

In many organisms studied from the chronobiological point of view there is a temporal organization of physiological and behavioural activities. This temporal organization enables the organism to foresee periodical physical changes in its envi-
environment. These include the day-night cycle, changes in temperature, and seasonal changes. With respect to the daily cycle, the temporal organization is composed of a circadian pacemaker centre that produces a rhythm with a period near to 24 h, an input pathway for synchronization with the Zeitgebers and a output pathway to the motor centres.

To determine if the circadian rhythm of an animal responds to physical changes in the environment the animal must be studied under constant conditions. The response most frequently studied in chronobiology has been the locomotory activity, because the motor nervous centres are believed to be controlled by the circadian pacemaker centre. To determine whether locomotory activity is only a response to the environmental change, the animals have been studied in constant darkness (DD) and in constant illumination (LL). In this way, it has been found that the typical rhythmicity of the circadian pacemaker centre is entrained through a Zeitgeber, for example the light-darkness (LD) cycle.

In order to synchronize with the environmental cycle, the circadian system must have a photoreceptor or photoreceptors, which may be either in the visual or in non-visual organs. The entrainment pathway has been elucidated in mammals (Moore et al., 1995), insects (cricket, Tomioka & Yukizane, 1997; Drosophila Helfrich-Förster et al., 2001; the cockroach, Nishitsutsuji-Uwo & Pittendrigh, 1968); and in scorpions (Fleissner & Fleissner, 1988). This question has not yet been studied in spiders.

Aschoff (1960) showed a relationship between the behavioural rhythmicity of the animals under DD or LL and its activity, whether it be predominantly diurnal or nocturnal. According to his ‘rule’ nocturnal animals continue their rhythmical activity under DD while in LL they become arrhythmic.

Locomotor activity rhythms in spiders have been studied both in the field (diel rhythms) and in the laboratory (diel and circadian rhythms). Although the majority of spiders are nocturnal, species of the families Salticidae, Oxyopidae, Thomisidae and Lycosidae are also active during the day (Foelix, 1996; see Cloudsley-Thompson, 2000, for a review). By means of pitfall traps, the diel activities of several species of Lycosidae have been assessed in the field.

A previous study on the wolf spider Lycosa tarentula (Araneae, Lycosidae) (Ortega et al., 1992) showed that adult females of this species submitted to a natural LD cycle changed the onset of their locomotor activity according to the time of sunset. When activity was investigated during summer, its onset was later than it was during the autumn months. Activity was also studied, although not extensively, under constant conditions. Daily and circadian changes in the size of the rhabdoms in the four pairs of eyes of Lycosa tarentula have also been studied (Kovoor et al., 1995, 1999).

The aim of the present work was to analyse the rhythm of locomotor activity of L. tarentula under laboratory conditions, both under DD and LL and under a light-darkness cycle (LD). The ocular pathway of entrainment to the LD cycle was also studied.
Material and Methods

Material

*Lycosa tarentula* (Araneae, Lycosidae) lives in a burrow nearly 15 cm in depth. As other members of the family, it has eight eyes arranged in three rows. The front row is composed of the anterior lateral eyes (ALEs) and anterior median eyes (AMEs). The middle row comprises the posterior median eyes (PMEs); and the posterior row, the posterior lateral eyes (PLEs). All the eyes, except for the AMEs have a tapetum that reflects light after it has passed through the receptors. The AMEs are called *direct eyes* because their rhabdoms are placed between the cell body and the lens; the PMEs, PLEs and ALEs are called *indirect eyes* because the cell body is placed between the lens and the rhabdomere.

Methods

Thirty-three adult females were studied. These came from populations located in the surroundings of Madrid. Immature specimens were collected and they completed their development in the laboratory. The rhythm of locomotory activity was studied in DD followed by entrainment to a LD (12:12 h) (lights on 08:00 h and at 25°C). The light intensity was 40 lx at the substratum of the cage. The spiders were fed with meat flies (*Calliphora vomitoria*). In seven other animals the locomotor activity rhythm was studied in LL followed by entrainment as the specified for DD.

The locomotor activity was studied in activity boxes with a 10 cm deep substratum of soil. In one corner was a artificial burrow. Locomotor activity was detected by four infrared photocells. Information like which photocell was affected and the timing of this was transmitted to a Tanron computer for storage and later analysis.

To calculate the period of the locomotory activity rhythm we determined whether a spider was active or not during 6-min time spans. Activity was scored as 1, no activity was scored as 0. In this way actograms were made in which each horizontal line represents 1 day and each vertical line the existence of locomotor activity during a 6-min time span. If there was not activity during that period, a white space appears. The period was analysed by periodogram analysis combined with a $P^{2}$ test with 5% significance level (Sokolove & Bushell, 1978).

To study the role of the four pairs of eyes in entrainment to LD, the prosoma and three pairs of eyes were painted, leaving only one pair of eyes functional. Painting was carried out as follows. First, the entire prosoma and the three corresponding pairs of eyes were painted with a layer of collodion. Three successive layers of water-soluble Pelikan paint were added afterwards. Finally, the paint was covered by another layer of collodion.

That part of the prosoma not occupied by the eyes was painted in order to avoid the possible interaction of cerebral extraocular photoreceptors. These have been involved in the entrainment to LD cycles of other arthropods (*Calliphora vicina*, Cymborowski, 1994; *Drosophila*, Helfrich-Förster, 1997; several species of ants, Felisberti & Ventura, 1996).
Results

Activity under DD

In most cases, all the animals showed locomotory activity only at the onset of the subjective night, although in one case (0.03%) there was also activity at the end of the subjective night. The mean value of tau ($\tau$) was $24.1 \pm 0.59$ h (range 23.3 h to 25.8 h, $n = 33$). Figure 1A and 1B show actograms of spiders with $\tau > 24$ h and an actogram of another with $\tau < 24$ h, respectively.

One spider (0.03%) had a complex rhythm of locomotory activity, with two periods separated by 2 h. In three animals (0.09%) the actogram was not constant under DD; it showed marked irregularities in such a way that two periods separated by 1 h could be distinguished (Fig. 1C). One of them was a female that had moulted 10 days before the beginning of the recording, while, in the other two animals the interval after moulting into the adult stadium and the beginning of the recording was more than 81 days.

Activity in LD

Locomotory activity was studied in 14 spiders after they had been kept in constant darkness for fourteen days. These animals showed entrainment to the new cycle, independently of the free-running rhythm in the first or second day. When illuminated before the beginning of the subjective night, the onset of locomotor activity was inhibited and the animal became active again when the light was switched off (Fig. 2).

Locomotor activity was generally restrained to the dark phase during the LD cycle, although several animals showed a small amount of diurnal activity (see Figure 2). However, the beginning of activity always occurred after light had been extinguished even after a phase shift engendered by light intensity as low as 1 lx (Fig. 3).

Table 1 shows the value of the phase angle difference for each one of the 14 animals. All of them showed a negative phase angle difference. They generally began their activity during the first hour after light off. Table 1 also shows the duration of nocturnal activity (mean $\pm$ SD) under LD, measured as % of the total of 6-min bins. As a rule, the spiders showed values not higher than 25% of the total time.

Ten males were also studied under LD. Table 1 also shows the phase angle difference and the nocturnal activity duration (mean $\pm$ SD). In general, males were active longer than females were. Six males showed considerable locomotory activity during the day.

Activity under LL

Seven animals were observed under LL. They all showed similar behaviour and, in none of the cases a clear period could be distinguished. These animals either showed a small amount of activity, distributed throughout the day, or they showed some activity during the first 2-3 days and afterwards none (Fig. 4). These animals were subsequently entrained by a LD cycle 12:12 (light intensity = 1 lx). Entrainment was
Figure 1. A: The freerunning locomotor activity of a spider with a period of 24.9 h. Right, its chi-square periodogram. B: The freerunning locomotor activity of a spider with a period of 23.7 h. Right, its chi-square periodogram. C: The freerunning locomotor activity of a spider with two periods, one of 23.5 h, the other of 24.5 h. Right, its chi-square periodogram.
observed from the first day in all of them (Fig. 4). They showed nocturnal locomotor activity similar to that of spiders which had been entrained after having been maintained under DD.

Role of the eyes in the entrainment to LD

Several animals in which the entire prosoma and all the eyes had been painted enabled us check the efficiency of the masking system. In this case, the spiders would be expected to behave as if they were under DD. Figures 5 and 6 show animals treated in this way on the first 35 days and studied under LD. It can be seen that animals had a free-running rhythm and that phase shifting of the light did not modify this.

Seven animals with only AMEs uncovered and four animals with only ALEs uncovered were studied. None of the animals with only AMEs uncovered showed an entrainment to LD cycle (Fig. 5), while all the animals with one or both ALEs uncovered showed a perfect entrainment to the cycle (Fig. 6). The animals shown in these figures seem to be entrained during the first 10-15 days and afterwards, they began to free-run; however, this kind of rhythm has also been remarked on animals observed under DD.

Covering the eyes may have caused modifications of the photoreceptors. To exclude this possibility, other spiders were studied in which only the prosoma and
three pairs of eyes were covered, leaving the remaining pair uncovered. In this case, only animals with AMEs uncovered showed a free-running rhythm (Fig. 7), while those with ALEs uncovered showed good entrainment.

Six animals with only PMEs uncovered and six animals with only PLEs uncovered were also investigated. In both cases, the remaining eyes were invariably able to entrain shift in the LD cycle (Figs. 8 and 9, respectively).

**Discussion**

These results show that, in the laboratory, *Lycosa tarentula* has a locomotor activity rhythm similar to that previously observed under natural conditions (Ortega et al., 1992). This locomotory activity takes place mainly in darkness and begins several
Table 1. Phase angle difference (min) and % nocturnal activity of females and males *Lycosa tarentula*.

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minutes after the light has been switched off. This rhythm can be observed even under conditions of very low luminosity (1 lx) as in other arthropods (e.g.: *Drosophila* can be entrained by a light intensity of 0.01 lx [cited by Helfrich-Förster et al., 2001]). Under DD almost all the animals showed a free-running rhythm with a single period; the exceptions were related to physiological effects such as moulting or aging. I have found only one animal with a complex period similar to that described by Helfrich (1986) for *Drosophila* and Hong and Saunders (1998) for *Calliphora vicina*. It is important to emphasize that none of the 33 spiders studied was arrhythmic under DD. Together with the low percentage of animals with complex rhythms, this seems to indicate that the circadian oscillator(s) of *L. tarentula* has (have) a robust organization.

Under LL a very marked inhibition of the locomotor activity was observed. Under DD and LL the rhythm of locomotory activity is in accordance with the ‘rule’ of Aschoff (1960) – the nocturnal animals should show a free-running rhythm under DD and be arrhythmic in LL.

Under natural conditions these spiders have been observed to walk a distance of 40–50 cm from their burrows during the day and, when they are in the burrow, they remain in its upper part. The small amount of diurnal activity observed in this study could be due to the low light intensity in comparison with that experienced in natural conditions. (This has been measured at more than 90,000 lx). The

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*Figure 4.* Representative actogram of *L. tarentula* under LL and its posterior entrainment under L (1 lx) D 12:12. Right, its chi-square periodogram.
absence of moving objects such as prey or mates in the activity cages could also be a factor.

The males showed higher nocturnal activity than the females. This can be explained by the fact that, during the reproductive season, males are wander in search of females as has been shown to take place in other species (e.g. *Cupiennius* spp. Schmitt et al., 1990). The large amount of activity of the six tested males during the artificial day correlates well with field observations (personal observations) during which males have been found courting females early in the morning and at midday.

The rhythm of locomotory activity of *L. tarentula* is circadian and not mere a response to the absence of light, as shown by the results obtained in DD and LL. The free-running rhythm is readily entrained by light of an intensity as low as 1 lx.

*Figure 5.* Actogram of a spider with the entire prosoma and all the eyes covered between days 1 and 35; on day 36 (*), AMEs were uncovered. The initial freerunning rhythm continued even when AMEs were uncovered. Light intensity = 1 lx.
Role of the different eyes in LD cycle entrainment

On account of the anatomical disposition of the optic nerves, it has not been possible to cut these surgically, as it has been done in the case of some insects (cockroach, Nishiitsutsuji-Uwo & Pittendrigh, 1968; Roberts, 1965; cricket, Tomioka & Yukizane, 1997; and the orthopteran Hemideina thoracica, Waddell et al., 1990). The AMEs and ALEs nerves are in a ventral position in relation to PMEs, so it is difficult to approach them (Kovoor et al., 1992). Consequently, they could not be cut without damaging those of the PMEs. I therefore decided to use a procedure of painting the eyes with black paint as has already been done in crickets (Nowosielski & Patton, 1963). This procedure did not allow entrainment of the LD cycle as is shown in the free-running rhythm during the first 35 days of animals shown in Figures 5 and

![Figure 6. Actogram of a spider with the whole prosoma and all the eyes covered between days 1 and 35; on day 36 (*), ALEs were uncovered. The free-running rhythm was entrained when the ALEs were uncovered. Light intensity = 1 lx.](image-url)
Figure 7. Actogram of an animal studied under LD 12:12. Between days 1 and 6 the animal experienced no experimental manipulation; after day 7, all the eyes except for the AMEs were covered. A freerunning rhythm can be observed after this procedure. Light intensity = 1 lx.

Figure 8. Actogram of an animal studied under LD 12:12 and a posterior shift of 6 h of this cycle. The entire prosoma and all the eyes except for the PMEs were covered. Light intensity = 1 lx.
6. At the light intensities used in my experiments, this procedure gives similar results to nerve cutting. It can be used as a control to exclude the possible presence of extraocular photoreceptors.

None of the animals in which AMEs were covered were able to entrain to the LD cycle. They showed a very clear free-running rhythm similar to that which they had displayed when the whole prosoma and eyes were covered. This result could not have been due to the previous covering of the eyes because animals in which all the eyes except for the AMEs were covered, showed a free-running rhythm when exposed to an environmental LD cycle. The result therefore cannot be due to a physiological change induced by previous painting of the eye.

All the eyes other than the AMEs were capable of entraining the rhythm of locomotion to the LD cycle, either after the entire prosoma and all the eyes had been covered, or after a phase shift of the LD cycle. It seems, therefore, that the photoreceptors for entrainment of the locomotor rhythm are distributed only in the indirect eyes.

Previous anatomical studies (Kovoor et al., 1992, 1993) have shown a different central projection of the AMEs on the one hand, and of the ALEs, PLEs, and PMEs, on the other. The latter (unpublished observations) connect with a neuroendocrine cell group which could function as the circadian oscillator of the species. The absence of a projection from AMEs to these neuroendocrine cells would explain the absence of

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entrainment to LD when these eyes alone are uncovered. The relationship between the eyes and the circadian oscillator seems to be reciprocal because the rhabdoms of the ALEs, PLEs, and PMEs showed a clear circadian rhythmicity, but this is less clear in the case of the rhabdoms of the AMEs (Kovoor et al., 1999).

My results show for the first time that a relationship exists between an ocular pathway and the synchronization of the locomotor rhythm of spiders. Entrainment of eye function, as shown by the electoretinogram (ERG) in all the eyes has previously been demonstrated in other species of the Subphylum Chelicerata (Limulus, Renninger et al., 1997; the scorpion Androctonus australis, Fleissner, 1974, 1977). However, this is the first evidence, to my knowledge, of a spider’s eye which does not function in the entrainment of the locomotor rhythm but in polarized-light orientation (Ortega-Escobar & Muñoz-Cuevas, 1999). My results suggest that there may be a behavioural specialization of L. tarentula eyes, the direct eyes (AMEs) functioning for orientation and the indirect (ALEs, PLEs, and PMEs) for LD cycle entrainment. This specialization can be added to the previous observed by Rovner (1993) on the sexual behaviour of another Lycosid spider, Rabidosa rabida. In this species, the PMEs are used to distinguish between a male and a female of the species.

Acknowledgments
This research was supported by DGICYT Grant PB94-0200 of the Spanish Ministry of Science. J.-J. Orgaz-Fraile helped me with some of the experiments. I thank Dr. John Cloudsley-Thompson for helpful comments on the manuscript. Furthermore, I thank Antoni Diez-Noguera for donating the periodogram analysis program. Figures have been made by Emilio Ortega-Escobar, graphic designer.

References


