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Seasonal changes of locomotor activity patterns in ruin lizards *Podarcis sicula*

II. Involvement of the pineal

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Abstract Seasonal differences in the locomotor activity pattern of *Podarcis sicula* held in constant conditions in the laboratory were found to be associated with systematic differences in both the freerunning period of locomotor rhythms (τ) and the circadian activity time (α). In order to establish whether the pineal played a role in the control of seasonal changes in circadian parameters, we examined the effects of pinealectomy in constant conditions on the locomotor behavior of lizards displaying the bimodal activity pattern typical of summer. In most lizards pinealectomy lengthened τ, shortened α and abolished the bimodal activity pattern. Pinealectomy induced a sudden transition from the typical locomotor behavior of summer, characterized by a marked bimodal pattern, short τ and long α, to the typical locomotor behavior of autumn, characterized by a unimodal pattern, a long τ and short α. These results demonstrate that the pineal plays a central role in the seasonal reorganization of the circadian system that occurs in *P. sicula*.

Key words Circadian rhythms · Locomotor activity
Pinealectomy · Seasonality · Lizards

Introduction

The pineal gland has been shown to play a central role in the regulation of circadian behavioral rhythms of lizards. Pinealectomy abolishes circadian rhythms of locomotor activity in *Anolis carolinensis* and produces marked changes in the freerunning period (τ) and circadian activity time (α) in *Sceloporus olivaceus, S. occidentalis* and *Podarcis sicula* (Underwood 1977, 1981, 1983; Foà 1991). Radioimmunoassay measurements in *P. sicula* have shown that circadian rhythms of melatonin in the blood are abolished after pinealectomy (Foà et al. 1992a). These data demonstrate that the pineal is the only source of rhythmic blood-borne melatonin in *P. sicula*, and are consistent with the hypothesis that changes in τ and α induced by pinealectomy in this lizard are due to the concomitant withdrawal of rhythmic melatonin from the blood.

Investigations in *A. carolinensis* demonstrated that 24-h cycles of both light and temperature can entrain the pineal melatonin rhythm and that differences in length of daily photoperiod or thermoperiod affect the phase, amplitude and duration of this rhythm (Underwood 1985; Underwood and Calaban 1987). Hence, the current ambient lighting and temperature conditions (and their seasonal change) are readily translated into an internal cue in the form of the pineal melatonin rhythm. This cue can be used to regulate both the daily and annual physiology of lizards (Underwood 1985).

Seasonal differences in the locomotor activity pattern of *P. sicula* held in constant laboratory conditions were found to be associated with systematic differences in both τ and α of the freerunning rhythm (Foà et al. 1994). Between June and August the locomotor records of most lizards are characterized by a marked bimodal pattern, a short τ and long α. In the remaining months the activity pattern is mainly unimodal and typically associated with a long τ and short α. These data demonstrate in *P. sicula* a reorganization of the circadian system on a seasonal basis (Foà et al. 1994).

The present investigation tested whether the pineal, which has already been shown to be involved in the control of circadian rhythms of locomotor activity in *P. sicula* (Foà 1991) plays a role in the control of seasonal changes in the circadian organization of this lizard species. As chronic administration of exogenous melatonin was effective in lengthening τ, shortening α and abolishing the bimodal activity pattern of *P. sicula* in summer (Foà et al. 1992b), we examined the effects of pinealectomy on the locomotor behavior of bimodal lizards collected in July and immediately placed in the laboratory in constant temperature and darkness.

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Methods

During July 1992 ruin lizards *Podarcis sicula campestris* De Betta 1857 (adult males only; 6.5–8 cm snout-vent length) were collected on sand dunes along the coast of the Ligurian sea (Marina di Vecchiano, Pisa, Italy). After capture each lizard was carried to the lab and immediately placed inside an individual tilt-cage (30 x 15 x 11 cm), connected to an Esterline Angus event recorder for monitoring locomotor activity. The tilt-cages were placed inside environmental chambers, that were kept in constant darkness (DD) and temperature conditions (29°C).

Surgery

Pinealectomy and sham pinealectomy were always performed during the lizard's subjective day. Cold anesthesia, pinealectomy and sham pinealectomy were performed as described in Foà (1991). Postexperimental histological evaluation of the brain of PIN-X lizards showed that the pineal was completely removed.

Experimental protocol

Lizards were allowed to freerun in DD for 20–30 days, after which pinealectomy or sham pinealectomy were performed. Activity in DD was recorded for several weeks after surgery.

Data evaluation

Estimates of the freerunning period (τ) and the circadian activity time (α) were made for the 10-day segment just before surgery and a 10-day segment 2–6 weeks after surgery. Values of τ and α before and after surgery were compared by means of the Wilcoxon matched-pairs test. Comparisons between experimental lizards (pinealectomized) and control lizards (sham pinealectomized) were made by Fisher's exact test. Bimodality or unimodality of the locomotor activity pattern before and after surgery was established by visual inspection and then checked by means of spectral analysis (periodogram), following the procedures described in Foà et al. (1994).

Results

Prior to being subjected to sham pinealectomy ($n = 4$) or pinealectomy ($n = 13$), all lizards used in the experiment displayed a clear bimodal pattern of circadian locomotor activity in constant temperature and DD (Figs 1–3). Values of τ and α of each individual lizard before and after sham or real pinealectomy are reported in Fig. 4.

Sham pinealectomy did not induce significant changes in τ or α ($P > 0.10$, Wilcoxon matched-pairs

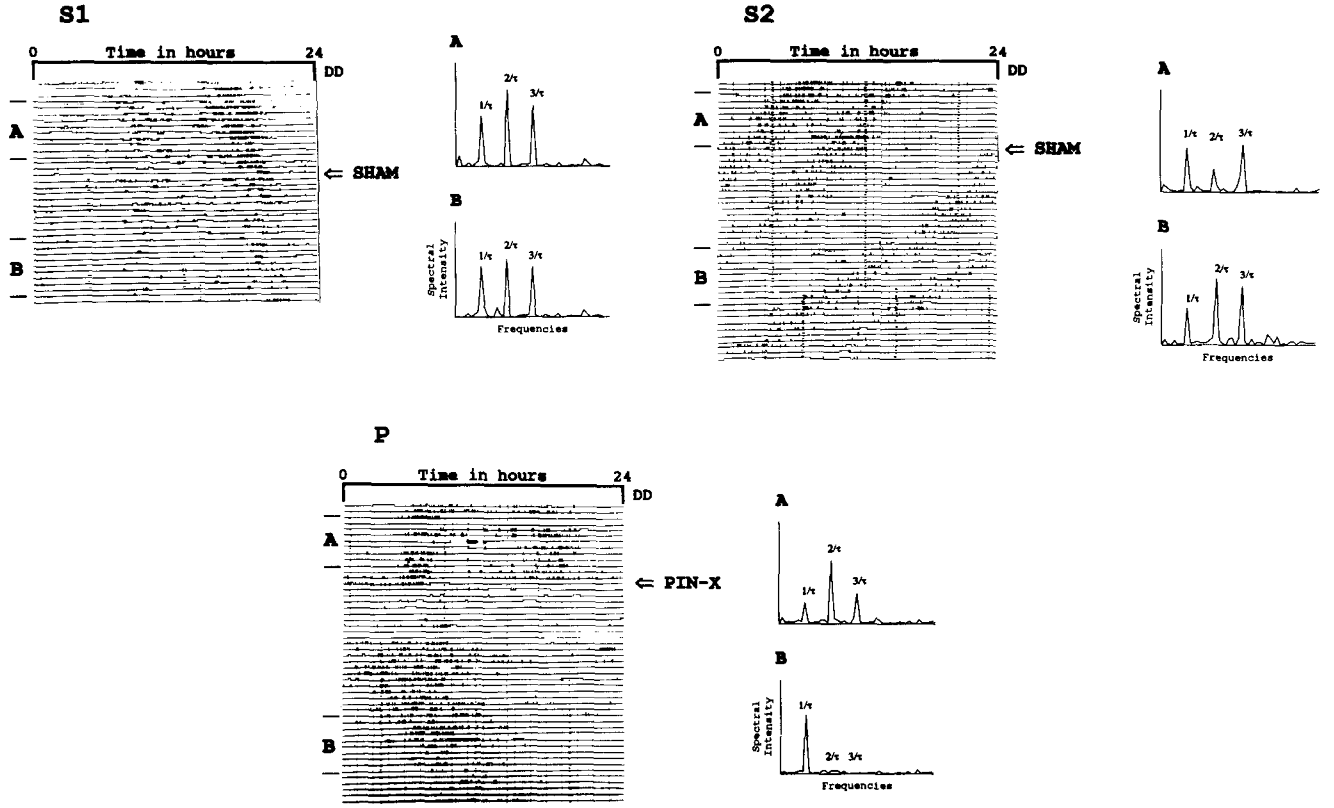


Fig. 1 Locomotor activity records of three bimodal lizards subjected to sham pinealectomy (*S*1, *S*2) or pinealectomy (*P*) while freerunning in constant temperature (29°C) and darkness. Each *horizontal line* is a record of 1 day's activity (0000–2400 hours), and consecutive days are mounted one below the other. *Arrows* indicate the day of surgery. *Capital letters* on the left margin of each record define 10-day segments that were subjected to spectral analysis. Results of spectral analysis are shown on the right of each record. *S*1, *S*2: sham pinealectomy did not affect locomotor rhythmicity. Frequency peaks at the $1/\tau$ submultiples $2/\tau$ and $3/\tau$, which characterize bimodal locomotor records before surgery, clearly persisted after sham pinealectomy. Note in record S2 the long-lasting bimodality (about 6 weeks) after sham surgery. *P*: pinealectomy induced lengthening in τ, shortening in α and the abolishment of the bimodal pattern, as the frequency peaks at $2/\tau$ and $3/\tau$ disappeared after this surgery

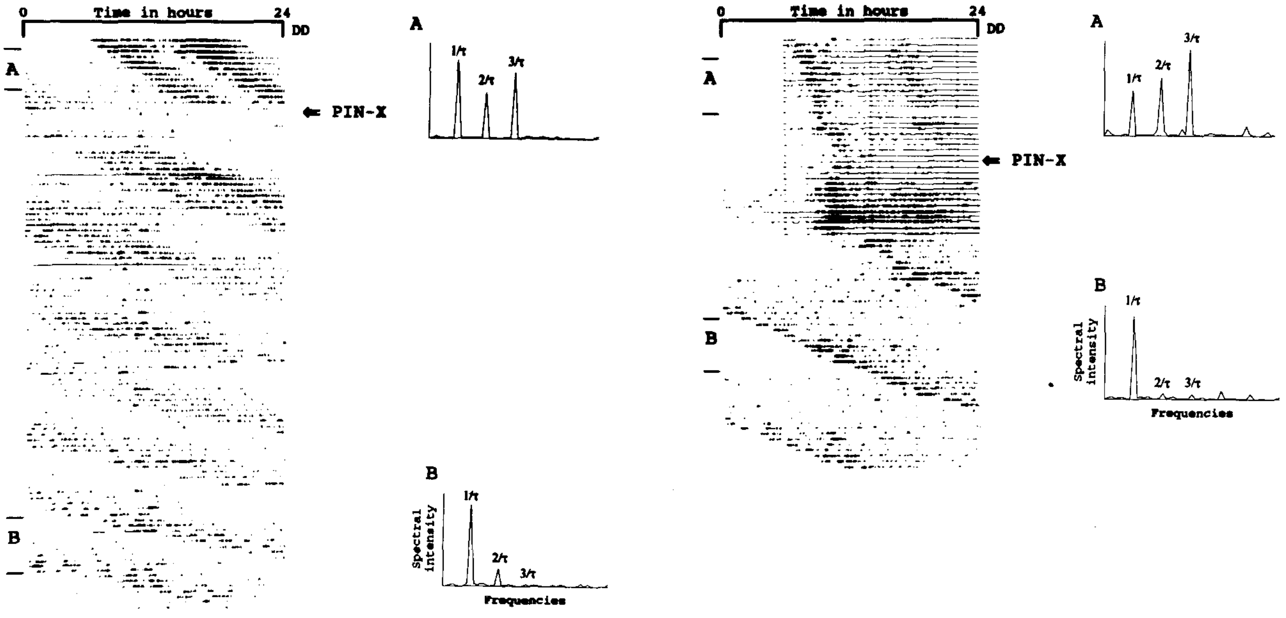


Fig. 2 Effects of pinealectomy in two bimodal lizards. Pinealectomy markedly lengthened τ and shortened α in both lizards. The abolition of the bimodal pattern of locomotor activity induced by pinealectomy was confirmed in both lizards by spectral analysis. (For other explanations, see Fig. 1)

test). Only low numbers of sham pinealectomized lizards were tested here, because a previous investigation based on higher numbers of such lizards ($n = 15$) in constant temperature and DD had already established the lack of any effect of sham surgery on either τ or α (Foà 1991). All sham pinealectomized lizards of the present experiment retained their bimodal activity pattern, as confirmed by spectral analysis (Fig. 1, top).

Pinealectomy induced a significant lengthening in τ ($P < 0.003$) and shortening in α ($P < 0.001$). Furthermore, in 12 out of 13 pinealectomized lizards the bimodal pattern of locomotor rhythms was completely abolished (Fig. 1 bottom, Fig. 2). This was confirmed by spectral analysis, as the frequency-peaks at the $1/\tau$ submultiples $2/\tau$ and $3/\tau$, which characterize the bimodal locomotor records before pinealectomy, disappeared after this surgery. Locomotor records of pinealectomized lizards were clearly unimodal and the only frequency-peak left was that at $1/\tau$ (Fig. 1 bottom, and Fig. 2).

In one lizard pinealectomy modified the bimodal locomotor pattern without abolishing bimodality completely (Fig. 3). After pinealectomy the interval between

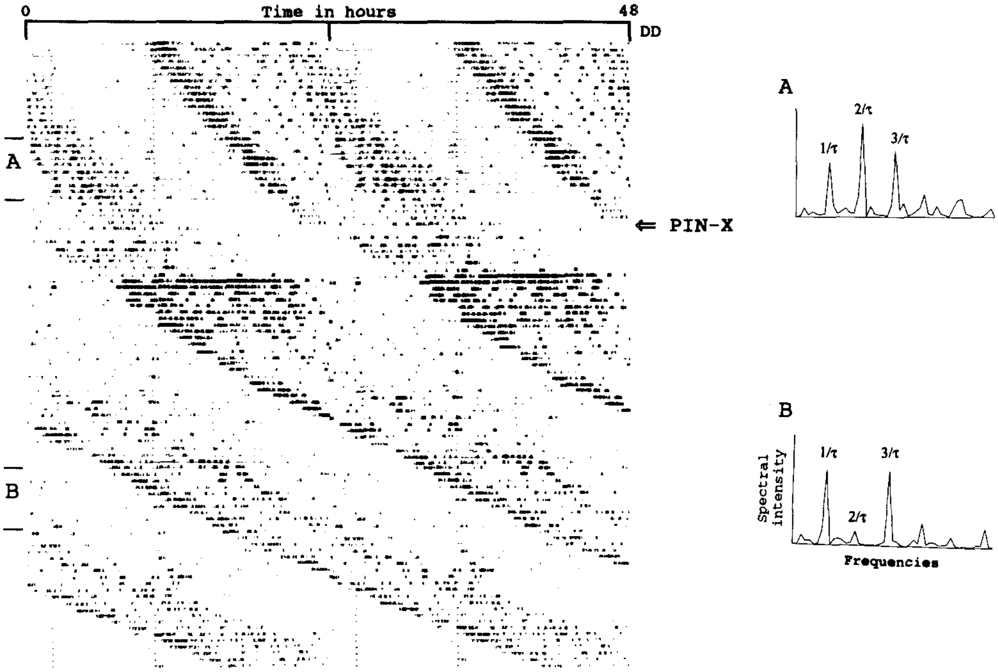
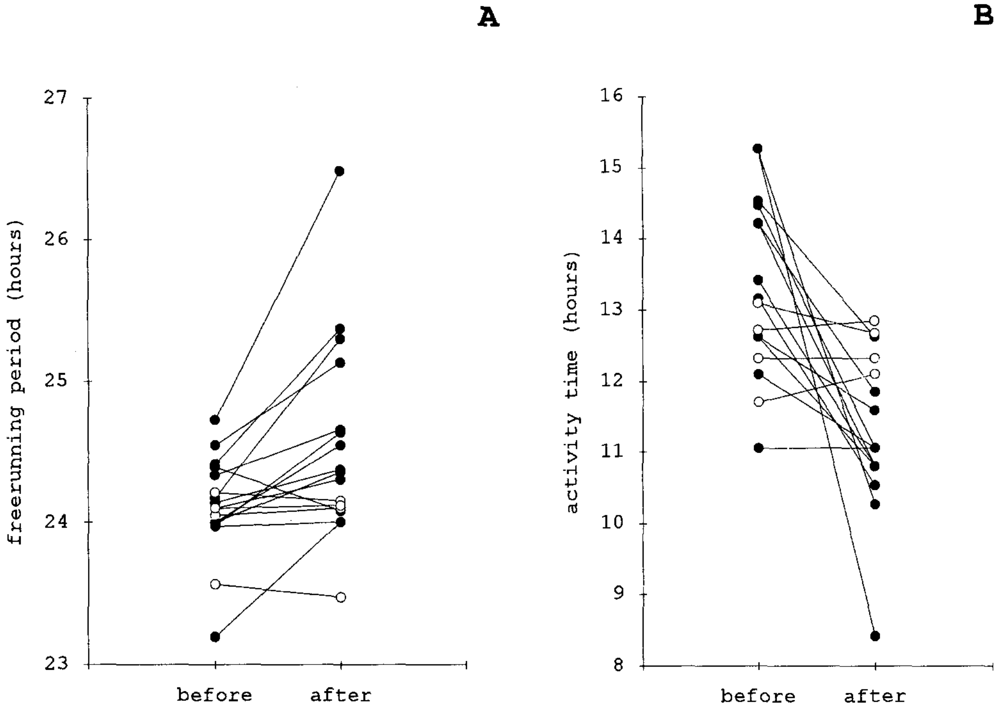


Fig. 3 Locomotor record of the only one lizard in which pinealectomy did not abolish bimodality completely. The record has been plotted on a 48-h time scale to aid interpretation. Although not abolished, the bimodal locomotor pattern of this lizard was modified by pinealectomy. In fact, spectral analysis (*right panels*) shows that the frequency peak at $2/\tau$, which was the highest frequency peak before pinealectomy, disappeared after this surgery. Note also the marked lengthening in τ and shortening in α after pinealectomy. (For further explanations, see Fig. 1)

Fig. 4A,B Estimates of the freerunning period (τ) and circadian activity time (α) before and after sham pinealectomy (*empty circles*) and pinealectomy (*filled circles*). Lines connect data points for all lizards before and after surgery. Sham pinealectomy did not significantly change **A** τ or **B** α ($P > 0.10$, Wilcoxon matched-pairs test). Pinealectomy induced (*A*) a significant lengthening in τ ($P < 0.003$) and (*B*) shortening in α ($P < 0.001$)



the two main bouts of circadian activity shortened by about 3 h and the frequency-peak at 2/τ (the highest frequency-peak revealed by spectral analysis in this locomotor record before surgery) was abolished.

Differences in the numbers of lizards whose locomotor rhythms were affected by the surgery (pinealectomy vs. sham pinealectomy) were found to be highly statistically significant ($P = 0.0025$, Fisher's exact test).

Discussion

Pinealectomy in summer had marked effects on the circadian locomotor behavior of *Podarcis sicula* held in constant temperature (29°C) and DD: in most lizards the freerunning period (τ) lengthened, the circadian activity time (α) shortened and the bimodal pattern of activity was abolished (Fig. 4). The locomotor behavior of pinealectomized lizards closely resembles that of most intact lizards tested in autumn (and part of those tested in spring) in constant temperature (29°C) and DD (Foà et al. 1994). Pinealectomy induced a sudden transition from the typical locomotor behavior of summer, characterized by a marked bimodal pattern, short τ and long α, to the typical locomotor behavior of autumn, characterized by a unimodal pattern with long τ and short α.

As expected, together with the appearance of the single-peaked pattern the frequency peaks at 2/τ and 3/τ (so typical of the double-peaked pattern) immediately disappeared from the locomotor records of pinealectomized lizards. In only one lizard did pinealectomy not abolish bimodality completely (Fig. 3). Residual bimodality in this lizard resembles the relatively weak bimodal pattern exhibited by some lizards in spring or early autumn (Foà et al. 1994: Fig. 2C). These results demonstrate that the pineal plays a central role in the seasonal reorganization of the circadian system that occurs in *P. sicula*.

The pattern of changes in circadian parameters observed in *P. sicula* after pinealectomy is compatible with the multioscillator model for the circadian system developed by Pittendrigh and co-workers (Pittendrigh 1974; Pittendrigh and Daan 1976; Daan and Berde 1978) and summarized in Foà et al. (1994). According to this model, the interdependent changes in τ and α of locomotor rhythms and the transition of the activity pattern from bimodal to unimodal are expected to arise from a change in phase relationship between the two circadian oscillators, or sets of oscillators, driving these rhythms. In the present case, the interdependent changes in τ and α and the abolition of the bimodal pattern in the same individual lizard were obviously caused by pinealectomy. Hence, the application of Pittendrigh's model to these data suggests that the phase relationship between circadian oscillators driving locomotor rhythms in *Podarcis sicula* changed in response to pinealectomy.

The behavioral effects of chronic implants of exogenous melatonin (via silastic capsules) in *P. sicula* are the same as those of pinealectomy in summer: the bimodal pattern of locomotor rhythms is abolished after application of the implants and, at the same time, a marked lengthening in τ and shortening in α invariably occurs (Foà et al. 1992b: Fig. 1C). Such a common situation is most probably because both treatments virtually abolish the circadian rhythms of blood-borne melatonin (Beldhuis et al. 1988; Janik and Menaker 1990; Foà et al. 1992a, b; Janik et al. 1992). This suggests a correlation in *P. sicula* between melatonin rhythms in the blood and the state of mutual coupling between oscillators: when melatonin rhythms become disrupted, due to pinealectomy or melatonin implants, a new phase relationship between oscillators driving locomotor rhythms is readily achieved. Altogether, these data support Underwood's idea that the pineal of lizards, through its output of melatonin into the blood, could act as a coupling device between mutually coupled circadian oscillators within a multi-oscillator system (Underwood 1979, 1981, 1992).

As pinealectomy or melatonin implants never abolished behavioral circadian rhythmicity in *P. sicula*, oscillators must exist elsewhere in the circadian system of this lizard which can drive locomotor rhythms independently of melatonin rhythms. Interestingly, the situation which most closely resembles that found in the lizard *P. sicula* is that of the starling (*Sturnus vulgaris*). Extensive investigations by Gwinner and co-workers demonstrated that circadian rhythms of perch-hopping and feeding generally persist in starlings either after pinealectomy or application of melatonin implants, despite the fact that both treatments abolish circadian rhythms of circulating melatonin (Gwinner 1978; Gwinner et al. 1987; Beldhuis et al. 1988; Janik et al. 1992).

Recently, lesions to the suprachiasmatic nuclei of the hypothalamus (SCN) of *P. sicula* have been shown to abolish circadian rhythms of locomotor activity (Minutini et al., in press). This suggests that the SCN contain the primary pacemaker(s) for locomotor rhythms in this lizard and confirms the results of previous investigations in the iguanid lizard *Dipsosaurus dorsalis*, which had indicated a crucial role for the SCN in the circadian system of these lizards (Janik et al. 1990).

Since laboratory experiments in *A. carolinensis* have unequivocally shown that the phase, amplitude and duration of the pineal melatonin rhythm vary depending on the length of daily photoperiod and thermoperiod (Underwood 1985; Underwood and Calaban 1987), seasonal changes in pineal melatonin synthesis and, consequently, blood-borne melatonin profiles are likely to occur in the natural environment of the lizard species inhabiting temperate zones. In Chelonia, namely the tortoise *Testudo hermanni*, annual changes in melatonin rhythms have been actually shown to occur under natural conditions with maximal amplitude of these rhythms in summer and their complete disappearance in winter (Vivien-Roels et al. 1979). If a similar situation were found in *P. sicula*, the role of melatonin output from the pineal into the blood in the context of the seasonal reorganization of the circadian system would be that of a coupling device. This would seasonally modulate the mutual phase relationship between oscillators driving locomotor rhythms in order to set the suitable activity pattern for the current season and recognize local time throughout the year.

Some experimental evidence has been gathered in both birds and mammals that suggests that seasonal changes in circadian parameters can be mediated by seasonal changes in the reproductive system (see Gwinner 1986, for review). In male starlings (*Sturnus vulgaris*), for example, α lengthens in parallel with testicular growth and shortens with testicular regression. The lengthening in α observed to occur with increasing testicular size is blocked by castration and re-established by testosterone injections (Gwinner 1975 1980). The fact that marked seasonal changes of locomotor activity patterns have been unequivocally found in *Podarcis sicula* (Foà et al. 1994) makes this lizard a particularly suitable model animal to ask whether the reproductive hormones interact with the circadian system and play a role in mediating seasonal changes in behavior. For instance, it may be interesting to test whether castration and/or testosterone administration in male *P. sicula* would induce the switch between bimodal and unimodal activity pattern and the changes in τ and α of locomotor rhythms.

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