

Pineal Gland and Circadian Locomotor Activity Rhythm in the Lacertid *Gallotia galloti eisentrauti*: Pinealectomy Induces Arrhythmicity

Miguel Molina-Borja

ABSTRACT

In order to contribute to a comparative view on lacertids, the effect of pinealectomy on the freerunning activity displayed under constant darkness and temperature ($27.5^{\circ}\text{C} \pm 0.5$) has been studied in the lizard *Gallotia galloti eisentrauti*. Animals showed an entrained motor activity rhythm under an initial light-dark (12:12 hours) routine and freerunning circadian periods ranging between 24.1 and 25.5 h during constant darkness (periodograms obtained by Sokolove & Bushnell's method). After pinealectomy, most animals showed no significant circadian rhythm, their locomotor activity becoming diffuse throughout the whole 24 h period. Thus, the pineal gland seems to play an important role as a main pacemaker regulating the endogenous activity rhythm under constant conditions. This result contrasts with that found in *Podarcis sicula* where after pinealectomy only changes in length of the freerunning period were found.

Abstracting keywords: Circadian rhythm, locomotor activity, pinealectomy, lacertid.

INTRODUCTION

The pineal gland is considered to be one of the main components of the circadian system in various nonmammalian vertebrates. This statement is based on two widely different types of study: on the one hand, pinealectomy has been reported to produce arrhythmicity in locomotor activity of different vertebrates including some birds (Ebihara and Kawamura 1981), lizards and fish (see Underwood 1990 for a review). On the other hand, in vitro pineal gland cultures show that a circadian pattern of melatonin synthesis is maintained even in constant darkness (DD) in some birds (Takahashi et al. 1980) reptiles (Menaker and Wisner 1983) and fishes (Falcón et al. 1989). All the above results support the idea that the pineal must be a circadian oscillator and that it has an important role in the central pacemaking mechanism (see Menaker 1982) regulating locomotor rhythmicity in these species.

However, for other species, the removal of the pineal gland only produced changes in the free-running circadian period of activity shown under constant conditions, as occurs in some fishes (Kavaliers 1980). Regarding lizards, pinealectomy induced this effect in *Sceloporus occidentalis* while it has no effect on the circadian period in *Dipsosaurus dorsalis* (Underwood 1981, 1983; Janik and Menaker 1990). Moreover, in pineal gland *in vitro* cultures, the melatonin production rhythm does not persist for a long time, being damped within a few days in some birds (Binkley et al. 1978), species of lizard (Janik and Menaker 1990) and fish (Gern and Greenhouse 1988). Taken together, the results of the two types of experiment suggest that the pineal gland is not the main oscillator of the circadian pacemaking mechanism.

Overall, it seems that the role played by the pineal in the circadian system is species-specific for birds, lizards and fishes and does not correlate with the phylogenetic relationships within each group (Underwood 1990).

It has been shown that the circadian system of many vertebrates also includes the suprachiasmatic nuclei and the eyes, and that these affect in different ways the expression of circadian activity rhythms (Underwood 1973; Rusak and Zucker 1979; Takahashi and Menaker 1982; Konishi et al. 1985; Cahill and Besharse 1990).

For lizards, most papers on the organization of the circadian mechanism have used several iguanids as experimental animals (Underwood 1977, 1981, 1983; Menaker and Wisner 1983; Janik and Menaker 1990), but for the lacertids, the effects of pinealectomy and retina removal on the locomotor activity rhythm (Foà 1991) and those of pinealectomy on the plasma melatonin levels (Foà et al. 1992) of *Podarcis sicula* were reported only recently.

The lizard *Gallotia galloti eisentrauti*, (Fam. Lacertidae) is one of two subspecies present in Tenerife island and has been studied from the behavioural point of view (Molina-Borja 1981, 1985) and its activity rhythms in different light conditions (Molina-Borja et al. 1986; García-Díaz et al. 1989). Considering the potential involvement of the circadian system in the daily and seasonal aspects of behaviour and in order to compare the role played by the pineal gland of this lacertid with that reported for *P. sicula* and other lizards, the circadian rhythms in activity were analysed in a light-dark cycle (LD) and before and after pinealectomy from animals maintained in continuous darkness (DD) and constant temperature.

MATERIAL AND METHODS

Animals

Adult male lizards of the subspecies *Gallotia galloti eisentrauti*, Fam. Lacertidae

(Bischoff 1982), were captured in their natural habitat in Bajamar (North of Tenerife). The animals (10.5-12.0 cm snout-vent length) were transported to the laboratory and maintained individually in cages under controlled light-dark (LD) and temperature conditions.

Locomotor recording

Locomotor activity of individual animals was recorded in cages (35 x 35 x 40 cm) with infrared cells (four per cage) connected to an interface which in turn was linked to a PC computer. A program in C language with assembler routine counted the beam interruptions every five minutes and recorded their numbers in a computer file. Data, in counts per hour, were analysed by the Sokolove and Bushell's (1978) periodogram method to determine the rhythm period. The cages were situated inside wooden chambers, located in small rooms completely isolated from the rest of the laboratory. Animals were initially maintained in a LD cycle of 12:12h during 15 to 20 days and afterwards in DD for a minimum of 60 days. Temperature was always kept constant at $27.5 \pm 0.5^\circ \text{C}$. Periodograms were calculated separately for the whole of the initial days (15) in LD and for the days in DD both prior to (25-30 days) and after pinealectomy (30-35 days). During the whole recording period the lizards were fed with cat food containing vitamins and with live meal worms (*Tenebrio molitor* larvae) and pieces of tomatoe. Food was administered every two to three days, at different times of the day, from the outside of their cages through cylindrical glass tubes. A few lizards showed some weight loss but most of them were in good health at the end of the experiment.

Surgery

Pineals were surgically extracted under ketamine anaesthesia during the animal's subjective day and after the animals had been at least 25 days in DD. The operations were performed under dim light outside the lizard cages and lasted 30-45 min. To extract the pineal, a small piece of the skull above the gland was removed and after making a small incision in the meninges, the organ was grasped and removed with micro-forceps. Small pieces of Gelfoam were placed on the brain and the skull piece was situated in place again and fixed with dental cement. Sham lesion consisted of removing a piece of the skull above the pineal that was fixed again in its initial position with dental cement. Immediately after surgery, animals were replaced in their recording cages. At the end of the whole recording experiment each animal was sacrificed by deep anaesthesia and its brain removed and fixed for histological examination. Sagittal brain sections (10 μ) were stained with hematoxylin-eosin 1% and their analysis showed that, except in two cases, the gland had been completely removed in all lizards.

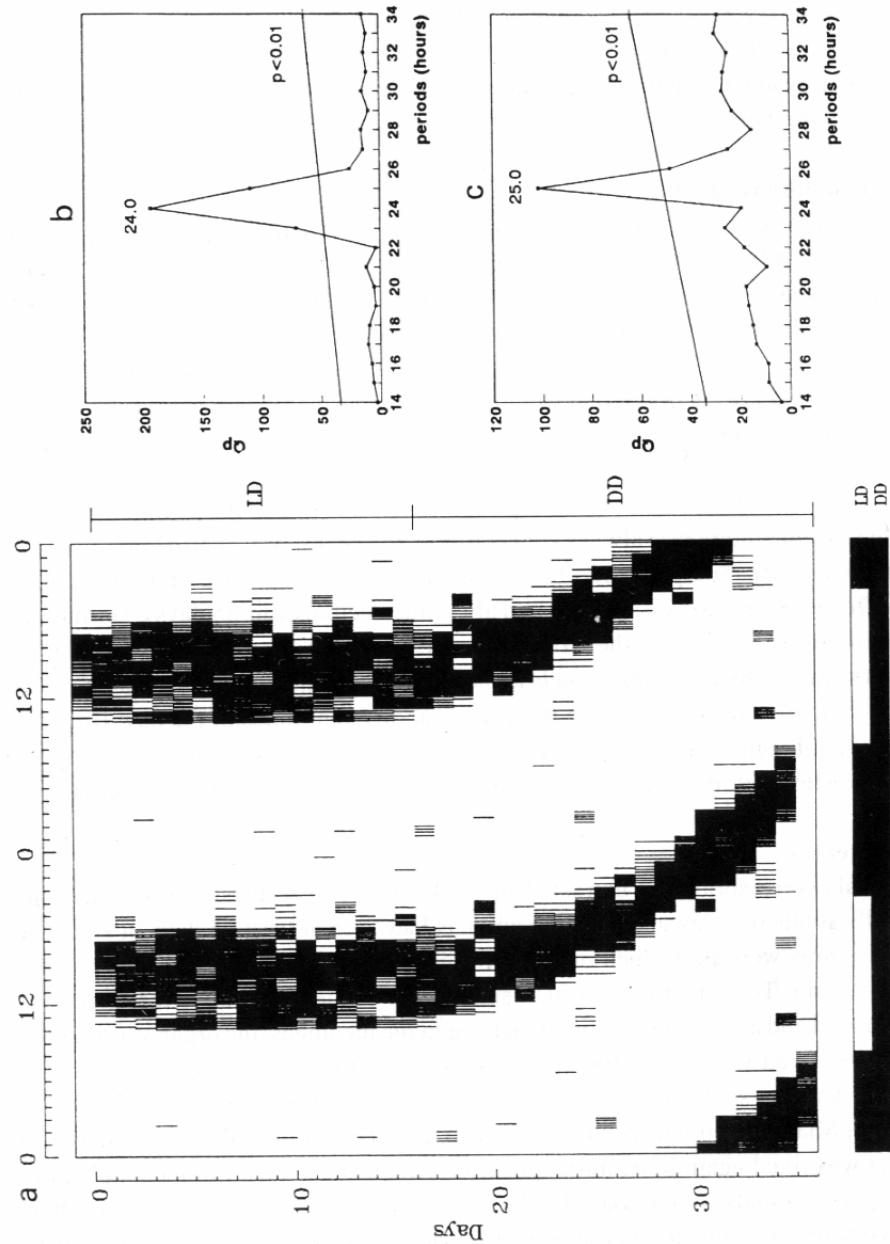


Fig. 1. a) Double plot (48 hrs) of locomotor activity from a lizard during the initial LD cycles and in DD. The morning surge in motor activity began with the light period. White and black bars at the bottom of the graph correspond to the light and dark periods, respectively. b) and c): Periodograms for the LD and DD data series shown in a) (Line: significance level at $p < 0.01$).

RESULTS

Activity rhythms in LD (12:12) and DD

All lizards (20) exhibited an entrained motor activity rhythm with a 24 h period during the first fifteen days of recording in the LD (12:12 h) cycle.

During DD and constant temperature conditions, almost all animals showed a free-running activity rhythm with circadian period (τ) greater than 24 h, ranging between 24.1 and 25.5 h. Only in two cases τ was less than 24 h. An example of a continuing circadian rhythm in DD after the LD cycles is shown in Fig. 1a and the corresponding periodograms for the LD and DD conditions are given in Figs. 1b and c, respectively. The activity onsets in the freerunning lizards in DD could always be extrapolated back to the onsets of the activity phases on the last day of LD, which shows that the latter are driven by the circadian clock and are not 'masked' by the external photoperiod.

Pinealectomy in DD

Sham animals (6) showed only very small changes of their circadian period ($0.1 \text{ h} \pm 0.05 \text{ h SEM}$) compared with that shown in the previous untreated condition. An example of circadian activity data in DD before and after sham-surgery is presented in Fig. 2a and the corresponding periodograms shown in Figs. 2b and 2c.

In contrast, of the fourteen pinealectomized lizards, twelve showed no circadian rhythmicity, as their locomotor activity was spread throughout all hours (Figs. 3a and b). The other two animals exhibited small changes (0.2 h) in their free-running periods after pinealectomy, but histological examination of their brains showed that some pineal tissue was still present. In Fig. 4a the changes in τ of every lizard in the different experimental conditions have been represented and in Fig. 4b those for the control group.

DISCUSSION

The present results show that the pineal is a very important component of the circadian activity organization in the lizard *G. g. eisentrauti* since even with the eyes and other possible pacemaking centers intact, the animals were unable to manifest a free-running (endogenous) circadian period in the absence of an intact pineal. This species, therefore exhibits a behaviour clearly different from that of the only other lacertid, *Podarcis sicula*, for which the effects of pinealectomy have been reported (Foà 1991). In the latter, pinealectomy produced changes in the circadian period under DD condition, but no arrhythmicity.

In iguanids, arrhythmicity and period changes of locomotor activity have also

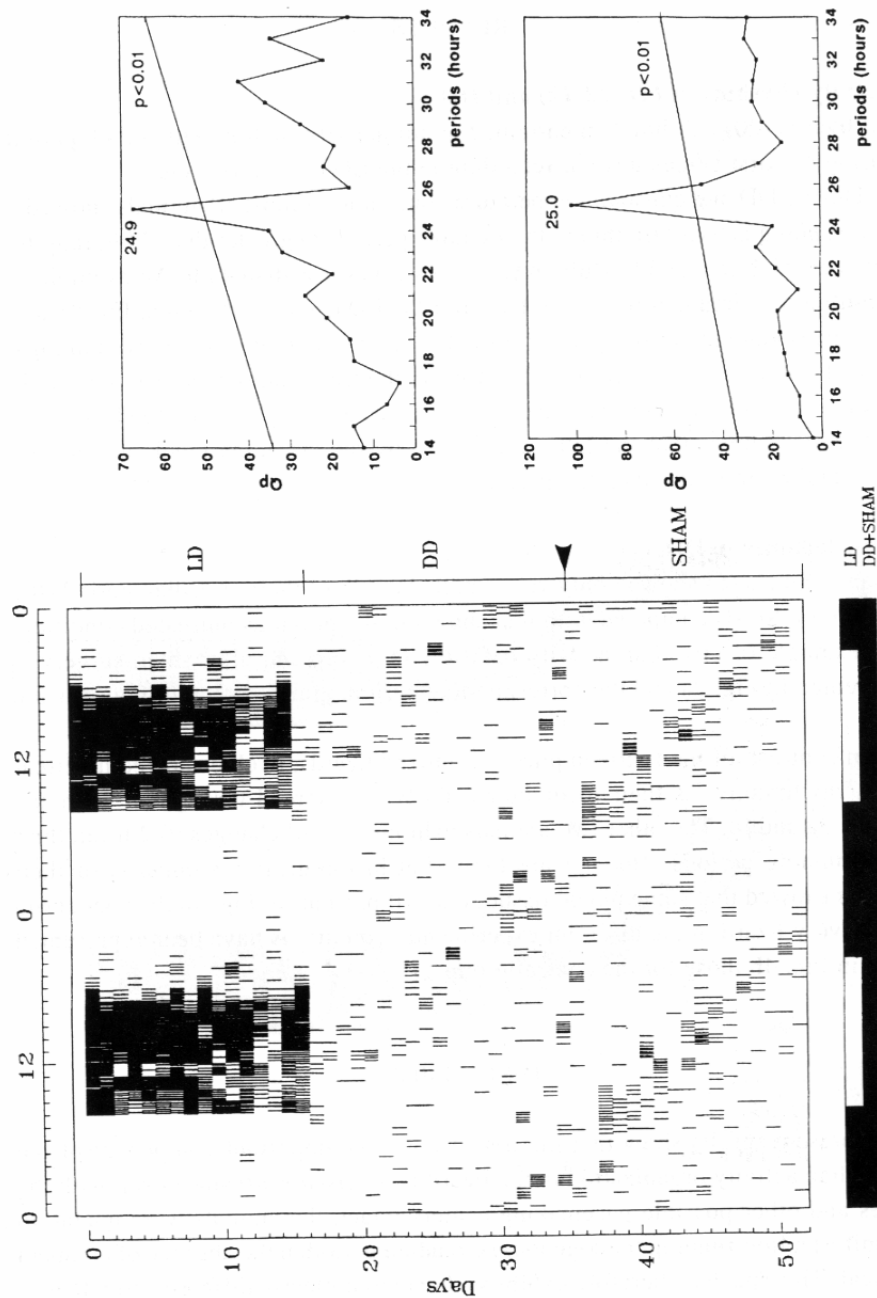


Fig. 2. a) Example of the motor activity recording from a sham-pinealectomized lizard showing no change in the circadian period. Arrow indicates the date of the sham-lesion. b) and c) Periodograms for data series shown in 2a before and after sham-surgery, respectively.

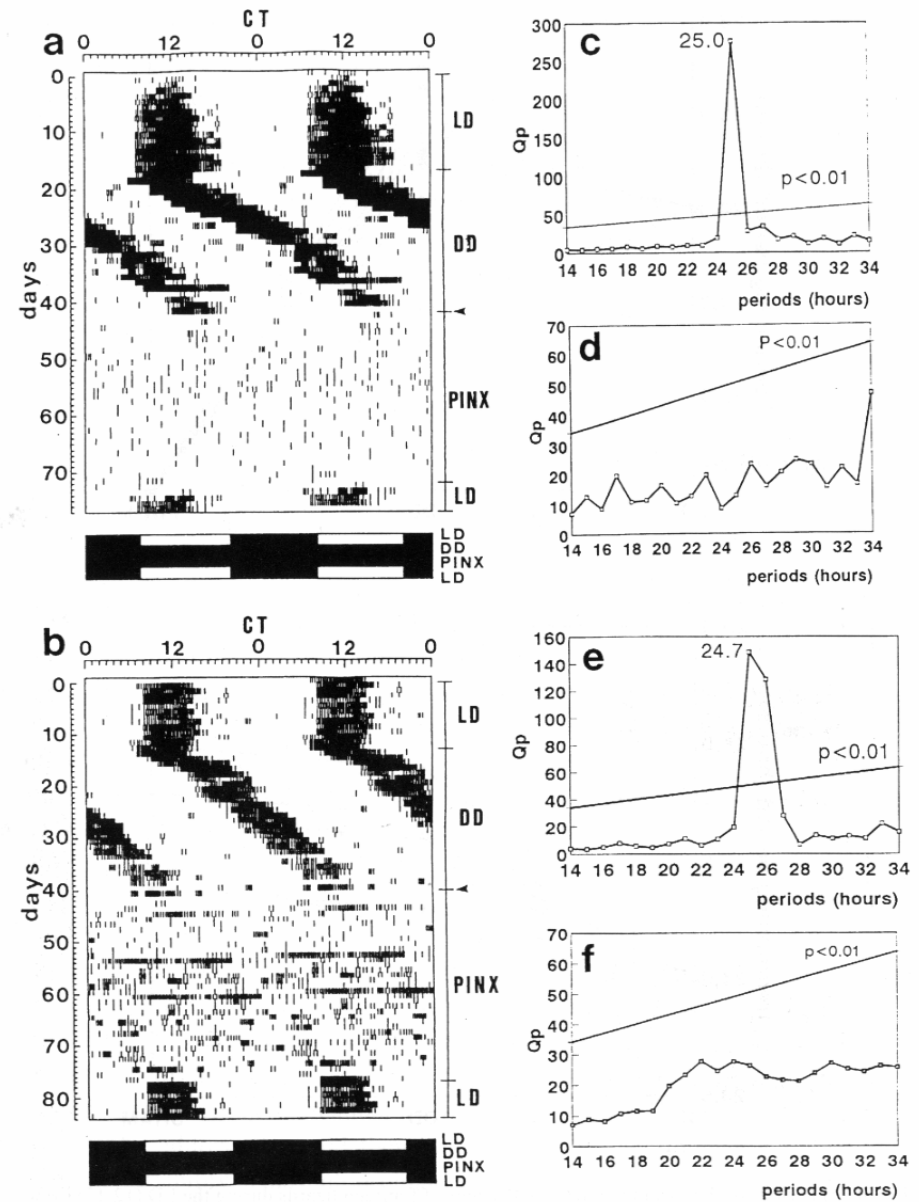


Fig. 3. a) and b) Two examples of the effect of pinealectomy on the free-running activity rhythm of the lizards. Arrhythmicity (activity spread throughout the whole recording) can be seen after the surgery (its date being indicated by an arrow). c) d) e) and f) Periodograms for the data series before and after pinealectomy of the two lizards, respectively. Lines: significance level at $p < 0.01$.

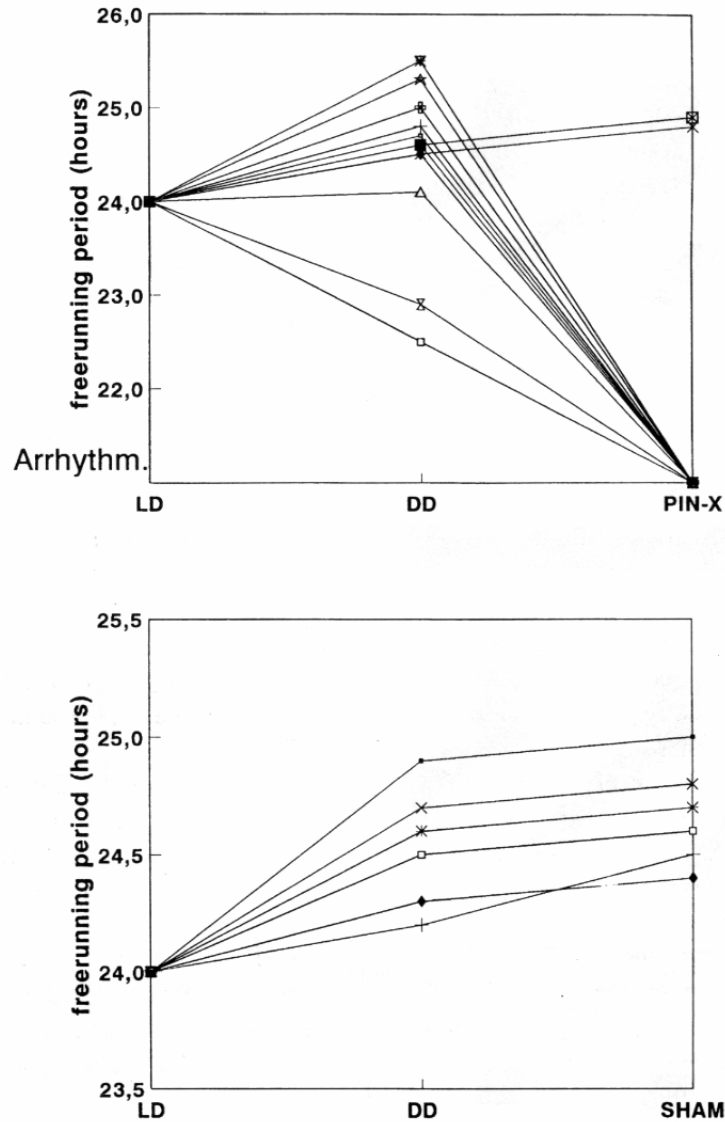


Fig. 4. a) Periods of the locomotor activity rhythms of fourteen lizards during the LD (12:12) cycle, before (DD) and after (PINX) pinealectomy in constant darkness. Lines connect period values in the three situations; Arrhythm. on ordinate represents arrhythmicity. Two individuals showed only small period changes after pinealectomy. Superimposed symbols in DD correspond to more than one animal with the same period. b) corresponding periods for six sham-operated animals in the three conditions.

been reported for different species. Thus, pinealectomy of *Sceloporus occidentalis* in DD induced changes of the free-running circadian period (Underwood 1981), while the same operation in *Anolis carolinensis* abolished circadian locomotor rhythmicity (Underwood 1983). Moreover, in the latter species, pineal culture studies showed undamped circadian rhythms of melatonin synthesis during 10 days in DD (Menaker and Wisner 1983). Based on these results, the pineal of *A. carolinensis* was hypothesized to have a main role in the "central rhythm generator" (see Menaker 1982). A multioscillator system controlling circadian activity and including (at least) the pineal and the suprachiasmatic nuclei was hypothesized by Underwood (1983). Later on, pinealectomy was shown not to affect locomotor rhythmicity in another iguanid, *Dipsosaurus dorsalis*, and *in vitro* pineals were unable to secrete melatonin rhythmically in DD (Janik and Menaker 1990). However, electrolytic lesions of the suprachiasmatic nuclei (SCN) abolished circadian locomotor rhythmicity in the same species which indicates that the SCN has a prominent role in its pacemaking mechanism (Janik et al. 1990). Based on this result, it has been suggested that the SCN could also drive the locomotor rhythms in *P. sicula* and *S. occidentalis* (where pinealectomy has little influence on circadian activity rhythms: Foà 1991). As pinealectomy abolished the circadian rhythm of plasma melatonin in *P. sicula*, Foà et al. (1992) suggested that changes in the free-running period of the locomotor rhythm induced by pinealectomy were due to withdrawal of rhythmic melatonin content of the blood in these lizards.

With respect to the effects of pinealectomy, *G.g. eisentrauti* is then more similar to *A. carolinensis* than to *S. occidentalis*, *D. dorsalis* or to *P. sicula*. The possible participation of the SCN in the general circadian system of our species remains to be assayed, but the pineal gland seems to have a key role in the central mechanism.

All the above results indicate that the role played by the pineal in the circadian organization of locomotor activity is different among the different lizard species. Underwood (1983) has hypothesized that the different patterns could be accounted for in terms of an interspecific variability in the strength of coupling among various oscillators within a circadian multioscillator system. The important question to be addressed would then be: what is the function/cause for that interspecific variability in the multioscillatory coupled system?. Underwood (1990) and Foà (1991) suggested that the phylogenetic incoherence of the circadian system in iguanids could be the result of an adaptation of this system to particular environmental niches occupied by the species concerned. Considering the different pineal involvements in the control of the circadian activity rhythms in *G.g. eisentrauti* and *P. sicula*, the same could be said for these lacertids. Nevertheless, the question still remains as to what are the environmental/evolutionary causes of these differences both in iguanids and lacertids.

ACKNOWLEDGEMENTS

I thank Dr. A. Díez-Noguera for his software support. Mr. Raides Rodríguez wrote the C program for the data recording and Felipe Padrón built the interface to connect infrared cells to the computer. Ana Lancha is thanked for her help with histological analysis. This study was supported by Grant no. 51/08.03.90 from the Gobierno Autónomo de Canarias.

REFERENCES

- BINKLEY, S.A., RIEBMAN, J.B. and REILLY, K.B. (1978): The pineal gland: A biological clock in vitro. *Science* 202: 1198-1201.
- BISCHOFF, W. (1982): Die innerartige Gliederung von *Gallotia galloti* (Duméril et Bibron 1839) (Reptilia: Sauria: Lacertidae) auf Teneriffa, Kanarische Inseln. *Bonn. zool. Beitr.* 33: 363-382.
- CAHILL, G.M. and BESHARSE, J.C. (1990): Circadian regulation of melatonin in the retina of *Xenopus laevis*: limitation by serotonin availability. *J. Neurochem.* 54: 716 - 719.
- EBIHARA, S. and KAWAMURA, K. (1981): The role of the pineal organ and the suprachiasmatic nucleus in the control of circadian locomotor rhythms in the Java sparrow, *Padda oryzivora*. *J. Comp. Physiol.* 141: 207-214.
- FALCON, J., BRUN-MARMILLON, J., CLAUSTRAT, B. and COLLIN, J. P. (1989): Regulation of melatonin secretion in a photoreceptive pineal organ: an in vitro study in the pike. *J. Neurosci.* 9: 1943-1950.
- FOA, A. (1991): The role of the pineal and the retinae in the expression of circadian locomotor rhythmicity in the ruin lizard, *Podarcis sicula*. *J. Comp. Physiol.* A 169: 201-208.
- FOA, A., JANIK, D. and MINUTINI, L. (1992): Circadian rhythms of plasma melatonin in the ruin lizard *Podarcis sicula*: effects of pinealectomy. *J. Pineal Res.* 12: 109-113.
- GARCIA-DIAZ, C., MOLINA-BORJA, M. and GONZALEZ-GONZALEZ, J. (1989): Circadian rhythm and ultradian oscillations in the motor activity of the lacertid lizard *Gallotia galloti* in continuous light. *J. Interdisc. Cycle Res.* 20: 97-105.
- GERN, W.A. and GREENHOUSE, S.S. (1988): Examination of in vitro melatonin secretion from superfused trout (*Salmo gairdneri*) pineal organs maintained under diel illumination or continuous darkness. *Gen. Comp. Endocrinol.* 71: 163-174.
- JANIK, D.S. and MENAKER, M. (1990): Circadian locomotor rhythms in the desert iguana I. The role of the eyes and the pineal. *J. Comp. Physiol.* A 166: 803-810.
- JANIK, D.S., PICKARD, G.E. and MENAKER, M. (1990): Circadian locomotor rhythms in the desert iguana. II. Effects of electrolytic lesions to the hypothalamus. *J. Comp. Physiol.* A 166: 811-816.
- KAVALIERS, M. (1980): Circadian locomotor activity rhythms of the Turbot, *Lota lota*: seasonal differences in period length and the effect of pinealectomy. *J. Comp. Physiol.* 136: 215-218.
- KONISHI, K., OHTA, M. and HOMMA, K. (1985): Important role of the eyes controlling the locomotor rhythm in the quail. *J. Interdisc. Cycle Res.* 16: 217-226.
- MENAKER, M. (1982): The search for principles of physiological organization in vertebrate circadian rhythms. In: *Vertebrate circadian systems*, ASCHOFF, J., DAAN, S. and GROOS, G.A. (eds.) Springer, Berlin, pp. 1-12.
- MENAKER, M. and WISNER, S. (1983): Temperature-compensated circadian clocks in the pineal of *Anolis*. *Proc. Natl. Acad. Sci. USA* 80: 6119-6121.
- MOLINA-BORJA, M. (1981): Etograma del lagarto de Tenerife, *Gallotia galloti galloti* (Sauria-Lacertidae). *Doñana Act. Vert.* 8: 43-78.
- MOLINA-BORJA, M. (1985): Spatial and temporal behaviour of *Gallotia galloti* in a natural population of Tenerife. *Bonn. zool. Beitr.* 36: 541-552.
- MOLINA-BORJA, M., GONZALEZ-GONZALEZ, J., GOMEZ-SOUTULLO, T. and GARCIA-DIAZ, C. (1986): 24 hr entrainment and ultradian fluctuations in the activity of the lizard *Gallotia galloti*. *J. Interdisc. Cycle Res.* 17: 295-305.
- RUSAK, B. and ZUCKER, I. (1979): Neural regulation of circadian rhythms. *Physiol. Rev.* 59: 449-526.
- SOKOLOVE, P.G. and BUSHHELL, W.N. (1978): The Chi-square periodogram: its utility for analysis of circadian rhythms. *J. Theor. Biol.* 72: 131-160.
- TAKAHASHI, J.S., HAMM, H. and MENAKER, M. (1980): Circadian rhythms of melatonin release from individual superfused chicken pineal glands in vitro. *Proc. Natl. Acad. Sci. USA* 77: 2319-2322.
- TAKAHASHI, J.S. and MENAKER, M. (1982): Role of the suprachiasmatic nuclei in the circadian system of the house sparrow, *Passer domesticus*. *J. Neurosci.* 2: 815-828.
- UNDERWOOD, H. (1973): Retinal and extraretinal photoreceptors mediate entrainment of the circadian locomotor rhythm in lizards. *J. Comp. Physiol.* 83: 187-222.
- UNDERWOOD, H. (1977): Circadian organization in lizards: the role of the pineal organ. *Science* 195: 587-589.
- UNDERWOOD, H. (1981): Circadian organization in the lizard, *Sceloporus occidentalis*: the effects of blinding, pinealectomy and melatonin. *J. Comp. Physiol.* 141: 537-547.
- UNDERWOOD, H. (1983): Circadian organization in the lizard *Anolis carolinensis*: a multioscillator system. *J. Comp. Physiol.* A 152: 265-274.
- UNDERWOOD, H. (1990): The pineal and melatonin: regulators of circadian function in lower vertebrates. *Experientia* 45: 914-922.