

## LIZARD HEART RATE AND MOTOR ACTIVITY RELATIONSHIPS IN THE CIRCADIAN AND ULTRADIAN RANGES

J. GONZÁLEZ-GONZÁLEZ\* and M. MOLINA-BORJA†‡

\*Lab. Biofísica, Dept. Fisiología, Fac. Medicina; †Lab. Fisiología Animal, Dept. Biología Animal, Fac. Biología, Univ. La Laguna, Tenerife, Canary Islands, Spain

(Received 25 February 1991)

**Abstract**—1. The heart rate and motor activity of lizards (*Gallotia galloti*) maintained in a light–dark cycle (12:12 hr) and with changing cyclic temperature were recorded. Both variables showed a circadian pattern, motor activity variations leading heart rate by approximately 110 min.

2. Results from cross spectral analysis showed that heart rate was highly correlated with temperature and both variables were changing practically in phase. However, motor activity and temperature were less correlated and in this case motor activity led temperature by approximately 100 min.

3. In the ultradian range, two periodicities could be distinguished in the power spectra of motor activity and heart rate variations during the day: one between 0.019 and  $3.9 \times 10^{-3}$  cycles/min (central peak around 85 min period) and another, of lower power, between 0.05 and 0.02 cycles/min (central peak around 34 min period). During the night, only the heart rate showed both periodicities but with a lower amplitude.

4. The presence of ultradian oscillations in heart rate during the night suggested that they do not depend on motor activity which was almost absent during that daily period.

5. Motor activity and heart rate were also highly correlated in the ultradian range, motor activity leading heart rate by around 5 min at the 85 min main peak.

### INTRODUCTION

The control of cardiovascular parameters has been shown to change in relation to the sleep–wakefulness states (Mancia and Zanchetti, 1980). These changes in the central control are considered to be the origin of daily fluctuations in heart rate.

Circadian rhythms in heart rate variability have been described for humans (Malpas and Purdie, 1989; Huikuri *et al.*, 1990) and in heart rate of goats (Matsui, 1990). A significant difference between the morning and night mean heart rates has also been obtained for toads (Dumsday, 1990).

Higher frequency oscillations in heart rate, close to the fundamental rate of breathing in cats (Chess *et al.*, 1975) and rats (Lacombe *et al.*, 1988) and with a period between 1 and 3 hr in humans and dogs (Orr and Hoffman, 1974; Shimada and Marsh, 1979; Broten and Zehr, 1988) have also been found.

Although in reptiles the circadian pattern in motor activity of certain iguanids (Underwood, 1977, 1983) and circadian rhythm and ultradian oscillations in a lacertid (Molina-Borja *et al.*, 1986; García-Díaz *et al.*, 1988) have been studied, no reference is known that is related to circadian and ultradian oscillations in heart rate. However, some authors have studied the influence of temperature on heart rate (Licht, 1965; De Vera and González, 1986) and short-term oscillations in the variability of heart rate (González and De Vera, 1988).

The aim of the present study is to analyse the presence of circadian rhythm (frequency around 1

cycle/day) and ultradian fluctuations (frequencies > 1 cycle/day) in the heart rate of specimens of a lacertid lizard from Tenerife (Canary Islands), maintained in a controlled environment and to see the temporal relationships between heart rate and motor activity within the circadian and ultradian ranges.

For this study we have used spectral analysis methods, including cross spectral relationships between lizard motor activity, heart rate and environmental temperature daily variations.

### MATERIALS AND METHODS

Adult male specimens (40–60 g weight) of the lizard *Gallotia galloti* were used in the study, the animals being captured in the field and transferred to laboratory cages situated within small isolated laboratory rooms.

The temperature inside the rooms was between 23 and 27°C (during the day) and 17 and 19 ± 1°C (during the night), following a cyclic variation. A light–dark cycle of 12:12 hr was also maintained, light period beginning at 8.00 a.m. (local time).

After a minimum of 4–5 days of acclimatization, the animals were surgically operated upon to implant electrodes under their skin. Surgery was made under ketamine chloride anaesthesia (50 mg/kg) and two silver electrodes were placed under the anterior and posterior dorsal skin. A third reference electrode was anchored on the skull. All electrodes were connected through thin and flexible wires to an oscilloscope and to a polygraph. The wire flexibility always permitted free movement of the animals. The polygraph was in turn connected to an automatic data acquisition and processing system, previously developed (González and De Vera, 1984) using a PC. The voltages coming from the polygraph were introduced into the system by means of an analogic-digital converter and treated afterwards by a program which

‡To whom correspondence should be addressed.

calculated the number of R pics per minute. These data were recorded in a computer file.

After at least 5 days following surgical operation, heart rate was continuously recorded up to a maximum of 10 days. During this period the animals were in cages situated on activity sensitive platforms. The latter were connected to the automatic data acquisition system and the activity data (in counts/minute) were also recorded in a separate file. And the same was done for the data of environmental temperature within the animal cage, the signal being sent to the data acquisition system by a thermistor.

*Calculations within circadian range*

To calculate the circadian periods the Fast Fourier Transform (FFT) was applied to data segments of 256 points, each point representing the mean value of the variable (heart rate, motor activity or temperature) in 30 min (data window of 7680 minutes width), beginning at 8.00 a.m.

For each data segment of any signal, mean and lineal trend were removed and a cosine tapering applied before FFT calculations. The spectral coefficients of the power spectra were smoothed to reduce their variance and an ensemble average power spectrum was obtained for all individuals.

We also obtained the cross power spectra density function between each pair of signals. In this case, after obtaining the amplitude spectrum of each signal, the real and imaginary components of cross spectra were computed and smoothed. The phase spectrum and coherence function were calculated from the corresponding power spectrum of the signal and the cross spectra. Finally, ensemble averages of power spectra, coherence functions and phase spectra were obtained for all animals. After smoothing and ensemble averaging, we got a number of degrees of freedom greater than 300. Taking this into account, 95% confidence intervals were obtained for coherence and phase values at the circadian peak. Procedures of these calculations can be found elsewhere (Jenkins and Watts, 1968; Bendat and Piersol, 1971).

A cross-spectrum between two signals consists of: the coherence spectrum and the phase spectrum. The (squared) coherence spectrum  $k^2(f)$ , have values between 0 and 1 and is a measure of the correlation between the variation of two signals at the frequency,  $f$ . It is similar to the squared correlation coefficient used in linear regression analysis.

The phase spectrum  $\Phi(f)$  indicates at each frequency  $f$  the phase difference (lead or lag) between the signals. All phase spectra we present have been scaled in the region of

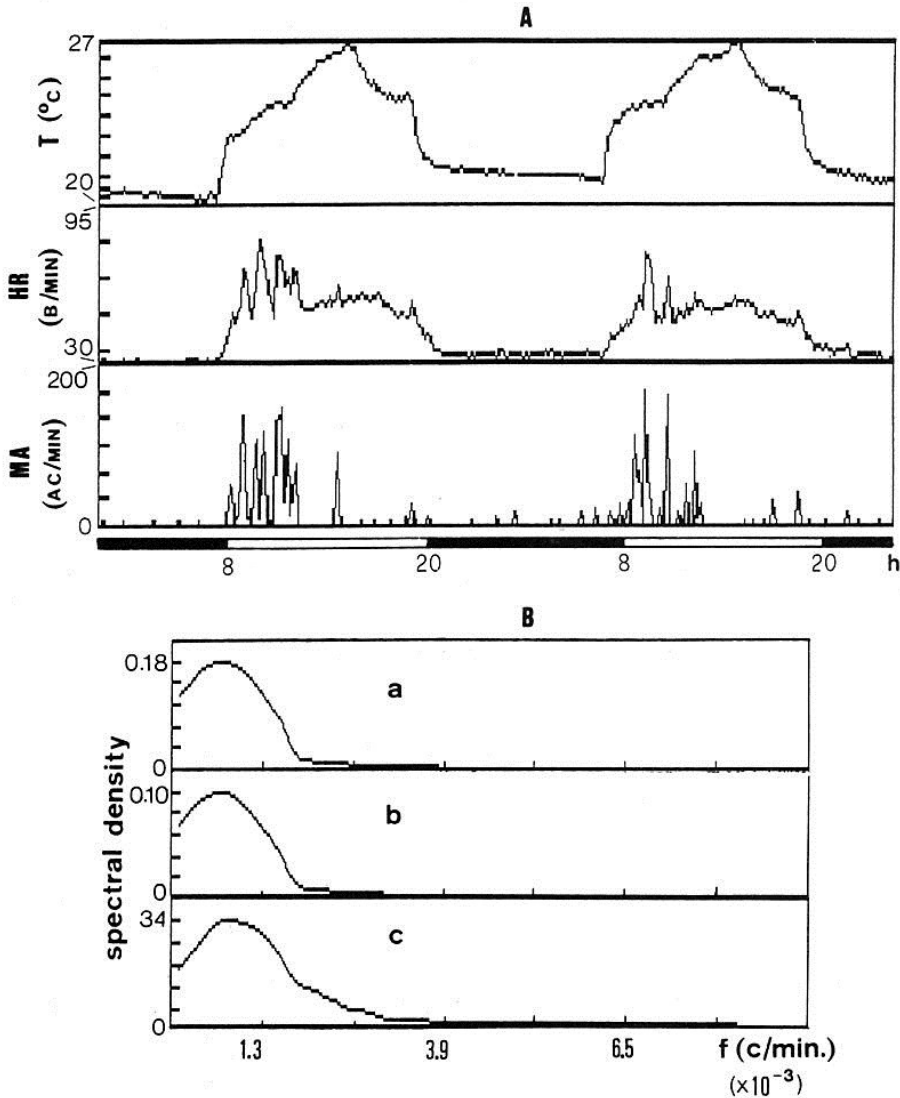


Fig. 1. (A) Daily evolution of temperature (T, in °C), heart rate (HR, in beats/min) and motor activity (MA, in activity cpm) throughout 2 consecutive days. Black and white bar at the bottom indicates dark and light daily periods. (B) Mean power spectra for environmental temperature (a), heart rate (b) and motor activity (c) data segments. Abscissa: frequency (cycles/min). Ordinate: spectral density in temperature<sup>2</sup> × min (a), (beat/min)<sup>2</sup> × min (b) and (activity counts)<sup>2</sup> × min (c).

$-180$  to  $+180^\circ$ . In our figures, a negative value of  $\Phi(f)$  implies that the second specified signal leads the first one at this frequency and for a positive value the reverse holds.

#### Calculations within ultradian range

To calculate ultradian frequencies, data segments of 256 points were used, each point corresponding to the mean value of the variable in 2 min (=512 min data window). Two separate data segments were analysed each day, one beginning at 10.00 a.m. ("morning" segment) and the other at 10.00 p.m. ("night" segment).

Each data segment was initially processed as described in the circadian range and ensemble averages of power spectra, coherence function and phase spectra were calculated separately for "morning" and "night" segments of all individuals. Confidence intervals of 95% for coherence and phase values were also calculated at the main peaks of the ultradian components.

## RESULTS

A 2 consecutive day record of heart rate (HR), motor activity (MA) and temperature (T) is shown in Fig. 1A. The three measurements follow a circadian pattern with maximum values during the light period. In Fig. 1B the ensemble average power spectra of the three signals have been represented, showing that all of them have a period around 24 hr ( $0.7 \times 10^{-3}$  cycles/min).

When the coherence function and phase relationship between the three different pairs of signals were calculated for the studied animals, the results expressed in Figs 2 and 3 were obtained. In the circadian range, the coherence was high (0.80) for the HR/MA signals but they have an average phase difference of  $-27.7^\circ$

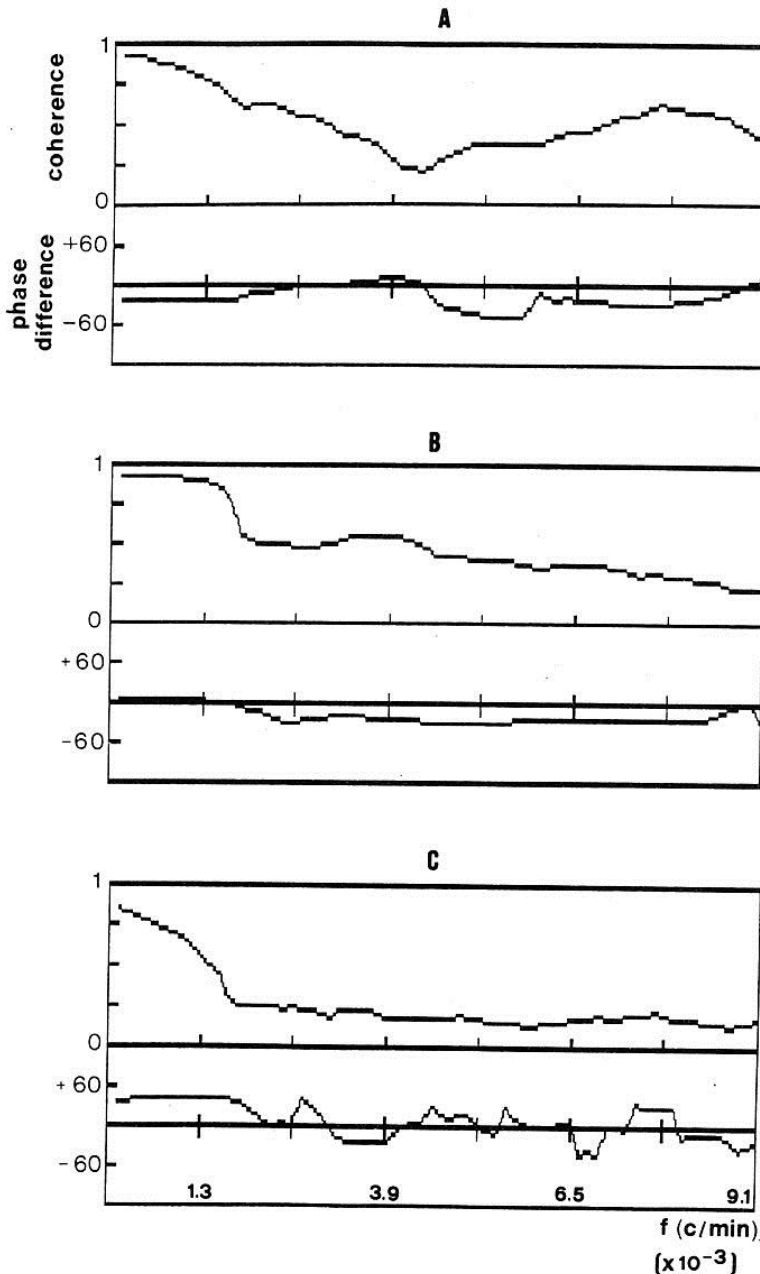


Fig. 2. Mean coherence function and phase difference (in degrees) between heart rate and motor activity (A), heart rate and temperature (B) and temperature and motor activity (C) in the circadian range. Abscissa: frequency in cycles/min.

(this value corresponds to a phase difference (Phd) of 110.8 min at the circadian frequency) which means that motor activity leads to the heart rate (Fig. 2A and Table 1). When comparing HR and T, a high coherence (0.94) was also found, but in this case a positive phase difference of  $3.45^\circ$  (Phd of 13.8 min at the circadian frequency) resulted between the signals and then HR leads T (Fig. 2B and Table 1). For the MA and T comparison a coherence of 0.78 was obtained, the two signals having a mean phase difference of  $+25.1^\circ$  (Phd of 100.4 min at the circadian

frequency), then MA leads T (Fig. 2C and Table 1). Coherence between HR and T was significantly higher ( $P < 0.05$ ) than that of HR/MA and MA/T, while coherence between HR/MA was not significantly different than that of MA/T.

In Fig. 2A, high coherence between HR/MA can also be appreciated within the ultradian range (around a frequency of  $7.8 \times 10^{-3}$ ) and the phase between the signals is also negative. The latter does not happen when comparing HR/T and MA/T (Fig. 2B and C). However, these results will be explained in the follow-

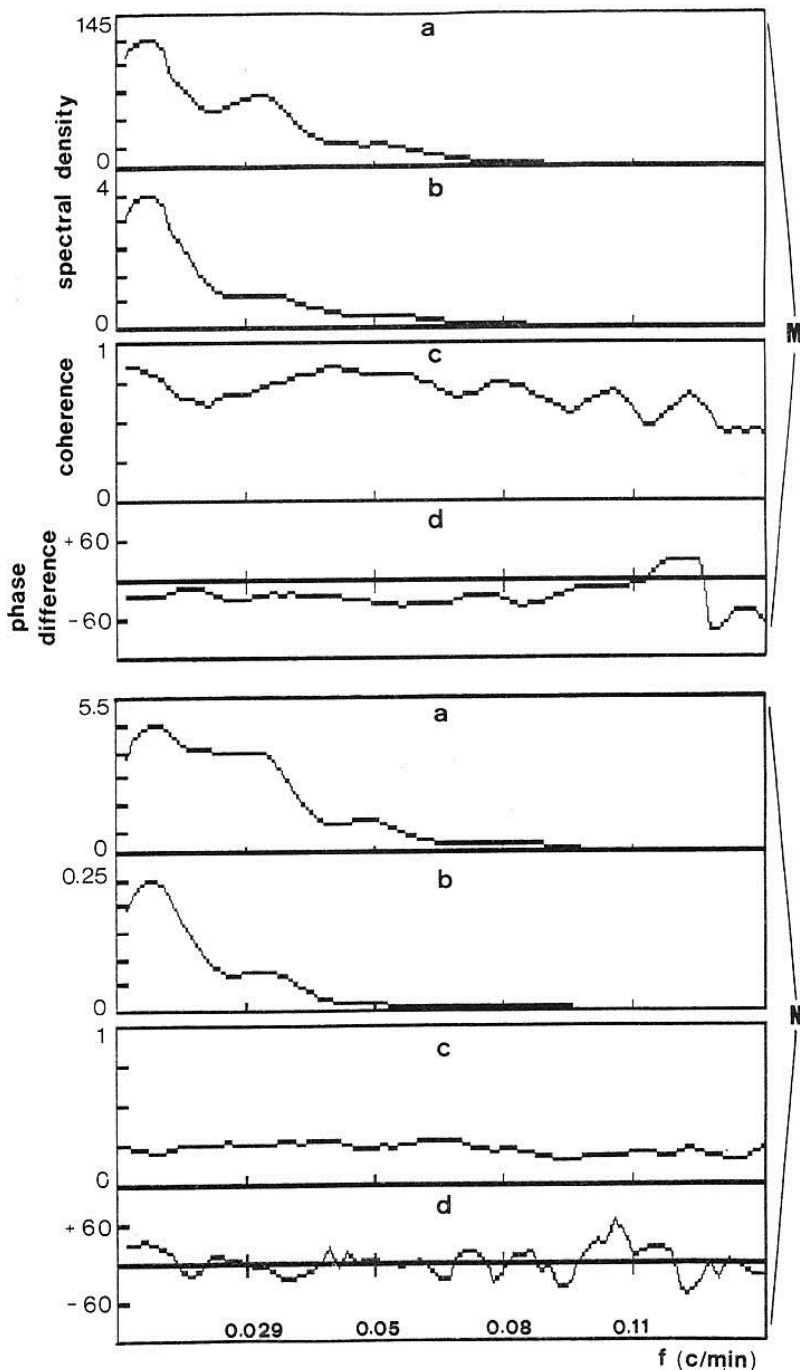


Fig. 3. Mean power spectra for motor activity (a) and heart rate (b) and mean coherence function (c) and phase difference (d, in degrees) in the ultradian range of daily (M) and nightly (N) data segments. Abscissa: frequency in cycles/minute. Ordinates in power spectra: spectral density in (activity counts)<sup>2</sup> × min (a) and (beat/min)<sup>2</sup> × min (b).

Table 1. Coherence and phase difference values between heart rate and motor activity (HR-MA), heart rate and temperature (HR-T) and motor activity and temperature (MA-T) in the circadian range

	Coherence coefficient	Phase difference	N
HR/MA	0.80 (0.76-0.83)	-27.7 (± 2.4)	11
HR/T	0.94 (0.92-0.95)	+3.4 (± 1.15)	13
MA/T	0.78 (0.73-0.82)	+25.1 (+ 2.7)	10

Values are means and confidence intervals at 95% in parentheses.

ing paragraph on the ultradian range. When analysing the ultradian range of the data segments, ultradian periodicities were found both in HR and MA during daily and nightly time periods. During "morning" segments, two spectral peaks appeared within the bands of 0.0039-0.0195 c/min (period between 256 and 51.2 min, with a peak around 85 min) and in the band 0.05-0.02 c/min (20-50 min period, with a peak around 34 min) (see Fig. 3 daily and nightly data). During "night" segments, while HR showed a similar spectral picture (with lower power), MA showed a wide ultradian band with no clear peak at any frequency.

For "morning" data segments the coherence between HR and MA in the first band was high (0.80) but the two signals had an average phase difference of  $-22.1^\circ$  (Phd of 5.2 min at the peak frequency), that is MA leads HR. For the second band, the coherence was 0.73 and Phd  $-21.8^\circ$  (2.05 min at the peak frequency) and again MA leads HR (see Fig. 3 daily data c, d and Table 2).

During "night" segments, coherence between MA and HR was very low and then phases have no significance (see Fig. 3 nightly data c, d). As temperature did not show ultradian oscillations, no spectral comparison between HR, MA and T were made.

## DISCUSSION

A clear circadian pattern (period around 24 hr) has been detected in heart rate and motor activity of freely moving lizards maintained in a light-dark cycle of 12:12 hr and with cyclic environmental temperature. Both heart rate and motor activity showed a higher amplitude during the morning period.

The comparison of HR and MA in the circadian range showed that both signals have a high correlation in the lizards studied but are out of phase: motor activity leading heart rate. This difference could be due to the heart rate being maintained at relatively

Table 2. Coherence and phase difference between heart rate and motor activity in the ultradian range (frequency in cycles per minute). Phase difference in degrees. C.I.: confidence intervals at 95%

Ultradian band (c/min)	Coherence coefficient	Phase difference
0.019-3.9 ( $\times 10^{-3}$ )	0.80 (0.76-0.83)	$-22.1^\circ$ (± 2.7°)
0.05-0.02	0.73 (0.70-0.75)	$-21.8^\circ$ (± 2.3°)

Coherence and phase values are means and 95% confidence intervals are between parentheses.

high values while temperature is increasing but motor activity is high during the first part of the light period and almost absent during the second one. Heart rate circadian rhythm seems to follow daily temperature variation (both signals showed a higher coherence and a small phase difference). That is, temperature could be considered as one of the main factors involved in the expression of heart rate circadian rhythm. Moreover, heart rate recordings of new animals (unpublished data) in a L:D cycle and constant temperature show that although a circadian rhythm in heart rate is also present, its amplitude is less than that found for animals maintained with a cyclic changing temperature. A cyclic temperature has been shown to synchronize motor activity in lizards while the animals had no other temporal information (Hoffman, 1968).

However, the coherence coefficient between motor activity and temperature, although also being high is the lowest value found for any pair of variables (see Table 1). This could be due to activity being drastically reduced during the afternoon while the temperature was progressively decreasing (see Fig. 1). The phase spectrum showed that motor activity leads temperature. Therefore, motor activity did not appear to precisely follow the gradual change in environmental temperature and is perhaps more dependent on central oscillators.

Ultradian oscillations in motor activity have previously been reported for the lizard *Gallotia galloti* (Molina-Borja *et al.*, 1986; García-Díaz *et al.*, 1989). The present results confirm those previously obtained with an ultradian periodicity about 85 min (main peak) and a second one with less amplitude around 34 min. Heart rate also showed ultradian periodicities around the same frequencies.

Orr and Hoffman (1974) had already described a 90 min oscillation (0.011 cycles/min) in heart rate of resting humans and Livnat *et al.* (1984) also found ultradian oscillations (periods between 1 and 2 hr  $0.016-8.3 \times 10^{-3}$  cycles/min) in the heart rate of free-running dogs. The results in our lizards showed that apart from ultradian peaks within this band, there was also a second ultradian periodicity around 34 min although with less power.

During the day, this ultradian peak appeared well defined in the motor activity power spectrum but poorly represented in that of heart rate. However, during the night while this peak could be distinguished in the heart rate power spectrum, it did not appear in that of motor activity. The latter result reflects that motor activity was almost absent during the night. As temperature was practically constant during that period, then ultradian oscillations in heart rate could be considered as endogenous, not related to the activity of the animals nor the environmental temperature. However, these oscillations in HR could be related to respiratory cycles or to a cyclic modulation of the autonomous nervous system. Livnat *et al.* (1984) suggested that the ultradian oscillations in heart rate and mean blood pressure of dogs were influenced by central cardiovascular centers. In fact ultradian oscillations in cardiovascular variables have been suggested as being the result of a complex multi-oscillatory system that could include: one or more central oscillators and a rhythmic release of hormones

acting on cardiocirculatory system (Lavie and Kripke, 1981; Broten and Zehr, 1988, 1989).

During the day, heart rate and motor activity in the lizards had a high coherence (0.80) and motor activity led (around 5 min) heart rate at the 85 min main peak. Coherence at the second ultradian frequency between MA/HR is lower (0.73) than that of the first one and phase difference is small (1.8 min). This result is in agreement with the general fact that elevated metabolic needs during activity are fulfilled with a higher cardiac output that can result from increases in heart rate. However, a higher metabolism can be supported by an increase in the extraction of oxygen by the tissues and not only with a higher heart rate (White, 1976). On the other hand, Blinowska and Marsh (1985) showed that ultradian oscillatory patterns in muscle activity and heart rate and blood pressure of dogs were not tightly coupled.

The cross spectral technique used in this work is considered to be useful to establish long term relationships (in the circadian and ultradian range) between motor activity and heart rate, as well as for future studies on possible nervous mechanisms regulating heart rate.

*Acknowledgements*—This work was supported by the Research Grant Pb86-0316 of the D.I.C.Y.T. of the Education Ministry to Dr J. González.

#### REFERENCES

- Bendat J. S. and Piersol A. G. (1971) *Random Data: Analysis and Measurement*. John Wiley, New York.
- Blinowska K. and Marsh D. J. (1985) Ultra- and circadian fluctuations in arterial pressure and electromyogram in conscious dogs. *Am. J. Physiol.* **249**, R720–R725.
- Broten T. P. and Zehr J. E. (1988) Baroreflex modulation of ultradian oscillations of blood pressure and heart rate in unanesthetized dogs. *Chronobiologia* **16**, 241–255.
- Broten T. P. and Zehr J. E. (1989) Autonomic modulation of ultradian blood pressure and heart rate oscillations in dogs. *Am. J. Physiol.* **256**, R1127–R1137.
- Chess C. F., Tam R. M. K. and Marsh D. J. (1975) Influence of cardiac neural input on rhythmic variations of heart period in the cat. *Am. J. Physiol.* **228**, 775–780.
- De Vera Porcell L. and González González J. (1986) Cardiac responses to temperature in the lizard *Gallotia galloti*. *Comp. Biochem. Physiol.* **85A**, 389–394.
- García-Díaz C., Molina-Borja M. and González-González J. (1989) Circadian rhythm and ultradian oscillations in the motor activity of the lacertid lizard *Gallotia galloti* in continuous light. *J. interdiscipl. Cycle Res.* **20**, 97–105.
- González-González J. and De Vera Porcell L. (1984) Sistema microcomputarizado de análisis de señales biológicas para su utilización en laboratorios de investigación animal. *Resúmenes Conf. Iberoamer. Bioingen.* pp. 114. Asturias, Spain.
- González-González J. and De Vera-Porcell L. (1988) Spectral analysis of heart rate variability of lizard *Gallotia galloti*. *Am. J. Physiol.* **254**, R242–R248.
- Hoffmann, H. (1968) Synchronisation der oiroadianen aktivitätsperiodik von Eidechsen durch temperaturcyclen verschiedener amplitude. *Z. Vergl. Physiol.* **58**, 225–228.
- Jenkins G. M. and Watts D. G. (1968) *Spectral Analysis and Its Applications*. Holden-Day, San Francisco.
- Lacombe J., Nosjean A., Meunier J. M. and Laguzzi R. (1988) Computer analysis of cardiovascular changes during sleep–wake cycle in Sprague–Dawley rats. *Am. J. Physiol.* **254**, H217–H222.
- Lavie P. and Kripke D. F. (1981) Ultradian circa 1 1/2 hour rhythms: a multioscillatory phenomena. *Life Sci.* **29**, 2445–2450.
- Licht P. (1965) Effects of temperature on heart rates of lizards during rest and activity. *Physiol. Zool.* **38**, 129–137.
- Livnat A., Zehr J. E. and Broten T. P. (1984) Ultradian oscillations in blood pressure and heart rate in free running dogs. *Am. J. Physiol.* **246**, R817–R824.
- Mancia G. and Zanchetti A. (1980) Cardiovascular regulation during sleep. In *Physiology of Sleep* (Edited by Orem J. and Barnes C. D.), pp. 2–50. Academic Press, New York.
- Molina-Borja M., González-González J., Gómez-Soutullo T. and García-Díaz C. (1986) 24 hr entrainment and ultradian fluctuations in the activity of the lizard *Gallotia galloti*. *J. interdiscipl. Cycle Res.* **17**, 295–305.
- Orr W. C. and Hoffman H. J. (1974) A 90 minute cardiac biorhythm: methodology and data analysis using modified periodograms and complex demodulation. *IEEE Trans. biomed. Eng.* **21**, 130–141.
- Shimada S. G. and Marsh D. J. (1979) Oscillations in mean arterial blood pressure in conscious dogs. *Circ. Res.* **44**, 692–700.
- Underwood H. (1977) Circadian organization in lizards, the role of the pineal organ. *Science* **195**, 587–589.
- Underwood H. (1983) Circadian organization in the lizard *Anolis carolinensis*, a multioscillator system. *J. comp. Physiol.* **152**, 265–274.
- White F. N. (1976) Circulation. In *Biology of the Reptilia* (Edited by Gans C. and Dawson W. R.), Vol. 5, pp. 225–334. Academic Press, London.