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Autonomic mediation in the interdependences between cardiocortical activity time variations and between cardiorespiratory activity time variations in the lizard, *Gallotia galloti*

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Abstract

Multivariate nonlinear analysis methods were applied to variability time series extracted from electrocardiographic, electrocorticographic and respiratory activities of *Gallotia galloti* lizards, to study interdependences between cardio-cortical activity time variations, and between cardio-respiratory activity time variations. Autonomic nervous system involvement in the mediation of such interdependences was investigated through pharmacological blockade. Cardiac variability was evaluated from the R–R intervals of the electrocardiogram (RRIv). Cortical (CORTv) and respiratory (RESPv) activity time variations were evaluated from power-data signals derived from both electrocorticogram and respiratory signal segments obtained within each R–R interval, respectively. A nonlinear index N to measure interdependence between the signals, and a surrogate data test to measure the significance and nature of the interdependences were used. A nonlinear dependence of RRIv vs. CORTv and of RRIv vs. RESPv was found. Both dependences seem to be unconnected with the functioning of both α_1-adrenoceptor and cholinoceptor systems, but appear to be mediated by β-adrenoceptor mechanisms. A linear dependence of CORTv vs. RRIv and of RESPv vs. RRIv was also found. Both dependences seem to be unconnected with the operation of α_1-adrenoceptor, β-adrenoceptor and cholinoceptor systems. It is suggested that both the cardiocortical and cardiorespiratory synchronizations studied seem to be mediated by β-adrenoceptor mechanisms.

Keywords: Reptiles; Nonlinear analysis; Multivariate analysis; Variability time series; Autonomic nervous system; Pharmacological blockade; Telemetry

1. Introduction

Coordination between physiological systems is important for the steady functioning of organisms. A part of the vertebrates' brain particularly involved in coordination is the brainstem that coordinates and regulates the activities of somatomotor, peripheral visceral and central nervous systems (Langhorst et al., 1981, 1983; Lambertz et al., 2000). Within the diverse range of research regarding the dynamic functional interactions in the brainstem, studies of cardiorespiratory and cardiocortical interactions could contribute to a deeper understanding of the coordinated dynamic functioning of peripheral and central physiological systems. Temporal cardiorespiratory interactions ensure adequate supply of oxygen and adjust clearance of metabolic waste products. These blood flow and gas exchange adjustments are coordinated by the autonomic nervous system (ANS) according to the information provided by receptors in the cardiovascular and respiratory systems and in the brainstem (Langhorst et al., 1975; Grönlund et al., 1991). Studies in mammals on cardiocortical interactions have described a close connection between the subcortical sources of some EEG rhythms and the autonomic control of the beat-to-beat heart rate variability (HRV) while awake (Heikkilä et al., 1987; Troncoso et al., 1995), as well as while asleep (Berlad et al., 1993; Ako et al., 2003; Jurysta et al., 2003).

Cardiorespiratory interactions have long been known in reptiles (White and Ross, 1966; Huggins et al., 1970; Shelton and Burggren, 1976; Wang and Hicks, 1996; Taylor et al., 1999). Although ventilatory influence on the heart rate pattern

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has been described in some snakes (*Crotalus durissus*, Wang et al., 2001), the existence of respiratory sinus arrhythmia (RSA) in reptiles, such as it is understood in mammals, is nowadays subject of controversy. This debate mainly takes place in the field of the power spectral analysis of time series. Thus González and De Vera (1988) reported that in the lizard *Gallotia galloti* there were no oscillations in the heart rate pattern at frequencies associated with breathing, which was interpreted as non-existence of RSA. Porges et al. (2003) maintain that the existence of RSA in lizards is variable and correlates depending on whether the lizard is an active forager, in which case it does exhibit RSA, or a sit-and-wait predator, in which case it does not exhibit RSA. Recently, Campbell et al. (2006) have demonstrated in *Crotalus durissus terrificus* that this phenomenon occurs in snakes in a similar manner to that described for mammals.

With regard to cardiocortical interactions and their autonomic control in reptiles, as far as the authors know, no investigation, except for De Vera et al. (2000) has been performed. In that study, by using methods based on Pearson's product moment correlation, it was suggested that the linear relationship between HRV and EEG activity in lizards is independent of both α_1-adrenergic and cholinergic mechanisms and mediated by β-adrenoceptor mechanisms.

One way of determining the dynamic properties of physiological systems is to study the complex, chaotic-like signals that they generate when functioning. Because of the intrinsic nonlinearity of some physiological activities (Elbert et al., 1994), a set of time series analysis methods derived from both the information theory and the nonlinear dynamical system theory have been applied since the 1990s to study the nature and behaviour of different physiological systems (Kantz et al., 1988; Galka, 2000). In particular, nonlinear multivariate time series analysis has recently begun to be used in physiology – mainly in neurophysiology – to study the relationship between simultaneously recorded signals (Arnhold et al., 1999; Le Van Quyen et al., 1999; Quian Quiroga et al., 2002; De Vera et al., 2005; Pereda et al., 2005b; De La Cruz et al., 2007). The underlying idea is that the assessment of the interdependence between signals can give new insights into the function of the systems that produce them. Thus, the multivariate analysis approach studies the interaction between signals to understand how changes in this interaction might affect the performance of the systems (Pereda et al., 2005b). In this context, the degree of interdependence between signals, i.e. the degree of interaction between them, has been quantified through different synchronization nonlinear indices (Arnhold et al., 1999; Quian Quiroga et al., 2002). Furthermore, some of these indices are asymmetric measures, thereby presenting the great advantage over the linear methods of giving information about driver–response relationships between systems (Arnhold et al., 1999; Quian Quiroga et al., 2000; Pereda et al., 2005a,b).

This unquestionable advantage of the nonlinear over the linear measures, has encouraged the authors to investigate whether the cardiorespiratory and cardiocortical relationships, that have been previously identified in *G. galloti* using linear methods (González and De Vera, 1988; De Vera et al., 2000), show directionality; i.e. whether a given system drives or is driven by the other system. This would in principle enable us to establish cause-effect relationships between the cardiac and respiratory systems, and also between the cardiac and the cortical electrical systems. Moreover, the study of cause-effect relationships between the cardiac and respiratory systems would provide new insights into the RSA controversy in reptiles. In this research report, cardiac, cortical and respiratory activity time variations were studied through variability signals derived from direct electrocardiograms (ECG), electrocorticograms (ECoG), and respiratory signals. Cardiac variability was evaluated from the series of R–R intervals of the ECG, and cortical and respiratory activity time variations were evaluated from power-data signals extracted from both ECoG and respiratory signal segments obtained within each R–R interval, respectively. Finally, once the cause-effect relationships between pairs of systems had been determined, their possible mediation by the ANS was studied by means of pharmacological blockade of α_1, β and muscarinic acetylcholine receptors.

In short, the goals of the present investigation are: 1) the examination of relationships between cardio and cortical activity time variations, and between cardio and respiratory activity time variations in *G galloti* lizards by means of the application of multivariate nonlinear time series analysis methods, and 2) to elucidate the role of the ANS in the mediation of the interactions found between the physiological systems involved.

2. Materials and methods

2.1. Animals

Fifty six lizards of the species *Gallotia galloti*, 11.0–14.2 cm in length (snout-vent), and 68.4–81.6 g (mean 73.1±3.4 s.d.) body mass from the island of Tenerife (Canary Islands, Spain) were used. The lizards were captured in their natural habitat and kept in terraria under a 12-h light (08:00–20:00 h; 23±1 °C ambient temperature)–12-h dark (20:00–08:00 h; 21±1 °C ambient temperature) cycle for at least 15 days before experiments. Water and food were available *ad libitum*. The Ethical Committee of the University of La Laguna approved all animal procedures described below.

2.2. Surgery

The experimental animals were anesthetized (ketamine hydrochloride–xylazine hydrochloride solution: 80 mg/kg–12 mg/kg, intramuscular; Sigma-Aldrich Co, Madrid, Spain) and electrodes were implanted using aseptic techniques, to simultaneously record continuous ECoG and ECG activity. Moreover, a thermistor placed outside a nasal opening was also used to simultaneously record respiratory activity.

One 3.5 mm long stainless steel Teflon coated electrode of 0.5 mm diameter, was used as active electrode to record monopolar ECoG. The tip of this electrode was stereotaxically implanted – through a hole drilled in the skull – so that it made contact with the surface of the left medial cortex. In addition,

one 4 mm long stainless steel screw of 1.15 mm diameter was secured to the left parietal bone outside the brain cavity, and served as reference electrode. The two chronically implanted ECoG electrodes – active and reference – were glued to the skull with acrylic cement.

The ECG was recorded by one 6 mm long stainless steel rolled wire electrode inserted subcutaneously in the dorsal lumbar region. The above mentioned stainless steel screw secured to the left parietal bone outside the brain cavity, which served as ECoG reference electrode, was also used as ECG reference electrode.

Respiratory activity was recorded by means of a small thermistor (RS 151-142; Amidata SA, Madrid, Spain) glued to the maxilla, in such a way that its tip was partially blocking the air flow through the left nasal opening. By means of this temperature-sensing element, it is possible to obtain a respiratory activity signal (RESP) in lizards, as nasal air flow changes, which occur in parallel with respiration, generate corresponding temperature changes in the air in the vicinity of the thermistor tip. A miniaturized Wheatstone bridge (Servicio de Electrónica, University of La Laguna, Spain) converted the temperature changes recorded by the thermistor to voltage variations. The reliability of this technique for recording the lizards' respiratory activity was satisfactorily proven by simultaneously recording and comparing the electromyogram of the intercostals muscles (De Vera and González, 1986; De Vera et al., 2005) with the RESP.

The cables coming from the ECG and ECoG electrodes and from the Wheatstone bridge were led to a transmitter, which was a part of a telemetry and data acquisition system for the measurement of biopotentials (described below). Mean duration of surgery was about 60 min. After surgery, each lizard was housed individually in a temperature-controlled terrarium (25±1 °C) and allowed to recover and get acclimatized for 7 days before recordings began. No postoperative drugs were used.

2.3. Experimental protocol

ECG, ECoG and RESP recordings were carried out in a noiseless thermostatically controlled (25±1 °C) chamber. The chamber was under a 12-h light (08:00–20:00 h)–12-h dark (20:00–08:00 h) cycle. Lizards were free of any restraint and therefore could move freely in their cages. The experiment consisted of a 5-h baseline recording session (under intraperitoneal injection of physiological saline — control group) and followed by a 5-h recording session under ANS blockade. The ANS blockers used were prazosin (selective α_1-adrenoceptor antagonist; 3 mg/kg; $N=18$), propranolol (β-adrenoceptor antagonist; 4 mg/kg; $N=20$) and atropine (non-selective muscarinic receptor antagonist; 3 mg/kg; $N=18$). All drugs (purchased from Sigma Chemical Co., St. Louis, MO, USA) were dissolved in saline and administrated by means of a 2-ml/kg bolus injected intraperitoneally. The effectiveness of blockade with prazosin, propranolol and atropine was verified by measuring cardiovascular responses to the agonists phenylephrine (3 μg/kg, Sigma Chemical Co., St. Louis, MO, USA), isoprenaline (1 μg/kg, Sigma Chemical Co., St. Louis, MO, USA) and methacholine (0.1 μg/kg, Sigma Chemical Co., St. Louis, MO, USA), respectively. Only the lizards in which the responses were abolish by the blocking drugs were accepted for the study.

Animal behaviour was continuously monitored by three CCD cameras (CE-7860C, Japan), provided by auto iris TV lenses (COMPUTAR, HG1214AFCS-3, Japan) that completely scanned the recording chamber. The output of the cameras was sent to the same computer that controlled the data acquisition system (see below). This experimental set up allowed the observer to select for analysis only the periods in which the lizard was at rest and with open eyes.

2.4. Measurements

ECG, ECoG and RESP signals (Fig. 1, left) were continuously and simultaneously measured by a telemetry and data acquisition system (Data Sciences International, St. Paul, MN, USA). The system consists of four main elements: transmitter, receiver, data exchange matrix, and data acquisition software. The transmitter (TL10M3-F50-EEE) measures the three biopotentials that are the object of study: ECG, ECoG and RESP signals. Due to its relatively large size (4 cm), this device is neither intraperitoneally nor subcutaneously implanted into the lizards, but dorsally attached by means of a piece of Velcro® (a tape glued to the flat side of the transmitter, and the other one silk-sutured to the skin). The receiver (RPC-1) detects the signals from the transmitter and is placed under the animal's cage. The data exchange matrix (DEM), among other functions, converts the telemetered digital signal from the receiver to binary words for communication with the acquisition program. The data acquisition software (Dataquest A.R.T. 2.3 Gold) detects, collects (samples) and analyzes the data signals sent to the computer from the receiver via the DEM.

The ECoG and RESP signals were recorded using a 30-Hz low-pass filter and were sampled at a frequency of 500 Hz. The ECG signal was recorded using a 10-Hz low-pass filter and was also sampled at a frequency of 500 Hz.

2.5. Signal analysis

The series of R–R consecutive intervals (RRI) from the ECG was calculated by means of the data acquisition software of the telemetry system. Simultaneous ECoG and RESP signal segments, whose length matched the corresponding durations of the successive RRI, were then obtained. Therefore, an ECoG signal segment and a RESP signal segment of the same duration (period) as the corresponding RRI were simultaneously obtained for each RRI.

Each of the individual segments extracted from the ECoG signal was spectrally analyzed using a FFT algorithm to obtain its power spectral density function (PSD). In order to perform this particular analysis, each signal segment was first linear-trend removed by means of the least squares fit, and cosine tapered over the first and last 10% of the samples to reduce leakage in the spectrum. Each ECoG spectrum was then analyzed by calculating the cumulative PSD in its very low frequency band (VLF: from the fundamental frequency to 7.5 Hz), because this is the band

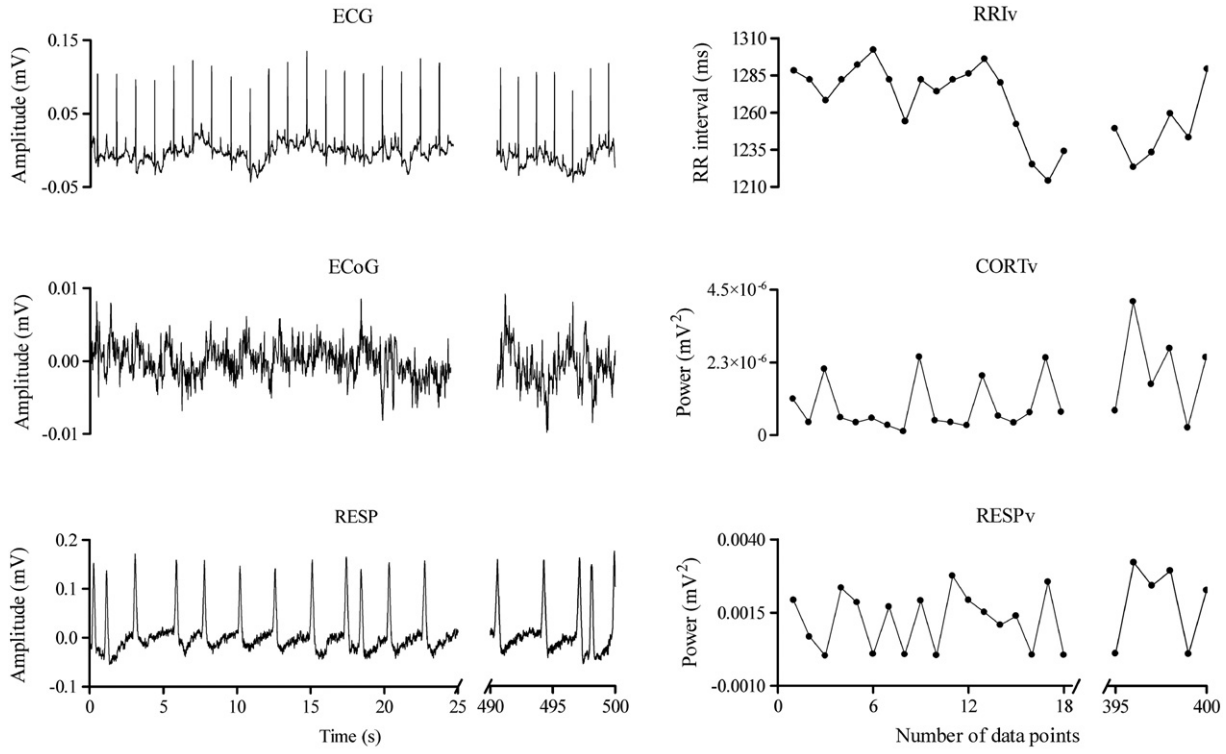

Fig. 1. An example of electrocardiographic (ECG), electrocorticographic (ECoG), and respiratory (RESP) signals (left) and the corresponding variability signals derived from them (right) from a 76.4-g male lizard in control condition. RRIv: R–R interval variability signal; CORTv: power-data variability signal built from the power spectral density values in the low frequency band of the ECoG spectra within each R–R interval; RESPv: power-data variability signal derived from the power values of the RESP signal within each R–R interval.

where most of the power is concentrated (Romo et al., 1978; Karmanova, 1982; Gaztelu et al., 1991; De Vera et al., 1994). Consequently, for each ECoG signal segment, one PSD value in the low frequency band was obtained. Similarly, each of the segments taken from the RESP signal was analyzed by calculating its variance — a measure that is equivalent to the total spectral power of any signal segment (Lynn, 1979). Thus, for each RESP signal segment, one variance (power) value was obtained. At the end of this procedure, it was possible to construct three simultaneous variability signals derived from the ECG, ECoG and RESP recordings: a RRI variability signal (RRIv), a power-data variability signal resulting from the PSD values in the VLF band of the ECoG spectra (CORTv), and a power-data variability signal derived from the power values of the RESP signal segments (RESPv) (Fig. 1, right). Each of the three simultaneous variability signals was formed by 400 data points: 400 RRI durations, 400 PSD values in the VLF band of the ECoG spectra, and 400 power values from the RESP signal. Logically, the time periods equivalent to these 400 data points were variable, because the RRI durations were also variable either in control condition or under prazosin, propranolol and atropine, respectively. The obtained CORTv and RESPv power-data signals reflect the functioning variability of the central nervous and respiratory systems within each cardiac cycle, respectively. Three variability signals – recordings – from each experimental animal were selected for analysis. Therefore 54, 60 and 54 recordings were analyzed in the control-prazosin, control-propranolol and control-atropine experiments, respectively. Nonstationary variability signals were detected and removed from analysis using methods suggested by Schreiber (1997). Selected signals were finally trend removed and normalized to zero mean and unit variance.

2.6. Nonlinear interdependence

Multivariate nonlinear analysis techniques were used to study the interaction between RRIv and CORTv and between RRIv and RESPv.

The mutual dependence between two signals X and Y coming from interacting nonidentical systems can be quantified starting from the concept of *generalized synchronization*: a state in which a functional dependence between the systems exists (Rulkov et al., 1995; Pikovsky et al., 2001; Pereda et al., 2005a). The first step consists of reconstructing the state spaces of each signal by means of time-delay embedding (Takens, 1980; Arnhold et al., 1999; Pereda et al., 2005b). The nth reconstructed state vectors are obtained as

$$\vec{x}_n = (x(n), x(n-\Delta\tau), ..., x(n-(m-1)\Delta\tau)$$

and (1)

$$\vec{y}_n = (y(n), y(n-\Delta\tau), ..., y(n-(m-1)\Delta\tau), \quad n=1, ..., N'$$

where N' is the total number of vectors, $\Delta\tau$ is the delay time — chosen as the first minimum of the mutual information function — and m is the embedding dimension — chosen as the dimension for which the percentage of false nearest

neighbours was lower than 5% (Pereda et al., 2003; Stam, 2005). The calculation of the interdependence between the two reconstructed state spaces is then carried out assessing the statistical dependence of the state-space structure of X on that of Y and *vice versa* (Quian Quiroga et al., 2000; Quian Quiroga et al., 2002). In order to do this, the set of nearest neighbours of each reconstructed vector $\overrightarrow{x_n}$ is compared with its set of mutual or conditioned neighbours, which are the vectors in its state-space that bear the same time indices as the nearest neighbours of $\overrightarrow{y_n}$. The difference between both sets of vectors is then quantified by using the mean squared Euclidean distance to calculate the index N, defined as:

$$N^{(k)}(X|Y) = \frac{1}{N'}\sum_{n=1}^{N'} \frac{R_n(X) - R_n^{(k)}(X|Y)}{R_n(X)} \quad (2)$$

where N' is the total number of delayed vectors such as Eq. (1), $R_n(X)$ is the average squared radius of the $\overrightarrow{x_n}$ points cloud, and $R_n^{(k)}(X|Y)$ stands for the average squared Euclidean distance from $\overrightarrow{x_n}$ to its k mutual neighbours. The mutual neighbours of $\overrightarrow{x_n}$ are those state vectors of X bearing the same time indices of the nearest neighbours of $\overrightarrow{y_n}$. In the presence of synchronization they are closer to the reference vectors than the average (Rulkov et al., 1995), so that the index is positive, and, the greater the synchronization, the greater its value. If the signals are independent, $R_n^{(k)}(X|Y) \approx R_n(X)$ and the index is close to zero. The index N is sensitive to both linear and nonlinear interdependencies, and has shown to be robust against noise and relatively insensitive to the complexity of the signals (Quian Quiroga et al., 2002). As Eq. (2) is normally asymmetric (i.e. in general, $N^{(k)}(X|Y) \neq N^{(k)}(Y|X)$), the index N can also be used to detect asymmetric couplings between signals. In fact, when $N^{(k)}(X|Y) > N^{(k)}(Y|X)$, this indicates that X depends more on Y (i.e. Y influences more on X) than *vice versa*; thus, the system that generates X is considered as the *driven response* system, while Y is considered as the autonomous *driver* system (Quian Quiroga et al., 2000; Quian Quiroga et al., 2002). In the present study $m=8$, $\Delta\tau=3$, and $k=9$ was taken for the three signals, according to previous investigations (De Vera et al., 2005).

2.7. Surrogate analysis

The statistical significance and nature – linear or nonlinear – of the interdependence was determined by using a variant of the surrogate data method proposed by Prichard and Theiler (1994). In this variant (Pereda et al., 2001), the index N between the signals X and Y is compared with those obtained between the original X and n surrogate versions of Y ($n=19$; 95% of significance level). These surrogate versions share all its properties with Y, but are completely independent from X. Surrogate signals were obtained according to the iterative amplitude adapted Fourier transform algorithm (IAAFT; Schreiber, 1998) by means of the TISEAN package (Hegger et al., 1999).

The Z-score obtained from the ratio of the difference between the original index and the average for the ensembles of surrogates to the s.d. for this ensemble is designated as *sigma univariate* (σ_u). A $\sigma_u \geq 2$ indicates statistically significant interdependence between the signals at the 95% level of confidence (Schreiber and Schmitz, 2000). Only recordings exhibiting a $\sigma_u \geq 2$ were included in the present study. Recordings exhibiting a $\sigma_u < 2$ were taken as having an index N equal to zero.

To assess the nature of the interdependence, the value of the index N for the original pair of signals was compared with n $X-Y$ surrogate pairs ($n=19$; 95% of significance level), where the linear dependencies between the signals were conserved, and the non-linear ones were removed. The ratio of the difference between the original index and the average for the ensembles of surrogates to the s.d. for this ensemble is designated as *sigma bivariate* (σ_b). A $\sigma_b < 2$ indicates linear nature of the interdependence between signals at the 95% level of confidence, whereas a $\sigma_b > 2$ indicates nonlinear nature of the interdependence between signals at the 95% level of confidence (Andrzejak et al., 2003).

Table 1
Index N of interdependence between RRIv and CORTv signals and between RRIv and RESPv signals

	N (RRIv vs. CORTv)	N (CORTv vs. RRIv)	P^1	N (RRIv vs. RESPv)	N (RESPv vs. RRIv)	P^2
Control	0.045±0.099	0.113±0.210	0.016	0.062±0.089	0.050±0.099	0.268
Prazosin	0.040±0.066	0.107±0.201	0.016	0.073±0.110	0.075±0.143	0.966
P*	0.891	0.888		0.642	0.493	
N_r	39			25		
Control	0.031±0.060	0.118±0.198	0.000	0.079±0.105	0.056±0.118	0.430
Propranolol	0.013±0.015	0.083±0.138	0.000	0.029±0.029	0.085±0.132	0.046
P*	0.035	0.239		0.040	0.465	
N_r	50			28		
Control	0.033±0.055	0.084±0.149	0.013	0.110±0.180	0.084±0.150	0.166
Atropine	0.022±0.046	0.121±0.216	0.001	0.078±0.119	0.053±0.087	0.232
P*	0.285	0.324		0.269	0.187	
N_r	42			32		

Values are means±SD.
N_r: number of recordings analyzed after having eliminated the non-stationary ones.
P*: P-value [control−blockade]: vertical comparison
P^1: P-value [N(CORTv vs. RRIv)−N(RRIv vs. CORTv)]: horizontal comparison.
P^2: P-value [N(RRIv vs. RESPv)−N(RESPv vs. RRIv)]: horizontal comparison.

Table 2
Index σ_b for the interdependence between RRIv and CORTv and between RRIv and RESPv

	σ_b (RRIv vs. CORTv)	σ_b (CORTv vs. RRIv)	σ_b (RRIv vs. RESPv)	σ_b (RESPv vs. RRIv)
Control	3.9 (3.1–4.7)	1.3 (1.0–1.5)	2.9 (2.2–3.5)	1.3 (1.0–1.7)
Prazosin	3.1 (2.6–3.7)	1.4 (1.1–1.7)	2.6 (1.7–3.5)	1.4 (0.9–1.9)
N_r	39		25	
Control	3.2 (2.8–3.6)	1.4 (1.1–1.6)	2.3 (1.8–2.8)	1.8 (1.4–2.2)
Propranolol	4.0 (3.6–4.4)	1.1 (0.9–1.3)	3.2 (2.6–3.8)	1.3 (0.9–1.7)
N_r	50		28	
Control	3.2 (2.9–3.6)	1.0 (0.8–1.2)	2.8 (1.9–3.7)	1.5 (1.1–1.9)
Atropine	2.7 (2.4–3.1)	1.4 (1.1–1.6)	2.4 (1.8–2.9)	2.1 (1.7–2.5)
N_r	42		32	

Values are means; numbers in parentheses indicate −95% and +95% confidence limits of the means.
Values <2 indicate linear nature of the interdependence.
Values >2 indicate nonlinear nature of the interdependence
N_r: number of recordings analyzed after having eliminated the non-stationary ones.

2.8. Statistical analysis

The statistical analysis of the differences between the indices N in control condition and under a particular ANS blockade was performed using a t-test for dependent samples. Equally, a t-test for dependent samples was also used to assess the existence of asymmetry in the interdependence between pairs of signals – i.e. to compare $N(X|Y)$ with $N(Y|X)$ – in control condition and under each of the ANS blockades. Comparisons were considered to be statistically significant at $P<0.05$.

3. Results

3.1. Interdependence between RRIv and CORTv

The index N revealed significant dependence of RRIv vs. CORTv and of CORTv vs. RRIv (Table 1, first and second columns). The mean value of the index N(CORTv vs. RRIv) was greater than that of the index N(RRIv vs. CORTv) both in control condition and under each of the ANS blockades (Table 1, first to third columns, horizontal comparisons). The dependence of RRIv vs. CORTv was of nonlinear nature both in control and under all the ANS blockades (Table 2, first column). The dependence of CORTv vs. RRIv was of linear nature both in control and under all the ANS blockades (Table 2, second column).

β-Adrenergic blockade with propranolol significantly decreased the index N(RRIv vs. CORTv), whereas both prazosin and atropine did not alter it (Table 1, first column, vertical comparisons). None of the ANS blockades significantly modified the value of the index N(CORTv vs. RRIv) (Table 1, second column, vertical comparisons).

3.2. Interdependence between RRIv and RESPv

The index N showed significant dependence of RRIv vs. RESPv and of RESPv vs. RRIv (Table 1, fourth and fifth columns). There was no difference between the index N(RRIv vs. RESPv) and the index N(RESPv vs. RRIv) in control condition. However, under β-adrenergic blockade the index N (RESPv vs. RRIv) was significantly greater than the index N (RRIv vs. RESPv) (Table 1, fourth to sixth columns, horizontal comparisons). The dependence of RRIv vs. RESPv was of nonlinear nature both in control and under each of the ANS blockades (Table 2, third column). The dependence of RESPv vs. RRIv was of linear nature in control and under both α_1-adrenergic and β-adrenergic blockades. Parasympathetic blockade changed the nature of this dependence from linear to nonlinear (Table 2, fourth column).

β-Adrenergic blockade with propranolol significantly decreased the index N(RRIv vs. RESPv), whereas both prazosin and atropine did not alter it (Table 1, fourth column, vertical comparisons). None of the ANS blockades significantly modified the value of the index N(RESPv vs. RRIv) (Table 1, fifth column, vertical comparisons).

4. Discussion

Coordination of both the cardiocortical and the cardiorespiratory control in lizards has been examined in the present study using a nonlinear index N of interdependence. This index, based on the concept of mutual neighbours, works by assessing the statistical interdependence between the reconstructed state vectors of the signals coming from the functioning of the physiological systems involved.

The index N revealed a nonlinear dependence of RRIv on CORTv that appears to be unaffected by both α_1-adrenoceptor and cholinoceptor systems, but mediated by β-adrenoceptor mechanisms — as shown above, only propranolol decreased the index N(RRIv vs. CORTv). The index N also showed the existence of a linear dependence of CORTv on RRIv. This dependence, however, appears to be unconnected with α_1-adrenoceptor, β-adrenoceptor and cholinoceptor systems — as is shown above, none of the ANS blockers modified the index N (CORTv vs. RRIv). These results suggest the existence of a generalized synchronization between the system that generates the low-frequency oscillations in the lizards' ECoG, and the one that produces oscillations in their cardiac interval. By taking into account both the nature of the dependences and the mediation of the ANS, it can be suggested that this synchronization may be achieved by means of two complementary ways: one of nonlinear nature acting through β-

adrenoceptor mechanisms, and the other of linear nature acting without the ANS involvement. However, it was found that *N* (CORTv vs. RRIv) was greater than *N*(RRIv vs. CORTv) both in control condition and under each of the ANS blockades, what means that CORTv always depended more on RRIv than *vice versa*. This circumstance indicates that the interdependence found must be considered asymmetric strictly speaking. In the light of the dynamical systems theory, this asymmetry would suggest that the system that generates the low-frequency oscillations in the lizards' ECoG is linearly driven by the one that produces the oscillations in their cardiac interval. Obviously, this conclusion must be examined very cautiously. In effect, according to Pereda et al. (2005a) the influence of the complexity of the signal – its degree of freedom in a broad sense – on the calculation of indices based on the mutual neighbours concept is one of its major disadvantages (Pereda et al., 2001; Quian Quiroga et al., 2000; Schmitz, 2000) and, although the index *N* has been refined to reduce this dependence (Quian Quiroga et al., 2002), it is not possible to assure that it has completely disappeared. In the present study, the complexity of CORTv fully surpasses the complexity of RRIv, which is why the results indicating asymmetry in the interdependence between CORTv and RRIv could be misleading. This is why we think that it would be inappropriate, due to methodological considerations, to establish any relationship of cause-effect, directionality or predominance (driving-driven) between the systems involved.

Out of the two suggested complementary ways in which these systems can relate to each other, we think that the one of nonlinear nature acting through β-adrenoceptor mechanisms would be the predominant from an evolutionary point of view. This choice is based on two main facts. On the one hand, anatomical studies in mammals have shown that neurons from the pedunculopontine tegmental nucleus and from the lateral hypothalamic area provide both ascending projections to the cerebral cortex and descending multisynaptic projections to the stellate sympathetic outflow (Krout et al., 2003). As far as the authors know, similar studies have not been performed in reptiles. However, given that it is well known that sympathetic nerve fibres from the stellate ganglia innervate the atria and ventricles, as well as the sinoatrial node in reptiles (Morris and Nilsson, 1994; Taylor et al., 1999), it could be that analogous neural circuits connecting the rudimentary neocortex and/or thalamic nuclei with the cardiosympathetic system could already be existing in reptiles. On the other hand, a study from De Vera et al. (2000) showed that the correlation found between the RR intervals coefficient of variation and the mean power frequency of the EEG spectra of *G. galloti* disappeared under propranolol, but not after prazosin or atropine. It can then be suggested that a kind of generalized synchronization through β-adrenoceptor mechanisms could exists between the rudimentary neocortex, striatum, and/or thalamic nuclei – possible generators of the reptilian slow telencephalic electrical activity – and the lizards' heart. Obviously, other physiological and/or behavioural factors besides those discussed in the present study could also be involved in the relationships between these systems.

The index *N* revealed a nonlinear dependence of RRIv on RESPv. This dependence appears to be unconnected with α_1-adrenoceptor and cholinoceptor systems, but to be mediated by β-adrenoceptor mechanisms –as shown above, only propranolol decreased the index *N*(RRIv vs. RESPv). The index *N* also showed the existence of a linear dependence of RESPv on RRIv. However, this dependence appears to be unconnected with α_1-adrenoceptor, β-adrenoceptor and cholinoceptor systems — as shown above, none of the ANS blockers modified the index *N*(RESPv vs. RRIv). These results suggest the existence of a generalized synchronization between the system that generates the power oscillations in the lizards' respiratory signal and the system that produces oscillations in their cardiac interval. Bearing in mind both the nature of the dependences and the mediation of the ANS, one can suggest that this synchronization could be achieved by means of two complementary ways: one of nonlinear nature acting through β-adrenoceptor mechanisms, and the other of linear nature acting without the ANS intervention. These results clearly demonstrate the existence of an evident degree of cardiorespiratory synchrony in *G. galloti*, in agreement with what has been found in this respect in other species/groups of reptiles (White and Ross 1966; Burggren, 1975; Wang and Hicks, 1996; Taylor et al., 1999; Porges et al., 2003). Cardiorespiratory coordination in rats seems to depend on parasympathetic mechanisms instead (Pereda et al., 2005a).

Recent findings in rats using nonlinear synchronization indices (Pereda et al., 2005a) have shown an asymmetry in the cardiorespiratory coordination in basal state. This asymmetry, with the heart rate variability signal depending more on the respiratory signal than *vice versa*, was attributed to the well-known phenomenon of RSA. RSA in mammals is characterized by an increase in heart rate during the inspiratory phase of respiration, probably due to an inhibition of the activity of the vagal preganglionic neurons, in such a way that the inhibitory vagal tone on the heart is decreased. Asymmetry in the interdependence between RRIv and RESPv was not detected in the control condition in the present investigation. Therefore, it is not possible to state any directionality relationship in the lizards' cardiorespiratory coordination. In effect, non-existence of asymmetry in this interdependence means that there is a *bidirectional coupling* between the system that generates the power oscillations in the lizards' respiratory signal and the system that produces oscillations in their cardiac interval. Bidirectional coupling between systems means that there are not relationships of cause-effect, directionality or predominance (driving–driven) between them. This finding supports the suggestion from a previous investigation (González and De Vera, 1988), where it was established, by means of power spectral analysis, that the beat-to-beat heart rate variability in this lizard does not seem to be correlated with their ventilatory activity. Our results, supporting the absence of RSA in *G. galloti*, are consistent with neuroanatomical studies that have not identified a cardioinhibitory function for the nucleus ambiguus in the lizard *Varanus exanthematicus* (ten Donkelaar et al., 1987).

As for the effects of the ANS blockade, β-adrenergic blockade caused RESPv to depend more on RRIv than *vice versa*. This result suggests that the bidirectional coupling

between the system that generates the power oscillations in the lizards' respiratory signal and the one that produces oscillations in their cardiac interval, typical of the basal condition, is achieved by means of the β-adrenergic nervous system involvement, preventing the RRIv generator system from taking control of the cardiorespiratory coordination.

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