

## Nonlinearity and fractality in the variability of cardiac period in the lizard, *Gallotia galloti*: effects of autonomic blockade

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**De Vera L, Santana A, Gonzalez JJ.** Nonlinearity and fractality in the variability of cardiac period in the lizard, *Gallotia galloti*: effects of autonomic blockade. *Am J Physiol Regul Integr Comp Physiol* 295: R1282–R1289, 2008. First published August 6, 2008; doi:10.1152/ajpregu.90391.2008.—Both nonlinear and fractal properties of beat-to-beat R-R interval variability signal (RRV) of freely moving lizards (*Gallotia galloti*) were studied in baseline and under autonomic nervous system blockade. Nonlinear techniques allowed us to study the complexity, chaotic behavior, nonlinearity, stationarity, and regularity over time of RRV. Scaling behavior of RRV was studied by means of fractal techniques. The autonomic nervous system blockers used were atropine, propranolol, prazosin, and yohimbine. The nature of RRV was linear in baseline and under  $\beta$ -,  $\alpha_1$ - and  $\alpha_2$ -adrenoceptor blockades. Atropine changed the linear nature of RRV to nonlinear and increased its stationarity, regularity and fractality. Propranolol increased the complexity and chaotic behavior, and decreased the stationarity, regularity, and fractality of RRV. Both prazosin and yohimbine did not change any of the nonlinear and fractal properties of RRV. It is suggested that 1) the use of both nonlinear and fractal analysis is an appropriate approach for studying cardiac period variability in reptiles; 2) the cholinergic activity, which seems to make the  $\alpha_1$ -,  $\alpha_2$ - and  $\beta$ -adrenergic activity interaction unnecessary, determines the linear behavior in basal RRV; 3) fractality, as well as both RRV regularity and stationarity over time, may result from the balance between cholinergic and  $\beta$ -adrenergic activities opposing actions; 4)  $\beta$ -adrenergic activity may buffer both the complexity and chaotic behavior of RRV, and 5) neither the  $\alpha_1$ - nor the  $\alpha_2$ -adrenergic activity seem to be involved in the mediation of either nonlinear or fractal components of RRV.

autonomic nervous system; fractal analysis; nonlinear analysis; recurrence plot; telemetry

THE CLASSICAL APPROACH to identify the sources of heart rate variability (HRV) uses methods based on the linear systems theory. This approach quantifies time series by means of tools based mainly on the classical theory of correlation, autoregressive modeling, or the Fourier analysis (11, 35). These tools have proven, and continue to prove, useful in assessing short-term cardiovascular control in vertebrates (4, 5, 10, 11, 17, 34). However, as the linearity and homeostaticity of physiological systems began to be challenged during 1990s, new methods, based on system dynamics, have been trying to provide since then an alternative/complementary explanation to the complexity of cardiovascular signals. This modern approach uses methods derived from both fractal and nonlinear dynamics (chaos) theories. Fractal analysis deals with time series that present temporal self-similarity: the same features repeat themselves

on different measurement scales. Scaling behavior, which can be considered as a specific form of chaos, is present in cardiovascular variability signals of mammals, and it is thought to be modulated by the autonomic nervous system (ANS) (16, 20, 33, 49, 50). The aim of the nonlinear approach is to study the dynamical properties of a signal as well as how the system that produces the signal works. Nonlinear dynamics makes it possible to establish a connection between the measured signal and its source (1, 20). Physiological control systems appear to be better modeled under the nonlinear than under the linear paradigm. In effect, it is more plausible that the output of a physiological system is the result of the joint action of the variables involved in its dynamics, so that the influence of each variable will be mediated by the values of the others (nonlinear behavior) than of the action of each variable taken separately (linear behavior) (14, 20, 27). Moreover, under the linear model, the irregularity present in most physiological signals is simply disregarded or considered as coming from an external random source, while under the nonlinear model this irregularity is thought to reveal a complex behavior of the system, and it is treated in terms of what is known as chaos theory (12, 20). In addition, a major part of HRV in vertebrates, which is located in a very low frequency band of the power spectra, i.e., fish (7), reptiles (18), and mammals (17), has not been satisfactorily dealt with by the classical approach. These oscillations have been attributed in mammals to slow oscillators with overlapping time scales as well as to nonlinear behavior (21, 30).

Numerous studies, using both classical or modern methods of time series analysis together with pharmacological blockade, both in humans (21, 31, 32, 35) and other mammals (2, 5, 6, 19, 53), have shown that the analysis of HRV may provide information on the function of the ANS in cardiac control. Since many structural and functional features of organs are common to all vertebrates, physiological research in reptiles could provide an evolutionary perspective on the origin(s) and maintenance of the different physiological mechanisms. Moreover, reptiles have a morphologically and functionally less intricate brain than mammals, which is interpreted as less diversity in the functioning and tasks of neurotransmitter receptor systems; this makes them, therefore, an ideal model for studying system dynamics. Cardiac period variability in mammals arises from the action and interaction of mechanisms which act through neural, mechanical, vascular and humoral factors as well as others. To know the particularities of neuroautonomic factors, and how they are involved in determining

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the R-R interval variability signal (RRV) in reptiles, would thus provide a foundation from which the mammalian cardiac autonomic regulation is derived. However, we are not ultimately concerned with what happens in mammals, but concerned with whether some characteristics present in mammals were already present in reptiles. Nevertheless, few studies applying classical methods to investigate the role of the ANS in the short-term cardiac control have been performed on reptiles (4, 36, 40). In this respect, previous investigations using Fourier analysis and pharmacological blockade (8, 9), have shown that the R-R interval low-frequency oscillations in the lizard *Gallotia galloti* appear to be powered by both  $\alpha_1$ -adrenergic and cholinergic activities and buffered by  $\beta$ -adrenergic activity.

To the best of the authors' knowledge there are no reports in the literature on neuroautonomic mechanisms regulating cardiac period variability in reptiles using fractal and/or nonlinear techniques. The present study is based on the hypothesis that the nature of lizard cardiac period oscillations is both nonlinear and fractal and that this is mediated by the ANS. This hypothesis is founded on a simple argument based on the fields of both evolutionary and comparative physiology: if normal heart rhythms in mammals seem to exhibit fractality, nonlinearity, and chaotic dynamics (3, 16, 20, 26, 27, 33, 42), and these characteristics seem to be mediated by the ANS, as is nowadays generally accepted (2, 6, 19, 21, 25, 28, 31, 37, 49, 50), then, as Reptilia is the sister group of Mammalia in the phylogeny of tetrapod vertebrates, it could thus be that fractality, nonlinearity, and chaotic dynamics are also already present, and also mediated by the ANS, in normal heart rhythms of reptiles. Therefore, the aim of the present work is to confirm this hypothesis by 1) investigating the components of lizard beat-to-beat RRV by means of both nonlinear and fractal methods, and by 2) quantifying the effects of ANS blocking agents on both nonlinear and fractal indices extracted from lizard RRV.

## MATERIALS AND METHODS

### Animals

Forty-eight lizards of the species *G. galloti*, 10.3–13.8 cm in length (snout to vent), and 66.1–79.1 g (mean  $70.3 \pm 3.1$  SD) body mass from the island of Tenerife (Canary Islands, Spain) were used. The Ethical Committee of the University of La Laguna approved all animal procedures described below.

### Surgery and Experimental Protocol

The lizards were anesthetized (ketamine hydrochloride-xylazine hydrochloride solution: 80–12 mg/kg, intramuscular; Sigma-Aldrich, St. Louis, MO) and electrodes were implanted using aseptic techniques to record continuous ECG activity. The ECG was recorded by two 6-mm long stainless steel rolled wire electrodes inserted subcutaneously, one near the nuchal region and the other near the dorsal lumbar region. No postoperative drugs were used.

The cables coming from the ECG electrodes were connected to a transmitter, which was part of a telemetry and data acquisition system for the measurement of biopotentials (Data Sciences International, St. Paul, MN). The system consists of four main elements: transmitter (TL10M3-F50-EEE), receiver (RPC-1), data exchange matrix, and data acquisition software (Dataquest A.R.T. 2.3 Gold). The ECG signal was recorded using a 10 Hz low-pass filter and was sampled at a frequency of 500 Hz.

ECG recordings were carried out in a noiseless thermostatically controlled ( $25 \pm 1^\circ\text{C}$ ) chamber that was under a 12:12-h light-dark cycle. Lizards were free of any restraint and could therefore move freely in the chamber. The experiment consisted of a 5-h baseline recording session [under injection of physiological saline (control group)] followed by a 5-h recording session under ANS blockade. The ANS blockers used were atropine (nonselective muscarinic receptor antagonist; 3 mg/kg;  $n = 14$ ), propranolol ( $\beta$ -adrenoceptor antagonist; 3.5 mg/kg;  $n = 13$ ), prazosin (selective  $\alpha_1$ -adrenoceptor antagonist; 3 mg/kg;  $n = 12$ ), and yohimbine (selective  $\alpha_2$ -adrenoceptor antagonist; 1.5 mg/kg;  $n = 9$ ). All drugs (purchased from Sigma) were dissolved in saline and administrated by means of an intraperitoneally injected 2-ml/kg bolus. The effectiveness of blockade with atropine, propranolol, prazosin, and yohimbine was verified by measuring cardiac responses to the following agonists: methacholine (0.1  $\mu\text{g}/\text{kg}$ , Sigma), isoprenaline (1  $\mu\text{g}/\text{kg}$ , Sigma), phenylephrine (3  $\mu\text{g}/\text{kg}$ , Sigma), and clonidine (3  $\mu\text{g}/\text{kg}$ , Sigma), respectively. Only the lizards in which the responses were eliminated by the blocking drugs were accepted for the study.

### Signal Preprocessing

The series of consecutive R-R intervals from the ECG was calculated by means of the data acquisition software of the telemetry system. From each recording, the best segments of 1,000 consecutive R-R intervals were selected after checking for stationarity and lack of artifacts. Stationarity was checked as follows: a 1,000-point window was moved along the data recording; each 1,000-data point segment was divided into 10 sequences of 100 points; and the mean of each sequence was calculated. The segment with the lowest coefficient of variation in its mean was selected. The selected time series were linearly detrended through a least square fit and then normalized to zero mean and unit variance. At the end of this procedure, several RRV of 1,000 data points each were ready for nonlinear and fractal analysis.

### Nonlinear Techniques

**Signal complexity.** The complexity of RRV was calculated from the correlation integral (CI) of the signal by calculating its correlation dimension ( $D_2$ ). The CI of the signal was calculated by using Takens' theorem (43) adapted to deal with series of time intervals between relevant events (22). First, the procedure requires the reconstruction of the signal attractor in the state space of the system. For such reconstruction, it is necessary to calculate the time delay vectors, whose components are the consecutive values of the RRV, and the dimension of the space. The delay time ( $\tau$ ) ensuring lack of temporal correlation between successive components was assessed from the mutual information function of the RRV (1) as the greater of the first minimum of the signal (here,  $\tau = 14$  in control and under all pharmacological blockades except propranolol, where  $\tau = 4$ ). The minimal embedding dimension ( $m$ ) necessary for a proper reconstruction of the dynamics of RRV was computed by means of the false nearest neighbor method (22) on average with  $m = 5$  in control and under all pharmacological blockades. Once the attractor was reconstructed, the CI was computed by using the Grassberger-Procaccia algorithm modified by Theiler (45).  $D_2$  was calculated as the mean slope of the linear region of the plots  $\log \text{CI}$  vs. the logarithm of the intervector distance in the three highest embedding dimensions (from  $m = 5$  to  $m = 6$ ). The  $D_2$  index, which is, in fact, a measure of the degrees of freedom of the system, estimates the number of independent regulating mechanisms in a generating system, which is necessary to describe each point of the attractor. This index is considered a measure of the static behavior of the system generating the signal.

**Quantifying chaotic behavior.** A measure for characterizing the dynamic behavior of a system, making it possible to distinguish deterministic-chaotic behavior from noisy behavior caused by random external influences, is the largest Lyapunov exponent ( $\lambda_1$ ). This

quantity is a measure of the convergence or divergence of proximate trajectories in the state space, and gives an idea of the system's sensitive dependence on initial conditions. An increasing  $\lambda_1$  indicates a progressive divergence of adjacent trajectories in an attractor and, therefore, a decrease in the predictability of the system. In short, if  $\lambda_1 < 0$ , the system is highly predictable (stable system) and, if  $\lambda_1 > 0$ , the system is not predictable (chaotic system).  $\lambda_1$  was calculated using the algorithm proposed by Rosenstein et al. (38), once the signals were embedded in phase space states of dimension  $m = 5$ . The average evolution of the intervector distances ( $\Delta S$ ) was computed as a function of the evolving time ( $t$ ).  $\lambda_1$  was then calculated as the average slope of the plots  $\log \Delta S$  vs.  $t$  in each  $m$ .

**Testing for nonlinearity.** The magnitudes of both  $D_2$  and  $\lambda_1$  are not per se indicative of system nonlinearity. Consequently, the presence of nonlinear components in the RRV was tested by means of a variant of the surrogate data method proposed by Theiler et al. (46). A surrogate signal is a modified version of the original signal that shares all its linear properties but is devoid of coherent phase relationship. Surrogates of the original RRV were obtained by randomizing the phases of the fast Fourier transform spectrum and then reconstructing the signal by the inverse fast Fourier transform. This procedure was performed using the iterative amplitude-adapted Fourier transform algorithm (39) by means of the TISEAN package (23). The next step consisted of calculating the  $D_2$  of the original signals and that of their corresponding surrogates to compare them by using a  $t$ -test for dependent samples. Significant differences ( $P < 0.05$ ) between the  $D_2$  of the original signals and their surrogates indicate nonlinearity.

**Stationarity and regularity of the signal over time.** These characteristics were assessed by means of the recurrence quantification analysis. By using this analysis, it is possible to detect hidden patterns and structural changes in data and perceive similarities across the time series being studied. Recurrence quantification analysis, which is based on recurrence plots (13, 47, 51), allowed the quantitative evaluation of %recurrence (%RC, percentage of the plot occupied by recurrent points), %determinism (%DT, percentage of recurrent points that appear in sequence forming diagonal lines in the distance matrix), and the length of the longest diagonal line in the recurrence plot ( $L_{max}$ ). Both %RC and %DT are a measure of the stationarity and regularity of the signal over time (52), whereas  $L_{max}$  is inversely related to the  $\lambda_1$  (6, 52). Both %RC and %DT have particular physiological significance: on the one hand, embedded processes manifesting periodic dynamics have higher %RC values than other embedded processes characterized by aperiodic dynamics; on the other hand, deterministic dynamics repeat themselves. Recurrence plots were constructed using the Euclidean norm for intervector distances lower than the square root of  $m$ .

#### Fractal Techniques

Fractal characteristics of the signals were determined by means of the coarse-graining spectral analysis (48). This method allows the separation of both harmonic and nonharmonic (fractal) components of the total spectral power of the signal. This separation is possible because the fractal component is scale invariant, i.e., when rescaled, it will still retain its power when cross correlated with the original. Coarse-graining spectral analysis enables the calculation of the %fractal component in total RRV power (%FP) as well as an index referred to as fractal exponent ( $\beta$ ). This index can be obtained as the absolute value of the slope of  $\log$  frequency vs.  $\log$  fractal power plot by a least-square fit. The frequency range for calculating the exponent was selected in the range in which the spectra presented the clearest  $1/f^\beta$  dependence.

#### Statistical Analysis

The statistical analysis of the differences between the RRV parameters in control conditions and under a particular ANS blockade was performed using a  $t$ -test for dependent samples or a Wilcoxon

matched paired test when appropriate. Comparisons were considered to be statistically significant at  $P < 0.05$ .

## RESULTS

Characteristic three-dimensional phase-space plots (scatter-plots) of 1,000 consecutive R-R intervals each, in baseline and under the different ANS blockades are shown in Fig. 1. Besides the expected different range in the values of the R-R intervals, it is visually observed that the dispersion degree of the cloud of points clearly depends on the type of blockade performed.

Table 1 summarizes the results of both nonlinear and fractal analyses of RRV in baseline conditions.

The results from the surrogate data test for nonlinearity in control and under the different ANS blockades are shown in Table 2, in the fourth and fifth columns. One can see that in the control condition, and during propranolol, prazosin, and yohimbine there were no significant differences between the  $D_2$  of the original RRV vs. the surrogate RRV. These findings indicate that the nature of RRV was linear in baseline and under  $\beta$ -,  $\alpha_1$ - and  $\alpha_2$ -adrenoceptor blockades, respectively. Atropine however, changed the linear nature of RRV to nonlinear. On the other hand, as expected, cholinoreceptor and  $\alpha_1$ -adrenoceptor blockades induced tachycardia, whereas  $\beta$ -adrenoceptor blockade induced bradycardia in the lizards.  $\alpha_2$ -Adrenoceptor blockade induced tachycardia (Table 2, second column).

The effects of the different ANS blockades on both nonlinear and fractal indices of RRV are shown in Table 2, in the fourth and the sixth to eleventh columns. The control values of the R-R interval duration, as well as the ones corresponding to both nonlinear and fractal RRV indices did not differ in the four groups of lizards used. The only index that remained unchanged regardless of the ANS limb that was pharmacologically blocked was %FP. The remaining indices changed depending on the pharmacological intervention. Cholinoreceptor blockade increased %DT,  $L_{max}$ , and  $\beta$ , respectively, and did not change  $D_2$ ,  $\lambda_1$ , and %RC, respectively.  $\beta$ -Adrenoceptor blockade increased  $D_2$  and  $\lambda_1$ , respectively, and decreased %RC, %DT,  $L_{max}$ , and  $\beta$ , respectively. Both  $\alpha_1$ - and  $\alpha_2$ -adrenoceptor blockades did not change any of the RRV nonlinear and fractal indices.

## DISCUSSION

The control-resting R-R interval values reported for *G. galloti* in this research, and the R-R interval values under cholinoreceptor, and  $\alpha_1$ - and  $\beta$ -adrenoceptor blockades are in accordance with values obtained by the authors in previous studies (8, 9). As far as the authors know, the effect of the  $\alpha_2$ -adrenoceptor blockade with yohimbine on the R-R interval value in a reptilian species is reported here for the first time, which means there are no data from other reptiles for comparison. Yohimbine easily enters the central nervous system in humans producing an increase in heart rate and blood pressure (24). A recent pharmacological study using spectral analysis showed that yohimbine decreased HRV and spontaneous baroreflex sensitivity in mice (44). The tachycardia observed in *G. galloti* under yohimbine resembles that observed during prazosin administration. Tachycardia in lizards under prazosin has been attributed to a circulatory effort to compensate for the parallel decrease in blood pressure incurred by vasodilatation

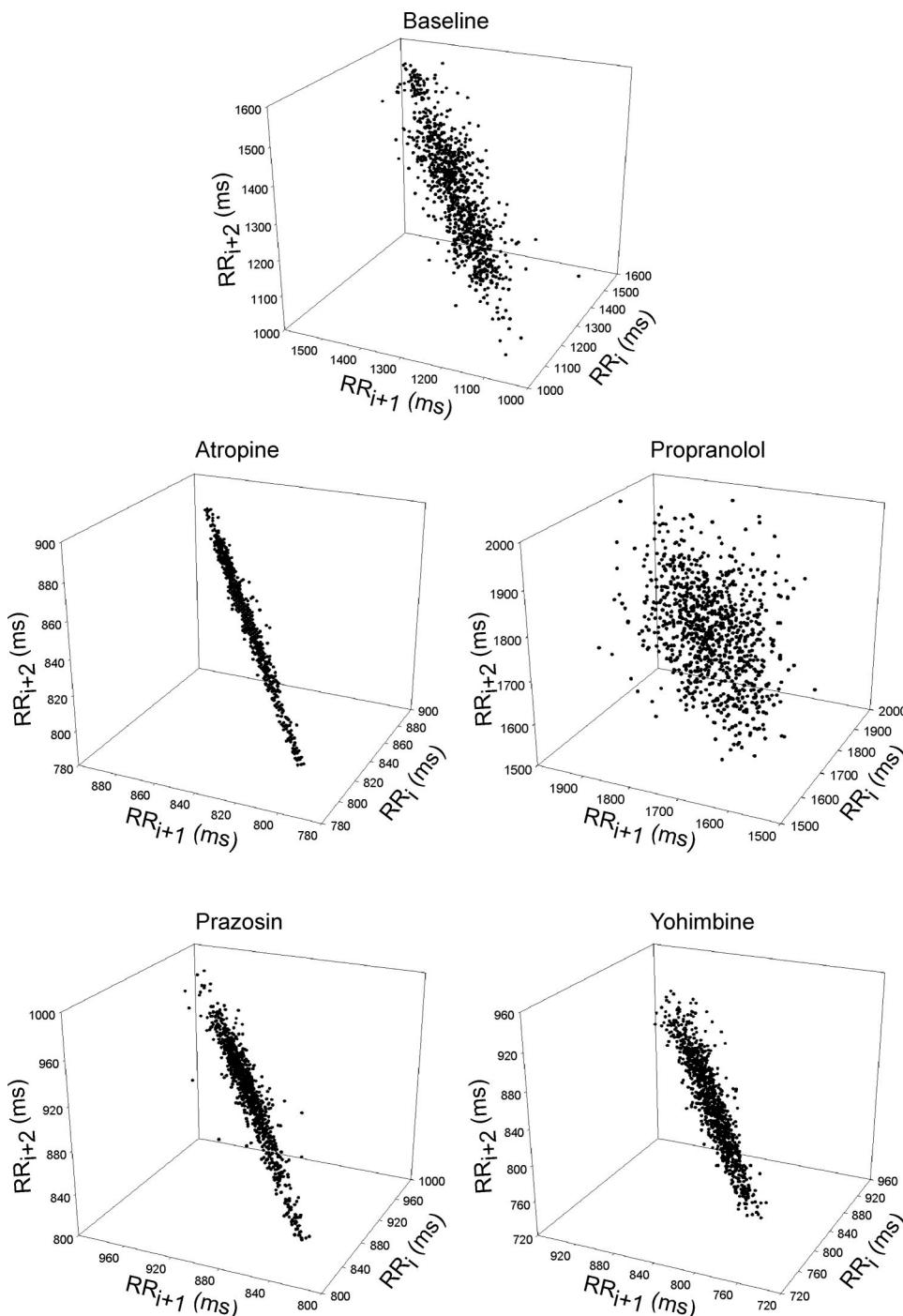


Fig. 1. Examples of three-dimensional phase-space plots of 1,000 consecutive beat-to-beat R-R intervals ( $RR_i$ ) in baseline (top, center) and under cholinoreceptor (middle, left),  $\beta$ -adrenoceptor (middle, right),  $\alpha_1$ -adrenoceptor (bottom, left), and  $\alpha_2$ -adrenoceptor (bottom, right) blockades.

of the resistance vessels (9). However, the lack of data on blood pressure in lizards under yohimbine prevents any further explanation about the mechanism(s) responsible for the observed tachycardia.

#### RRV Nonlinear and Fractal Indices in Baseline

The mean values of RRV nonlinear and fractal indices obtained in *G. galloti* are not directly comparable with the ones obtained in mammals, which is, to the authors knowledge, the only animal group where this kind of study has been carried out to date. Comparisons are extremely problematic because liz-

ards belong to a different animal group and because authors use different algorithms to calculate those indices from cardiovascular signals. However, in spite of this, some similarities with mammals have been found in this respect. Thus, the lizard's  $D_2$ , %RC, %DT, and  $L_{max}$  values are in line with values obtained in rats (19), and the lizard's %FP and  $\beta$  values agree with values reported in humans (49, 50). With regard to differences, by way of example,  $D_2$  values reported in humans (26, 30) and rabbits (54) are somewhat greater than the lizard values, while values reported in mice (28) and piglets (53) are slightly smaller than the lizard values. The  $\lambda_1$  was positive at

Table 1. Nonlinear and fractal indices of RRV in baseline

	RRV Baseline
RR	1271±263
D <sub>2</sub>	4.7±0.9
λ <sub>1</sub>	0.147±0.035
%RC	57±5
%DT	78±16
L <sub>max</sub>	537±308
%FP	84±3
β	1.21±0.43

Values are means ± SD; n = 48 lizards. RR, duration of R-R interval of ECG in ms; D<sub>2</sub>, correlation dimension; λ<sub>1</sub>, largest Lyapunov exponent in s<sup>-1</sup>; %RC, %recurrence; %DT, %determinism; L<sub>max</sub>, length of the longest diagonal line in the recurrence plot; %FP, %fractal component in total power spectra; and β, fractal exponent.

baseline and remained positive during the different autonomic blockades. This positive λ<sub>1</sub> indicates sensitive dependence on initial conditions and therefore loss of predictability, which is indicative of deterministic-chaotic behavior in all states of RRV in lizards. The lizard's λ<sub>1</sub> value was rather smaller than values reported for humans (21, 30), mice (28), piglets (53), and rabbits (54). The lizard resting RRV contained a high percentage of random fractal components; the remaining part was harmonic oscillations with characteristic frequencies previously studied (18). The mean value for β indicates the existence of 1/f noise in lizard RRV.

#### RRV Dynamics from a Nonlinear Technique Point of View

The results from the surrogate data test for nonlinearity suggest that the nature of RRV in lizards in the basal state is linear. This result strongly contrasts with those of several studies in mammals such as rats (19), rabbits (25, 54), piglets (53), and humans (3, 29, 41, 42) that support the existence of nonlinear components in their HRV in the basal state. It is currently thought that this nonlinearity could arise from the interaction of the parasympathetic and β-sympathetic activities in a typically nonlinear way to modulate the duration of the cardiac period (20). This conclusion is supported by studies that have reported a decrease of nonlinearity in the HRV after parasympathetic blockade in rabbits (25, 54), piglets (53), rats (19), and humans (42) and by others reporting a further decline in the nonlinear characteristics of HRV in β-adrenoceptor-blocked animals whose parasympathetic system had been previously blocked (53). In contrast, Porta et al. (37) have recently found that the short-term RRV is mostly linear in humans at

rest, and that the activation of the parasympathetic or sympathetic nervous systems does not produce nonlinear dynamics. In the opinion of these authors, the use of nonlinear models in short-term RRV is not supported by their data except under controlled respiration at slow respiratory rates. As mentioned above, the RRV in lizards in the basal state seems to be linear by nature. According to the linear model, the output of a physiological system results from the independent action of each of the variables that participate in its dynamics. This would imply that, in lizards in the basal state, the cholinergic, α<sub>1</sub>-, α<sub>2</sub>-, and β-adrenergic activities act separately, without interacting with each other when modulating the R-R interval durations. A possible explanation for this linear behavior is founded on the fact that cardiac period oscillations in *G. galloti* are mainly mediated by cholinergic activity as previous studies have shown (8, 9). The role of the vagal activity in RRV modulation is so preponderant in this animal that the adrenergic activities, which are also involved in RRV modulation, although with a much lower participation, would not need to consequently exchange information between one another, and even much less so with vagal activity, to accomplish their comparatively simpler task. Moreover, from an evolutionary point of view, this linear behavior could indicate that an exchange of information among the studied components of the neuroautonomic mechanisms regulating the lizards' cardiac period is not necessary to deal with the dynamics that could be involved in their life style. Mammals, which have to cope with more sophisticated dynamics typical of more evolved stages, seem to demand, on the contrary, an exchange of information among the components of their neuroautonomic network. The necessity for exchanging information between the components of physiological systems in vertebrate evolution, probably secures a successful coordination and functioning of an increasing number of highly sensitive organ systems. On the other hand, nonlinearity in the HRV of mammals has also been associated with the presence of a respiratory component in the signal that is mainly mediated by the parasympathetic nervous system in rats (19) and humans (15, 26, 29). In agreement with this argument, the absence of respiratory sinus arrhythmia in *G. galloti* that we have suggested in previous studies (10, 18), would also contribute in parallel to the absence of nonlinearity in lizard RRV in the basal state.

Given that a nonlinear dynamic is only established in RRV after cholinergic blockade, it must be inferred that, in the absence of vagal activity, the α<sub>1</sub>-, α<sub>2</sub>-, and β-adrenergic activities interact with each other, which is how inputs to the

Table 2. Nonlinear and fractal indices of RRV in control and under the different autonomic nervous system blockades

No.	RR	D <sub>2</sub>		λ <sub>1</sub>	%RC	%DT	L <sub>max</sub>	%FP	β	
		Original	Surrogate							
Control	14	1344±261	4.6±0.6	5.0±1.0	0.151±0.026	59±7	77±15	539±322	84±5	1.19±0.50
Atropine	14	1112±236†	4.3±1.0	5.0±1.0§	0.153±0.023	59±3	92±7†	824±191†	84±8	1.87±0.47‡
Control	13	1334±153	4.5±0.9	4.4±0.7	0.131±0.033	58±4	80±17	581±315	85±9	1.17±0.46
Propranolol	13	1899±346‡	5.3±0.6*	5.0±0.6	0.162±0.008†	53±2*	34±11‡	118±78‡	83±5	0.38±0.29‡
Control	12	1196±187	5.0±0.5	4.9±0.9	0.164±0.023	57±5	76±13	521±290	82±6	1.23±0.32
Prazosin	12	935±231‡	4.5±1.1	4.7±0.6	0.156±0.030	59±5	84±13	650±224	85±8	1.27±0.38
Control	9	1284±360	4.5±1.7	4.4±1.5	0.137±0.053	57±5	80±20	528±328	84±11	1.24±0.46
Yohimbine	9	938±268†	3.4±1.5	3.9±1.0	0.122±0.062	60±5	91±7	616±182	82±10	1.51±0.49

Values are means ± SD. No., number of lizards. Control-blockade statistical significance, vertical comparison: \*P < 0.05, †P < 0.01, ‡P < 0.001. Surrogate data test for nonlinearity, original-surrogate statistical significance, horizontal comparison, §P < 0.01.

sinoatrial node will behave under a nonlinear dynamics. A possible explanation for this behavior could be that, under this circumstance of absence of vagal activity, the role of each of the studied adrenergic activities in RRV modulation acquires such importance that they must interact with each other to exchange information, to accomplish their now comparatively more complex task. It could, therefore, be said that, because of its preponderant role in RRV modulation, parasympathetic activity makes the  $\alpha_1$ -,  $\alpha_2$ -, and  $\beta$ -adrenergic activity interaction unnecessary when modulating RRV. In other words, the parasympathetic role seems to consist of keeping a linear status in RRV modulation by means of the cardiac neuroreceptors involved. As the RRV behaves in such a linear way, the suitability of nonlinear techniques of providing new information about the cardiac period control system in lizards could be questioned. In this respect, although a physiological system seems to have a linear behavior, its study by means of nonlinear methods can help not only to confirm its linearity but also to observe possible deviations from this linearity. In effect, nonlinear analysis methods based on the values of both  $D_2$  and  $\lambda_1$ , together with the surrogate data test may predict not only a linear behavior in a given system, but also provide a more detailed description of its underlying dynamics than the linear methods do. The rationale for this is that linear traditional methods characterize a state of a given system by averaged quantities like mean values and/or variances, among others, while the nonlinear ones try to characterize it by a whole set of quantities like complexity, predictability, and/or scaling exponents, among others (27).

#### *Effects of Cholinceptor Blockade*

Cholinceptor blockade did not alter the complexity or the chaotic behavior of the RRV. These results suggest that both the complexity and chaotic behavior of lizards' RRV are not mediated by cholinergic activity. The comparison of our results with the ones obtained in mammals under atropine entail a certain difficulty because there is not a general consistency regarding this in this animal group. The findings, to give an example, differ as follows: no alteration of  $D_2$  in humans (30); decrease of  $D_2$  in rats (19), rabbits (54), piglets (53), and humans (31); increase of  $D_2$  in mice (28); no alteration of  $\lambda_1$  in rabbits (54) and humans (30); decrease of  $\lambda_1$  in rats (2) and piglets (53), and increase of  $\lambda_1$  in mice (28). Besides the disagreement among species, it is however, currently accepted that in most mammals both the deterministic-chaotic behavior and complexity of HRV are vagally mediated. It is clear from the data above that this is not the case in lizards, where cholinergic activity does not seem to play any role in modulating either the complexity or the chaotic behavior of RRV. Cholinceptor blockade increased the stationarity and regularity of lizard RRV over time. This result, which agrees with findings in rats, where an increase in %RC, %DT, and  $L_{max}$  has been reported during atropine (6, 19), suggests that the parasympathetic system may buffer both the stationarity and regularity of lizard RRV. Fractality also increased under atropine. This result agrees with findings in humans (50), and proves that the fractal components of RRV in lizards are buffered via cardiac parasympathetic neural activity.

#### *Effects of $\beta$ -Adrenoceptor Blockade*

As opposed to cholinceptor blockade,  $\beta$ -adrenoceptor blockade increased both the complexity and chaotic behavior of RRV. This result indicates that propranolol enhances both the number of degrees of freedom as well as the unpredictability of the system that controls the RRV. Therefore, it could be suggested that the  $\beta$ -adrenergic activity may buffer both the complexity and chaotic behavior of lizard RRV. Instead, most findings in mammals show no alteration of either  $D_2$  [rats (19), rabbits (54), piglets (53)] or  $\lambda_1$  [rats (2), rabbits (54), piglets (53), humans (21)] after  $\beta$ -adrenoceptor blockade with propranolol. Also, in contrast to cholinceptor blockade,  $\beta$ -adrenoceptor blockade decreased both the stationarity and regularity of lizard RRV over time. A similar effect of propranolol on %RC, %DT, and  $L_{max}$  has been found in rat RRV (19). Lizard data suggest that the  $\beta$ -adrenergic activity, in contrast to cardiac parasympathetic neural activity, may enhance both the stationarity and regularity of RRV over time. Fractality also decreased under propranolol. This result does not agree with findings in humans, where the  $\beta$ -adrenergic activity seems to play a minor (49) or no role (21) in modulating the fractal HRV dynamics. Lizard data show that fractal components of RRV are enhanced via cardiac  $\beta$ -sympathetic neural activity.

#### *Effects of $\alpha_1$ - and $\alpha_2$ -Adrenoceptor Blockades*

Both  $\alpha_1$ - and  $\alpha_2$ -adrenoceptor blockades did not alter any of the measures regarding either the nonlinear or fractal structure of RRV in lizards. These results suggest that both  $\alpha_1$ - and  $\alpha_2$ -adrenergic activities do not appear to be involved in the generation of either nonlinear or fractal components of lizard RRV. In this respect, a certain parallelism may be established between reptiles and mammals about the noninvolvement of the  $\alpha_1$ -adrenergic activity in the generation of some nonlinear components of RRV, because prazosin did not change the recurrence quantification analysis indices in either rats (6, 19) or in lizards.

#### *Conclusions*

In summary, it is suggested that in lizards: 1) the RRV in the basal state is linear by nature possibly due to the fact that the cholinergic activity seems to make the interaction between  $\alpha_1$ -,  $\alpha_2$ -, and  $\beta$ -adrenergic activities unnecessary; 2) fractality, as well as both RRV regularity and stationarity over time, may result from the balance between the opposing actions of cholinergic and  $\beta$ -adrenergic activities; 3)  $\beta$ -adrenergic activity may buffer both the complexity and chaotic behavior of RRV, and 4) neither the  $\alpha_1$ - nor the  $\alpha_2$ -adrenergic activities seems to be involved in the mediation of either the nonlinear or fractal components of RRV.

#### *Perspectives and Significance*

The present study provides evidence that both nonlinear and fractal analysis are a valid approach for studying the control of cardiac period in reptiles. These techniques complement the traditional spectral and time-domain analysis and are able to provide an alternative explanation to the complexity of cardiac signals. Comparison with results from other reptilian species/groups was not possible because, to the authors' knowledge, to date there are no studies on reptiles about the application of

these modern methods to investigate their cardiac period variability.

Multivariate nonlinear analysis applied to simultaneous recordings of beat-to-beat R-R intervals and arterial blood pressure could be of great interest to assess short-term cardiovascular control in this species, because the mechanisms involved in cardiovascular regulation could very likely interact with each other in a nonlinear way. To this effect, the assessment of interdependence between heart rate and arterial blood pressure by means of nonlinear tools based on the concepts of both phase synchronization and joint state space (20) could be of use for understanding the complexity of the cardiovascular dynamics of reptiles.

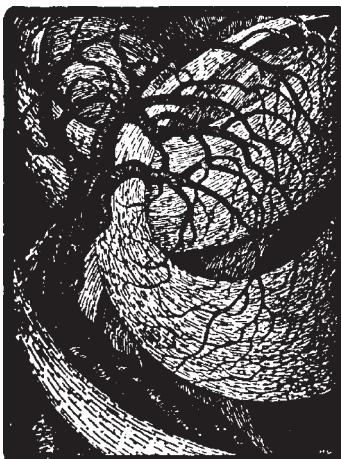
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