

Nonlinear, fractal, and spectral analysis of the EEG of lizard, *Gallotia galloti*

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González, Julián, Antoni Gamundi, Rubén Rial, M. Cristina Nicolau, Luis De Vera, and Ernesto Pereda. Nonlinear, fractal, and spectral analysis of the EEG of lizard, *Gallotia galloti*. *Am. J. Physiol.* 277 (Regulatory Integrative Comp. Physiol. 46): R86–R93, 1999.—Electroencephalogram (EEG) from dorsal cortex of lizard *Gallotia galloti* was analyzed at different temperatures to test the presence of fractal or nonlinear structure during open (OE) and closed eyes (CE), with the aim of comparing these results with those reported for human slow-wave sleep (SWS). Two nonlinear parameters characterizing EEG complexity [correlation dimension (D2)] and predictability [largest Lyapunov exponent (λ_1)] were calculated, and EEG spectrum and fractal exponent β were determined via coarse graining spectral analysis. At 25°C, evidence of nonlinear structure was obtained by the surrogate data test, with EEG phase space structure suggesting the presence of deterministic chaos (D2 \sim 6, $\lambda_1 \sim$ 1.5). Both nonlinear parameters were greater in OE than in CE and for the right hemisphere in both situations. At 35°C the evidence of nonlinearity was not conclusive and differences between states disappeared, whereas interhemispheric differences remained for λ_1 . Harmonic power always increased with temperature within the band 8–30 Hz, but only with OE within the band 0.3–7.5 Hz. Qualitative similarities found between lizard and human SWS EEG support the hypothesis that reptilian waking could evolve into mammalian SWS.

correlation dimension; largest Lyapunov exponent; fractal exponent; harmonic power; reptile telenchephalic activity

EVOLUTIONARY STUDIES searching for the origin of mammalian and avian sleep using reptiles as experimental animals proliferated in the sixties and in the seventies. Unfortunately, the results were discouraging, because neither slow-wave sleep (SWS) nor rapid eye movement sleep (REMS) was unquestionably found in these animals. However, recent works (27) have demonstrated unequivocal REMS in the platypus, a primitive mammal, although it showed rather deviant characteristics from the well-known REMS of mammals and birds. After this result, it seems compulsory to analyze reptilian neurophysiology, trying to find the minimum set of characteristics needed to define both SWS and REMS. Using signal analysis techniques (10), our group showed that the reptilian electroencephalogram (EEG) exhibited slow-wave spindles and high-voltage spikes that could appear both spontaneously and after sensory

stimulation, a set of traits very similar to those found in mammals during SWS. This evidence was used to support the idea that the reptilian waking, being mainly due to the activity of brain stem structures, could evolve into the mammalian SWS. As a result, the true evolutionary acquisition of mammals would not be sleep, but cortical wakefulness achieved after the development of multisensory motor processing areas in the neocortex.

The development of new methods for time series analysis could help to overcome the problems found in earlier studies. In particular, mathematical tools for the study of nonlinear dynamical systems have made possible the analysis of complex signals, formerly regarded as stochastic, from a different point of view. In regard to the EEG, they are contributing to a better characterization of the integrative brain operation (22). The two nonlinear measures mostly calculated have been the correlation dimension (D2), a measure of the EEG complexity that estimates its degrees of freedom, and the largest Lyapunov exponent (λ_1), an index of the sensitive dependence of the temporal evolution of the system on initial conditions. When λ_1 is greater than zero, different states of the system that are arbitrarily close will become separated after sufficiently long times, as it happens in chaotic systems (11). These measures have been used to clarify many different aspects of human and other mammal EEGs (12, 19, 22) and also as a sign of chaotic behavior in the system producing the signal (3). Therefore, it seems interesting to use them in search of similarities among physiological signals from different animal species.

A limitation to the usefulness of D2 and λ_1 comes from the fact that, when calculated from linearly correlated noise, the later can be misinterpreted as a nonlinear signal (23). Consequently, it is essential to test whether nonlinearity is present in a given time series before carrying out its analysis. One way to achieve this goal consists of comparing the value of a nonlinear parameter (i.e., D2) from the original signal with those calculated from an ensemble of surrogate signals (32). Surrogate signals are obtained from the original in such a way that they preserve most of its properties but are devoid of coherent phase relationships. Recent results (19) showed that human EEG exhibits a nonlinear characteristic mainly during SWS, presenting the lowest values for D2 in this state. Similar outcomes have been reported in regard to λ_1 (12, 25).

Another way to characterize the complexity of a time series involves the determination of its fractal dimen-

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sion without carrying out a previous reconstruction of its dynamic in the phase space. This method has many operational advantages over those described above (7). A recent procedure for this purpose is called coarse graining spectral analysis (CGSA) (33), which is based on the calculation of the fractal exponent β from the power spectral density (PSD) function of signals that exhibit frequency power-law dependence $1/f^\beta$. In addition, this algorithm allows us to split the total PSD in two components: harmonic and fractal. Although the presence of deterministic chaos (with a broad-band spectrum) is incompatible with this type of fractal, theoretical studies have demonstrated that random fractal noise can fool the algorithms for D2 estimation (18). Thus a linear correlation between D2 and β has been found in human EEG for all states except during SWS, and, as a consequence, β has been proposed as a useful measure to describe the human EEG in these stages (19).

The three main goals of this work are 1) to establish whether the reptilian EEG exhibits fractal or nonlinear characteristics in different experimental situations and, if so, to describe them by calculating β , D2, and λ_1 , 2) to determine the changes in the harmonic components of the EEG among these situations, and 3) to compare the results obtained from those reported for human SWS EEG.

METHODS

Animals and data acquisition. EEG signals were registered in six lizards (*Gallotia galloti*) from the Canary Islands chronically implanted with electrodes symmetrically placed on the surface of the dorsal cortex of both hemispheres. To analyze an ample choice of different activity states, EEG recordings were obtained during waking with open (OE) and closed eyes (CE) at temperatures of 25 ± 1 and $35 \pm 1^\circ\text{C}$, as well as during night rest (NR) at $20 \pm 1^\circ\text{C}$ while the animals were kept in a soundproof, thermostated chamber. Before the diurnal recordings, animals were maintained in the chamber during an adaptation period of 8 h at the selected temperature. NR recordings were collected 4 h after the light was turned off, with temperature maintained at 20°C . The EEG signals corresponding to the left (LH) and right (RH) hemispheres were digitized at 256 samples/s by means of a 12-bit analog/digital card and stored in a personal computer for further analysis. Five nonoverlapping segments of 4,096 samples (16 s) from each lizard in every temperature and experimental situation were chosen for the analysis after careful inspection for stationarity and lack of artifacts from the measurement devices or animal movements. The resulting EEG signals were preprocessed to achieve a zero mean and unity variance. Linear trend was removed through the least squares fit method.

Nonlinear techniques. Once the EEGs were embedded in phase space states of dimension m (from 2 to 20) according to Takens (29), λ_1 was calculated using the algorithm of Rosenstein et al. (26), which has been shown to be accurate and robust (i.e., not sensitive to the initial choice of parameters in the calculation) dealing with short time series. The average evolution of intervector distances (ΔS) was computed as a function of the evolving time (t) for m ranging from 7 to 10, different delay times (10–20 sample units), and neighborhood sizes. λ_1 was then calculated as the average slope of the plots $\log\Delta S$ vs. t in each m . As for D2, the correlation integral $C(r)$

for distances r] in each embedding space was obtained using the Grassberger-Procaccia algorithm slightly modified after Theiler (31) to reduce the effect of temporal correlations. The correlation time was taken as the time for the autocorrelation function to drop to $1/e$. The limits of the linear region in the five highest dimensions (16–20) were determined for the ensembles of segments in each experimental situation by visual inspection of $\log C(r)$ vs. $\log(r)$ curves. Then, the slopes $[\delta \log C(r)/\delta \log(r)]$ of these curves were plotted against $\log(r)$, and their mean and SD were calculated for each of the $\log(r)$ values between the fixed limits. D2 was estimated as the mean value with minimal SD, but saturation was considered satisfactory only when SD was <0.1 .

To obtain statistical evidence whether the EEG presented nonlinear structure, 39 surrogates from each segment were constructed as follows: 1) a Gaussian-distributed set of random numbers with the same mean, SD, and rank structure as the original data was generated; 2) a random-phase surrogate of this Gaussian-distributed set was constructed by taking the Fourier transform of the original data, randomizing the phases of this transform, and taking the inverse transform; and 3) the original data were shuffled so that they had the same rank structure as the random-phase surrogate constructed in step 2. This variant of the original data is the Gaussian-scale random-phase surrogate (32). Data sets constructed in this way avoid the spurious identification of nonrandom structure that the simpler phase-randomized surrogates can produce (24). Then, the value $S = |Q - \hat{Q}_s|/\sigma_s$ is estimated, where Q is the Takens best estimator of D2 (21) for the original signal, and \hat{Q}_s and σ_s are the mean value and the SD of the estimator for its 39 surrogates. The units of S are commonly called sigmas (32). When the number of sigmas is greater than two, the time series is considered nonlinear (with a level of significance $\alpha = 0.05$). Otherwise, the null hypothesis could not be rejected, i.e., the original was not different from linearly correlated noise transformed by a static, monotone nonlinearity (24).

Spectral techniques. Spectral characteristics of the time series were determined via the CGSA method (33). Briefly, if the total spectral power of a signal consists of both harmonic and nonharmonic (i.e., fractal) components, they can be separated. This is so because the fractal component is scale invariant, i.e., when rescaled, it will still retain its power when cross correlated with the original data (16). In contrast, rescaling of harmonic components causes a complete loss of spectral power when cross correlated with the original. β can be obtained then as the absolute value of the slope of the fractal power vs. frequency in log-log scale. The frequency range of 3–30 Hz was selected because the spectra presented the clearest $1/f^\beta$ dependence within it. The spectral power within each band was calculated as the sum of the harmonic power accumulated in the ranges 0.3–7.5 Hz [low-frequency band (LF)], and 8–30 Hz [high-frequency band (HF)]. The choice of these ranges for each frequency band was made according to the characteristic shape of the EEG spectra in these animals.

Statistical comparisons. Multivariate ANOVA with repeated measures, with temperature (25 and 35°C), experimental situation (OE and CE), and hemisphere (LH and RH) as dependent factors, was used to determine differences among the mean values of the parameters. Least significant difference post hoc test was used to compare pairs of means. NR was compared only with the experimental situation 25°C CE by using a t -test for dependent samples. In addition, Pearson correlation coefficient was used to establish whether there was any linear dependence between D2 and β . In every case,

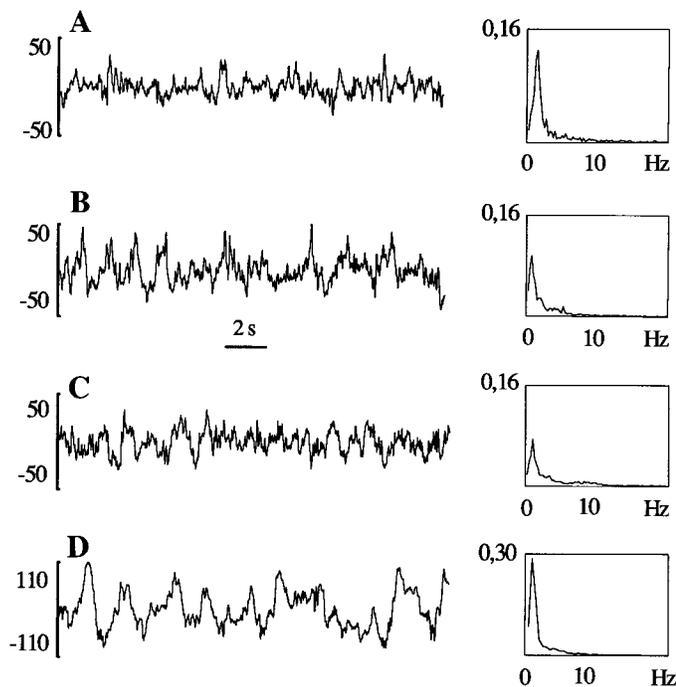


Fig. 1. Original electroencephalogram (EEG) traces (*left*) and corresponding normalized power spectral density (PSD; *right*). Lizard EEG records at 20 (A), 25 (B), and 35°C (C) [left hemisphere (LH), closed eyes (CE)], and 1 human EEG record during slow-wave sleep (D). In all traces, vertical axis is in mV.

statistical evidences were considered significant when $P < 0.05$.

RESULTS

EEG records. Figure 1, *left*, shows examples of EEG traces from one lizard during NR, 25, and 35°C (CE, LH). In addition, a trace of human EEG during SWS (C3-A2 derivation) recorded in our laboratory is shown for comparison. The presence of slow waves is evident in both reptilian and human EEG records.

Nonlinear measures. Figure 2 shows an example of a plot of the slope $[\delta \log C(r) / \delta \log(r)]$ vs. $\log(r)$ in the range $m = 16$ to $m = 20$. The slope exhibited a clear plateau, indicating saturation of D2 vs. increasing m . Table 1 summarizes the percentage of segments (%S) showing this behavior in each situation. It can be observed that

this value was always high ($>65\%$). This result would suggest the existence of a strange attractor with $D2 \sim 6$ and greater in LH than in RH in both awake states only at 25°C ($P < 0.001$; see Table 1). Temperature increased D2 in both hemispheres during waking ($P < 0.001$). The difference between OE and CE was only significant at 25°C in both LH and RH ($P < 0.001$), with D2 greater in OE. No differences were found between NR and 25°C (CE). As for the dynamic parameter, λ_1 was clearly greater than zero in every experimental situation. It was always higher in RH when compared with LH ($P < 0.001$) and increased with temperature only in the CE state ($P < 0.001$ in RH, $P < 0.01$ in LH; see Table 1). There were differences between OE and CE at 25°C ($P < 0.001$ in RH, $P < 0.05$ in LH), but not at 35°C. Furthermore, this parameter was significantly lower ($P < 0.01$) during NR than at 25°C, CE in RH, but not in LH.

Figure 3 presents the results of the surrogate data test in both NR and OE (LH) experimental situations. Only at 25°C was the mean number of sigmas (and its 95% confidence limit) >2 , the statistical threshold to reject the null hypothesis of linearly correlated noise for some embedding dimensions. At 35°C, although for these dimensions the mean value remained >2 , the confidence limit included this value, indicating that at this temperature it is not possible to assert statistically the presence of nonlinear structure in the EEG. Similar results were reached for CE and RH. In addition, during NR the mean value was <2 , so in this case it was not possible to reject the null hypothesis.

Spectral measures. The power spectra corresponding to each of the EEG traces of Fig. 1 are shown in Fig. 1, *right*. In all the spectra, the existence of slow waves is clearly stressed for the presence of a main peak in the 1- to 3-Hz frequency range. Figure 4 illustrates the ensemble average of both fractal (Fig. 4, *left*) and harmonic (Fig. 4, *right*) power spectra at 25 and 35°C (OE) of the LH obtained via CGSA. Fractal spectra exhibited a clear power-law dependence, with $\beta \sim 2$ and, unlike the nonlinear parameters, decreased equally in both hemispheres from 25 to 35°C ($P < 0.001$). There were differences neither between both hemispheres nor between OE and CE states. β was also unable to distinguish between NR and 25°C CE. In regard to the

Fig. 2. Plots of $\log C(r)$ vs. $\log(r)$ (*left*) and slope vs. $\log(r)$ (*right*) for 5 highest embedding dimensions. There is a range of $\log(r)$ values where slope is constant, corresponding to linear region on *left*. In addition, slope does not depend on embedding dimension and, so, saturation is reached. See *Nonlinear techniques* for more information.

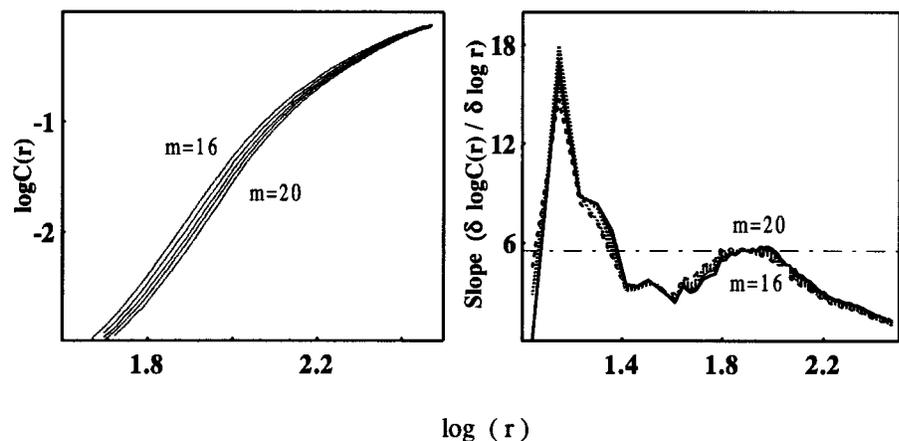


Table 1. Values of the parameters in each experimental situation

Hem	State	T, °C	%S	D2	λ_1 , s ⁻¹	%FP	β	r (D2 vs. β)
LH	NR	20	63.8	6.01 ± 0.20	1.22 ± 0.12	80.3 ± 0.6	2.15 ± 0.05	-0.61 ($P < 0.01$)
	CE	25	90.3	5.63 ± 0.24	1.18 ± 0.09	78.5 ± 2.0	2.12 ± 0.05	-0.19 (NS)
		35	83.7	6.49 ± 0.25*	1.43 ± 0.09†	82.2 ± 0.9†	2.04 ± 0.04†	-0.19 (NS)
RH	OE	25	97.8	6.15 ± 0.07	1.39 ± 0.05	79.5 ± 0.6	2.15 ± 0.06	-0.35 (NS)
		35	84.7	6.57 ± 0.04*	1.55 ± 0.09	79.6 ± 0.9	2.04 ± 0.04*	-0.35 (NS)
	NR	20	84.0	5.76 ± 0.32	1.22 ± 0.12	84.2 ± 1.5§	2.11 ± 0.10	-0.84 ($P < 0.001$)
RH	CE	25	72.9	6.11 ± 0.17	1.71 ± 0.12	81.6 ± 1.0	2.13 ± 0.09	-0.20 (NS)
		35	76.4	6.31 ± 0.33*	2.09 ± 0.15*	83.0 ± 1.5‡	2.04 ± .05*	-0.20 (NS)
	OE	25	91.1	6.29 ± 0.13	2.15 ± 0.15	82.7 ± 0.7	2.12 ± 0.08	-0.06 (NS)
		35	73.3	6.68 ± 0.27*	2.08 ± 0.12	83.4 ± 0.7	1.99 ± .08*	-0.35 ($P < 0.001$)

Values are means ± SE of percent saturation for correlation dimension (D2) in last 5 embedding dimensions (%S). D2, largest Lyapunov exponent (λ_1), percent fractal power (%FP), fractal exponent (β), and D2 vs. β Pearson correlation coefficient (r) for left (LH) and right hemispheres (RH) during open eyes (OE), closed eyes (CE), and night recording (NR). For P , statistical significance is shown in parentheses, whereas in other cases * $r < 0.001$, † $r < 0.01$, and ‡ $r < 0.05$ indicate statistical comparisons between 25 and 35°C, and § $r < 0.01$ between 25°C (CE) and NR. For CE-OE and LH-RH comparisons, see RESULTS. NS, not significant.

harmonic spectra, they always exhibited a main peak with center frequencies of 1.3 ± 0.3 Hz at 20°C, 1.6 ± 0.1 Hz at 25°C, and 2.1 ± 0.2 Hz at 35°C, with statistical differences only between 20 and 35°C ($P < 0.05$). The harmonic spectral power within the LF and HF bands is shown in Fig. 5; because no differences were found between hemispheres, they were considered together. The power during OE was greater than during CE at 25°C ($P < 0.001$) only in the HF band and in both LF ($P < 0.001$) and HF bands ($P < 0.05$) at 35°C. Furthermore, as can be seen in Fig. 5, the power increased globally with temperature ($P < 0.001$). This increment affected both bands in OE, but it was only evident in the HF band during CE records. In addition, the power decreased significantly in both bands during the night ($P < 0.01$).

In Fig. 6, global linear correlation between β and D2 for every segment analyzed is shown. There was a negative correlation, with β decreasing for increasing D2, but when each situation is considered separately, this correlation was significant only for NR and at 35°C (RH, OE). Results from every experimental situation are shown in Table 1.

DISCUSSION

The results from the nonlinear measures suggest that the EEG of lizards possesses a structure in its phase space resembling what we can find in systems with a strange attractor and thus the presence of deterministic chaos. In fact, most of the EEG records showed saturation of the plots D2 vs. $\log(r)$ when increasing m , suggesting the existence of a chaotic process with a relatively high complexity (D2 ~6). Furthermore, in every experimental situation the dynamic parameter λ_1 was >0 ($\lambda_1 \sim 1.5$), apparently supporting this later conclusion. Nevertheless, a surrogate data test, used to check if these outcomes were spurious, showed that the null hypothesis of linearly correlated noise could only be rejected at 25°C in both OE and CE, whereas at 35°C the results were not so conclusive. Anyway, the evidence of nonlinearity was never too strong: the mean value of sigmas (and its 95% confidence interval) was slightly >2 only at certain values of m . It is well known that an increase of the

time series complexity (D2 at 35°C was greater than at 25°C) decreases the ability of the surrogate data test to distinguish from linearity (32). However, this increment was not large enough here to produce this methodological misinterpretation. In fact, other EEG characteristics (such as band spectral power) also changed with temperature, suggesting that this exogenous factor greatly conditioned the structure of the signal. In this context it is worthy to mention that, as is well known, the reptilian physiology is strongly dependent on temperature. In moderately favorable environmental conditions, reptiles tend to maintain their body temperatures within certain limits in what is called their mean selected (20) or preferred temperature. Pilot experiments, in which the *Gallotia* lizards were able to select their exposure to heat in their home cages, rendered 26–29°C as the self-selected cephalic temperature, this coincides with earlier studies carried out in the same species (13). Thus, at the experimental temperature closer to the preferred one (25°C), the integrated activity of the lizard telencephalic cortical neurons (estimated via the EEG) showed a nonlinear structure with different characteristics in the two awake states analyzed, waking (OE) and rest (CE). However, the scenario changed when temperature increased up to 35°C: the differences between both states disappeared and the evidence of nonlinear structure became weaker. Moreover, D2 increased in every case and λ_1 only in CE. Because D2 gives an estimation of the signal complexity (11) while λ_1 has recently been used to quantify morphological regularity ratings in the EEG signal regardless of its level of chaos (15), this indicates that at 35°C the EEG became more complex and less regular and predictable (greater D2 and λ_1). A possible explanation of these results could be that the lizards might control their physiological activity, for instance, by changing their central nervous system level of activation to succeed in meeting changing demands, as long as their body temperature is kept within the range of preferred temperature. This is shown in the modified structure of the EEG according to the eye state, which should follow both environmental and internal factors. On the contrary, at 35°C (well over the preferred temperature range), a reduced efficiency of the regula-

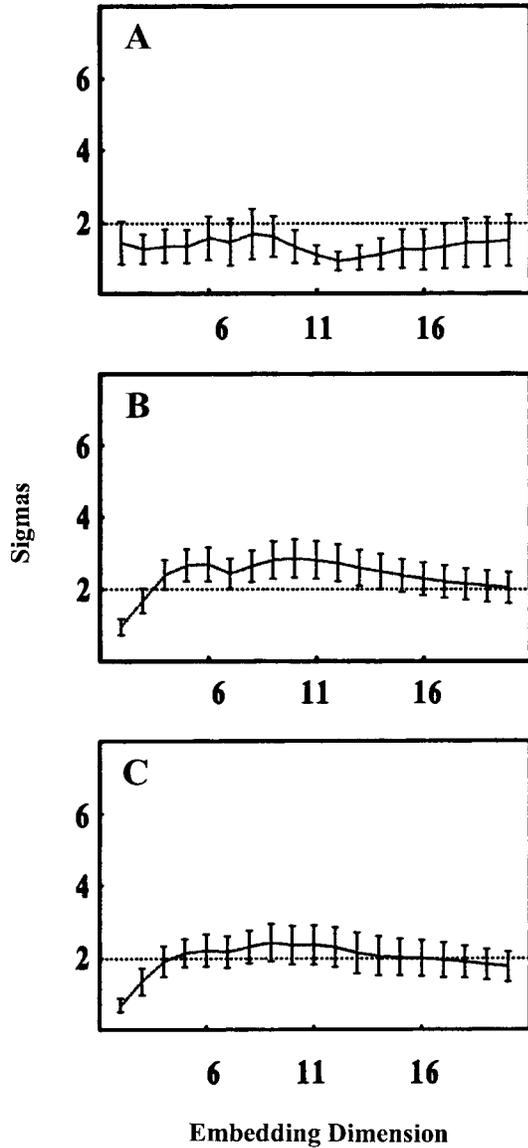


Fig. 3. Number of sigmas vs. embedding dimension for ensemble of EEG analyzed during night (A), 25°C (B), and 35°C [C; LH, open eyes (OE)]. Bars indicate 95% confidence limit for mean. Only at 25°C is mean value above limit of 2 for a certain range of embedding dimensions, allowing us to reject the null hypothesis of linearly correlated noise.

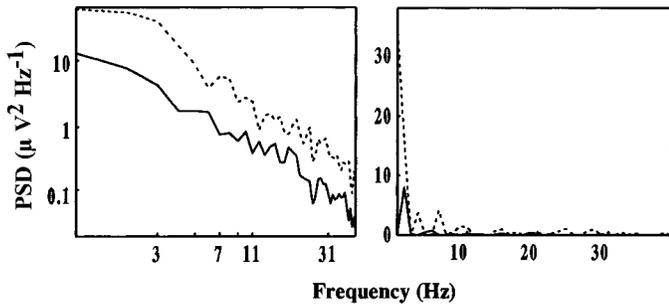


Fig. 4. Both components of EEG PSD isolated via coarse graining spectral analysis: fractal (left) and harmonic (right) at 25 (solid lines) and 35°C (dashed lines). It is noteworthy that slope of fractal power (and in consequence fractal exponent β) is slightly greater (in absolute value) at 35°C.

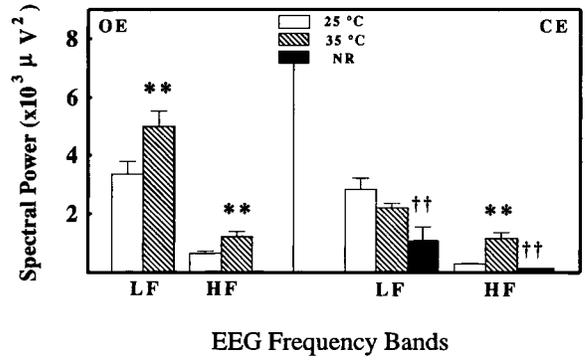


Fig. 5. Total harmonic power in each band at 25 and 35°C during OE (left) and CE [right; note this panel includes night recording (NR)]. ** Differences between states at each temperature ($P < 0.01$); †† differences between 25°C CE and NR ($P < 0.01$).

tory mechanisms is shown by the lack of difference between OE and CE. The importance of eye state in determining the EEG activity is well known in mammals (arousal reaction) and also in birds (1).

The effects of temperature could consist of a simple metabolic increase: heating the body tissues produced a nonspecific increment of the nervous activity regardless of the activation or inactivation needs. This is congruent with some results reported for evoked responses (ER) in reptiles (2), which presented the best ER (signal)-to-EEG (background) ratio at the preferred temperature range, whereas it decreased when body temperature was moved to extreme values. It is well established that maintaining the body temperature within narrow limits (as happens in homeotherms, but also to a limited extent in reptiles) serves to maintain an effective endogenous control of physiological variables in front of changing external factors. On the other hand, the increasing of D2 with the temperature never reached values comparable to those from waking mammal ($D2 \sim 8$), remaining instead much closer to those from mammalian SWS ($D2 \sim 5$). Increasing the temperature in reptiles is always correlated with an increase of their activity. On the contrary, a high temperature causes sleepiness and also the production of slow-wave EEG in mammals (6), which means that these animals show mixed features between waking and SWS. Thus,

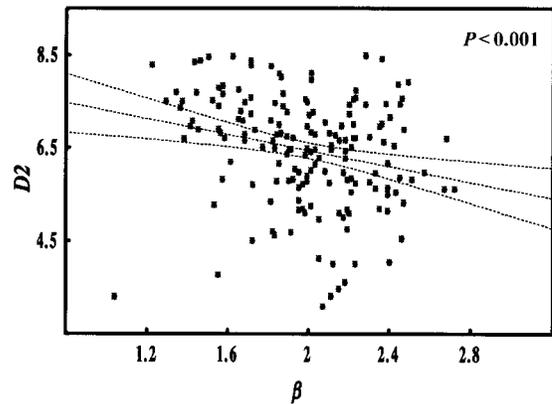


Fig. 6. Correlation dimension D2 vs. fractal exponent β (all segments included). P value for linear correlation is indicated top right. As can be seen, this correlation was inverse and significant.

although nonlinear analysis has not yet been performed in mammals under hyperthermia, a D2 lower than that of waking but higher than that of SWS should be expected. Therefore, hyperactivity in reptiles increases the complexity of their EEG as measured by D2, but the values reached are still far from those of waking mammals and are similar to those that can be expected in mammals under hyperthermia. Considering both the D2 increment and the weak evidence of nonlinear structure at 35°C, the changes caused by hyperthermia are probably due to an increase of EEG randomness instead of a true increase in alertness. The same could be expected from the corresponding values of mammals under hyperthermia.

NR results deserve a closer look. Although there was a significant correlation between D2 and β (see Table 1) and the results from the surrogate data test did not allow us to assert that nonlinearity was present, the values of the nonlinear measures (D2 and λ_1) were not different from those obtained at 25°C CE. This seemingly paradoxical outcome can perhaps be explained by taking into account the signal-to-noise ratio, considering the background EEG as "noise" opposed to the goal-directed activity that should be dominant during waking. During NR, EEG amplitude reached the lowest values, and, most likely, the low level of neural information processing (with nonlinear dynamic) would have been masked in a high percentage of background activity. It is also noteworthy that in human EEG, the transition from awake to SWS is characterized by a drastic decrease in the absolute value of nonlinear estimators, parallel with a change from linear to nonlinear structure (19). However, in lizards there were no differences between diurnal rest at 25°C with CE and NR (a state in which the animal is clearly at rest with its eyes closed, but where no additional evidence of real mammal-like sleep was obtained). Further studies are being carried out at present in our laboratories to obtain a deeper insight into this complex situation.

The fractal EEG spectra obtained via CGSA in every experimental situation showed power-law dependence $1/f^\beta$ with $\beta \sim 2$. In accordance with the requirements found by Yamamoto and Hughson (33) for a signal to be a random fractal, in addition to the former condition, the EEG might present a percentage of fractal power near 100%. These restrictions were fulfilled for all the records analyzed (see Table 1). It means that, at any temperature, a great percentage of the EEG power was not due to the harmonic components (with well-determined discrete and harmonic frequencies) but spread out in a wide range. The negative linear correlation found between D2 and the fractal parameter β at 35°C (RH, OE) suggested that the behavior of the EEG of lizards at this temperature could be compatible with those from a linear system with power-law spectra, which exhibits a well-described dependence between these measures (18). On the contrary, the lack of correlation at 25°C supported the nonlinear character of the EEG already suggested above. In fact, at this temperature, D2 was sensitive enough to detect differences between both diurnal states and hemispheres,

whereas β was not. In those cases where both measures are correlated, it is possible to obtain an estimation of the system complexity through the fractal exponent (33), and this information is essentially the same that can be obtained from D2.

In addition, the CGSA also allowed us to analyze the harmonic power after removing the fractal contribution to the total spectrum. The increase of the EEG power with temperature reported here was in good agreement with previous studies (2, 14) that dealt with the total spectral power. However, the present approach has the important advantage of allowing the study of these two components (harmonic and nonharmonic) and their variation within each band separately. A temperature increase produced an effect on harmonic power during diurnal states depending on the eye state. The harmonic power in the HF band was modified by the temperature regardless of the state, whereas the power in the LF band increased only in OE, indicating that this increase was only due to the processing of the additional sensory information (likely visual) reaching the telencephalic cortex. This statement was supported by the fact that, at 25°C, differences between states were significant only concerning the HF band: no increase of the LF band was necessary. General power increase and synchronization have been reported in other submammalian vertebrates as the normal arousal reaction (4, 5), contrariwise to mammals and birds, in which activation is reflected as a reduction of EEG power and synchrony. To summarize, an increase of the temperature produced an unspecific response (independent of the activation) in the HF band and a specific response in the LF when processing sensory information (OE). Thus the results from nonlinear analysis were confirmed: global physiological differences between both temperatures can be detected and characterized in the EEG signal from both its complexity and its morphological regularity as well as from the amount of harmonic and nonharmonic power in the LF and HF bands. In addition, the EEG measures showed that the animals had better control of their activation level at temperatures close to 25°C.

Previous evidence also exists on temperature-dependent changes in EEG activity in mammals. Thus it has been reported that a reduction in temperature causes an exponential decrease of the predominant EEG frequencies in the Djungarian hamster during entrance into daily torpor (9). The $\text{Na}^+\text{-K}^+$ pump was suggested there as the rate-limiting step in determining EEG frequency. Here we only considered the temperature effect on the main component of the spectrum (1–3 Hz), but the shift observed was not significant and far from those reported there. However, in a previous study (10) we described a linear reduction of the frequencies of EEG spindles in *Gallotia* lizards with temperature. This is reflected here in the unspecific decrease of the HF power from 35 to 25°C mentioned above, suggesting that this effect could be associated with a temperature-dependent physiological mechanism, as happens in the Djungarian hamster.

Only D2 and λ_1 were able to detect the interhemispheric differences: both parameters were greater in the RH than in the LH. Brain asymmetries have been documented in many mammalian species and birds (8). Moreover, a measure of the global complexity in the human EEG of healthy subjects was higher over the RH during waking (28) but up to now this is the first objective description of a functional asymmetry in a reptilian species. This suggests that lateral differentiation could be widespread in the vertebrate group. The most robust interhemispheric differences were found in λ_1 . These could mean that a state divergence might be possible between the two hemispheres, as has been found in some birds (1) and in marine mammals (17). Were this effect confirmed, the basis for unihemispheric sleep would be traceable to reptiles.

Finally, it should be mentioned that human EEG (C3-A2) during SWS and lizard EEG at 25°C exhibited similar qualitative characteristics from the spectral and nonlinear analyses. Indeed, they both presented a main spectral peak with a center frequency ~ 1 Hz (see Fig. 1), with the presence of nonlinear structure confirmed by the nonlinear test and the lack of correlation between D2 and β . This gives greater support to the hypothesis outlined in our previous study (10) that both EEGs present similarities. After the present results, we can extend these similarities to their structure in the phase space. Quantitatively, however, D2 and λ_1 of lizards (25°C) were slightly higher and β slightly lower than for human SWS (19). Furthermore, it is clear that the physiological role of the proposed similarities might be different in each case: in mammals, cortical EEG reflects the state of the most important processing region, whereas in reptiles, although the cortex has some associative ability, the main sensorimotor processing is elaborated in mesencephalon and rombencephalon, including the main visual centers as well as the final motor common path (4, 30). Thus, whereas mammalian cortical EEG exhibits a nonlinear structure in SWS but is not different from linearly correlated noise during waking, equivalent conclusions could not be drawn in reptiles. In effect, in these animals nonlinearity appears to be present in their telencephalic EEG during waking at the preferred temperature, whereas it is not so clear when the temperature increases above its optimal limits. In other words, similar changes should have opposite meaning, from low to high vigilance in mammals but from efficient to less efficient processing in reptiles.

In summary, the present study demonstrated that the EEG of lizards exhibits nonlinear characteristics at 25°C (D2 ~ 6 , $\lambda_1 \sim 1.5$) as could be inferred from the results concerning the surrogate data test and from the lack of correlation between D2 and the fractal exponent β . This nonlinear structure would coincide with the highest effectiveness in sensory processing at the preferred temperature range. So, although the evidence of this structure was weak and seemed to be strongly dependent on temperature (i.e., an increasing of this exogenous factor changed the scenario), it was possible to extract information about the neurophysiological

activity of these animals and its variations in response to internal and external changes. These changes were not reduced to nonlinear characteristics, but included variations in both the fractal and harmonic powers.

Perspectives

The nonlinear analysis of EEG is well documented mathematically (11), but the meaning of the terms complexity (D2) and predictability (λ_1) associated to brain function is not yet entirely clarified. Nevertheless, both D2 and λ_1 were able to provide insight about reptilian EEG features that would have remained undisclosed otherwise. These features showed small changes among different states, maybe because the cortex is practically absent in reptiles and therefore reptilian wakefulness would be speculated to be under brain stem control.

More advanced nonlinear tools currently in production (such as cross nonlinear prediction) should help to validate the reported nonlinear structure of lizard EEG. This would represent an important step in our knowledge of brain function, because it would demonstrate that several neural networks are able to couple with each other in a nonlinear way to produce the EEG. Furthermore, the presence of this structure, as well as its qualitative characteristics close to those from human SWS, would reinforce our hypothesis of homology between mammalian SWS and reptilian waking. The assessment of mammalian EEG under hypo- and hyperthermia using nonlinear methods would likely provide further evidence of this similarity. On the other hand, it would be worthy to extend the temperature range, trying to settle the role of the temperature-dependent mechanism whose influence in lizard EEG is suggested by the results. Finally, the interhemispheric asymmetry in EEG activity, which was highlighted especially by λ_1 , is also a striking result that deserves further investigation to elucidate its relevance in animal evolution.

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