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Time-Related Interdependence Between Low-Frequency Cortical Electrical Activity and Respiratory Activity in Lizard, *Gallotia galloti*

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ABSTRACT Electroencephalograms of medial cortex and electromyograms of intercostal muscles (EMG-icm) were simultaneously recorded in the lizard, *Gallotia galloti*, during two daily time periods (at daytime, DTP: 1200–1600 h; by night, NTP: 0000–0400 h), to investigate whether a relationship exists between the respiratory and cortical electrical activity of reptiles, and, if so, how this relationship changes during the night rest period. Testing was carried out by studying interdependence between cortical electrical and respiratory activities, by means of linear and nonlinear signal analysis techniques. Both physiological activities were evaluated through simultaneous power signals, derived from the power of the low-frequency band of the electroencephalogram (pEEG-LF), and from the power of the EMG-icm (pEMG-icm), respectively. During both DTP and NTP, there was a significant coherence between both signals in the main frequency band of pEMG-icm. During both DTP and NTP, the nonlinear index N measured significant linear asymmetric interdependence between pEEG-LF and pEMG-icm. The N value obtained between pEEG-LF vs. pEMG-icm was greater than the one between pEMG-icm vs. pEEG-LF. This means that the system that generates the pEEG-LF is more complex than the one that generates the pEMG-icm, and suggests that the temporal variability of power in the low-frequency cortical electrical activity is driven by the power of the respiratory activity. *J. Exp. Zool. 303A:217–226, 2005.* © 2005 Wiley-Liss, Inc.

INTRODUCTION

The main regions of the vertebrate brain are functionally subdivided. The functional system that regulates and integrates respiratory, cardiovascular, and somatomotor systems and the adjustment of central nervous activity in mammals is the common brainstem system (Langhorst et al.,'83; Rittweger,'99; Lambertz et al., 2000). A system of similar characteristics has been described in different reptilian groups (Naifeh et al.,'71; Takeda et al.,'86; Douse and Mitchell,'91; Taylor et al.,'99).

Brain-lung interactions in mammals have largely been investigated using linear and nonlinear time series analysis techniques. Linear measures have revealed that EEG parameters change synchronously with respiration (Pfurtscheller,'76), and that higher centers in the brain affect the breath-to-breath variability of the respiratory pattern (Mador and Tobin,'91). Moreover, breathing differs deeply in wakefulness and sleep. Both frequency and amplitude of respiration decrease during sleep (Phillipson and Bowes,'86), and the variability of the breathing pattern decreases during deep slow-wave sleep and increases during REM sleep (Tabachnik et al.,'81). It has recently been demonstrated, using nonlinear measures, that the EEG correlation dimension (a measure of the signal complexity) is higher during REM than during sleep stages I or IV (Burioka et al., 2001; Sako et al., 2001). Also, respiratory movement and EEG signals are more regular during deep slow-wave sleep (stage IV) than

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during other states of consciousness (Burioka et al., 2003).

Interactions between cortical electrical and respiratory activities in reptiles have not been described yet. The presence of olfactory bulb high-frequency spindles evoked by passage of air through the nasal cavity, during inspiration or during sniffing in *Caiman sclerops* (Huggins et al.,'68; Verlander and Huggins,'77), has been reported.

Time series analysis techniques have been largely used to describe the behavior of many physiological systems of vertebrates. When these systems come into operation, they generate complex, chaotic signals, which are a manifestation of their dynamic properties. The traditional approach to the analysis of these signals has been based on the dynamics of linear systems, primarily through the spectral analysis. As many biological signals apparently present a chaotic behavior, this approach has recently been improved with the introduction of techniques based on the dynamics of nonlinear systems. Furthermore, both the linear and the nonlinear approaches allow the study of the behavior of a given physiological system, and the relationships between two different systems.

The authors are unaware of any mathematical tools, either linear or nonlinear, that have been used until now with the purpose of characterizing relationships between cortical electrical and respiratory activities in reptiles. For that reason, the aim of the present study is to carry out research on lizards, by means of linear and nonlinear signal analysis techniques, to determine the possible interdependence between the low-frequency cortical electrical activity–where most of the EEG's power is concentrated–and the respiratory activity. This possible interdependence is analyzed at two different daily time periods: diurnal (light-activity-wakefulness) and nocturnal (dark-rest-sleep), to assess the influence of the degree of alertness on the relationships between the systems under study. The study is performed at a constant body temperature to avoid metabolic changes related to variations in body temperatures.

MATERIALS AND METHODS

Animals

Eight lizards of the species *Gallotia galloti*, 9.6 – 12.1 cm in length (snout to vent) and 38.6 – 50.7 g (mean 42.8 ± 4.8 g) 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 (0800–2000 h; 23 ± 1°C ambient temperature) – 12–h dark (2000–0800 h; 20 ± 1°C ambient temperature) cycle for at least 15 days before experiments. Water and food was available ad libitum. The Ethical Committee of the University of La Laguna approved all animal procedures described below.

Surgery

The experimental animals were anesthetized and two pairs of electrodes were implanted using aseptic techniques, to simultaneously record continuous EEG and respiratory activity. Lizards were anesthetized with ketamine hydrochloride/ xylazine hydrochloride solution (80/12 mg/kg, im.; SIGMA-ALDRICH Co., Madrid, Spain). One 3.5 mm length stainless steel Teflon coated electrode of 0.5 mm diameter was used as active electrode to record monopolar EEG. 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; one stainless steel screw was secured to the left parietal bone outside the brain cavity, and served as reference electrode. The two chronically implanted EEG electrodes, active and reference, were glued to the skull with acrylic cement. Two 6 mm length stainless steel rolled wire electrodes were inserted subcutaneously on both lateral sides of the thorax over the ribs, in order to record respiratory activity through the electromyogram of the intercostal muscles (EMG-icm). The activity of intercostal muscles of *G. galloti,* in relation to breathing, consisted of two bursts of electrical activity on the EMG-icm, separated by a muscular resting period of about 260 ms duration (Figure 1). The bursts correspond to the active expiration (about 430 ms duration) and active inspiration (about 340 ms duration) periods characteristic of the electromyographic ventilatory cycle of this lizard (De Vera and González,'86). After surgery, each lizard was housed individually in a temperature-controlled terrarium (25 ± 1°C) and allowed to recover and acclimatize for 5 days before recordings began.

Experimental protocol

Simultaneous EEG and EMG-icm recordings were uninterruptedly carried out for two days, keeping the lizard in a thermostatically controlled chamber (25 ± 0.5°C) placed in a room far away

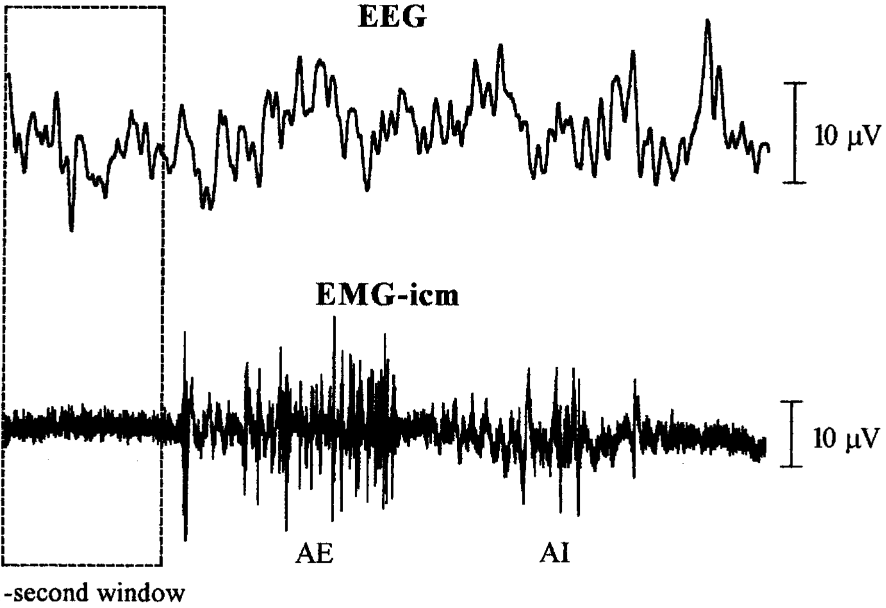

Fig. 1. Simultaneous signal segments of electroencephalogram (EEG) and electromyogram of intercostal muscles (EMG-icm). A data window of 1–second duration was moved along the complete recordings, to calculate successive simultaneous values of power in the low frequency band of EEG, and of variance of the EMG-icm, respectively. The bursts of activity that are seen in the EMG-icm correspond to the periods of active expiration (AE) and active inspiration (AI) described in the ventilatory pattern of *Gallotia galloti* (De Vera and González,'86).

from any sensorial disturbance. The chamber was under a 12–h light (0800–2000 h) – 12–h dark (2000–0800 h) cycle and provided with an electronic mechanism to gradually achieve full brightness or darkness at the start or the end of the light-dark cycle, to achieve a sunrise-sunset effect. Water and food were provided daily at 0900 h through a special hole in the door's chamber. Lizards were free of any restraint other than the lead wires.

Measurements

During the course of the experiment, EEG and EMG-icm signals were continuously and simultaneously measured by a recording system (Nihon Kohden RM–85, Tokyo, Japan). The EEG signal was recorded using a 0.3–s time constant and a 30 Hz high filter, and the EMG-icm was recorded using a 0.003–s time constant and a 1 kHz high filter. The analog signals from the recording system were led to a 14–bit A/D converted card, controlled by a PC for on-line processing. This operation was performed by means of a computer assembler program, which sampled EEG and EMG-icm signals simultaneously at a frequency of 100 Hz and 1 kHz, respectively.

Signal analysis

The analysis of the continuous recording of EEG and EMG-icm signals was restricted to two different daily time periods: day time period (DTP: 1200–1600 h) and night time period (NTP: 0000–0400 h). This time selection was made in order to compare the mean values of the different parameters studied, corresponding to two clearly different environmental conditions: light and activity as opposed to darkness and rest (Molina-Borja et al.,'86).

The signal analysis was carried out only in those recordings where the typical electromyographic ventilatory pattern of these animals appeared clearly and continuously. Moreover, if one EEG recording and/or one EMG-icm recording exhibited any artifactual alteration due to the animal's movements, neither recording was taken into account for the analysis.

During both DTP and NTP, both EEG and EMG-icm signals were first analyzed as 1–s duration segments (Figure 1). The results coming from this analysis were then grouped every 600 data points, to generate derived signals—from both EEG and EMG-icm—of 10–min duration each. By doing this, 24 derived signals were obtained from every experimental animal in each one of the two time periods selected.

Each one of the 1–s duration segments of the EEG signal were spectrally analyzed using a FFT algorithm to obtain their power spectral density function (PSD). In order to perform this 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. A total of 49 spectral coefficients with a spectral resolution of 1 Hz were obtained from each EEG spectrum. Each EEG spectrum was then analyzed by calculating the cumulative PSD in its low frequency band (LF: 0.5–7.5 Hz); therefore, for each 1–s duration EEG signal, one PSD value in the low frequency band was obtained.

Each one of the 1–s duration segments of the EMG-icm signal were analyzed by calculating their variance–a measure that is equivalent to the total PSD of any signal segment (Lynn,'79); therefore, for each 1–s duration EMG-icm signal, one variance (power) value was obtained.

The next step consisted of creating derived power-data signals using the above-mentioned power values over a period of 10–min. By doing this, each one of both 10–min duration derived signals was formed by 600 data points. The power-data signal derived from the 600 PSD values in the LF band of EEG spectra was named pEEG-LF. Similarly, the power-data signal derived from the 600 power values of EMG-icm segments was named pEMG-icm, and gives a measure of the lizard's respiratory activity. Nonstationary signals were detected, and removed from analysis, using methods suggested by Schreiber ('97). At this point, several 600 data point pEEG-LF and pEMG-icm were ready for linear and non-linear analysis.

Under the linear systems' model, the study of interdependencies between simultaneously recorded physiological variables has been traditionally accomplished by using the cross-correlation or coherence functions. Both functions are symmetrical with regard to the variables under study and can only detect linear correlations, hence presenting serious limitations. In contrast, the nonlinear approach, which comprises the calculation of different multivariate nonlinear indexes, can get around those restrictions. In effect, these indexes are sensitive not only to linear and nonlinear interactions, but can also detect asymmetric interdependencies, in which the dependence of a given signal on another differs from the reverse (Schiff et al.,'96; Le Van Quyen et al.,'98; Arnhold et al.,'99; Quian Quiroga et al., 2002). Therefore, the nonlinear approach permits a unique insight into the physiological control systems, because it can detect directionality in the information flow between the systems under study. These indexes can indicate which system is more active (i.e., more complex or with more degrees of freedom). This fact can be extended to propose which one of the implicated systems is working as the *driver* of information, that mainly influences the other, and which one is operating as the *driven response* of information, that receives the influence of the other. This nonlinear characterization of interdependencies in physiological systems has been recently introduced, mainly in studies on relationships between different brain areas in humans (Le Van Quyen et al.,'98,'99; Arnhold et al.,'99; Quian Quiroga et al., 2000, 2002; Pereda et al., 2001, 2003) and, to a lesser scale, in reptiles (Pereda et al., 2002).

Coherence function

The coherence function (COH) between pEEG-LF and pEMG-icm was obtained from the ratio between their smoothed cross and autospectra (Bendat and Piersol, 2000). Then, the mean COH value in the frequency band delimited by the frequency corresponding to the center of gravity of pEMG-icm spectrum ± 0.01 Hz, was calculated. The purpose of this operation was to get a representative single value of COH for each pEEG-LF/pEMG-icm segments pair analyzed. In order to test the significance of the actual pEEG-LF/pEMG-icm COH values, they were then compared with those COH values obtained between the present pEMG-icm and a 600 data point shifted pEEG-LF.

Nonlinear interdependence

To calculate the mutual dependence between both pEEG-LF (X) and pEMG-icm (Y) using nonlinear methods, it is first necessary to reconstruct the state spaces of each signal by means of time-delay embedding (Arnhold et al.,'99; Pereda et al., 2002).

The calculation of the interdependence between the two reconstructed state spaces was carried out assessing the statistical dependence of the state-space structure of X on that of Y and vice versa (Quian Quiroga et al., 2002). In order to do this, the set of nearest neighbors of each reconstructed vector $\vec{X}_i$ is compared with its set of mutual or conditioned neighbors, which are the vectors in its state-space that bear the same time indexes as the nearest neighbors of $\vec{Y}_i$. The difference between both sets of vectors is then quantified by using the mean squared Euclidean distance to calculate the index N, defined as:

$$N^{(k)}(X|Y) = \frac{1}{N}\sum_{i=1}^{N}\frac{R_i(X) - R_i^{(k)}(X|Y)}{R_i(X)} \qquad (1)$$

where $R_{\mathrm{i}}\,(X)$ is the average squared radius of the $\vec{X}_i$ points cloud, and $R_{\mathrm{i}}^{(\mathrm{k})}\,(X|Y)$ stands for the average squared Euclidean distance from $\vec{X}_i$ to its k mutual neighbors. The index N equals one for identical signals, whereas for completely independent signals it is normally close to zero; moreover, as Equation (1) is normally asymmetric (i.e., $N^{(\mathrm{k})}\,(X|Y) \neq N^{(\mathrm{k})}\,(Y|X)$), the index N can also be used to detect asymmetric couplings. In fact, when $N^{(\mathrm{k})}\,(X|Y) > N^{(\mathrm{k})}\,(Y|X)$, the system that generates X is said to be more active or complex than the system generating Y. This is so because the complexity of the system X results from both its own complexity and from that introduced by the system Y. Thus, the system X is considered as the *driven response* system, while Y is considered as the autonomous *driver* system (Quian Quiroga et al., 2000). Contrary to other closely related nonlinear interdependence indexes such as the Arnhold index S (Arnhold et al.,'99), index N is less sensitive to the difference in the complexity of both analyzed signals, thus making it especially suitable for the analysis of signals of different degrees of freedom, as is the case here (see the Results section).

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 ('94). In this variant of Pereda et al. (2001), the index N between 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 with Y all its properties, but are completely independent from X. At the given level of statistical significance, the value of the index N is due to the interdependence between X and Y if and only if it is greater for the original pair than for the other n pairs. 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 SD for this ensemble is designated by σ_{b}. A $\sigma_{\mathrm{b}} < 2$ indicates linear nature. Surrogate signals were obtained according to the IAAFT algorithm (Schreiber,'98) by means of the TISEAN package (Hegger et al.,'99).

From the coherence function alone, it is not possible to obtain further information about the way in which two physiological activities interact with each other. In effect, the function of coherence per se is unable to detect asymmetries in the interdependence. However, using the more recent nonlinear index N to study the interdependence between two activities, it is possible to see not only whether this correlation is linear or not, but also whether it is asymmetrical.

Statistical analysis

The statistical analysis of the differences between mean values of two particular signals' indexes or parameters in any experimental situation (DTP or NTP), was performed using a Student's *t*-test for dependent samples or a Wilcoxon signed rank test, as appropriate. These tests were also performed to assess significant differences between the mean values of two particular signals' parameters or indexes from different experimental situations (DTP opposite to NTP). Statistical analyses were weighted according to the number of effective recordings–those remaining after removing nonstationary ones–obtained in each experimental animal. Comparisons were considered statistically significant if $P < 0.05$.

RESULTS

Figure 2 shows ten-minute segments of simultaneous pEEG-LF and pEMG-icm from lizard L07 during DTP and NTP. The main feature of most pEEG-LF segments was the presence of very high-PSD peaks, clearly distinguished from the base line. These high-power peaks can sometimes reach values of PSD up to 20 times the mean value of a particular signal segment. Instead, the main feature of the pEMG-icm was its irregularity and the absence of very high-power peaks. Nevertheless, some minor peaks could sometimes be seen in the pEMG-icm in synchrony with some very high-PSD peaks in the pEEG-LF.

The mean amplitude value of the pEEG-LF during DTP was greater ($P < 0.001$) than during NTP (Figure 3 left). Equally, the mean amplitude

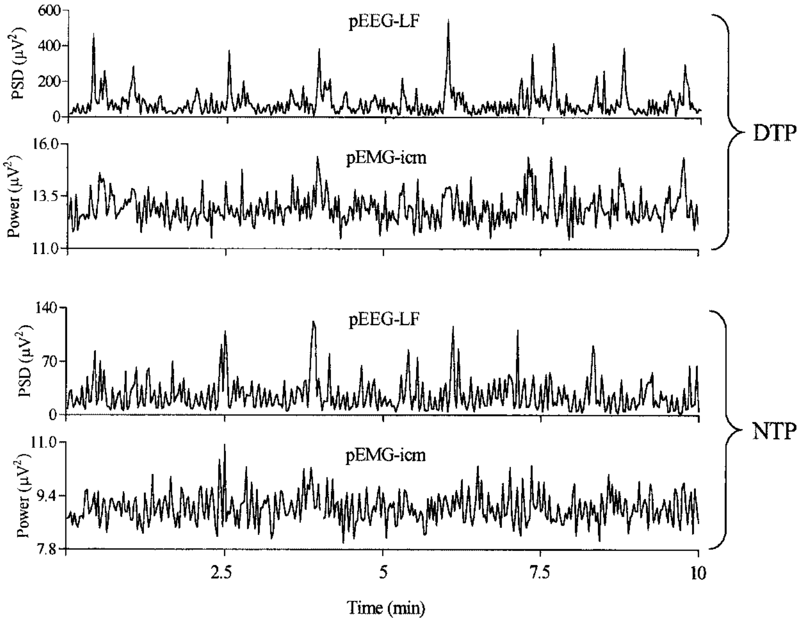

Fig. 2. An example of simultaneous pEEG-LF and pEMG-icm segments from lizard L07 (male, 47.3–g) at the selected DTP and NTP.

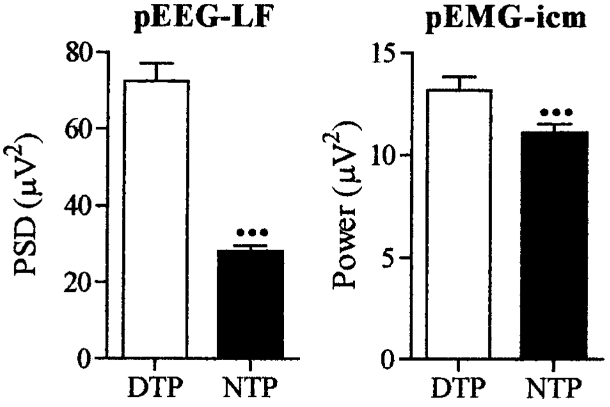

Fig. 3. PSD mean values of pEEG-LF (left) and power mean values of pEMG-icm (right) calculated at the selected DTP and NTP. Symbol ● ● ● indicates statistically significant differences ($P < 0.001$) of the corresponding parameters' mean values between DTP and NTP. Error bars correspond to the standard error of mean. N° of computed signals = 163.

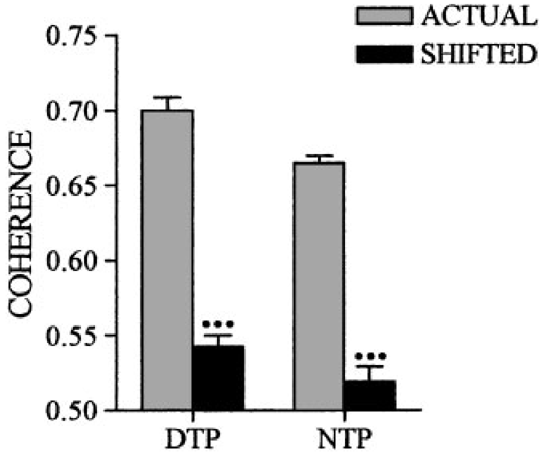

Fig. 4. Actual and shifted coherence mean values between pEEG-LF and pEMG-icm calculated at the selected DTP and NTP. Symbol ● ● ● indicates statistically significant differences ($P < 0.001$) between actual and shifted coherence mean values. Error bars correspond to the standard error of mean. N° of computed signals = 163.

value of the pEMG-icm during DTP was greater ($P < 0.001$) than during NTP (Figure 3 right). The percentage of day/night change of pEEG-LF was 59.7% (median), whereas that of the pEMG-icm was 24.3% (median). There were statistically significant differences between both percentages of change ($P < 0.001$).

The mean coherence value calculated between pEEG-LF and pEMG-icm during both DTP and NTP was greater ($P < 0.001$) than the one obtained between the pEMG-icm and the shifted version of pEEG-LF (Figure 4). This indicates a significant coherence between both signals during the two periods of time studied. The mean coherence value between both signals obtained during the DTP was greater ($P < 0.001$) than the one obtained during the NTP.

TABLE 1. Mean values of index σ_b

Inderdependence	No. signals	DTP	NTP
pEMG-icm *vs.* pEEG-LF	163	0.57 (0.42–0.72)	−0.63 (−0.83–−0.43)
pEEG-LF *vs.* pEMG-icm		1.33 (1.23–1.43)	0.94 (0.74–1.13)

Values <2 indicate linear nature of the interdependence.
Numbers in parentheses indicate −95% and +95% confidence limits of the means.

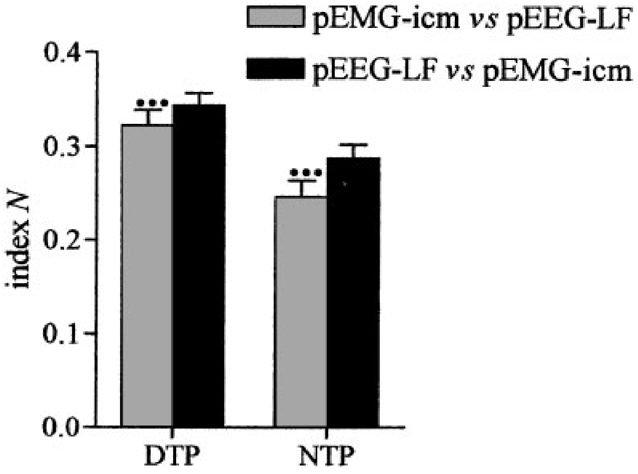

Fig. 5. Nonlinear index N mean values computed between pEEG-LF vs. pEMG-icm and between pEMG-icm vs. pEEG-LF, calculated at the selected DTP and NTP. Symbol ●●● indicates statistically significant differences ($P<0.001$) between pEEG-LF vs. pEMG-icm and pEMG-icm vs. pEEG-LF index N mean values. Error bars correspond to the standard error of mean. N° of computed signals = 163.

The variant of the surrogate data method used showed that, in both DTP and NTP, the index N measured significant interdependence between pEEG-LF and pEMG-icm. It also showed that the interdependence was of linear nature (Table 1). The mean value of the index N between pEEG-LF and pEMG-icm was greater ($P<0.001$) than that between pEMG-icm and pEEG-LF, in both DTP and NTP (Figure 5). This result indicates that there is an asymmetric coupling between both signals, and that this asymmetry is present both during DTP and NTP. According to the criterion stated in the Materials and Methods section, in both DTP and NTP, the pEEG-LF is more complex than the pEMG-icm. The asymmetry found between pEEG-LF and pEMG-icm during DTP was 12.8 % (median), whereas during NTP it was 19.7% (median). There were statistically significant differences between both percentages of asymmetry ($P<0.001$).

DISCUSSION

This study has been performed to investigate the existence of interdependence between the slow telencephalic electrical activity (assessed from the EEG of the medial cortex) and the respiratory activity (assessed from the EMG of the intercostal muscles) in lizards (*Gallotia galloti*) during long time periods, at daytime and nighttime. To do this, simultaneous computations and comparisons of successive power spectral values of the low-frequency EEG and power values of the EMG-icm signal have been made. These power values are proportional to the amplitude of the slow cortical electrical activity and respiration, respectively. Traditional spectral techniques such as the coherence function, and more modern techniques, coming from the dynamics of nonlinear systems, were used to study the interdependence between both signals.

The presence of very high-power peaks in most pEEG-LF could reflect the existence of time periods of particularly intense cortical electrical activity of low frequency. These periods, which can last up to 10 seconds, occur with an arrhythmic pattern, and are present both during DTP and NTP. The fact that similar high-power peaks in the simultaneous pEMG-icm signals are not apparently present, and that their amplitude decreases during NTP, as also occurs with the mean amplitude of the pEEG-LF, could indicate that these peaks are not motion artifacts but a peculiar characteristic of the low-frequency cortical electrical activity of lizards. However, from the data available, which do not include any independent measure of the animal movements, it is not possible to ascertain the precise nature of such peaks.

Both the spectral function of coherence and the nonlinear index N showed that there is a significant linear correlation/interdependence between the power of the slow telencephalic electrical activity and the power of the respiratory activity. The index N, furthermore, showed that the interdependence found is asymmetrical; the pEEG-LF vs. pEMG-icm interdependence is greater than the pEMG-icm vs. pEEG-LF one.

According to the terminology proposed by Arnhold and collaborators ('99), this means that the system that generates the pEEG-LF power oscillations, probably the rudimentary neocortex, striatum, and/or thalamic centers, is more active or complex, than the system that generates the oscillations in the power of the respiratory activity–probably the respiratory center of the brainstem. By interpreting this result in the light of the dynamics system terminology used by Quian Quiroga et al. (2000), it can then be proposed that the rudimentary neocortex, striatum, and/or thalamic centers are driven by the power oscillations originated in the respiratory center of the brainstem, through interconnection with thalamic centers. Evidently, other physiological and/or behavioral factors, besides respiration, could also mediate the EEG activity through other neural connections.

In reptiles, the importance of a brainstem site of central respiratory rhythm generation, which integrates afferent inputs from central and peripheral receptors, has been stated in a number of studies performed in different reptilian groups (Naifeh et al.,'71; Takeda et al.,'86; Douse and Mitchell,'91; Taylor et al.,'99). In mammals, the common brainstem system regulates and integrates respiratory, cardiovascular and somatomotor systems, and the adjustment of central nervous activity (Rittweger,'99). In addition, the subcortical origin of slow EEG waves has been suggested in different studies carried out in mammals: these waves have been recorded in the ventro-lateral thalamic nucleus in close time relation with the activity of deep cerebellar nuclei (Steriade et al.,'71), in the reticular thalamic nucleus after disconnection from the cerebral cortex (Steriade et al.,'87), and in hyperpolarized thalamic neurons (Steriade et al.,'91).

A linear relationship between the brain and the lungs has also been reported in humans (Burioka et al., 2003) by calculating the *approximate entropy* (a measure of system complexity) of respiratory movement and EEG signals during different stages of consciousness. That study takes into consideration the possibility of the involvement of nonlinear dynamic processes in the coordination between brain and lungs, because the change in complexity of respiration depends in part on the complexity of the EEG. Moreover, in that study a linear relationship between brain and lungs was found, and it was explained by considering the existence of an independent third system (e.g. brainstem systems including that of the cholinergic system (Lydic,'96)) affecting both the brain and the lungs. These results from the present study can neither affirm nor reject the existence of that third system. However, as stated above, evidence suggests the existence of two asymmetric interdependent systems, so that the low-frequency cortical electrical activity is driven by the respiratory activity. Moreover, it was found that the characteristics of the interdependence between cortical electrical and respiratory activities remain during NTP. This finding means that the underlying physiological process that governs the interdependence under study is also active during NTP, thus being independent of the day-night clock.

It seems that the alert state of lizards modulates the amplitude of pEEG-LF peaks, since during NTP they are of lower amplitude than during DTP. In fact, at night the absence of light can reduce the alert's threshold, although the metabolic demands must be similar during DTP. This must be so because the lizards are at the same temperature during DTP as during NTP. Supporting this argument is the fact that the DTP/NTP change in pEEG-LF is considerable (approximately 60%), in comparison to the DTP/NTP change in the respiratory power (around 24%). In relation to the DTP/NTP change in pEEG-LF, a number of authors have reported that the EEG's amplitude of reptiles decreases as the activity is reduced, and is maximal during alert waking and minimal at rest (Tauber et al.,'68; Luttges and Gamow,'70; Flanigan et al.,'73; Hartse and Rechtschaffen,'82; Ayala-Guerrero et al.,'88; Huitrón-Reséndiz et al.,'97; Nicolau et al., 2000). In contrast to mammals, the increase of EEG's amplitude during alert periods has also been reported in fish (Enger,'57; Schad and Weiler,'59; Barthelémy et al.,'75; Shapiro and Hepburn,'76; Bullock and Corwin,'79; Laming,'80) and amphibians (Segura and De Juan,'66; Hobson,'67; Colombo et al.,'72; Huntley et al.,'78; Laming and Evert,'84). Therefore, rejecting the idea that the high-power peaks in the pEEG-LF signals are motion artifacts, it can be said that the time periods corresponding to the appearance of high-power peaks in pEEG-LF found in *Gallotia* during the DTP, could correspond to alert periods, where there is great sensory stimulation and/or complex behaviors are taking place. Power peaks of the pEEG-LF signals during the NTP decrease by approximately 60%, signifying much reduced nighttime stimuli or complex behaviors.

In conclusion, the respiratory activity variations of *G. galloti* appear to drive the dynamic activity of its rudimentary neocortex, striatum, and/or thalamic centers. In addition, the state of alert of the animal contributes fundamentally to modulation of the power of the pEEG-LF signal.

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LITERATURE CITED

Arnhold J, Grassberger P, Lehnertz K, Elger CE. 1999. A robust method for detecting interdependences: application to intracranially recorded EEG. Physica D 134:419–432.

Ayala-Guerrero F, Calderón A, Pérez M. 1988. Sleep patterns in a chelonian reptile (*Gopherus flavomarginatus*). Physiol Behav 44:333–337.

Barthelémy L, Peyraud C, Belaud A, Mabin D. 1975 Etude electroencephalographique de l'anguille (*Anguilla anguilla* L). J Physiol (Paris) 70:173–185.

Bendat JS, Piersol AG. 2000. Random data: analysis and measurements procedures. New York: Wiley.

Bullock TH, Corwin JT. 1979. Acoustic evoked activity in the brain of sharks. J Comp Physiol 129:223–234.

Burioka N, Cornélissen G, Halberg F, Kaplan DT. 2001. Relationship between correlation dimension and indices of linear analysis in both respiratory movement and electroencephalogram. Clin Neurophysiol 112:1147–1153.

Burioka N, Cornélissen G, Halberg F, Kaplan DT, Suyama H, Sako T, Shimizu E. 2003. Approximate entropy of human respiratory movement during eye-closed waking and different sleep stages. Chest 123:80–86.

Colombo JA, Negroni JA, Segura ET. 1972. Behavioral and EEG patterns of the female toad *Bufo arenarum* during artificial clasping. Physiol Behav 9:21–25.

De Vera L, González JJ. 1986. Effect of body temperature on the ventilatory responses in the lizard *Gallotia galloti*. Respir Physiol 65:29–37.

Douse MA, Mitchell GS. 1991. Time course of temperature effects on arterial acid-base status in *Alligator mississippiensis*. Respir Physiol. 83:87–102.

Enger PS. 1957. The electroencephalogram of codfish (*Gadus callarias*). Acta Physiol Scand 39:55–72.

Flanigan WF, Wilcox RH, Rechtschaffen A. 1973. The EEG and behavioral continuum of the crocodilian, *Caiman sclerops*. Electroenceph Clin Neurophysiol 34:521–538.

Hartse KM, Rechtschaffen A. 1982. The effect of amphetamine, nembutal, alpha-methyl thyrosine, and parachlorophenylalanine on sleep-related spike activity of the tortoise, *Geochelone carbonaria,* and on the cat ventral hippocampus spike. Brain Behav Evol 21:199–222.

Hegger R, Kantz H, Schreiber T. 1999. Practical implementation of nonlinear time series methods: the TISEAN package. Chaos 9:413–435. Software publicly available at the Internet site http://www.mpipks-dresden.mpg.de/~tisean/TISEAN_2.1/index.html

Hobson JA. 1967. Electrographic correlates of behavior in the frog with special reference to sleep. Electroenceph Clin Neurophysiol 22:113–121.

Huggins SE, Parsons LC, Peña RV. 1968. Further study of the spontaneous electrical activity of the brain of *Caiman sclerops*: Olfactory lobes. Physiol Zool 41:371–383.

Huitrón-Reséndiz S, Ríos C, Rojas P, Mexicano G, Ayala-Guerrero F. 1997. Effect of *p*-chlorophenylalanime (PCPA) on sleep and monoamines content in the brain of a lizard species. Brain Res 761:19–24.

Huntley A, Donnelly M, Cohen HB. 1978. Sleep in the western toad (*Bufo boreas*) Sleep Res 7:141.

Lambertz M, Vandenhouten R, Grebe R, Langhorst P. 2000. Phase transitions in the common brainstem and related systems investigated by nonstationary time series analysis. J Auton Nerv Syst 78:141–157.

Laming PR. 1980. Electroencephalographic studies on arousal in the goldfish (*Carassius auratus*) J Comp Physiol Psychol 94:238–254.

Laming PR, Evert JP. 1984. Unit activity, EEG changes and slow potentials in the brain of toads (*Bufo bufo*) in response to biologically significant stimuli. EEG synchrony and negative potential shifts as possible mediators of responsiveness. J Comp Physiol 154:89–101.

Langhorst P, Schulz B, Schulz G, Lambertz M. 1983. Reticular formation of the lower brainstem. A common system for cardiorespiratory and somatomotor functions: discharge patterns of neighbouring neurons influenced by cardiovascular and respiratory afferents. J Auton Nerv Syst 9: 411–432.

Le Van Quyen M, Adam C, Baulac M, Martinerie J, Varela FJ. 1998. Nonlinear interdependences of EEG signals in human intracranially recorded temporal lobe seizures. Brain Res 792:24–40.

Le Van Quyen M, Martinerie J, Adam C, Varela FJ. 1999. Nonlinear analysis of interictal EEG map the brain interdependences in human focal epilepsy. Physica D 127:250–266.

Luttges MW, Gamow RI. 1970. Garter snakes: studies on spontaneous and evoked brain responses with electroencephalography. Comm Behav Biol 5:115–130.

Lydic R. 1996. Reticular modulation of breathing during sleep and anesthesia. Curr Opin Pulm Med 2:474–481.

Lynn PA. 1979. An introduction to the analysis and processing of signals. London: The MacMillan Press Ltd. p. 80–84.

Mador MJ, Tobin MJ. 1991. Effect of alterations in mental activity on the breathing pattern in healthy subjects. Am Rev Respir Dis 144:481–487.

Molina-Borja M, González J, Gómez T, García C. 1986. 24–H entrainment and ultradian fluctuations in the activity of the lizard *Gallotia galloti galloti* (Sauria-Lacertidae). J Interdiscipl Cycle Res 17:295–305.

Naifeh KH, Huggins SE, Hoff HE. 1971. Effects of brain stem section on respiratory patterns of crocodilian reptiles. Respir Physiol 13:186–197.

Nicolau MC, Akaârir M, Gamundí A, González J, Rial RV. 2000. Why we sleep: the evolutionary pathway to the mammalian sleep. Prog Neurobiol 62:379–406.

Pereda E, Rial R, Gamundí A, González JJ. 2001. Assessment of changing interdependencies between human electroencephalograms using nonlinear methods. Physica D. 148:147–158.

Pereda E, Gamundí A, Nicolau MC, De Vera L, González JJ. 2002. Evidence of state-dependent interhemispheric relationships in lizard EEG during the awake state. IEEE Trans Biomed Eng 49:548–555.

Pereda E, Mañas S, De Vera L, Garrido JM, López S, González JJ. 2003. Non-linear asymmetric interdependencies in the electroencephalogram of healthy term neonates during sleep. Neurosci Lett 337:101–105.

Pfurtscheller G. 1976. Ultra-slow changes in the rhythmic activity within the alpha band and their probable origin. Pflugers Arch 367:55–66.

Phillipson EA, Bowes G. 1986. Control of breathing during sleep. In: Handbook of Physiology. The Respiratory System. Control of Breathing, vol. II. Bethesda, MD: American Physiological Society. p 649–689.

Prichard D, Theiler J. 1994. Generating surrogate data for time series with several simultaneously measured variables. Phys Rev Lett 73:951–954.

Quian Quiroga R, Arnhold J, Grassberger P. 2000. Learning driver-response relationships from synchronizations patterns. Phys Rev E 61:5142–5148.

Quian Quiroga R, Kraskov A, Kreuz T, Grassberger P. 2002. Performance of different synchronization measures in real data: a case study on electroencephalographic signals. Phys Rev E 65:041903.

Rittweger J. 1999. Common slow modulation of respiration, arterial blood pressure, and cortical activity during sleep onset while napping. Clin Physiol 19:221–229.

Sako T, Burioka N, Suyama H, Nomura T, Takeshima T, Shimizu E. 2001. Nonlinear behavior of human respiratory movement during different sleep stages. Chronobiol Int 18:71–83.

Schad H, Weiler IJ. 1959. Electroencephalographic patterns of the goldfish. (*Carassius auratus* L.). J Exp Biol 36:435–452.

Schiff SJ, So P, Chang T, Burke RE, Sauer T. 1996. Detecting dynamical interdependence and generalized synchrony through mutual prediction in a neural ensemble. Phys Rev E 54:6708–6715.

Schreiber T. 1997. Detecting and analyzing nonstationarity in a time series using nonlinear cross prediction. Phys Rev Lett 78:843–884.

Schreiber T. 1998. Constrained randomization of time series data. Phys Rev Lett 90:2105–2108.

Segura ET, De Juan A. 1966. Electroencephalographic studies in toads. Electroenceph Clin Neurophysiol 21:373–380.

Shapiro CM, Hepburn HR. 1976. Sleep in a schooling fish, *Tilapia mossambica*. Physiol Behav 16:613–615.

Steriade M, Apóstol V, Oakson G. 1971. Control of unitary activities in cerebellothalamic pathway during wakefulness and synchronized sleep. J Neurophysiol. 34:389–413.

Steriade M, Domich L, Oakson G, Deschenes M. 1987. The deafferented reticular thalamic nucleus generates spindle rhythmicity. J Neurophysiol. 57:260–273.

Steriade M, Dossi RC, Núñez A. 1991. Network modulation of a slow intrinsic oscillation of cat thalamocortical neurons implicated in sleep delta waves: cortically induced synchronization and brainstem cholinergic suppression. J Neurosci 11:3200–3217.

Tabachnik E, Muller NL, Bryan AC, Levison H. 1981. Changes in ventilation and chest wall mechanics during sleep in normal adolescents. J Appl Physiol 51:557–564.

Takeda R, Remmers JE, Baker JP, Madden KP, Farber JP. 1986. Postsynaptic potentials of bulbar respiratory neurons of the turtle. Respir Physiol 64:149–160.

Tauber ES, Rojas-Ramírez J, Hernández-Peón R. 1968. Electrophysiological and behavioral correlates of wakefulness and sleep in the lizard, *Ctenosaura pectinata*. Electroenceph Clin Neurophysiol. 24:424–433.

Taylor EW, Jordan D, Coote JH. 1999. Central control of the cardiovascular and respiratory systems and their interactions in vertebrates. Physiol Rev 79:855–916.

Verlander JM, Huggins SE. 1977. Computer analysis of the electroencephalographic activity of the caiman olfactory bulb. Electroenceph Clin Neurophysiol 42:829–834.