XV Latin American Symposium
on Chronobiology 2019
Colonia del Sacramento - Uruguay

# Social modulation on daily variability in electric behavior

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## ABSTRACT

Daily rhythms of behavior often result from the expression of a circadian rhythm modulated and synchronized by abiotic and biotic cues from the environment. Circadian rhythms allow living organisms to anticipate changes and allocate energy in order to cope with predicted events as well as to time behavioral displays in social contexts. Understanding the complexity of behavioral rhythmicity requires a more comprehensive analysis that takes into consideration the intricacy of natural environmental constrains, in the wide range of modulating factors operating in nature. Gymnotus omarorum is a pulse type gymnotiform widely distributed in Uruguay, which constantly displays an electric behavior, with a circadian rhythm of nocturnal increases in its rate of emission, that serves communicative and perceptual purposes. Given its fundamental role, the electric behavior needs to be a reliable signal especially in social contexts. In this report, we aim at analyzing the daily changes in the variability of the electric behavior as well as the modulatory effect of the social context on this variability.

**Keywords:** Circadian Rhythms; Electric Behavior; Electric Fish; Social Synchronization

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DOI: 10.5935/1984-0063.20200012

## INTRODUCTION

Daily rhythms of behavior often result from the expression of a circadian rhythm modulated and synchronized by abiotic and biotic cues from the environment. Light and temperature, but also predatory activity and conspecific interactions, are important synchronizers of endogenous rhythms[1,2]. Circadian rhythms allow living organisms to anticipate changes and allocate energy in order to cope with predicted events as well as to time behavioral displays in social contexts. Classical studies in precisely controlled laboratory conditions have led to a simplified, dichotomic view, of either nocturnal or diurnal patterns. This is especially so when reducing behavior to a single variable, the most commonly used being locomotor activity. Understanding the complexity of behavioral rhythmicity requires a more comprehensive analysis that takes in into consideration the intricacy of natural environmental constrains in the wide range of modulating factors operating in nature, as well as the different expressions of behavior displayed by individuals of the species[3].

Nocturnal fishes of the South American order Gymnotiformes are characterized by the emission of species-specific weak electric discharges that serve electrosensory and electrocommunication purposes[4,5]. These typical electric behavior consists of pulse discharges (the "electric organ discharge", EOD) continuously emitted by a specialized electric organ. A medullary pacemaker nucleus commands these emissions, setting the basal rate of the EOD (EOD-BR)[6] while modulated by central connections from pre-pacemaker structures. The EOD is a behavioral display that encodes information (in the waveform and frequency domains) about an individual's species identity, sex, and physiological state[7]. In addition, the EOD is the physical carrier of perceptually relevant sensory information[8]. Arousal state in weakly electric fish implies an increase in EOD basal rate hence increasing the availability of sensory information per unit of time. Exploratory movements, novelty detection, escape responses and even volition are associated with increases in EOD-BR[1]. As arousal coincides with the active phase of the day, the EOD-BR in these nocturnal animals increases during the night[1, 9-12].

*Gymnotus omarorum* is a pulse type gymnotiform widely distributed in Uruguay[13]. The nocturnal increase in EOD-BR is a circadian rhythm[14], depends on melatonin[15] and is expressed in the natural habitat even though the surrounding vegetation generates a constantly dark environment[1]. Moreover, the rhythmic nocturnal rise in EOD-BR is precisely timed to the daily maximum temperature of the water, rendering this cue the most likely environmental zitegeber. Social context is a fundamental circadian synchronizer in natural populations, however overlooked when analyzing the rhythmic expression of natural behaviors. The nocturnal increase in EOD-BR is indeed strongly influenced by the social context, which exerts a potent synchronization effect among animals in the same population[1].

Given its fundamental role the EOD needs to be a reliable signal, yet modifiable to accommodate the aforementioned necessary changes. Previous reports for this species have shown that the EOD-BR has an intrinsic low variability when measured in isolated animals in a resting condition at laboratory settings. Moreover, EOD-BR variability shows an exact correlation with the variability of the spontaneous discharge of the central pacemaker that triggers de EOD[16]. It is interesting to consider how is this system behaving in the natural habitat, in which individuals are challenged by a more complex and changing environment. A high variability in the rate of emission of the EOD likely increases its uncertainty as electrosensory signal, and hinders the performance of both, communicative and perceptual channels. In this report, we aim at analyzing the daily changes in the variability of the electric behavior and the modulatory effect of the social context on this variability.

## MATERIAL AND METHODS

Adult *G. omarorum*[13] (n=11) were used in natural settings including the recording of 6 individuals in natural conditions and 5 individuals isolated in shelters within the natural habitat (seminatural condition). All specimens were collected in Laguna del Sauce, Maldonado, Uruguay (34º 48' S, 55º 18' W). Fish were located using a fish detector, consisting in an electronic audio amplifier connected to a pair of electrodes, as described elsewhere[17]).

The experiments were conducted during the non-breeding season at the peri-equinox period, under a natural light-dark cycle of 12:12. Periodic light and temperature measures were taken each 30 minutes: a) inside the water under the natural vegetation and b) outside the water (HOBO-MicroDAQ: UA-002-08). Measurements range: Temperature: -20° to 70°C (-4° to 158°F); Light: 0 to 320,000 lux (0 to 30,000 lumens/ft2).

All research procedures complied with ASAP/ABS Guidelines for the Use of Animals in

Research and were approved by the Institutional Ethical Committee (Comisión de Ética en el Uso de Animales, Instituto Clemente Estable, MEC, 008/11).

### EOD-BR recordings in social context

EOD-BR was recorded during 72 hs from fish (n=6) placed in individual plastic nets with electrodes, under the natural vegetation, in their natural habitat. 30s recordings were made once an hour. Fish in these conditions are almost always detectable. As fish can move around while the electrodes in the plastic nets remain in a fixed position, the amplitude and waveform of their EOD usually changes. The natural social context is preserved as plastic meshes are electrically transparent, enabling the perception of conspecifics' electric signals. Conspecifics in this setting might be freely moving fish or other enclosed fish placed nearby.

### EOD-BR recordings in isolated animals

Fish (n=5) were placed in 50 L individual plastic tanks containing a shelter and equipped with a pair of electrodes attached to its sides. Given the size of the shelters, when recordings show stable EOD amplitudes, it can be reliably assumed that fish are still while sheltered and hence in a voluntary locomotor rest. EOD-BR was recorded for 30 seconds per hour during 24 h, only if fish were sheltered and hence still. Fish in this condition are isolated from the influence of conspecifics. Light and temperature were monitored as described earlier.

## Data processing and statistical analysis

The EOD was recorded through electrodes placed in the water, digitalized using standard computer soundcards and recorded with a custom developed Matlab (The MathWorks, Inc.) program which detects the moment of EOD occurrence.

EOD-BR was calculated as the inverse of the inter EOD intervals in the recordings and expressed in terms of the median ± MAD values. As fish differ in their individual EOD-BR an index (BRIn) was calculated to determine the increase between the EOD-BR measured an hour before sunset ($BR_{-60}$) and the values measured at sunset ($BR_{sunset}$), regardless absolute values for each fish. The global BRIn for the whole group was calculated as the median ± MAD value of individual indexes.

BRIn = (BR-60-BRsunset)/BR-60

In order to normalize the effect of water temperature on EOD-BR, values were corrected to a constant 20°C temperature by using the $Q_{10}$ value of 1.5 as calculated for electric fish[11]. $Q_{10}$ is a unitless quantity calculated as the factor by which the rate increases when the temperature (T) is raised by ten Celsius degrees.

Q10=EOD-BR*(T)/EOD-BR*(T+10)

Paired non-parametrical two-tailed Wilcoxon test was used for statistical analysis. Data are shown as median ± MAD.

We studied the differences in variability between day and night for all fish (social and isolated). Qualitative and quantitative analysis were performed to each set of data by implementing an ad hoc routine in Matlab. We calculated variance, and coefficient of variation according to the following equations:

Variance

$$\sigma^2 = \frac{1}{N-1}\sum_{i=1}^{N}(x_i - \overline{X_N})^2$$

Coeficient of variation

$$CV = \frac{\sigma}{\overline{X_N}}$$

Poincaré diagrams were plotted as a means to obtain a qualitative analysis of dispersion. Each observed value of a set of data is plotted against the following, i.e. each EOD's frequency value (BR) against the following (BR+1). These types of diagrams are intimately related to the system's variability and can be quantified. To mathematically characterize the information given by this diagram, an ellipse if fitted to the graph and the longitudinal and perpendicular axes (SD1 and SD2) of such ellipse are measured. The perpendicular (SD1) and longitudinal (SD2) radius of this ellipse represent long and short term variability of the system respectively. Poincaré diagrams were plotted separately for each individuals' day and night values of EOD-BR. Longitudinal and perpendicular axes were measured separately for each diagram giving a value for long term variability during night and day ($SD1_{night}$, $SD1_{day}$) and for short term variability during night and day ($SD2_{night}$, $SD2_{day}$).

## RESULTS

The EOD-BR of *G. omarorurm* consistently increases during the night in the natural habitat, with a mean EOD-BR 12% higher at sunset than 60 min before (EOD-BrIn=0,12 ± 0,05) ($FB_{sunset}$ vs FB-60; n=6; Wilcoxon test, p=0.02). Figure 1a shows the nocturnal increase EOD-BR for a single individual along the 72 hs recorded. This increase is paired with a decrease in variability as shown in the Poincare plot in 1b. Ellipses are fitted at the mean EOD-BR both for the night (29.3) and the day hours (27.1). Long and short term variability are measured by the longitudinal (SD1) and perpendicular (SD2) axes of the ellipses. SD1 values are is lower during the night ($SD1_{night}$ vs $SD1_{day}$, Wilcoxon n=6, p=0.02) and the same holds true for SD2 axes ($SD2_{night}$ vs $SD2_{day}$, Wilcoxon n=6, p=0,05) as shown in figure 1c. Night and day variance was calculated in order to quantify variability. Variance is also significantly lower at night across the population (Wilcoxon n=6, p=0.02) (figure 1d).

When individuals are recorded in the natural habitat inside plastic tanks social context is removed, not affecting the nocturnal increase in EOD-BR. Animals in this group show a mean EOD-BR 17.5% higher at sunset than in the previous hour (EOD-BrIn=0,175 ± 0.09) ($FB_{sunset}$ vs $FB_{-60}$; n=5; Wilcoxon test; p=0.04). Figure 2a shows the nocturnal increase EOD-BR for a single isolated individual along 24 hs. Poincare plots for isolated individuals show the aforementioned difference in EOD-BR, since the ellipse fitted to day values is centered at a lower (31.4) position that the ellipse fitted to the night values (34.1). Figure 1b shows an example individual. However, an analysis of both axes of the ellipses shows no difference in variability in these two conditions (figure 1c). Variance calculated for all the individuals shows no difference between day and night (figure 2d).

The EOD-BR variability decreases during the night exclusively in animals in social context. In order to compare this set of data, which do not have the same mean, we calculated the coefficient of variation. Figure 3 shows the variation coefficient for each condition both during the night and the day. In social animals the coefficient of variation is significantly lower during the night than during the day (Wilcoxon, n=6, p=0.02). Moreover, variability during the night is significantly lower in social animals than in isolated animals (Mann-Whintney, p=0.04).

## DISCUSSION

Natural behavior relays on a precise timing that allows synchronization among conspecifics and with the natural cycles of the environment. Circadian clocks alone are not enough to sustain these adaptive rhythms and need to be successfully modulated by the environment and social context. In nature, the behavioral repertoire has a wider range[3] and social interactions are a fundamental source of behavioral diversity. Social context exerts a synchronization effect on the dynamics of the daily cycle of electric behavior[1].

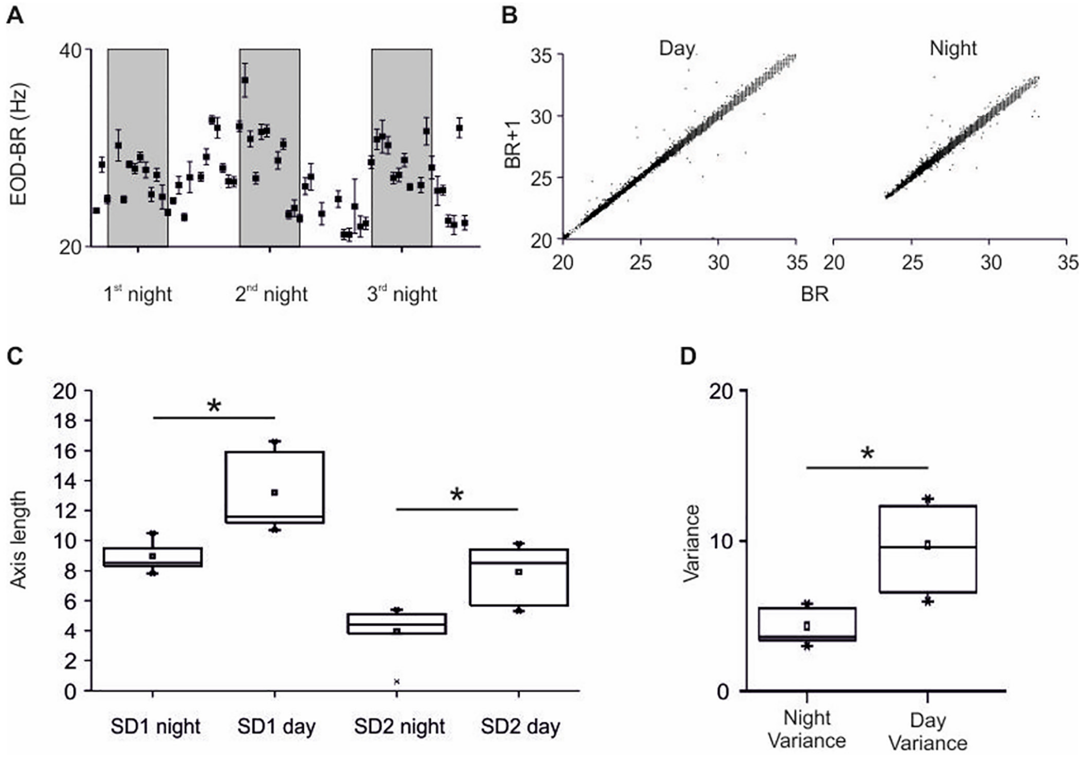

**Figure 1.** Daily analysis of the variability of EOD-BR in social fish. A) EOD-BR of a fish recorded in its natural habitat for 72 hs. Mean frequency values calculated for each hour swing daily with higher values at night (dark rectangles, sunset 7pm, sunrise 7 am). B) Poincaré plots for the day and night EOD-BR values of a single fish, showing the higher dispersion of daily values. C) Quantitative analysis of Poincaré plots based on the comparison of the longitudinal (SD1) and perpendicular (SD2) axes of an ellipse fitted around the points of the graphical representation shown in B. Variability in significantly lower during the night regardless the axis considered (SD1night vs SD1day, Wilcoxon n=6, p=0.02; SD2night vs SD2day, Wilcoxon n=6, p=0,05). D) Variance, a quantitative measure of variability also shows the lower night time variability (Wilcoxon n=6, p=0.02).

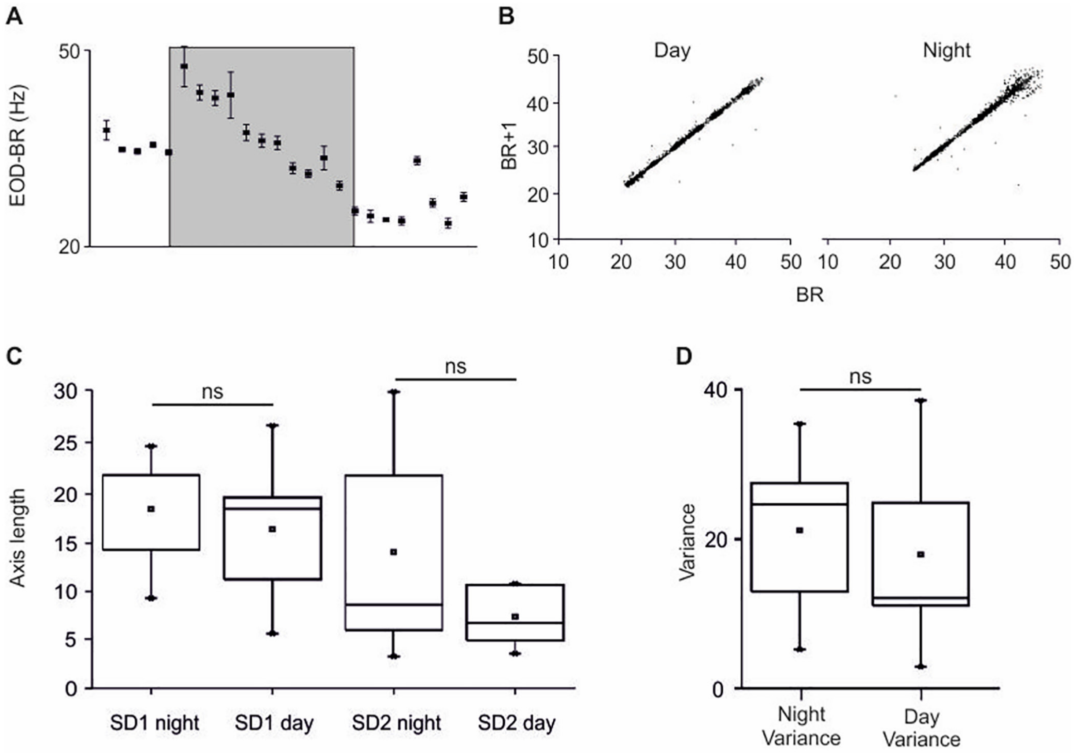

**Figure 2.** Daily analysis of the variability of EOD-BR in isolated fish. A) EOD-BR of a fish recorded in its natural habitat in isolation conditions for 24 hs. Mean frequency values calculated for each hour swing daily with higher values at night (dark rectangle, sunset 7pm, sunrise 7 am). B) Poincaré plots for the day and night EOD-BR values of a single fish, showing the relative dispersion of frequency values. C) Quantitative analysis of Poincaré plots based on the comparison of the longitudinal (SD1) and perpendicular (SD2) axes of an ellipse fitted around the points of the graphical representation shown in B. Variability is not significantly during the different phases of the day regardless the axis considered. D) Variance, a quantitative measure of variability also shows same variability during day and night.

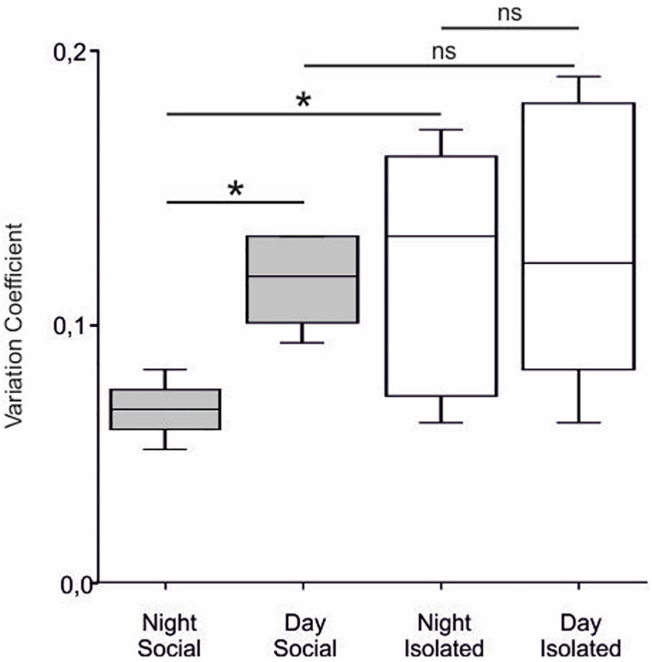

**Figure 3.** Coefficients of variation for night and day values of EOD-BR in social and isolated individuals. Social context lowers variability during the night (Night social vs Night isolated, Mann-Whintney, p=0.04) and allows the emergence of a daily difference of this trait (Night social vs Day social, Wilcoxon, n=6, p=0.02).

We show in this work that synchronization is paired by a reduction in variability which is particularly evident at night, when social interactions and exploratory activity tend to be more frequent[9]. At night, animals inhabit an enriched environment where information needs to be conveyed in a reliable and efficient way.

The EOD-BR of *G. omarorurm* increases during the night in both social and isolated animals. This increase, which has also been reported for animals in laboratory conditions[15], is sustained by a circadian rhythm of nocturnal increase[14]. The nocturnal increase in EOD-BR in laboratory settings is milder and shorter lasting than the one recorded in nature, evidencing the enhancing effect exerted by this enriched natural context. Moreover, social interactions, even the ones occurring in laboratory settings, have been shown to potentiate the nocturnal increase in electric behavior on the related species *Brachyhypopomus gauderio*[11].

A more detailed analysis of electric behavior reflects further effects of social context on its expression. When EOD-BR variability is assessed in a natural context there is a clear daily rhythm, with a significant nocturnal reduction in variability accompanying nocturnal increase in EOD-BR. However, when animals in the natural habitat are isolated from their conspecifics, time intervals between EODs became more variable regardless of daytime. This finding adds new evidence to the claim for the importance of taking into account different behavioral parameters for temporal analysis, as well as to establish the role of the multiple different factors that play a relevant timing role in the natural habitat.

The EOD is a complex signal that carries information serving communicative and perceptual goals. Since the variability of a signal correlates with its entropy[18] or uncertainty, a greater regularity in nocturnal EOD-BR might be ensuring a more reliable scenario for electrocommunication and electroreception. Moreover, social context in other teleosts, as well as in mammals, has been shown to induce a reduction in variability and quantity of locomotor and exploratory activity[19, 20], suggesting a common effect of conspecific presence on motivation for behavioral displays. Unpublished data from our group shows the same effect of social context on locomotor activity, in the annual fish *Austrolebias reicherti*. Behavior, in order to provide an advantage for the species displaying it, needs to be adequately timed, a challenging task in a variable environment. However, the natural variability of environmental clues and social context yields a coordinating and timing effect on behavioral displays, which, when expressed in nature, tend to be more organized and less variable giving rise to a more adaptive repertoire.

## ACKNOWLEDGEMENTS.

The authors wish to thank the organization of the XV Latin American Symposium on Chronobiology for including this work in the Symposium's special issue

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