Evidence for age-related changes in the circadian activity rhythm of the diurnal primate Callithrix jacchus: a case report


To cite this article: Fabiana B. Gonçalves, Galileu R. Borges, Bruno S.B. Gonçalves, Jeferson S. Cavalcante, Alexandre A.L. Menezes & Carolina V.M. Azevedo (2015): Evidence for age-related changes in the circadian activity rhythm of the diurnal primate Callithrix jacchus: a case report, Biological Rhythm Research

To link to this article: http://dx.doi.org/10.1080/09291016.2015.1129695

Accepted author version posted online: 09 Dec 2015.

Submit your article to this journal

View related articles

View Crossmark data
Evidence for age-related changes in the circadian activity rhythm of the diurnal primate *Callithrix jacchus*: a case report

Fabiana B. Gonçalves\textsuperscript{a,b}, Galileu R. Borges\textsuperscript{a}, Bruno S.B. Gonçalves\textsuperscript{a,c}, Jeferson S. Cavalcante\textsuperscript{d}, Alexandre A.L. Menezes\textsuperscript{a} and Carolina V.M. Azevedo\textsuperscript{a}

(Received 22 October 2015; final version received 29 November 2015)

\textsuperscript{a}Laboratório de Cronobiologia, Departamento de Fisiologia, Universidade Federal do Rio Grande do Norte, Natal, RN, Brazil.
\textsuperscript{b}Escola Multicampi de Ciências Médicas do Rio Grande do Norte, Universidade Federal do Rio Grande do Norte, Caicó, RN, Brazil.
\textsuperscript{c}Laboratório Interdisciplinar de Neurociências Clínicas, Universidade Federal de São Paulo, SP, Brazil.
\textsuperscript{d}Laboratório de Estudos Neuroquímicos, Departamento de Fisiologia, Universidade Federal do Rio Grande do Norte, Natal, RN, Brazil.

Fabiana B. Gonçalves  
Escola Multicampi de Ciências Médicas do Rio Grande do Norte  
Universidade Federal do Rio Grande do Norte  
Post Office Box 1511; Zipcode 59300-000. Caicó/RN- Brazil  
Tel: +55 84 3342-2337  
E-mail: fabiana@emcm.ufrn.br

Galileu R. Borges  
Departamento de Fisiologia – Centro de Biociências  
Universidade Federal do Rio Grande do Norte  
Post Office Box 1511; Zipcode 59078-970. Natal/RN - Brazil  
Tel: +55 84 3215-3409  
Fax: +55 84 3211-9206  
E-mail: galileuborges@yahoo.com.br

Bruno S. B. Gonçalves  
Departamento de Psiquiatria  
Universidade Federal de São Paulo  
Zipcode: 04039-032. São Paulo/SP - Brazil  
Tel:+55 11 5576-4845  
E-mail: brunocrono@hotmail.com

Jeferson S. Cavalcante  
Departamento de Fisiologia – Centro de Biociências  
Universidade Federal do Rio Grande do Norte  
Post Office Box 1511; Zipcode 59078-970. Natal/RN - Brazil  
Tel: +55 84 3215-3409  
Fax: +55 84 3211-9206  
E-mail: jefsc@uol.com.br

Alexandre A.L. Menezes  
Departamento de Fisiologia – Centro de Biociências  
Universidade Federal do Rio Grande do Norte
Abstract
Many cross-sectional studies have shown that circadian rhythms change with age, but such age-related modifications are gradual and may be insufficiently described by cross-sectional studies. In the present case study, circadian activity rhythm (CAR) was evaluated longitudinally, in both LD (12:12) and LL conditions, on two occasions in a single male marmoset: when “adult” (3 y.o.) and when “old” (9 y.o.). When adult, the CAR synchronized with positive phase angles for the onset and offset of activity. In LL, the rhythm free-ran with $\tau < 24$ h. When old, the animal showed a significant phase delay of its activity rhythm with respect to the LD cycle (t-test, $p<0.01$) and a reduction on total daily activity (t-test, $p<0.01$), with signs of lesser stability, greater fragmentation and some loss of photic synchronization. In LL, the period free-ran with $\tau > 24$ h. We conclude that aging attenuates photic synchronization and the expression of a circadian activity rhythm in LL in the marmoset. Further studies with a larger number of individuals are needed to confirm these findings.

Key Words: Aging; Synchronization; Ontogenesis; Non-human primates; Marmoset.

1. Introduction
The circadian activity rhythm (CAR) shows age-related changes in rodents and primates that are characterized by a reduction in amplitude, an increase in activity fragmentation (Aujard et al. 2007; Gutman et al. 2011; Zhdanova et al. 2011; Gomez et al. 2012), and variation in the intrinsic free-running period which is associated with different phase relationships between activity onset/offset and the light-dark cycle (Valentinuzzi et al. 1997; Aujard et al. 2007; Zhdanova et al. 2011). Considering the inter- and intra-specific differences in the aging process, a longitudinal chronobiological study may contribute to a more reliable description of age-related and gradual changes in biological rhythms than a transverse study.

The close phylogenetic relationship between marmosets and humans, as well as their similarity in development and lifetime, make marmosets a good model for studies of aging (Fischer & Austad 2011). In the first weeks of life, this diurnal primate shows a progressive increase in the power of circadian activity that becomes stable around 16 weeks after birth (Menezes et al. 1996). After puberty onset, the juveniles show higher levels of activity and a phase delay of their activity phase relative to the light phase (Melo 2012). In adulthood, the CAR has been well characterized, with an endogenous
period less than 24 h (23.2 ± 0.3h) and an activity phase which lasts around 11 h and has a positive phase relationship to the LD cycle (Erkert 1989). However, the circadian pattern of activity during aging is unknown.

Despite the potential advantages of using the marmoset as an experimental model for aging and of the longitudinal approach to ontogenetic studies, long-term follow-ups upon a large number of individuals is very challenging. Here, we present the first longitudinal description of the circadian activity rhythm in one marmoset, measured in adulthood and old age. The results suggest age-related modifications in the generation and photic synchronization of circadian activity in this primate.

2. Methods

The project was approved by the Ethics Committee on Animal Use of UFRN - protocol nº 022/2011.

The data were obtained from one male marmoset, when adult (3 y.o.; Gonçalves et al. 2009) and again when older (9 y.o.). On both occasions, the animal was studied: A. in an LD cycle (12:12 ~350:2lx; when adult, for 25 days; when old, for 20 days); B. in LL (~350lx; when adult, for 10 days; when old, for 50 days); and C. during resynchronization to the LD cycle (for 10 days at both ages). Recordings of motor activity were made as in previous studies (Gonçalves et al. 2009; Silva et al. 2014).

In addition to the methods previously used in adult marmosets to assess the activity rhythm - $\psi_{\text{onset}}$, $\psi_{\text{offset}}$ and $\alpha$, see Gonçalves et al. 2009, Silva et al. 2014 - we propose to use other phase markers in the current study due to the impossibility to calculate the $\psi_{\text{onset}}$ and $\psi_{\text{offset}}$ in old age. These new parameters included the Center of Gravity (CoG) of the activity phase that was obtained by the program El temps (A. Díez-Noguera, Universitat de Barcelona, http://www.el-temps.com), and corresponds to the point when the sum of activity from the onset of active phase to this point is the same of the sum of activity from this point to the end of the active phase. The other phase marker was the time of start of the most active 10-hour interval (M10) that was obtained from the total of activity during the most active 10-hour interval (M10). In addition, there were calculated the interdaily stability (IS) and the intradaily variability (IV) (see Aujard et al. 2007 for more details); and the total daily activity. The Hurst coefficient (H) was used to evaluate the fragmentation of rhythm. This parameter is a...
measure of randomness in a data series with values varying between 0 and 1, values near to 0.5 indicate aleatory noise, i.e., higher fragmentation.

The period in LD and LL conditions was obtained by Lomb-Scargle periodogram using the *El temps*. The rate of resynchronization to LD after LL was evaluated from the number of transient days until the reestablishment of a phase relationship between the onset of active phase and lights-on ($\psi_{\text{onset}}$) of ±30min.

For analysis, 10-days intervals of each light condition were compared between ages by dependent Student t-test ($p \leq 0.05$). At the transition of LD and LL, the last 10 days before and the 10 days after were used. Results are presented as mean ± SD.

3. Results

In adult age, the CAR synchronized to the LD cycle ($T = 24h$) with positive angles at the onset ($\psi_{\text{onset}} = 13.5 \pm 9.6\text{min}$) and offset of the active phase ($\psi_{\text{offset}} = 209.5 \pm 57.1\text{min}$) with respect to lights-on and lights-off, respectively (Figure 1A). In LL, the rhythm free-ran ($\tau = 23.8h$ - Figure 1B) and, after re-exposure to an LD of 24h, there were five transient days until photic resynchronization, with positive phase angles of $\psi_{\text{onset}} = 38.0 \pm 41.6\text{min}$ and $\psi_{\text{offset}} = 250.5 \pm 84.0\text{min}$ with lights-on and lights-off, respectively, being established (Figure 1C). In all three conditions, the duration of the active phase was approximately 9 h.

In old age, it was impossible to calculate the $\psi_{\text{onset}}$ and $\psi_{\text{offset}}$, and consequently, the duration of active phase. In the initial LD condition, the animal synchronized to LD ($T = 24h$), but a different phase relationship was established between the active and the light phases. From the new phase markers used, it was possible to observe a delay in the phase angle differences between CoG and lights-on (adult: $-267.2 \pm 42.5\text{min}$; old: $-726.8 \pm 86.5\text{min}$; t-test = 15.5; $p < 0.01$), and the diurnal activity moved partly into the dark phase (Figure 1D). Also, a delay was observed in the *M10*, which occurred around 6 h ($395.5 \pm 37.6\text{min}$) after lights-on in the marmoset when old, but only about 30 min ($25.7 \pm 17.4\text{min}$) after lights-on when adult (t-test = -32.08; $p < 0.01$). In LL, the period lengthened to 24.1h (Figure 1E) and, on being re-exposed to a 24-h LD cycle, activity did not resynchronize, showing a period of 24.8h (Figure 1F).

In old age, there was reduction in the total daily activity compared to adulthood in all three conditions (Figure 1G). From a qualitative description, there was observed a great reduction on *M10* in LD (19657 vs. 5855u.a.) and LL (11493 vs. 1960u.a.), and an increase on rhythm fragmentation (LD - IV: 0.51 vs. 1.01u.a; H: 0.9 vs. 0.8u.a and LL -
IV: 0.66 vs. 1.07u.a.; H: 0.9 vs. 0.8u.a) and a decrease on rhythm stability (LD - IS: 0.55 vs. 0.18u.a. and LL - IS: 0.58 vs. 0.21u.a.).

4. Discussion

The longitudinal data obtained from this animal showed that the pattern of activity rhythm observed in adult marmosets (Erkert 1989, Gonçalves et al, 2009) changed significantly over the course of six years. In old age, this diurnal primate expressed lower levels of activity as seen from the difference in the total daily activity and the qualitative analysis of M10. A similar reduction in activity rhythm with aging has been observed in rodents (Valentinuzzi et al. 1997) and other non-human primates (Aujard et al. 2007; Zhdanova et al. 2011). This age-related attenuation may be associated with a reduction in rhythm stability (IS) and increase in its fragmentation (IV and H), as suggested from a qualitative analysis where we observed signs of a fall in IS, a rise in IV and fall in H, similar to findings in another non-human primate (Aujard et al. 2007).

With regard to the new phase markers that were investigated (CoG and M10), aging was associated with a significantly delayed rhythm and higher instability during photic synchronization. In addition, the free-running period lengthened, probably due to age-related changes on rhythmicity generation (Gutman et al. 2011). These modifications may be due to specific effects of aging on synchronization and the generation of biological rhythms, as evidenced in previous studies on middle-aged rats (Weinert & Weinert 1998).

The phylogenetic proximity and the diurnality of marmosets highlight this species as a good animal model for aging studies in humans. Despite the advantages of a longitudinal approach when describing the development of age-related physiological changes on rhythmicity, the present results obtained should be interpreted cautiously since they were limited to data obtained from one animal. The hypotheses raised in this exploratory study need to be tested in more animals to confirm the observations as well as to establish causal relationships.

References

Figure 1: Actograms of the circadian activity rhythm of the same male marmoset when 3 y.o. (adult: parts A, B and C) and 9 y.o. (old: parts D, E and F) in LD 12:12 (parts A and D), LL (parts B and E) and during LD resynchronization (parts C and F). The Center of Gravity is marked by lines on the actograms. The total daily activity (mean ± SD) is represented in part G of figure. *Between-age comparisons were analyzed using dependent Student’s t-test with p < 0.01.