



Circadian activity rhythm in pre-pubertal and pubertal marmosets (*Callithrix jacchus*) living in family groups



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HIGHLIGHTS

- In marmosets, entering puberty is associated to changes on circadian activity rhythm.
- Puberty is associated to a phase delay on active phase and an increase in total daily activity.
- There are sex differences on the changes on the activity offset and in the total daily activity after entering puberty.
- The juveniles show a delayed active phase in relation to adults.
- The juveniles have a higher total daily activity and higher evening activity than adults.

ARTICLE INFO

Article history:

Received 22 October 2015

Received in revised form 4 December 2015

Accepted 23 December 2015

Available online 24 December 2015

Keywords:

Puberty

Ontogenesis

Phase delay

Sex differences

Non-human primates

ABSTRACT

In marmosets, a phase advance was observed in activity onset in pubertal animals living in captivity under semi-natural conditions which had stronger correlation with the times of sunrise over the course of the year than the age of the animal. In order to evaluate the effect of puberty on the circadian activity rhythm in male and female marmosets living in family groups in controlled lighting conditions, the activity of 5 dyads of twins (4 ♀/♂ and 1 ♂/♂) and their respective parents was continuously monitored by actiwatches between the 4th and 12th months of age. The families were kept under LD 12:12 h with constant humidity and temperature. The onset of puberty was identified by monitoring fecal steroids. Juveniles showed higher totals of daily activity and differences in the daily distribution of activity in relation to parents, in which the bimodal profile was characterized by higher levels in evening activity in relation to morning activity. Regarding the phase, the activity onset and offset, occurred later in relation to parents. After entering puberty, the activity onset and offset occurred later and there was an increase in total daily activity. On the other hand, when assessing the effect of sex, only females showed a delay in the activity offset and an increase in total daily activity. Therefore, the circadian activity rhythm in marmosets has peculiar characteristics in the juvenile stage in relation to the total of daily activity, the onset and offset of the active phase, and the distribution of activity during this phase. Besides, the entering puberty was associated with a phase delay and increase on total daily activity, with differences between sexes, possibly due to hormonal influences and/or social modulation on rhythm.

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1. Introduction

In mammals, the temporal allocation of biological rhythms in relation to the main *zeitgeber*, the light–dark cycle, can be characterized by changes during ontogenesis. In relation to adulthood in which the circadian rhythm is well established, the first days and months after birth are marked by rhythms with ultradian periodicity [1–3]. At the other end of the development, senescence is characterized by

modifications in the expression of circadian rhythms, such as a shortening of the endogenous period [4–7], advance in the beginning and end of the active phase [8], decrease in the amplitude [9–8] and an increase in fragmentation [8]. Many studies consider that once circadian rhythmicity is established in infancy, rhythms do not change until senescence. However, there is evidence that there are temporal changes in the allocation of circadian rhythms in relation to the LD cycle associated with puberty [10–13]. In humans during adolescence for example, there is a tendency to sleep and wake up later than in childhood and adulthood, which features a phase delay [10–11].

The phase changes during puberty are not unique to the human species, since phase delays are observed during puberty in the circadian rhythms of different variables and species such as 1) activity, in rhesus

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monkeys [12], mice [15–16], laboratory rats [17–18] and degus [14,19,20]; 2) temperature, in mice [14–15] and fat sand rats (*Psammomys obesus* [21]; and 3) oxygen consumption, in fat sand rats [21].

In contrast, marmosets living in semi-natural environmental condition showed a phase advance on circadian activity rhythm after entering in puberty. In this condition, the beginning of active phase had a stronger correlation with the sunrise times than with age. Thus, this advance may have been due to a seasonal modulation [23]. In this species, the effect of seasonality was also described on the diurnal distribution of activity which is characterized by a bimodal profile due to the presence of two activity peaks; one in the morning and one in the afternoon, with reduced activity around the mid-day [24]. This profile becomes more pronounced in the warmer months of the year both in adult animals [25] and juveniles [23]. Therefore, to evaluate the effect of puberty on the circadian activity rhythm in this diurnal primate, it is necessary to isolate the seasonal cues by keeping the animals under controlled conditions of light, temperature and humidity.

Few studies evaluated the effect of sex on changes in circadian activity rhythm during puberty. Sex differences have been observed in degus [14] and humans [22]. In degus, the onset of activity is more delayed in females than males after 7 months of age [14]. In humans, girls have a greater delay in their sleep/wake cycle than boys in their early teens. As puberty progresses, the boys' cycle becomes later than girls [22]. Therefore, it is important to analyze the effect of sex on changes of circadian activity rhythm during puberty.

Marmosets are diurnal neotropical primates that present the pubertal development slower than rodents and faster than the old world primate, the rhesus monkey. This aspect associated to 1) the social characteristics of this species, 2) the phylogenetic proximity to humans, 3) the low maintenance cost and 4) the easy adaptation to captivity [24,26,27], makes the marmoset an animal model used in various researches in the biomedical field [26] and a good model for analyzing changes on circadian activity rhythm in puberty.

In this species, the circadian activity rhythm stabilizes at around 4 months of age [28], with the active phase characterized as starting just before the light and finishing within 2 h before dark in adult animals, living in the wild [29] and in artificial light–dark cycles of 12:12 [7,30]; and in juveniles [23] and adults [24–25], living in semi-natural conditions. However, the characteristics of activity rhythm in juveniles under controlled environmental conditions are unknown.

Regarding the social characteristics of the species, marmosets live in groups of up to 15 individuals in the natural environment [31]. In these groups, there is a breeding pair that exerts dominance over the other individuals [32], including reproductive suppression of subordinates [33–34]. Females have stronger hierarchical relationships than males. Between females, there is inhibition of sexual behavior and neuroendocrine inhibition of ovulation. Between males, this suppression can be only behavioral, when occurs between fathers and sons, but also involves neuroendocrine suppression of testicular function, when occurs between unrelated males [26]. In addition, gestations (usually of twins) last about 5 months and the parental care is shared by all members of the group [35–38,27]. Based on behavioral and physiological aspects, Abbott et al. categorized 3 stages from infancy to adulthood: infants (0–3 months), juvenile (3–12 months) and subadult (13–18 months); puberty is allocated between the juvenile and subadult stages, with differences between sexes, starting at around 6 months in females and 7 months in males, ending at approximately 16 months in both sexes [26].

Puberty is associated to phase changes in circadian activity rhythms in rodents [14–21], an old world primate specie [12] and humans [10–11], that are characterized by a phase delay. Besides, sex-related differences were observed in rodents and humans. In contrast, we observed a phase advance in common marmosets, but it was not possible to affirm if this was due to seasonal or pubertal effects [23]. Also, the social context of marmoset raises the possibility of sexual differences in rhythm associated to puberty. Therefore, to evaluate the effect of

puberty and sex-related differences in circadian activity rhythm in common marmosets, we analyzed the activity patterns of juveniles and adults of both sexes living in family groups in a controlled light–dark cycle of 12:12 h under constant temperature and humidity conditions. We hypothesize that in marmosets 1) the parameters of circadian rhythm changes after puberty onset, with differences between sexes; and 2) the juveniles show differences in the rhythmic parameters in relation to parents.

2. Methods

2.1. Subjects and maintenance conditions

The experiment was conducted with five families, consisting of a dyad of twins (4 ♀/♂ and 1 ♂/♂) and their parents. During data collection another dyad of twins was born in family 2 which also became part of the group, but was not included in data collection (Table 1). The families were kept in a laboratory at the Primatology Colony of Universidade Federal do Rio Grande do Norte under a light–dark cycle of 12:12 h (lights on: 07:00 h, ~350 lx; lights off: 19:00 h, ~8 lx), and controlled temperature (27.3 ± 0.7 °C) and relative humidity ($58.2 \pm 8.3\%$) conditions. These lighting conditions were provided by white fluorescent bulbs at light phase and by an incandescent yellow light at dark phase. Each family was housed in a cage measuring $1.2 \text{ m} \times 1.0 \text{ m} \times 1.0 \text{ m}$ in a room of measuring $2.95 \text{ m} \times 2.77 \text{ m} \times 5.70 \text{ m}$. Families were visually isolated, but with acoustic and olfactory contact. Families were introduced to the experiment room when the juveniles turned 3 months and 15 days, and remained in the experimental room for 8 months at different times over the two years of the experiment as entrance into the room was determined from the date of the birth of juveniles. For most of the months during the experiment there were 3 families simultaneously sharing the room (Table 1). For enriching their environment, each cage contained a nest box ($25 \text{ cm} \times 30 \text{ cm} \times 21 \text{ cm}$), and perches and swings made of wood and plastic.

Water and raisins were available ad libitum. Additionally, other food items were available during the light phase. In the early light phase between 07:00 and 09:00 h, the animals received a nutritional mixture (milk, boiled eggs, bread, bananas and vitamins A, C, E and D supplements). Food leftovers were replaced between 13:00 and 15:00 h by fruit, shredded chicken or tropical sweet potatoes according to a weekly

Table 1
Composition of families.

Family	Animals	Sex	Age group	Dates of data collection	Dates and age of puberty onset
1(F1)	Genilson	♂	Juvenile	June/2009 to February/2010	September 16 (7 months)
	Geraldo	♂	Juvenile		
	Giva	♀	Adult	September 2 (6 months)	
	Guará	♂	Adult		
2(F2)	Florise	♀	Juvenile	September/2009 to May/2010	November 9 (5 months)
	Floriano	♂	Juvenile		
	Flor	♀	Adult	December 23 (7 months)	
	Crivo	♂	Adult		
	Francinilda	♀	Infant		
3(F3)	Francil	♂	Infant	February to October/2010	June 21 (7 months)
	Alma	♀	Juvenile		
	Alisson	♂	Juvenile		
	Andréia	♀	Adult		
	Fontes	♂	Adult		
4(F4)	Amália	♀	Juvenile	April to December/2010	May 25 (5 months)
	Amaro	♂	Juvenile		
	Afrodite	♀	Adult		
	Ravi	♂	Adult		
	Oboé	♀	Juvenile		
5(F5)	Oboé	♀	Juvenile	June/2010 to February/2011	September 9 (7 months)
	Odara	♂	Juvenile		
	Ozelita	♀	Adult		
	Zonta	♂	Adult		

menu. In addition, supplements such as arabic gum, granola, condensed milk, and insects (*Tenebrio molitor*) were added to these portions at this time. At the end of the light phase between 16:30 h and 18:00 h, leftover food was removed from the cage. The experiment was conducted in accordance with the rules of Animal Research of the Universidade Federal do Rio Grande do Norte, the Brazilian Society of Neuroscience and Behavior, and International Ethical Standards for Animal Rhythm research [39].

2.2. Experimental procedures

The motor activity of the animals was continuously monitored by actigraphy (Actiwatch model AW 16 MINIMITTER, Mini Mitter Company, Bend, Oregon, USA, containing 16 kb on-board memory). The actimeter was placed in a plastic container with the whole unit weighing 23 g, corresponding to a maximum of 10% of the weight of the juveniles. The assembly was placed on the animal's back [40] after their transfer to the experimental room. After a 15-day interval of adapting to using the actimeter and to the other experimental conditions, data collection was conducted for 8 consecutive months between the 4th and 12th months of the juvenile's life. Motor activity was binned and recorded at 5-min intervals, and the data were transferred to a file through the *Mini Mitter ACTIWATCH Reader version 3.4* software [Mini Mitter Company] each 30 days. For data transferring, the animal was attracted to a small cage and then, contained by a technician in marmoset husbandry to remove the actiwatch from the plastic container that remained in the back of animal. During data transferring, each animal received a positive reinforcement as arabic gum. After that, the actiwatch was placed in the plastic container and the animal was placed back in original cage.

To determine hormone levels and characterize the onset of puberty, feces were collected twice a week between 07:00–09:00 h between the 4th and the 12th months of the juveniles' life. During the interval of feces collection, a piece of clean paper was placed below the cage. Then, one of researchers positioned behind one-way glass waited for the evacuation of each juvenile. After that, the observer entered the experimental room and performed the collection of the sample. The concentration of fecal progesterone and estrogen in females and the metabolites of testosterone (androsterone, androstenedione, dehydroepiandrosterone, dehydroandrosterone, and epiandrosterone) in males was measured using the enzyme-linked immunosorbent assay (ELISA) developed by Munro et al. [41] and modified for use in feces by Sousa and Ziegler [42] in the Laboratory of Hormonal Measurements of the Universidade Federal do Rio Grande do Norte. Males were considered pubescent when the androgen level reached 1.5 times baseline and for females when the level of estrogen and progesterone reached 1.5 times baseline, remaining high for at least 10 days [23,43]. The presence of the ovarian cycles was utilized to confirm the beginning of puberty in females and was identified according to the criterion adopted by Ziegler and Sousa [44]. According to this criteria, the sustained rise at 1.5 times in relation to baseline levels of metabolites of gonadal hormones in the feces of adult female marmosets was used to determine the stage of ovarian cycle, reflecting the levels of gonadal hormones in the bloodstream showed by Harlow et al. 1984 [45]. The intra-assay variation coefficients for progesterone, estrogen and androgen were 0.86%, 0.26% and 1.28% and inter-assay coefficients of variation were 13.39%, 5.80% and 4.08%, respectively.

2.3. Data analyses

The following parameters were defined from the time series of motor activity composed by each 24 h-interval collected on a 5 min basis: (a) the onset and (b) the offset of the active phase, determined using the option *Serial Analysis of El Temps* program (A. Díez-Noguera, Universitat de Barcelona, <http://www.el-temps.com>) that calculate the two flanks of activity phase (the onset and the offset of active phase)

by a Heaviside function on continuous data [46]; (c) the duration of the active phase (α), obtained by the difference between the offset and the onset of the active phase; (d) the ratio EA/MA, that is the ratio between the amount of activity in the 3 consecutive hours of greatest activity (M3) between 15 and 21 h (evening activity – EA), and the M3 of the interval between 5 and 11 h (morning activity – MA) and (e) the total daily activity, which is the sum of the activity levels over 24 h.

Given that temporal profiles of daily activity in marmosets are usually bimodal, the ratio EA/MA allows a quantitative analysis of this profile. This parameter expresses the relation between the two peaks of activity of the animal in the activity phase, that is, identifies the moment that the animal concentrates most of the activity. Higher values in the ratio EA/MA represent higher levels of the activity in the evening peak in relation to the morning peak.

In order to assess age-related changes in the circadian activity rhythm in juveniles, three 30-day intervals were established along the juvenile phase (1–30 days, 60–90 days, 145–175 days) in all animals. In this analysis, the selected intervals represent different stages of youth development as are allocated in the beginning and middle of the juvenile phase. All parameters were compared, in a daily basis, between the sexes and ages (juveniles and adults) among the intervals of juvenile phase by Repeated Measures ANOVA, to better characterize ontogenetic differences. The animals of the families 1 and 2 were excluded from this analysis because of problems in night lighting during the intervals evaluated in this section which could interfere with the parameters of motor activity. Considering that the data collected in this range did not occur around puberty of the juveniles of these families, the data from these animals were included in the analysis of puberty.

To evaluate the effect of puberty and the sex-related differences on the circadian activity rhythm parameters, 2 intervals were compared before and after puberty in both sexes in all juveniles. To establish these intervals, the day of entering puberty (defined according to the hormonal criteria mentioned above) was considered as day 0. Data collected 10 days before and 10 days after that day were removed to minimize the effects of instability around the entrance into puberty for analysis. Therefore, two intervals were obtained: 10–40 days before puberty and 10–40 days after puberty. The parameters of circadian activity rhythm were compared between sexes before and after puberty, using Repeated Measures ANOVA.

In all cases, Tukey's test was applied as post-hoc procedure in the presence of statistically significant differences ($p < 0.05$).

3. Results

3.1. Characterization of the circadian activity rhythm along the juvenile stage

Juveniles showed higher levels of total daily activity than parents (Figs. 1D and 2 – $F_{(1, 335)} = 1408.8$, $p < 0.05$), in all intervals of juvenile phase (Fig. 1D – Tukey, $p < 0.05$). Furthermore, only juveniles increased the total daily activity along the intervals ($F_{(2, 670)} = 99.27$, Tukey, $p < 0.05$). Also, males showed higher levels of total daily activity than females ($F_{(1, 335)} = 52.01$, $p < 0.05$), in all intervals of juvenile phase (Tukey, $p < 0.05$) in both ages (Tukey, $p < 0.05$).

The onset ($F_{(1, 330)} = 14.16$, $p < 0.05$) and the offset of activity phase ($F_{(1, 330)} = 3.51$, $p = 0.06$) occurred later on juveniles in relation to adults. However, there is interaction between ages and sexes on these parameters (onset of activity phase: $F_{(1, 330)} = 4.46$; offset of activity phase: $F_{(1, 330)} = 16.84$, $p < 0.05$ – Fig. 1). This interaction is characterized by earlier onset and offset of activity phase in the adult male in relation to the other animals of the group (Tukey, $p < 0.05$).

Juveniles showed higher values in the ratio EA/MA than adults (Fig. 1 – $F_{(1, 330)} = 48.47$, $p < 0.05$), in all intervals (Tukey, $p < 0.05$) in both sexes (Tukey, $p < 0.05$). This result reflects the differences in the characteristics of the bimodal activity pattern between juveniles and adults. In

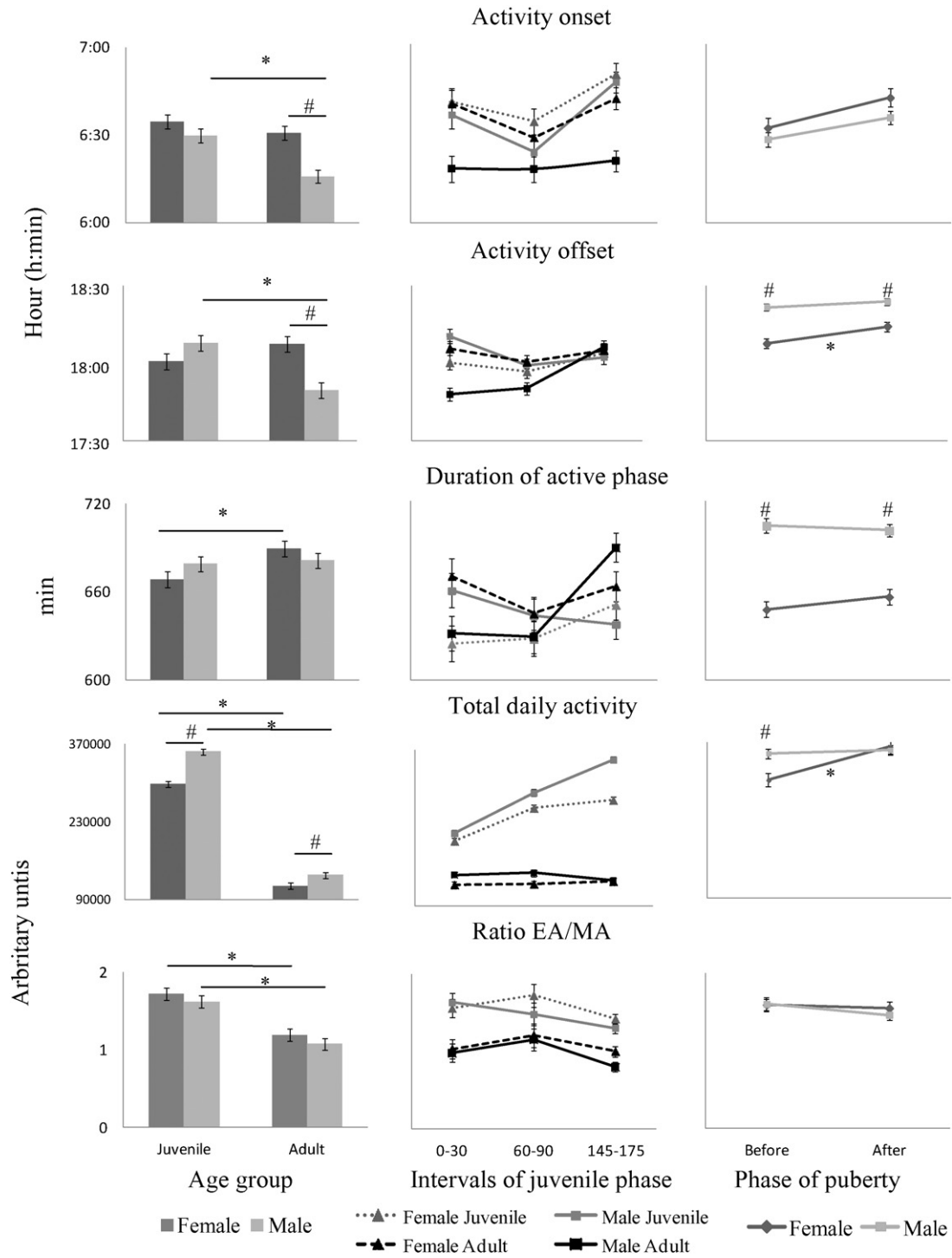


Fig. 1. Means (\pm standard error) of the onset, offset and duration of active phase (α); the total daily activity and the ratio EA/MA of each group age in both sex, of each group age and sex over the intervals of juveniles' age and of juveniles according to sex before and after puberty onset. * represents significant differences between the group age or phases in each sex and # represents significant differences between the sexes in each group age or phase of puberty, ANOVA, Tukey, $p < 0.05$.

juveniles, this pattern is characterized by higher levels of activity in the peak of evening in relation to morning, while adults show higher levels of activity in the morning peak (Fig. 3).

3.2. The effect of sex on circadian activity rhythm in pubertal animals

There was an increase in the total activity after entering puberty ($F_{(1, 269)} = 13.62$; $p < 0.05$). In addition, there is an interaction between sex and phase of puberty ($F_{(1, 257)} = 9.08$; $p < 0.05$ — Fig. 1). This interaction

is characterized by the higher levels of activity in males in relation to females before puberty (Tukey, $p < 0.05$). After puberty, only females increased the total activity reaching values similar to that found in males (Fig. 1D — Tukey, $p < 0.05$).

After entering puberty, the onset ($F_{(1, 257)} = 9.39$; $p < 0.05$) and the offset ($F_{(1, 257)} = 7.67$; $p < 0.05$) of active phase occurred later. In relation to sex differences, the onset of the activity ($F_{(1, 257)} = 3.44$, $p = 0.06$) showed a tendency and the offset of activity phase (Fig. 1 — $F_{(1, 257)} = 46.00$; $p < 0.05$) occurred later in males in relation to females.

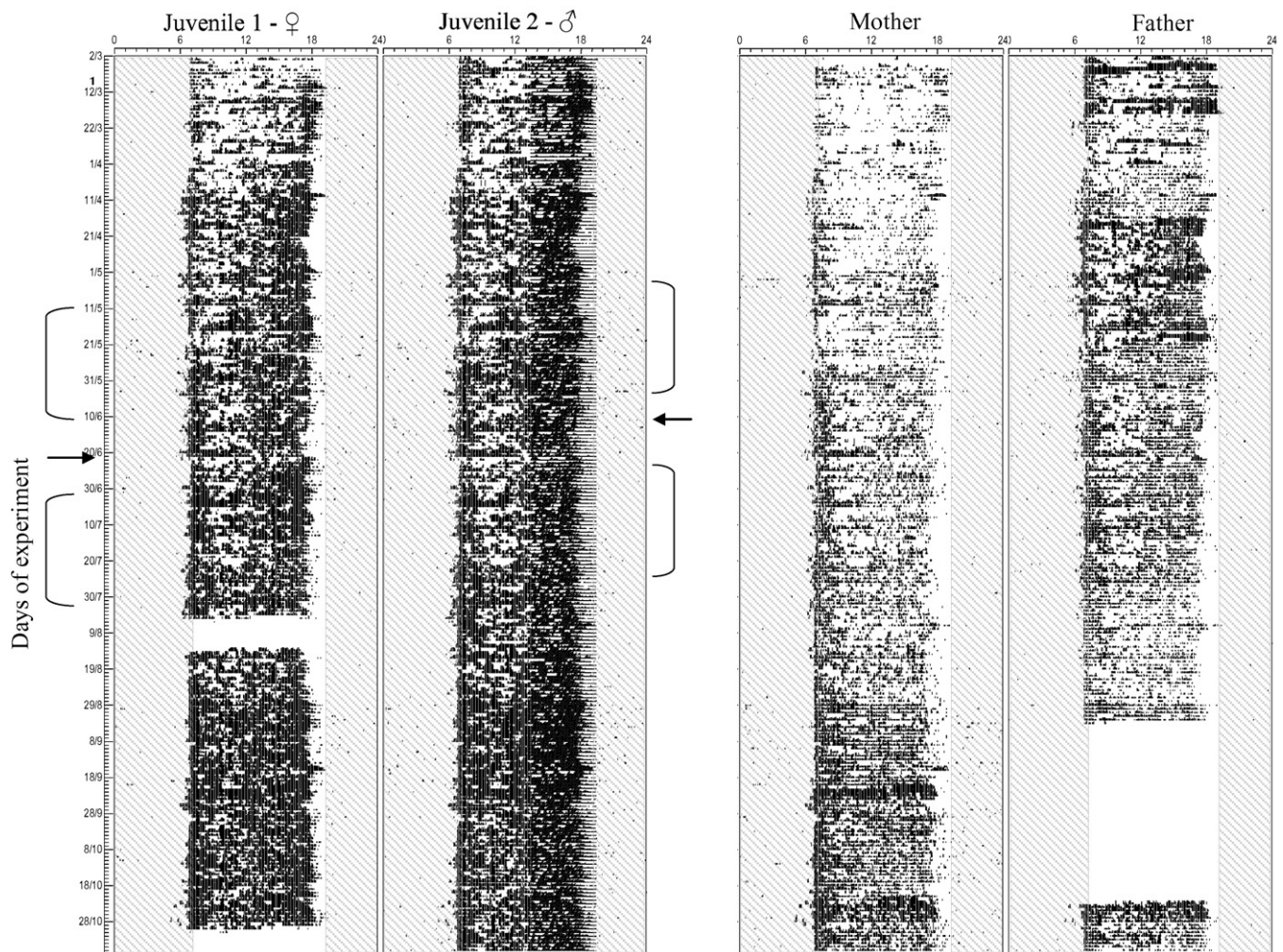


Fig. 2. Actograms of each animal of family 3 as representative of recordings before and after the puberty onset in all families. The blank spaces indicate missing data due to data collection problems. The arrows indicate the onset of puberty of each juvenile and lines represent the windows used for analysis of the puberty effect. Gray bars represent the dark phase.

Despite the absence of interaction between phase of puberty and sex, only females showed a delay on the offset of active phase ($F_{(1, 257)} = 2.10$; $p > 0.05$; Tukey, $p < 0.05$ – Fig. 1) after entering puberty.

In addition, the duration of activity phase ($F_{(1, 257)} = 0.09$; $p > 0.05$) did not change after entering into puberty. In relation to sex differences, females exhibited a shorter duration of activity phase than males ($F_{(1, 257)} = 80.80$; $p < 0.05$), independent of the phase of the puberty (Fig. 1 – $F_{(1, 257)} = 1.18$; $p > 0.05$).

Also, no differences in the ratio EA/MA occurred before and after entering puberty (Figs. 1 and 3 – ($F_{(1, 257)} = 1.04$; $p > 0.05$). This ratio is characterized by bimodal activity patterns with higher levels of activity in the peak of evening in relation to morning in both sexes and phases (Fig. 3).

4. Discussion

In this study, there was a delay in the onset and offset of the active phase after entering puberty in marmosets maintained in controlled light–dark cycle of 12:12 h under constant temperature and humidity conditions. This contrasts with the phase advance observed in a previous study in this specie under seminatural condition [23] that may have been due to seasonal modulation. The phase change observed in marmosets in the present study is in the same direction to that described for the end of the active phase in rhesus monkeys [47,12]; at the start and the end of the active phase in degus [19–20] and in lab

rats [17]; and the start and end of sleep in humans [10–11]. In humans, it is suggested that the phase delay in puberty is associated with pubertal development [11] and the influence of social factors [48–50]. In rats and degus, experimental manipulations indicate a modulation of circadian rhythmicity in puberty related to the increase of gonadal hormones [17,20].

Differently of previous studies [12,17,20,47], we evaluated the effect of puberty in animals maintained in their social context, i.e., in family groups. In this context, juveniles showed later activity onset and offset in relation to parents, particularly to the father. Different phase relationships to light–dark cycle are also observed between adults and adolescent in humans. In adolescent humans, the puberty-related phase delay can be of different magnitudes according to social influences associated to different patterns of light exposure at evening [48–49,51] and morning.

On the other hand, the social context of marmosets can act reducing the phase differences between juveniles and parents that were of small magnitude. In this species, the existence of differences at the beginning and end of the active phase between juveniles and adults of the same group cannot be adaptive to the social organization since temporarily displaced animals could be isolated from the group making them more susceptible to predators. The social synchrony between members of a marmoset group was observed in previous work in our laboratory, where the activity profiles of animals within a group were more correlated than between individuals among families [52]. The evaluation of

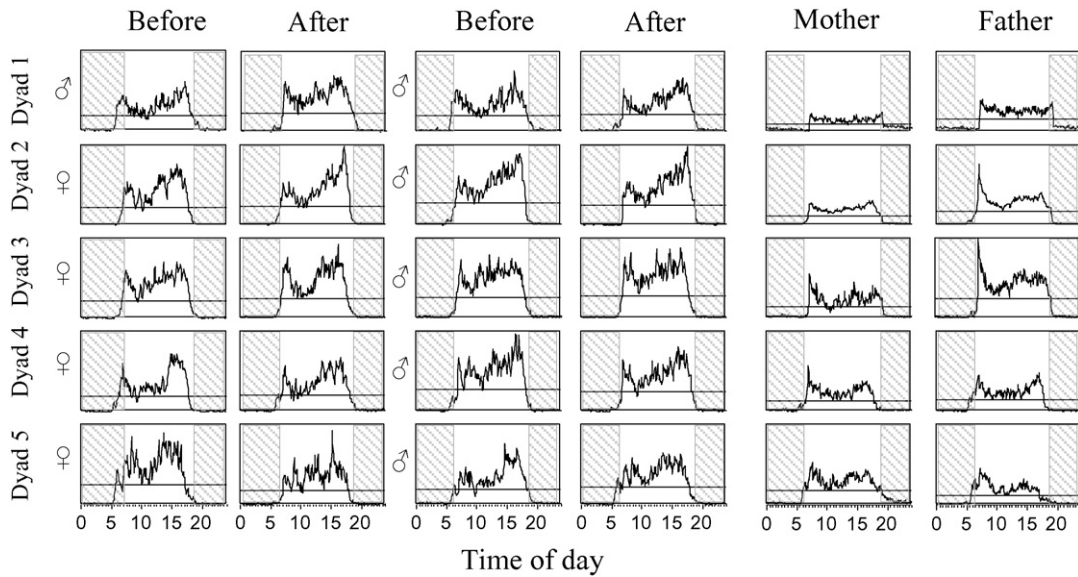


Fig. 3. Profiles of mean hourly activity of each juvenile before and after entering puberty and their respective parents. Gray bars represent the dark phase.

the effect of puberty on rhythms without the influence of social factors is difficult in marmosets because long-term social isolation in early development can negatively influence the behavioral development of animals in that stage [53]. Moreover, as these diurnal primates live in groups in their natural environment, it may be that the effect of puberty will be very subtle with respect to the influences of social factors in this condition.

In a different way, the phase delay in adolescent humans in nowadays is a feature that, although it brings harm to social and school life due to partial sleep deprivation, is not related directly to survival as it would be in marmosets. In the ancestral environment where the solar day exerted a stronger pressure in sleep-wake cycle in humans, this delay could be subtle, similar to that observed in nowadays in adolescents that live in rural areas without lighting at home [49–50]. The increase of arousal activities associated to light exposure at night by the use of electronic devices in parallel with a reduction of direct risks to survival in humans might be contributing to increasingly later bedtimes in nowadays.

Regarding sex differences on circadian activity rhythm, the increase in activity and the delay at the end of the active phase in females were changes in the expression of rhythm associated with entering puberty that are likely associated with the effect of gonadal hormones on the circadian timing system. In intact and sterilized degus, Hummer et al. [14] observed that there was a delay in the start of the activity after entry into puberty only in intact animals and the delay was more pronounced in males which promoted a difference from the beginning of the active phase of females. In degus, sexual differences in the daily distribution of activity are established with age; in early development both have concentrated activity in the evening and with age, males concentrate activity in the light phase [20]. In marmosets, further studies are needed to evaluate the effect of gonadal hormones on the rhythm and to observe what happens in these parameters in later stages of development.

Independently of puberty, some rhythm parameters differed between juveniles and adults. The total activity were higher in juveniles than in adults, a result similar to that found in previous studies in juvenile marmosets [28,23]. This result may be related to an increase in independence of the animal with age [54,55], which favors the occurrence of behaviors that affect the motricity of animals, such as foraging [54] and playing [56–57], raising activity levels. In addition to the increase with age, the differences in activity levels between juveniles and adults may be associated with differences in the behavioral

repertoire of each age group. In adults, in addition to foraging, behaviors involving little movement, such as grooming predominate [57,54].

It was also observed that the EA/MA ratio was higher in juveniles than in adults as a result of higher levels of activity during the evening hours than in the morning. The evening and morning activity peaks characterize a bimodal profile, similar to that observed in free-ranging animals [58], indicating that the bimodal distribution may be a species-specific pattern in marmosets. The calculation of the ratio EA/MA is an alternative used in this work to evaluate this distribution of activity throughout the day. In this, this bimodal distribution of activity would be related to the model proposed by Pittendrigh & Daan [59], in which the expression of the circadian activity rhythm would be regulated by two oscillators; the morning (M) related to sunrise, and the evening (E) related to sunset. Thus, considering that the evening activity was higher than the morning for juvenile marmosets and the opposite relationship is observed in adults, we suggest that the functioning of the M and E oscillators could change during ontogenesis.

Changes in the daily activity profile during ontogenesis were observed in degus [20] and rats [18]. In these species, the bimodal activity profile changes to a unimodal one from the juvenile phase to adulthood. In degus, the unimodal profile of adult is characterized by the concentration of activity in the light phase and in rats in the dark phase. Additionally longitudinal studies are necessary to evaluate the bimodal pattern in marmosets from the juvenile to the adult stage to identify the moment when the daily pattern changes and also, to confirm if these changes are associated to the functioning of the M and E oscillators during ontogenesis.

Martynhak et al. [61] proposed the inclusion of a bimodal diurnal preference category in the classification of chronotype for humans, in which individuals exhibit preference to conduct some activities in the morning and others in the evening. However, the distribution of activity during the activity phase was not evaluated in adolescent humans. Thus, it is interesting to evaluate whether the later bedtimes observed in human adolescents [10–11] are associated to differences in the distribution of activity during the activity phase with higher levels of activity at evening, similar to that observed in marmosets.

Besides the evidences of ontogenetic changes in the distribution of activity during the active phase in marmosets, we cannot exclude that the bimodal pattern observed in the experimental animals can be influenced by feeding effects on activity levels during the active phase. In addition to the raisins available ad libitum, the animals received a

nutritional mixture at irregular times at the beginning of light phase whose leftover was replaced by fruits in irregular times at the second half of light phase. Since the feeding time can act as a zeitgeber is several species [60], future studies are necessary to evaluate the effect of feeding in circadian activity rhythm in marmosets.

In this study, besides the beginning and end of the active phase, the use of the ratio EA/MA may help to assess other phases of rhythms, and a better understanding of the modifications in the expression of circadian rhythmicity during puberty. Furthermore, it is possible to make comparisons from the phylogenetic and ontogenetic point of view when comparing these parameters with other ages and other species of primates and humans. As the marmoset presents a phylogenetic proximity to humans and several physiological and behavioral aspects have been well characterized [26], this species would be a good animal model for better understanding human circadian rhythmicity.

In summary, the beginning and end of the active phase, the total daily activity and the ratio EA/MA showed differences in relation to age and puberty, and the total daily activity also showed an increase along age in marmosets. Also, sex-related changes were observed, such as a delay at the end of the active phase and an increase in the total activity after entering puberty only in females. Therefore, although puberty exerts an effect on some parameters of circadian activity rhythm, the influence of social dynamics and sex can be factors that influence this process in marmosets. Further studies are needed to evaluate the effect of gonadal hormones on the rhythm in this species.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Acknowledgments

We would like to thank our graduate students Henrique James, Fernanda Kolodiuk, Kahena Florentin, Luiz Cândido da Silva Júnior, Deyse Bezerra, Diego Fernandes, Camila Bessa and Bruna Wanderley for assisting in data collection. Also, we thank Flávio Coutinho, Edinólia Câmara and Antônio Barbosa for technical support at the Primateology Center of UFRN, and Dr. Maria de Fátima Arruda, Dr. Nicole Galvão-Coelho and Dr. Maria Bernardete Cordeiro de Sousa for suggestions made during the investigation and data analysis. We are also grateful to *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES) and *Fundação de Apoio à Pesquisa do Rio Grande do Norte* (FAPERN) for financial support.

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