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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.

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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.

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 ± 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 m × 1.0 m × 1.0 m in a room of measuring 2.95 m × 2.77 m × 5.70 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 cm × 30 cm × 21 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	September 16
	Geraldo	♂	Juvenile	February/2010	(7 months)
	Giva	♀	Adult		September 2
	Guará	♂	Adult		(6 months)
2(F2)	Florise	♀	Juvenile	September/2009 to	November 9
	Floriano	♂	Juvenile	May/2010	(5 months)
	Flor	♀	Adult		December 23
	Crivo	♂	Adult		(7 months)
	Francinilda	♀	Infant		
	Francil	♂	Infant		
3(F3)	Alma	♀	Juvenile	February to October/2010	June 21
	Alisson	♂	Juvenile		(7 months)
	Andréia	♀	Adult		June 15
	Fontes	♂	Adult		(7 months)
4(F4)	Amália	♀	Juvenile	April to December/2010	May 25
	Amaro	♂	Juvenile		(5 months)
	Afrodite	♀	Adult		October 5
	Ravi	♂	Adult		(9 months)
5(F5)	Oboé	♀	Juvenile	June/2010 to February/2011	September 9
	Odara	♂	Juvenile		(7 months)
	Ozelita	♀	Adult		July 27
	Zonta	♂	Adult		(5 months)