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Changes in circadian rhythms during puberty in *Rattus norvegicus*: Developmental time course and gonadal dependency

Megan Hastings Hagenauer [a,b,c,*], Andrea F. King [c], Bernard Possidente [d], Marilyn Y. McGinnis [e], Augustus R. Lumia [e], Elizabeth M. Peckham [c], Theresa M. Lee [a,b,c]

[a] Neuroscience Program, University of Michigan, Ann Arbor, MI 48109-0520, USA
[b] Reproductive Sciences Program, University of Michigan, Ann Arbor, MI 48109-0520, USA
[c] Department of Psychology, University of Michigan, Ann Arbor, MI 48109-1043, USA
[d] Biology Department and Neuroscience Program, Skidmore College, Saratoga Springs, NY 12866, USA
[e] Department of Pharmacology, University of Texas Health Science Center, San Antonio, TX 78229, USA

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ABSTRACT

During puberty, humans develop a later chronotype, exhibiting a phase-delayed daily rest/activity rhythm. The purpose of this study was to determine: 1) whether similar changes in chronotype occur during puberty in a laboratory rodent species, 2) whether these changes are due to pubertal hormones affecting the circadian timekeeping system. We tracked the phasing and distribution of wheel-running activity rhythms during post-weaning development in rats that were gonadectomized before puberty or left intact. We found that intact peripubertal rats had activity rhythms that were phase-delayed relative to adults. Young rats also exhibited a bimodal nocturnal activity distribution. As puberty progressed, bimodality diminished and late-night activity phase-advanced until it consolidated with early-night activity. By late puberty, intact rats showed a strong, unimodal rhythm that peaked at the beginning of the night. These pubertal changes in circadian phase were more pronounced in males than females. Increases in gonadal hormones during puberty partially accounted for these changes, as rats that were gonadectomized before puberty demonstrated smaller phase changes than intact rats and maintained ultradian rhythms into adulthood. We investigated the role of photic entrainment by comparing circadian development under constant and entrained conditions. We found that the period (τ) of free-running rhythms developed sex differences during puberty. These changes in τ did not account for pubertal changes in entrained circadian phase, as the consolidation of activity at the beginning of the subjective night persisted under constant conditions in both sexes. We conclude that the circadian system continues to develop in a hormone-sensitive manner during puberty.

During adolescence, humans develop a propensity towards "night-owl" behavior or "evening chronotype", exhibiting a later, or delayed, timing of many daily rhythms including rest and activity (Crowley et al., 2007; Roenneberg et al., 2004; Thorleifsdottir et al., 2002; Yang et al., 2005). These developmental changes in chronotype are likely to be partially rooted in hormonal influences on the body's circadian timekeeping system, as they persist under controlled, laboratory conditions (Carskadon et al., 2004; Carskadon et al., 1997), exhibit pronounced sex differences in timing and magnitude (Roenneberg et al., 2004) and correlate with secondary-sex development, even after taking into account age and related social influences (Carskadon et al., 1993; Sadeh et al., 2009). The purpose of this study, as well as another study published in Hormones and Behavior (Hagenauer et al., 2011), is to determine whether similar changes in chronotype can be reliably observed during puberty in laboratory rodent species, and, if so, to elucidate their physiological mechanism.

Circadian rhythms in mammals are generated by an endogenous pacemaker (Ralph et al., 1990) that must be entrained by external time cues (or "zeitgebers") such as light to maintain a stable phase relationship with the outside world (Moore-Ede et al., 1982). Under conditions in which there are no time cues from the outside world (also referred to as constant or "free-running" conditions), the circadian system continues to generate daily rhythms with a period (or day length, τ) that only approximates 24 h (Moore-Ede et al., 1982). Under entrained conditions, the phase relationship between the solar day and the circadian system's output rhythms, such as daily rest/activity and hormonal cycles, is used to characterize an individual's chronotype. Thus, changes in chronotype, such as those seen during adolescence in humans, can be caused by changes in the entrainment of the circadian pacemaker (due to altered photic sensitivity or endogenous period) or changes in the phase relationship between the circadian pacemaker and its output rhythms.

* Corresponding author at: Department of Psychology, 530 Church St., Ann Arbor, MI 48109-1043, USA. Fax: +1 734 763 7480.
E-mail address: hagenaue@umich.edu (M.H. Hagenauer).

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It is already well-documented that the circadian system is sensitive to gonadal hormones during early development and adulthood. The timing of daily rhythms is shifted during different stages of the menstrual cycle (Manber and Bootzin, 1997; Parry et al., 1994; Parry et al., 2000). In adult laboratory rodents, the gonadal hormones that affect the phase and period of circadian rhythms include estrogens, progestins, androgens, and non-traditional neuroactive steroids (Albers, 1981; Axelson et al., 1981; Kent et al., 1991; Li and Satinoff, 1996; Davis et al., 1983; Morin et al., 1977; de Tezanos Pinto and Golombek, 1999; Daan et al., 1975; Iwahana et al., 2008; Karatsoreos et al., 2007; Jechura et al., 2000; Labyak and Lee, 1995). The sensitivity of the adult circadian system to these steroidal hormones is determined in some rodent species by the organizational effects of gonadal hormones during the perinatal period (*rat*: Albers, 1981; *hamster*: Zucker et al., 1980).

The influence of gonadal hormones on the circadian system during puberty is less understood, although it is reported that pubertal hormones can alter circadian phase (*degu*: Hummer et al., 2007) as well as produce organizational effects on the circadian system (*hamster*: Davis et al., 1983). Indeed, in some species there is a critical window of sensitivity to the organizational effects of gonadal hormones as late as young adulthood (*degu*: Hummer et al., 2007). There has been little attempt to determine how common pubertal changes in chronotype are across mammalian species or to elucidate their hormonal or neural bases using animal models (Hagenauer et al., 2009). Evidence from five species suggests that pubertal changes in circadian phase are not uniquely human (*rhesus macaque*: Golub et al., 2002; *laboratory mouse*: Weinert et al., 1994; Weinert and Waterhouse, 1999; *laboratory rat*: McGinnis et al., 2007; Kittrell and Satinoff, 1986; *Octodon degus* (degu): Hummer et al., 2007; Tate et al., 2002; and *Psammomys obsesus*: Neuman et al., 2005). However, only three of the studies (using the slow-developing, diurnal species of the macaque and degu) have attempted to thoroughly characterize the developmental progression of circadian phase change in relation to secondary-sex development (Golub et al., 2002; Tate et al., 2002; Hummer et al., 2007), and only one study directly examined the role of pubertal hormones (Hummer et al., 2007). Similar to humans, the macaque and degu show a delayed circadian phase during puberty (around the time of first menarche in the rhesus macaque, and first vaginal or prepucial opening in the degu) that reverses by adulthood. These developmental changes do not occur following pre-pubertal gonadectomy (Hummer et al., 2007). Therefore, pubertal elevations in sex hormones are likely to drive circadian phase changes.

Another mechanism underlying pubertal changes in circadian phase may be an elongation of the endogenous period of the circadian pacemaker (τ; Carskadon et al., 2004). In support of this theory, τ appears to elongate during puberty in humans and shorten during adulthood in a manner that parallels changes in chronotype (Carskadon et al., 1999; Carskadon et al., 2004). In rodent studies, male rats had a longer τ during late puberty (postnatal age P47–59 days, $\tau = 23.89$ h) than during adulthood (age P105–P115, $\tau = 23.75$ h; McGinnis et al., 2007). This theory needs to be explored further using additional timepoints and a comparison of the sexes because data from the diurnal degu indicated that pubertal changes in circadian phase are not dependent on changes in τ (Hummer et al., 2007).

The rat is a useful animal model for examining the mechanism underlying circadian chronotype change during puberty because there is previous indication that male rats undergo a 3 h magnitude phase change during puberty (McGinnis et al., 2007). Secondary-sex characteristics typically first appear around the postnatal age (P) of P30 in females and P45 in males, with mature sexual characteristics evident by around P60 (Ojeda and Urbanski, 1994). We hypothesized that pubertal rats would show a delay in circadian phase that peaks around mid-puberty, similar to what is observed in slow-developing mammalian species (the degu and macaque; Golub et al., 2002; Hummer et al., 2007). We further hypothesized that, as in the degu, these changes in phase would be dependent on gonadal hormones but independent of pubertal changes in τ. Finally, we expected that the development of delayed phase during puberty might be accompanied by a decrease in the ultradian components prevalent in the rhythms of newly-weaned young rats (Cambras and Diez-Noguera, 1988; Castro and Andrade, 2005; Diez-Noguera and Cambras, 1990; Ibuka, 1984; Joutsiniemi et al., 1991; Kittrell and Satinoff, 1986). To test these hypotheses, we first evaluated the correlation between changes in activity distribution and sexual maturation in both sexes, and then determined the dependency of changes in activity distribution on pubertal exposure to gonadal hormones (Experiment 1). We then examined the relationship between developmental changes in circadian phase and changes in the endogenous period of the circadian pacemaker under constant conditions (τ) in both sexes (Experiment 2).

Methods

All procedures were conducted in accordance with the guidelines established for the care and use of laboratory animals by the National Institute of Health and under approved by local animal use and care committees (IACUC). Sprague–Dawley rats were housed during testing in individual plastic cages ($47 \times 27 \times 20$ cm) with a Nalgene running wheel (9×34.5 cm, Mini Mitter, Bend, OR) and free access to food (5001 Rodent Diet, PMI Nutrition) and water. They were maintained under a 12:12 light–dark (LD) cycle at 20 °C. During the lighted part of the LD cycle, the testing environment was dimly lit (40–60 lx measured at cage level, provided by fluorescent house light) to reduce photic inhibition of activity (masking). Running wheel activity data was collected in 10-min bins using VitalView software (Minimitter, Bend, OR) and analyzed using ActiView software (Minimitter, Bend, OR). To prevent circadian disruptions, routine procedures occurred at random times during the lighted period of the LD cycle.

Experiment 1: pubertal changes in circadian rhythms under entrained conditions

Animals

Four iterations of the same experiment were conducted with a total sample size of 62 rats. These rats were obtained from breeding colonies at the University of Michigan, comprising eight litters from eight dams and sires. The dams and sires were purchased from Charles-River Laboratories (Wilmington, MA). Litters were reduced to 8 rats by postnatal day 3 (P3) with roughly balanced sex ratio.

The rats were placed in the testing environment before weaning. The pups used in iteration 1 were raised under a 14:10 LD cycle (lights on 05:00–19:00) until the age of P8–P14, when they were moved to the testing environment (12:12 LD, lights on 06:00–18:00). All other rats were raised and tested on the 12:12 LD cycle. Within the testing environment, cages were kept on tables to standardize light exposure. At weaning (age P19–P22), rats were placed in individual opaque plastic cages. Wheel-running data from the first two days after weaning was not used to avoid transient artifacts. Thus, all rats had been exposed to the testing environment for 5–16 days before their activity data was used for analysis (beginning P22–P24).

Surgery

Approximately one third of the rats underwent gonadectomy (GDX) or SHAM GDX surgery prior to puberty at age P12–P15 (conditions were roughly balanced within a litter). The remaining rats underwent no surgery. Any GDX animals that developed secondary-sex characteristics were removed from the analysis ($n = 3$), as were two females (no surgery) that exhibited low wheel-running counts,