

Testicular Hormones Modulate Circadian Rhythms of the Diurnal Rodent, *Octodon degus*

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Sex differences have been identified in a variety of circadian rhythms, including free-running rhythms, light-induced phase shifts, sleep patterns, hormonal fluctuations, and rates of reentrainment. In the precocial, diurnal rodent *Octodon degus*, sex differences have been found in length of free-running rhythm (τ), phase response curves, rates of reentrainment, and in the use of social cues to facilitate reentrainment. Although gonadal hormones primarily organize circadian rhythms during early development, adult gonadal hormones have activational properties on various aspects of circadian rhythms in a number of species examined. Gonadectomy of adult female *O. degus* did not influence τ , phase angle of entrainment, or activity patterns in previous experiments. The present experiment examined the role of gonadal hormones in adult male degus' circadian wheel-running rhythms. We predicted that male gonadal hormones would have an activational effect on some aspects of circadian rhythms, particularly those in which we see sex differences. Phase angles of entrainment, τ , length of the active period (α), maximum and mean activity levels, and activity amplitude were examined for intact and castrated males housed in LD 12:12. Responses to light pulses while housed in constant darkness (DD) were also compared. Castration had no significant effect on τ or light-induced phase shifts. However, castration significantly increased phase angle of entrainment and decreased activity levels. The data indicate that adult gonadal steroids are not responsible for the sex differences in endogenous circadian mechanisms of *O. degus* (τ , PRC), although they influence activity level and phase angle of entrainment. This is most likely due to masking properties of testosterone, similar to the activity-increasing effects of estrogen during estrus in *O. degus* females. © 2000 Academic Press

Key Words: circadian rhythms; *Octodon degus*; sex differences; testosterone; castration.

Circadian rhythms are critical mechanisms for an organism's adaptation to its environment (Daan and Aschoff, 1982). They guide the majority of behaviors observed in a vast array of plants and animals from single-celled algae (Hastings and Sweeney, 1958) to complex vertebrates, including humans (Aschoff, 1976; Czeisler, Richardson, Zimmerman, Moore-Ede, and Weitzman, 1981). From basic biological processes such as cell division and hormonal fluctuations to complex behaviors such as foraging and the timing of parturition, circadian rhythms regulate and organize an organism's life so as to occupy a specific niche, including a time niche.

The study of circadian rhythms has revealed a number of sex differences in a variety of species including rats, mice, hamsters, and humans. Sex differences have been identified in almost every aspect of circadian rhythms including free-running rhythms, light-induced phase shifts, sleep and activity patterns, hormonal fluctuations, and rates of reentrainment. Rats display circadian sex differences in their activity levels and length of free-running periods (τ), with females having a shorter τ than males and higher activity levels (Schull, Walker, Fitzgerald, Hiilivirtti, Ruckdeschel, Schumacher, Stanger, and McEachron, 1989). Female rats also have greater circadian fluctuations of plasma corticosterone than males (Critchlow, Lieberit, Bar-Sela, Moutcastle, and Lipscomb, 1963). Human females have a shorter τ than males (Wever, 1984). Human males and females differ in their temperature rhythms, sleep patterns, and timing of corticosteroid peaks (Wever, 1984). There is also a sex difference in responsiveness to corticotropin-releasing hormone, with women showing longer periods of ACTH eleva-

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tion (Gallucci, Baum, Laue, Rabin, Chrousos, Gold, and King, 1993).

Sex differences in the circadian system of hamsters have been extensively documented. Similar to rats, female hamsters have a shorter τ than male hamsters (Davis, Darrow, and Menaker, 1983). In addition to the difference in τ , male and female hamsters display a marked difference in their ability to entrain to photoperiods over 24 h long, with males entraining to these photoperiods more quickly and with more stability than females (Davis *et al.*, 1983). Unlike females, males tend to show split rhythms when exposed to constant light conditions (Morin and Cummings, 1982) and are insensitive to the effects of estradiol treatment on circadian rhythms (Zucker, Fitzgerald, and Morin, 1980). There also are sex differences in the entrained rhythms of hamsters, with females displaying a phase angle of entrainment (time of activity rhythm onset relative to the environmental time cue) that begins earlier in the subjective day (Davis *et al.*, 1983) and a difference in circadian responsiveness to pulses of light while housed in constant darkness, measured as phase shifts in the days following the light pulses (phase response curve, or PRC) (Daan and Pittendrigh, 1976).

Octodon degus is a highly social, diurnal South American precocial rodent that shares several important characteristics with humans that could be advantageous for laboratory research of circadian rhythms (Labyak, 1993). Besides their diurnal nature, degus show individual variation in rhythms similar to humans (Labyak, Lee, and Goel, 1997; Labyak and Lee, 1997; Lee and Labyak, 1997). Like humans, degus display sex differences in their circadian rhythms. The τ for females is longer of that of males (23.7 vs 23.2 h) (Labyak and Lee, 1995; 1997). Males also reentrain 15–20% faster than females after 6-h phase shifts in the light:dark cycle (Goel and Lee, 1995a), although when housed with a previously entrained female, females are quicker to reentrain than males (Goel and Lee, 1995b).

Sex differences generally arise as a result of gonadal hormones acting in an organizational manner during early development (Breedlove, 1993) and/or in an activational manner in adulthood (Baum, 1993; Carter, 1993). However, sex differences can also be generated without hormonal input by other genetic differences (Beyer, Eusterschulte, Pilgrim, and Reisert, 1992; Sibug, Koppers, Beyer, Maxson, Pilgrim, and Reisert, 1996; Gahr and Metzendorf, 1999). Furthermore, organizational processes may not be limited to early development, but may occur during puberty (Eichmann and Holst, 1999). It is unclear when and where

changes take place in degus to result in differences in circadian rhythms between males and females. When adult female degus were ovariectomized, phase angle of entrainment, activity level and pattern, and τ were unchanged (Labyak and Lee, 1995). This indicates that activational effects of ovarian hormones in adult females are not responsible for observed sex differences. Since ovarian hormones do not appear to affect sex differences in female degus, the sex difference in τ of degus could be the result of adult male hormones, particularly testosterone.

Castration of adult male hamsters and rats does not have a significant effect on τ . However, castration of adult male mice significantly lengthens τ (Daan, Damassa, Pittendrigh, and Smith, 1975) and ovariectomy of female hamsters eliminates the sex difference in phase angle of entrainment (Davis *et al.*, 1983). In addition, testosterone is reported to increase running wheel activity in male rodents (voles: Rowsemitt, 1986, 1988; hamsters: Ellis and Turek, 1983; mice: Daan *et al.*, 1975; rats: Roy and Wade, 1975).

Although some species display activational effects of adult hormones on the circadian system, data generally suggest that testosterone primarily causes an organizational change in the circadian system during perinatal development (Zucker *et al.*, 1980). Because of the precocial nature of the degus and their long gestation period of 3 months, it is likely that many of the fundamental characteristics of the circadian clock are sexually differentiated prior to birth. However, given the information from adult male mice and other examples, we predicted that manipulations of testicular hormones would alter some properties of circadian rhythms as seen in some other species.

MATERIALS AND METHODS

Animals and Housing

Ten adult male degus (age 6 months to 2 years, average life span 5–7 years) were obtained from an outbred colony maintained at The University of Michigan. The animals were housed individually in $48 \times 26.7 \times 20.3$ cm Nalgene cages with running wheels (9 cm wide, 34.5 cm diameter). Each cage was placed within a light-tight enclosure that allowed for individual manipulation of lighting for each animal. Prior to entering the experiment, degus were maintained at $20^\circ \pm 1^\circ\text{C}$ in LD 12:12, with lights on at 06:00 and off at 18:00. Cages were cleaned weekly during the light phase of the LD cycle or with red light illumination for

animals in DD. Rodent chow (Purina 5001) and water were available *ad libitum*. All procedures involving animals were approved by the Animal Care and Use Committee (IACUC) at the University of Michigan.

Phase 1 protocol. Phase angles of entrainment (Ψ), α , amplitude, activity levels, period of free-running rhythms (τ), and phase changes after light pulses were obtained from each animal both pre- and postcastration, allowing each animal to serve as its own control. Activity data were collected throughout all phases of the experiment as wheel rotations per 10-min interval with Dataquest III (Minimitter, Inc.) software.

To obtain baseline phase angle data, degus were maintained with running wheels in LD 12:12 with lights on at 06:00 and off at 18:00. Data collection began 3 days after introduction of the wheels, allowing the animals to become familiar with the apparatus. Phase angle of activity onset was determined after an animal displayed at least 2 weeks of entrained rhythms and was calculated by examining 24-h activity frequency histograms for the time of activity onset. Activity onset was defined as at least 40 min of consecutive activity with a minimum of 40 wheel revolutions per 10-min block of activity preceded by a lack of activity of at least 4 h. The time of activity onset over a period of 4 to 7 days was averaged and compared to the LD cycle to obtain phase angle of activity onset. The same method was used to calculate phase angle of activity offset over the same time period. Both activity onset and offset were measured relative to the onset of the light cue. A positive number indicated an activity onset or offset prior to the light cue (lights on or off), while a negative number indicated activity beginning after the light cue. Activity duration (α) was determined by subtracting phase angle of onset from phase angle of offset. Mean daily activity levels were calculated by averaging activity levels across 10-min bins over a 24-h period. Maximum activity level was defined as the greatest number of wheel rotations per 10-min bin in a 24-h period. Activity rhythm amplitude was obtained by subtracting the activity mean from the maximum activity level.

Phase 2 protocol. After circadian parameters during LD 12:12 were obtained, the animals were released into conditions of total darkness (DD) and allowed to establish a free-running rhythm. Period length (τ) was determined after an animal maintained a steady free-running rhythm for at least 2 weeks by examining 24-h frequency histograms to determine time of activity onset over a period of at least 7 days and averaging the difference in time of onset from one day to the next (regression).

While in DD, the intact animals' responses to 20-min light pulses (250 lux) during a phase delay portion of the PRC (CT4) and a phase advance portion (CT20) were also calculated. CT4 and CT20 were chosen as the target times for light pulses based on PRC data indicating that the largest delays and advances are generally seen at those times in degus (Lee and Labyak, 1997). Frequency histograms were used to determine activity onset for an individual animal on the day of the light pulse administration and the animal's calculated τ was used to determine timing of CT4 or CT20. Following the first light-induced phase shift, the animal was allowed to resume and maintain a steady free-running rhythm and was then exposed to the second 20-min light pulse so that each animal received light pulses at both CT4 and CT20. The light-induced phase shift was defined as the average difference between the expected and actual times of activity onset over the 4–5 days following the light pulse. Expected time of activity onset was determined by using the previously calculated τ and determining where activity would be expected to begin if the animal remained constant in its free-running rhythm and did not respond to the light pulse. The magnitude of the phase shift was measured by comparing the expected time of activity onset under constant conditions with the actual time of onset following the light pulse.

Phase 3 protocol. After the light-induced phase shifts, the animals were once again maintained in LD 12:12 (lights on at 06:00 and off at 18:00) and were castrated during their active phase. Surgical procedures for castration were carried out with Ketamine HCl (30 mg/kg body wt) and Xylazine (2.5 mg/kg body wt) anesthesia. Yohimbine (2.5 mg/kg body wt) and lactated ringers (3 ml, i.p.) were used to aid recovery. All degus were allowed 1 week for recovery before resuming running wheel activity.

Three days after reinstallation of running wheels, data collection resumed and the animals were subjected to each of the conditions previously experienced as intact animals. Phase angles of entrainment, α , τ , activity levels, and magnitude of light-induced phase shifts were obtained in the castrated animals and compared to intact states using paired *t* tests.

Statistical Analyses. Paired *t* tests were used to compare animals in the intact vs castrated conditions for each circadian parameter examined, with $P < 0.05$ considered significant. Data are presented as means \pm SEM.

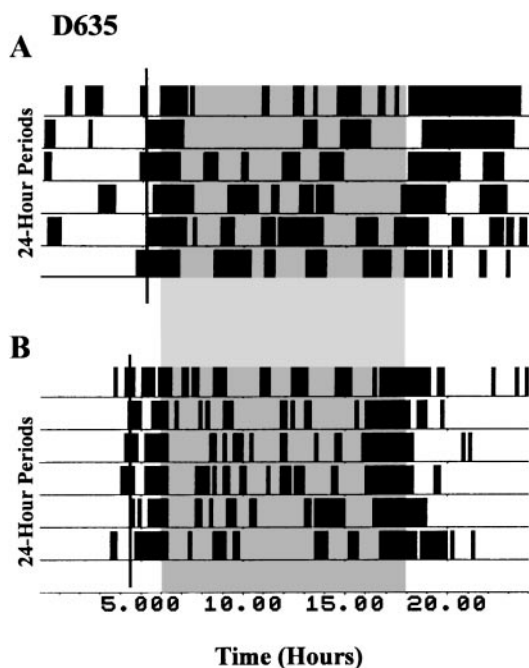


FIG. 1. Double-plotted actogram of a representative adult male degu's entrained rhythms in gonadally intact (A) and castrated (B) conditions. Thin lines represent phase angle of activity onset. Shaded areas represent the light period (0600 to 1800).

RESULTS

Castration did not affect τ or the magnitude of light-induced phase shifts. The τ was 23.58 ± 0.093 h prior to castration and 23.52 ± 0.018 h after castration. Light-induced phase shifts at CT4 were -1.119 ± 0.198 h in the intact animals and -1.351 ± 0.384 h in the castrates. Responses to light pulses at CT20 were 1.005 ± 0.407 h prior to castration and $.842 \pm .281$ h after castration.

Figure 1 shows a typical animal's entrained rhythms before and after castration. Castration significantly increased phase angle of activity onset in adult males ($P < 0.002$, Fig. 2A). Phase angle of offset and activity level were also affected. Phase angle of activity offset was advanced ($P = 0.003$, Fig. 2B). This corresponded with a shortened α ($P = 0.026$, Fig. 2C). Mean activity rhythm levels decreased ($P = 0.008$, Fig. 2D) along with activity amplitude ($P = 0.024$, Fig. 2E). Maximum activity level also decreased significantly ($P = 0.017$, Fig. 2F).

DISCUSSION

The data indicate that core properties of degus' circadian system are hormonally independent in the

adult male degu; castration had no effect on τ or phase responses to light pulses, indicative of the PRC. This contrasts with mice, in which castration of adult males significantly lengthens τ (Daan *et al.*, 1975). There also was no difference in the number of transients, or days of disrupted rhythms following light pulses, between intact and castrated animals. The stability of τ and the PRC in the absence of gonadal hormones of both male and female degus (Labyak and Lee, 1995) reflects fundamental mechanisms of the circadian pacemaker of *O. degus* that are sexually dimorphic but are not influenced by changes in adult hormone levels. Given this information, it is likely that organizational effects of steroid hormones take place earlier in the developmental period, possibly at puberty, but more likely during gestation or around the time of parturition. It is also possible that these sex differences are independent of testosterone.

In contrast to τ and PRC, the phase angles of entrainment, α , and all measures of activity were affected by castration. Phase angle of onset of wheel-running activity was increased; castrated males began activity earlier than when intact. However, even with the earlier onset of activity, the combination of change in activity offset and decreased α resulted in centering the active period more within the confines of the light period, with the cumulative effect of a more strictly diurnal pattern of activity (Fig. 1). Kas and Edgar (1999) reported that many male degus housed with running wheels readily increase activity during the nocturnal phase. Perhaps high testosterone levels interact with housing conditions to alter activity pattern. A similar analysis has not been done with female degus. The effect of testosterone on the confinement of activity into either the light or dark portion of the light cycle has also been observed in voles. Castrated voles concentrated their activity in a more diurnal pattern than intact animals and testosterone replacement reinstated a nocturnal preference for wheel-running activity (Rowsemitt, 1986, 1988).

The change in phase angle of entrainment and activity duration and levels as a result of castration may be interpreted as a loss of activational properties of gonadal hormones as seen in other rodent species (voles: Rowsemitt, 1986, 1988; hamsters: Ellis and Turek, 1983; mice: Daan *et al.*, 1975; rats: Roy and Wade, 1975). This is similar to the way in which estrogen increases the activity rhythms of adult female degus (Labyak and Lee, 1995). The presence of gonadal hormones in males and in females (on the day of estrus when estrogen is high) stimulates running

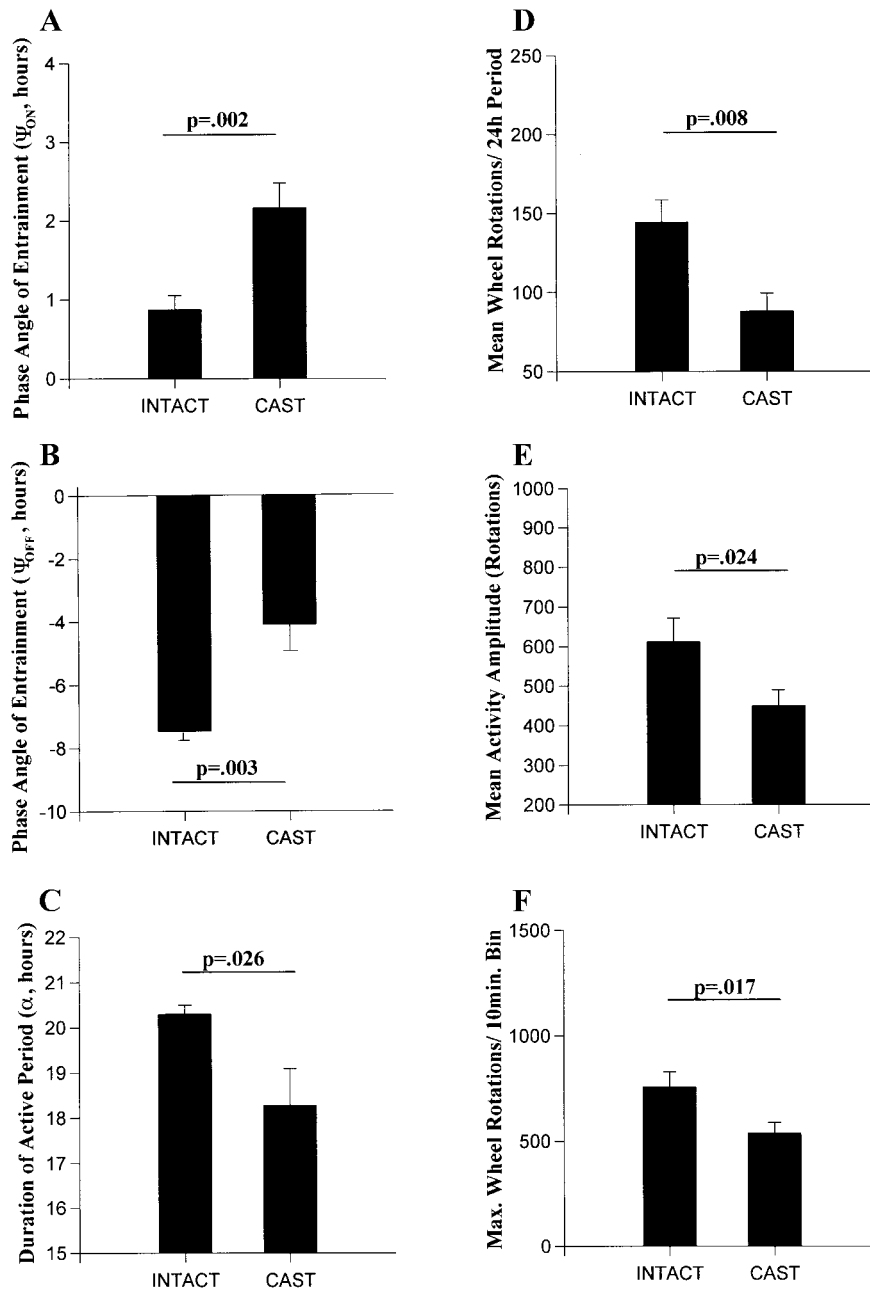


FIG. 2. Circadian wheel-running activity patterns of males in an intact then castrated state described by (A) phase angle of activity onset, Ψ_{ON} ; (B) phase angle of activity cessation, Ψ_{OFF} ; (C) duration of activity, α ; (D) mean number of wheel revolutions in 24 h; (E) activity rhythm amplitude; and (F) daily maximum number of wheel revolutions in a 10-min interval.

wheel activity and castration attenuates the effect in both sexes.

The relationship between activity stimulation by gonadal hormones in males and females may not be surprising. Roy and Wade (1975) examined the role of testosterone on the regulation of running wheel activity in rats and proposed that testosterone is converted

into estrogen to stimulate activity. Testosterone significantly increased activity, but estradiol benzoate was 100 times as effective as testosterone. The fact that dihydrotestosterone had no effect on running wheel activity suggests that the conversion of testosterone to estrogen is important for the stimulating effects on activity.

The brain areas responsive to testosterone and responsible for certain aspects of sexually differentiated circadian rhythms have not been clearly identified yet. Receptors for testosterone in the SCN have only been identified in adult ferrets (Kashon, Arbogast, and Sisk, 1996), while mRNA for ER β has been reported in adult SCN of female rats (Shughrue, Lane, and Merchenthaler, 1997). However, testosterone may be exerting its effects indirectly through hormone receptors in sexually dimorphic areas neurally connected with the SCN, such as through the amygdala and/or paraventricular nucleus of the thalamus (PVT).

The intergeniculate leaflet (IGL) of the thalamus also projects to the SCN (e.g., Morin, Blanchard, and Moore, 1992) and has estrogen receptors in the female guinea pig (DonCarlos, Monroy, and Morrell, 1991). Most recently, IGL lesions in degus have been found to increase α and Ψ_{onset} (Goel, Governale, Jechura, and Lee, 2000). Because of the similarity of these findings with the current study, it would be interesting to look for hormone receptors in the IGL of the degu. Testosterone receptors in the IGL could possibly mediate these effects through an inhibitory connection in the IGL.

Interestingly, Moga and Moore (2000) found that lesions of the paraventricular thalamus (PVT) of blinded rats resulted in a concentration of activity in late subjective night compared to controls. It is possible that the PVT is responsible for the timing of activity placement and that testosterone exerts its effects through the amygdala, which contains androgen receptors in hamsters (Clancy, Bonsall, and Michael, 1992; Simerly, Chang, Muramatsu, and Swanson, 1990; Wood and Newman, 1993, 1995) and is reciprocally connected with the PVT (Gomez and Newman, 1992; Moga, Weiss, and Moore, 1995). Androgen receptors have been reported in the PVT of the male ferret (Kashon *et al.*, 1996), so it is also possible that testosterone is directly exerting its effects on the timing of activity placement in the degu through androgen receptors in the PVT. Gonadal hormones could also be exerting their actions further into the limbic system (Amir, Cain, Sullivan, Robinson, and Stewart, 1999), such as through the amygdala or other steroid receptor-rich areas, such as the raphe.

In rats and hamsters, both the amygdala and PVT are sensitive to steroid hormones and both have connections to the SCN; the PVT has a direct and reciprocal connection with the SCN, while the amygdala has indirect connectivity to the SCN and a direct reciprocal projection to the PVT (Gomez and Newman, 1992; Moga *et al.*, 1995; Moga and Moore, 2000). A

more detailed analysis of steroid receptor availability and neural connectivity to the SCN in degus may help to clarify some of the possible areas involved in sex differences in degus.

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