Period gene expression in the diurnal degu (Octodon degus) differs from the nocturnal laboratory rat (Rattus norvegicus)

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Vosko AM, Hagenauer MH, Hummer DL, Lee TM. Period gene expression in the diurnal degu (Octodon degus) differs from the nocturnal laboratory rat (Rattus norvegicus). Am J Physiol Regul Integr Comp Physiol 296: R353–R361, 2009. First published November 26, 2008; doi:10.1152/ajpregu.90392.2008.—Recent data suggest that both nocturnal and diurnal mammals generate circadian rhythms using similarly phased feedback loops involving Period genes in the suprachiasmatic nuclei (SCN) of the hypothalamus. These molecular oscillations also exist in the brain outside of the SCN, but the relationship between SCN and extra-SCN oscillations is unclear. We hypothesized that a comparison of “diurnal” and “nocturnal” central nervous system Per rhythms would uncover differences in the underlying circadian mechanisms between these two chronotypes. Therefore, this study compared the 24-h oscillatory patterns of Per1 and Per2 mRNA in the SCN and putative striatum and cortex of Octodon degus (degu), a diurnal hystricognath rodent, with those of the nocturnal laboratory rat, Rattus norvegicus. The brains of adult male degus and rats were collected at 2-h intervals across 24 h in entrained light-dark and constant darkness conditions, and sections were analyzed via in situ hybridization. In the SCN, degu Per1 and Per2 hybridization signal exhibited 24-h oscillatory patterns similar in phasing to those seen in other rodents, with peaks occurring during the light period and troughs during the dark period. However, Per1 remained elevated for five fewer hours in the degu than in the rat, and Per2 remained elevated for two fewer hours in the degu. In brain areas outside of the SCN, the phase of Per2 hybridization signal rhythms in the degu were 180° out of phase with those found in the rat, and Per1 hybridization signal lacked significant rhythmicity. These results suggest that, while certain basic components of the transcriptional-translational feedback loop generating circadian rhythms are similar in diurnal and nocturnal mammals, there are variations that may reflect adaptations to circadian niche.

clock genes; Period; diurnality; chronotype; striatum; cortex; suprachiasmatic nucleus

WHILE THE SUPRACHIASMATIC nuclei (SCN) of the hypothalamus are integral both for generating and synchronizing circadian rhythms in mammals, their role in influencing chronotype is not well understood. In the few diurnal mammals that have been systematically compared with nocturnal mammals, the phasing of SCN activity appears surprisingly similar (52). This has led many researchers to believe that, instead of a “diurnal signal” coming from the SCN, there are “diurnal interpretations” of the SCN’s signal that may account for mammalian diurnality (13, 29, 43, 56). However, a growing number of studies indicate consistent differences between the SCN neural activity of diurnal and nocturnal species (25–26, 34, 40, 57). Therefore, continued research may yet elucidate a relationship between SCN function and chronotype.

The discovery of a self-sustaining molecular feedback loop within SCN cells has provided a direct means to examine the circadian pacemaker’s phase in relation to physiological and behavioral chronotype. Among the players in this feedback loop, the period genes Per1 and Per2 are of particular interest in relation to circadian niche because of their integral involvement in generating rhythmicity under constant conditions (9, 68) and in entraining circadian phase to time cues (zeitgebers) such as light (4–5, 35, 42, 53, 55). Another period gene, Per3, does not play a direct role in the circadian clock mechanism regulating the locomotor activity of nocturnal mice (9, 53); however, polymorphisms in this gene have been linked to extreme diurnal preference in humans (6, 16, 48), suggesting that it may play a significant role in the circadian organization of diurnal species.

To date, there have been no studies directly comparing Period gene expression in diurnal and nocturnal species. There have been several studies that have independently examined Period mRNA rhythms in species of both chronotypes (e.g., Refs. 10, 29, 35, 37, 40, 55, 59). However, the methodology used in these studies varied greatly, particularly in terms of lighting condition and tissue collection interval (which were typically large, e.g., 4–6 h). Therefore, although we can confidently argue that in both nocturnal and diurnal chronotypes, Per1 and Per2 mRNA in the SCN peak during midsubjective day and fall to baseline levels during the night, it is difficult to assess less robust differences in peak duration or timing, or the precise phase of the rise and fall. It is important to investigate these possible differences, since SCN zeitgeber sensitivity is thought to reflect Period gene expression levels (21, 32). Furthermore, PER induction in response to light has been hypothesized to mediate photic phase-shifts of behavioral rhythms (4, 5, 55, 62), and so differences in Per transcript levels may reflect differences observed in daytime zeitgeber sensitivity and “dead zones” in the phase response curves (PRC) between diurnal and nocturnal mammalian species (24, 56).

Daily rhythms in clock gene expression also occur in brain areas besides the SCN, and the phase relationship between SCN and extra-SCN oscillators may reflect differences in chronotype. Previous work in rats and mice has indicated that the phase of Per mRNA expression in brain areas outside of the

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SCN closely relates to the phase of behavioral activity rhythms (3, 31, 37–38, 63). In the diurnal ground squirrel, Per1 rhythms in the motor cortex peak during the light period, a complete phase-reversal compared with Per1 rhythms in brain areas outside the SCN in nocturnal rodents (37). Therefore, the differences in phase of the molecular clocks within the brain might also be an important factor in organizing a mammal’s temporal phenotype.

To better address the relationship between the circadian pacemaker and chronotype, we systematically compared Per1 and Per2 expression profiles inside and outside of the SCN in the diurnal Octodon degus (degu) and nocturnal Rattus norvegicus (rat) using frequent sampling intervals (2 h), species-specific probes, and identical in situ and analysis methodology. Within the SCN, we hypothesized that subtle differences would exist between degus and rats in Per1 and Per2 peak timing and duration, so as to account for the lack of a daytime dead zone in the PRC of the diurnal species. However, we expected that the basic waveforms of mRNA expression would still be quite similar. In contrast, outside of the SCN, we expected that the basic waveforms of mRNA expression would exist between degus and rats in Per1 and Per2 mRNAs waveforms in degus and rats would be 180° out of phase with each other, reflecting the differential phasing of their behavioral rhythms. Additionally, because no study had examined the circadian expression pattern of Per3 in a diurnal mammal, we sought to characterize its expression in the degu.

MATERIALS AND METHODS

Animals and Housing

Adult male degus (n = 82, age 2–4 yr; average lifespan 5–7 yr) were obtained from a breeding colony at the University of Michigan (Ann Arbor, MI), and adult male Sprague-Dawley rats (n = 56, age 3–7 mo) were obtained from Charles River Laboratories (Wilmington, MA). All animals were housed in 48 × 26.8 × 20.3 cm opaque plastic cages under a 12:12-h light-dark (LD) cycle (250 lux, lights on from 0600 to 1800) with food and water available ad libitum. Room temperature was maintained at 18°C. Degus were defined as diurnal and included in the study if screening showed that the majority of their daily activity occurred during the light period of the LD cycle, as characterized by any particular riboprobe.

Isolation of degu Per1, Per2, and Per3 cDNA sequences. Primers were used in RT-PCR to isolate fragments from the Per cDNA sequences. The DePer1 cDNA sequence (GenBank accession no. EU715821) is a 892-bp fragment 89% identical to the corresponding region of mouse Per1 (GenBank NM 011065). The DePer2 cDNA sequence (GenBank EU590918) is a 532-bp fragment 87% identical to the corresponding region of mouse Per2 (GenBank NM 011066). The DePer3 cDNA sequence (GenBank EU590919) is a 204-bp fragment 84% identical to the corresponding region of mouse Per3 (GenBank NM 011067).

Production of radioactive riboprobes. Degu Per1, Per2, and Per3 fragment-containing vectors (courtesy of Dr. Jeremiah Shepard and Dr. Steven McKnight of Southwest Medical School) and rat Per1 and Per2 fragment-containing vectors (courtesy of Dr. Stanley Watson and Dr. Huda Akil, University of Michigan) were linearized with restriction enzymes and used as templates for hybridized RNA probes. The sequences of the degu DNA fragments used to produce the antisense RNA probes corresponded to nucleotides 194–1082 of mPer1, 491–1022 of mPer2, and 1057–1260 of mPer3. The sequences for the rat DNA fragments used to produce the antisense RNA probes can be found elsewhere (54). Linearized plasmid was purified and incubated at 37°C for 1.5 h in a transcription buffer that included RNA polymerase and 35S-labeled UTP and CTP (for all reagents, see Ref. 8). The resulting probe was treated with DNase and separated from free nucleotides using Micro Bio-Spin Chromatography Columns (Bio-Rad Laboratories, Hercules, CA).

Tissue preparation. Brain sections (16 μm) were sliced on a cryostat into four series of coronal sections through the SCN and stored on slides at −80°C until hybridization. Tissue sections were fixed with 4% paraformaldehyde for 1 h, rinsed, and incubated in 0.1 M triethanolamine with 0.25% acetic acid to reduce nonspecific binding. The tissue was then dehydrated through a series of alcohols and allowed to air-dry for 1–3 h.

Hybridization. The 35S-labeled probe was diluted in hybridization buffer to yield a concentration of 1.5–3 million counts–min⁻¹–100 μl⁻¹. This diluted probe (100 μl) was applied to cover slips that were placed on the slides. The slides were incubated overnight at 55°C in sealed boxes containing filter paper soaked in 50% formamide. After incubation, cover slips were removed, and slides were rinsed. To reduce nonspecific binding, sections were incubated in RNase A solution, briefly washed, and then incubated in a high-stringency saline solution at 65°C for 1 h. Tissue was finally dehydrated through a series of alcohols and exposed to Biomax film (Kodak, Rochester, NY) in light-tight boxes for 14–72 h (depending on the probe). To verify the absence of nonspecific binding, sense probes were run using tissue from several representative time points.

Analysis

 Autoradiographs were digitized using MCID software (Imaging Research, St. Catharines, Ontario), and the magnitude of the hybridization signal was determined by experimenters blind to the group identity of the tissue using Scion Image software. This software analyzed the density of grayscale units for any particular brain region of interest relative to the background threshold signal measured from the corpus callosum (macro written by Dr. S. Campeau, University of Michigan). This measurement was then divided by the total area of the region of interest to produce a mean hybridization signal per unit area (we refer to this as the “hybridization signal” for any particular brain region).

Measurements taken for each animal were represented by the average hybridization signal of three mid-SCN sections (Fig. 1). The location of the SCN was determined by comparing the shape of the optic chiasm and other ventral landmarks with a published brain atlas (rat, see Ref. 47) or with our own previous anatomical characterization of the SCN (degu, see Ref. 18). Outside of the SCN, measurements taken for each animal were represented by the average
hybridization signal of two sections using 1.3-mm-diameter bilateral samples (Fig. 2). In the rat, these samples were taken from the striatum, parietal cortices, and cingulate cortices at 0.30–1.30 mm posterior to bregma (47). In the degu, samples were taken from regions showing analogous structure (e.g., cortical layering) or similar placement in relationship to local landmarks (e.g., the lateral ventricles, the corpus callosum, the optic chiasm). Because these regions have not been rigorously anatomically verified in this species, we refer to them as “putative”. These particular brain regions outside the SCN were selected for analysis because of (1) previous evidence that they contain rhythmic Period gene expression (31, 36) and (2) reliable presence in coronal tissue sections containing the SCN.

Statistics

Daily changes in the hybridization signal for mRNA probes were determined statistically using between-subject analyses. For within-species comparisons, nonparametric analyses were performed using the k-independent samples Kruskal-Wallis H Test because some data did not meet the parametric statistical assumptions of normal distribution and homogeneous variance. For between-species comparisons, we normalized the amplitude of the Per1 and Per2 hybridization signal rhythms because each species required a separate in situ hybridization. To perform this normalization, we defined “0” as the average hybridization signal of the trough time point and “100” as the average hybridization signal of the peak time point for each species. Between-subjects ANOVA was used to determine if there was a main effect of species on normalized hybridization signal in the SCN or an interaction between time point and species. Post hoc independent samples Student’s t-tests compared the normalized hybridization signal rhythms because each species required a separate in situ hybridization.

RESULTS

**Period Gene mRNA in the SCN of the Degu**

A strong hybridization signal for antisense Per1, Per2, and Per3 mRNA probes was observed in the SCN of the degu under 12:12-h LD and DD conditions (Figs. 1 and 3). No hybridization signal for sense Per1, Per2, and Per3 mRNA probes was observed in any brain region (data not shown).

**Degu Per1**. Per1 hybridization signal under LD and DD conditions showed a significant change over time [LD: n = 54, $\chi^2(11) = 37.479, P < 0.001$; DD: n = 27, $\chi^2(5) = 15.154, P = 0.010$; Fig. 3A]. Per1 was elevated during the light phase of the LD cycle [n = 54, T(23.64) = 6.322, P < 0.001] and the subjective day of the DD cycle (n = 27, U = 21, P = 0.003). Both conditions produced peak Per1 levels at ZT4/CT4.

**Degu Per2**. Per2 hybridization signal under LD and DD conditions showed a significant change over time [LD: n = 55, $\chi^2(11) = 49.205, P < 0.001$; DD: n = 26, $\chi^2(5) = 14.571, P = 0.012$; Fig. 3B]. Per2 was elevated during the light phase of the LD cycle [n = 55, T(38.013) = 3.252, $P = 0.002$] and demonstrated a nonsignificant trend toward elevation during the subjective day of the DD cycle (n = 26, U = 37, $P = 0.088$). The LD condition produced peak Per2 at ZT8.

**Degu Per3**. Per3 hybridization signal under LD and DD conditions showed a significant change over time [LD: n = 51, $\chi^2(11) = 37.029, P < 0.001$; DD: n = 5, $\chi^2(5) = 16.038, P = 0.007$; Fig. 3C]. Per3 was elevated during the light phase of the LD cycle [n = 51, T(23.368) = 6.853, $P < 0.001$] and the subjective day of the DD cycle [n = 25, T(22.79) = 3.464, $P = 0.002$]. The LD condition produced peak Per3 at ZT2. Under DD conditions, CT2 was not sampled, and a peak was found at CT8.

**Period Gene mRNA in the SCN of the Rat**

A strong hybridization signal for antisense Per1 and Per2 mRNA probes was observed in the SCN of the rat under 12:12-h LD conditions (Fig. 4). No hybridization signal for
found a significant main effect of species on normalized Per1 hybridization signal \( [n = 109, F(1, 85) = 24.907, P < 0.001; \text{Fig. 4A}] \), as well as an interaction between species and time point \( [n = 109, F(11, 85) = 3.402, P = 0.001; \text{Fig. 4A}] \). Following correction for multiple comparisons, post hoc analyses did not have enough power to reveal which specific time points were responsible for these species differences \( (P > 0.05) \). However, the species differences at several time points surrounding the peak had large \((r > 0.80, \text{ZT6})\) or moderate \((r > 0.50, \text{ZT2, -10, -12, and -20})\) effect sizes. Using 50% peak values as a relative measure of elevated mRNA levels, these results suggest that Per1 in the rat is elevated for twice as long as Per1 in the degu.

Similarly, we found a significant main effect of species on normalized Per2 hybridization signal \( [n = 91, F(1, 71) = 4.650, P = 0.034] \) as well as an interaction between species and time point \( [n = 91, F(9, 71) = 5.358, P < 0.001] \). Post hoc analyses revealed that these species differences were most significant at ZT14 \( (P = 0.020) \), and several time points surrounding the peak had large \((r > 0.80, \text{ZT2 and -14})\) or moderate \((r > 0.50, \text{ZT8 and -10})\) effect sizes. These results suggest that Per2 in the rat peaks later in the day than Per2 in the degu and is elevated above 50% of peak for at least two

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\text{Fig. 3. Period gene mRNA levels in the SCN of the degu show similar daily fluctuation under 12:12-h LD and constant darkness (DD) conditions. Relative mRNA levels in the SCN at each time point were compared using in situ hybridization. Separate in situ hybridizations were performed on LD and DD tissue; therefore, the mean hybridization signal for each time point \( (n = 3-5, \pm \text{SE}) \) is normalized as %peak, with trough given the value of “0.” Zeitgeber time (ZT)/circadian time (CT) 0 is double-plotted as CT/ZT24. Each of the three Period genes, Per1 (A), Per2 (B), and Per3 (C), exhibits significant daily fluctuation \((P < 0.02)\) in transcript level in the degu SCN under both conditions.}
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\text{sense Per1 and Per2 mRNA probes was observed in any brain region (data not shown).}
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Rat Per1. Per1 hybridization signal under LD conditions showed a significant change over time \( [n = 55, \chi^2(11) = 43.404, P < 0.001; \text{Fig. 4A}] \). Per1 was elevated during the light period of the LD cycle \( [n = 55, T(35.081) = 10.097, P < 0.001] \), with the peak occurring at ZT2.

Rat Per2. Per2 hybridization signal under LD conditions showed a significant change over time \( [n = 46, \chi^2(9) = 34.908, P < 0.001; \text{Fig. 4B}] \). Unlike in the degu, Per2 in the rat was not significantly elevated during the light period of the LD cycle \( [n = 46, T(33.793) = 0.381, P = 0.705] \). Peak Per2 occurred at ZT10.

Comparison of Period Gene mRNA in the SCN of the Degu and Rat

The monophasic rhythms of Per1 and Per2 hybridization signal in the SCN of degus and rats were grossly similar. However, differences existed in peak duration and timing. We

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\text{Fig. 4. Comparison of daily Period gene mRNA levels in the SCN of the nocturnal rat and diurnal degu. Relative Period gene mRNA levels in the SCN at each time point were compared using in situ hybridization. The mean hybridization signal for each time point \( (n = 3-5, \pm \text{SE}) \) is normalized as %peak, with trough given the value of 0. ZT0 is double-plotted as CT/ZT24. Both species demonstrate daily fluctuation (degu: \( P < 0.001 \); rat: \( P < 0.001 \)) in Per1 (top) and Per2 (B) mRNA levels in the SCN that differed between the two species \( (P < 0.05) \) and varied by time point \( (P < 0.001) \). In the rat Per2 dataset (bottom), ZT16 is absent, and ZT18 is not shown due to only having data from one animal.}
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hours longer. Because of data loss, rat Per2 was unable to be characterized at ZT16 and ZT18; therefore, it is possible that species differences in Per elevation could be greater than depicted.

**Period Gene mRNA Outside of the SCN in the Degu**

Outside of the SCN in the degu, strong hybridization signals for Per1 and Per2 were observed in the putative cortex, striatum, lateral septal nuclei, several thalamic nuclei, and the cornus ammonis (CA)1, CA2, CA3 and dentate gyrus regions of the hippocampus (Fig. 2). Strong hybridization signals for Per3 were observed in the putative cortex, striatum, and the CA1, CA2, CA3, and dentate gyrus regions of the hippocampus (data not shown).

**Putative cingulate cortex.** Per2 hybridization signal under LD conditions showed a significant change over time \( n = 49, \chi^2(11) = 22.037, P = 0.024; \text{Fig. 5A} \). Per2 was elevated during the light period of the LD cycle \( n = 49, T(30.75) = 2.754, P = 0.010; \text{Fig. 5B} \). Peak Per2 occurred at ZT0. Per1 and Per3 hybridization signals under LD conditions did not show a significant change over time \( \text{[Per1: } n = 46, \chi^2(11) = 6.524, P = 0.836; \text{Fig. 6, Per3: } n = 47, \chi^2(11) = 13.200, P = 0.280; \text{data not shown]} \) nor elevation during the light period of the LD cycle \( \text{[Per1: } n = 46, T(44) = 0.206, P = 0.838; \text{Fig. 6, Per3: } n = 47, T(45) = 0.841, P = 0.405; \text{data not shown}] \).

**Putative parietal cortex.** Per2 levels under LD conditions showed a nonsignificant trend toward change over time \( n = 54, \chi^2(11) = 19.391, P = 0.054; \text{Fig. 5A} \). Per2 was elevated during the light period of the LD cycle \( n = 54, T(40.953) = 2.405, P = 0.021; \text{Fig. 5B} \). Peak Per2 occurred at ZT0, and post hoc analysis indicated that ZT0 was significantly different from ZT12 \( (P < 0.05) \). Per1 and Per3 levels under LD conditions did not show a significant change over time \( \text{[Per1: } n = 53, \chi^2(11) = 11.946, P = 0.368; \text{Fig. 6, Per3: } n = 47, \chi^2(11) = 5.882, P = 0.881; \text{data not shown]} \) nor elevation during the light period of the LD cycle \( \text{[Per1: } n = 53, T(51) = 0.849, P = 0.400; \text{Fig. 6, Per3: } n = 47, T(45) = 0.266, P = 0.791; \text{data not shown} \). 

**Putative striatum.** Per2 levels under LD conditions did not show a significant change over time \( n = 54, \chi^2(11) = 11.088, P = 0.436; \text{Fig. 5A} \). However, Per2 was elevated during the light period of the LD cycle \( n = 54, T(31.728) = 2.367, P = 0.024; \text{Fig. 5B} \). Peak Per2 occurred at ZT6. Per1 and Per3 levels under LD conditions did not show a significant change over time \( \text{[Per1: } n = 53, \chi^2(11) = 9.286, P = 0.596; \text{Fig. 6, Per3: } n = 49, \chi^2(11) = 5.622, P = 0.897; \text{data not shown]} \) nor elevation during the light period of the LD cycle \( \text{[Per1: } n = 53, T(51) = 0.393, P = 0.696; \text{Fig. 6, Per3: } n = 49, T(25.408) = 1.232, P = 0.229; \text{data not shown} \).

**Period Gene mRNA Outside of the SCN in the Rat**

Outside of the SCN in the rat, strong hybridization signals for Per1 and Per2 were observed in the cortex, striatum, several thalamic nuclei, and the CA1, CA2, CA3, and dentate
showed a significant change over time [$n = 45$, $\chi^2(11) = 25.359$, $P = 0.008$], showing a peak at CT16 but were not significantly elevated during the dark period of the LD cycle [$n = 45$, $T(33.966) = -0.774$, $P = 0.444$; Fig. 6].

**DISCUSSION**

To date, this is the only study to document *Period* gene expression in the brain of the *O. degus*, a useful diurnal laboratory species, as well as the first to examine *Per3* expression in any diurnal animal. Examination of *Period* gene expression in the degu under both a 12:12-h LD cycle and constant conditions suggests that these rhythms are endogenously generated, as in other mammals. Within the SCN, degu *Per1* and *Per2* rhythms appear similar to those reported for the diurnal ground squirrel (*Spermophilus tridecimlineatus*), grass rat *ansorgei* (*Arvicanthis ansorgei*), and grass rat niloticus (*Arvicanthis niloticus*), (10, 37, 40, although see Ref. 29). Each of these species shows a peak in *Per1* expression during the early subjective day and a peak in *Per2* expression during the late subjective day.

We had originally hypothesized that differences in *Per3* might be related to diurnal chronotype because polymorphisms in *Per3* in humans correlate with delayed sleep phase syndrome and extreme diurnal preference (6, 16, 48). Indeed, the daily pattern of *Per3* mRNA in the SCN of the diurnal degu was several hours phase-advanced compared with previously published studies of *Per3* mRNA in the SCN of the nocturnal laboratory rat (12, 49), showing transcript levels that peaked during the day (ZT/CT2–8) and declined between ZT/CT10–14. However, we did not explicitly compare *Per3* mRNA in the two species and, the timing of the degu *Per3* peak and trough is similar to *Per3* rhythms in the nocturnal mouse (27, 60). Therefore, any differences in *Per3* waveform that may exist between the degu and rat are unlikely to be related to chronotype.

A detailed comparison of *Per1* and *Per2* waveforms in the SCN of the degu and rat revealed several differences. Rat *Per1* mRNA levels in the SCN peaked early in the day and maintained elevated levels for nearly two times as long as found in the degu (Fig. 4). *Per2* levels in the rat SCN also appeared to fall noticeably later in the evening than *Per2* in the degu SCN, although the full extent of this duration difference was not completely captured because of missed evening sample points. These trends are corroborated by our data from degus kept under DD conditions, as well as data from other rat studies (7, 39, 66–67). Furthermore, a review of current literature suggests that our data may identify a consistent difference in the duration of *Per* elevation in the SCN of diurnal and nocturnal mammals (Fig. 7 and Refs. 7, 9, 10, 22, 29, 33, 36–37, 39–40, 45, 61, 64, and 66–67). Therefore, we believe it is unlikely that these species differences are due to random chance or an artifact of the thresholding procedure used in our analysis. However, replication is desirable using additional diurnal and nocturnal species.

Outside of the SCN, the phasing of *Per2* rhythms of degus and rats differed by 180°, correlating with the differential phasing of their behavioral rhythms. Degu *Per2* mRNA in the putative cingulate cortex, parietal cortex, and striatum was found to be elevated during the early part of the light period. In contrast, *Per2* mRNA in these same regions in the rat was...
The function of period genes outside the SCN is currently a matter of speculation. Masubuchi et al. (31) have argued that the Per mRNA rhythms outside of the SCN are unlikely to be the result of locomotor activity, since the rhythms anticipate the beginning of activity onset and gradually decrease throughout the day while activity remains elevated. Our findings are similarly incongruent with the idea that Per outside of the SCN is a result of activity, since the trough in Per2 mRNA rhythms occurred at a time point (ZT10–ZT12) that is typically a period of vigorous activity in the degu (44). Therefore, our data support the proposition that Period gene expression outside of the SCN may represent slave oscillators that under normal conditions are driven by the master circadian oscillator in the SCN (19, 31). We propose that these slave oscillators are differentially phased in diurnal and nocturnal species.

In conclusion, the aim of this study was to correlate chronotype with the daily pattern of core clock gene expression within the SCN and external to the core circadian pacemaker. Because we considered relative mRNA levels in the SCN to reflect the phase of the circadian pacemaker, we believe that our study indirectly compared clock phase between a diurnal and nocturnal mammal. However, before we can draw any strong conclusions regarding the functional significance of these results, several parameters remain to be compared between diurnal and nocturnal species. For instance, although the mRNA level of analysis is useful, protein expression profiles are ultimately needed. Furthermore, a more careful characterization of mRNA expression in the core and shell regions of the SCN could yield further differences between diurnal and nocturnal species. Last, the in situ hybridization technique may not be sensitive enough to detect the low amplitude rhythms outside of the SCN. Replication of these results using quantitative RT-PCR may be a useful alternative.

**Perspectives and Significance**

Our study adds to growing evidence that the SCN may be adapted for a diurnal or nocturnal lifestyle (14, 25–26, 28, 34, 40, 51, 57). However, whether these differences in SCN function are sufficient to “cause” diurnal phasing of behavioral output or reflect an adaptation to a light-intense niche remains unresolved (20). Diurnal species contain many adaptations to circadian rhythms with a particular phase. For example, the degu has color vision and an increased threshold for circadian photic sensitivity (as reviewed in Ref. 20). Interestingly, both Per1 and Per2 are hypothesized to be insensitive to photic induction during midday elevation (32, 21). Therefore, our observation that the degu SCN shows a shorter duration of Per1 elevation could be related to the shorter duration of midday photic insensitivity that arguably characterizes diurnal mammals (23–24, 56).

Our data indicating that Per2 rhythms outside of the degu SCN are 180° out of phase with those rhythms in the rat are more likely to relate to questions regarding the origin of diurnal phasing of behavioral output. Nocturnal species with diurnally phased activity rhythms have been shown to exhibit similar changes in extra-SCN phasing (19, 31).

Finally, it is important to acknowledge that the direct application of information about mammalian core circadian mechanisms taken from nocturnal animal models may not be en-
tirely translatable to human circadian systems. Diurnal mammalian circadian systems have been found to be highly variable (54); therefore, it is vital that more species be examined to reach generalizable conclusions.

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