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Exploring determinants of behavioral chronotype in a diurnal-rodent model of human physiology

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Abstract

Numerous studies conducted with human participants have shown that differences in chronotype, defined as individual patterns of early or late beginning of daily activity, have implications for many biobehavioral processes, such as cognitive performance, mood, impulsivity, academic achievement of college students, and mental health. However, the determinants of individual variation in chronotype have not been investigated. Basic research on circadian rhythms has provided a basis for investigating the causes of chronotype variation, but experimental tests of pertinent hypotheses are difficult to conduct with human subjects. This limitation can be overcome by use of animal models. This study was conducted with a rodent species, the antelope ground squirrel (*Ammospermophilus leucurus*), that, like humans, is active during the daytime, exhibits a spread of chronotypes, and has a similar average free-running circadian period. We found chronotype to be a stable trait within individuals based on strong consistency of separate determinations made six months apart (correlation r = 0.91). We also found a moderate correlation of chronotype with the duration of the active phase (r = -0.51) and with free running period (r = 0.34), but weak correlation with rhythm robustness (r = 0.16), and no correlation with photic responsiveness or with masking responses. The best multiple regression model, incorporating the duration of the active phase, free-running period, and rhythm robustness, explained 38% of the variance in chronotype. Although 62% of the variance in chronotype remained unaccounted for, the results are encouraging because they document the possibility of using a diurnal rodent as a model for the investigation of the determinants of chronotype variation in humans.

Keywords: chronotype, circadian rhythm, locomotor activity, circadian entrainment, circadian masking, animal personality

1. Introduction

Four decades ago, Horne and Östberg [1] developed a "morningness-eveningness inventory" to classify people along a continuum from "morning types" or "larks" (people who usually wake up early and are more productive in the morning) to "evening types" or "owls" (people who usually wake up late and are more productive later in the day). Similar inventories have been developed since then [2-4], particularly the Munich Chronotype Questionnaire [5, 6], which has become popular in recent years [7-11].

Morning types usually wake up several hours earlier than evening types, for example an average of 07:20 versus 09:20 in a study in England [12], although the full spread of chronotypes (95% confidence interval of the mean) was found to be about 6 hours in a more extensive study [13], indicating that most people wake up within a 6-hour window in the morning, with "larks" as early as 6 o'clock and "owls" as late as noon. Consistently with these differences in wake-up time, the daily rhythms of body temperature [1, 14, 15], heart rate [16, 17], and melatonin secretion [15, 18-20] peak earlier in the day in morning types than in evening types. Morning types are more alert at wake-up time [3, 21], are better at recognition of sentences presented in the morning than in the afternoon [22], are less stressed out by morning commute driving than evening types [23], and prefer having sex earlier in the day than evening types [24]. Morning types tend to perform better academically than evening types both in high school [25-27] and in college [28-30]. Morning types also tend to be generally happier than evening types [31-33], whereas evening types tend to be more impulsive than morning types [34-37].

It seems likely that differences in chronotype may have implications for human health, both physical and mental. Disruption of the relationship between the internal circadian clock and the synchronizing environmental cycle, such as the disruption observed after transcontinental travel, with shift work, or even with the extensive use of artificial light in the modern 24-hour

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society, has been shown to have serious negative health effects. These include cardiovascular disease [38], higher incidence of breast cancer [39], development of metabolic syndrome [40], and increased occurrence of psychiatric disorders [41]. Specifically regarding mental health, a study in Taiwan found that, among college students, evening types displayed more psychopathology than morning types [42]. In Finland, a study of 10,500 adults found that eveningness was associated with increased odds of diagnosis of depressive disorder [43]. A study in Australia found that young people with various mental disorders, particularly affective disorders, tended more towards eveningness than did control individuals [44]. Among patients with major depressive disorder in Korea, evening types showed greater suicidal ideation than morning types [45].

Although not explicitly acknowledged by Horne and Östberg nor by most researchers investigating the implications of morningness or eveningness, the variation in chronotype corresponds to differences in the phase angle of entrainment of the circadian system with respect to the synchronizing environmental cycle. Animals, including humans, have an endogenous pacemaker that generates circadian rhythmicity but that is modulated by environmental stimuli, particularly the light-dark cycle. Given the natural frequency (speed) of the pacemaker, the frequency of the entraining environmental cycle, and the species-specific sensitivity of the pacemaker to the environmental cycle, the oscillatory pattern of the pacemaker establishes a predictable temporal relationship with the environmental cycle that is called the "phase angle of entrainment" [46, 47]. The phase angle of entrainment can be defined in reference to any stage of the environmental cycle, although it is often defined in reference to either lights-on (sunrise) or lights-off (sunset). "Morning types" tend to wake up and be more productive early in the day (and, therefore, have an advanced phase angle of entrainment), whereas "evening types" tend to

wake up later and be more productive in the afternoon and evening (and, therefore, have a delayed phase angle of entrainment).

When it is recognized that interindividual differences in chronotype are reflections of differences in the phase angle of entrainment, it becomes apparent that differences in chronotype should be present not only in humans but also in other animal species. A few studies of chronotype in individual animal species have been conducted over the years [48-54], and in a recent study the activity rhythms of individuals of 16 mammalian species, ranging in size from mice to cattle, were examined under comparable environmental conditions [55]. The full spread of individual chronotypes within each species was computed as the interval containing 95% of the chronotypes. This measure of spread was as narrow as 40 min in sheep and as wide as 23 h in cats. This means that all individual sheep initiated activity each day within a short, 40-min window, whereas individual cats initiated activity over the full course of the day. Importantly, the relatively intermediate human chronotype spread of 6 h was the same as that of the laboratory rat, being wider than those of 7 of the other species and narrower than those of 8 of the other species [55].

In the present study we document individual variation in time of daily activity onset in a laboratory population of 52 antelope ground squirrels, *Ammospermophilus leucurus*, a species that we have previously shown to be an excellent diurnal rodent model, more reliably diurnal than the Mongolian gerbil, the degu, and the Nile grass rat [56]. Our measurements allow us to chronotype the individuals and assess further relationships between the range of chronotypes and other parameters of circadian rhythmicity. The novelty of our study resides not only in a systematic search for the determinants of behavioral chronotype based on previously neglected knowledge of the basic mechanisms of entrainment of circadian rhythms, but also in the application of recent understanding of chronotype variation in non-human species. Because

human chronotype variation has additionally been associated with particular personality traits [34, 37, 42, 57-59], we also attempted to conduct behavioral tests that might illuminate the relationship between personality scores and chronotype in humans. Although personality traits are traditionally recognized only in humans, several recent studies have attempted to measure such attributes in animals [60-65].

2. Materials and method

2.1. Rationale

Basic research on circadian rhythms has established that the phase angle of entrainment depends on three variables: the natural frequency of the circadian pacemaker (or its reciprocal, circadian period), the frequency of the entraining environmental cycle (or its reciprocal, zeitgeber period), and the species-specific sensitivity of the pacemaker to the environmental stimulus (phase-shifting response curve) [46, 66]. Because the period of the entraining environmental cycle (the light-dark cycle) is constant on Earth, that is, each day lasts exactly 24.0 h, only the other two parameters need to be manipulated. The period of the pacemaker affects the phase angle of entrainment in a relatively simple way: if the clock is slow, it must be advanced each day, which means that a larger section of the phase-advance region must be exposed to light when the clock is slower; and, similarly, if the clock is fast, it must be delayed each day, which means that a larger section of the phase-delay region must be exposed to light. The third parameter is the sensitivity of the pacemaker to the environmental stimulus: if the pacemaker is very sensitive to stimulation, that is, if large phase-shifts can be evoked, then the section of the phase-advance or phase-delay region that must be stimulated will be smaller, and the phase angle of entrainment will be adjusted correspondingly.

In addition to the phase angle of entrainment, the distribution of activity over the course of a day can be affected by "masking". In diurnal animals, activity is usually favored by the presence of light during the night ("positive masking") and inhibited by darkness during the day ("negative masking") regardless of circadian time [67]. Thus, masking must also be considered as a potential predictor variable of chronotype.

Despite the accumulated knowledge about variation among individuals of a species in the time at which they begin activity and about mechanisms of synchronization of circadian rhythms, no previous study has investigated the relationship between chronotype and entrainment. Because this process takes place at the individual level, the investigation must focus on isolated individual subjects, which has not been done so far. Ideally, such an investigation should be conducted with human participants; however, because of the long time in isolation required of subjects in these types of study, it is important to first conduct studies in animal models to determine whether studies in humans are justified. We chose antelope ground squirrels (*Ammospermophilus leucurus*) as an animal model because this species is consistently and virtually exclusively diurnal, has very robust activity rhythms, exhibits a chronotype distribution as wide as that of humans, has a mean free-running period similar to that of humans, and is easily housed in the laboratory [56].

2.2. Subjects

Adult antelope ground squirrels of both sexes (29 males and 23 females) were either captured in the field in Owyhee County, Idaho (under Idaho Department of Fish and Game Permit No. 160812, n = 10) or born in captivity to wild-caught pregnant females (n = 42). Adult body masses ranged from 106 to 145 g with a mean of 123 g.

During the study, the animals were housed individually in polypropylene cages with wire tops (36 cm length, 24 cm width, 19 cm height). The cages were lined with wood bedding (Aspen Shavings, Northeastern Products Corporation, Warrensburg, NY) and were kept inside light-tight, ventilated individual chambers maintained in a room kept at 25 °C. Purina rodent chow (Rodent Diet 5001, Lab Diet, St. Louis, MO) was provided *ad libitum* on the metal cage top, which also held a water bottle with a sipping tube extending into the cage. All animals were housed under a light-dark cycle with 12 hours of light per day (12L:12D), which was controlled by a programmable electronic timer (ChronTrol XT, ChronTrol Corp., San Diego, CA) that activated white fluorescent bulbs (General Electric F4T5CW) generating an illuminance of approximately 360 lux (range: 340 to 390 lux across chambers), as measured 8 cm above the cage floor.

2.3. Procedure

Experimental procedures were reviewed and approved by the local Institutional Animal Care and Use Committee (Protocol No. 006-AC16-014) in accordance with the regulations of the *Guide for the Care and Use of Laboratory Animals* (U.S. National Research Council, 2011).

2.3.1. Chronotype

Each animal cage was equipped with a metallic running wheel (15 cm diameter) with a 5-mm wire mesh running surface (Small Run Around, Pets International, Elk Grove, IL). A small magnet attached to the wheel activated a magnetic switch affixed to the top of the cage and connected to data acquisition boards (Digital Input Card AR-B2001, Acrosser Technology, Taiwan) linked to desktop computers, and activity counts were saved in 6-min bins (0.1 h intervals).

In human studies, which are predominantly based on surveys rather than actual activity records, it has been suggested that chronotype could be defined as the time of mid-sleep on free (non-working) days [13]. In animal studies [55] and human studies with actigraphic data [68], the time of activity onset on free days (every day for laboratory animals) has been of practical value and was chosen for this study.

For determination of the average daily time of activity onset (chronotype), the squirrels were kept under a 12L:12D cycle for at least 4 consecutive weeks, and data from the second 2 weeks were used for analysis. Onset time was determined by a computer algorithm, as described previously [55]. For the computation, the program first smoothed the time series by means of a 7-h moving-averages procedure and phase-advanced it by 3.5 h to correct for the 3.5-h phase-delay caused by the moving-averages procedure. Then, for each 24-h interval, the onset time was computed as the time when the activity level rose above the daily mean. Occasionally (i.e., in fewer than 3% of the data sets), the algorithm failed to identify an onset for a given day. In these cases, the missing value was replaced with a random number within the range of the remaining onsets. Chronotype was computed twice for each animal, approximately six months apart, to verify reproducibility.

2.3.2. Free-running period

For the determination of free-running period, the animals were kept in constant darkness for 2 or more weeks after having been under 12L:12D for at least 2 weeks. Circadian period was computed using 10 consecutive days in constant darkness. Computation of free-running period was conducted by the chi square periodogram procedure [69, 70].

2.3.3. Phase shifting

The animals were kept under 12L:12D for 4 weeks before being placed in constant darkness and receiving a single light pulse 10 days later. Single 3-h pulses of white light were administered in each animal's own isolation chamber (360 lux) without physical disturbance of the animals. After the pulse, another 10 days were spent in darkness so that the new circadian phase could be determined. By convention, the time of activity onset is designated as CT 0 ("circadian time zero") for diurnal species. Pulses were administered at CT 20, i.e., approximately 20 h after activity onset, which corresponds to the peak of the phase-advance region of the antelope ground squirrel's phase-response curve [71]. Phase shifts were determined by drawing separate eye-fit lines through the onsets for 7 days before and 7 days after the pulse and calculating the time between the 2 lines on the first cycle following the pulse.

2.3.4. Photic masking

Masking was evaluated after the animals had been under the standard 12L:12D cycle, with lights on from 07:00 to 19:00, for at least two weeks. Masking was produced by presentation of 2 h of light in the middle of the night ("positive masking," with lights on from 00:00 to 02:00) or by presentation of 2 h of darkness in the middle of the day ("negative masking," with lights off from 12:00 to 14:00). A masking index was computed by subtracting the number of wheel revolutions during the 2-h window from the number of wheel revolutions during the same 2-h window on the previous day, when the standard LD cycle prevailed, and dividing the difference by the average number of wheel revolutions per 2 h during the previous 10 days.

2.3.5. Rhythm robustness

Rhythm robustness is a pertinent characteristic of a rhythmicity, even though not a parameter of entrainment. Robustness refers to the strength of rhythmicity and is closely related to the stationarity of the time series [72]. Robustness is independent of amplitude, except at the extreme low end of the range, because a rhythm with zero amplitude also has zero robustness. Rhythm robustness under 12L:12D was computed as the percentage of "perfect" rhythmicity, as calculated by the chi-square periodogram procedure, over 10 or more consecutive days [72].

2.3.6. Alpha

The duration of the active phase of the daily rhythm is called alpha. We calculated alpha by subtracting the activity offset time from the onset time. We calculated the offset time using a procedure similar to that used for the calculation of the onset, as described in section 2.3.1. Because alpha can be affected by the presence and duration of a light-dark cycle, we calculated alpha both under the 12L:12D cycle and in constant darkness.

2.3.7. Body temperature rhythm

To confirm that the onsets of the locomotor activity rhythm were adequate indices of chronotype, we submitted a subsample of animals to the monitoring of body temperature. Each one of 10 squirrels (5 males and 5 females) was injected intra-abdominally with a temperature-sensitive microchip (LifeChip PIT tags, Destron Fearing, Eagan, MN). The signal from the microchip was monitored by a custom-designed antenna system connected to an RM310 reader and an SM303 multiplexer (Biomark, Boise, ID). Locomotor activity was monitored with Konlen passive infrared motion sensors (Light in the Box, Seattle, WA). Temperature and activity data were continuously recorded with a desktop computer and saved in 6-min bins (0.1 h intervals). For standardization, 20-day segments were selected for analysis after the animals had

adjusted to the experimental conditions. Individual time series were analyzed by cosinor rhythmometry [73, 74].

2.3.8. Traits that may represent behavioral phenotypes of personality

Each animal participated in 3 behavioral tests, at least a week apart from each other, intended to imitate a simplified personality test. Each behavioral test was conducted in duplicate (on different days), so that the reproducibility of the results could be evaluated. The three tests were designed to simulate 3 of the "Big 5" personality traits [75], as follows:

1) The open-field test aimed to simulate the measurement of the personality trait of neuroticism (anxiety). For this test, a squirrel was removed from its home cage and placed in a clear plastic cage (52 cm length, 36 cm width, 30 cm height, with a perforated top to prevent the animal from jumping out of the cage but still allowing for ventilation) with 35 equal squares of approximately 8 x 8 cm drawn on the floor. Between 10:00 and 14:00 on a given day, an animal was placed in the open field and allowed to explore it. The variable recorded was the number of square borders crossed during 5 min. A lower number of crossings was interpreted as greater anxiety. The cage floor was wiped with a 75% alcohol solution and allowed to dry between tests.

2) The fruit-attraction test aimed to simulate the measurement of the personality trait of openness to experience. For this test, the animals were left undisturbed in their cages. Between 10:00 and 14:00 on a given day, half a green grape (3.5 g) was dropped inside an animal's cage. The variable recorded was the time, in seconds, that it took the animal to hold the grape in its forepaws and start eating it. A shorter latency was interpreted as more openness to experience, i.e., more sensation seeking. If the animal took more than 3 min to grab the grape, the time was recorded as 3 min. Although the test was conducted always at the same time of day (which could imply different states of satiety for animals with different chronotypes), we conducted further

tests at different times of day later on and were able to confirm that some animals were

consistently more attracted to fruit than others at various times of the day.

3) The cage-neatness test aimed to simulate the measurement of the personality trait of

conscientiousness. For this test also, the animals were left undisturbed in their cages. Between

10:00 and 14:00 on a given day, 10 days after the previous cage change, the cage was inspected

for neatness. The cage floor was visually divided into 8 equal segments, and the experimenter

counted the number of segments containing noticeable feces and urine. A lower number of soiled

segments was interpreted as more conscientiousness, i.e., more attention to cage neatness.

Whereas the open-field test has been widely used as an anxiety test in rodents [76], the

other two tests were developed specifically for the present study as a preliminary evaluation of

potential personality traits in antelope ground squirrels. The investigation of animal personality

is a new field of inquiry without well-established assessment instruments [60-65].

3. Results

The chronotype of most of our 52 animals was easily identified by visual inspection of

their daily records of running-wheel activity. The two animals with records exemplified in Fig. 1

had the most extreme chronotypes of all animals tested. The earliest animal (Fig. 1 A) started

running approximately half an hour before lights-on and continued, with occasional

interruptions, until approximately 2 h before lights-off. The latest animal (Fig. 1 B) started

running approximately 9 h after lights-on and continued well into the dark phase of the light-dark

cycle, although with a noticeable reduction of intensity after lights-off.

The unusually late squirrel (Fig. 1 B) stood out as an apparent outlier in the frequency

distribution of all chronotypes plotted in Fig. 2 A. All other squirrels initiated activity between

06:30 and 11:00 under the 12L:12D light-dark cycle. Because the distribution is right skewed

(mode at 07:30 and mean at 08:30), the apparent outlier may not be an actual outlier and may legitimately represent the long right tail of the distribution in the antelope squirrel population as a whole. A t test comparing the chronotypes of males (08:24) and females (08:30) revealed no significant difference between the sexes, $t(50) = 0.238$, $p = 0.813$, for which reason we have not indicated sex in our displays of the data.

Circadian period ranged from 23.6 to 24.8 h with a mode of 24.1 h (Fig. 2 B). Phase shifts evoked by a 3 h light pulse at CT 20 were modest, ranging from 0 to 2 h (Fig. 2 C). Few animals became active when the lights were turned on for 2 h in the middle of the night, reflecting weak positive masking (Fig. 2 D). Darkness during 2 h in the middle of the day reduced activity in some animals, as expected, but actually enhanced activity in other animals, resulting in positive rather than negative masking for these animals (Fig. 2 E). Rhythm robustness had a relatively symmetrical distribution with mode and mean of 50% (Fig. 2 F). Alpha (duration of the activity phase) ranged from 7.5 to 14 h, with a mean of 11.1 h under the 12L:12D cycle (Fig. 2 G); this mean was not significantly different from the mean alpha of 11.3 h determined in constant darkness, $t(51) = 1.018$, $p = 0.314$.

The chronotypes shown in Fig. 2 A were calculated as the means of two determinations conducted approximately 6 months apart for each animal. The strong correlation between the values obtained in the 2 determinations is shown in Fig. 3 ($r = 0.91$, $p < 0.001$). The correlation remains strong if the outlier is excluded ($r = 0.82$, $p < 0.001$), confirming that chronotype is a stable attribute of individual animals. Differences in chronotype due to aging could not be evaluated in detail because all the 42 animals born in the laboratory were the same age, with less than a week difference, and the age of the 10 animals caught in the wild could not be accurately determined, though at least one year older than those born in the laboratory. For a broad comparison, we used a t test to compare the mean chronotype of laboratory-born animals (08:25,

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age = 13 months) with the mean chronotype of wild-caught animals (07:55, age = 26 or more months). The 2 means were not significantly different, $t(50) = 1.387$, $p = 0.172$.

To confirm that the records of locomotor activity provided an adequate measure of chronotype, we submitted a subsample of 10 animals to the monitoring of body temperature. The average daily patterns of body temperature and locomotor activity of a representative animal show that the waveforms are very similar (Fig. 4). For all 10 animals, the acrophases of the temperature and activity rhythms were strongly correlated ($r = 0.92$, $p < 0.001$).

The bivariate correlations for chronotype and circadian parameters show that chronotype was significantly correlated with alpha (duration of the activity phase), with a correlation coefficient of $r = -0.512$ (Table 1). The negative correlation indicates that individuals with longer alphas had earlier chronotypes. Chronotype was also significantly correlated with circadian period, although with a smaller correlation coefficient of $r = 0.343$. The positive correlation indicates that individuals with longer free-running periods had later chronotypes. None of the other circadian parameters tested was significantly correlated with chronotype, but circadian period was negatively correlated with positive masking, meaning that individuals with longer free-running periods were less likely to exhibit an increase of activity evoked by exposure to light during the night, and alpha was positively correlated with positive masking, meaning that animals with longer active phases were more susceptible to positive masking.

Although bivariate analysis did not show a connection between rhythm robustness and chronotype, multiple regression analysis indicated that robustness ($\beta = 0.183$) contributed significantly, along with alpha ($\beta = -0.469$) and circadian period ($\beta = 0.334$), to the variance in chronotype. The regression with the 3 predictors yielded a multiple correlation of $R = 0.620$, $p < 0.001$, thus accounting for 38% of the variance in chronotype. The variance inflator factors ranged from 1.02 to 1.04.

For all 3 behavioral tests intended to measure personality traits, the 2 separate measurements were significantly correlated, indicating that the responses of the animals were stable (r = 0.75 for neuroticism, r = 0.69 for openness, and r = 0.54 for conscientiousness). The bivariate correlations for chronotype and putative personality traits are shown in Table 2. The absence of significant correlations between pairs of traits confirms that the 3 tests were measuring independent patterns of behavior, as intended. However, the absence of significant correlations between chronotype and personality traits indicates a lack of relationship between chronotype and putative personality traits.

4. Discussion

Most squirrels initiated activity between 06:30 and 11:00 under a 12L:12D light-dark cycle with lights on from 07:00 to 19:00. This chronotype spread of 4.5 h is a little wider than the spreads for fox squirrels and horses [55] and a little narrower than the 6 h spread for human subjects as determined by questionnaires [13] or by the acrophase (peak) of activity data obtained with accelerometers [68]. For a subsample of 10 antelope squirrels, we found that chronotypes specified by the acrophase of the body temperature rhythm and by the acrophase of the activity rhythm were strongly correlated (r = 0.92), thus confirming that behavioral chronotype is consistent with physiological circadian organization. Behavioral chronotypes for all squirrels determined twice six months apart were also strongly correlated (r = 0.91), which confirms the long-term stability of chronotype in antelope ground squirrels, as recently also demonstrated for humans [11]. Over longer periods of time, chronotype varies with age in humans [13], but we were not able to evaluate such variation in our animals because we did not have a known range of ages wide enough for a meaningful comparison.

Regarding correlations between the variables we measured, we must recognize that correlation per se does not demonstrate causation. However, many of the bivariate relationships have been previously studied in laboratory models and are known to represent causal links. For example, genetic mutations that promote a shorter circadian period have been shown to result in earlier chronotypes in hamsters [77]. Experimental reduction of photic sensitivity has been shown to affect the phase angle of entrainment in mice [78]. Negative photic masking has been shown to obscure the activity onsets under standard light-dark cycles in mice [79], thus affecting the determination of chronotype.

Our squirrels exhibited a moderate but significant correlation between chronotype and circadian period (r = 0.34), which means that interindividual variation in circadian period accounted for 12% of the variance in chronotype. Although the extreme, and infrequent, case of Advanced Sleep Phase Disorder in human patients has been associated with a shortening of circadian period in several studies [80-82], only one study has conducted a preliminary investigation of the determinants of chronotype in normal human subjects, in which the authors found a moderate correlation (r = 0.50) between habitual wake time and circadian period in 17 individuals studied in the laboratory under a forced desynchrony protocol [83]. Because the phase angle of entrainment to a 24-h light-dark cycle depends on circadian period, the shorter circadian period of morning chronotypes could explain why morning chronotypes have phase-advanced rhythms and why evening chronotypes, who have longer circadian periods, have phase-delayed rhythms. That study investigated only the effect of variation in circadian period and found 75% of the variance in chronotype to be unaccounted for [83]. Our study involved many more prospective predictors than that study, although 62% of the variance we observed in chronotype remained unaccounted for. In addition to circadian period, we found that activity duration (alpha) and rhythm robustness had significant effects on chronotype variation. We

found that masking and photic sensitivity did not contribute significantly to chronotype variation. The lack of contribution from photic sensitivity is consistent with our finding of a weak phase-shifting response to 3-h light pulses of 360 lux. Very few of our animals displayed a shift greater than 1 h, and some animals displayed no shift at all (Fig. 2 C). This weak responsiveness to photic stimulation is comparable to that of humans. For example, St. Hilaire and colleagues obtained maximum phase advances of only 1 h in human subjects stimulated with 8,000 lux for an hour [84]. Other researchers have obtained larger phase shifts but only by using pulses of 10,000 lux for up to 7 h [85-87]. Because the free-running periods of humans and antelope ground squirrels do not deviate much from 24.0 h, small daily shifts are sufficient for entrainment to 24-h light-dark cycles.

The mean alpha of 11.1 h in our squirrels fit well within the 12 h duration of light in the daily light-dark cycle and was not significantly different from the alpha determined in constant darkness. Alpha is not a functional parameter in the non-parametric theory of entrainment, and yet it was the variable most strongly correlated with chronotype in our study. Shorter alphas were associated with later chronotypes. This was possibly the case because alpha can theoretically affect both masking and photic responsiveness. Masking and photic responsiveness were not significant predictors themselves, but a shorter alpha allows more time for positioning of activity (and awareness) during the light phase of the light-dark cycle. A long alpha results in exposure to light both early and late in the light phase, thus potentially overlapping the phase-advance and phase-delay regions of the photic phase-response curve and consequently modifying the phase angle of entrainment.

Rhythm robustness was the third variable included in our multivariate regression model, despite not being considered in the non-parametric theory of entrainment. There is no obvious

reason why animals with stronger rhythmicity should have later chronotypes. We note that the correlation was weak (r = 0.16) and not significant in the bivariate analysis.

It was unexpected that even the extended set of prospective predictors (circadian period, alpha, and rhythm robustness) accounted for only 38% of the variance in chronotype. We cannot exclude the possibility that our experimental design may have missed an important predictor or misevaluated one or more predictors that were measured, but we believe that the issue resides in the expectation itself. That is, the expectation that the phase angle of entrainment (chronotype) could be fully explained by variations in circadian period and photic responsiveness, with modulation by photic masking, may not be justified. Although the non-parametric theory of entrainment has been widely accepted [46, 65], strong arguments have been made concerning its incompleteness, in the sense that entrainment to full light-dark cycles must involve parametric effects in addition to the non-parametric effects [88, 89]. Additional predictor variables not yet recognized could presumably account for the chronotype variation that was unaccounted for by the variables we investigated.

Given the results we obtained, it seems clear that further animal research would be useful to develop needed background information for designing human studies of the determinants of chronotype variation. Such knowledge will eventually provide strategies to improve human performance and combat disease. Once it is known how chronotype is determined in laboratory rodents, human studies can be designed in a manner that minimizes participant discomfort and optimizes data collection. Variations in circadian period, alpha, and rhythm robustness are strong candidates for determinants of individual variation in human chronotype, but other predictors must be identified.

Because various human chronotypes are associated with particular human personality traits [34, 37, 42, 57-59], we conducted some initial behavioral tests that we believed might

correspond to personality measures in humans, and we examined the correlation between our test scores and chronotype. However, we found no significant correlation between chronotype and the 3 traits we attempted to test: neuroticism, openness, and conscientiousness. Our tests had good test-retest consistency, but the lack of correlation with chronotype could have many reasons, including the validity of our tests as measures of putative personality traits. We note that a recent meta-analysis of the human literature on personality and chronotype revealed that conscientiousness is the only 1 of the 5 personality traits that correlates significantly with chronotype, with a modest $r = 0.29$ [90]. Although we found no significant correlation between chronotype and the 3 behavioral traits that we tested, we did find a marginal correlation between our estimates of openness and neuroticism ($r = -0.25$). This finding is consistent with findings that openness is not fully independent of neuroticism in human subjects [91].

In this study we documented individual variation in time of daily activity onset, or chronotype, in a laboratory population of diurnally active rodents, antelope ground squirrels. We found chronotype to be a stable trait within individuals. Chronotype was correlated most strongly with duration of the active phase of the activity cycle, alpha, and less strongly with free-running period and rhythm robustness, which together explained 38% of the variance in chronotype. We suggest that further animal research would be helpful to identify variables that account more extensively for the variance in chronotype, thus paving the way for effective studies in human subjects.

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Figure Captions

Fig. 1. Daily running-wheel activity rhythms (actograms) of squirrels with the earliest (A) and latest (B) average times of activity onset, representing two extreme "chronotypes." Time of day is indicated on the horizontal axis and number of days on the vertical axis. Raw data are plotted with 6-min resolution. The horizontal white and black bars above the actograms indicate duration of the light and dark parts of the light-dark cycle, respectively (lights on at 07:00 and off at 19:00).

Fig. 2. Frequency distribution of chronotypes and other circadian parameters of 52 antelope ground squirrels. (A) Chronotype, expressed as average time of day of activity onset. (B) Free-running period of circadian rhythm, duration in hours. (C) Phase shift, in hours. (D) Positive masking. (E) Negative masking. (F) Robustness. (G) Duration of daily activity (alpha), in hours.

Fig. 3. Consistency of chronotype, as a correlation of activity onset time, in hours, of individual squirrels measured twice, six months apart. Correlation coefficient, r = 0.91 (p < 0.001).

Fig. 4. Averaged records of body temperature (A) and locomotor activity (B) for an individual antelope ground squirrel with a chronotype (average time of onset of activity) of 07:42 while housed under a 12L:12D cycle with lights on at 07:00. The data are plotted with 6-min resolution. Each data point is the mean of 30 consecutive days. The error bars denote the standard errors of the mean plotted in 2-h intervals to avoid cluttering of the figure. The horizontal white and black bars at the top indicate duration of the light and dark parts of the light-dark cycle, respectively.

Table 1. Bivariate correlation table for chronotype and circadian parameters.

	Chronotype	Period	Shift	Masking +	Masking −	Robustness	Alpha
Chronotype	1						
Period	0.343 *	1					
Shift	0.071	0.249	1				
Masking +	−0.123	−0.298 *	0.039	1			
Masking −	0.049	0.098	−0.232	0.142	1		
Robustness	0.163	−0.169	−0.139	−0.024	0.147	1	
Alpha	−0.512 *	−0.082	0.220	0.275 *	−0.047	−0.081	1

* Without correction for multiple testing, $|r| > 0.273$ is significant for $p < 0.05$.

Table 2. Bivariate correlation table for chronotype and putative personality traits.

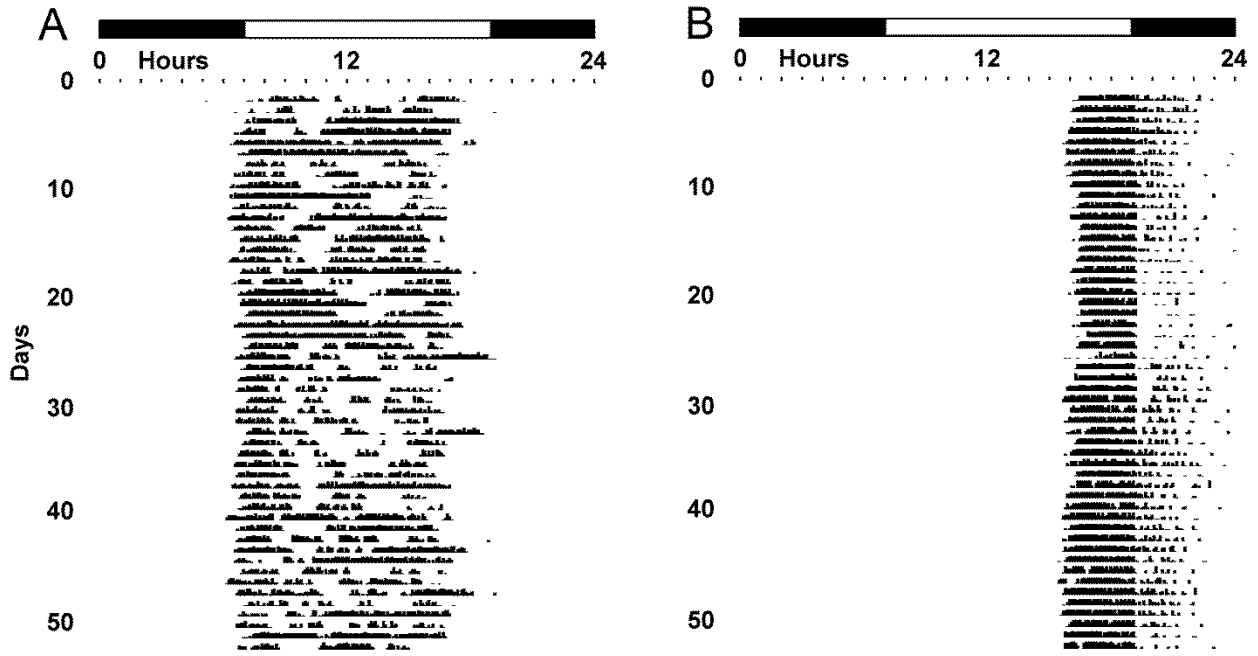|	Chronotype	Neuroticism	Openness	Conscientiousness
Chronotype	1			
Neuroticism	−0.031	1		
Openness	0.134	−0.252	1	
Conscientiousness	−0.141	0.146	−0.002	1

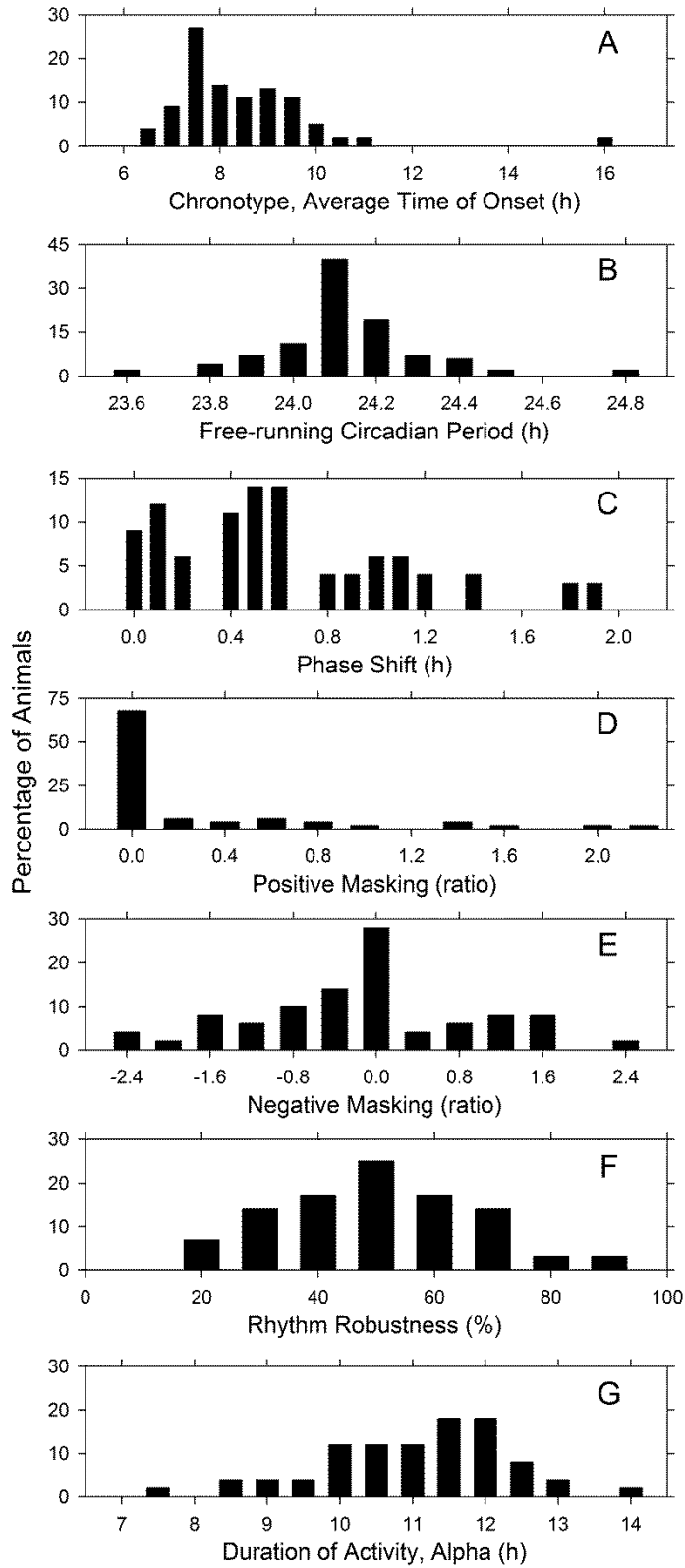* Without correction for multiple testing, $|r| > 0.273$ is significant for $p < 0.05$.
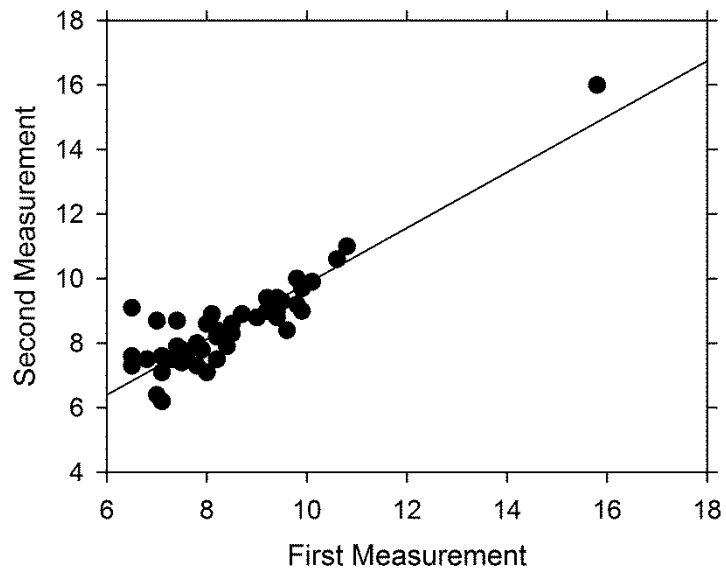
Figure 1

Figure 2

Figure 3

Figure 4