# Natural entrainment of circadian systems

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# Natural entrainment without dawn and dusk: the case of the European ground squirrel (*Spermophilus citellus*)

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## ABSTRACT

Observational data collected in the field and in enclosures show that diurnal, burrow-dwelling, European ground squirrel (*Spermophilus citellus*) never were above ground during twilight at dawn, or at dusk. The animals emerged on average 4.02 (sd = 0.45) hours after civil twilight at dawn, and retreated in their burrows on average 2.87 (sd = 0.47) hours before civil twilight at dusk. Daily patterns of light perceived by these burrowing mammals were measured with light-sensitive radio collar transmitters in an enclosure (the Netherlands) and in the field (Hungary). The observational data are corroborated by the telemetry data which show clear daily patterns of timing of light perception including light perceived from the burrow entrance. The first light was observed by the animals on average 3.54 (enclosure, sd = 0.45) and 3.60 (field, sd = 0.31) hours after civil twilight at dawn, while the final observed light was on average 3.04 (enclosure, sd 0.64) and 2.02 (field, sd 0.72) hours before civil twilight at dusk. Thus, the animals do not perceive the rapid natural light-dark transitions that occur at civil twilight. Instead, they generate their own pattern of exposure to light, within the natural light-dark cycle. The classical phase response model for entrainment by light or dark pulses can not explain how the circadian system of this species remains entrained to the external, natural light-dark cycle while the major light-dark transitions are created by its own behavior.

## INTRODUCTION

The process of entrainment of circadian systems by light has been studied intensively under laboratory conditions using unnatural, usually rectangular light-dark (LD) cycles (Pittendrigh 1981a). Few studies have inquired about the LD patterns and light intensities actually seen by animals (DeCoursey 1989) and humans (Savides *et al*. 1986) in their natural environment. According to the generally accepted theory on circadian entrainment by instantaneous phase shifting in response to light (Pittendrigh and Daan 1976b), animals in nature should theoretically (1) observe at least one of the two daily twilight transitions and (2) respond with their activity patterns to the day-to-day variations in light intensity. In this chapter these questions are adressed for the diurnal, burrow-dwelling, European ground squirrel (*Spermophilus citellus*) in an outdoor enclosure. Field observations on *S. saturatus* (Kenagy *et al*. 1989) and *S. citellus* (Chapter 2) have shown that these species are never seen above ground at the time of major light-dark transitions at dawn and dusk. Because the phase response curve (PRC) model for circadian entrainment attributes primary significance to the LD transitions (*e.g.* Pittendrigh & Daan 1976b), it is important to assess whether the animals perceive these transitions inside their burrows. Therefore, the light environment of individual ground squirrels was measured qualitatively using light-sensitive collar transmitters. The telemetric measurements were collected in outdoor enclosures in the Netherlands throughout the active season as well as in the squirrels' natural habitat in the Hungarian puszta. The data allow a precise assessment of the time of day when the animals perceive light information. The existence of a correlation between the annual change in timing of civil twilight and the onsets and offsets of activity was investigated. In addition, photometric measurements were used to estimate the light intensity perceived by the animals while being above ground. We tested whether day to day changes in light intensity at different times during the day affect the timing of the subsequent onset or offset of activity. The PRC model predicts that an increase in light intensity at the beginning of activity would result in a subsequent phase advance, whereas an increase in light intensity at the end of activity would result in a phase delay. This study is a first attempt to investigate whether the PRC model is sufficient to explain entrainment under natural conditions.

## METHODS

### Visual Observations

A total of 9 European ground squirrels (4 males; 5 females) were released in two enclosures (11 x 22 m each) at the Biological Center in Haren, the Netherlands (53°10´N; 6°36´ E) on September 7, 1994. In each enclosure 8 artificial burrows were provided but most animals used burrows dug by themselves. After the hibernation season, the daily timing of emergence above ground, and final departure below ground was studied by visual observations from February 21 until July 7, 1995. The animals could be individually recognized upon capture by Passive Identification Transponders (PIT tags, Trovan®, EID Aaltenbu, Aalten, the Netherlands) injected subcutaneously on the back. No PIT tag losses or infections at the injection site occurred, corresponding with the findings of Schooley *et al*. (1993) in *S. townsendii*.

During the observations the animals could be identified from a distance by black hair dye marks on their backs. When the dye marks became faint, the animals were trapped and repainted on days without observations. The ground squirrels fed on natural vegetation, but extra food (Teurlings® rabbit breeding chow, Ø 3 mm, Teurlings, Waalwijk, the Netherlands) was provided at four sites in each enclosure from May 15, 1995 onward. Observations were made from an observation hide 1.5 m above ground, that provided a good view over both enclosures. After March 28, 1995, the observations extended each week over at least two morning periods and two evening periods (Figure 3.3, horizontal lines). A morning observation started at civil twilight at dawn and did not end before all individuals had been seen at least once above ground. An evening observation started while all animals still were active (before 17:00 h) and ended at civil twilight at dusk. Every minute, the presence of each individual above ground was recorded.

### Telemetry

In a follow-up study, the light environment for each individual was recorded by measuring the light environment qualitatively with light-sensitive radio collar transmitters (Televilt; TXH-2/L; weight = 10 g, Televilt, International AB, Lindesberg, Sweden). In the enclosures in Haren, the Netherlands, 9 animals (4 males; 5 females) were studied from March 16 to October 29, 1996, and 5 animals (2 males; 3 females) were studied from April 18 to July 14, 1997. A further 8 animals (4 males; 4 females) were studied in the field at Bugac Puszta, Hungary (46˚38' N; 19˚40' E) from July 25 to August 7, 1996. A loop of the flexible antenna was led through a plastic tube to attach the transmitter to the neck of the animal, where as the free end of the antenna was straightened along the back of the animal. The transmitters were attached while the animals were lightly anesthetized with halothane for a few minutes.

Each transmitter could be identified by a radio frequency between 148 and 150 MHz that was at least 0.01 MHz different from the next transmitter. The pulse intervals of the transmitters varied between one and two seconds, depending on the light environment. The calibration of the transmitters showed steep sigmoidal curves with an inflection point around 0.03 lux. Hence, the signal could be interpreted as a yes or no signal (yes: light intensity > 0.03 lux, pulse interval ≈ 1 sec.; no: light intensity < 0.03 lux, pulse interval ≈ 2 sec.). The pulse interval data were received and stored by a receiver/data logger (Televilt; RX900-C). The receiver automatically sampled up to 12 pre-programmed channels in a continuous loop, staying tuned to each channel for 10 seconds. Thus, the sampling rate for each transmitter was at least one sample bout every two minutes. The logger was connected to a computer about once every week in the enclosure and twice every week in the field to download the data. The pulse intervals stored by the logger occasionally included some isolated aberrant values. These were due to positional changes of the animals, general noise on the frequency band employed, and occasionally missed pulses that caused the stored pulse interval to be twice as large as the actual transmitted pulse interval. The aberrant values were filtered out, separately for each channel, by custom made software on the basis of two criteria: (1) the pulse interval was outside the range from minimal (light) to maximal (dark) values, (2) the pulse interval deviated more than 50 ms from the preceding or following series of 50 measurements. When the proportion of deleted values in a moving window of 50 data points exceeded 70%, the whole data window was rejected.
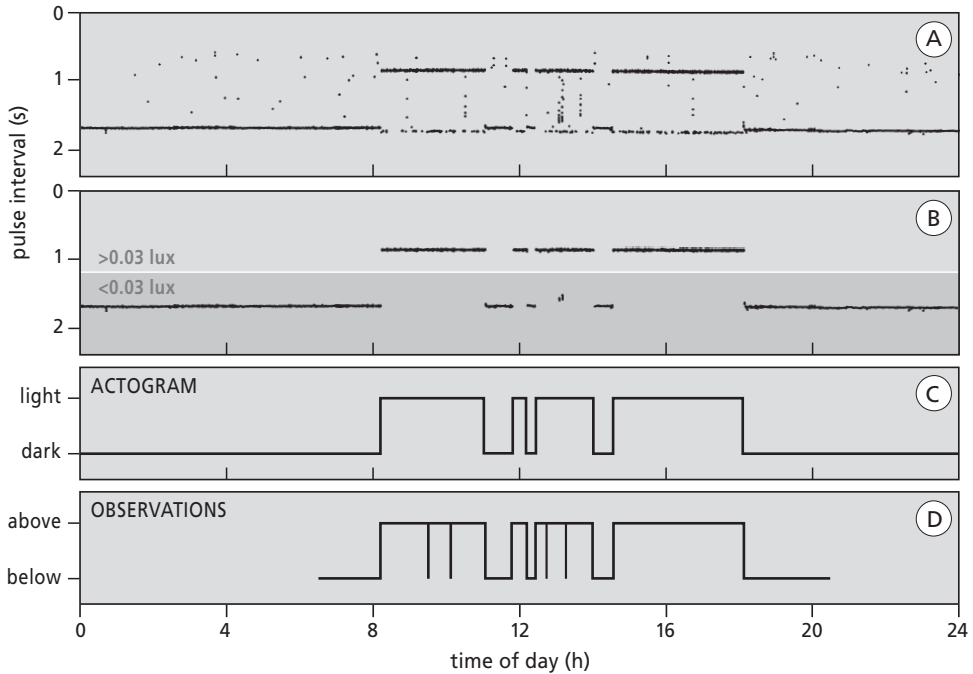
**Figure 3.1** Telemetry filtering example with simultaneous observations of female #86, during May 6, 1996. (A) Raw pulse interval data of a light-sensitive radio collar transmitter (148.075 MHZ). Interval data are plotted on an inverse Y-axis. (B) Same data without points rejected by the filtering program (see text). The horizontal line is indicating the inflection point of the calibration curve at 0.03 lux. Points above the line indicate the animal being in a light environment. (C) Actogram constructed from the data of panel B reducing the pulse interval to a yes or no signal. (D) Simultaneous observations with a one minute sampling rate of above-ground activity. Observations started at 6:30 h and ended at 20:30 h.

Figure 3.1 provides an example of a telemetry record obtained over one day. Figure 3.1A shows the raw, unfiltered pulse interval data as recorded, figure 3.1B the corresponding signal after filtering out the aberrant intervals, and figure 3.1C the resulting LD signal pattern. In addition, simultaneous visual observations were made every minute (Figure 3.1D). Both the telemetry actogram (Figure 3.1C) and the actogram based upon simultaneous observations (Figure 3.1D) show several short visits to a burrow. These visits often were observed to last less than a minute. Therefore, they occasionally might have been missed by the telemetry system, by the observer, or by both. Differences between visual observations and the telemetry data might also originate from the fact that animals may be located in a light environment where they cannot be detected by our visual observations (*e.g.* burrow entrances). A 95% correspondence was found between observational data and telemetry data that were simultaneously collected for six animals over one complete daily activity phase. The daily onset and offset of above ground activity as derived from light-sensitive radio telemetry records and visual observations showed close correspondence in this example as well as in other simultaneously collected data sets.

Missing data in the light telemetry data set occurred for several reasons other than the filtering technique described above. Regular checks of the animals revealed, in some cases, irritation of the skin after wearing the transmitters for about 1 month. In these cases, the transmitters were removed and replaced only after the skin had healed. Fluctuations in the frequency of the transmitters or the receiver, in combination with the narrow band width of the transmitters, sometimes caused loss of the transmitted signal. In those cases, the signal was restored by retuning of the receiver.

### Activity

Onset of above ground activity was defined as either the first visual observation or, in the case of the telemetry data, the first light signal of an animal for that day. Offset of activity was defined as either the final observation or the final light signal of an animal for that day. Only onsets and offsets on days without missing data during the inactive phase between civil twilight and onset or offset of activity were included in further analyses. Civil twilight (sun position 6° below the horizon) was used as a reference point in evaluating phase angle differences ($\psi$) of activity onsets and offsets. At this time, the logarithm of solarirradiance level is changing most steeply and is about half way between the night and day values (Daan and Aschoff 1975). Phase angle differences were calculated as the difference in time between the onset of activity and civil twilight at dawn, derived from the *Nautical Almanac*, or between the offset of activity and civil twilight at dusk. $\psi_{onset}$ was negative whenever activity started after civil twilight at dawn, and $\psi_{offset}$ was positive whenever activity ended before civil twilight at dusk.

### Light Intensity

To correlate daily activity patterns with light intensities in the course of the day, vertical solar radiance (in $W.m^{-2}$) measurements, averaged over each hour, were obtained from the Royal Netherlands Meteorological Institute (KNMI). These measurements were taken at Groningen airport Eelde, at 6 km distance from the enclosure. The sensor diffusor was half spherically shaped and pointed upward, perpendicular to the earth's surface. For calculating the correlations with activity onset or offset, average light intensity in the morning (8:00 - 9:00 h), around noon (12:00 - 13:00 h), and in the afternoon (16:00 - 17:00 h) were derived from this data set.

## RESULTS

### Visual Observations

An example of a representative individual above-ground activity pattern is presented in figure 3.2. The timing of the observations, indicated by horizontal lines, was organized to measure the first emergence (onset) and final retreat (offset) in the daily above-ground activity patterns. The data show that the animals never were observed above ground at the times of civil twilight. The animals emerged on average 4.02 (sd = 0.45) hours after civil twilight at dawn, and they retreated on average 2.87 (sd = 0.47) hours before civil twilight at dusk.
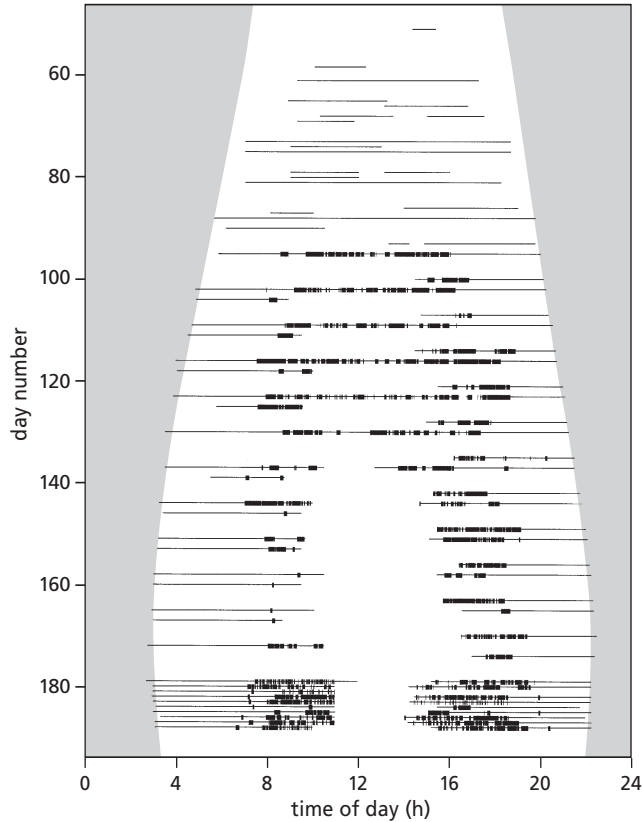
**Figure 3.2** Above-ground activity record of one animal (female #69). Data were collected by visual observations with a frequency of one scan every minute. Observation periods are indicated with horizontal lines; minutes in which the animal was observed above ground are indicated with a vertical marks. Shaded areas indicate night phases bordered by civil twilight times.

### Ligh-Sensitive Radio Telemetry

Figure 3.3 shows records of above ground activity patterns of 2 male and 3 female ground squirrels measured with light-sensitive radio collar transmitters in 1996. The data show that the animals did not perceive light around civil twilights at dawn and dusk. In only one case (female #86; July 13, 1996; not shown) the telemetry system detected above ground activity starting 5 minutes before and ending 23 minutes after civil twilight at dusk. This shows that the telemetry system is able to detect above-ground activity around civil twilight. The timing of onset and offset of activity as measured with light-dependent telemetry is in line with the visual observations presented in figure 3.2. The first light detected by the animals is on average 3.54 (sd = 0.45) hours after civil twilight at dawn, the final light observed is on average 3.04 (sd = 0.64) hours before civil twilight at dusk. The telemetry data collected in the field (Figure 3.4) showed similar patterns with first observed light on average 3.60 (sd = 0.31) hours after dawn civil twilight, and final observed light on average 2.02 (sd = 0.72) hours before dusk civil twilight.
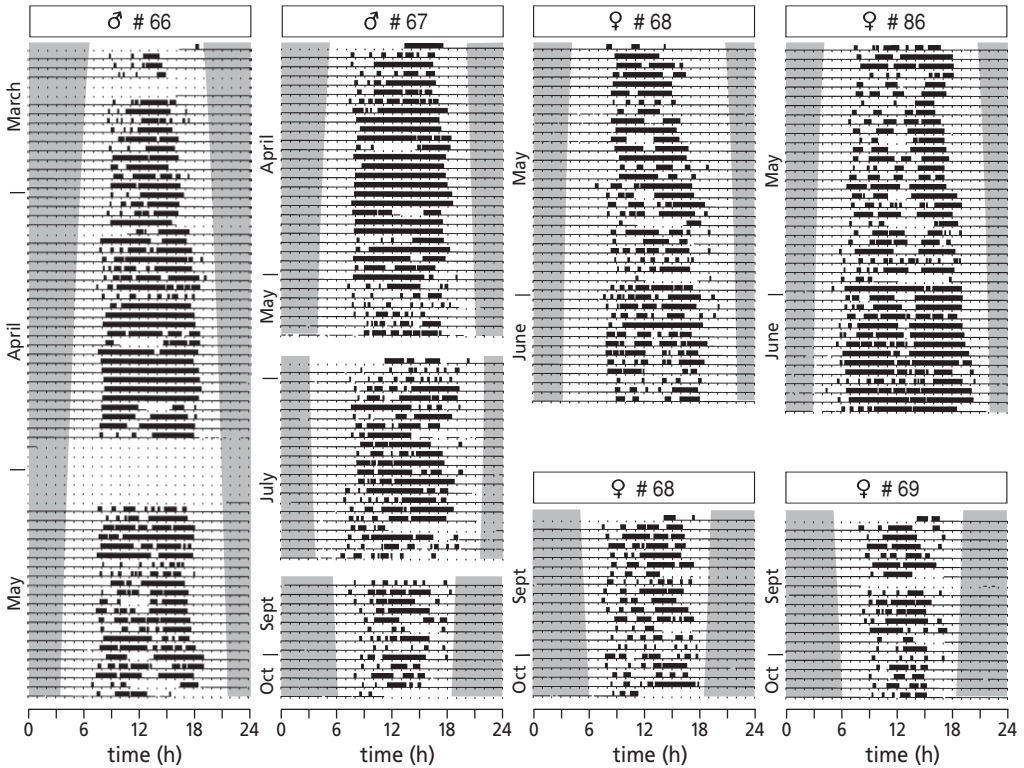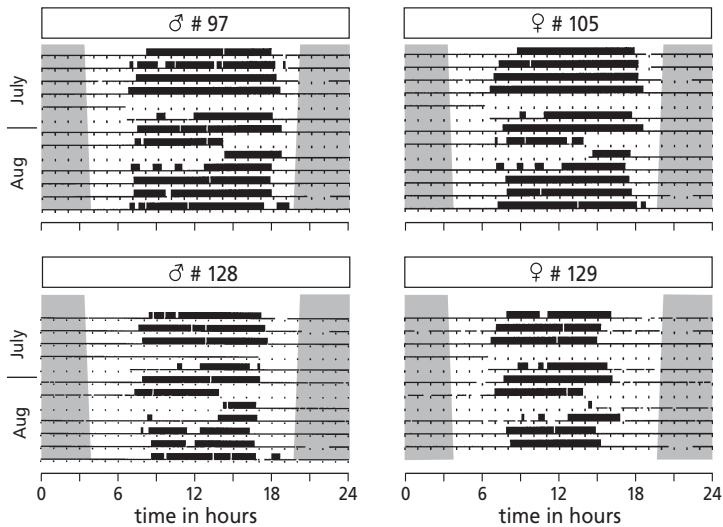
**Figure 3.3** Actograms based upon light-sensitive radio telemetry recordings at the enclosure in Haren. For each 2 minute bin, a vertical mark indicates when the animal was in a light environment (above ground), a horizontal line indicates when the animal was in a dark environment (below ground). Missing data are indicated by the absence of a horizontal line. Shaded areas indicate night phases bordered by civil twilight times.

### Phase angle difference

The average phase angle differences for 10-day bins of the onset of activity and civil twilight at dawn ($\psi_{onset}$), and the offset of activity and civil twilight at dusk ($\psi_{offset}$) are presented in figure 3.5 for both the visual observations and telemetry data. Overall, the observational data and telemetry data correspond with each other.

The telemetry data collected at the field site show a slightly longer activity phase when compared with the data obtained in the enclosure. Both observations and telemetry data show that $\psi_{offset}$ was fairly constant throughout the active season. This indicates that the offsets follow a curve that is of similar shape as the yearly changes in civil twilight at dusk but about 3 hours advanced. $\psi_{onset}$ was minimal around the start of the summer, with emergences above ground approximately 5 hours after dawn. The data further suggest a local minimum in $\psi_{offset}$ in April. During this period, mating and gestation takes place (Millesi *et al.*, 1999b) which might cause the ground squirrels to stay above ground slightly longer than in March and May.

**Figure 3.4** Actograms based upon light-sensitive radio telemetry recordings in the field at Bugac Puszta National Park, Hungary: for a juvenile male (#97), juvenile female (#105), adult male (#128) and an adult female (#129). For each two minute bin, a vertical mark indicates when the animal was in a light environment, a horizontal line indicates when the animal was in a dark environment. Shaded areas indicate night phases bordered by civil twilight times. Missing data are indicated by the absence of a horizontal line. Downloading the data on July 30 and August 2 caused approximately one day of missing data, gaps occurring at the same time in all four actograms indicate periods in which data logging was stopped to re-tune the receiver.

## Light Profile

The onsets and offsets of a representative animal were plotted on corresponding curves of hourly variation in solar radiance for 7 consecutive days in mid-May, 1996 (Figure 3.6). The curves indicate the day to day variation in light intensity which is perceived by the animal during its activity phase. Minor differences in radiance levels during above ground activity are in the order of 1 log unit. These differences are mainly due to variation in cloud cover. The animal may also perceive a slight change in radiance level due to solar altitude just after activity onset and before activity offset. In general, these differences are less than 1 log unit. The telemetry data, which indicate when the animal was exposed to sunlight, were combined with simultaneously measured light intensity, to reconstruct the actual light intensity perceived by the animal (Figure 3.7). Such a plot indicates the variation in solar radiance due to differences in overcast (< 1 log unit) and movements in and out of the burrow (> 7 log units).
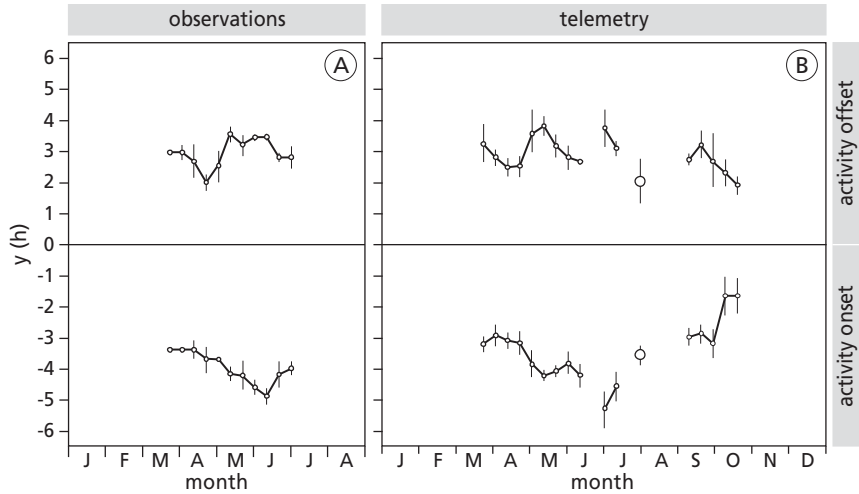
## Activity Onset and Offset

Multiple regression analyses were used to test whether differences in light intensity between days affect the subsequent activity onset or offset as measured with the light-dependent telemetry system in 1996 and 1997 (Table 3.1). In the first regression model, average activity onset of several individuals per day was analysed as a dependent variable. Civil twilight times at dawn and dusk, and light intensities of the previous day (average radiance in the

**Table 3.1** Daily onset (in h) and offset (in h) of activity explained, in multiple regression models, by time of civil twilight at dawn ($T_{ct\ dawn}$, in h) and dusk ($T_{ct\ dusk}$, in h), light intensity in the morning (average radiance between 8:00 and 9:00 h ($I_m$, in $^{10}log(W.m^{-2})$)), light intensity around noon (average radiance between 12:00 and 13:00 h ($I_n$, in $^{10}log(W.m^{-2})$)), and light intensity in the afternoon (average radiance between 16:00 and 17:00 h ($I_a$, in $^{10}log(W.m^{-2})$)). Timing of individual onsets and offsets of activity were measured by light dependent telemetry. Onsets and offsets were calculated as population averages for each day in two years (1996 and 1997). Light intensities of the previous day (i-1) were correlated with (subsequent) activity onset on day i, whereas light intensities of the same day (i) were correlated with (subsequent) activity offset on day i. Changes in residual sum of squares (Δ residual SS) and significant levels (p) were computed by excluding significant variables from, or including rejected variables in the final model. Onset final model: $r^2 = 0.27$; offset final model: $r^2 = 0.58$.

| dependent variable | parameter | Δ residual SS | Δ d.f. | p | coefficient |
|---|---|---|---|---|---|
| ONSET | null model | 61.79 | 122 | | |
| | final model | 45.30 | 120 | | |
| | constant | | +1 | | -27.93 |
| | $T_{ct\ dawn}$ | +10.47 | +1 | <0.0001 | +1.73 |
| | $T_{ct\ dusk}$ | +7.80 | +1 | <0.0001 | +1.37 |
| rejected terms | $I_{m,\ i-1}$ | -0.10 | -1 | 0.60 | |
| | $I_{n,\ i-1}$ | -0.13 | -1 | 0.57 | |
| | $I_{a,\ i-1}$ | -0.25 | -1 | 0.42 | |
| OFFSET | null model | 156.99 | 111 | | |
| | final model | 65.32 | 109 | | |
| | constant | | +1 | | -0.23 |
| | $T_{ct\ dusk}$ | +61.49 | +1 | <0.0001 | +0.73 |
| | $I_{m,\ i}$ | +7.56 | +1 | 0.0006 | +1.09 |
| rejected terms | $T_{ct\ dawn}$ | -0.45 | -1 | 0.39 | |
| | $I_{n,\ i}$ | -1.54 | -1 | 0.11 | |
| | $I_{a,\ i}$ | -0.04 | -1 | 0.80 | |

morning, at noon, and in the afternoon) were used as independent variables. In the second regression model, average activity offset of several individuals per day was analysed as a dependent variable. Civil twilight times at dawn and dusk, and light intensities of the same day (average radiance in the morning, at noon, and in the afternoon) were used as independent variables. European ground squirrels are rarely seen above ground during rainfall which might affect subsequent timing of onset or offset. Therefore, onsets following days with rainfall between 6:00 h and 20:00 h and offsets on days with rainfall between 6:00 h and 20:00 h were excluded from the analyses.

Only light intensity at noon was found to have a significant positive effect on the timing of offset on that day. Average light intensity at noon was found to be 486.44 (sd = 212.26) W.m$^{-2}$, ranging from 58 to 861 W.m$^{-2}$. Using the coefficients given in Table 3.1, we calculated that variation in light intensity at noon of ±1 sd would result in a variation in offset of ±0.51 h. This effect is considerable when compared with a standard deviation of ±0.81 h of the residual offset when light intensity at noon is excluded from the regression model (Table 3.1).

**Figure 3.5** Phase angle difference (ψ) between civil twilight and the onset (negative ψ) and offset (positive ψ) of activity during the course of the year. Data are average values over 10 days, based upon daily population averages. Between-days standard deviations are indicated by error bars. Direct observations of surface activity, collected in 1995 in the enclosure (A). Telemetry data of 1996 and 1997, collected in the enclosure (B, line) and telemetry data collected in the field (B, large circle).

## DISCUSSION

Observations of above ground activity and measurements by light-sensitive radio telemetry showed that European ground squirrels were always below ground in the darkness of their burrows at the times of the major light transitions during twilight. The squirrels potentially could have watched dawn and/or dusk by choosing positions in the burrows where light intensity was attenuated below 0.03 lux. Here they would not have been detected either by our visual observations or by the telemetry system. Similar light-sampling around dusk has been described in nocturnal bats (Voûte *et al*. 1974) and flying squirrels (DeCoursey 1986). We consider it most improbable that the ground squirrels showed such behavior. One should first expect that the threshold of 0.03 lux eventually will be exceeded which would indicate light perception well before the animal emerges above ground. The close correspondence between observations and telemetry shows that this is not the case. In addition, European ground squirrels block the entrance tunnels to their burrows with soil after their final daily entry into the burrows (Hut and Scharff 1998), which surely prevents light from penetrating into the burrow system. Thus, the animals do not perceive the natural LD transitions during twilight, but instead create their own self-selected light regime. Figure 3.7 provides an example of the deduced pattern of light intensity perceived by a ground squirrel over a day. Most of the variance in this example was due to the animal going in and out of its burrow and only a minor part originated from the natural LD cycle.

In the widely accepted PRC model for circadian entrainment (Pittendrigh 1981a) perception of the twilight LD transitions is crucial. In this theory, the discrepancy between the endogenous circadian period (τ) and the period of the LD cycle (T) is corrected each day by instan-
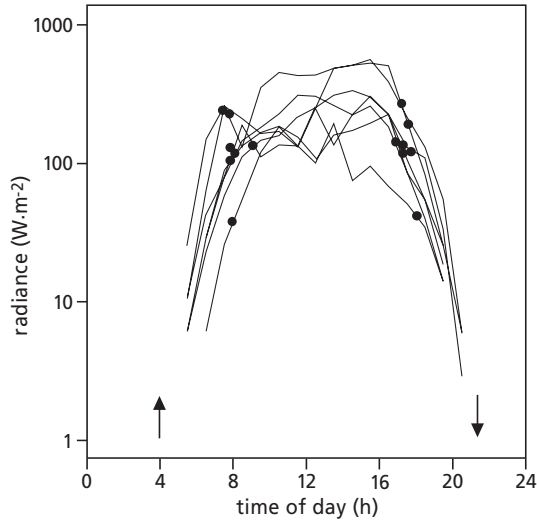
**Figure 3.6** Radiance profiles for seven consecutive days (May 11 - 17 , 1996). As an example, the onsets and offsets of male #66, simultaneously, measured with light-dependent telemetry, are plotted on the radiance curves of the corresponding day to indicate the variation in light intensity between days that was perceived by the animal during its first and final daily presents above ground. Arrows indicate timing of civil twilight at dawn and dusk.
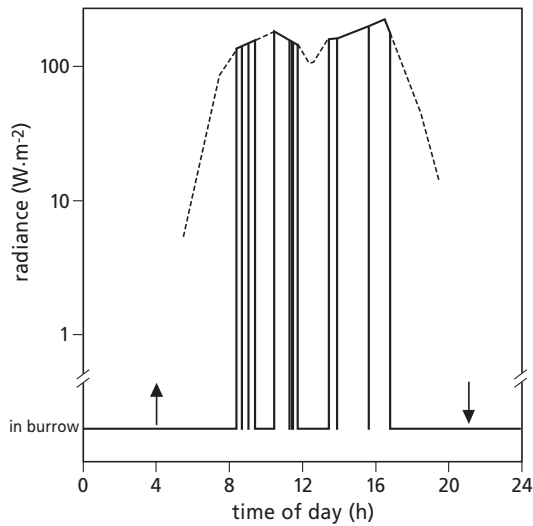


**Figure 3.7** Example of a daily pattern of radiance as perceived by female #70. The solar radiance curve for May 13, 1996 is indicated with a dotted line. The light environment of the ground squirrel, as measured with light-dependent telemetry, is depicted with a solid line, indicating those parts of the solar radiance curve that are perceived by the ground squirrel while being above ground. The radiance levels inside the burrow are lower than 0.03 lux ($\approx 1.10^{-4}$ W.m$^{-2}$), as measured with the light dependent collar transmitter. Arrows indicate timing of civil twilight at dawn and dusk.

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taneous phase shifts occurring at dawn and/or dusk as the light-sensitive portion of the PRC drifts into the light. If the animal itself generates the LD transitions by emerging long after dawn and retiring long before dusk, it can not use these LD transitions as a zeitgeber. Thus, in the European ground squirrel entrainment under natural conditions can not be explained by a model that is solely based upon instantaneous phase shifts due to LD transitions.

The PRC model would further make it likely that increased intensity of light at the beginning of the subjective day would cause an advance in the circadian rhythm, whereas an increased intensity of light at the end of the subjective day would cause a delay. In our data set, no evidence was found that such relationships actually occur under natural entrained conditions. The only effect of solar radiance levels on activity patterns was the positive association between solar radiance at noon and subsequent activity offset. Solar radiance at noon may decrease in the order of 1 log unit due to cloud cover, which correlates with an earlier activity offset (Table 3.1). This indicates that ground squirrels stay above ground longer when the conditions during daytime are favorable. This tendency might well represent a masking effect rather than a shift of the internal clock.

Positive correlations between times of civil twilight and onset or offset of activity do not imply a causal role of civil twilight in the process of entrainment. On the contrary, civil twilight is not even perceived by the animals. Obviously other factors are responsible for entrainment. We discuss several cues that might be used as a zeitgeber in the ground squirrels.

First, there are potential nonphotic zeitgebers. For example, the ground squirrels might use acoustic information, such as bird song, as a zeitgeber. We consider this unlikely because the environmental noise patterns and bird fauna in our enclosures in a suburban area in the Netherlands must be very different from the open Hungarian puszta. Also, the presence of bird song, or other sounds, varies in the course of the year and, therefore will not provide a reliable time cue. Alternatively, the ground squirrels might have learned to use acoustic information as a predictor variable for twilight times. Indeed, Amir and Steward (1996) reported that the activity rhythm in rats can be shifted by a nonphotic conditioned stimulus; these results, however, could not be reproduced by De Groot (1998). Such a mechanism would require at least some days on which conditioning or reinforcement takes place. We consider such speculations implausible since we never measured regular reoccurrence of activity around civil twilight.

Daily temperature cycles might also act as a zeitgeber. Laboratory studies in squirrels have shown large individual variations in responses to temperature cycles (Pohl 1998; Rajaratnam and Redman 1998). In these studies, only a few individuals actually entrained to temperature cycles, and some of them were found to be 180° out of phase with the temperature cycle. Similar results were obtained in the European ground squirrel (personal observations). Temperature cycles can at best be considered to be a weak zeitgeber in ground squirrels. Furthermore, daily temperature variation in the field is not very reliable and strongly buffered in the ground squirrel burrows. Temperature measurements in a vertical burrow entrance on the Hungarian puszta revealed the absence of any daily temperature fluctuations below a depth of 25 cm (personal observation). Ground squirrels which sampled temperature fluctuations also would receive light information coming from the burrow entrance. We measured light intensities inside burrow tunnels on a cloudy day around noon at distance of 25 cm from the entrance. These were found to be in the range of 10 to 100 lux and, therefore, would have

been detected by the light-sensitive radio collars. In addition, the largest temperature transitions are those generated by the animal itself when emerging and retreating underground. Possibly the strongest argument against nonphotic entrainment to natural cues in ground squirrels comes from the finding that bilaterally enucleated golden mantled ground squirrels do not show entrainment in outdoor cages (Nelson and Zucker 1981).

Second, there are several possible photic zeitgebers. Changes in spectral composition of sunlight have been suggested to be important for entrainment (Krüll *et al*. 1985; Roenneberg and Foster 1997; Von Schantz *et al*. 1998). Ultraviolet (UV) light increases sharply several hours after dawn and decreases well before dusk, and might be surmised to act as a zeitgeber. We have found, however, that the circadian system of the European ground squirrel is insensitive to UV light (Chapter 7).

Roenneberg and Foster (1997) suggested a possible role for a time-compensated sun compass orientation like mechanism that might be used for entrainment. The position of the sun relative to certain landmarks in principle can provide information on the time of day. Although theoretically possible, there are no data to show the presence of such a mechanism in mammals. This hypothesis surely would require an entrainment mechanism very different from phase response entrainment as measured under laboratory conditions. Such a mechanism also would involve some type of learning of the positions of landmarks and, therefore of conditioning with respect to LD transitions.

Although European ground squirrels do not perceive the major changes in radiance level during twilight, the changes in radiance levels in the course of the day show that the ground squirrels might perceive a decrease in solar radiance of about 1 log unit just before they retire into their burrows at the end of their activity phase, about 3 hours before civil twilight at dusk (Figure 3.6). Similar changes in light intensity might be perceived by some individuals at the beginning of their activity phase. These changes do not exceed the variations in radiance levels in the middle of the light phase, which also are in the order of 1 log unit. Around noon these changes are erratic due to cloud cover, whereas at the end of activity, this decrease in light intensity reflects a more regular time of day dependence. Yet, they form only a reliable signal only if averaged or integrated over several days, suggesting that entrainment in the ground squirrel might involve more than simple phase shifts each day. This argument does not necessarily hold for nocturnal burrowers. They would not see any light (except moon- or starlight) if their activity above ground does not extend into the twilights. Indeed, there is evidence that the twilight transitions actually are used by nocturnal rodents (DeCoursey 1986; DeCoursey 1989). Also, LD cycles with simulated twilights have proved to form stronger zeitgebers in natural rodents than ad square wave LD cycles (Boulos *et al*. 1996a; 1996b; Roenneberg and Foster 1997; Wever 1967). We do not pursue the modes of entrainment of diurnal and nocturnal animals further here. In a companion paper (Chapter 9) we explore the possibility that adjustment of period in response to light contributes to the entrainment process by allowing integration of light information over several cycles (Beersma *et al*. 1999).

Whatever cue the animals are using, entrainment must be a great deal more complicated than is indicated by models based solely on instantaneous phase shifts. This study illustrates the importance of measuring behavior under natural conditions for theories based on laboratory measurements.

## ACKNOWLEDGMENTS