The European souslik in the natural light-dark cycle: what is the signal for afternoon retreat?

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Abstract: European sousliks (*Spermophilus citellus*) are strictly diurnal with a very precise daily activity rhythm in field conditions. This activity rhythm is regulated by the internal biological clock which in turn is synchronized by the natural light/dark cycle (entrainment). Due to their regular retreats into the darkness of their burrows, fast light intensity changes in natural light cannot be used for resetting of their biological clock. Careful analysis of retreat behaviour at the end of the day shows that neither a specific light intensity level nor a specific light intensity decrease can explain the day-to-day variations in the timing of retreat. If a general decrease in light intensity is a signal indeed, its effect must be phase dependent since a light intensity decrease by the solar eclipse on August 11, 1999, in the middle of the day failed to induce retreat behaviour.

Keywords: *Spermophilus citellus*, European souslik, entrainment, behaviour, solar eclipse, light intensity, telemetry.

Introduction

European sousliks (*Spermophilus citellus*) in their natural habitat emerge from their burrows around four hours after twilight at dawn and disappear around three hours before twilight at dusk (Everts et al. 2004). This poses a problem for circadian entrainment. They might see the dusk and dawn transitions if they would observe the outside light from the entrance of their burrows. This is, however, prevented by the complete blocks the sousliks make in their entrance tunnels after their last afternoon retreat (Hut & Scharff 1998). Entrainment to daily temperature fluctuations can be excluded since they are absent in the burrows underground (Hut et al. 1999a). The largest temperature changes are induced by emergence and retreat itself. In addition, temperature cycles have shown to be unreliable as a Zeitgeber for squirrels (Pohl 1998, Rajaratnam & Redman 1998) and for sousliks (Hut et al. 1999a).

Due to late emergence and early retreat, the most precise signals indicating the time of day, the fast changes in light intensity during dawn and dusk twilight, are not available for synchronization of their circadian system. Sousliks might, however, respond to the very slow afternoon decrease in light intensity related to the gradual decrease in the sun's azimuth above the horizon rather than its disappearance under the horizon. If they would respond to this decrease by retreating into the darkness underground (Hut et al. 1999b), they would generate a secondary lights-off signal, to be used for entrainment of their endogenous circadian clock. In this study we closely studied properties in the afternoon light decrease and phase dependent sensitivity for natural light decrease.

Methods

Afternoon light decrease

Four male and five female sousliks were maintained in an outdoor enclosure in Haren, The Netherlands (53°10' N, 6°36' E). They were equipped with light sensitive radio-transmitter collars (Televilt, International AB, Lindesberg,

Sweden; see Hut et al. 1999b) from March 16 to October 29, 1996. The presence above ground was indicated by the pulse interval of the transmitter signals. The transmitter generated a radiopulse signal of 1 Hz when the collar sensor was exposed to a light intensity of more than 0.03 Lux, and a pulse signal of 2 Hz when the collar sensor received less than 0.03 Lux. Signals of individual sousliks could be identified by frequency. All transmitter signals were received and stored in 2-minute intervals by a receiver (Televilt, RX900-C). From the entire course of these recordings, all individual retreats (66 in total) on precipitation-free days were selected. Concurrent light intensity measurements in 60-minute intervals were obtained from Groningen airport Eelde, at 6-km distance. We determined daily light intensity ($W*m^{-2}$) and rate of change in light intensity ($W*m^{-2}*s^{-1}$) at the precise time of each souslik's final afternoon retreat in its burrow.

Solar eclipse

We exploited the solar eclipse of August 11, 1999, to test whether sousliks respond to a decrease in light intensity independently of circadian phase. This was done in a field population of European sousliks near Vienna, Austria (48°18' N, 16°22' E) (Millesi et al. 1999). At this location the moon covered 99% of the sun's disk at 12:46 h local time. On August 10, 11 and 12, we recorded the number of sousliks above ground every 5 minutes in a 1-ha focal area, from circa 06:00 h until circa 20:00 h. Light intensity was measured every 10 minutes, around the partial solar eclipse data were recorded every single minute. To validate visual observations, on August 9 five sousliks were equipped with light-sensitive radiotransmitter collars as described above. Their presence above ground during August 10-12 was recorded every 10 minutes, and every single minute around the time of the eclipse. The results of the solar eclipse study have been published elsewhere (Spoelstra et al. 2000).

Results

Afternoon light decrease

Figure 1 shows the timing of all 66 retreats recorded and the corresponding curves of declining light intensity in the afternoon. For all animals, retreat occurred within a range of 2 log units. This does not impress as a constant light intensity. However, if one looks at

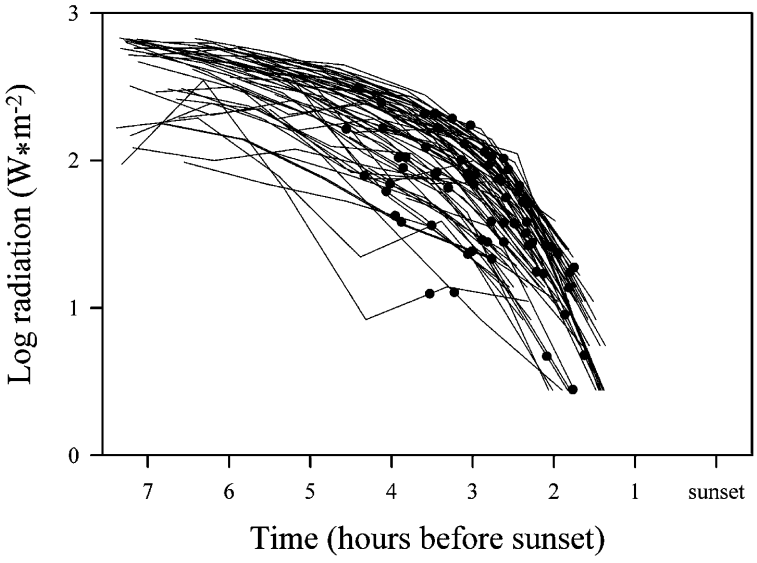

Figure 1. Daily curves describing radiation and the times of souslik afternoon retreat in their burrows (dots).

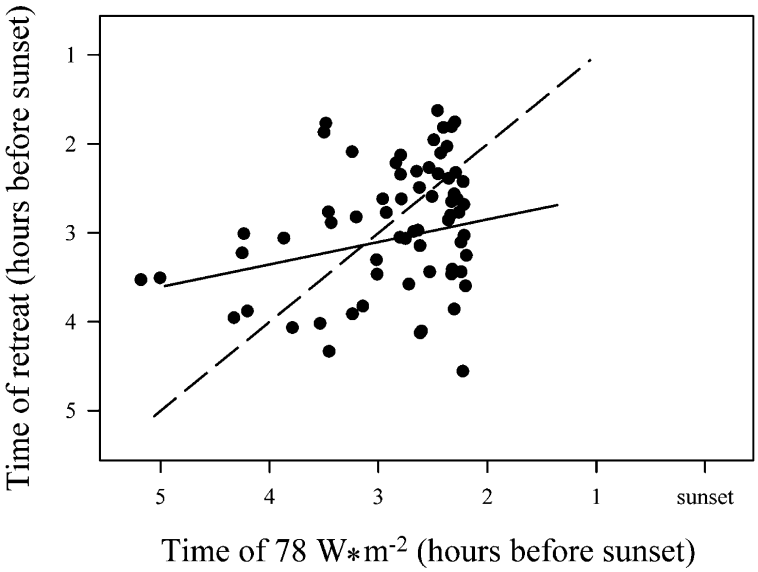

Figure 2. Occurrence of average retreat light intensity (78 $W*m^{-2}$) and average retreat time (in hours before sunset) for each subjective day. Dashed line: y = x (if animals on average would retreat exactly at average retreat light intensity).

figure 1 in more detail, it appears as though the earliest retreats occurred at light intensities that were at least sub-maximal, while the latest retreats tend to be seen on the brightest days.

We further analysed this by first calculating the average light intensity at the time of afternoon retreat (78 $W*m^{-2}$). Then we plotted retreat time against the time at which this light intensity was reached on the same day (figure 2). Although the timing of 78 $W*m^{-2}$ is not the only determinant of retreat, the later this intensity occurs the later the animals retreat into their burrows (P=0.017).

It might also be that the sousliks wait for a particular rate of change of the light intensity to make their final afternoon retreat. To determine whether there is such a specific decline in light intensity that triggers the afternoon retreat,

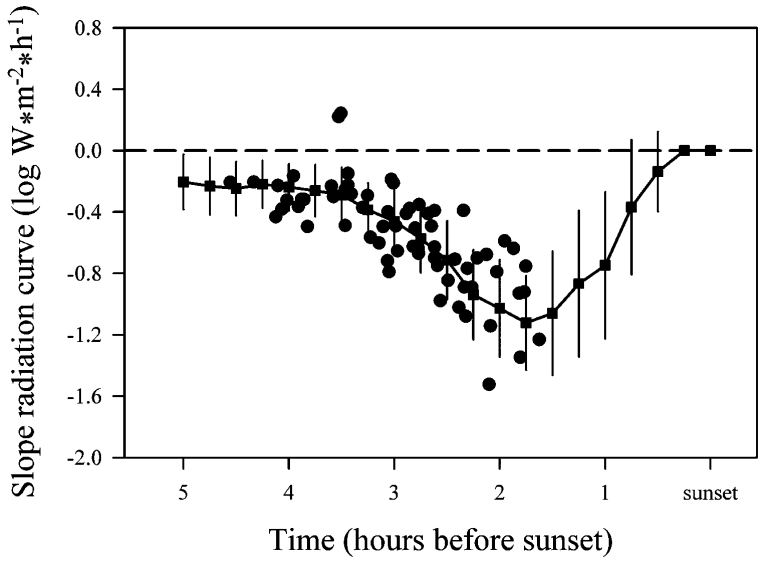

Figure 3. Average decline in light intensity of all subjective days (±SD, solid line) and moments of retreat (dots) with corresponding momentary decline in light intensity.

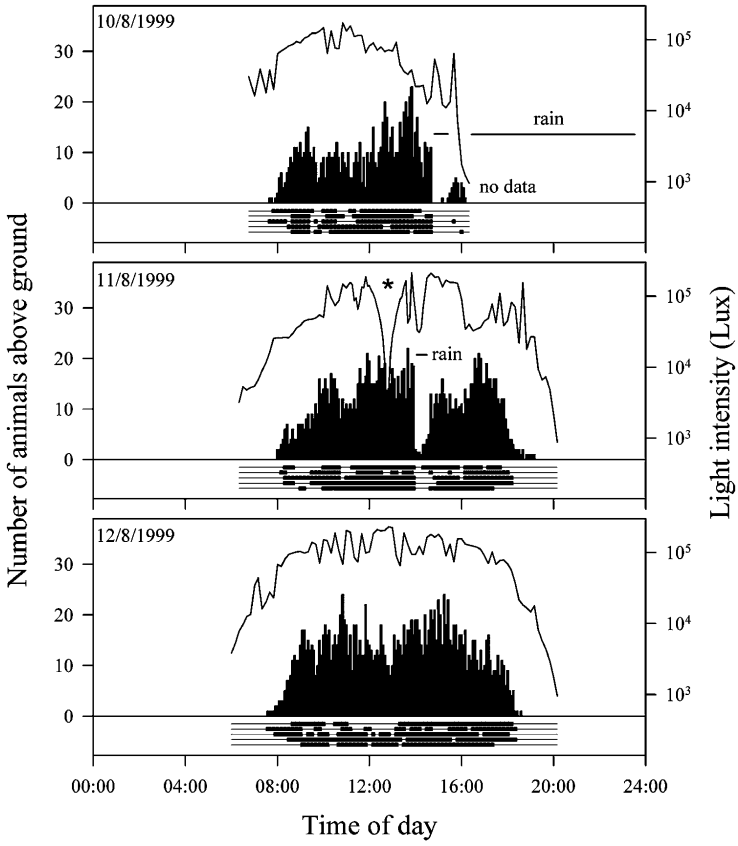

Figure 4. Number of sousliks active above ground (grey bars) and concurrent light intensity (thin black line), observed in a 1-ha focal area in a population near Vienna, Austria. Thick horizontal lines indicate presence of five animals with radio transmitters above ground. Asterisk denotes timing of the partial (99 %) solar eclipse at 12:46 h local time, on August 11, 1999 (civil twilight dawn 5:07 h; civil twilight dusk 20:51 h). (Spoelstra et al. 2000).

we plotted the actual times of retreat along with the light intensity decrease (figure 3, which also shows the average values for the rate of decrease starting five hours before sunset). We conclude from figure 3 that there is no association of the final retreat with light intensity decline, since afternoon retreats are distributed between intensity decreases ranging from 0.2 to 1.1 $W*m^{-2}*h^{-1}$.

Solar eclipse

During the three observation days, light intensity was $24*10^3$-$37*10^3$ Lux at the appearance of the first souslik above ground, and $20*10^3$-$27*10^3$ Lux when the last animal retreated, as reported by Spoelstra et al. (2000) (figure 4). Rain always suppressed aboveground activity, and persistent rain on August 10 precluded data acquisition from 16:20 h onwards. The sky was clear during the partial solar eclipse. Light intensity before the eclipse (11:16 h-11:46 h) was circa $110*10^3$ Lux and dropped more than 2 log units to 1039 Lux at 12:46 h. From 12:16 h to 13:16 h on average 16.3 animals were active above ground. This number was similar to either the hour before (15.4) or the hour after the eclipse (16.4).

Four of the sousliks equipped with radio-transmitters were above ground for most (156-160 minutes, 20 minutes absence by rain) of a three-hour period around the eclipse. The remaining animal was underground for two brief episodes of 21 and 12 minutes, i.e., it behaved indistinguishably from its activity pattern during the other days.

Conclusion

The afternoon retreat data indicate that neither a specific light intensity nor a specific rate of light

A European souslik *(Spermophilus citellus)* collects nest material. Langenzersdorf, Vienna, August 1999. *Photograph: Kamiel Spoelstra.*

intensity decrease solely determine the timing of the sousliks' afternoon retreat in their burrows. If a combination of both is responsible for the timing of the afternoon retreat, the response to this signal is apparently circadian phase dependent. This conclusion is based on an experiment under natural conditions with a solar eclipse around the middle of the day, where low levels of light intensity (well below levels normally perceived at the end of activity) and sharp rates of light intensity decrease failed to induce retreat behaviour.

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Samenvatting

De Europese siesel in de natuurlijke licht-donkercylcus: wat is het signaal voor terugtrekking in de middag?

Siesels *(Spermophilus citellus)* zijn uitsluitend dagactief en hebben een zeer precieze timing van hun gedrag. Dit wordt geregeld door de interne circadiane pacemaker die wordt gesynchroniseerd door de natuurlijke licht-donkercyclus ('entrainment'). Deze synchronisatie kan niet plaatsvinden aan de hand van snelle veranderingen in lichtintensiteit, omdat deze onregelmatig voorkomen doordat siesels zich overdag vaak terugtrekken in de duisternis van het ondergrondse hol.

Deze studie analyseert de lichtcondities op het moment dat de siesels stopten met hun dagelijkse activiteit en tot de volgende ochtend onder de grond bleven. Een specifieke lichtintensiteit noch een specifieke snelheid in afname van deze lichtintensiteit blijkt hiervoor het signaal te zijn. Als het zo is dat een afname van lichtintensiteit in het algemeen een signaal is om voor de rest van de dag te stoppen met bovengrondse activiteit, dan is deze bovendien afhankelijk van de circadiane fase.
De sterke afname van het daglicht door de zonsverduistering van 11 augustus 1999 midden in de activiteitsperiode had geen effect op het activiteitspatroon en het circadiane ritme van de siesels.

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