ספרת צורי

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אין לעשות כל שימוע מסחרי بمאמורים.
IN VolVEMENT OF THE PINEAL GLAND IN DAILY SCHEDULING OF THE GOLDEN SPINY MOUSE

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Summary

The light-dark cycle is the major time cue for daily and seasonal scheduling of physiological activities. However, non-photic cues (e.g. environmental and social constraints) may also play a significant role. A natural model exists in the golden spiny mouse (Acomys russatus) which is nocturnal when maintained alone but diurnal when sharing a habitat with its congener, the common spiny mouse (A. cahirinus). We have recently observed that the presence of A. cahirinus provokes a major change in the daily rhythms of body temperature (Tb) and urine volume without affecting the melatonin rhythm and photoperiod-induced responses. The apparent lack of interaction between the daily and photoperiodic scheduling was further investigated by studying the significance of the pineal to the modification of A. russatus daily rhythms induced by the presence of A. cahirinus. Lesion of A. russatus pineal gland resulted in diminution of urinary 6-sulfatoxymelatonin (6-SMT) and modification of Tb and urine volume rhythms. However, the modification of Tb and urine volume rhythms provoked by the presence of A. cahirinus were similar in pineal lesioned and sham-operated A. russatus. The non-photic signals released by A. cahirinus did not significantly affect glucose utilization in the suprachiasmatic nucleus of pineal- as well as sham-lesioned A. russatus. Thus, the modification of the daily scheduling of A. russatus by the photoperiod involves the pineal and/or the melatonin rhythm whereas non-photic cues effect a direct (perhaps masking), pineal-independent response to the competitor.

Key Words: melatonin, circadian rhythms, pineal gland, non-photic signals, body temperature, 6-sulfatoxymelatonin, Acomys russatus, Acomys cahirinus, rodent

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The nocturnal synthesis of melatonin by the pineal gland and its release into the circulation provide a signal of darkness in the organism (1). In mammals that use changes in the photoperiod to time their reproduction and thermoregulation, temporal signals to the reproductive and thermoregulatory system are controlled by the phase and duration of the daily pineal melatonin production. In addition, recent research indicates that daily melatonin administration can entrain the circadian clock of several mammalian species (rats, Djungarian hamsters and humans) (2). Under normal circumstances, the habitual activity pattern of an animal maintains a constant phase relationship with the external day/night variations and, consequently, with the nocturnal melatonin rhythm. However, competition, food availability constraints, or social interactions (including shift work in humans), can impose a shift in the activity pattern. Consequently the phase relationship between the activity/rest cycle and the nocturnal melatonin peak is changed. Does the melatonin signal maintain its role in the daily and seasonal scheduling under such circumstances? The case of the golden spiny mouse Acomys russatus, provides a natural example for such a situation.

The golden spiny mouse and the common spiny mouse (A. cahirinus) are two rodent species which coexist in sympatric relations in the arid and hot parts of Israel and are well adapted to their environment (3). A shift from diurnal into nocturnal activity was noted in A. russatus under field conditions when A. cahirinus was removed from the common habitat (4). This suggested that interspecific relations determine the diurnal activity of A. russatus. Moreover, the odor of urine and feces of A. cahirinus, suffice to force A. russatus to increase diurnal activity under laboratory conditions (5). We have recently observed (unpublished) that the presence of A. cahirinus, resulted in a change in the waveform of the body temperature (Tb) rhythm with an additional Tb peak at daytime, delays excretion of the major melatonin metabolite, 6-sulfatoxymelatonin (6-SMT) and increases 2-deoxyglucose uptake by the suprachiasmatic nuclei in A. russatus. Nevertheless, the duration of the 6-sulfatoxymelatonin peak and the responses of A. russatus to day length were not impaired. Based on these data we hypothesized that the modification of the diurnal rhythms represent a masking effect of the forced increase in diurnal activity of A. russatus. Such a hypothesis would imply that the change in waveform of various circadian rhythms by the non photic cues would be independent of the melatonin rhythm. To investigate this issue we have compared the effects of A. cahirinus on the daily rhythms of pineal-lesioned and sham-operated A. russatus.

Glucose utilization, which provides an effective assay for oscillatory activity of the circadian pacemaker located in the suprachiasmatic nuclei (SCN) of the hypothalamus (6,7), was also assessed. The studies were performed in mice acclimated to short days, namely when melatonin production is highest so as to accentuate the impact of removal of the pineal.

Materials and Methods

**Animals and surgical procedures:** Male A. russatus (mean body weight 57±8 g) were obtained from the breeding colonies (The University of Haifa at Oranim) established by animals brought from the Dead Sea area. The animals were acclimated to short days (SD) (8 hr. light: 16 hr. dark cycles; lights-on at 08:00-16:00 hr.) for 3 weeks at a constant ambient temperature of 28°C. Cool-white fluorescent illumination and dim red light were on during the light period whereas during the dark period, only the dim red light was on. Following acclimation for 3 weeks, the mice were anesthetized (0.3 ml saline containing 8 mg ketamine and 2.5 µl Rompun per 100 g body weight) and pineal-lesioned (PL) by applying electrical current to the gland, or sham-lesioned (SL) by applying electrical current close to the gland and left to recover for 2 weeks. This method was selected because removal of the pineal by the traditional pinealectomy methods (surgical removal or aspiration of the gland) was lethal in 100% of the animals.
Body temperature (Tb): Following the 2-week recovery period, rectal temperature was measured every 4 h for 48 h to establish the daily Tb rhythms in these mice. The mice were then exposed to A. cahirinus (1 mouse/3 A. rassatus) by placing the cages with animals of both species close to each other in the same room for 5 days and body (rectal) temperature (Tb) recorded for an additional period of 30 h. Tb was measured by use of a copper-constantan thermocouple connected to a TH-65 Wescor digital thermometer (±0.1°C). The thermocouple was inserted 3 cm into the rectum for a period of 30 s as described (8). It should be noted that the impact of photoperiod and proximity to A. cahirinus on Tb rhythms of A. rassatus measured by rectal thermometry were similar to those measured by us by a transmitter implanted into the abdominal cavity (9). Rectal measurements of Tb were preferred to avoid excessive surgical procedures which are often lethal in these animals.

Urine excretion rate (V): Animals were maintained in separate metabolic cages made of mesh nets mounted above a paraffin sheet and were treated as above. The urine excreted by each animal was collected by micro pipette at 4 h intervals for 24-36 hr. The urine excretion rate was calculated from the volume excreted per collection divided by the collection time interval.

Urinary 6-sulfatoxymelatonin excretion rate (6-SMT): Mice were treated as above. Urine was collected separately from each animal every 4 hr for 48 hr, urine volumes recorded and aliquots analyzed for 6-SMT (the major melatonin metabolite which accounts for 85-90% of the circulating melatonin; 10) in duplicates by radioimmunoassay (Stockgrand Ltd, Surrey, UK). The 6-SMT excretion rate was calculated from the total amount of metabolite in each urine sample divided by the collection time interval.

2-Deoxy-glucose uptake in the suprachiasmatic nuclei (2DG): Male A. rassatus were acclimated to SD for three weeks at 28°C (2 subgroups, 4 mice each). The mice of one subgroup were kept in the absence of A. cahirinus whereas those of the second subgroup were exposed to A. cahirinus (3 A. rassatus/A. cahirinus) by placing the cages containing the mice of two species close to each other in the same room for 5 days. The animals in each subgroup were injected with 14C-2-DG (200 uCi/Kg in 50 ul saline, i.p.) at 11:00 hr. (i.e. mid light period at which 2-DG uptake has been shown to be highest in a number of species) (6,7). After 60 min. these mice were killed by decapitation and the brains removed rapidly and frozen in dry ice. The Frozen brains were sliced by microtome and the slices containing the SCN were mounted on glass slides and subjected to autoradiography (Kodak Direct Exposure film). The autoradiographs were analyzed by image analyzer. The location of the SCN in the sections used to generate the autoradiographs was verified by cresyl violet staining.

Data and statistical analyses: Periodic functions were fitted for each set of data (mean values of all individual animals) collected over a period of 24 or 48 hr. As most rhythms were sharply peaked, or indicated more than one peak, the function was fitted to the baseline cosine function (11).

\[ y(t) = M + A_1 \cos(2\pi t/24 (t - \phi_1)) + A_2 \cos(2\pi t/24 (t - \phi_2)) \]

where \( t \) represents the time of day (in hr.), \( M \) the mean of data points, \( A_1 \) the amplitude of the rhythm, \( \phi_1 \) the acrophase (the phase of the crest), \( A_2 \) and \( \phi_2 \) are the corresponding values of the 1st harmonic (12 hr.) and \( y(t) \) the predicted value of the variable at time \( t \). The results of the best fit regressions (Table I) are represented as solid lines in the Figures. The effects of treatment (SL or PL), congener exposure (A. cahirinus or alone), and time of day (hour) were analyzed by 3-way analysis of variance (ANOVA) with repeated measurement on the last two factors (exposure, hour).
Results

The urinary excretion of the melatonin metabolite, 6-SMT, in PL and SL mice at various times of the day are shown in Fig 1. A robust nocturnal rhythm in 6-SMT was observed in the sham-lesioned animals. Pineal lesion resulted in a decrease of 80% of urinary 6-SMT and abolition of the 6-SMT diurnal rhythm.

![Graph showing urinary 6-sulfatoxymelatonin rhythms of A. rassatus.](image)

Body temperature (Tb) rhythms of SL and PL A. rassatus acclimated to short (SD) photoperiod are shown in Figure 2. In SL animals, Tb exhibited a daily rhythm, with a peak in the dark phase. In PL mice, the mesor (M) decreased and the amplitude (A) of the Tb rhythm increased (by a mean of 0.6 and 0.4°C respectively) without affecting the waveform and phase of the rhythm. Exposure to A. cahirinus markedly modified Tb waveform in both SL and PL mice. Three-way ANOVA indicated that Tb rhythm was significantly affected by each of the three variables (pinealectomy, exposure and hour) (Table 2). No interactions were found.

![Graph showing body temperature rhythms of A. cahirinus.](image)

Fig. 2

Effects of A. cahirinus on body temperature (Tb) rhythms in a) SL and b) PL A. rassatus before (*) and after (+) exposure to A. cahirinus. Mean±SD values of 6 animals are presented. The solid lines are theoretical curves reconstructed from the best fit parameters obtained by analysis of the data and presented in Table 1.
Table 1

Best Fit Regression Parameters of the Baseline Cosinor Function

<table>
<thead>
<tr>
<th>Parameter</th>
<th>AC</th>
<th>M</th>
<th>A2</th>
<th>a1</th>
<th>a2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tb-SL</td>
<td>-</td>
<td>36.5±0.18</td>
<td>0.51±0.27</td>
<td>22±0.5</td>
<td>0</td>
</tr>
<tr>
<td>Tb-SL</td>
<td>+</td>
<td>36.8±0.09</td>
<td>0.86±0.14</td>
<td>20.6±0.9</td>
<td>0.50±0.14</td>
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<tr>
<td>Tb-PL</td>
<td>-</td>
<td>35.9±0.18</td>
<td>0.93±0.26</td>
<td>22±0.9</td>
<td>0</td>
</tr>
<tr>
<td>Tb-PL</td>
<td>+</td>
<td>36.7±0.12</td>
<td>0.66±0.17</td>
<td>21±0.6</td>
<td>0.34±0.18</td>
</tr>
<tr>
<td>V-SL</td>
<td>-</td>
<td>604±69</td>
<td>160±97</td>
<td>4.8±3.1</td>
<td>143±53</td>
</tr>
<tr>
<td>V-SL</td>
<td>+</td>
<td>403±53</td>
<td>82±60</td>
<td>11.4±3.3</td>
<td>97±66</td>
</tr>
<tr>
<td>V-PL</td>
<td>-</td>
<td>472±73</td>
<td>139±94</td>
<td>21.5±2.4</td>
<td>121±59</td>
</tr>
<tr>
<td>V-PL</td>
<td>+</td>
<td>716±288</td>
<td>378±117</td>
<td>18±3.1</td>
<td>175±110</td>
</tr>
</tbody>
</table>

SL and PL A. rassatus mice were acclimated to SD in the absence (-) and presence (+) of A. californicus (AC). Tb (in C) and V (in µL) were measured at 3-4 hr. intervals over 24-48 hr. Mean values were fitted by non-linear regression. The mean±S.D. values of the variables obtained by the regression analyses are depicted. The fitted functions are presented as solid lines in Figures 2 and 3.

Table 2

Statistical Analyses (three-way ANOVA)

<table>
<thead>
<tr>
<th>SOURCE</th>
<th>df</th>
<th>DEPENDENT</th>
<th>VARIABLES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>1</td>
<td>Body temperature (Tb)</td>
<td>Urine volume (V)</td>
</tr>
<tr>
<td>Exposure</td>
<td>1</td>
<td>8.41**</td>
<td>3.72*</td>
</tr>
<tr>
<td>Hour</td>
<td>5</td>
<td>10.71***</td>
<td>2.45*</td>
</tr>
<tr>
<td>Treatment X Exposure</td>
<td>5</td>
<td>2.56 NS</td>
<td>1.86 NS</td>
</tr>
<tr>
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<td>5</td>
<td>0.06 NS</td>
<td>0.57 NS</td>
</tr>
<tr>
<td>Exposure X Hour</td>
<td>5</td>
<td>0.59 NS</td>
<td>0.81 NS</td>
</tr>
<tr>
<td>Treatment X Exposure x Hour</td>
<td>5</td>
<td>0.78 NS</td>
<td>0.32 NS</td>
</tr>
</tbody>
</table>

F-test from three-way ANOVA analyses of the data depicted in Figs. 2 and 3 for significant effects of treatment, congener exposure and time of day, with repeated measures on the last two factors. **P<0.01; *P<0.05; NS P>0.05.

The daily rhythm in urine volume (V) of SL and PL A. rassatus are shown in Figure 3. In both PL and SL animals, there was a significant day-night rhythm in urine volume, with peaks at the beginning and end of the dark period. Minimal urine volume was excreted at 14:00 hr in SL and 10:00 hr in PL mice. Three way ANOVA indicated that both treatment and hour significantly affected V rhythms whereas the effect of the presence of A. californicus was not significant (Table 2). No interactions were found.

3H-labeled 2DG accumulation by the SCN of SD-acclimated A. rassatus at 11:00 hr in both PL and SL animals (Mean±S.D. 0.48±0.25 and 0.43±0.32, respectively n= 4) was low compared to incorporation in adjacent brain areas. Exposure to A. californicus tended to enhance 2-DG uptake.
into the SCN in the PL animals (0.81±0.31; n=4) but not in the SL animals (0.27±0.22; n=4). The increase in 2DG accumulation by the SCN in the PL animals did not reach statistical significance.

Fig. 3

Effects of A. coharinus on urine volume (V) rhythms in a) SL and b) PL A. russatus before(-) and after (+) exposure to A. coharinus. Mean±SD values of 6 animals are presented. The solid lines are theoretical curves reconstructed from the best fit parameters obtained by analysis of the data and presented in Table 1.

Discussion

The results obtained indicate a role for the pineal gland in photoperiod-related alterations in thermoregulation of A. russatus. The decrease in average Tₘ under short days in PL mice indicates that melatonin has a thermotropic effect in these animals. A hyperthermic action of melatonin is also consistent with the increase in mean Tₑ in intact A. russatus under SD as compared to LD and with the increase in melatonin output and elevation of mean Tₑ in propranolol-injected mice (12,13). This is also consistent with our previous data showing that injection of melatonin elevated the mean Tₑ values and obliterated the decrease in Tₑ in the late afternoon in LD as well as SD acclimated A. russatus (8). It is also compatible with studies on the effect of melatonin injections on mammalian thermoregulatory responses which indicated that melatonin increased heat production by non-shivering thermogenesis in several diurnal and nocturnal rodents (14) and provoked hyperthermia in rats (15).

In contrast, an increase in Tₑ in the presence of A. coharinus was observed in both SL (at 16:00 hr.) and PL (at 12:00-16:00 hr.) mice. This indicates that the modification of the Tₑ rhythm by the non-photic cues is independent of the pineal melatonin and may perhaps reflect a masking effect of the modified activity rhythm. Notably, the melatonin-metabolite levels and daily rhythms of the SL animals were similar to those previously observed in intact A. russatus (13), thus indicating that application of the electrical current per se did not affect circadian rhythmicity.

The effects of pineal-lesion on the urine volume rhythms of the SD-acclimated A. russatus suggest a role for the pineal in photoperiodic adjustment of urine excretion rates. This is compatible with recent finding in rats in which pinealectomy has been shown to ablate the increase in plasma oxytocin and vasopressin seen over the hours of daylight suggesting a role for melatonin in fluid
balance (16). Previous studies in rats have indicated that some 70-90% of solute and water are excreted during the hours of activity (17,18).

Unlike hamsters and rats (6) and as in the case of the 13-line ground squirrel (Citellus tridecemlineatus) (7), the uptake of 2-DG into the SCN of A. russatus during daytime was low. The low 2-DG uptake into the SCN during daytime might be species specific, or indicate that the electrical activity of the SCN is not augmented during daytime. The tendency of non-photic cues to increase 2-DG uptake by the SCN in PL but not SL mice may reflect changes in exposure to photic stimuli due to increased activity of A. russatus during light hours. It may also be explained by phase shift in the circadian clock, as in rats rendered diurnal by restricted-schedule feeding (19), or in oscillators outside or in the immediate vicinity of the SCN as in SCN-ablated hamsters shown to be entrained by daily feeding but not LD cycles (20).

The case of A. russatus thus indicates that non-photic (olfactory and perhaps auditory) stimuli influence the daily scheduling by masking rather than by entraining the biological clock. Unlike the effects of photoperiod, the effects of the non-photic signals on circadian physiology are independent of the melatonin rhythm. This apparent lack of circadian adaptation to social (competitive) cues may be necessary to maintain proper photoperiodic responsiveness.

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References