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Hicham Farsi, Driss Harti, Mohamed R Achaâban, Mohammed Piro, Véronique Raverot, et al.. Melatonin rhythm and other outputs of the master circadian clock in the desert goat (*Capra hircus*) are entrained by daily cycles of ambient temperature. *Journal of Pineal Research*, 2020, 68 (3), 10.1111/jpi.12634 . hal-03010494

HAL Id: hal-03010494

<https://cnrs.hal.science/hal-03010494>

Submitted on 17 Nov 2020

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Article type : Original Manuscript

Melatonin rhythm and other outputs of the master circadian clock in the desert goat (*Capra hircus*) are entrained by daily cycles of ambient temperature

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Running title: Ambient temperature cycle as a *zeitgeber* in goat.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1111/jpi.12634](https://doi.org/10.1111/jpi.12634)

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Abstract

In desert areas, mammals such as camel and goat are exposed to harsh environmental conditions. The ambient temperature (Ta) cycles have been shown to entrain the circadian clock in the camel. In the present work, we assumed that, in the goat living in a desert biotope, Ta cycles would have the same synchronizing effect on the central clock. Therefore, the effects of Ta cycles on body temperature (Tb), locomotor activity (LA) and melatonin (Mel) rhythms as outputs of the master circadian clock have been studied. The study was performed on bucks kept first under constant conditions of total darkness (DD) and constant Ta, then maintained under DD conditions but exposed to Ta cycles with heat period during subjective day and cold period during subjective night. Finally, the Ta cycles were reversed with highest temperatures during the subjective night and the lowest temperatures during the subjective day. Under constant conditions, the circadian rhythms of Tb and LA were free-running with an endogenous period of 25.3 and 25.0h, respectively. Ta cycles entrained the rhythms of Tb and LA to a period of exactly 24.0h; while, when reversed, the Ta cycles led to an inversion of Tb and LA rhythms. Similarly, Ta cycles were also able to entrain Mel rhythm, by adjusting its secretion to the cooling phase before and after Ta

cycles inversion. All together, these results show that the Ta cycles entrain the master circadian clock in the goat.

Keys word: Goat, entrainment, body temperature, melatonin, locomotor activity, ambient temperature, circadian clock.

Introduction

In mammals, the circadian organization of physiological and behavioural functions is under the control of the master clock, located within the hypothalamic supra-chiasmatic nuclei (SCN), and of secondary clocks within peripheral organs^{1,2,3}. The light-dark (LD) cycle was found to be the most powerful *zeitgeber* for the SCN (For review see^{2,3}). How ambient light synchronizes the circadian system is now well documented^{4,5,6,7}. However, little is known about the ability of ambient temperature (Ta) cycles to entrain circadian rhythms. Such entrainment is well established for the circadian system in non-mammalian vertebrates (For review see⁸). In laboratory mammals, despite the responsiveness of peripheral clocks to the synchronizing effects of ambient temperature cycles^{9,10,11}, experiments showed either a complete absence of entrainment of the master clock in some species^{11,12} or a partial entrainment of locomotor activity (LA) rhythm in only some individuals^{13,14,15,16,17,18}. In such conditions, it would be very difficult to conclude of a real synchronization of the master circadian clock since, as reported^{15,16,17,18}, such entrainment of LA rhythm could be a result of a masking effect of Ta. Accordingly, Ta cycles are assumed to have a weak effect on the master circadian clock at least in endothermic mammals (For review see⁸). Within mammals, a group of several species including camel, display heterothermy phenomenon, which means that under special conditions (e.g. dehydration and exposure to high Ta), Tb can follow Ta¹⁹. Moreover, it should be noted that the circadian system of heterothermic animals was shown to be more sensitive to environmental Ta^{20,21,22}. Thereby, in a previous study²³, our team has shown that, in the camel, the Ta cycles are a strong *zeitgeber* for the circadian clock in the SCN. Two outputs of the master clock, plasma melatonin (Mel) and body temperature (Tb) rhythms were indeed found to be synchronized by Ta cycles. To our knowledge, this finding was the first demonstration in mammals of such non-photic entrainment to Ta.

In camels under heat stress and dehydration, heterothermy corresponds to a switching from a perfect endothermic state to a heterothermic state^{19,24}. The Ta synchronization of the circadian

system described in the camel could be related to this specific thermoregulation process. Recently, such heterothermy has been also shown in other desert ungulates, including oryx and goat^{25,26,27}. Thus, the raised question is whether the observed entrainment of the circadian clock by Ta cycles is specific to camel or is a mechanism shared by other desert large species.

To test this hypothesis, we have studied three outputs of the master clock, namely: Tb, LA and Mel rhythms, in the desert goat.

Experimental procedures

Animals

Seventeen healthy adult male desert goats (*Capra hircus*) weighing 26 ± 0.3 kg and aged around 3.5 years were used in this study. Animals were housed in specific facilities at the Hassan II Agronomy and Veterinary Medicine Institute of Rabat (Latitude: 34°01'N, Longitude: 6°50'W). Males were used instead of females to avoid eventual effects of oestrous and gestation on circadian rhythms.

This study was carried out in accordance with the International Ethical Standards²⁸ and the national recommendations for animal farm husbandry, experimentations and surgeries of the Hassan II Agronomy and Veterinary Medicine Institute of Rabat.

Experimental Design

Experiment 1 was intended to verify if Ta cycles could entrain the Tb and Mel rhythms in the goat. To avoid the masking effect of LA on the Tb rhythm^{23,29}, bucks (n=10) were maintained in forced inactivity but allowed an easy standing, moving around and sitting in sternal and lateral recumbency. Animals received a complete compound food (Maraa[®], Alf-Sahel, Morocco) and hay 4 times a day to avoid a possible entrainment by feeding^{23,30}. Water was *ad libitum*. The air relative humidity during this experiment was around 23%.

This experiment was carried on five stages as follows:

Stage 1 (5 days): is an adaptive period to indoor conditions with an artificial LD cycle (12L-12D) and uncontrolled Ta (17.00°C-25.50°C).

Stage 2 (21 days): this step was designed to demonstrate the existence in the goat of a free-running rhythm of Tb. Animals were placed under constant conditions of total darkness (DD) and constant ambient temperature (CTa) of $23\pm0.37^{\circ}\text{C}$ to avoid any temporal perception from the environment. This CTa was chosen as thermo-neutral Ta for goats.

Stage 3 (21 days): the DD conditions were maintained and a 24-h artificial daily Ta cycle (NRT) with a heating-period applied during the subjective day (maximum 31.93°C , minimum 16.17°C).

Stage 4 (29 days): correspond to the same DD conditions but a 12-h phase advance of Ta cycle (RT) (maximum 32.47°C , minimum 16.30°C) was realized with a heating-period shifted to occur during the subjective night.

Stage 5 (21 days): a return to the former conditions of stage 3 with Ta cycle peaking under the subjective day (NRTr).

Experiment 2 was conducted on 7 bucks and designed to check if Ta cycles could synchronize the LA rhythm. Each buck was able to move freely in an individual controlled sheltered stable of 36m^2 . The same feed diet of experiment 1 and water were provided *ad libitum*; while the relative humidity was around 21%. To avoid the possible masking effect of the social behaviour of bucks on LA rhythm, experiment was conducted individually and lasts each 125 days. Each individual experiment consisted of the following stages:

Stage 1 (5 days): is an adaptive period to indoor conditions with an artificial LD cycle (12L-12D) and uncontrolled Ta cycle (19.05°C - 24.60°C).

Stage 2 (26 days): This stage was designed to demonstrate the existence in the goat of a free-running rhythm of LA rhythm. Constant conditions of DD and CTa ($23\pm0.40^{\circ}\text{C}$) were applied.

Stage 3 (27 days): The DD conditions were maintained and a 24-h artificial Ta cycle (NRT, maximum: 31.38°C and minimum: 15.98°C) with a heating-period during the subjective day was applied.

Stage 4 (44 days): Animals were kept under the same conditions of stage 3 but a 12-h phase advance of Ta cycle (RT, maximum 32.68°C and minimum 15.01°C) was realized, with a heating-period during the subjective night.

Stage 5 (23 days): corresponds to a return to the previous conditions of stage 2.

Details of these experimental procedures are detailed in **Figure 1**.

Temperature measurements

The rhythm of Tb was measured during experiment 1 by using data logger, Ibuttons® (DS1922L, Dallas Semi-Conductor, USA). The devices were programmed for 16-bit with a resolution of 0.0625°C and to record Tb every 12 min. Then, the Ibuttons were protected with silicone elastomer (Rhodorsil RTV141) before their surgical implantation in the abdominal cavity of bucks. This site has been widely used to record Tb in several species^{31,32,33}.

Locomotor activity measurements:

The LA rhythm of bucks was recorded during experiment 2 using the validated video-locomotion scoring technique (For more details, see³⁴) which is based on a visual attribution of scores to activity, where 0: animal on a sitting position and not moving; 1: animal on a sitting position and moving lightly its limbs beneath the body; 2: animal on standing position without movements; 3: animal on standing position moving its limbs but without locomotion; 4: animal walking and exploring the environment; 5: animal in intense locomotor activity or agitated.

The LA rhythm was measured separately in each animal using a time set of 5 minutes. This time interval is imposed by the used software which provides activity as actograms. The recorded video sequences were visually analysed on a computer screen by two well-trained evaluators who assigned a score to each activity state (For details, see³⁴).

Melatonin assay

To determine the plasma concentration of Mel under various conditions of experiment 1, blood was sampled every 2-hours during the last 24-hours of stages 3, 4 and 5. Blood was collected in 10 ml lithium heparin BD-Vacutainer tubes by puncturing alternatively the right and left external jugular veins. To minimize stress, bucks were accustomed to handling and sampling procedures for several weeks. The blood sampling was performed under complete darkness without any source of light by well-trained operators. Samples were immediately centrifuged at 1760 g for 30 min and then plasma was stored at -20°C for subsequent Mel assay.

Mel concentrations were measured using an in-house radioimmunoassay after extraction from plasma with diethylether³⁵. Duplicated extracted samples were mixed with in-house antibody and labelled 125Iodomelatonin (Perkin Elmer NEX2360), incubated overnight at 4°C. The radioactivity of the pellet obtained after precipitation by anti-rabbit γ -globulin solution was counted. The intra-assay CV was 9.5% at levels 39 and 57ng/L. The inter-assay CV was 12.7% at levels 50 ng/L and 12.4% at 100 ng/L. The quantification limit was of 3.7 ng/L.

Data Analysis

Temperature data. Tb data was presented as means \pm Standard Errors of the Means (SEM) and plotted in simple curves using the SigmaPlot[®] software (Systat. Sigmaplot v12.0, Systat, Chicago, IL). The period, acrophase, mesor, amplitude and the robustness of Tb rhythm were calculated by nonlinear least squares regression using Acro[®], Cosinor[®] and LSP[®] softwares (Refinetti R, Circadian Rhythm Laboratory, University of South Carolina, <http://www.circadian.org/software.html>). Cosinor analysis was performed according to the equation:

$$f=y\theta+a*\cos[2\pi*(t-\phi)/\tau]$$

Where f is Tb at time t , $y\theta$ is the mesor, a is the amplitude, ϕ is the acrophase and τ the period. For each parameter, a confidence interval of 95% probability is given. Similarly, the degree of significance of the regression is calculated. The One-Way ANOVA test was used to determine if there are statistical differences in the periods and the acrophases of Tb between the different stages of experiment 1, mainly between NRT, RT and NRTr conditions.

Locomotor activity data. LA rhythm data was presented as individual actograms using the Actogram Plotter[®] software (Refinetti R, Circadian Rhythm Laboratory, University of South Carolina, <http://www.circadian.org/software.html>). As for Tb, the rhythm parameters of LA were calculated by the same Cosinor analysis equation.

Similarly, a one-way ANOVA test was also used to compare periods and acrophases of LA rhythm between stages of experiment 2 and also, to verify the equality of means of the daily activity onsets and offsets around the daily Ta transition.

Melatonin data. Results data of Mel were presented as means \pm SEM and plotted using the SigmaPlot® software. The Mel concentrations under different Ta treatments were evaluated separately for each individual and then for the mean of all animals. Accordingly, the Mel rhythm secretion for stages 3, 4 and 5 were fitted using a nonlinear regression of the following exponential mathematical equation^{36,37,38}:

$$f=y0+(ymax/((1+exp(slope1*(phi1-x)))*(1+exp(slope2*(x-phi2)))))$$

Where f is the concentration of Mel variation at time x , $y0$ is the basal level, $ymax$ is the amplitude of the Mel peak concentration, $phi1$ and $phi2$ are the inflection points and represent the time points at which 50% of the maximal increase and 50% of the decline were reached respectively. $Slope1$ and $slope2$ are the slopes of the onset and the offset (inflection points at half the peak height) and were assigned with arbitrary constant values. The equation was chosen because it minimizes the dispersion of the abscissas of the inflection points and their collinearities with other parameters. The algorithm provides an ANOVA for the mathematical regression which was tested using F-test. It also provides an asymptotic estimate (by excess) of the standard error for each parameter. Shapiro-Wilk's test was used to check the homogeneity of residuals and Bartlett's test for the homogeneity of variances²³. The following step was designed to compare the obtained $phi1$ and $phi2$ between NRT, RT and NRTr conditions of Ta cycles corresponding respectively to stages 3, 4 and 5 of experiment 1. A paired t-test was performed twice to determine the $phi1$ and $phi2$ statistical differences, first between NRT (stage 3) and RT (stage 4) conditions and then between RT (stage 4) and NRTr (stage 5) conditions.

$P \leq 0.05$ was considered statistically significant in all statistical tests.

Results

Under stage 1 conditions, Tb showed a clear daily rhythm (Figure 2) characterized by a robustness of $40 \pm 2.7\%$, a mesor of $38.74 \pm 0.12^\circ\text{C}$, an amplitude of $0.69 \pm 0.36^\circ\text{C}$, an acrophase at $17:23 \pm 0.28\text{h}$ and a period of exactly 24.0h (Table 1). Tb was lowest around 08h00 am, started then its ascending phase during the early morning achieving high value in the early evening. After

Light/Dark transition, Tb dropped across the scotophase achieving its lowest value at Dark/Light transition.

Under similar environment (stage 1, experiment 2), LA rhythm showed intense daily activity during the photophase (Figure 3). Cosinor regression of LA rhythm shows that the robustness was of $32.0 \pm 1.8\%$, the acrophase occurred at the middle of the photophase, exactly at $12:33 \pm 0.30\text{h}$, the period was exactly of 24.0h and the mesor and the amplitude were respectively 1.59 ± 0.08 and 1.7 ± 0.09 (Table 2).

The existence of circadian rhythms in goat was inferred by stage 2 of both experiments 1 and 2. We observed that both Tb and LA rhythms (24.0h period) first shown under a cyclic indoor environment of stage 1, persisted when goats were transferred under constant conditions (stage 2) with a period different from 24.0h (Figures 2 and 3; Tables 1 and 2): exactly of $25.3 \pm 0.09\text{h}$ for Tb rhythm and $25.0 \pm 0.10\text{h}$ for LA rhythm. The one-way-ANOVA showed that the periods of constant conditions (stage 2) were statistically different (Tb $F_{(4,45)}=53.36$; $P < 0.0001$; and LA $F_{(4,30)}=39.23$; $P < 0.0001$) from those under indoor cyclic conditions (stage 1).

A slight reduction of the shape regularity of the Tb and LA curves has been noticed during constant conditions (stage 2: Figures 2 and 3). Moreover, animal activity on double-plot LA actograms appears to be daily shifted indicating a free-running state of LA. In fact, the two rhythms were shifted since acrophases were delayed after transferring animals from cyclic environment to constant conditions (Tables 1 and 2). The acrophase was shifted by 21.5h for Tb and by 22.4h for LA during DD and CTa. Under free-running conditions, the robustness also changed, decreasing significantly from 40.0% to 27.1% for Tb and from 32% to 7.3% for LA (Tables 1 and 2). These results, together with the persistence of rhythmicity under constant conditions, confirm the existence of a circadian clock that drives these two rhythms.

The next step of this study was to verify if daily Ta cycles are able to synchronize the two outputs of the master circadian clock, the Tb and LA rhythms. This investigation was carried out under DD and Ta cycles of stages 3, 4 and 5 of experiment 1 and stages 3 and 4 of experiment 2. Under DD conditions, the Ta cycles of stage 3 were able to generate regular rhythms of Tb and LA (Figures 2 and 3). The period changed from 25.3h (Tb) and 25.0h (LA) during constant conditions

to become exactly 24.0h, indicating that Ta daily cycle entrained both Tb and LA rhythms. Indeed, an increase of the robustness was observed ranging from 27.1% and 7.3% under constant conditions to 58.3% and 15.3% under cycling Ta, respectively for Tb and LA rhythms (Tables 1 and 2).

In any case, when Ta rose, Tb gradually increased and its acrophase occurred during the heat phase, (Figures 4A-B, 5A-1 and 5B-1). For LA rhythm, actograms showed also high activity during heat phase while inactivity coincides with the cooling phase of Ta cycle (Figure 3, stage 3 and 4). Acrophases occurred at $15:39 \pm 0.21$ h for Tb rhythm and at $13:12 \pm 0.12$ h for LA rhythm (Tables 1 and 2).

The entrainment by the Ta cycles was confirmed by the fact that a 12-h phase advance of its cycle (stage 4) led to a significant phase advance of the Tb and LA rhythms (Figures 3, 4A, 5A-2 and 5B-2). All the ten bucks of experiment 1 showed a clear entrainment of Tb rhythm by Ta cycles while in experiment 2 which was performed on 7 bucks, six exhibited an entrainment of LA rhythm by Ta cycles as can be shown in Figure 3; while only one animal displayed a weak response to the entrainment effect by this cue.

The shifting of the rhythm was only within 6 ± 0.26 days for Tb (Figures 5A-2 and 5B-2) while it took 10 ± 0.26 days for the LA (Figure 3). The final pattern of LA rhythm was completely synchronized to the new Ta cycle within 12.5 ± 1.07 days, having therefore a peak of activity during heat-phase at the subjective night. Accordingly, acrophase of LA occurred at $02:49 \pm 0.27$ h and that of Tb at $02:20 \pm 0.16$ h (Tables 1 and 2). The one-way ANOVA confirmed the existence of a significant (Tb $F_{(5,40)} = 1114$; $P < 0.0001$ and LA $F_{(2,50)} = 874.9$; $P < 0.0001$) shifting in both acrophases.

When NRT and RT phases were separately analysed, LA onsets and offsets did not show statistical differences between successive days. This indicates a significant equality of the means around each Ta cycle transition (Figure 6). As for acrophase, the onset and the offset of LA were shifted and seemingly following the Ta cycle conditions (Figure 6). Indeed, the two parameters coincide respectively with the cold-heat and heat-cold transitions, whatever the nature of the Ta cycle.

When the goats returned to the former Ta cycle with maximum of heat during subjective day (stage 5, experiment 1), (Figures 4 B, 5A-3 and 5B-3), the Tb rhythm was delayed and went back to its initial pattern observed in stage 3. Acrophase was delayed by 12.56h to occur at 14:54±0.26h (Table 1). The ascendant and descendant phases of Tb follow those of the Ta cycles as previously seen in stage 3 (Figure 4 and 5-A).

Concerning LA rhythm, a return from an inversed Ta cycle (stage 4) to the constant conditions (stage 5) induced a free-running state showing a daily delay of its phase (stage 5: Figure 3). Excluding the Ta cycling under DD conditions, the period becomes significantly different ($F_{(4,30)} = 39.23$; $P < 0.0001$) from 24.0h, precisely of 24.9±0.12h. The acrophase was also shifted to occur at 03:35±0.32h. Moreover, the regularity of the rhythm decreased with a robustness that also dropped to 9.2±0.48% (Table 2).

All together, these changes emphasize once again the entrainment effect of Ta cycles.

From experiments 1 and 2, Ta seemed to be a *zeitgeber* capable of synchronizing the circadian clock of the goat. Even if two outputs of the clock (Tb, LA) are driven by Ta, as demonstrated previously, it remains critical to ascertain that these results correspond to a real entrainment of the circadian clock and not to a masking effect. So, we verified if Ta is able to entrain the endogenous rhythm of Mel, one of the most robust outputs of the clock activity of SCN. An endogenous rhythm of Mel under DD and constant Ta (stage 2) was observed in all animals (data not shown). No major changes were noticed in the Mel rhythm when Ta cycle with heating period during the subjective day was applied (stage 3). However, when daily Ta cycle was 12-h phase advanced (Maximum of Ta at the end of subjective night, Stage 4), the Mel rhythm was shifted (phase delay, Figure 7). A Mel phase advance was then observed when animals returned to the former Ta cycle with maximum of Ta during the subjective day (stage 5, Figure 7).

The paired t-test showed that Ta treatment induced significant phase differences for both onsets (Phi1) and offsets (Phi2) of Mel rhythm, except for offset in the return from RT (stage 4) to NRTr (stage 5) (Table 3). Under all these conditions, Mel rhythm peaked when the Ta cycle was in the cold-phase.

Discussion

The daily variations of Tb and LA in goat were previously recorded in several thermoregulatory^{39,40,41} or chrono-biological^{42,43,44,45} studies. They were clearly identified, but their circadian nature was not established yet, until we did in the present study. Indeed, our results demonstrate that both rhythms persist under constant conditions with a period close to 24.0h. This indicates that in the goat, as in other mammals^{23,46,47,48,49,50,51}, Tb and LA comply with the rules defining circadian rhythms.

Under their desert biotope, goats are frequently exposed to a very wide range of daily Ta cycles. Such variations are suspected to strongly affect several systems and circuits in the brain, particularly, the circadian system as demonstrated in the camel²³. Based on these specific findings, we have hypothesized that in the desert goat, cyclic fluctuations of Ta could also synchronize its master circadian clock. Our results clearly show that, under 24.0h applied DD, Ta cycle entrains the Tb and LA rhythms to exactly 24.0h. A 12-h switch of the daily Ta cycle induced a clear shift of both rhythms. Similarly, the return to the former Ta cycle induced a few days later a return to the initial phase (phase delay) of the Tb rhythm. Such result raises a pertinent question regarding the interpretation of the observed effect: is it a real entrainment of the master clock or a masking effect of Ta on Tb and LA rhythms?

Zeitgebers are known to act on the period and phase of the rhythm¹⁸, while masking cues strengthen or weaken the rhythm and affect generally the amplitude and the shape of the overt rhythm^{29,52,53}. A masking effect of Ta on Tb is unlikely since the observed Tb peak in the goat, as in the camel²³, is delayed from that of Ta. This delay indicates that there is no important radiant heat load (radiant, conductive and convective) occurring at the moment of Ta peak. Tb is also modified by evaporative water loss, thermal conductance and metabolic heat production.

Moreover, in our study any significant heat production from locomotion during the experiment 1 is very unlikely since the LA of animals was restrained. The animals were also in perfect endothermy and any mechanisms of evaporative water loss, exogenous heat loading and dissipation are unlikely to occur.

When applying Ta cycle in experiment 2, a transient and progressive period before final synchronization of the LA rhythm is seen after several days. This transition is typical of an

entraining effect on the circadian clock. Moreover, the observed changes in the period and phase led to infer that Ta does not induce the LA rhythm directly, but entrained it through a circadian clock. In natural conditions, the goat is a diurnal species^{34,44,54}, showing its maximal daily activity during the heat phase (the day). During Ta entrainment, bucks were also active during the heat period, regardless of Ta cycle applied. The phase relationship of activity with Ta cycle, as seen in natural conditions, is therefore maintained under Ta entrainment. In all species studied until now, in which an entrainment of LA rhythm was shown, animals keep their phase relationship with Ta cycle, nocturnal species being active during the cold phase of Ta while diurnal species being active during the warm phase^{13,17,18, 20,22,55}.

The temperature range used in the present study (from cold: 16°C to heat: 32°C) is seemingly strong enough to provide a temporal information for synchronizing LA rhythm of the goat. Similarly, close oscillations of Ta were able to induce an entrainment of LA rhythm in rodents¹⁸. It is also established that the thermo-neutral zone in the goat is between 13°C and 21°C⁵⁶. We believe that in desert goat breeds, adapted to their biotope high temperatures, this interval would be higher. As previously reported, the used range of Ta cycle between 16° to 32°C induced a stronger entrainment of LA rhythm. However, narrower ranges would probably provide a weaker *zeitgeber*. No attempt was made till now to investigate such intensity-response curve for temperature entrainment.

In mammals, Mel secretion from the pineal gland is known to be the most powerful phase-marker of the master circadian clock. It depends much more on LD than on Ta cycles⁸. In goat, as in the camel²³, under DD conditions, plasma Mel follows Ta cycle and its peak occurred only in the cryophase. Analysis of plasma Mel combined to Tb and LA under DD conditions has, therefore, confirmed that the daily Ta cycle entrain three outputs of the master circadian clock, which precludes any masking effect. Thus, Ta cycle is a true *zeitgeber* in the goat. Beside the camel, this finding constitutes the second demonstration of such clear entrainment of the circadian system by Ta cycles in mammalian species. How the non-photoc information of Ta reaches the SCN through central and/or peripheral thermo-receptors, and how it could presumably change its molecular machinery still need further investigation.

Conclusion

The present work demonstrates that daily Ta cycle, a strong environmental cue in the desert habitat, is able to synchronize the central clock of the goat by entraining the Tb, LA and Mel rhythms. Until now, a similar effect has been described in only two mammalian species, namely the camel and herein the goat. This finding raises enquiry on whether such phenomenon represents common process of adaptation of the circadian system in heterothermic species to the desert or it is specific to these two species only. Accordingly, further investigations in other desert species are needed to elucidate the prevalence of this peculiar thermoregulatory feature in mammals.

Acknowledgments

The authors are grateful to Hassan II Agronomy and Veterinary Medicine Institute (Rabat, Morocco), the Institute of Cellular and Integrative Neurosciences (Strasbourg, France), the Laboratory of Hormonology (Lyon, France) and the Moroccan AVPN Association (Association de Vulgarisation et Promotion des Neurosciences) for supporting this study.

Funding

This study was funded by the International Foundation for Science (IFS: Research Grant Agreement No. I-3-B-6082-1).

Conflict of Interest Statement

The authors declare no actual or potential conflict of interest.

Author Contributions

Hicham Farsi: Performed the experiments; Analyzed and interpreted the data and drafted the manuscript. Driss Harti: Performed the experiments. Mohamed Rachid Achaâban: Analyzed and interpreted the data and drafted the manuscript. Mohammed Piro, Mohammed Ouassat, Véronique Raverot, Béatrice Bothorel: Contributed reagents, materials, analysis tools or data and drafted the manuscript. Paul Pévet, Etienne Challet: Conceived and designed the experiments; Analyzed and interpreted the data and drafted the manuscript. Khalid El Allali: Conceived and designed the

experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data and drafted the manuscript.

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Table 1: Parameters of the Tb rhythm during different stages of experiment 1.

Experiment 1	Period	Acrophase	Mesor	Amplitude	Robustness
Stage 1: LD uncontrolled Ta	24.0±0.09 h ^a	17:23±0.28 h ^a	38.74±0.12°C	0.69±0.36°C	40.0±2.7%
Stage 2: DD+CTa	25.3±0.09 h ^b	14:55±0.14 h ^b	38.82±0.06°C	0.23±0.18°C	27.1±1.8%
Stage 3: DD+NRT	24.0±0.02 h ^a	15:39±0.21 h ^c	39.18±0.10°C	0.43±0.24°C	58.3±3.0%
Stage 4: DD+RT	24.0±0.02 h ^a	02:20±0.16 h ^d	39.34±0.15°C	0.55±0.38°C	55.7±2.3%
Stage 5: DD+NRT _r	24.0±0.03 h ^a	14:54±0.26 h ^c	39.18±0.18°C	0.50±0.29°C	57.3±1.9%

Note: One-way ANOVA analysis was used for the calculation of statistical differences between periods and acrophases across different stages of the experiment 1. Different superscript lowercase letters indicate significant differences between different stages ($p \leq 0.05$). Similar superscript lowercase letters indicate no statistically significant differences between compared stages ($p > 0.05$).

Table 2: Parameters of the LA rhythm during different stages of experiment 2.

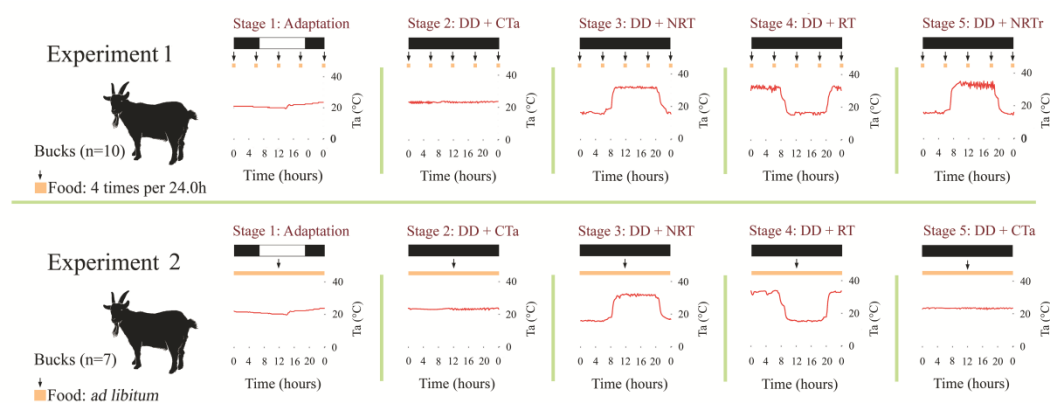
Experiment 2	Period	Acrophase	Mesor	Amplitude	Robustness
Stage 1: LD uncontrolled Ta	24.0±0.04 h ^a	12:33±0.30 h ^a	1.59±0.08°C	1.70±0.09°C	32.0±1.8%
Stage 2: DD+CTa	25.0±0.10 h ^b	10:58±0.30 h ^{aA}	1.20±0.90°C	0.60±0.02°C	07.3±0.3%
Stage 3: DD+NRT	24.0±0.05 h ^a	13:12±0.12 h ^{aAb}	1.60±0.06°C	1.00±0.03°C	15.3±1.3 %
Stage 4: DD+RT	24.0±0.03 h ^a	02:49±0.27 h ^{bAcB}	1.53±0.10°C	0.86±0.07°C	16.4±1.1 %.
Stage 5: DD+CTa	24.9±0.12 h ^b	03:35±0:32 h ^{cAdB}	1.31±0.06°C	0.47±0.10°C	09.2±0.5 %

Note: One-way ANOVA analysis was used for the calculation of the statistical differences between periods and acrophases across different stages of the experiment 2. Different superscript lowercase or uppercase letters indicate significant differences between different stages ($p \leq 0.05$). Similar superscript lowercase or uppercase letters indicate no statistically significant differences between compared stages ($p > 0.05$).

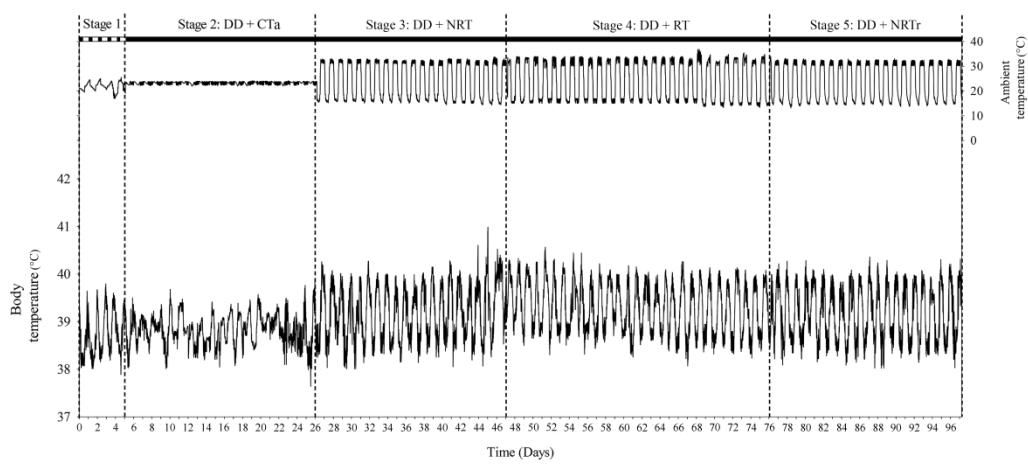
Table 3: Mean values \pm S.E.M. of individual phi1 and phi2 estimated with the nonlinear regressions applied for each animal in NRT, RT and NRTr conditions of Ta cycle respectively during stages 3, 4 and 5.

Phi 1			Phi 2		
NRT (Stage 3)	RT (Stage 4)	NRTr (Stage 5)	NRT (Stage 3)	RT (Stage 4)	NRTr (Stage 5)
16,57 \pm 1,80 ^a	13,21 \pm 2,07 ^{bA}	13,99 \pm 2,29 ^B	10,24 \pm 1,65 ^a	14,17 \pm 1,94 ^{bA}	8,96 \pm 2,02 ^A

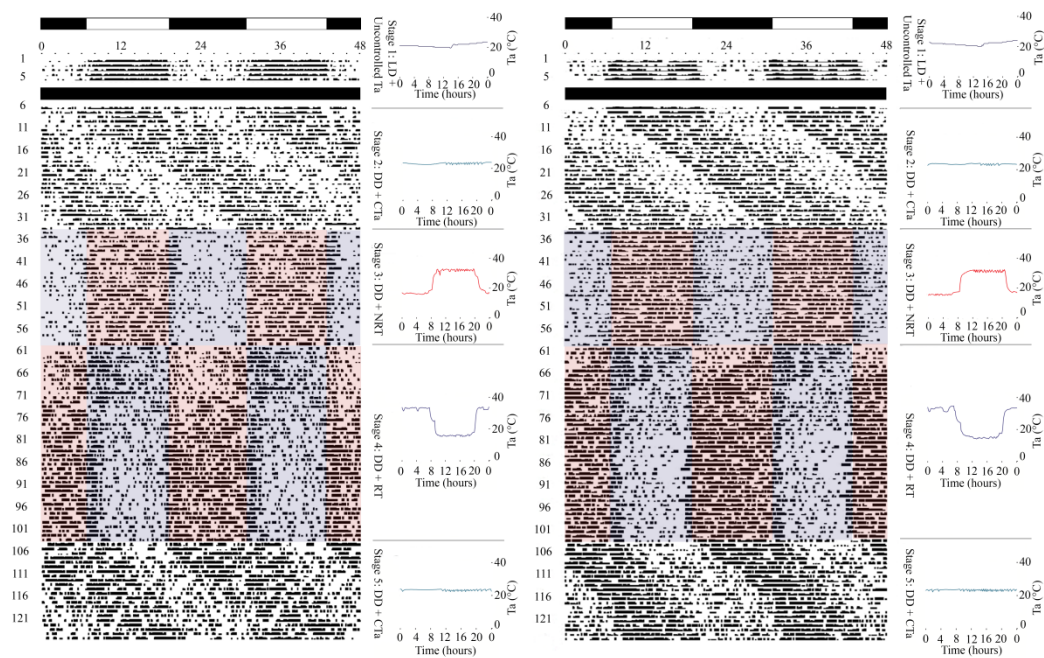
Note: Paired t-test analysis for the estimated Phi1 and Phi2 were performed for NRT and RT and then for RT and NRTr treatments: 1) Different superscript lowercase letters indicate significant differences between NRT and RT treatment for both Phi1 and Phi2 ($P \leq 0.05$). 2) Different superscript uppercase letters indicate significant differences between RT and NRTr treatment in Phi1 ($P \leq 0.05$). 3). Similar superscript uppercase letters indicate no statistically significant differences between RT and NRTr treatment in Phi2 ($p > 0.05$).



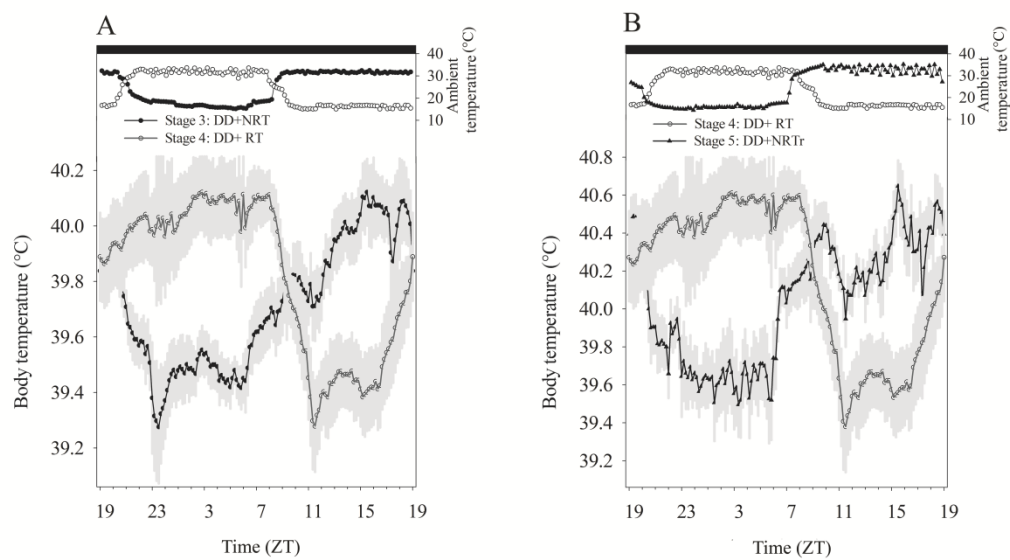
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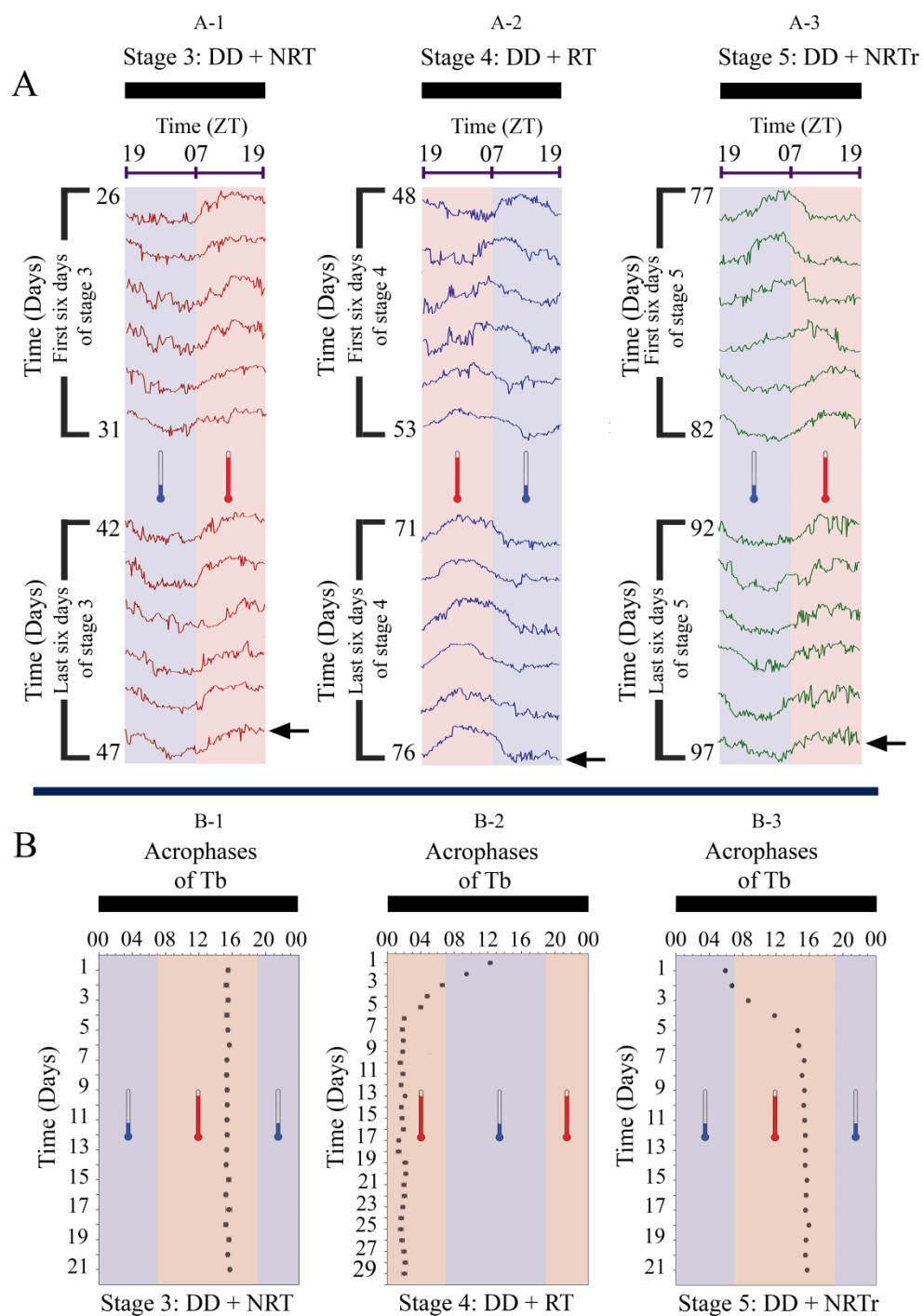
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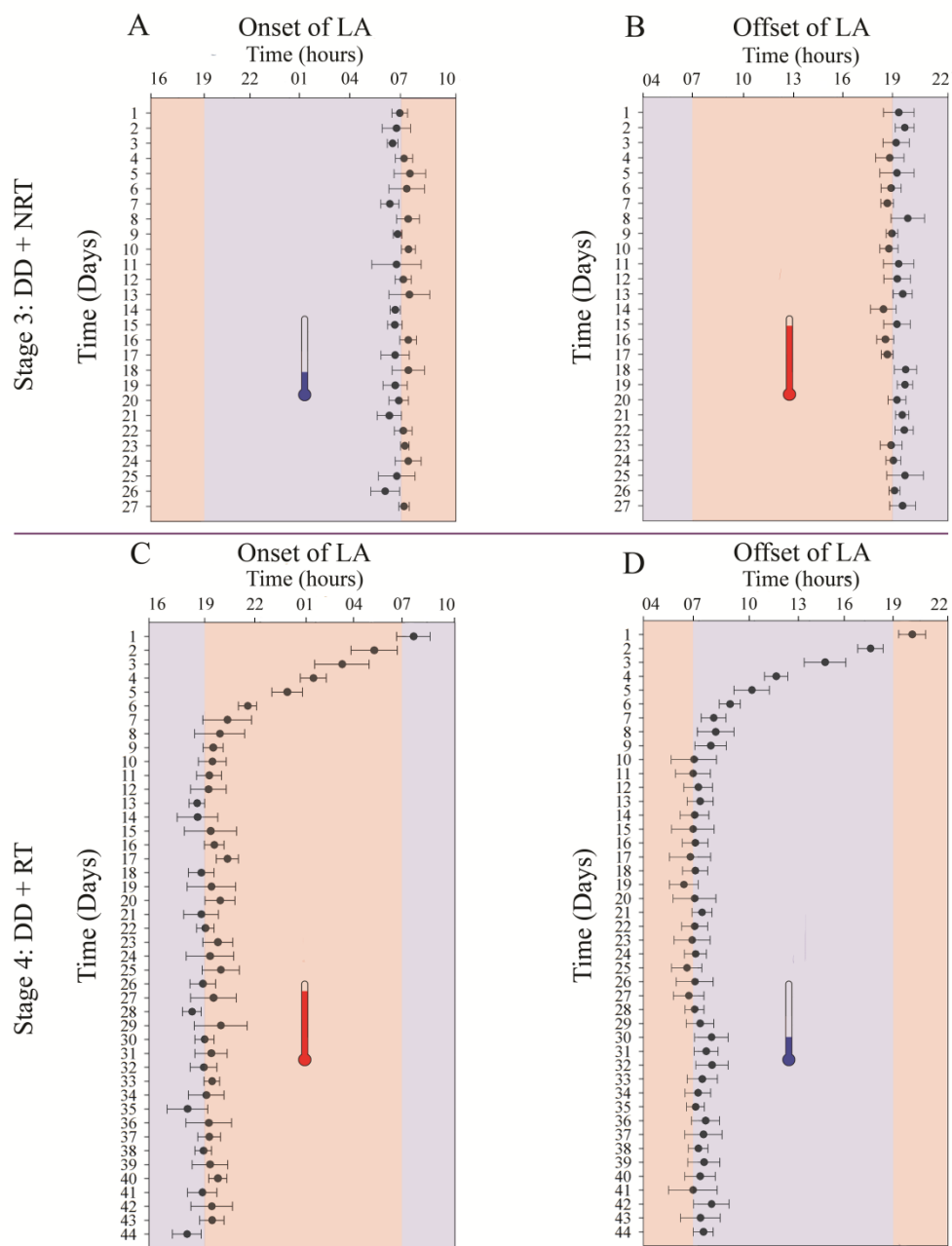
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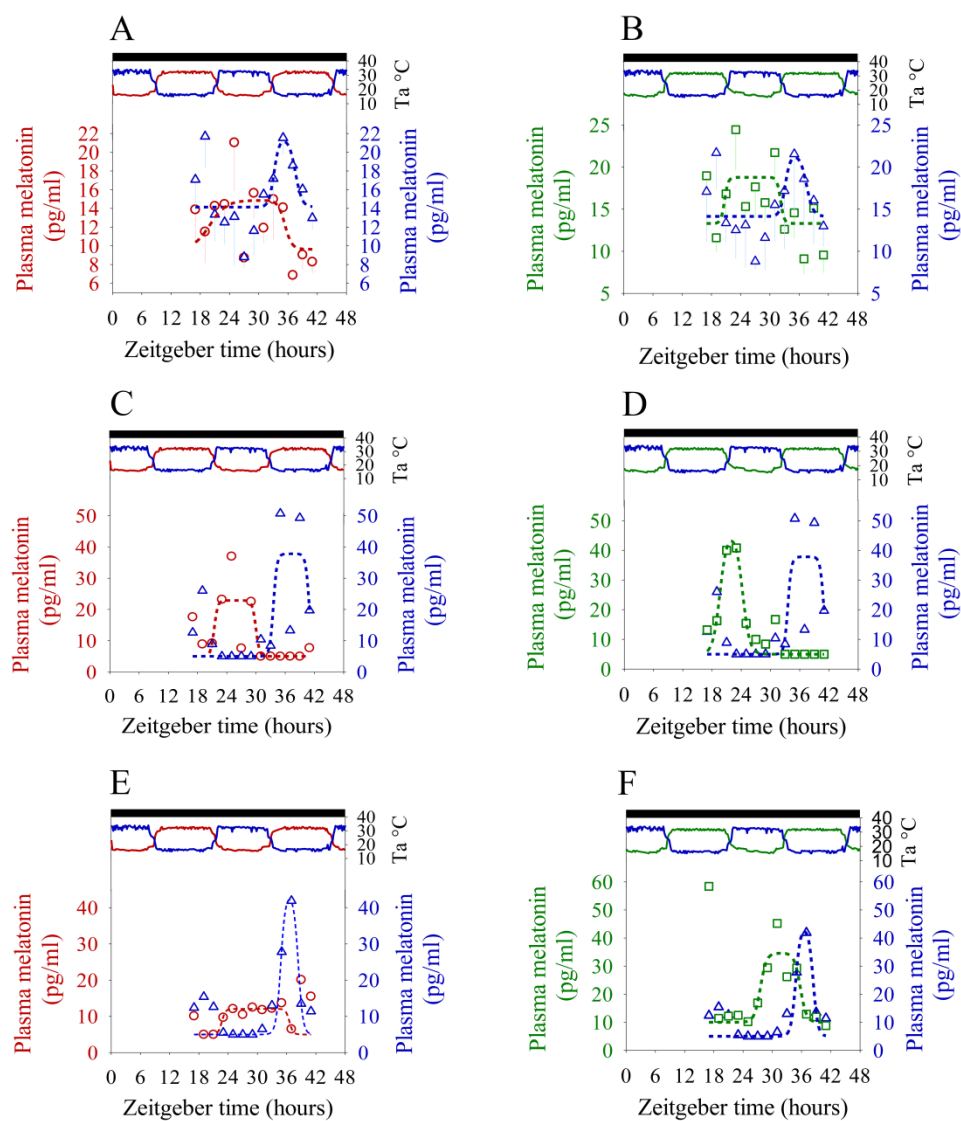
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