Physiology & Behavior 103 (2011) 352–358

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/phb

The circadian body temperature rhythm of Djungarian Hamsters (*Phodopus sungorus*) revealing different circadian phenotypes

Konrad Schöttner [a], Jim Waterhouse [b], Dietmar Weinert [a,*]

[a] Institute of Biology/Zoology, Martin-Luther-University Halle-Wittenberg, Germany
[b] Research Institute for Sport and Exercise Science, Liverpool John Moores University, Liverpool, UK

ARTICLE INFO

Article history:
Received 3 November 2010
Received in revised form 3 February 2011
Accepted 9 February 2011

Keywords:
Djungarian hamster
Circadian rhythm
Body temperature
Motor activity
Arrhythmic activity patterns
Unmasking

ABSTRACT

Djungarian hamsters (*Phodopus sungorus*) of our breeding stock show three rhythmic phenotypes: wild type (WT) animals which start their activity shortly after "lights-off" and are active until "lights-on"; delayed activity onset (DAO) hamsters whose activity onset is delayed after "lights-off" but activity offset coincides with "lights-on"; and arrhythmic hamsters (AR) that are episodically active throughout the 24-h day. The main aim of the present study was to investigate whether the observed phenotypic differences are caused by an altered output from the suprachiasmatic nuclei (SCN). As a marker of the circadian clock, the body temperature rhythm purified from masking effects due to motor activity was used.

Hamsters were kept singly under standardized laboratory conditions (L:D = 14:10 h, T: 22 °C ± 2 °C, food and water ad libitum). Body temperature and motor activity were monitored by means of implanted G2-E-Mitters and the VitalView® System (MiniMitter).

Each phenotype showed distinctive rhythms of overt activity and body temperature, these two rhythms being very similar for each phenotype. Correcting body temperatures for the effects of activity produced purified temperature rhythms which retained profiles that were distinctive for the phenotype. These results show that the body temperature rhythm is not simply a consequence of the activity pattern but is caused by the endogenous circadian system. The purification method also allowed estimation of thermoregulatory efficiency using the gradients as a measure for the sensitivity of body temperature to activity changes. In WT and DAO hamsters, the gradients were low during activity period and showed two peaks. The first one occurred after "lights-on", the second one preceded the activity onset. In AR hamsters, the gradients did not reveal circadian changes.

The results provide good evidence that the different phenotypes result from differences in the circadian clock. In AR hamsters, the SCN do not produce an obvious circadian signal. With regard to DAO hamsters, it remains to be investigated whether the clockwork itself or the afferent entraining pathways are abnormal in comparison with the WT hamsters.

1. Introduction

Circadian rhythms are normally stable and synchronized to the rhythms of the organism's environment. This enables an organism to adapt to day–night changes and to prepare for, rather than merely react to, cyclic events in the environment, and thus guarantees an optimal functioning of the biological system [1–3]. In addition, a stable circadian system seems to be a prerequisite for proper photoperiodic time measurement and, therefore, for seasonal adaptation [4–7]; this seems particularly true for species like the Djungarian hamster (*Phodopus sungorus*) which lives in an environment with extreme seasonal changes [8].

In Djungarian hamsters (*P. sungorus*) of our Institute, a number of animals show activity patterns that seem incompatible with proper adjustment to a periodic environment [9]. The activity onset in these animals is delayed by several hours. As the activity offset is still coupled to lights-on, the activity time (α) is strongly compressed. In these, so-called, delayed activity onset (DAO) animals, the activity onset can be shifted until α reaches a critical value (3:02 ± 0:12 h). Passing this critical value, the animals start to free run despite the presence of a periodic light–dark regimen. Finally, the rhythm breaks down, and the animals become arrhythmic.

The delayed activity onset indicates an attenuated ability to synchronize with the periodic environment and may result from an aberration in the endogenous circadian system, particularly the circadian clock and its interaction with the photic zeitgeber [10]. Arrhythmicity may originate from a "collision" of two extremely loosely coupled oscillators; thus, a zero phase difference between them (synchrony), together with reciprocal inhibition between the oscillators, might lead to a combined output with zero amplitude [7].

* Corresponding author at: Martin-Luther-Universität Halle-Wittenberg, Institut für Biologie/Zoologie, Domplatz 4, D-06108 Halle, Germany. Tel.: +49 345 5526464; fax: +49 345 5527152.
E-mail address: weinert@zoologie.uni-halle.de (D. Weinert).

0031-9384/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.physbeh.2011.02.019

However, one should also consider the possibility that processes downstream from the SCN may be involved in the expression of the observed rhythmic phenotypes, and that differences in these, rather than the SCN, might account for the observed variation. One of the aims of the present paper is to investigate in more detail if these observed phenotypic differences are associated with altered output from the SCN.

The output from the SCN cannot be measured directly in freely moving animals, but the body temperature rhythm is an appropriate marker of the circadian clock, particularly when it has been purified to remove masking effects which are mainly due to motor activity [11]. In the present study, Djungarian hamsters of three rhythmic phenotypes were investigated: (1) wild type (WT) hamsters, which start their activity immediately after "lights-off" and show activity offset at "lights-on"; (2) DAO hamsters, which show a delayed activity onset whereas activity offset remains entrained to "lights-on" (see above); and (3) arrhythmic hamsters (AR), which are active throughout the 24-h day and thus show a behavior like SCN-lesioned animals. The following issues have been concentrated upon: Do the overt activity and body temperature rhythms show similar patterns within each rhythmic phenotype? Does this pattern apply also with the endogenous (purified) temperature rhythm? Are there changes in thermoregulatory efficiency during the course of the day, as observed previously [12,13]?

2. Material and methods

2.1. Experimental animals: breeding and housing conditions

The animals were taken from two breeding lines that have been established at our Institute. In one case, least related individuals (showing a wild-type, WT, activity pattern) were mated; this led to offspring with a high percentage of WT animals. In the other case (DAO animals) more closely related, and preferably brothers and sisters, were paired; this led to an inbred lineage with a high percentage of DAO hamsters [9].

Animals were kept in air-conditioned, windowless rooms in standard plastic cages (Macrolon®, Type III) with wood shavings (Allspan®, The Netherlands) as nesting material. Animal bedding was renewed every two weeks. Food pellets (breeding diet Altromin® 7014, maintenance diet Altromin® 7024, mixed in the ratio 1:2; Altromin GmbH, Lage, Germany) and tap water were provided ad libitum. Room temperature was 22 °C ± 2 °C, and relative humidity varied between 60 and 65%. The LD regimen was 14:10 h, with light on from 04:00 to 18:00 h Central European Time and a light intensity 80–100 lx during L and 0 lx in D.

Experiments were performed with 7 WT, 5 DAO and 4 AR hamsters. Animals were selected on the basis of their daily activity pattern, obtained over a 3-week period by passive infrared motion detectors (see below). This procedure of establishing an individual's phenotype was necessary since not all animals of the inbred lineage were DAO hamsters and DAO hamsters were found occasionally in the WT lineage. All animals were female, except two DAO hamsters. So far, no sex-specific differences have been found in the activity rhythms and their sensitivity to photic cues in either phenotype [8–10]. Also, the daily means of body temperature did not show the regular changes which would indicate the presence of a sexual cycle. Female Djungarian hamsters nearly always reveal estrous cycles only in the presence of a male animal but not if they are kept individually, as in the present experiments (unpublished own results).

All experimental procedures were conducted according to the German law for animal protection.

2.2. Motor activity and core body temperature registration

Body temperature and motor activity were measured by means of G2-E-Mitters and the VitalView® System (MiniMitter, Bend, Oregon, USA). The E-Mitters were implanted into the peritoneal cavity. For this purpose the animals were anesthetized by an i.p. injection of 125 mg/kg ketamine hydrochloride (Ketanest® S (5 mg/ml), Parke-Davis GmbH, Berlin, Germany). An abdominal incision was made, the transmitter implanted and the incision closed using sutures [14]. Following transmitter implantation the hamsters were housed singly.

The E-Mitters work without batteries and are energized by the receiver (ER-4000 Energizer Receiver, MiniMitter) placed under the home cage. The receiver picks up the transmitted signal and converts it to a computer-readable form. Recordings were performed at 5-min intervals, each recording being the summed activity impulses over the 5 min, and the body temperature was recorded at the end of each of these 5-min intervals.

In some cases (for details, see Results), motor activity was measured additionally by means of passive infrared (PIR) motion detectors (Wizard, Guardall Ltd., Scotland). They were mounted above the cage roof in such a way that they detected motions of the hamsters in all sectors of the cage. The correct position was estimated in preliminary studies by visual observation. The impulses from the PIR detectors were stored and analyzed using the Chronobiological Kit® (Stanford University, Stanford, California, USA).

Following 10 days for recovery after surgery, animals were monitored for a month, to get the baseline patterns of daily activity and body temperature.

2.3. Data analysis

The counts detected by PIR motion sensors were depicted as double-plotted actograms using the software provided in the Chronobiology Kit®. The activity and temperature data obtained with the Vital View system were also depicted as actograms using Microsoft Excel® and showing only the values above the respective daily mean. In the case of body temperature, the absolute deviations were depicted; in the case of motor activity, the relative deviations from unity, a value indicating the daily mean activity. The actograms not only allowed a visual analysis of the data but also selection of intervals with stable rhythms for unmasking body temperature (see below) and the estimation of certain rhythm parameters like activity onset and offset, its duration etc.

To investigate the endogenous component of the temperature rhythm, the raw temperature data were unmasked, or "purified", by the method of Weinert and Waterhouse [13]. In brief, the method is as follows. As a preliminary step, the "integration time" for activity counts had to be estimated (which was the period of time before a temperature reading for which the activity counts needed to be summed). This estimate was performed using 10-day data sets from 6 WT hamsters and two 4-h "windows" (middle of the light time and middle of the dark time), giving a total of 120 windows. For each window, activity was summed over 5, 10… 60 min and these sums were correlated with the temperature measured at the end of these integration intervals. Considering all windows, the highest positive coefficients of correlation were found between 20 and 40 min (mean: 28.8 min). Accordingly, an integration time of 30 min was used for the analysis.

The purification method uses 10 days of data. It calculates the linear regression of raw temperatures on activity using a 3-h "window" (36 data pairs, each consisting of activity counts summed over the previous 30 min, see above, and the temperature measured at the end of this 30-min interval). If the correlation between temperature and activity is statistically significant, then the intercept (when activity equalled zero) is taken as a better estimate of the endogenous temperature at that time, and the gradient as a measure of the sensitivity of temperature to activity. If the correlation between temperature and activity is not significant, then the average temperature over this 3 h-window, rather than the intercept, is calculated, and the gradient is taken as zero. The 3-h window is then