Physiology & Behavior 105 (2012) 835–840

Contents lists available at SciVerse ScienceDirect

Physiology & Behavior

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

Social interaction with a rhythmic rat enhances the circadian pattern of the motor activity and temperature of LL-induced arrhythmic rats

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ARTICLE INFO

Article history:
Received 13 July 2011
Received in revised form 20 October 2011
Accepted 25 October 2011

Keywords:
Circadian rhythm
Constant light
Arrhythmicity
Social interaction
Isolation

ABSTRACT

Although light is the main factor that influences circadian rhythms, social interaction may also have a role on their regulation. Here, the influence of social interaction on rat circadian behavior was investigated, addressing the question of whether cohabitation would induce the appearance of a circadian rhythm in arrhythmic rats due to constant light. To this end, circadian rhythms of motor activity and body temperature of male and female LL-induced arrhythmic rats were studied before, during and after a 20-day period in which rats stayed in the same cage with a rat of the same sex but with stronger rhythm. Results showed that the manifestation of the circadian motor activity rhythm of LL-induced arrhythmic rats increased after cohabitation. In the case of the expression of the body temperature rhythm, there was a progressive daily increase in the power content of a daily 24 hour pattern throughout the cohabitation days, which remained when animals were again isolated. Thus, the presence of a rhythmic rat increases the strength of the circadian behavior of rats showing a weak circadian rhythm.

1. Introduction

Circadian rhythms are endogenous fluctuations that in mammals appear in most of the biological functions and that are regulated by the action of the main pacemaker situated in the suprachiasmatic nuclei in the hypothalamus [1]. Circadian rhythms play an important role in animals' lives, since animals carry out their activities at the time which is favorable and enhance the efficiency of many physiological or behavioral functions. The adaptation to the external changes provides a temporal organization for physiological processes and animal behavior in relation with the environment. Since circadian rhythms have evolved as adaptation to the daily lighting fluctuations, it is not surprising that the main adjustment of the pacemaker is due to light changes and that light intensity produces strong effects on the period length of the endogenous rhythm [2]. However, in addition to light, other, nonphotic factors have also been reported to influence the mammals' pacemaker, such as motor activity [3–5] or food restriction [6,7].

Social interaction is another factor that may modulate the circadian behavior. As the circadian adaptation to the external light–dark cycle, the adaptation to live with other animals is also needed for survival. However, the role of social stimuli on the circadian system has not been elucidated. Several reviews summarize the current knowledge of social interactions [8–10]. In general, social interactions are considered to be, at best, weak Zeitgebers on circadian rhythms, although in the field they seem to be more important than under lab conditions.

The role of social cues has been considered as a mechanism for adjusting the phase of individuals within a population [11]. In the lab, several experiments in different species have been carried out to attempt to know the influence of the other individuals living in the same cage. Group housed flies may display more coherent or converging activity rhythms than isolated animals [12]. Moreover, tau changes have been found in hamsters living in pairs, although usually only one of each pair modifies the period length of the circadian rhythm [11] and in Octodon degus, which partially entrain their rhythms in the absence of light by social information [13].

It is not clear whether all species have the same sensitivity to social interaction. It has been suggested that rat is far less sensitive to nonphotic stimuli than other species as the hamster [14,15]. However, we recently found that rats living together increase the coherence of their endogenous rhythm of body temperature [16] suggesting the action of social interaction as a weak coupling agent.

In this paper we studied the effect of social interaction in rats, under the hypothesis that arrhythmic animals could improve their circadian behavior if they were housed together with rhythmic rats. We took advantage of the fact that rats are very sensitive to the effects of constant light and become arrhythmic under LL [17,18]. However, when they are raised under bright LL during suckling, they develop a clear stable circadian rhythm with a period close to 25.5 h [19,20]. Thus, LL provides a unique opportunity to have intact adult rats showing different behavioral patterns under the same environmental

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doi:10.1016/j.physbeh.2011.10.027

condition, that is, rats raised under LL with clear expression of the rhythms in adulthood and rats raised under LD, with very weak or complete absence of rhythms when placed under LL as adults. Therefore, we here studied the effect of the interaction between LD- and LL-raised rats on their circadian rhythms of motor activity and temperature, with special emphasis in the appearance of any possible circadian pattern in the initially arrhythmic rats.

2. Material and methods

2.1. Animals: breeding and housing conditions

7 pregnant female Wistar rats (Charles River, France) arrived at our lab at 17 days of gestation and were maintained under LD (12 h:12 h). The day of delivery, pups were cross-fostered, in such a way that each litter had a similar number of male and female pups coming from different dams. 3 of the new litters were transferred to LL conditions while the other 4 remained under LD. After 24 days, pups were weaned and isolated in individual cages (25×25 cm). For this experiment we used 6 male and 4 female pups raised under LL and 6 males and 4 females raised under LD. Each group of rats was formed by rats coming from the different litters. The rest of the pups were used for other experiments.

Since the day of weaning (day 1 of the experiment), all the rats were isolated in individual cages into the same room under constant bright light (LL) which was provided by two fluorescent tubes with an intensity of 300 lx at cage level. Motor activity was recorded since the day of weaning. On day 47, the circadian pattern of each motor activity rhythm was clearly established, as it was earlier described according to the lighting conditions during suckling [19,20]: pups raised under LL showed a clear circadian rhythm under LL after weaning whereas animals raised under LD showed an arrhythmic pattern under LL after weaning. Then, all the rats were implanted with a temperature sensor into the abdominal cavity. The sensor was programmed to start recording data three days later, once animals were recovered from surgery. After recovery (about day 50), one LL-raised rat was housed together with a LD-raised rat belonging to the same sex. Thus, in total we studied 10 pairs of rats (6 pairs of males and 4 of females). To decrease the possibility of fighting, the rats were co-housed in a fresh cage, bigger than the previous one, sized 25×50 cm. Rats interacted for 20 days. Afterwards, rats were again isolated in individual cages for other 20 days. During all the experiment, animals had always free access to food and water. Animal handling procedures were in accordance with the institutional guidelines for the care and use of laboratory animals established by the Ethical Committee for Animal Experimentation of the University of Barcelona.

2.2. Motor activity and core body temperature registration

Motor activity (MA) was continuously recorded for 92 days, since the day of weaning until 13 days after cohabitation. The activity meters consisted of two crossed perpendicular infrared beams crossing the cage 7 cm above the floor, in the case of small cages for isolated rats and of 2 parallel infrared beams for the cages used for one pair of rats. In any case, every time the animal crossed one beam a count was detected. The number of counts was accumulated and recorded every 15 min.

Body temperature was measured by data loggers (Thermochron®, iButton type DS1921H-FS with a resolution of 0.125 °C) implanted intraperitoneally to rats under isoflurane anesthesia. This variable was recorded for 20 days when the animals were housed together in the same cage and for 20 days after they went back to the individual cages. At the end of the experiment animals were sacrificed by CO_2 inhalation and the temperature sensors were removed. Temperature measurements were recorded every 30 min.

2.3. Data analysis

For comparisons between groups, motor activity (MA) and temperature (T) data were analyzed separately in different stages (see Fig. 1): Individual pattern of MA was analyzed for 13 days before (MA "before") and for 13 days after (MA "after") cohabitation. Although this variable was also recorded when the animals were housed together, these data were not used for the analysis, since they are due to the simultaneous activity of the two rats, and thus not useful for comparison among groups. Individual patterns of temperature were analyzed in two stages: 20 days during cohabitation (T "during") and 20 days after (T "after"). In all the stages, the rhythm manifestation was studied by means of the chi-squared periodogram applied to the data [21], which provides the period of the most significant rhythm and the percentage of variance (PV) explained by this rhythm. PV was used as a marker of the presence and stability of the rhythm.

To study the evolution of the daily rhythmic pattern, serial section analyses were carried out to test the daily fitting to a sinusoidal curve of consecutive and non overlapping series of data corresponding to 24 h. In this way, the first harmonic of the spectrum has a 24 hour period and its power content (power content of the first harmonic, PCH1) indicates the variance of the data explained by the curve.

Daily mean values of PCH1 (mean of each group) was plotted versus time (days of the experiment) as an indicator of the evolution of the circadian rhythm throughout the experiment.

Moreover, the individual mean value of the PCH1 for each stage was calculated by averaging, for each animal, the power content of the harmonic of the days of each studied stage (before, during or after cohabitation). By this procedure a daily circadian measure for each animal was obtained. It should be noted that for the calculation of the mean PCH1 the phases were not considered.

Since, the evolution of PCH1 in LL- or LD-raised rats had been widely studied in our lab, in the same strain of rats, we used as control, motor activity data of two groups of 6 rats each (3 females and 3 males), one group raised under LL and the other under LD. MA data were obtained equally to the data of the current experiment (infrared beams, 25×25 cm size cage and 15 minute sampling interval) and reanalyzed according to the procedure described above.

To explore the direct behavioral interaction between the animals of a pair, we carried out a cross-correlation between the data records of each pair of animals, for motor activity and temperature separately. To eliminate the similarity derived from the presence of a common rhythm, we applied a high pass filter by subtracting the smoothed series (moving average with an interval of 2 h) from the original series. The interval of displacements was set from minus to plus 24 h, and the maximum value of the cross-correlation function (MCC) in this interval was taken as the indicator of the existing correlation. To establish the level of significance the Bonferroni correction was applied based on the number of lags calculated.

Time series analyses were conducted by means of an integrated package of tools for chronobiology "El Temps©" v. 251 (A. Díez-Noguera, Universitat of Barcelona, http://www.el-temps.com) and statistical analysis was carried out with SPSS® (v. 18) package.

3. Results

At the beginning of the experiment, the circadian pattern (Fig. 1) of each animal was studied in order to quantify differences between LD- and LL-raised rats, before the iButton implantation. To do so, periodogram analysis was applied to data corresponding to days 17 to 47 since weaning. As expected, all rats raised under LL were rhythmic under LL with a mean period of 25 h 26 min (s.e. 3.3 min). In the LD-born group, all the females as well as 1 out of 6 males showed no significant rhythm. The other males showed a weak, although significant, rhythm with a period of 25 h 13 min (s.e. 29.5 min). The mean percentage of variance explained by the rhythm had a mean