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Motor activity rhythms of forced desynchronized rats subjected to restricted feeding

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Abstract

Although light is the strongest zeitgeber for the circadian pacemaker, other stimuli can also produce entrainment. In the rat, periodic restricted feeding (RF) is a weak stimulus that may act as a zeitgeber. We tested the effect of RF on the motor activity rhythms of rats subjected to forced dissociation. In this situation two components, supposed to be related with the ventrolateral and dorsomedial areas of the suprachiasmatic nucleus, are detected in their motor activity. One component is entrained to the external light–dark cycle (Light Dependent Component, LDC) and thus has the same period, while the other has a period longer than 24 h (Non-Light Dependent Component, NLDC). This experiment examined whether RF can act on one or both of these two rhythms. Rats were maintained under the light–dark cycles of 22 h (T22) or 23 h (T23) for 44 days with food available for four hours per day. Afterwards the rats received food ad libitum, to test the effect of the previous RF condition. Results show that RF modifies the manifestation of the two initial rhythms, being this effect stronger under T23 than under T22. However RF does not affect the NLDC period. The results reveal that the animal can manifest simultaneously several rhythmic patterns.

Keywords: Restricted feeding; Suprachiasmatic nucleus; Motor activity; Circadian

1. Introduction

The suprachiasmatic nuclei of the hypothalamus (SCN) are considered to be the most important structure of the mammal circadian system, since they generate and coordinate the circadian rhythmicity of the organisms. The SCN generate the circadian expression in many behavioral and physiological variables such as locomotor activity, body temperature, plasma corticosterone and pineal melatonin [1]. Moreover, the circadian system allows synchronization with the environmental time cues. Light is the strongest zeitgeber since it shifts the clock in a circadian time-dependent way and produces the entrainment to the external light–dark cycle. However, this is not the only zeitgeber. Nonphotic stimuli such as increased locomotor activity, restricted feeding, social cues, and exposure to novelty can phase shift and/or entrain the circadian pacemaker of mammals [2]. One of the nonphotic stimuli that can affect the organization of activity, independently of the LD cycle, is restricted access to food. However, this is quite controversial. Although survival may depend on behavioral adaptation to periodic restricted feeding (RF), the extend to which RF acts as a zeitgeber varies among species [3–5]. Food availability may affect the circadian organization of daily rhythms [6]. Two behavioral activity components will result in case of a nocturnal animal submitted to an LD cycle, if food is only available during the day. One component is entrained by the feeding cycle, observed by anticipatory activity 2–3 h before the onset of the food availability, referred to as the food anticipatory activity (FAA) [7,8]. The other is the normal nocturnal activity entrained by the light–dark cycle. Although the rat prefers to feed at night, it will exhibit diurnal FAA if food is only available during the day [7]. Other variables such as serum corticosterone [9,10], free fatty acids, and core temperature are associated with FAA, and their values rise in anticipation of the daily meal. The SCN has been identified as the light-entrained pacemaker (LEP), whereas the mechanisms for the food-entrained oscillator (FEO) remain elusive. The FEO is thought to be a circadian oscillator independent of the SCN, because FAA is also present in animals with bilateral lesions of the SCN [11,12]. However, the precise

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anatomical location of the FEO remains to be identified [13,14,8]. Recent studies emphasize the importance of peripheral oscillators, placed outside the SCN, as possible components of the FEO. Studies of clock gene expression in mice and rats indicate that the phase of the genes in the SCN is not affected by the restricted feeding under LD cycle, while clock genes expressed in peripheral tissues are phased shifted with the feeding restriction [15]. Special attention has been given to the liver as a possible peripheral clock [13,8,15]. These peripheral organs interact with central nervous structures involved with ingestive behavior and energy balance. It has been suggested that FEO is a distributed system of interacting structures that require an integrative approach [16].

In the absence of other zeitgebers, for instance under constant lighting conditions, RF may entrain the circadian rhythm driven by the SCN. However, the entrainment depends on the species [17,7] on the closeness of the period of the RF cycle to the free-running period [12] and also on the duration of the RF [18].

All this seems to indicate that under an LD cycle, the SCN is not usually entrained by RF, but that, under certain circumstances, RF can act as a zeitgeber. It has been suggested that the coupling between the FEO and the light entrainable pacemaker (LEP) of the SCN is asymmetrical, as FEO is usually coupled to LEP, but FEO has a weak effect on the LEP. It is no clear how feeding activity affects the circadian pacemaker, although the two parts of the SCN, dorsomedial or ventrolateral, may not be equally affected by the feeding restriction [19].

When rats are exposed to T22 cycles, their motor activity rhythm can be dissociated and two simultaneous circadian rhythms appear in behavior: One, with a period equal to that of the external light–dark cycle (Light Dependent Component —LDC) that seems to be related to the function of the ventrolateral part of the SCN and the other, with a period of more than 24 h (Non-Light Dependent Component—NLDC), associated to the dorsomedial part [20]. This situation implies that the circadian clock is not as strongly coupled as it is under free-running or 24 h LD cycles and that the two main oscillator populations in the SCN can be functionally differentiated. Forced dissociation could provide an opportunity to study the effect of a weak zeitgeber, such as RF, on the motor behavior of the rat. This experiment was designed to test whether RF may affect the circadian pacemaker under the paradigm of forced dissociation by modifying one or the two circadian components which may correspond to the two anatomical parts of the SCN.

2. Material and methods

20 female and 20 male Wistar rats were used for the experiment. They were purchased from Charles River Laboratory (France) at the age of 3 months. When they arrived at our laboratory, they were housed individually in transparent cages measuring 25×25×12 cm and placed into two sound-proof rooms. During the first five days of the experiment, all the animals were kept under 12 h-light and 12 h-darkness cycles to synchronize their rhythms. Afterwards half of the rats were submitted to symmetrical cycles of 22 h: 11 h-light and 11 h-darkness (11:11 LD; T22) and the other half to an 11.5:11.5 LD cycle (T23) throughout the experiment. For each T, 6 males and 6 females were submitted to RF with a period of 24 h 12 min, with 4 h of food access per day (experimental rats), while 4 males and 4 females were maintained in the same room with food ad libitum (control rats). RF was applied by a researcher entering the room every 1452 min (24 h 12 min). A period different than 24 h was chosen as the periodicity of RF, to avoid potential coincidences with external and involuntary influences. RF was maintained from day 5 to day 66 (RF stage) excepting days 49–52 and 59–62, when rats were food deprived to test the endogenous character of the RF rhythm. Finally, the experiment ended with one stage of feeding ad libitum (aL stage) for a further 31 days. In all cases, lighting was provided indirectly by two fluorescent tubes with an intensity of 300 lux at cage level. Darkness was attained using a dim red light with an intensity of less than 0.1 lux. During the experiment, all the rats had free access to tap water, and control rats had free access to food. All rats were fed commercial rat chow (A04 for rat and mouse, Panlab).

The motor activity rhythm was recorded throughout the experiment by activity meters, which consisted of two crossed perpendicular infrared beams crossing the cage 7 cm above the floor. The number of movements was accumulated and recorded every 15 min.

2.1. Data analysis

The rhythmic behavior of the rats was analyzed separately for motor activity data corresponding to the two stages: stage 1 and stage 2. The first one corresponds to the time in which experimental rats were submitted to RF and the second, to aL. Control rats were used to test the effect of possible environmental factors apart from food during RF, and the effect of time (duration of the experiment) on the motor activity rhythms, and thus, had always free access to food.

We calculated the mean value of the motor activity of each animal in each stage, the food anticipatory activity (FAA) measured as the percentage of the total motor activity per cycle that occurs during the 2 h before the restricted food availability and also the activity during the 4 h of restricted feeding (4 h-RF), also calculated as a percentage of the total motor activity per cycle.

For each stage, the Sokolove–Bushell periodogram [23] was used to calculate the periods of the significant rhythms using the motor activity data smoothed by a moving average of 3 data points to reduce non-significant noise. To carry out the periodogram analysis, 42 days data were used in stage 1 for all rats. However for stage 2, 31 days data were used for T22 group and 22 days data for T23 group. In most of the rats 3 peaks were detected by the periodogram, one with the period of the external light–dark cycle (Light Dependent Component, LDC), another with the period of the RF cycle (Food Dependent Component FDC), and the third with a period that was not dependent on the light or on food availability (Non-Light Dependent Component, NLDC).