Available online at www.sciencedirect.com
SCIENCE DIRECT
Neuroscience Research 52 (2005) 166–173
Neuroscience Research
www.elsevier.com/locate/neures

Altered food-anticipatory activity rhythm in *Cryptochrome*-deficient mice

Michihiko Iijima [a], Shun Yamaguchi [b], Gijsbertus T.J. van der Horst [c], Xavier Bonnefont [c], Hitoshi Okamura [b], Shigenobu Shibata [a,*]

[a] *Department of Pharmacology, School of Science and Engineering, Waseda University, Nishitokyo, Tokyo 202, Japan*
[b] *Division of Molecular Brain Science, Department of Brain Sciences, Kobe University Graduate School of Medicine, Kobe 650-0017, Japan*
[c] *MGC, Department of Cell Biology and Genetics, Erasmus MC, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands*

Received 19 October 2004; received in revised form 25 February 2005; accepted 2 March 2005
Available online 28 March 2005

Abstract

In nocturnal rodents, restricted feeding to daytime (RF) causes feeding-associated diurnal locomotor activity that persists for the next 1–2 days when food is withheld. Along with this anticipatory behavior, the expression pattern of clock genes such as *mPer1* and *mPer2* changes from a nocturnal to diurnal pattern in the liver and cerebral cortex but not in the suprachiasmatic nucleus (SCN). Whether the molecular clockwork, in which *mCry1* and *mCry2* genes are essential components, is involved in food-anticipatory circadian rhythms is unknown.

In this study, we investigated the impact of the absence of mCRY products upon the locomotion pattern induced by RF. RF caused an increase in daytime activity that lasted even for 2 days after food was withheld, in wild-type and *mCry1*−/− *mCry2*−/− mice. However, RF-induced activity was less stable and appeared more gradually in mutant mice. Similar results were obtained with mice housed under constant darkness or with SCN-lesioned wild-type and mutant mice. Our data reveal that *mCry* proteins are basically dispensable for food-entrainable oscillation. However, it is also important to note that *mCry* deficiency affects the stability and development of RF-induced anticipatory locomotor activity.

Keywords: Suprachiasmatic; Anticipation; Clock gene; Circadian; Locomotor activity

1. Introduction

The suprachiasmatic nucleus (SCN) houses a master pacemaker that regulates behavioral and physiological circadian rhythms such as locomotor activity, body temperature, and endocrine release (Moore, 1997; Hastings, 1997). Recent studies unraveled the molecular mechanisms responsible for circadian rhythmicity: a number of putative clock genes (*Per1*, *Per2*, *Per3*, *Clock*, *Bmal1*, *Cry1*, *Cry2*, *Rev-erbα*, and *Ck1ε*) regulate each other's expression levels according to a subtle interplay between transcription/translation feedback loops (Dunlap, 1999; Reppert and Weaver, 2001). This molecular clock is not only present within the SCN (Kume et al., 1999), but also in peripheral organs (Yamazaki et al., 2000; Damiola et al., 2000) and even in cultured fibroblasts (Balsalobre et al., 1998; Yagita and Okamura, 2000; Yagita et al., 2001). However, the central SCN clock plays an important role in governing peripheral clocks since the destruction of SCN abolishes the circadian rhythms of glucocorticoid secretion (Meyer-Bernstein et al., 1999), locomotor activity (Moore, 1997), and also rhythms in *Per* gene expression in peripheral tissues (Hara et al., 2001; Sakamoto et al., 1998).

When food is available for only a limited period of daytime (restricted feeding, RF, during 4–7 days), mice, and rats show increased diurnal locomotor activity, 2–4 h before feeding time (Mistlberger, 1994; Hara et al., 2001).

Abbreviations: CT, circadian time; RF, restricted feeding; SCN, suprachiasmatic nucleus; ZT, zeitgeber time

* Corresponding author. Tel.: +81 424 61 1291; fax: +81 424 50 2271.
E-mail address: shibatas@waseda.jp (S. Shibata).

0168-0102/$ – see front matter
doi:10.1016/j.neures.2005.03.003

Furthermore, this daytime increase of locomotion continues over the next several days under fasting conditions. This food-associated rhythm is SCN-independent since it is preserved in SCN-lesioned animals (Mistlberger, 1994; Wakamatsu et al., 2001). Interestingly, RF is able to reset the circadian oscillations of clock genes in peripheral organs but not in the SCN (Damiola et al., 2000; Stokkan et al., 2001; Hara et al., 2001). Thus, RF seems to strongly entrain the peripheral circadian clocks without the participation of SCN clock. However, whether the circadian clock present in extra-SCN tissues is required for RF-induced resetting of locomotor activity remains unknown.

In the present study, we investigated the RF-induced entrainment of locomotor behavior in *mCry1*−/− *mCry2*−/− mice. In order to avoid any influence from the SCN, we also conducted experiments in SCN-lesioned animals.

2. Materials and methods

2.1. Animals

We used 6–8-week-old male ICR mice (Takasugi, Saitama, Japan) and *mCry1*−/− *mCry2*−/− double-mutant mice (*mCry*-deficient mouse, van der Horst et al., 1999) backcrossed in ICR background for two times. In our preliminary experiment, *mCry*-deficient mice ($N = 3$) carrying original background genes (C57black6j) exhibited a similar RF-associated rhythm (data not shown). Moreover, we observed a similar RF-associated rhythm between ICR mice (present results) and C57black6j mice (Akiyama et al., 2004). We prepared more available strain of *mCry*-deficient mice for this experiment. All animals were maintained under a 12-h light:12-h dark cycle and allowed free access to food and water. Experimental animal care was conducted under the permission of the ''Experimental Animal Welfare Committee in the School of Human Sciences of Waseda University'' (Permission #01–10), the Law (no. 105), and Notification (no. 6) of the Japanese Government.

2.2. Locomotor activity measurement

Mice were housed individually in transparent plastic cages (31 cm × 20 cm × 13 cm) and locomotor activity was measured using an infrared area sensor (Omron F5B, Tokyo, Japan). The sensitivity of each infrared area sensor was slight difference. Actually locomotor activity counts were differences among mice (5087–10024 for wild, 4497–12435 for mutant). Then, in regard to the sensitivity, we calculated normalized activity levels. The normalized absolute value of activity was slightly changed (5087–9452 for wild, 4497–8866 for mutant). Results are expressed as normalized absolute value. The activity counts (number of movements) were recorded by computer and stored on disk at 6-min intervals.

2.3. RF schedule and RF-associated entrainment

RF was scheduled as previously described (Wakamatsu et al., 2001). Mice were housed under light–dark (LD) conditions or constant darkness and were fed ad libitum for 3 days (days 1–3). After 1 whole day of fasting (day 4), mice were given access to food for 4 h from ZT 5 (Zeitgeber time; ZT 0 is defined as the lights-on time) to ZT 9 for 6 consecutive days (days 5–10). In order to elucidate whether food-associated rhythm is derived from internal clock system, on days 11 and 12, food was again totally withdrawn.

2.4. SCN lesion

Bilateral thermal lesion of the SCN was performed as described (Wakamatsu et al., 2001). A stainless steel electrode (0.35 mm i.d.) was inserted into the SCN (0.5 mm posterior and 0.0 mm lateral to the bregma at a depth 5.3 mm below the skull surface) using a thermal lesion device (RFG-4A, Muromachi Medical Co., Tokyo, Japan) under xylazine (20 mg/kg, i.p.) and ketamine (50 mg/kg, i.p.) anesthesia. The lesion was made by maintaining a temperature of 55 °C for 15 s via a current path. After recovery from anesthesia, animals were transferred to a locomotor activity device. One month after surgery, behavioral arrhythmicity was assessed by a Chi-square periodogram (Sokolove and Bushell, 1978) in the range of 20–28 h. Lesion sites were confirmed histologically at the end of the experiment. We excluded the data from one out of five mice in the mutant group and one out of eight mice in the wild-type group, because these animals exhibited a significant ($p < 0.05$) 24-h oscillation rhythm on Chi-square periodogram and incomplete SCN lesioning with Nissl staining.

2.5. Statistics

Results are expressed as the mean ± S.E.M. One-way analysis of variance (ANOVA) was used to determine the RF-induced oscillation. Chi-square periodogram was also used for determination of significant oscillation. Two-way ANOVA was applied to determine the significant difference between daily activity pattern on the fasting days before and after RF (Figs. 1–3), and also between developmental pattern of anticipatory activity and mutation (Fig. 4). Developmental pattern of anticipatory among group differences were tested by Student's *t*-test.

3. Results

3.1. RF under a light–dark cycle

Upper panel of each figure shows the representative double-plotted actograms for each experimental group. In order to observe the difference of spontaneous activity level in details among wild-type and *mCry*-deficient mice, the activity