The circadian clock and behavior

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Abstract

Temporal organization is a fundamental feature of all living systems. Timing is essential for development, growth and differentiation and in the mature organism, it is essential to maintain normal physiology and behavior. The biological entity that permits an organism's day/night organization is the circadian system. In the following, we describe how daily or circadian activity is measured in mice, and what such activity measurements can tell us about the state of the animal. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

Activity in rodents is evaluated either by monitoring general movement (e.g., with photosensors), or by measuring wheel-running activity. Assessment by photosensors or infrared sensors registers general activity including movement that occurs during feeding or grooming. In contrast, wheel-running activity represents only intended running of the animal and therefore provides activity patterns lacking background activity. This is one of the reasons, why this type of activity measurement is preferred over infrared monitoring. In the following, we will focus only on wheel-running activity and describe general protocols.

In our wheel-running experiments, animals are housed individually in transparent plastic cages (280 mm long × 105 mm wide × 125 mm high, Techniplast 1155M) that are equipped with a steel running wheel of 115 mm in diameter. The axis of the wheel is equipped with a magnet that opens and closes a magnetic switch upon rotation of the running wheel. The switch is hooked up to a computer that counts the revolutions of the wheel. Twelve cages are placed in a black, light-tight box, which is ventilated and containing fluorescent light that can be controlled via a timer from the outside. The box is placed in a room that is controlled for temperature, humidity, light and free of noise and vibrations. If environmental noise is not well under control, a constant background sound (white noise) should be installed to drown any outside noise, e.g., traffic or trains. Living conditions that lead to disease can be readily observed. Outbursts of viral infections are reflected in daily wheel-running activity. After the viral burst, the circadian activity returns to the normal nocturnal activity until the next viral burst occurs. Hence, abnormal activity patterns can be a result of infections.

Wheel-running activity in mice is measured under a standard light–dark (LD) cycle that consists of 12 h of light (we use 500 lux, but 50 lux is sufficient, light intensity is measured with a lux-meter, Testo 0500, Lenzkirch, Germany) and 12 h of darkness, e.g., lights on 07:00, lights off 19:00. Activity recording is usually started just before lights off (e.g., 19:00) when mice are at the beginning of their activity phase. Hyperactivity due to the placement in a novel environment (arousal) has then a minimal effect on the animals clock [3]. It is important that at this point the data transmission is checked to correct problems that occur with data acquisition. If adjustments are necessary later on in the experiment, it is advisable to do them just before lights off, e.g., 19:00. This is also the guideline for food and
water supply, which is necessary every 3–4 weeks. Important to note here is that the cage and the wheel should not be replaced by a new cage, because a new wheel is a novel stimulus that can phase-shift the clock [5]. After the animals have been held in the LD 12/12 cycle for at least 10 days, they are ‘entrained’ and adjusted to this particular LD cycle and the measurements from that point on are reliable. These animals are still under the daily influence of light and therefore can use the light as a Zeitgeber or timegiver. Measurements under these conditions are determined by the Zeitgeber time (ZT), where ZT0 is lights on. The specification of the LD cycle determines the ZT for lights off, e.g., ZT12 in an LD 12/12 cycle. Animals held under these conditions can also be used for molecular analysis. Good markers for the circadian clock are the mPer genes [1,7], which are expressed in a diurnal manner and remain expressed in a circadian manner under constant conditions (see next paragraph).

To investigate the circadian clock, mice have to be held under constant darkness conditions. Their clock is under constant conditions (see next paragraph). mPer analysis. Good markers for the circadian clock are the genes [1,7], which are expressed in a diurnal manner and remain expressed in a circadian manner under constant conditions (see next paragraph).

![Fig. 1](image_url)

Fig. 1. (a) Wheel-running activity of WT and NOS1−/− mice before and after a 15 min light pulse. Mice were entrained for 10 days to an LD 12/12 cycle and then released into constant darkness (DD). Shown are 10 days of activity in DD before a light pulse was applied at CT14 (top arrow) or at CT22 (bottom arrow), respectively. The vertical grey lines indicate activity onset. The light pulse at CT14 causes a delay of wheel-running activity (vertical line moves to the right), whereas a light pulse at CT22 causes onset of activity at earlier times (vertical line shifted to the left). WT and NOS1−/− mice did not behave differently in response to a light pulse, but wheel-running activity was more fragmented in NOS1−/− mice. (b) Induction of mPer1 and mPer2 in NOS1−/− mice by a light pulse. NOS1−/− and WT mice were exposed to 500 lux of light for 15 min at ZT14 and ZT22, respectively. Induction of mPer1 and mPer2 after 1 h in NOS1−/− animals was comparable to WT animals indicating that lack of NOS1 has no influence on mPer gene light inducibility. Induction of mPer1 and mPer2 in WT animals at ZT14 is similar to mPer1 induction at ZT22. mPer2 is not inducible by light at ZT22 [1].

left undisturbed for 28 days in DD. For example, an mPer2 mutant animal can take 14–20 days until its circadian rhythm is lost [9]. After the period length has been determined, the circadian times (CT) for an animal are defined and time measurements under DD conditions are described by CT. For example, CT24 is at the end of the period of the animals clock, hence, after 23.8 h for a WT animal or after 22 h for an mPer2 mutant animal. Times given in CT indicate constant darkness conditions and are strictly speaking animal specific. Given that the individual variation in tau for a strain is not very large, the CT is based on the average tau of that strain and can be used for all individuals. Strains that immediately lose circadian rhythmicity in DD, like the mPer1/mPer2 double mutants [8] cannot be temporally defined by a CT, because no tau can be determined.

Phase shifts are the consequence of disturbance of the clock by an external signal, such as light, noise or providing a new running wheel. To study the effects of light on the phase shifting of the circadian clock, light pulses are applied to the animals. We apply a light pulse that has a duration of 15 min and an intensity of 500 lux. The animals that are investigated must establish a clear stable rhythm in DD for 10–12 days before a light pulse is applied. Animals that become arrhythmic in DD have to establish a stable rhythm in LD. A light pulse has only an effect on the clock if it is applied in the activity phase of the mouse, i.e., between CT12 and CT24 (subjective night) or ZT12 and ZT24, respectively [3]. To evoke a phase delay of the clock, the light pulse must be applied in the early night (between CT12 and CT18–20). For phase advances, the light pulse is applied in the late night (between CT18–20 and CT24). These are just approximate CT values, because they...
vary depending on the mouse strain [2,6]. After the light pulse, the animals are left in DD. Phase delays can already be recognized 1 day after the light pulse has occurred, but phase advances take up to 6 days to be recognizable and they are smaller than phase delays. At least seven consecutive days have to be measured to determine the amount of the phase advance or delay, respectively. Fig. 1 shows that NOS1 knock-out animals behave like WT animals in clock resetting (3, respectively. Fig. 1 shows that NOS1 knock-out animals behave like WT animals in clock resetting (4, Fig. 1(a)). In molecular terms, NOS1 mals behave like WT animals in clock resetting (5, respectively. Fig. 1 shows that NOS1 knock-out animals behave like WT animals in clock resetting (6, respectively. Fig. 1 shows that NOS1 knock-out animals behave like WT animals in clock resetting (7, respectively. Fig. 1 shows that NOS1 knock-out animals behave like WT animals in clock resetting (8, respectively. Fig. 1 shows that NOS1 knock-out animals behave like WT animals in clock resetting (9, respectively. Fig. 1 shows that NOS1 knock-out animals behave like WT animals in clock resetting.

The mPer1 and mPer2 genes are important components of the mammalian circadian clock [1,7–9]. Disturbance of the circadian clock can lead to a derailment of an organism’s physiology. To illustrate this point, we examined the transcriptional regulation of the clock-controlled vasopressin prepropressophysin in the suprachiasmatic nucleus (SCN). We found that the vasopressin mRNA rhythm was severely blunted in the SCN of mPer2 mutant and in mPer1/2 double mutant animals, but not in mPer1 mutants (Fig. 2). This indicates that vasopressin mRNA is positively regulated by mPer2, but not by mPer1. Vasopressin is well known for its peripheral effects on salt and water balance and has a number of distinct actions within the central nervous system. Therefore, a number of physiological abnormalities can be expected in mPer2 and mPer1/2 mutants. This clearly shows that in a WT animal the physiological state of the organism is not constant throughout the day. We recommend that behavioral studies are accompanied by a ZT with the corresponding LD cycle to ensure reproducibility of behavioral measurements.

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**References**