Physiology & Behavior 130 (2014) 66–74

Contents lists available at ScienceDirect

Physiology & Behavior

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

Dual control of seasonal time keeping in male and female juvenile European hamsters ☆

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HIGHLIGHTS

- Untypical for a circannual species, European hamsters have several litters per year.
- How offspring integrates at the proper position in the annual cycle was studied.
- Pups adopt the appropriate seasonal activity pattern of adults at an age of 78 days.
- The timing of puberty and the excretion of aMT6s depended on the season of birth.
- A dual control ensures proper seasonal short-term and long-term timing.

ARTICLE INFO

Article history:
Received 10 February 2014
Accepted 13 March 2014
Available online 26 March 2014

Keywords:
Ontogeny
Activity
aMT6s
Circannual
Cricetus cricetus
Seasonal entrainment

ABSTRACT

In contrast to photoperiodic rodent species, adult circannual European hamsters (*Cricetus cricetus*) do not rely on melatonin as transducer of the photoperiodic message. Instead, seasonal entrainment involves a special circadian organisation which characterizes a photoperiod-sensitive phase. When days shorten a precise activity pattern ("summer pattern") switches to a weak or arrhythmic "winter pattern". At the very same day gonadal regression is initiated and the circannual clock is reset. In contrast to this difference in photoperiodic time measurement, the broad time span in which offspring are born and the birth-season dependent timing of puberty is similar to photoperiodic rodents. We investigated how juvenile European hamsters measure photoperiod to situate themselves at the proper position in the annual cycle. Activity and 6-sulphatoxymelatonin (aMT6s) excretion were recorded in pups of five litters born at different seasons. Pups of all litters showed an activity pattern identical with the adults' summer pattern until postnatal day 78, suggesting that the pathway known to reset the circannual clock in adults is functional. The synchronous start of reproduction in yearlings supports this. However, since puberty and gonadal regression occurred before the switch in the activity pattern, the timing of reproduction in the birth year must be controlled by other means. As in photoperiodic species melatonin might be involved, since the aMT6s excretion showed daily and seasonal rhythms from early life on.

1. Introduction

In seasonally varying environments many mammalian species show seasonal rhythms of reproductive competence and quiescence so that the offspring are born at the most favourable seasons. The main *zeitgeber* (timeteller) ensuring proper synchronisation to the natural year are the reliable seasonal changes in photoperiod [1–3]. Two fundamentally different strategies have evolved for processing photoperiodic information.

In photoperiodic species the changes in photoperiod directly drive the seasonal changes between reproduction and sexual quiescence. For example, in Syrian hamsters (*Mesocricetus auratus*) or Siberian hamsters (*Phodopus sungorus*) a shortening of photoperiod induces gonadal atrophy and its lengthening gonadal development [4–6]. Photoperiodic species are unable to complete such a cycle without photoperiodic input, while circannual species show repetitive complete physiological cycles in constant conditions. In the latter, changes in photoperiod entrain an endogenous circannual clock, which then drives the reproductive rhythm with a period length of ca. 1 year [7–9]. Furthermore, the photoperiodic signal resetting the circannual clock is only effective during certain phases of the circannual cycle [10–13]. In the European hamster (*Cricetus cricetus*), a true circannual species [14], such a phase is the so-called sensitive phase to short photoperiod from mid May to

☆ We dedicate this article to the memory of Franziska Wollnik, who passed away recently. She greatly inspired our research on ontogeny and seasonal rhythms in European hamsters and will be deeply missed as a colleague and friend.

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http://dx.doi.org/10.1016/j.physbeh.2014.03.020
0031-9384/

mid July [15]. During this phase the animals "wait" for a shortening of the photoperiod below the critical value of 15.5 h (at 48°N) [16] which occurs naturally in mid July when it induces gonadal regression [15,17] and resets the circannual clock [13].

Another difference between photoperiodic species and the circannual European hamster is that in the former the photoperiodic message is predominantly transduced by the nocturnal peak of the pineal hormone melatonin [18–22] whose duration varies with the night length [23–25]. In European hamsters, however, melatonin is not necessary for entrainment [26] albeit not completely ineffective as a photoperiodic agent [27].

While most photoperiodic species show a severe impairment of seasonal entrainment after pinealectomy [28,19,22,29] most pinealectomized circannual species [30–34] including European hamsters [26] entrain without any problem to changes in photoperiod. European hamsters show extreme seasonal changes in the nightly melatonin peak [35,36] with such low levels during the short nights between mid May and mid July, that – especially in males – no nightly elevation is detectable [17]. Thus, in natural conditions, a melatonin peak is lacking exactly when photoperiodic information needs to be transported towards the circannual clock. European hamsters thus use predominantly a melatonin-independent pathway to transduce photoperiodic information [26].

This melatonin-independent pathway is reflected in seasonal changes of the activity pattern. During most parts of the year European hamsters show a weak or even arrhythmic activity pattern (referred to as "winter pattern"). Between mid May and mid July, however, i.e. during the sensitive phase to short photoperiods [37,17], the rhythm becomes robust, the activity onset precise, the phase angle of entrainment positive (the activity starts before sunset), the activity duration shorter and the activity amounts elevated [37,17]. This special circadian organisation will in the following be referred to as "summer pattern". The early and precise activity onset is needed as stable reference point to detect the tiny daily advances of sunset after the summer solstice [38] in mid July [15], when photoperiod has decreased only by half an hour from maximal values. At the very same day the activity pattern switches back to the weak "winter pattern", gonadal atrophy is initiated [38], and the circannual clock is reset [13]. Thus in the European hamster, the photoperiodic resetting of the circannual clock is based on a uniquely tight interaction of behaviour and physiology.

Most mammalian circannual species such as deer, sheep or wolves usually have offspring only once a year [3]. The timing of puberty and the initial setting of the circannual clock may thus follow a fixed programme. The European hamster is a circannual species [14], but a female might have up to three litters a year [39–41] which consequently are born at different seasons. This is similar to other rodents (for review see [42]), though they are predominantly photoperiodic species. For most of them the age at which puberty sets in depends on the photoperiod in which the animals are born [43–48]. This applies also to pups of the circannual European hamster [49].

While during juvenile development European hamsters show great similarities to photoperiodic species, the photoperiodic time measurement in adults profoundly differs. That raises the question, how juvenile European hamsters process photoperiodic information early in life and how they integrate in the annual cycle. Thus, this study searches for characteristic elements of the melatonin-dependent and the melatonin-independent pathway in juvenile European hamsters born at different seasons. For this purpose activity, urinary aMT6s excretion (6-sulphatoxymelatonin, the main catabolite of melatonin) and the reproductive state were analysed in pups of five asynchronously born litters of European hamsters.

2. Materials and methods

The studies were performed in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and German law and were approved by the proper authorities (Regierungspraesidium Stuttgart).

2.1. Animals

A total of 34 European hamster pups (18 males and 16 females) of five litters born at different seasons between April 7 and July 26 were investigated (Table 1). They were born and raised in our breeding colony in Stuttgart (latitude 48.46°N) and were maintained under constant temperature (18 ± 2 °C) and humidity (55 ± 5%), but natural light conditions (LDnat) with twilights and gradual changes of photoperiod provided by a clear window along the upper part of the rooms south side. Light intensity varied with season, time and weather and was at daytime between ca. 100 and 500 lx at cage level. After weaning at postnatal day (PD) 20–23, the animals were housed individually in Macrolon cages (type IV, 35 × 57 cm, Becker, Castrop Rauxel, Germany) equipped with a nest box and a running wheel (diameter 35 cm, width 10 cm). Food (Altromin 1314, Lage) and tap water were given ad libitum. Additionally, the animals got a daily piece of fruit and vegetable flakes (Matzinger) until PD 50.

A group of yearlings (11 males and 21 females) in which the onset of the reproductive phase was followed were kept under identical conditions.

2.2. Experimental design

2.2.1. Reproductive state

In the yearlings the reproductive state was checked every three weeks in a short Enflurane flush (Ethrane, Abbot). However, young juveniles were too nervous so that anaesthesia and repeated handling to check gonads regularly would have lead to profound disturbances in the activity pattern (our main interest). To avoid this, the reproductive state of the animals was initially checked only when a strong increase in body weight (data not shown) suggested that the animals became reproductive or at the latest when they were two months old. Thus the accurateness of the date of gonadal development was limited. Henceforth, the reproductive state was checked every 2–3 weeks. The animals were considered reproductive when scrotal testes of ≥1.8 cm were found, since then testosterone levels are at the maximum [50], or when the vagina was completely open. Scrotal testes of smaller size or a partially open vagina were considered as developing or regressing gonads. Sexual quiescence was defined as the time when testes were completely vanished in the abdomen or when the vagina was completely closed.

2.2.2. Activity recording

To follow age related changes in the 24 h activity rhythm, the running-wheel activity of the European hamster pups was registered from weaning until PD 140. This record was only interrupted by four 24 h-urine collections at four different age classes (PD 26–32, PD 36–38, PD 47–51 and PD 86–124, see below). The day after the urine collection was excluded from the activity analysis. A magnetic reed switch mounted on the wheel axle detected the revolutions. These impulses

Table 1
Investigated animals. The number of males in which not only the activity pattern but also the urinary excretion of aMT6s was analysed is indicated in parentheses. *PP* stands for photoperiod and *LD* for the ratio of the light phase to the dark phase.

Litter number	Date of birth	Number of pups		~PP at birth date
		Males	Females	
L18	07.04.2000	2 (2)	2	LD13:11 increasing
L19	22.05.2000	4 (4)	3	LD15.5:8.5 increasing
L20	05.06.2000	4	4	LD16:08 increasing
L21	19.06.2000	5 (4)	3	LD16:08 stable
L24	26.07.2000	3 (3)	4	LD15.5:8.5 decreasing
	Total	18 (13)	16	