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Seasonal Molecular Timekeeping Within the Rat Circadian Clock

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Summary

In temperate zones duration of daylight, i.e. photoperiod, changes with the seasons. The changing photoperiod affects animal as well as human physiology. All mammals exhibit circadian rhythms and a circadian clock controlling the rhythms is located in the suprachiasmatic nucleus (SCN) of the hypothalamus. The SCN consists of two parts differing morphologically and functionally, namely of the ventrolateral (VL) and the dorsomedial (DM). Many aspects of SCN-driven rhythmicity are affected by the photoperiod. The aim of the present overview is to summarize data about the effect of the photoperiod on the molecular timekeeping mechanism in the rat SCN, especially the effect on core clock genes, clock-controlled genes and clock-related genes expression. The summarized data indicate that the photoperiod affects i) clock-driven rhythm in photoinduction of c-fos gene and its protein product within the VL SCN, ii) clock-driven spontaneous rhythms in clock-controlled, i.e. arginine-vasopressin, and in clock-related, i.e. c-fos, gene expression within the DM SCN, and iii) the core clockwork mechanism within the rat SCN. Hence, the whole central timekeeping mechanism within the rat circadian clock measures not only the daytime but also the time of the year, i.e. the actual season.

Key words
Suprachiasmatic nucleus • Rat • Photoperiod

Introduction

In temperate zones, daylength, i.e. the photoperiod, changes during the course of the year. The changes in the photoperiod affect mammalian and marginally even human behavior and physiology. Animals prepare for a forthcoming season by modulating their fur, feeding, reproductive, metabolic and locomotor activity, etc. In nocturnal rodents, the duration of locomotor activity reflects the photoperiod as being shorter on long summer than on short winter days (Puchalski and Lynch 1991, Elliot and Tamarkin 1994). The rhythmic production of the pineal hormone melatonin which is a part of the timekeeping system is also highly photoperiod-dependent, both in nocturnal as well as in diurnal animals; the high nocturnal melatonin production is shorter on long summer than on short winter days (Illnerová and Vaněček 1980, Illnerová 1988, 1991). In humans, a shortening of daylight in the fall together with a decrease in outdoor light intensity is often accompanied by fatigue and seasonal affective disorder (Terman *et al.* 1989). Human physiology may apparently also adapt to changes in daylength if human subjects experience natural photoperiods; under the long summer

days the melatonin signal may be shorter similarly as is the case with other mammals (Vondrašová *et al.* 1997, Vondrašová-Jelínková *et al.* 1999). Thus, the photoperiod may affect significantly the mammalian timekeeping system.

In mammals, light is perceived solely by the retina (Moore 1996). Besides cones and rods mediating vision, cells in the deep retinal ganglion layer containing photopigment melanopsin appear to be involved in light perception as well (Provencio *et al.* 1998, Berson *et al.* 2002, Menaker 2003). The ganglion cells may mediate the "circadian vision", i.e. inform the circadian system about outdoor lighting conditions in order to keep the endogenous system in proper phase with the outside world. The information about light is transduced to the principal generator of circadian rhythmicity in the mammalian body located within the suprachiasmatic nuclei (SCN) of the hypothalamus. The connection between the retina and the SCN is performed predominantly through a monosynaptic retinohypothalamic tract or, less extensively, through a polysynaptic geniculohypothalamic tract (Harrington and Rusak 1986).

In a non-periodic environment, e.g. under constant darkness, the SCN is able to generate synchronous oscillations with a period close, but not equal to 24 h. The actual length of the endogenous period is genetically given and is species specific. When dissociated in an *in vitro* culture, most of the SCN cells are able to generate independent rhythmic output with different period lengths (Welsh *et al.* 1995). Moreover, individual SCN cells differ in their morphological parameters, peptidergic phenotypes and in function. Whereas cells located predominantly in the ventrolateral (VL) part receive photic information from the retina and express light-dependent rhythmicity, cells located prevalently in the dorsomedial (DM) part do not receive any direct retinal input and are spontaneously rhythmic (Van den Pol 1991, Sumová *et al.* 1998, Guido *et al.* 1999a,b, Schwartz *et al.* 2000). Thus, communication not only between individual SCN cells but also between both parts of the SCN is important for the integration of all rhythmicity into the resulting output. The output rhythmic signal is transduced *via* humoral and neuronal pathways and controls rhythms in peripheral tissues. Cells of peripheral tissues are able, to some extent, to produce local oscillations independent of the SCN (Balsalobre *et al.* 2000, Yamazaki *et al.* 2000, Stokkan *et al.* 2001, McNamara *et al.* 2001, Terazono *et al.* 2003).

The basic molecular core clock mechanism responsible for generation of rhythmicity within the SCN and peripheral rhythmic cells became to be partly elucidated by the cloning of mammalian clock genes (Tei *et al.* 1997, Shearman *et al.* 1997, Sun *et al.* 1997, Gekakis *et al.* 1998, Kume *et al.* 1999). Eight genes, namely three Per (Per1,2,3), two Cry (Cry1, Cry2), Clock, Bmal1 and casein kinase 1 epsilon (CK1ε), are thought to be involved in interactive transcriptional-translational feedback loops that form the basic molecular clockwork (for review see King and Takahashi 2000, Reppert and Weaver 2001). Briefly, the protein products CLOCK and BMAL1 heterodimers positively activate rhythmic expression of Per and Cry genes and their protein products PER and CRY are produced thereafter. In cytoplasm, the PER and CRY proteins form complexes important for nuclear translocation of both proteins; the phosphorylation of PER protein monomers by CK1ε may also regulate their cellular location and stability. After shuttling into the nucleus, the PER:CRY complexes directly interact with the CLOCK:BMAL1 heterodimer and *via* chromatin remodeling inhibit the CLOCK:BMAL1 mediated transcription (Etchegaray *et al.* 2003). Moreover, BMAL1 is negatively autoregulated, and CRY1, CRY2 and PER2 proteins positively activate the Bmal1 transcription (Bae *et al.* 2001, Zheng *et al.* 2001, Yu *et al.* 2002). The regulation of Bmal1 transcription is likely mediated by REV-ERBα, a protein product of gene Rev-erbα that is controlled by CLOCK:BMAL1 (Preitner *et al.* 2002). Apparently, the rhythm generating mechanism is very complex and many processes which fine-tune the "orchestra" are expected to be revealed in the near future. To convey the rhythmic signal to periphery, the components of the core clock mechanism serve as transcription factors switching on the rhythmic transcription of a great array of clock controlled genes within the SCN and peripheral tissues (Jin *et al.* 1999, Oishi *et al.* 2000).

Even less is known about the mechanism involved in how light resets the self-oscillating molecular clockwork. Under light-dark conditions, the circadian clock is entrained by the light period of the day (Pittendrigh 1981). Using the clock controlled rhythmicity as a marker, it was shown that a light stimulus administered in the evening and early night delays, and a light stimulus administered in the late night and early morning advances the rhythmicity (Daan and Pittendrigh 1976, Illnerová and Vaněček 1982, 1987, Sumová and Illnerová 1998). Entrainment keeps the endogenous clock in proper phase with the light-dark

cycle of the outside world. Information about light is conveyed to retinorecipient SCN cells and mostly glutamate *via* glutamatergic NMDA and non-NMDA receptors induces different cascades of second messengers in dependence on the time of day (Ebling *et al.* 1991, Ding *et al.* 1994). Consequently, third messengers, namely phosphorylated CREB, are activated. However, the link connecting the third messenger activation and molecular core clock mechanism is still not completely understood. Probably, induction of light-sensitive clock genes Per1 and Per2 may proceed through CRE elements of their promotors (Trávníčková-Bendová *et al.* 2002, Tischkau *et al.* 2003). Even less is known about the mechanism of how the induced Per1 and Per2 mRNAs are involved in resetting the aforementioned molecular clockwork. Moreover, in mice phase shifts of output rhythms occur only when light-induced Per1 and Per2 expression spreads from the VL to the DM parts of the SCN (Yan and Silver 2002). As production of PER1 protein is delayed by several hours relative to the light-induced transcription of Per1 mRNA and the production is increased only by light of a substantially long duration (Hastings *et al.* 1999, Field *et al.* 2000), the actual role of the protein in the instantaneous resetting by short-lasting light pulses during the night remains unclear.

As already mentioned, the duration of daylight may vary substantially with the season of the year, e.g. at the latitude 50 ^{o}N, the duration of daylight in summer is double that in winter. The aim of this overview is to summarize data about the effect of the photoperiod on the molecular processes in the rat SCN, i.e. on expression of clock genes, clock-controlled genes and clock-related genes. Special attention is paid to distinct responses within the VL and DM parts of the nucleus. Rats are chosen as model animals because they are not, as with humans, considered to be truly photoperiodic and their circadian period length is similar to that in humans.

Photoperiod and clock-driven molecular mechanisms in the rat ventrolateral SCN

Photic stimuli induce rapid expression of immediate early genes, namely c-fos, jun-B etc. (Kornhauser *et al.* 1992, 1993). The most intensively studied c-fos encodes a sequence specific DNA-binding protein cFos that affects the expression of "late response" target genes by regulating their transcription. Levels of c-fos mRNA and cFos protein immunoreactivity (cFos-ir) are dramatically elevated by light in the retinorecipient VL part of the rat SCN but not in the DM SCN (Jáč *et al.* 2000b). Importantly, this photic stimulation is phase-dependent and hence the circadian clock controls the light-induced expression of c-fos. The pathway by which the molecular clockwork mediates gating of the photic response is, however, unclear. During the subjective night, the clock-controlled gate is open and light stimuli evoke high c-fos expression in the VL SCN as well as phase shifts of overt rhythms. During the subjective day, the gate is closed and light stimuli evoke neither c-fos expression nor shifts of the overt rhythms. The time interval of high photoinduction of c-fos mRNA and cFos-ir may thus serve as a SCN marker of duration of the subjective night (Sumová *et al.* 1995b, Trávníčková *et al.* 1996, Illnerová *et al*. 2000). Though it appears that circadian oscillations in the rat SCN start prenatally (Reppert and Schwartz 1984, Reppert and Uhl 1987, Shibata and Moore 1987), the rhythm in cFos photoinduction in the VL SCN develops only postnatally; this was observed in 10-day-old but not yet in 3-day-old rat pups (Bendová *et al.* 2004). Until at least 3 days of age, light induced high cFos-ir at any daytime and duration of the subjective night was thus not defined at this early stage.

In adult rats entrained to a long photoperiod with 16 h of light and 8 h of darkness per day (LD16:8) and then released into darkness, the interval permitting high light induced c-fos expression and cFos-ir was significantly shorter than in those entrained to a short photoperiod with 8 h of light and 16 h of darkness (LD8:16) (Sumová *et al.* 1995b). The molecular clock-controlled mechanism in the VL SCN was thus affected by the length of the previous photoperiod and the duration of subjective night was adjusted accordingly (Illnerová *et al*. 2000, Jelínková *et al.* 2000). Apparently, under long days, light intruding late into evening hours delayed slightly the evening rise in c-fos photoinduction, whereas light intruding into the early morning hours phase advanced markedly the morning decline. As a result, under the long photoperiod the interval permitting high c-fos photoinduction was compressed by about 5 h compared to that under the short photoperiod. Importantly, the compression was not due to any acute masking effect of light but rather true entrainment of the molecular clockwork. The interval enabling phase shifting the rhythm in cFos photoinduction by light pulses was shortened under the long photoperiod as well (Sumová and Illnerová 1998). After transferring rats from the long to the short photoperiod, it took about two weeks

before the compressed waveform of the rhythm in cFos photoinduction in the VL SCN fully decompressed (Sumová *et al*. 1995a). During the postnatal development, the photoperiod started to modulate the interval permitting high cFos photoinduction at 10 days of age, but even at this age the interval difference between the short and the long photoperiod did not yet attain that in adult animals (Bendová *et al.* 2004).

All the above mentioned data indicate that the functional state of the circadian clock within the rat VL SCN is affected by the photoperiod.

Photoperiod and clock-driven molecular mechanisms in the rat dorsomedial SCN

Whereas the cells of the VL part of SCN exhibit mostly light-dependent rhythms, the cells of the DM part are spontaneously rhythmic. One of the rhythms specific for the DM SCN is the rhythmic expression in arginine vasopressin (AVP) mRNA and peptide production (Cagampang *et al.* 1994). It has been proved that this rhythm is under the control of the transcriptional machinery of the core clockwork as the same elements that drive the core feedback loops control expression of the AVP gene (Jin *et al.* 1999). Another rhythm specific for the DM SCN of the rat (Sumová *et al.* 1998, Guido *et al.* 1999a) as well as the hamster (Guido *et al.* 1999b) is the rhythmic expression of c-fos and production of the protein product cFos. Importantly, the latter rhythm persists in constant darkness for at least two cycles, i.e. it is spontaneous and independent of light induction in contrast to the rhythm in c-fos photoinduction in the VL SCN. The spontaneous cFos rhythm may thus represent the expression of molecular clock-driven rhythmic intrinsic neuronal activity (Sumová *et al.* 1998). The rhythm is already expressed in 3-day-old rat pups with the same amplitude of oscillation as in adult animals (Bendová *et al.* 2004). In contrast to the rhythm in AVP expression, no link connecting the core clock machinery to the spontaneous c-fos expression has so far been uncovered. Therefore, c-fos should be considered rather a clock-related than a clock-controlled gene.

Both the above mentioned DM SCN rhythms are photoperiod-dependent. Under a long, LD16:8, photoperiod the morning rise in AVP mRNA expression and cFos-ir occurred significantly earlier than under a short, LD8:16, photoperiod. As the evening decline in AVP expression and cFos production occurred at about the same time under both photoperiods, the interval of high AVP mRNA expression and cFos-ir was longer by several hours under the long than under the short photoperiod (Jáč *et al.* 2000a, Sumová *et al.* 2000). Even under a light-dark cycle, extension of the interval of elevated cFos-ir was not due to an earlier light exposure under long days as during the morning hours light did not elevate cFos-ir in the DM SCN (Jáč *et al.* 2000b). Both the AVP mRNA and cFos-ir rhythms thus may serve as light-independent markers of the duration of the subjective day (Illnerová *et al*. 2000). Dependency of the cFos-ir rhythm on the photoperiod appears to develop slower in the DM than in the VL part of the SCN; even 10 days after birth the photoperiod did not yet affect duration of the interval of spontaneous cFos-ir (Bendová *et al.* 2004). The adjustment of timing of the morning rise in cFos-ir to a transfer from a long to a short photoperiod was roughly accomplished only within 3-6 days, whereas a fine adjustment continued even after 2 weeks following the transfer (Sumová *et al.* 2000). This finding might suggest the existence of a not yet recognized link between core clock mechanism and the rhythm in spontaneous cFos-ir. Moreover, as rhythmicity in both parts of the SCN is affected by a previous photoperiod in a similar manner, an internal processing of the entraining photoperiodic signal between the VL and the DM part of the rat SCN is highly plausible.

Photoperiod and core-clock mechanism in both parts of the rat SCN

All data summarized in the two previous chapters suggest that the photoperiod affects the molecular core clock transcriptional-translational machinery underlying oscillations in the SCN cells. Decoding the mechanism on how the clockwork processes information about photoperiod may show how the circadian clock measuring daily time serves also as seasonal timer in the mammalian body. Moreover, revealing the mechanism might help in our understanding of the photic resetting of the molecular clockwork.

Studies using Siberian and Syrian hamsters and mice have indicated that the photoperiod modulates expression of light-sensitive Per genes and levels of their protein products in the SCN (Messager *et al.* 2000, Nuesslein-Hildesheim *et al.* 2000, Tournier *et al.* 2003, Steinlechner *et al.* 2002). Similarly, in the rat SCN the PER1-ir (Sumová *et al.* 2002a,b) and Per1 mRNA expression (Sumová *et al.* 2003) are photoperiod-dependent. In contrast to the other species, in rats the

photoperiodic modulation was demonstrated under the conditions excluding any masking effect of light because after entrainment to photoperiod rats were released into constant darkness. The spontaneous daytime rise in Per1 mRNA expression and PER1-ir occurred earlier under a long than under a short photoperiod and, consequently, the interval of high Per1 mRNA expression and PER1-ir was significantly extended in long days (Fig. 1a,f and b,g, respectively). Moreover, the study in rats has revealed that not only the light-sensitive but also the light-insensitive clock genes expression is affected by the photoperiod (Sumová *et al.* 2003). The interval of high night Bmal1 mRNA expression was longer under a short than under a long photoperiod (Fig. 1d,i). The waveform of the rhythm remained in antiphase to the rhythm in Per1 mRNA be it under a long or a short photoperiod similar to the case in constant darkness. Thus, under a long photoperiod the high daytime Per1 mRNA expression was lengthened and the high nighttime Bmal1 expression shortened as compared to a short photoperiod. The change in the timing of Bmal1 mRNA expression might be related to a change in PER2 production that has also been shown to be modulated by the photoperiod (Nuesslein-Hildesheim *et al.* 2000, Lee *et al.* 2001, Shearman *et al.* 2000). Expression of another light-insensitive clock gene Cry1 was affected by the photoperiod as well but in a different way. Under a long photoperiod, both the rise and decline in Cry1 mRNA expression were advanced by about 4 h as compared to those under a short photoperiod and therefore the interval of high Cry1 expression did not change (Fig. 1c,h). The obvious consequence of the different effect of the photoperiod on the waveform of the rhythm in Per1 and Cry1 mRNAs expression was a distortion in contemporaneous availability of their protein products that is a prerequisite for forming heterodimers fulfilling the role of negative elements in the transcriptional machinery (Hastings 2001). Assuming that translation of CRY1 protein starts soon after beginning of its mRNA transcription (Reppert and Weaver 2001), we may hypothesize that in long days the interval when both proteins are produced together might be significantly shortened due to an earlier decline in Cry1 transcription (Fig. 1c,h). Similar dissociation of Per and Cry expression has been reported during a circadian resetting in mice subjected to an advance of a light-dark cycle (Ready *et al.* 2002) and in Syrian hamsters kept under LD14:10 (Tournier *et al.* 2003).

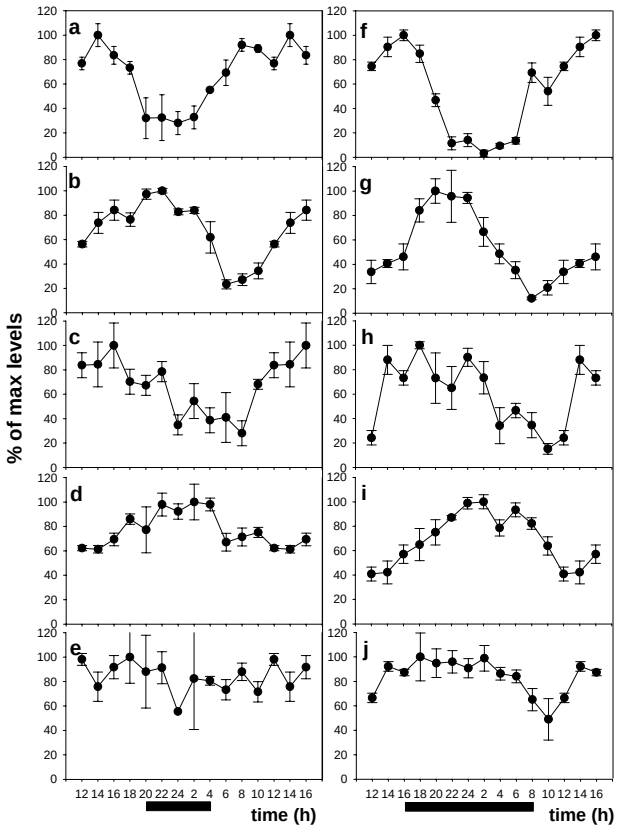

Fig. 1. Daily profiles of Per1 (a,f), Cry1 (c,h), Bmal1 (d,i), Clock (e,j) mRNA, and PER1-ir cells (b,g) in rats maintained either in LD16:8 (a-e) or in LD8:16 (f-j) and released into constant darkness. Data taken from Sumová *et al.* (2002a) and Sumová *et al.* (2003) represent a percentage of the maximal value. Solid bars indicate dark periods in LD16:8 and LD8:16, respectively.

The mechanism of linking the photoperiod-shaped waveform of the Per mRNA rhythm to a change in phasing of Cry expression is unknown. In the rat study, Per1, Cry1 and Bmal1 mRNA in the SCN oscillated with the higher amplitudes under a short than under a long photoperiod (Sumová *et al.* 2003). It is possible that dissociation in phasing between PER and CRY proteins under long days may account for the amplitude reduction. With the higher amplitude of rhythms in clock gene expression under short days, even the Clock mRNA was expressed in a rhythmic way (Fig. 1e,j, Tournier *et al.* 2003), although its non-rhythmic expression under constant darkness has been reported (Reppert and Weaver 2001). The amplitude of clock genes oscillations increases also during postnatal development: for Per1, Per2 and Bmal1 it was significantly higher in 10-day than in 3-day old rat pups (Sládek *et al.*, data to be published). The photoperiod affects clock gene expression not only in the rodent SCN but the ovine SCN as well (Lincoln *et al.*

2002). It is therefore obvious that information about the photoperiod is encoded in the core clockwork, namely in the waveform of the rhythmic expression of clock genes but also in the phasing and amplitude of the rhythms. Our next studies will be aimed at elucidating further inter-loop communication that underlies the basic mechanism of photic and photoperiodic entrainment.

Conclusions

Nocturnal rodents live under conditions where predicting the timing of natural daylight seems to be advantageous for their survival. In nature, they perceive information about daylight at limited time windows of the day, i.e. around sunset just after emerging from their burrows and/or around sunrise before they fall asleep. The circadian clock provides the animals with information about daytime so that they wake up at a proper time. The summarized data demonstrate that changes in the photoperiod affect the rat circadian clock at a molecular level. Photoperiodic modulation of the circadian clockwork provides thus the animals with information about the forthcoming season. Such information might be of importance also for humans if they lived in wild, while urban residents are only marginally photoperiodic.

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