

CIRCADIAN PROFILE AND PHOTIC REGULATION OF CLOCK GENES IN THE SUPRACHIASMATIC NUCLEUS OF A DIURNAL MAMMAL *ARVICANTHIS ANSORGEI*

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Abstract—The molecular mechanisms of the mammalian circadian clock located in the suprachiasmatic nucleus have been essentially studied in nocturnal species. Currently, it is not clear if the clockwork and the synchronizing mechanisms are similar between diurnal and nocturnal species. Here we investigated in a day-active rodent *Arvicanthis ansorgei*, some of the molecular mechanisms that participate in the generation of circadian rhythmicity and processing of photic signals. *In situ* hybridization was used to characterize circadian profiles of expression of *Per1*, *Per2*, *Cry2* and *Bmal1* in the suprachiasmatic nucleus of *A. ansorgei* housed in constant dim red light. All the clock genes studied showed a circadian expression. *Per1* and *Per2* mRNA increased during the subjective day and decreased during the subjective night. Also, *Bmal1* exhibited a circadian expression, but in anti-phase to that of *Per1*. The expression of *Cry2* displayed a circadian pattern, increasing during the late subjective day and decreasing during the late subjective night. We also obtained the phase responses to light for wheel-running rhythm and clock gene expression. At a behavioral level, light was able to induce phase shifts only during the subjective night, like in other diurnal and nocturnal species. At a molecular level, light pulse exposure during the night led to an up-regulation of *Per1* and *Per2* concomitant with a down-regulation of *Cry2* in the suprachiasmatic nucleus of *A. ansorgei*. In contrast, *Bmal1* expression was not affected by light pulses at the circadian times investigated.

This study demonstrates that light exposure during the subjective night has opposite effects on the expression of the clock genes *Per1* and *Per2* compared with that of *Cry2*. These differential effects can participate in photic resetting of the circadian clock. Our data also indicate that the molecular mechanisms underlying circadian rhythmicity and photic synchronization share clear similarities between diurnal and nocturnal mammals. © 2003 IBRO. Published by Elsevier Science Ltd. All rights reserved.

Key words: light, period genes, cryptochrome genes, circadian rhythms, diurnality.

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Abbreviations: CT, circadian time, N.S., nonsignificant; ROD, relative optical density.

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A considerable amount of physiological, biochemical and behavioral parameters display circadian fluctuations which are generated and maintained by an endogenous time-keeping system (Weaver, 1998). In mammals, the master circadian clock has been identified in the suprachiasmatic nucleus of the anterior hypothalamus (Moore and Eichler, 1972; Stephan and Zucker, 1972; Schwartz and Gainer, 1977; Inouye and Kawamura, 1979; Drucker-Colin et al., 1984), which is constituted by a population of autonomous single-cell circadian oscillators (Welsh et al., 1995). Recently, a model has been proposed to explain the core of the circadian clock, which consists of autoregulatory transcription-translation feedback loops (Shearman et al., 2000; Reppert and Weaver, 2001). This model involves a set of clock genes, including three *Period* genes (*Per1*, *Per2* and *Per3*), two *Cryptochrome* genes (*Cry1* and *Cry2*) and two genes coding for transcription factors, *Clock* and *Bmal1*. All these genes are expressed abundantly in the suprachiasmatic nucleus of nocturnal rodents (Albrecht et al., 1997; King et al., 1997; Shearman et al., 1997; Sun et al., 1997; Tei et al., 1997; Takumi et al., 1998; Gekakis et al., 1998). In the current model of the circadian clockwork, intercalating negative and positive feedback loops produce 24-h oscillations. CLOCK/BMAL1 heterodimers drive the transcription of *Per* and *Cry* genes (Reppert and Weaver, 2001). The CRY and PER proteins form complexes allowing their nuclear translocation. CRY can interact with CLOCK/BMAL1 to negatively regulate *Per* and *Cry* transcription while PER2 has been shown to positively regulate *Bmal1* transcription (Reppert and Weaver, 2001).

Besides the fact that circadian rhythmicity is endogenously generated, the circadian clock is also sensitive to external cues to ensure a proper synchronization to the timing of environmental cycles. It is well known that light is the most powerful stimulus able to reset the circadian clock, as demonstrated by analyzing the phase shifting effects of exposure to brief light pulses at different circadian times (DeCoursey, 1960; Daan and Pittendrigh, 1976). In nocturnal rodents, light exposure during the subjective night is susceptible to shift the phase of the clock, inducing behavioral phase shifts and also producing modifications in the expression of some of the clock genes within the suprachiasmatic nucleus. In addition to altered responses to light in *Per1* and *Per2* mutant mice (Albrecht et al., 2001), studies on photic induction of clock genes (Zylka et al., 1998; Yan et al., 1999; Miyake et al., 2000; Wilsbacher et al., 2001) and their inhibition with antisense oligonucleotides (Akiyama et al., 1999; Wakamatsu et al., 2001) indicate that a transient induction of *Per1* and *Per2*

may be a critical step in the photic synchronization of the clock in nocturnal rodents. Also, *Bmal1* RNA has been shown to be modulated by light in the rat suprachiasmatic nucleus (Abe et al., 1998). Moreover, incidental observations in mice suggest that the expression of *Cry2* could also be regulated by light (Okamura et al., 1999).

The functional properties of the mammalian circadian time-keeping system have been extensively studied in night-active rodents. Currently, there is only limited information in the literature about the molecular mechanisms that underlie the functioning of the circadian clock in day-active rodents. Recently, a daily fluctuation of *Per1* and *Per2* expression in the suprachiasmatic nucleus has been described in diurnal ground squirrels (*Spermophilus tridecemlineatus*) maintained under a light/dark cycle (Mrosovsky et al., 2001). Also, there is evidence that light exposure during the subjective night induces an increase of c-Fos immunoreactivity in the suprachiasmatic nucleus of the diurnal rodent *Arvicanthis niloticus*, when light also induces shifts in activity rhythms (Mahoney et al., 2001). There is, however, no information about the circadian expression of clock genes in day-active rodents and it remains to be clarified if the expression of these genes is modified by light exposure, as already demonstrated in nocturnal rodents.

Arvicanthis ansorgei is a rodent species that displays a diurnal pattern of wheel-running activity (Challet et al., 2002; Sloten et al., 2002). To further investigate the molecular mechanisms underlying circadian rhythmicity and processing of photic signals in diurnal mammals, we first analyzed circadian profiles of the clock genes *Per1*, *Per2*, *Bmal1* and *Cry2* in the suprachiasmatic nucleus of *A. ansorgei*. Second, we determined the phase responses to light at behavioral and molecular levels in that species.

EXPERIMENTAL PROCEDURES

Animals

Adult male and female *Arvicanthis ansorgei* obtained from our breeding colony in Strasbourg were used in the present experiments. The animals were genetically selected for general diurnal activity (Challet et al., 2002). All experiments were performed in accordance with the *Principles of Laboratory Animal Care* (NIH published 86-23, revised 1985) and the French national laws.

Animals were individually housed in Plexiglas cages equipped with Nalgene running wheels (9-cm wide × 34.5-cm diameter). The wheel-running rhythm was continuously recorded and the data were stored in 5-min time bins by Dataquest III (Mini-mitter Inc., Sunriver, OR, USA). Prior to the start of the experiments, the animals were maintained under a 12-h light/12-h dark cycle for 2–3 weeks, to establish the activity profiles. After this time, the animals were released into continuous dim red light conditions until the end of the experiments. During all the experimental procedures, food and water were available *ad libitum*.

Phase responses to light

In order to obtain the behavioral phase responses to light, 48 *A. ansorgei* were used. The baseline of the free-running rhythm was obtained during 20 days. The onset of the active period in diurnal species is defined as circadian time 00 (CT 00). This was estimated by fitting a line throughout the activity onsets of at least 10 consecutive cycles and extrapolated for one additional day. The animals were repeatedly scheduled to receive a light pulse at

different circadian times (CT 00, 02, 04, 06, 08, 10, 12, 14, 16, 18, 20 and 22). For exposure to light pulse, animals were removed from the recording room into an adjacent room. The animal cages were placed inside of a light pulse chamber. Then, animals were exposed to a 100 lux fluorescent white light during 1 h. After that time, the animals were sent back to the recording room to continue behavioral recording during at least 15 days, in order to determine the direction and magnitude of the elicited phase shifts.

As a control procedure, animals were transferred to the adjacent room, placed in the light pulse chamber during 1 h, but without exposure to any photic stimulus. Thereafter, the animals were returned to the recording room to determine the phase shifting effect of animal manipulation. Control animals were tested at three circadian times (CT 06, 14 and 22).

The magnitude and direction of the elicited phase shifts were estimated by linear regressions (Clocklab Software, Actimetrics, Evanston, IL, USA) considering 10 cycles before and 15 cycles after light pulse administration, excluding of these regressions the transitory cycles (most frequently, the first two cycles after light exposure). The phase shifts were calculated as the difference between the fitted lines (activity onsets) before and after light exposure. Finally, we obtained the phase response curve to light by plotting the elicited phase shifts as a function of the circadian phases when light pulses were delivered (Daan and Pittendrigh, 1976). The endogenous period (τ) was determined using a χ^2 periodogram (ClockLab) over the 10 days before and after treatment at CT 06, CT 14 or CT 22 in 12 light-pulsed and 12 control animals ($n=4$ per experimental group for a given CT).

In situ hybridization

In order to assess the profile of expression and photic induction of clock genes in the suprachiasmatic nucleus, 84 *A. ansorgei* were used for the *in situ* hybridization experiments. The animals were kept in the same conditions as described above, and the wheel-running rhythm was also recorded.

After obtaining basal recordings of wheel-running activity in constant dim red light conditions, CT 00 was estimated and the animals were randomly assigned to an experimental group. To obtain the circadian levels of each transcript, a first group of 42 animals was deeply anesthetized with isoflurane and decapitated in darkness at CT 01, 05, 09, 13, 17, 19 or 21 ($n=6$ for each circadian point). The brains were removed, frozen with isopentane and stored at -80°C until *in situ* hybridization procedure. A second group of 42 animals was exposed to a light pulse (100 lux for 1 h) starting at CT 00, 04, 08, 12, 16, 18 or 20 ($n=6$ for each circadian point). At the end of the light exposure, the animals were killed as above, the brains were removed and frozen as mentioned above.

Antisense and sense cRNA probes were generated by *in vitro* transcription (Maxiscript kit, Ambion Inc., Austin, TX, USA) using [^{35}S]UTP as radiolabel. The riboprobes used were *rPer1*, *rPer2*, *mCry2* (kindly provided by Dr. H. Okamura, Kobe University Graduate School of Medicine, Kobe, Japan) and *rBmal1* (kindly provided by Dr. M. Ikeda, Saitama Medical School, Saitama, Japan). The pre-hybridization, hybridization and post-hybridization procedures were performed according to the method previously described in detail (Gauer et al., 1998), except that coronal sections of 20 μm including the suprachiasmatic nucleus were hybridized overnight at 62°C . After high stringency post-hybridization washes and dehydration, the sections were exposed to Kodak Biomax films during 8 days at room temperature, with microscale standards. The *rPer1*, *rPer2*, *rBmal1* and *mCry2* sense probes revealed no hybridization signal in brain sections.

The hybridization signal was quantified in the suprachiasmatic nucleus by means of an image analyzing system (Biocom, Imagerie Instrumentation Biotechnologique, Ullis, France), the signal values being expressed in relative optical density (ROD). The data were calculated as the difference between the signal in identical

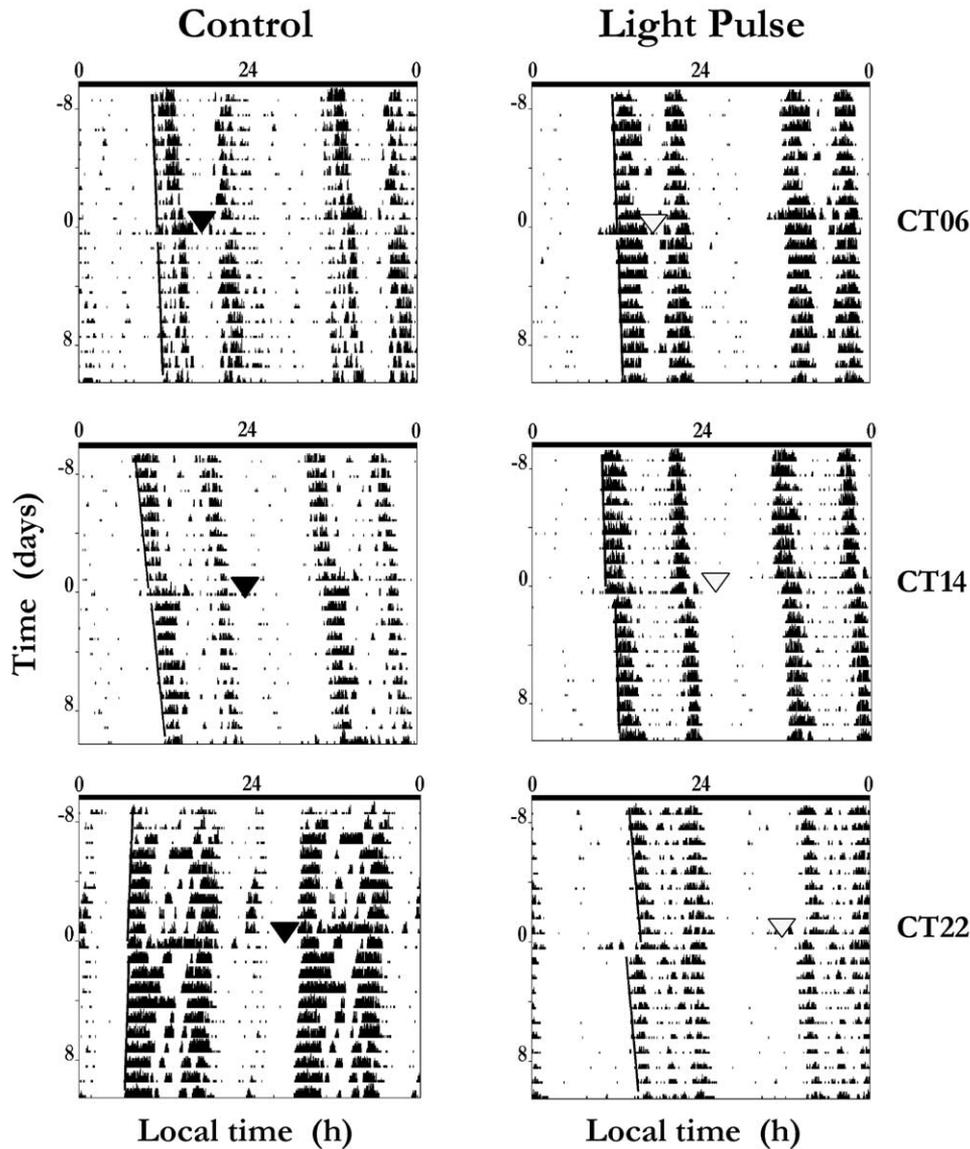


Fig. 1. Representative actograms of wheel-running rhythm in *Arvicantis ansorgei*. In the left panels, the control animals maintained under constant dim red light, were transferred at CT 06 (top), CT 14 (middle) or CT 22 (bottom) as indicated with the closed triangles, to a light chamber without light exposure. In the right panels, animals otherwise maintained under constant dim red light were exposed to a light pulse during 1 h, as indicated with the open triangles, started at CT 06 (top), CT 14 (middle) or CT 22 (bottom). During the subjective day (top) the light pulse administration had no effect on the activity onset. In contrast, in the early and late subjective night, light induced phase delays (middle) and phase advances (bottom), respectively. The day 0 indicates the day of light pulse administration or manipulation of the animals.

areas of the suprachiasmatic nucleus and the adjacent anterior hypothalamic area. The individual mean values were obtained from three to four sections of each *arvicantis* brain.

Data are presented as means \pm S.E.M. Analyses of variance with (τ) or without (clock gene expression) repeated measures were used for statistical comparisons. If significant effects were found, multiple comparisons were done with the student-Newman-Keuls test (Minitab, Minitab Inc.). $P > 0.05$ is considered as nonsignificant (N.S.).

RESULTS

Behavioral phase responses to light

To characterize photic resetting of the *Arvicantis ansorgei* circadian clock, we first determined the behavioral phase

response curve to light pulses. In this part of the study, we found that light pulse administration was able to induce noticeable phase shifts mainly during the subjective night. The exposure to light at the beginning of the subjective night (CT 12–16) induced behavioral phase delays (Figs. 1 and 2). The magnitude of the phase shifts were between 34 and 76 min, the maximal phase delays occurring at CT 14. On the other hand, the light pulse administration during the late subjective night and early subjective day (CT 20–02) induced phase advances. The magnitude of the elicited phase shifts ranged between 35 and 74 min, and the maximal phase advances were induced at CT 20 and 22 (Figs. 1 and 2). The breakpoint of the curve (i.e. the

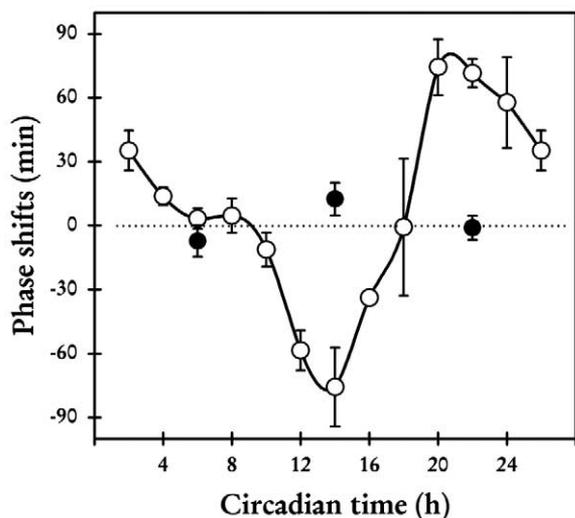


Fig. 2. Phase response curve to light pulses (indicated by open circles) in the diurnal rodent *Arvicantis ansorgei* maintained in constant dim red light and exposed to 1-h light pulses (100 lux). Results of the control procedure (indicated by the closed circles), in which the animals were transferred to a light chamber without exposure to a photic stimulus. The elicited behavioral phase shifts were measured in minutes (mean \pm S.E.M.; $n=6-8$). Data for CT 02 were double-plotted.

circadian phase when the transition from phase delays to phase advances takes place) was observed at CT 18. During the subjective day (CT 04–10), light pulse administration had no significant effect on the phase of the clock (Figs. 1 and 2). In the control procedure for light pulse administration, the manipulation of the animals had no effect on the phase of the wheel-running rhythm in all the circadian phases under study (Figs. 1 and 2).

Measured in 24 individuals housed in constant darkness, τ averaged 24.14 ± 0.03 h (range: 23.83–24.5 h). Neither the circadian time (CT 06, CT 14 and CT 22), nor the treatment (light exposure vs. control procedure) affected significantly τ ($P > 0.1$). The experimental procedure, however, shortened slightly but significantly τ (before vs. after treatment: 24.17 ± 0.03 vs. 24.11 ± 0.03 h, respectively; $P < 0.01$).

Circadian profiles of clock genes in the suprachiasmatic nucleus

Strong expression of *Per1* and *Cry2* mRNA was observed in the suprachiasmatic nucleus (Fig. 3), hippocampus (Fig. 4) and piriform cortex (data not shown) in *A. ansorgei* while moderate levels of these genes were noted in the paraventricular thalamic nucleus (Fig. 4). In contrast, *Per2* and *Bmal1* were predominantly expressed in the suprachiasmatic nucleus (Fig. 3), without noticeable expression in the hippocampus and the piriform cortex (data not shown).

In the assessment of clock gene expression under constant lighting conditions, we observed that *Per1* and *Per2* levels in the *A. ansorgei* suprachiasmatic nucleus showed a prominent circadian oscillation (main effect of time: $P < 0.0001$ and $P < 0.0001$ for *Per1* and *Per2*, respectively), the highest transcript levels being observed

during the subjective day (around CT 04 and CT 08 for *Per1* and *Per2*, respectively) and the lowest levels during the subjective night (around CT 16 and CT 18 for *Per1* and *Per2*, respectively). The peak level of *Per1* was 2.4 fold higher than the nadir value (Fig. 3 and 5). For *Per2*, the peak value was 3.6-fold higher in comparison with the nadir value (Figs. 3 and 5). Similarly, *Bmal1* expression displayed a well-defined circadian oscillation (main effect of time: $P < 0.0001$), the highest and lowest values being observed between CT 12 to CT 18 and from CT 20 and CT 08, respectively (Figs. 3 and 5). Finally, *Cry2* expression also displayed a circadian rhythmicity in the suprachiasmatic nucleus of *A. ansorgei* (main effect of time: $P < 0.0001$). We observed an increase in the transcript levels between CT 08 and CT 20. The peak value of expression occurring at CT 16 was 1.7 fold higher than the nadir value at CT 04 (Figs. 3 and 5).

Effect of light on clock gene expression in the suprachiasmatic nucleus

In the characterization of the phase responses of clock gene expression to light, we observed that a light pulse administration during the subjective night produced a remarkable increase of *Per1* expression in the suprachiasmatic nucleus of *A. ansorgei* (main effect of light: $P < 0.0001$; time \times light interaction: $P < 0.0001$). Changes in *Per1* mRNA levels were observed during all the subjective night (Figs. 3 and 6) and the increment induced by light at CT 12, 16, 18 and 20 ranged from 50 to 60% with reference to their basal levels at the same circadian phases. Also, at the beginning of the subjective day (CT 24), light induced a significant increase of *Per1* reaching about 40% of the basal levels (Fig. 6). In the remaining circadian times during the subjective day, light exposure had no effect on *Per1* expression.

A similar pattern of response to light pulse administration was found in *Per2* expression. Light exposure also induced a significant increment in the mRNA level (main effect of light: $P < 0.0001$; time \times light interaction: N.S.). The photic induction of *Per2* occurred specially at CT 12, 16 and 18 by about 35 to 60% compared with the basal levels at the same circadian phases (Fig. 3 and 6). Light exposure during the late subjective night and subjective day had no effect in the expression of *Per2* in the *A. ansorgei* suprachiasmatic nucleus (Figs. 3 and 6).

In contrast, the effects of light on *Cry2* expression were opposite to the ones previously described for *Per* genes. Indeed, the *Cry2* levels displayed a significant decrease in the suprachiasmatic nucleus after light pulse administration (main effect of light: $P < 0.0001$; time \times light interaction: N.S.). In response to the light pulse administration at CT 12, 16, and 20, this decrease of *Cry2* mRNA ranged from about 20 to 30% compared with the basal levels at the same circadian phases (Figs. 3 and 6).

Finally, *Bmal1* expression was not affected by light exposure at the circadian times under study (main effect of light: N.S.; time \times light interaction: N.S.), the levels of ex-

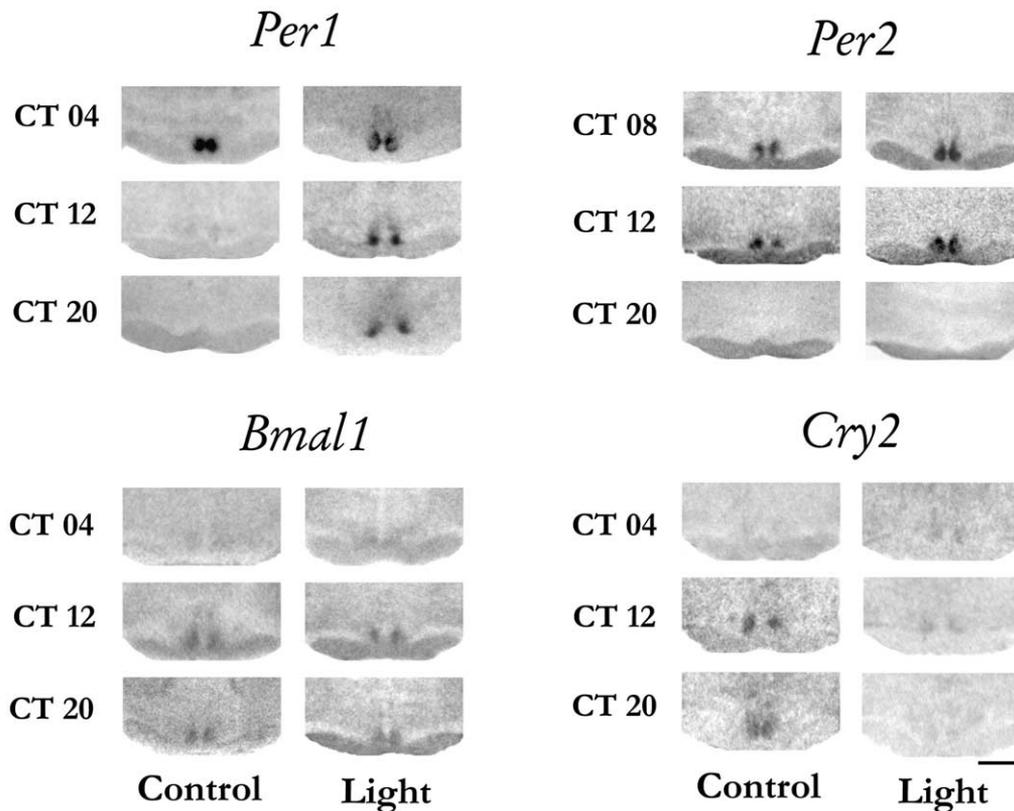


Fig. 3. Circadian expression and photic modulation of *Per1*, *Per2*, *Bmal1* and *Cry2* mRNA in coronal sections of the suprachiasmatic nucleus in *Arvicanthis ansorgei* exposed to light (Light: 100 lux during 1 h) or not (Control) during the subjective day (CT 04 or CT 08), the early (CT 12) and late (CT 20) subjective night. The time points have been chosen to show at least one section per gene close to the circadian peak of expression (i.e. at CT 04 for *Per1*, CT 08 for *Per2*, and CT 12 for *Bmal1* and *Cry2*) and to show light modulation of gene expression over the subjective night. Scale bar = 1 mm

pression of the light pulsed groups being similar to those kept in darkness (Figs. 3 and 6).

DISCUSSION

The present study describes the circadian profiles of expression of the clock genes *Per1*, *Per2*, *Bmal1* and *Cry2*, in the suprachiasmatic nucleus of *Arvicanthis ansorgei*, a diurnal rodent, maintained in constant dim red light. *Per1* and *Per2* genes display circadian variations, increasing their abundance during the subjective day and decreasing during the subjective night. Thus, our data confirm and extend previous findings on the daily variations of *Per1* and *Per2* genes in the suprachiasmatic nucleus of another diurnal mammal the ground squirrel, studied under a light/dark cycle (Mrosovsky et al., 2001). Similar circadian profiles for these clock genes under constant lighting conditions have been described in nocturnal rodents, such as mice (Takumi et al., 1998; Bae et al., 2001) in which the peak levels of expression occur at the same circadian times as in *A. ansorgei*, that is, around CT 04 for *Per1* and CT 08 for *Per2*. Nevertheless, in comparison with Wistar rats (Yan et al., 1999), the peak expression of these *Per* genes occurs earlier in *A. ansorgei*. Also, *Bmal1* exhibits a circadian expression in the suprachiasmatic nucleus of *A.*

ansorgei, the highest values being observed during the subjective night, in anti-phase with those of *Per1*, as previously described in nocturnal rodents (Abe et al., 1998; Tamaru et al., 2000; Bae et al., 2001). Finally, *Cry2* also exhibits a circadian pattern of expression in the suprachiasmatic nucleus of *A. ansorgei*: its levels increase at the end of the subjective day and peak during the early subjective night. Similar results have been described in the mouse suprachiasmatic nucleus at the transcript and protein levels (Kume et al., 1999; Okamura et al., 1999; Field et al., 2000). Therefore, the overall circadian pattern of clock genes in the suprachiasmatic nucleus of the diurnal rodent *A. ansorgei* is quite similar to that previously described in nocturnal rodents.

Our study suggests that there is a differential expression of clock genes in brain structures other than the suprachiasmatic nucleus. As observed in nocturnal mammals like mice or hamsters (e.g. Sun et al., 1997; Miyamoto and Sancar, 1998; Yamamoto et al., 2001), *Per1* and *Cry2* were abundantly expressed in the hippocampus and piriform cortex of *A. ansorgei*. In this species, we also noted moderate level of expression of *Per1* and *Cry2* in the paraventricular thalamic nucleus, which is one of the most important target of suprachiasmatic nucleus efferents (e.g.

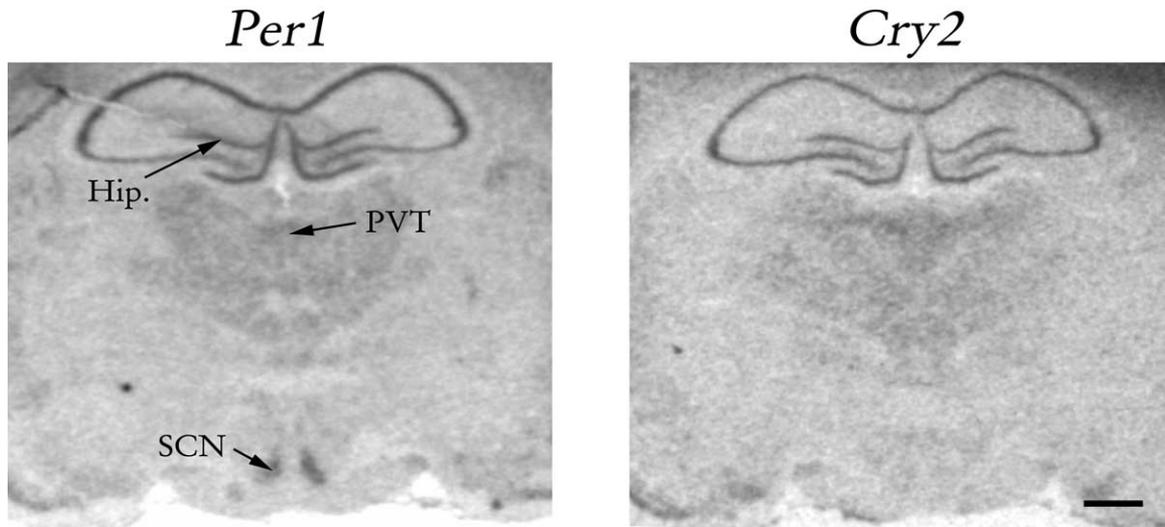


Fig. 4. Representative examples of *Per1* and *Cry2* mRNA expression in coronal sections at the level of the suprachiasmatic nucleus (SCN), hippocampus (Hip.) and paraventricular thalamic nucleus (PVT) in *Arvicanthis* at CT 04. Scale bar=1 mm.

Abrahamson and Moore, 2001). Moderate levels of expression in that thalamic structure have been already mentioned in nocturnal rodents for *Per1* (Yamamoto et al., 2001) and possibly for *Cry2* (Miyamoto and Sancar, 1998). Further studies are needed to determine the contribution of the paraventricular thalamic nucleus in the regulation of overt rhythmicity in day- and night-active mammals.

The present study shows that light exposure to *A. ansorgei* housed in constant dim red light has analogous

effects on the behavioral phase responses in respect to other species. Indeed, the shape of the phase response curve to light obtained in *A. ansorgei* is qualitatively similar to those found in nocturnal (e.g. DeCoursey, 1960; Daan and Pittendrigh, 1976; Pittendrigh and Daan, 1976; Takahashi et al., 1984; Honma et al., 1985) and diurnal (e.g. Navaneethakannan and Chandrashekar, 1986; Kas and Edgar, 2000; Mahoney et al., 2001) rodents. The major responses to light occur during the subjective night, induc-

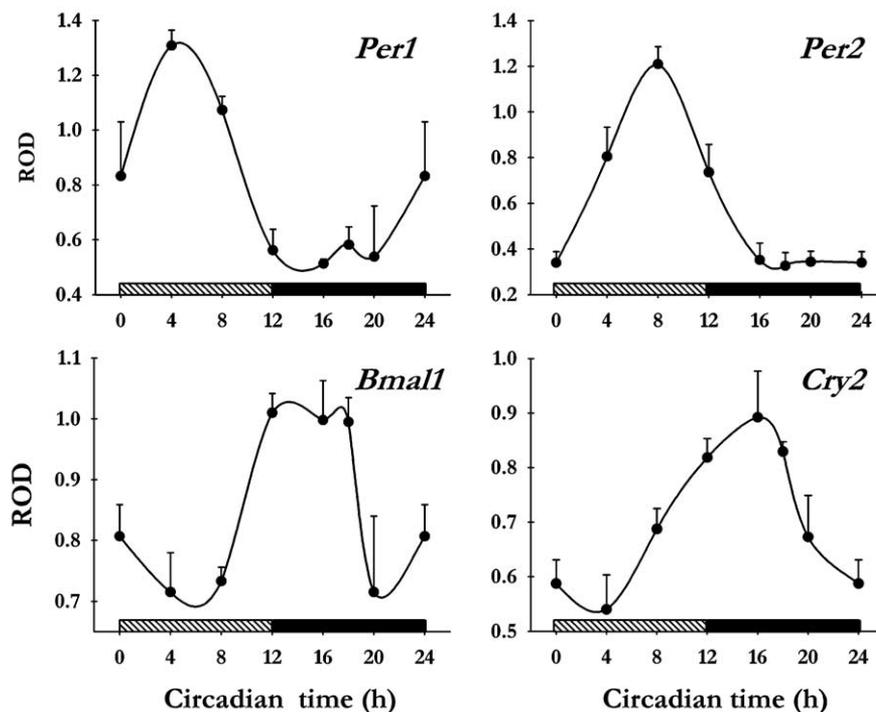


Fig. 5. Circadian expression of *Per1*, *Per2*, *Bmal1* and *Cry2* in the suprachiasmatic nucleus of the diurnal rodent *Arvicanthis ansorgei* maintained under constant dim red light. ROD values are expressed as means \pm S.E.M. ($n=6$ per circadian point). Data for CT 00 were double-plotted (CT 24). The effect of time was highly significant ($P<0.0001$) for the four genes studied.

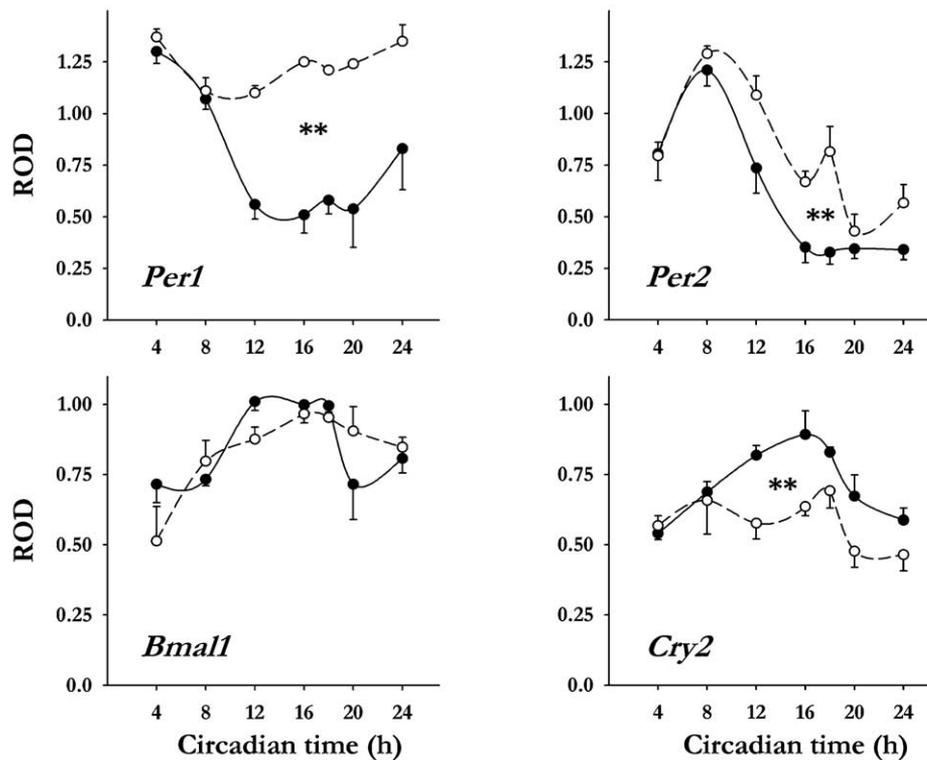


Fig. 6. Effects of light pulse exposure (100 lux during 1 h) on *Per1*, *Per2*, *Bmal1* and *Cry2* mRNA levels in the *Arvicanthis ansorgei* suprachiasmatic nucleus, as determined by quantitative *in situ* hybridization. ROD values in darkness (closed circles, data redrawn from Fig. 5) and after the light exposure (open circles) are expressed as means \pm S.E.M. ($n=6$ per circadian point). The effect of light was highly significant ($P<0.0001$, indicated by **) for *Per1*, *Per2* and *Cry2*, but not for *Bmal1* (N.S.).

ing phase delays of the circadian rhythm of locomotor activity in early night and phase advances in late subjective night. During the subjective day, light pulse administration has only slight effects on the behavioral phase. The phase response curve to light reported here shows close similarities to that previously observed in *A. niloticus* (Mahoney et al., 2001), another *Arvicanthis* species that can display diurnal or nocturnal behavioral patterns (Blanchong et al., 1999). These phase response curves differ in the magnitude of the elicited phase shifts: in *A. niloticus*, shifts were of smaller amplitude with reference to those induced in *A. ansorgei*. This could be due to differences in the intensity and duration of the administered light pulses, these parameters being known to affect the magnitude of the phase response curve to light (Takahashi et al., 1984). In addition, we observed that the onsets of phase delaying and phase advancing zones occur earlier in *A. ansorgei* in comparison with diurnal individuals of *A. niloticus* (Mahoney et al., 2001).

Our data provide clear evidence that light pulse administration has differential effects on clock gene expression in the suprachiasmatic nucleus of *A. ansorgei*. In constant dim red light, light exposure during the night induces an increase in the expression *Per1* and *Per2* to levels similar to those observed during daytime. Furthermore, this up-regulation is dependent on the circadian time when the photic stimulation is delivered. During all the subjective night and early subjective day, light exposure induces an

increase of *Per1* expression. This temporal window is similar to circadian phases during which the circadian clock is susceptible to delay or advance its phase in response to photic cues, as evidenced by the behavioral phase response curve to light (see Fig. 2). Similar effects of light exposure on *Per1* expression in the suprachiasmatic nucleus have been reported in rats (Miyake et al., 2000) and mice (Takumi et al., 1998). Nevertheless, light pulses delivered at CT12 to rats induce behavioral phase delays (Honma et al., 1985), but without effect on *Per1* expression (Miyake et al., 2000). By contrast, in both *A. ansorgei* and mice, at all the circadian phases when light is able to induce behavioral phase shifts, it also stimulates *Per1* expression. Furthermore, light pulse administration during the night also increases *Per2* expression in the suprachiasmatic nucleus of *A. ansorgei*, with a lower amplitude than that of *Per1*. A similar effect of light on *Per2* has been found in rats at CT16 (Miyake et al., 2000). In keeping with our findings in *A. ansorgei*, the influence of light in rats occurs in a shorter temporal window for *Per2* compared with *Per1* (Miyake et al., 2000). In mice, light induced expression of *Per2* differs somewhat from the behavioral phase responses to light. In this species, *Per2* induction occurs from late afternoon to the first half of the subjective night (Albrecht et al., 1997; Takumi et al., 1998; Zylka et al., 1998), whereas light exposure during the afternoon has no effect at behavioral level. The similar effects of light on *Per1* and *Per2* expression between the diurnal rodent *A.*

ansorgei and nocturnal rodents suggest that an induction of these genes could also be involved in phase resetting by light in *A. ansorgei* circadian clock. Further experiments are necessary to determine the effects of light exposure on protein levels of PER1 and PER2 in that species.

Moreover, light has no effect on *Bmal1* expression in the suprachiasmatic nucleus of *A. ansorgei*, since the levels of expression in the light pulsed group remain similar to the basal conditions at all the circadian phases studied. Different effects of light have been found on *Bmal1* regulation in nocturnal rodents, light being able to stimulate *Bmal1* expression especially in early subjective day (Abe et al., 1998). In contrast, expression of BMAL1 protein was reduced after exposure to light during the night (Tamaru et al., 2000). *Bmal1*^{-/-} (*Mop3*^{-/-}) mice exhibit alterations in photic synchronization to a light/dark cycle (Bunger et al., 2000). These changes could be due to a lack of *Bmal1* expression or to indirect effects on other clock genes, like *Per1* and *Per2*. Nevertheless, *Bmal1* does not appear to be directly involved in phase resetting by light in diurnal *A. ansorgei*.

In the suprachiasmatic nucleus of *A. ansorgei*, we observed a down-regulation of *Cry2* expression induced by light, which is striking during the subjective night. The fact that light can decrease *Cry2* expression has been incidentally mentioned in the mouse suprachiasmatic nucleus in the late night only (Okamura et al., 1999). Further studies are therefore needed to determine if light has a similar down-regulatory effect on *Cry2* over the subjective night in nocturnal rodents. Also, in *A. ansorgei* the levels of *Cry2* expression after the photic stimulation are similar to those observed in darkness during daytime. Expression of *hCry2* in transfected cells is not directly sensitive to light (Griffin et al., 1999). Unlike *in vitro* conditions, the light-induced decrease of *Cry2* expression *in vivo* could be important in photic resetting mechanisms of the clock, since CRY proteins constitute the negative limb of the feedback loop. Therefore, it is possible that the decrease induced by light on *Cry2* expression leads to a decreased level of CRY2, which would reduce its inhibition on the transcription activity of CLOCK/BMAL1. This action would help to maintain high levels of transcription of *Per* genes that are induced by light. Further experiments have to be performed to investigate the effects of light exposure on protein level of CRY2. Taken together, our results demonstrate that light affects the clockwork during the night by acting in synergy to increase the expression of *Per1* and *Per2* and reduce that of *Cry2*, in such a way that these clock genes reach, at least temporarily, their daytime levels to reset the phase of the clock.

In comparison with previous works in nocturnal species, our study in *A. ansorgei* shows clear similarities between diurnal and nocturnal rodents in the phase responses to light at both behavioral and molecular levels, indicating that the mechanisms of photic entrainment are phylogenetically highly conserved. It is thus possible that diurnal and nocturnal behavioral patterns are regulated rather at the level of circadian effectors, than at the level of the clock synchronization. Moreover, this study provides

the first detailed report of the opposite effects of light during the night on clock gene expression *in vivo*, with an up-regulation of *Per1* and *Per2*, and a down-regulation of *Cry2*.

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