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Compartmentalized expression of light-induced clock genes in the suprachiasmatic nucleus of the diurnal grass rat (*Arvicanthis niloticus*)

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Abstract

Photic responses of the circadian system are mediated through light-induced clock gene expression in the suprachiasmatic nucleus (SCN). In nocturnal rodents, depending on the timing of light exposure, *Per1* and *Per2* gene expression shows distinct compartmentalized patterns that correspond to the behavioral responses. Whether the gene- and region-specific induction patterns are unique to nocturnal animals, or are also present in diurnal species is unknown. We explored this question by examining the light-induced *Per1* and *Per2* gene expression in functionally distinct SCN sub regions, using diurnal grass rats *Arvicanthis niloticus*. Light exposure during nighttime induced *Per1* and *Per2* expression in the SCN, showing unique spatiotemporal profiles depending on the phase of the light exposure. After a phase delaying light pulse (LP) in the early night, strong *Per1* induction was observed in the retinorecipient core region of the SCN, while strong *Per2* induction was observed throughout the entire SCN. After a phase advancing LP in the late night, *Per1* was first induced in the core and then extended into the whole SCN, accompanied by a weak *Per2* induction. This compartmentalized expression pattern is very similar to that observed in nocturnal rodents, suggesting that the same molecular and intercellular pathways underlying acute photic responses are present in both diurnal and nocturnal species. However, after a LP in early subjective day, which induces phase advances in diurnal grass rats, but not in nocturnal rodents, we did not observe any *Per1* or *Per2* induction in the SCN. This result suggests that in spite of remarkable similarities in the SCN of diurnal and nocturnal rodents, unique mechanisms are involved in mediating the phase shifts of diurnal animals during the subjective day.

Keywords

Per1; *Per2*; *Avp*; circadian rhythms; photic entrainment; diurnality

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INTRODUCTION

Circadian rhythms are endogenously generated and are entrained to the environmental light-dark cycle (Pittendrigh, 1993). The temporal organization of circadian rhythms varies between species. For example, diurnal animals are active, while nocturnal animals are inactive during the light phase, suggesting different preferences or responses to light cues across species (Smale et al., 2003, Challet, 2007, Smale et al., 2008). However, relatively little is known about the neural mechanisms responsible for the differences in photic responses between diurnal and nocturnal animals.

Light can influence rhythmic activity patterns through two processes: masking and entrainment (Mrosovsky, 1999, Challet, 2007). Masking is mediated by direct clock-independent pathways, while entrainment involves resetting the phase of the master circadian clock, which in mammals resides in the suprachiasmatic nucleus (SCN) of the hypothalamus (Klein et al., 1991). Differences in masking may contribute to diurnality (Redlin and Mrosovsky, 2004), and differences in entrainment between diurnal and nocturnal animals have also been seen (Hagenauer and Lee, 2008).

Light entrains circadian rhythms by both acute non-parametric and tonic parametric mechanisms (Daan, 1977). Although the tonic effects of light are not well understood, acute light effects have been intensively studied, and some differences between diurnal and nocturnal animals have been revealed. For behavioral rhythms of nocturnal animals, light produces phase delays in the early night, phase advances in the late night, and has no effect during the subjective day (Daan and Pittendrigh, 1976, Schwartz and Zimmerman, 1990). Diurnal species show similar phase-dependent changes in responses to light during the subjective night, but generally differ from nocturnal ones in that they either lack or only possess a very small non-responsive interval during the subjective day (Kramm, 1975, Pohl, 1982, Lee and Labyak, 1997, Mahoney et al., 2001, Caldelas et al., 2003, Lahmam et al., 2008). The physiological responses of SCN neurons to light also differ between some diurnal and nocturnal animals (Meijer et al., 1989, Jiao et al., 1999). After a brief light exposure in the night phase, the majority of SCN neurons show decreased firing rates in diurnal animals, while showing increased firing rate in nocturnal animals.

Photic responses in behavior and physiology have a molecular basis. Although challenged by the studies using *Per1* and *Per2* deficient animals (Cermakian et al., 2001, Bae and Weaver, 2003, Bae and Weaver, 2007), it is generally recognized that the photic entrainment of an animal's behavioral rhythms is mediated by light-induced expression of clock genes in the SCN (Reppert and Weaver, 2001). A brief light pulse (LP) at the appropriate circadian phase induces the expression of the clock genes *Per1* and *Per2* in the SCN of both nocturnal (Shigeyoshi et al., 1997, Yan et al., 1999, Miyake et al., 2000) and diurnal species (Calderas et al., 2003, Novak et al., 2006).

It is noteworthy that in nocturnal animals, the light-induced *Per1* and *Per2* expression is compartmentalized in specific sub-regions of the SCN, and that this compartmentalization has functional/behavioral consequences (Shigeyoshi et al., 1997, Yan et al., 1999, Hamada et al., 2001, Yamamoto et al., 2001, Yan and Okamura, 2002, Yan and Silver, 2002). Following light exposure at night, *Per1* and *Per2* genes are initially expressed in the retinorecipient (or "core") region of the SCN. Then either *Per1* or *Per2* expression spreads from the retinorecipient region into the entire SCN depending on the timing of the light exposure. The propagation of the *Per1* gene expression corresponds to phase advances and that of *Per2* gene expression corresponds to phase delays (Yan and Silver, 2002, Yan and Silver, 2004). These results are consistent with studies of mutant animals that point to non-redundant roles of *Per1* and *Per2*

genes in photic entrainment (Albrecht et al., 2001), and also highlight the significance of the functional compartmentalization within the SCN (Yan et al., 2007).

In diurnal animals the light-induced expression of *Per1* and *Per2* genes has only been examined in the SCN as a whole (Caldelas et al., 2003, Novak et al., 2006). It is unclear whether the light-induced expression of *Per* genes in the SCN of diurnal animals shows the specific regional distribution that, in nocturnal animals, correlates with the direction of the behavioral phase shifts. It is also unclear whether changes in *Per* gene expression mediate some of the responses of behavioral rhythms to light during the subjective day in diurnal animals. To explore these issues, we utilized the diurnal grass rats *Arvicanthis niloticus*, which are originally from sub-Saharan Africa and have been bred in our research colony since 1993. The behavioral responses induced by a LP in these animals have been characterized (Mahoney et al., 2001), and they include phase advances in response to light exposure early in the subjective day. In the present study, we examined the spatiotemporal patterns of *Per1* and *Per2* expression in distinct SCN sub-regions following LPs that induce phase delays or advances in this diurnal species.

EXPERIMENTAL PROCEDURES

Animals

Adult female grass rats (*Arvicanthis niloticus*, n=60) from our breeding colony were kept in a 12:12h light-dark (LD) cycle. Food and water were provided *ad libitum*. The room was entered at irregular intervals to replenish food and water without disturbing the animals. Animals were reproductively mature adults, but there was no attempt to monitor reproductive conditions, because female grass rats in our colony do not show signs of estrous cycles in vaginal smears; furthermore, ovarian histology and daily tests of mating behavior reveal no evidence of a spontaneous estrous cycle (T. L. McElhinny and L. Smale unpublished observations). To investigate the photic response of their SCN, animals were divided into three groups. The first two groups were exposed to a LP (~500 lux, 30min) at Zeitgeber time (ZT) 14 and ZT22 (lights on is defined as ZT0). To test the effects of a LP during the subjective day, a third group was kept in constant darkness for 4 hours by extending the dark phase of the previous LD cycle and then exposed to a LP at the projected ZT4 of the first day in DD (Aschoff type II protocol). Animals were sacrificed at 0, 30, 90, and 140 min after the beginning of the LP. To determine whether the changes in gene expression after the LP were light-induced or endogenous, i.e., caused by the different times at which the animals were sacrificed, no-LP control groups were included. Animals from these control groups were sacrificed at either ZT4, 14 and 22, at the same time as the 140 min LP animals from those ZTs, but without being exposed to a LP. The experiment was performed in compliance with guidelines established by the Michigan State University All-University Committee on Animal Use and Care, and the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

In situ hybridization using Digoxigenin-labeled cRNA probes

Animals (n=4/ZT) were deeply anesthetized with an overdose of sodium pentobarbital and perfused transcardially with ice-cold saline, followed by a fixative (4% paraformaldehyde in 0.1M phosphate buffer). Brains were removed, post-fixed and then cryo-protected in 20% sucrose.

Serial coronal sections (40 μ m) were obtained from the rostral to the caudal end of the SCN using a cryostat (Reichert-Jung, Heidelberg, Germany). Every third section was collected for each of the following probes: *Per1*, *Per2*, and Arginine Vasopressin (*Avp*). *Avp* is a neuropeptide that is present primarily in the dorsomedial or shell region of the SCN (Van den Pol and Tsujimoto, 1985, Smale and Boverhof, 1999), and was used as a regional marker to delineate the boundary and sub-regions of the SCN. To minimize technical variations in the

hybridization procedure, sections from each temporal series across different experimental conditions were processed simultaneously.

The *in situ* hybridization histochemistry was performed using rat probes as described previously (Yan et al., 1999, Yan and Okamura, 2002). We were able to use rat probes because of the high sequence homology for *Per1* and *Per2* between grass rats and lab rats (Yan et al., 1999, Lambert et al., 2005). Briefly, tissue sections were processed with proteinase K at 37 °C and 0.25% acetic anhydride for 10 min. The sections were then incubated in hybridization buffer containing the Dig-labeled *Per1*, *Per2*, and *Avp* antisense cRNA probes (0.1 µg /1 ml) overnight at 60 °C in a water bath shaker. After a high-stringency post hybridization wash, sections were treated with RNase A, and then were further processed for immunodetection with a nucleic acid detection kit (Boehringer Mannheim, Indianapolis, IN, USA). Sections were incubated in a blocking reagent diluted 1: 100 in buffer 1 for 1 h at room temperature, then incubated at 4°C in an alkaline phosphatase-conjugated digoxigenin antibody diluted 1:2000 in buffer 1 for 3 days. On the following day, sections were then incubated in a solution containing nitroblue tetrazolium salt (0.34 mg/mL; Roche, Indianapolis, IN, USA) and 5-bromo-4-chloro-3-indolyl phosphate toluidinium salt (0.18 mg/mL; Roche, Indianapolis, IN, USA) for 14 hr. The colorimetric reaction was halted by immersing the sections in buffer 4 (10 mM Tris-HCl containing 1 mM EDTA, pH 8.0). Sections were mounted on slides and coverslipped with Permount (Fisher Scientific, Fair Lawn, New Jersey, USA).

Quantitative analysis

The SCN borders and sub regions were delineated based on *Avp* staining in adjacent sections. For quantification of optical density (OD), images of serial sections through the SCN were captured using a CCD video camera (CX9000, MBF bioscience, Williston, Vermont, USA) attached to a light microscope (Zeiss, Gottingen, Germany). Mid-SCN sections that could be clearly divided into an *Avp*-containing shell region and a non-*Avp* core region were chosen and the mRNA expression was quantified using the NIH Image J program (version 1.61). Relative OD, assessing the mean gray value per pixel, was used to quantify the intensity of the signal in the SCN sub-regions compared with the adjacent hypothalamic area. The difference between the density measurement of the SCN and the background was the relative OD value for each SCN. Images were processed using Adobe Photoshop 7 (Adobe Systems, Mountain View, CA, USA).

Statistical analysis

For *Per1* and *Per2* expression two-way ANOVA (4-time points; 0, 30, 90, and 140 min X 2-regions; shell and core) was used at each ZT (4, 14 and 22) to assess patterns of change after the LPs, followed by one-way ANOVAs when the interaction between the two factors was significant. The Dunnett post hoc test was used to assess the differences between individual survival times after the LPs. To test whether differences between 140 and 0 min was due to the effect of the LP or in turn due to an endogenous rhythm, we compared the 140 min LP groups for each ZT with their corresponding no-LP controls using 2 (LP vs no-LP) X 2 (region) AVOVAs. For the data on *Avp* expression, one-way ANOVAs were used to assess the effects of the LPs at each ZT and after the three survival times, and t-tests were used to compare the *Avp* expression of the 140 min LP groups to those of the no-LP control groups for each ZT. In all cases, differences were considered significant when $p < 0.05$.

RESULTS

Per1, *Per2* and *Avp* expression following phase delaying LP at ZT14

At ZT14, *Per1* staining (Figure 1 A, left column) was sparse before the LP (0 min), with only few SCN cells being labeled. After the LP, strong *Per1* staining was seen at 30 and 90 min,

with the intensity decreasing at 140 min. The non-LP control animals displayed very little *Per1* staining at 140 min (Figure 1 B). Compared to *Avp* staining in adjacent sections (Figure 1 A, right column), light-induced *Per1* was concentrated in the non-*Avp* core SCN region. On the other hand, *Per2* staining (Figure 1 A, middle column) was minimal before the LP, moderate at 30 min and very strong at 90 and 140 min in the entire SCN. The non-LP control animals at 140 min displayed no *Per2* staining (Figure 1 B). *Avp* staining was very faint at all time points examined.

The time course and the region-specific expression patterns of these genes were further analyzed quantitatively in the core SCN region as well as the *Avp*-containing shell region (Figure 1 C). For *Per1*, there was a significant effect of time ($F = 9.572$, $df = 3$, $P < 0.0001$), a significant effect of region ($F = 64.930$, $df = 1$, $P < 0.0001$) and a significant interaction between time and region ($F = 6.103$, $df = 3$, $P < 0.003$). One-way ANOVAs revealed that the time-dependent change was significant in the core ($F = 8.177$, $df = 3$, $P < 0.003$) but not in the shell ($F = 2.490$, $df = 3$, $P > 0.05$). In the core region, post hoc analysis indicated that the values for the 30, 90 and 140 min groups were significantly different from that of the 0 min group ($p < 0.05$). Comparing the 140 min LP and the non-LP controls, a significant effect of LP was observed (LP effect: $F = 9.481$, $P < 0.01$; region: $F = 10.919$, $P < 0.006$; interaction: $F = 5.274$, $P < 0.040$; two-way ANOVA). And the *Per1* level at 140 min-LP was significantly higher than that of the no-LP controls in both shell ($F = 8.064$, $df = 1$, $P < 0.030$; one-way ANOVA) and core regions ($F = 7.364$, $df = 1$, $P < 0.035$, One way-ANOVA). For *Per2*, time-dependent changes were also observed in both the core and shell regions (time: $F = 37.002$, $df = 3$, $P < 0.0001$; region: $F = 7.681$, $df = 1$, $P < 0.011$; interaction: $F = 1.429$, $df = 3$, $P > 0.05$; two-way ANOVA). Post hoc analysis revealed that *Per2* levels at 30, 90, and 140 min were significant higher than that at 0 min in both core and shell regions ($p < 0.05$). At 140 min, *Per2* expression in the LP group was significantly higher than that in the controls (LP effect: $F = 31.871$, $P < 0.0001$; region: $F = 1.482$, $P > 0.05$; interaction: $F = 0.297$, $P > 0.05$; two-way ANOVA). For *Avp*, there were no time-dependent changes ($F = 2.053$, $df = 3$, $P > 0.05$; one-way ANOVA), and no significant difference between the 140 min LP group and the no-LP controls ($t = 1.090$, $df = 6$, $P > 0.05$; t-test).

***Per1*, *Per2* and *Avp* expression following phase advancing LP at ZT22**

The expression of *Per1*, *Per2* and *Avp*, after a phase advancing LP at ZT22 is shown in Figure 2. *Per1* staining (Figure 2 A, left column) was absent before the LP (0 min), and increased at 30 min with intensely labeled cells in the non-*Avp* region. But at 90 and 140 min, strong *Per1* staining was seen throughout the SCN. The non LP 140 min controls displayed only moderate *Per1* staining (Figure 2 B). In contrast, *Per2* staining was absent at 0 and 30 min, and very faint at 90, 140 (Figure 2 A, middle column) and in the no-LP controls (Figure 2 B). The level of *Avp* staining (Figure 2 A, right column) also increased with a similar time course, being low at 0 and 30 min and high at 90 and 140 min. The no-LP controls showed high *Avp* staining as well (Figure 2 B).

Quantification of these results is shown in Figure 2 C. For *Per1*, a significant effect of time ($F = 31.805$, $df = 3$, $P < 0.0001$), a significant effect of region ($F = 18.536$, $df = 1$, $P < 0.0001$) and a significant interaction between time and region ($F = 3.452$, $df = 3$, $P < 0.032$) were revealed by two-way ANOVA. One-way ANOVAs revealed a significant effect of time on *Per1* expression in both the shell ($F = 21.835$, $df = 3$, $P < 0.0001$) and the core region ($F = 15.908$, $df = 3$, $P < 0.0001$). In the shell, post hoc analysis showed that *Per1* expression at 90 and 140 min was significantly higher than that at 0 min ($p < 0.05$), while in the core, *Per1* expression at 30, 90, and 140 min was significantly higher than that at 0 min ($p < 0.05$). *Per1* expression in the 140 min LP group was significantly higher than in no-LP controls in both core and shell regions (LP effect: $F = 23.998$, $df = 1$, $P < 0.0001$; region effect: $F = 1.327$, df

=1, $P > 0.05$; interaction: $F = 0.234$, $df = 1$, $P > 0.05$; two-way ANOVA). For *Per2*, two-way ANOVA revealed a significant effect of time ($F = 10.303$, $df = 3$, $P < 0.001$) and region ($F = 11.614$, $df = 1$, $P < 0.002$), with no interaction between time and region ($F = 0.689$, $df = 3$, $P > 0.05$). Post hoc analysis indicated that *Per2* expression at 90 and 140 min was significantly higher than at 0 min in both the core and the shell regions ($p < 0.05$). At 140 min, the *Per2* expression in the LP group was significantly higher than that of the non-LP controls, with more *Per2* induction in the core than that in the shell (LP effect: $F = 5.897$, $P < 0.05$; region: $F = 8.015$, $P < 0.015$; interaction: $F = 0.707$, $P > 0.05$; two-way ANOVA). For *Avp*, there was a significant effect of time ($F = 31.580$, $df = 3$, $P < 0.0001$; one-way ANOVA). Post hoc comparison showed that *Avp* expression at 90 and 140 min were significantly higher than that at 0 min. The difference between the LP group at 140 min and the no-LP controls was also significant ($t = 2.659$, $df = 6$, $P < 0.038$; t-test).

***Per1*, *Per2* and *Avp* expression following a phase advancing LP at ZT4**

Before and after the LP at ZT4, strong staining of *Per1*, *Per2* and *Avp* were observed at all time points examined (Figure 3 A, B). Quantitative analysis is shown in Figure 3 C. For *Per1*, there were no time-dependent changes in its expression in both core and shell regions (time: $F = 3.019$, $df = 3$, $P > 0.05$; region: $F = 0.790$, $df = 1$, $P > 0.05$; interaction: $F = 0.961$, $df = 3$, $P > 0.05$; two-way ANOVA). There were no differences in gene expression between the 140 min LP group and the no-LP controls (time: $F = 0.047$, $df = 1$, $P > 0.05$; region: $F = 2.976$, $df = 1$, $P > 0.05$; interaction: $F = 1.313$, $df = 1$, $P > 0.05$; two-way ANOVA). For *Per2*, there were time-dependent changes in both regions (time: $F = 8.268$, $df = 3$, $P < 0.001$; region: $F = 1.761$, $P > 0.05$; interaction: $F = 0.2166$, $df = 3$, $P > 0.05$; two-way ANOVA). Post hoc analysis showed that *Per2* expression at 90 and 140 min were significantly higher than that at 0 min in both core and shell regions. However, there were no differences between the 140 min LP group and the no-LP controls (time: $F = 1.230$, $df = 1$, $P > 0.05$; region: $F = 1.103$, $df = 1$, $P > 0.05$; interaction: $F = 1.093$, $df = 1$, $P > 0.05$; two-way ANOVA). For *Avp* expression, there were no differences across time points ($F = 1.367$, $df = 3$, $P > 0.05$, one-way ANOVA) or between the 140 min LP group and the no-LP controls ($t = 0.007$, $df = 6$, $P > 0.05$; t-test).

DISCUSSION

We examined the effects of LPs at different ZTs on clock gene *Per1* and *Per2* expression in distinct sub-regions of the SCN of diurnal grass rats. The results for ZTs 14 and 22 show unique spatiotemporal profiles of *Per1* and *Per2* gene expression that depend on the phase of the LP, and that correspond to the behavioral responses of the grass rats to timed exposure to light (Figure 4). After a phase delaying LP in the early night, both *Per1* and *Per2* were induced in the SCN, with strong *Per1* induction explicitly in the core and strong *Per2* induction throughout the entire SCN. After a phase advancing LP in the late night, *Per1* was strongly induced in both core and shell of the SCN, while *Per2* was only weakly induced in both regions.

This work is the first to explore the distribution of light-induced *Per*-gene expression in SCN sub-regions following a time course. These results demonstrate clear compartmentalized expression patterns of light-induced *Per* genes in the SCN, with the distribution of each being associated with the direction of behavioral phase shifts after a LP at ZT14 and 22. Although the up-regulation of *Per1* and *Per2* genes in the SCN initiated by a LP has been reported in diurnal rodents (Caldelas et al., 2003, Novak et al., 2006), the present study provides the first evidence for gene-and region-specific patterns of clock gene induction in the SCN, that is specific to the behavioral responses to LPs in a diurnal species. This compartmentalized expression pattern of the SCN of the diurnal grass rats is similar to that observed in nocturnal rodents (mouse: (Shigeyoshi et al., 1997, Yan and Silver, 2002); rat: (Yan et al., 1999, Yan and Okamura, 2002); hamster: (Hamada et al., 2001).

In nocturnal rodents, it has been well documented that the SCN is comprised of anatomically and functionally distinct sub-regions (Antle and Silver, 2005). Although there are species differences in the topography of the SCN that complicate the universal characterization of the SCN (Morin and Allen, 2006), there is general agreement that the SCN contains two major compartments. One compartment is located in the core or ventrolateral region, receives intense retinal input and shows low amplitude circadian oscillations; the other compartment is located in the shell or dorsomedial region which shows high amplitude circadian oscillations (Antle and Silver, 2005, Yan et al., 2007). The peptidergic phenotypes of SCN cells within each subregion differ significantly among species (Morin and Allen, 2006, Morin, 2007), but in the well-studied nocturnal rodents, such as mouse, rat and hamster, there is a shell region that contains neurons expressing AVP, and a core area rich in vasoactive intestinal polypeptide (VIP) and gastrin releasing peptide (GRP) (Morin et al., 1992, Moore, 1996, Abrahamson and Moore, 2001). In the diurnal grass rats, the SCN is also organized into distinct subdivisions that differ in their neuropeptide contents and in the distribution of retinal innervations (Smale and Boverhof, 1999). For example in grass rats, *Avp*-containing neurons are more concentrated in the dorsal region, and retinal fibers are most dense in the ventral portion of the SCN, where the *Avp* neurons are sparse. Similar patterns of neuropeptide expression in the SCN have been shown in other diurnal species as well (Lahmam et al., 2008).

Accumulating evidence indicates that the primary response of the SCN to an acute photic stimulus occurs in the VIP and GRP rich core region (Yan et al., 2007). Furthermore, light-induced *Per* gene expression has been found to colocalize with VIP (Shigeyoshi et al., 1997) and GRP in cells of the SCN (Dardente et al., 2002b, Karatsoreos et al., 2004). Interestingly, VIP and GRP are colocalized in some of neurons within the SCN (Romijn et al., 1998, Kawamoto et al., 2003). In the diurnal grass rats, the initial induction of *Per1* gene by a LP (Figure 1, 2) occurred in the non-*Avp* region, which overlaps with a cell population that expresses *Vip* mRNA (Yan, unpublished results), and that also contains GRP (Katona et al., 1998). Thus, it is likely that, as in nocturnal rodents, the cells showing acute light responses in the SCN of grass rats are ones that contain GRP and/or VIP. *In vitro*, applying either VIP or GRP induces phase-dependent shifts of rhythms in the electrical activity of the SCN (Piggins et al., 1995, Reed et al., 2001), suggesting that VIP and GRP share similar functions and cooperate in altering the phase of rhythms. Several other lines of evidence also suggest that VIP and GRP signaling is critical in maintaining the synchrony of SCN cellular oscillators and the flow of information within the SCN network (Aton et al., 2005, Maywood et al., 2006, Vosko et al., 2007). It should also be noted that there are other light-responsive cells that can not be attributed to any one identified peptidergic phenotype (Castel et al., 1997, Romijn et al., 1998).

The temporal profile of clock-gene expression in diurnal species is similar to that in nocturnal ones, with *Per* genes showing highest expression during the day and lowest during the night (Mrosovsky et al., 2001, Lincoln et al., 2002, Caldelas et al., 2003, Novak et al., 2006). Furthermore, Dardente et al (2002a, b) have found that similar to the nocturnal lab rats, the diurnal *Arvicanthis ansorgei* has a daily peak expression of *Per1* and *Per2* genes which is concentrated in the dorsomedial part of the SCN, and often co-expressed with *Avp*. Additionally, in the diurnal *A. niloticus*, the protein products of *Per1* and *Per2* genes also show spatial-temporal distributions that are similar to those seen in nocturnal rodents (Ramanathan et al., 2006). These results suggest a similar spatial organization of the SCN oscillators underlying circadian rhythm generation in diurnal and nocturnal rodents. The present study provides further evidence supporting the claims that the two subdivisions of the SCN play distinct roles in mediation of responses to photic stimuli, and that the pattern of functional heterogeneity of the SCN is similar in diurnal and nocturnal species.

Although our results document many similarities between diurnal and nocturnal animals in the responses of their SCN to acute light cues, it is still debatable whether or not the two groups possess the same mechanism of photic entrainment. It has been documented that there are differences in the light sensitivity and photic responses of the SCN neurons of diurnal and nocturnal rodents (Meijer et al., 1989, Jiao et al., 1999). The diurnal rodents are less sensitive to light, and after exposure to photic stimuli, the majority of SCN neurons show decreased firing rates in diurnal rodents while nocturnal rodents show an increased rate of neuronal firing. Using melanopsin deficient animals, it has recently been shown that changes in the retinal input to the SCN can lead to acute temporal-niche switching (Doyle et al., 2008). However, at present there is no evidence that evolutionary changes in genes regulating retinal functions have been responsible for the emergence of diurnality in naturally occurring mammalian species.

Although the effects of non-parametric light exposure during the dark phase appear to be very similar between the diurnal and nocturnal animals in both behavioral (reviewed by (Smale et al., 2003) and molecular levels (present study), relatively little is known about parametric light effects. Recent reports have demonstrated that different molecular and neural mechanisms underlying photic entrainment in response to discrete non-parametric and continuous parametric light cues (Duffield et al., 2009, Evans et al., 2009). In nature, diurnal animals receive more light exposure with respect to intensity and duration, and are never or rarely exposed to twilight, which provides a phasic, non-parametric effects (Hut and Scharff, 1998, Hut et al., 1999). Thus, in diurnal animals, the parametric effects of light that they experience during the day may play a particularly important role in synchronizing their internal clocks to the natural environment.

Another interesting finding of present study is the significant induction of *Per2* after the phase-advancing LP at ZT22 (Figure 2). In another diurnal rodent, the *A. ansorgei*, a LP in the advance phase increases the expression of *Per1* but has no effect on *Per2* (Caldelas et al., 2003). This apparent discrepancy could be caused by differences in the experimental conditions between these two studies, such as the LP duration, light intensity, and the time course. It should be noted, however, that in our grass rats, the level of light-induced *Per2* in the advance phase was relatively low compared to that of *Per1*, as well as to that of *Per2* after a phase-delaying LP. This suggests a limited functional significance for *Per2* induction in behavioral phase advances. The results on *Per2* induction in the advance phase are variable in nocturnal species. In mice and rats, it has been reported that light has no effect on *Per2* expression in the SCN in the late night (Albrecht et al., 1997, Miyake et al., 2000, Yan and Silver, 2002), while in hamsters, *Per2* is induced after a LP in the advance phase (Hamada et al., 2001). Thus while a LP in the late night always results in *Per1* expression, species differences, apparently unrelated to diurnality or nocturnality, exists for the effects of late-night LPs on *Per2* expression.

In many diurnal rodents, including the diurnal grass rats of the present study and *Arvicanthis ansorgei*, a LP in the early subjective day causes behavioral phase advances (Mahoney et al., 2001, Caldelas et al., 2003). It is well accepted that behavioral phase shifts result from the resetting of the central pacemaker of the SCN; and that this resetting involves changes in the expression level of genes that are molecular components of the clock machinery. However, after the LP in subjective day (ZT 4), we did not detect any light-dependent changes in either *Per1* or *Per2* expression in the SCN (Figure 3, 4). It should be noted though, that because we only have a no-LP control for the 140 min survival time after the LP, we can not exclude the possibility of transient light-dependent changes in the expression of *Per* gene at earlier time points. Interestingly, Caldelas et al (2003) examined *Per1* and *Per2* after an LP at CT4 in the entire SCN of the diurnal *A. ansorgei*, and did not detect any differences between the LP group and controls at 60min. Thus, the phase advances during the subjective day in diurnal grass rats may be mediated by translational or post-translational regulation of *Per* genes, or mediated through other clock genes. However, we can not rule out the possibility that our method lacks

the sensitivity necessary to detect small changes in gene expression. Since the expression of *Per1* and *Per2* around midday is already high, a ceiling effect could also make it difficult to detect a significant change.

The dissociation between behavioral phase shifts and acute clock gene induction has been seen in other paradigms. For example, melatonin injection can shift the phase of circadian rhythms without immediate induction of clock genes in the SCN (Poirel et al., 2003). Also, it has been shown that timed γ -radiation in the subjective day causes phase advances in cultured cell lines and in mouse wheel-running-behavior (Oklejewicz et al., 2008). Although the mechanism by which radiation causes behavioral phase shifts is not known, it has been determined that the phase shifts induced by radiation in cultured cell line do not require up-or down-regulation of the core clock genes (*Per1*, *Per2*, *Clock* and *Bmal1*) or their protein synthesis, indicating the involvement of post-translational mechanisms as mediators of phase shifts.

In addition to examining the expression of *Per* genes, we used the expression of *Avp*, one of the clock-controlled genes that is expressed in the shell of the SCN, to delineate the boundary and sub-regions of the SCN. The circadian profile of *Avp* expression is similar between nocturnal and diurnal rodents; it is high during the day and low at night (Dardente et al., 2004). After the LP in the advance phase (at ZT22), the temporal profile of *Avp* expression paralleled that of *Per1* in the shell region. As was the case for *Per1*, *Avp* expression was higher than control levels 140 minutes after the ZT22 LP. Also similar to the pattern of *Per1* expression in the shell of the SCN, *Avp* levels remained unchanged after LPs presented in the early night or subjective day. These results indicate that the same intracellular signaling pathways may be responsible for both *Per1* and *Avp* expression in the shell of the SCN, not only under circadian oscillations (Jin et al., 1999), but also after photic stimuli. It is likely that the up-regulated *Avp* represents light-induced expression of the gene. However, as the results did not extend into the declining phase and the next rising phase of the rhythm, making it impossible to rule out the possibility that the increase in *Avp* expression represents a phase-advance in the rhythm in expression of the gene (Hamada et al., 2004).

In summary, we found that in the diurnal grass rats, there was a compartmentalized pattern of light-induced *Per1* and *Per2* expression in their SCN. The results support the findings of work on the acute light responses of *Per* genes in the SCN of *A. ansorgei* (Caldelas et al., 2003), and provide a more detailed spatial and temporal analysis of light-induced *Per* gene expression in diurnal rodents. The results also demonstrate the functional heterogeneous organization of the SCN in a diurnal rodent. The response of the SCN in diurnal grass rats is very similar to those observed in nocturnal rodents (reviewed in (Yan et al., 2007), suggesting that the same molecular and intercellular pathways are being activated in responding to an acute light exposure. These data add to the accumulating evidence suggesting that the most basic difference between diurnal and nocturnal animals arises from mechanisms operating downstream of the SCN. However, our results also indicate that the unique phase advances of diurnal animals that occur in response to photic input during the early subjective day may be mediated by cellular mechanisms different from those described for the SCN of nocturnal species. Further, light entrains circadian rhythms by both parametric and non-parametric mechanisms (Daan, 1977) and the question of whether or not the rhythms of diurnal and nocturnal species respond in the same way to tonic, parametric light exposure remains open.

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A comprehensive list of abbreviations

AVP	Arginine Vasopressin
GRP	gastrin releasing peptide
LD	light-dark cycle
LP	light pulse
OD	optical density
SCN	Suprachiasmatic nucleus
VIP	vasoactive intestinal polypeptide
ZT	Zeitgeber time

Figure 1.

Representative photomicrographs (A, B) and quantitative analysis (C) of expression of *Per1*, *Per2* and *Avp* in the SCN after a LP at ZT14. (A), *Per1*, *Per2* and *Avp* expression in adjacent mid SCN sections before (0), and at 30, 90 and 140 min after the LP. (B) Gene expression in a non-LP control sacrificed 140 min after ZT14, but without being exposed to the LP. Scale bar = 100 μ m. (C) Quantitative analysis of *Per1* (top panel) and *Per2* (middle panel) in the shell (*Avp*-containing region) and core (non *Avp*-containing region) of the SCN; the bottom panel shows the quantitative analysis for *Avp* expression. The data are presented as mean $\pm$ SEM. For *Per1* (top), as indicate significant differences in the core region between pre-pulse and time points following the LP. For *Per2* (middle) as indicate significant differences between pre-pulse and time points following the LP in both the core and the shell regions. For both *Per1* and *Per2*, * indicates a significant difference between the LP 140min and the non-LP 140min groups in both the core and the shell regions.

Figure 2.

Representative photomicrographs (A, B) and quantitative analysis (C) of expression of *Per1*, *Per2* and *Avp* in the SCN after a LP at ZT22. (A), *Per1*, *Per2* and *Avp* expression in adjacent mid SCN sections before (0), and at 30, 90 and 140 min after the LP. (B) Gene expression in a non-LP control sacrificed 140 min after ZT22, but without being exposed to the LP. Scale bar = 100 μ m. (C) Quantitative analysis of *Per1* (top panel) and *Per2* (middle panel) in the shell (*Avp*-containing region) and core (non *Avp*-containing region) of the SCN; the bottom panel shows the quantitative analysis for *Avp* expression. The data are presented as mean $\pm$ SEM. For *Per1* (top) as or As indicate significant differences between pre-pulse and time points following the LP within the core (a) or the shell (A). For *Per2* (middle) as indicate significant differences between pre-pulse and time points following the LP in both the core and the shell regions. For *Avp* (bottom) as indicate significant differences between pre-pulse and time points following the LP. * indicates a significant difference between the LP 140min and non-LP 140min groups in the expression of *Per1* and *Per2* (core and shell regions) and *Avp*.

Figure 3.

Representative photomicrographs (A, B) and quantitative analysis (C) of expression of *Per1*, *Per2* and *Avp* in the SCN after a LP at ZT4. (A), *Per1*, *Per2* and *Avp* expression in adjacent mid SCN sections before (0), and at 30, 90 and 140 min after the LP. (B) Gene expression in a non-LP control sacrificed 140 min after ZT22, but without being exposed to the LP. Scale bar = 100µm. (C) Quantitative analysis of *Per1* (top panel) and *Per2* (middle panel) in the shell (*Avp*-containing region) and core (non *Avp*-containing region) of the SCN; the bottom panel shows the quantitative analysis for *Avp* expression. The data are presented as mean ± SEM. For *Per2* (middle), **a**s indicate significant differences between pre-pulse and time points following the LP in both the core and the shell regions.

Figure 4.

Spatial distribution of light-induced *Per1* and *Per2* expression in the SCN of *A. niloticus*. At ZT14, light-induced *Per1* expression was present only the core, but light-induced *Per2* expression was present in both the core and the shell regions of the SCN. At ZT22, light-induced *Per1* and *Per2* expression was present in both core and shell regions of the SCN. But the induction of *Per2* was much lower than that of *Per1*. At ZT4, there was no significant induction of *Per1* and *Per2* expression in both regions of the SCN.