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Rhythms in expression of PER1 protein in the amygdala and bed nucleus of the stria terminalis of the diurnal grass rat (*Arvicanthis niloticus*)

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Abstract

In the diurnal rodent *Arvicanthis niloticus* (grass rats) the pattern of expression of the clock genes and their proteins in the suprachiasmatic nucleus (SCN) is very similar to that seen in nocturnal rodents. Rhythms in clock gene expression have been also documented in several forebrain regions outside the SCN in nocturnal *Ratus norvegicus* (lab rats). To investigate the neural basis for differences in the circadian systems of diurnal and nocturnal mammals, we examined PER1 expression in the oval nucleus of the bed nucleus of the stria terminalis (BNST-OV), and in the basolateral (BLA) and the central (CEA) amygdala of male grass rats kept in a 12:12 light/dark cycle. In the BNST-OV, peak levels of PER1 expression were seen early in the light phase of the cycle, 12 hours out of phase with what has been reported for nocturnal lab rats. In the BLA the pattern of PER1 expression featured sustained high levels during the day and low levels at night. PER1 expression in the CEA was also at its highest early in the light phase, but the effect of sampling time was not statistically significant ($p < 0.06$). The results are consistent with the hypothesis that differences between nocturnal and diurnal species are due to differences in neural systems downstream from the SCN.

Keywords

oval nucleus of the bed nucleus of the stria terminalis; central nucleus of the amygdala; basolateral amygdala; PERI; diurnality; grass rats

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Introduction

Most of what we know about the circadian system of mammals comes from research with nocturnal species [21], yet, many mammals including humans are day active. The mechanisms responsible for such species differences are not completely understood [20, 21]. Daily rhythms of a variety of markers of neural activity in the mammalian master clock of the suprachiasmatic nucleus (SCN) and their phases with respect to the light-dark cycle are very similar in diurnal and nocturnal species [4, 21]. The same degree of similarity exists for the mechanisms responsible for the phase-setting effects of light [4], though the effects of serotonin on light-induced phase shifts are different [5]. Interestingly, mice lacking gene products involved in photoreception have been reported to adopt a diurnal or partially diurnal pattern of activity [10]. Thus in principle, a change in the type of retinal input that the SCN receives could result in a switch of temporal niche. However, at present there is no evidence that mutations that affect retinal functions have been responsible for the emergence of diurnality in naturally-occurring mammalian species.

One landmark achievement in the study of circadian biology has been the identification of the core molecular clock mechanism of the mammalian SCN. This mechanism involves the rhythmic expression of several clock genes and their proteins, which interact via positive and negative feedback loops in pacemaker neurons within the SCN [16]. Studies by us and others [21] have shown that the rhythmic expression of clock genes and their proteins is also remarkably similar in the SCN of diurnal and nocturnal rodents. In sharp contrast with the similarities in the intrinsic properties of the SCN, the rhythms in neural activity of several targets of the axonal output of the SCN are out of phase when species with different activity patterns are compared [21]. Thus, it is most likely that the differences between diurnal and nocturnal mammals stem from differences in the circadian system downstream from the SCN [20].

In nocturnal lab rats, clock genes are expressed rhythmically in several brain regions outside the SCN [1, 2, 8]. In our diurnal animal model, the grass rat (*Arvicanthis niloticus*), the brains of adults males show evidence of immunocytochemical labeling for the Period 1 protein (PER1) in the central and basolateral amygdala (CEA; BLA) and substantial labeling for PER1 in the oval nucleus of the bed nucleus of the stria terminalis (BNST-OV). Here we report the patterns of PER1 expression across the day/night cycle in these three brain regions of the grass rat brain. In lab rats [22] and grass rats [18], these brain regions are not direct targets of the axonal outputs of the SCN, but may be controlled by the SCN via multi-synaptic pathways [13, 23] or diffusible signals [19]. The CEA, BLA and BNST-OV play critical roles in the control of behaviors and autonomic functions [9, 12, 17], that differ in their temporal profiles between diurnal and nocturnal species, raising interesting questions about their possible roles in the mediation of differences between diurnal and nocturnal mammals.

Materials and Methods

We used immunocytochemistry (ICC) to evaluate the expression of PER1 protein in the CEA, BLA and BNST-OV of diurnal male grass rats kept on a 12:12 light/dark cycle and

perfused at 4-hr intervals from Zeitbeger Time (ZT) 2-ZT 22. All experimental procedures were in compliance with Michigan State University and NIH guidelines and regulations for the care and use of laboratory animals. The grass rats were obtained from our institution's breeding colony, which was established over 15 years ago from animals trapped in East Africa. The animals were housed individually in Plexiglass cages (34 × 28 × 17 cm) with food (PMI Nutrition Prolab RMH 2000, Brentwood, MO) and water provided *ad libitum*. Under these laboratory conditions [11] as well as in their natural habitat [3] grass rats are clearly day active. At each of the 6 ZTs, groups of animals (n = 6/ZT) were deeply anesthetized with sodium pentobarbital and perfused transcardially with 0.01M phosphate buffered saline (PBS; pH 7.2) followed by 4% paraformaldehyde (Sigma, St Louis, MO USA). Brains were removed and post-fixed for 4 hr and then transferred to 20% sucrose overnight and then stored in cryoprotectant at − 20° C until sectioned (30μ; coronal plane) using a freezing microtome. ICC for PER1 was performed on every third section exactly as previously described [15] using the primary antibody against mPER1 #1177, made in rabbit and graciously provided by Dr. D.R. Weaver from the University of Massachusetts. Counts of PER1 immunoreactive cells were obtained using an Axioscop 2 plus Zeiss microscope at 40X magnification. One section was counted bilaterally for the CEA and the BLA and five sections were counted bilaterally for the BNST-OV by individuals blind to the times at which animals were sacrificed. The data were analyzed using SPSS 15 software. For the BNST-OV, the cell counts were analyzed using a two-way analysis of variance (ANOVA) with ZT as a between-subjects factor and level of section as a within-subject factor. For the BLA and CEA, one-way ANOVAs were used to evaluate the effects of ZT, since the cell counts for these areas came from a single section/animal. Significant main effects were followed by individual group comparisons using Fisher's LSD test. Differences were considered statistically significant when $p < 0.05$.

Results

Figure 1 summarizes the data for the three areas. For the BNST-OV, there was a significant main effect of ZT ($F = 15.8$, df =5, $p < 0.001$) with no significant effect of level of section and no interaction. Individual comparisons showed that PER1 expression was significantly higher at ZT 2 compared to all the other ZTs and also ZT6 was higher than ZT14 (Fig. 1A). There was a significant main effect of ZT on PER1 expression in the BLA ($F = 2.66$, df = 5, $p < 0.04$) with values for ZTs 2 and 6 significantly higher than those for ZT 18 and 22 (Fig. 1E). In the CEA, PER1 expression was at its highest at ZT 2, but the main effect of ZT just missed statistical significance ($p = 0.06$) (Fig. 1I).

Discussion

Nocturnal lab rats (*Rattus norvegicus*) show rhythmic expression of PER1 in the BNST-OV and the same was true for the diurnal grass rats (*Arvicanthis niloticus*) of the present study. However, the peak levels occur at opposite phases of the light/dark cycle in these two species. Specifically, in the BNST-OV of lab rats, rhythms in expression of PER1, peak between ZT 12 and ZT 18 [2], whereas in diurnal grass rats the rhythm of PER1 expression in the BNST-OV featured a salient peak at ZT 2. These results raise the questions of how the mechanisms regulating the phase of PER1 rhythms differ in the two species and how these

differences affect the circadian regulation of physiology and behavior in diurnal and nocturnal species.

PER1 expression in the BLA is also different in grass rats and lab rats. In male nocturnal lab rats with free access to food, there is no rhythm in PER1 expression in the BLA [2]. By contrast, in male grass rats the BLA showed a significant rhythm, with peak levels occurring early in the light phase. It is possible that these two species differ with respect to the influence of PER1 rhythms on the circadian control of behavioral functions mediated by the BLA, which include emotional learning and the effects of emotions on memory consolidation [9, 12]. The emergence of a rhythm of PER1 in may have contributed to the switch to a day-active behavioral profile in grass rats. In the CEA, lab rats do not show a rhythm of PER1 expression [2], whereas grass rats showed a near significant trend ($p < 0.06$) for an effect of time of day, which may become more robust with a larger sample size. Thus, the species differences seen in the BLA could generalize to the CEA, which also plays a key role in emotional learning [9].

In the BLA and CEA of lab rats, the expression of PER2 is rhythmic in both males and females [8, 14]. Thus, for these nocturnal rodents, the coupling between rhythms in expression of PER1 and PER2 typical of the SCN is absent in these regions of the amygdala. It is possible that PER2 rhythms are enough to sustain a circadian oscillator in the amygdala of lab rats (i.e. without rhythms in PER1). One interesting feature of PER2 rhythms in these two areas of the amygdala of lab rats is that they are out of phase with each other, such that the peak in the CEA at ZT13 coincides with the trough in the BLA [8]. Thus in lab rats, the pattern of PER2 expression in the BLA is similar to that of grass rats for PER1 in the same region of the amygdala. Data on the expression of PER2 in the amygdala of grass rats are needed to further evaluate the differences and possible similarities between the circadian systems of diurnal and nocturnal species.

In summary, we have described rhythms of PER1 in the amygdala and BNST-OV in male grass rats. Of particular interest are two key differences that emerged between these diurnal grass rats and nocturnal lab rats. One is that within the BLA rhythms in PER1 expression are present in grass rats (present data) and absent in lab rats [2]. Another is that the rhythm of PER1 expression in the BNST-OV of grass rats is about 12 hr out of phase in reference to the pattern reported for nocturnal lab rats [2]. Thus, the molecular oscillator in this key region appears to be coupled very differently to the SCN and to the light/dark cycle in these nocturnal and diurnal rodents. The data for the BNST-OV show that whereas rhythms in the expression of clock genes in the SCN are the same in nocturnal and diurnal species, the phase of extra-SCN molecular oscillators can be quite different between these two groups of animals (see also [7]). Species differences in the phase of the molecular oscillator of the BNST-OV may play a role in the generation of temporally inverted patterns of rhythmicity in autonomic functions, such as blood pressure and heart rate [6, 17], known to be controlled in part by this region of the forebrain.

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References

1. Amir S, Lamont EW, Robinson B, Stewart J. A circadian rhythm in the expression of PERIOD2 protein reveals a novel SCN-controlled oscillator in the oval nucleus of the bed nucleus of the stria terminalis. J Neurosci. 2004; 24:781–790. [PubMed: 14749422]
2. Angeles-Castellanos M, Mendoza J, Escobar C. Restricted feeding schedules phase shift daily rhythms of c-Fos and protein Per1 immunoreactivity in corticolimbic regions in rats. Neuroscience. 2007; 144:344–355. [PubMed: 17045749]
3. Blanchong JA, Smale L. Temporal patterns of activity of the unstriped Nile rat, Arvicanthis niloticus. Journal of Mammalogy. 2000; 81:595–599.
4. Challet E. Minireview: Entrainment of the suprachiasmatic clockwork in diurnal and nocturnal mammals. Endocrinology. 2007; 148:5648–5655. [PubMed: 17901231]
5. Cuesta M, Mendoza J, Clesse D, Pevet P, Challet E. Serotonergic activation potentiates light resetting of the main circadian clock and alters clock gene expression in a diurnal rodent. Exp Neurol. 2008; 210:501–513. [PubMed: 18190911]
6. Day HE, Badiani A, Uslaner JM, Oates MM, Vittoz NM, Robinson TE, Watson SJ Jr, Akil H. Environmental novelty differentially affects c-fos mRNA expression induced by amphetamine or cocaine in subregions of the bed nucleus of the stria terminalis and amygdala. J Neurosci. 2001; 21:732–740. [PubMed: 11160452]
7. Lambert CM, Weaver DR. Peripheral gene expression rhythms in a diurnal rodent. J Biol Rhythms. 2006; 21:77–79. [PubMed: 16461987]
8. Lamont EW, Robinson B, Stewart J, Amir S. The central and basolateral nuclei of the amygdala exhibit opposite diurnal rhythms of expression of the clock protein Period2. Proc Natl Acad Sci U S A. 2005; 102:4180–4184. [PubMed: 15746242]
9. LeDoux J. The amygdala. Curr Biol. 2007; 17:R868–R874. [PubMed: 17956742]
10. Mrosovsky N, Hattar S. Diurnal mice (Mus musculus) and other examples of temporal niche switching. J Comp Physiol A Neuroethol Sens Neural Behav Physiol. 2005; 191:1011–1024. [PubMed: 16163543]
11. Novak CM, Smale L, Nunez AA. Fos expression in the sleep-active cell group of the ventrolateral preoptic area in the diurnal murid rodent, Arvicanthis niloticus. Brain Res. 1999; 818:375–382. [PubMed: 10082823]
12. Pare D. Role of the basolateral amygdala in memory consolidation. Prog Neurobiol. 2003; 70:409–420. [PubMed: 14511699]
13. Peng ZC, Bentivoglio M. The thalamic paraventricular nucleus relays information from the suprachiasmatic nucleus to the amygdala: a combined anterograde and retrograde tracing study in the rat at the light and electron microscopic levels. J Neurocytol. 2004; 33:101–116. [PubMed: 15173635]
14. Perrin JS, Segall LA, Harbour VL, Woodside B, Amir S. The expression of the clock protein PER2 in the limbic forebrain is modulated by the estrous cycle. Proc Natl Acad Sci U S A. 2006; 103:5591–5596. [PubMed: 16554373]
15. Ramanathan C, Nunez AA, Martinez GS, Schwartz MD, Smale L. Temporal and spatial distribution of immunoreactive PER1 and PER2 proteins in the suprachiasmatic nucleus and peri-suprachiasmatic region of the diurnal grass rat (Arvicanthis niloticus). Brain Res. 2006; 1073–1074:348–358.
16. Reppert SM, Weaver DR. Molecular analysis of mammalian circadian rhythms. Annual Review of Physiology. 2001; 63:647–676.
17. Saper, CB. Central autonomic system. In: Paxinos, G., editor. The rat nervous system. second edition. Academic press; 1995. p. 107-128.

18. Schwartz, MD. Arvicanthis niloticus. East Lansing: Michigan State University; 2006. Neural substrates of diurnality in the Nile grass rat.

19. Silver R, LeSauter J, Tresco PA, Lehman MN. A diffusible coupling signal from the transplanted suprachiasmatic nucleus controlling circadian locomotor rhythms. Nature. 1996; 382:810–813. [PubMed: 8752274]

20. Smale L, Lee T, Nunez AA. Mammalian diurnality: some facts and gaps. J Biol Rhythms. 2003; 18:356–366. [PubMed: 14582852]

21. Smale L, Nunez AA, Schwartz MD. Rhythms in a diurnal brain. Biological Rhythm Research. 2008; 39:305–318.

22. Watts AG, Swanson LW, Sanchez-Watts G. Efferent projections of the suprachiasmatic nucleus: I. Studies using anterograde transport of Phaseolus vulgaris leucoagglutinin in the rat. J Comp Neurol. 1987; 258:204–229. [PubMed: 3294923]

23. Yamazaki S, Kerbeshian MC, Hocker CG, Block GD, Menaker M. Rhythmic properties of the hamster suprachiasmatic nucleus in vivo. J Neurosci. 1998; 18:10709–10723. [PubMed: 9852606]

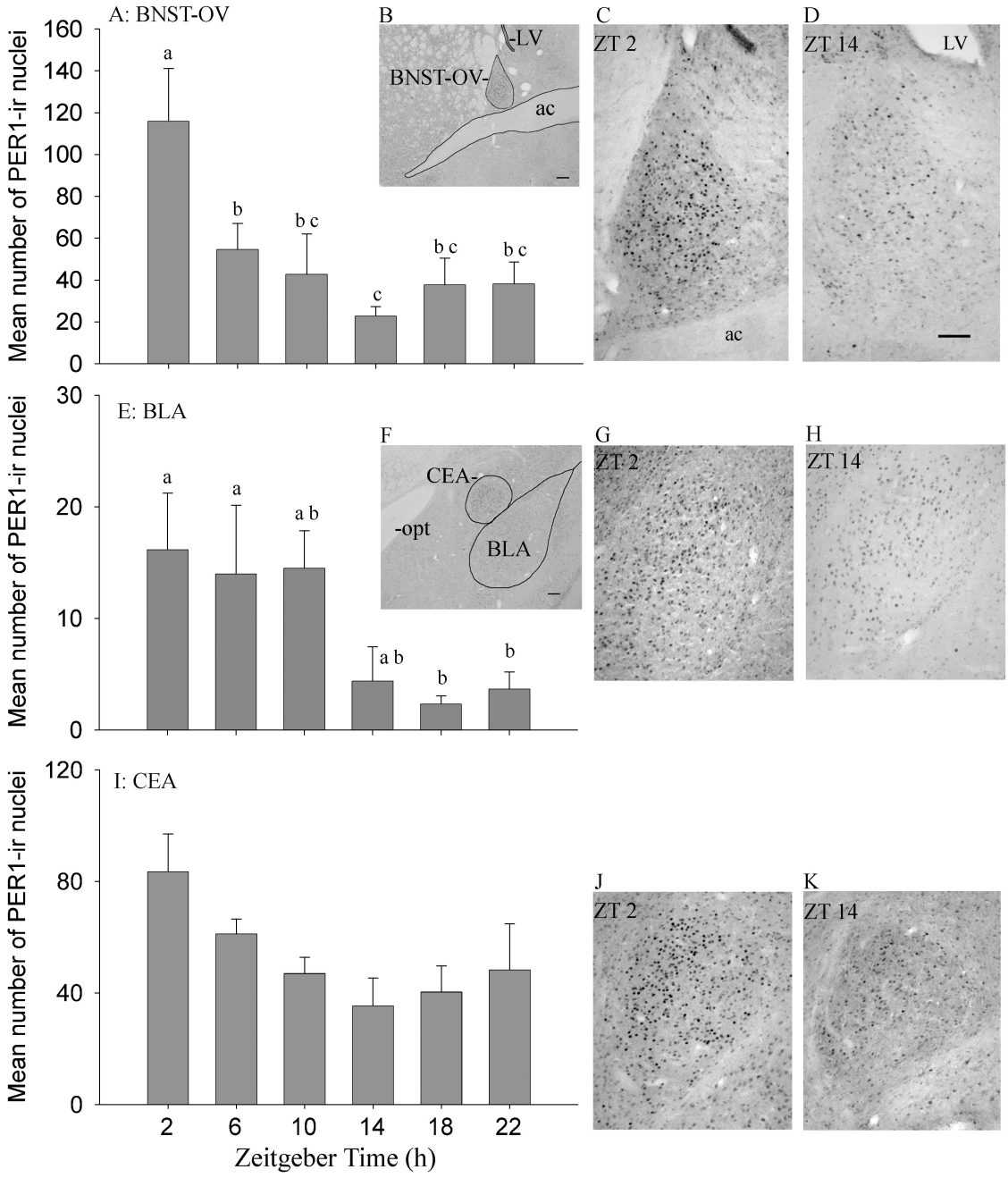

Figure 1.

Bar graphs showing the mean (±S.E.M.) number of cells immunoreactive for PER1 protein for each Zeitbeger time (ZT) in (A) the oval nucleus of the stria terminalis (BNST-OV; based on the average of 5 sections/animal), (E) the basolateral amygdala (BLA; based on total counts for one section/animal), and (I) the central amygdala (CEA; based on total counts for one section/animal). There was a significant main effect of ZT for both the BNST-OV and the BLA. For the CEA, the main effect of ZT missed statistical significance ($p = .06$). Significant differences ($p<0.05$) for the comparisons of individual ZTs for each

brain region are indicated by different letters. The inserts are photomicrographs showing key landmarks and line drawings depicting and the boundaries of the BNST-OV (B) and the BLA, and the CEA (F). The panels to the right show photomicrographs of the BSNT-OV (C, D), BLA (G, H), and CEA (J, K) of representative animals perfused at ZT 2 and ZT 14. Scale bars = 200μm for the inserts and 100μm for the photomicrographs. LV, lateral ventricle; ac, anterior commissure; opt, optic tract.