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RESPONSES OF BRAIN AND BEHAVIOR TO CHANGING DAY-LENGTH IN THE DIURNAL GRASS RAT (*ARVICANTHIS NILOTICUS*)

G. LEACH,[a] C. RAMANATHAN,[a,b] J. LANGEL[b] AND L. YAN[a,b]*

[a] *Department of Psychology, Michigan State University, East Lansing, MI 48824, USA*

[b] *Neuroscience Program, Michigan State University, East Lansing, MI 48824, USA*

Abstract—Seasonal affective disorder (SAD) is a major depressive disorder that recurs in the fall and winter when day-length gets short. It is well accepted that day-length is encoded by the principal circadian clock located in the suprachiasmatic nucleus (SCN), but very little is known about day-length encoding in diurnal mammals. The present study utilized the grass rat, *Arvicanthis niloticus*, to investigate how the circadian system responds to photoperiodic changes in a diurnal mammal that shows day-length-dependent mood changes. The animals were initially housed in equatorial day-length (12 h, EP) followed by either long (16 h, LP) or short (8 h, SP) photoperiods. The LP animals showed an expansion of the peak phase of the PER1 and PER2 rhythm in the SCN as well as an extended behavioral active phase. In contrast, the SP animals did not show any compression of their active phase nor a change in the peak duration of PER1 or PER2 expression, compared to those in EP. The results suggest that the circadian system in the diurnal grass rats is less responsive when day-length gets short compared to when it gets longer. The depression-like behaviors were assessed using sweet solution preference (SSP) and forced swimming test (FST). Animals in the SP group showed decreased SSP and increased immobility time in FST as compared to the EP group, suggesting a depressive phenotype. The present study serves as the first step toward exploring the role that the circadian system plays in SAD using a diurnal rodent model.
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Key words: photoperiods, diurnality, suprachiasmatic nucleus, PER1, PER2, seasonal affective disorder.

INTRODUCTION

Seasonal affective disorder (SAD) is one of the most striking seasonal changes observed in humans (Rosenthal et al., 1984). SAD is a major depressive disorder that involves recurring depressive episodes in the fall and winter when day-length gets progressively shorter (Howland, 2009). A link between SAD and circadian dysfunction has been suggested by the fact that light is the most salient synchronizer for circadian rhythms and that light therapy is often effective in treating SAD (Rosenthal et al., 1984; Pittendrigh, 1993). Several hypotheses, such as the photoperiod hypothesis, melatonin hypothesis and phase-shifting hypothesis, have been put forward that focus on the putative circadian underpinnings of this disorder (Levitan, 2007). However, how the circadian system in humans responds to photoperiodic changes is not well understood due to ethical and technical difficulties.

In mammals, the principal circadian clock is located in the suprachiasmatic nucleus (SCN) of the anterior hypothalamus (Klein et al., 1991). The SCN is also a fundamental element of a seasonal timer regulating annual rhythms in behavior and physiology (Goldman, 2001). Lesions of the SCN abolish both the daily rhythms in behavior and physiology, and the responses in reproductive function to changes in day-length (Rusak and Morin, 1976). Based on studies conducted mainly on nocturnal species, it is well accepted that in mammals, day-length information is encoded in the SCN (Hofman, 2004; Sumova et al., 2004; Johnston, 2005; Meijer et al., 2007, 2010). However, changes in day-length have a very different impact on behavioral rhythms between nocturnal and diurnal species (Refinetti, 2004). Therefore, a diurnal model should provide further insight into the photoperiodic responses of the circadian system in humans. The only work addressing the response of the SCN to photoperiodic changes was from Soay sheep, a seasonal breeding species living at temperate latitude (Lincoln et al., 2002). Almost nothing is known about how the circadian system responds to photoperiodic changes in non-seasonally breeding diurnal species.

To fill this gap, we utilized a diurnal equatorial rodent species, the unstriped Nile grass rat, *Arvicanthis niloticus*. The grass rats show diurnal patterns of general activity and do not show seasonal changes in their reproductive functions (McElhinny et al., 1997;

*Correspondence to: L. Yan, Department of Psychology & Neuroscience Program, 108 Giltner Hall, East Lansing, MI 48824, USA. Tel: +1-517-432-8189; fax: +1-517-432-2744.
E-mail address: yanl@msu.edu (L. Yan).

Abbreviations: ANOVA, analysis of variance; DAB, diaminobenzidine; EP, equatorial photoperiod; FST, forced swimming test; ICC, immunocytochemistry; LD, light/dark; LP, long photoperiod; SAD, seasonal affective disorder; SCN, suprachiasmatic nucleus; SP, short photoperiods; SP/AD, short photoperiods by advancing dark onset; SP/DL, short photoperiods by delaying light onset; SSP, sweet solution preference; ZT, Zeitgeber time.

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Blanchong et al., 1999). More interestingly, the grass rats show depression-like behaviors under winter-like lighting conditions, when the day-length is shortened to 5 h or the daytime light intensity is reduced to 50 lux (Ashkenazy-Frolinger et al., 2010; Yan et al., 2011). The light-dependent depressive responses suggest that the grass rat is a potential animal model of SAD (Workman and Nelson, 2011). In the present study, the daily profile of the clock proteins PER1 and PER2 in the SCN, activity rhythms and depression-like behaviors were examined in grass rats housed in different photoperiods. The results reveal unique patterns of response of the circadian system to changes in photoperiods that are associated with the emergence of depressive behaviors in this diurnal species.

EXPERIMENTAL PROCEDURES

Animals

The grass rats (*A. niloticus*) were from a laboratory colony established with animals imported from East Africa. These animals are equatorial; 12 h light is the typical photoperiod they experience in their natural habitat. Adult male grass rats ($n = 82$) were kept in a 12:12 h light/dark (LD) cycle (equatorial photoperiods, EP), with lights on at 6:00 and lights off at 18:00. Food (Prolab 2000 #5P06, PMI Nutrition LLC, MO, USA) and water were provided *ad libitum*. All experimental procedures were approved by the Michigan State University Animal Use and Care Committee.

Immunocytochemistry (ICC) of PER1 and PER2 in the SCN

Animals ($n = 48$) were randomly assigned into two groups, with one group moved to long photoperiods (LP, 16:8 h LD) and the other to short photoperiods (SP, 8:16 h LD), by either delaying or advancing the dark onset. Following 4 weeks of housing in their respective photoperiods, animals were anesthetized (pentobarbital, 200 mg/kg, ip) and perfused intracardially with saline followed by fixative (4% paraformaldehyde in 0.1 M phosphate buffer) every 4 h ($n = 4$/time point) throughout the day starting at 2 h after the dark onset time, which was defined as Zeitgeber time 12 (ZT12). Following the perfusion, brains were removed and post-fixed overnight in the same fixative, then cryoprotected in 20% sucrose for 2 days. ICC was carried out as described previously (Yan and Silver, 2004; Ramanathan et al., 2006). Alternate sets of coronal sections (30 μm) were cut through the entire SCN using a cryostat. Sections were incubated with either PER1 (1:5000, gift of Dr. D.R. Weaver, University of Massachusetts, MA, USA, now available as Millipore antibody AB2201) or PER2 (1:5000, Millipore antibody AB2202) for 48 h at 4 °C. Sections incubated with PER1 antibody were processed with avidin–biotin-immunoperoxidase technique using diaminobenzidine (DAB) as the chromogen. Sections incubated in PER2 antibody were processed with avidin–biotin-immunoperoxidase technique using DAB enhanced with 4% nickel sulfate as the chromogen. Brains from each time point were marked with unique cuts on the cortex, and sections at six different time points under same photoperiods were processed together as one time series. After the ICC reaction, sections were mounted on slides, dehydrated with alcohol rinses, cleared with xylene, air dried, and coverslipped with Permount (Fisher Scientific, NJ, USA).

Quantitative analysis of ICC results

For quantification, images of serial sections through the SCN were captured using a CCD video camera (CX9000, MBF bioscience, Williston, VT, USA) attached to a light microscope (Zeiss, Gottingen, Germany). Numbers of PER1- or PER2-immunoreactive (ir) nuclei were counted bilaterally in three mid-SCN sections using NIH Image J program. The counting regions for the SCN were delineated as in previous studies (Ramanathan et al., 2006). The counts from each side of the SCN was expressed as the percentage of the maximum count from the same time series, then the average counts from three bilateral regions were calculated to represent the value for each animal. A two-way analysis of variance (ANOVA) (time of the day × photoperiods) was first performed to assess the effect of photoperiods on the expression for each protein. If a significant interaction was detected, a one-way ANOVA followed by Tukey post hoc comparison was used to assess the effect of time of the day on the daily expression of PER1 or PER2. In all cases, differences were considered significant when $p < 0.05$.

Daily rhythms of locomotor activity

Male adult grass rats (4–6 month-old, $n = 18$) were singly housed in plexiglass cages (34 × 28 × 17 cm) with motion sensors mounted on top of the cage. General locomotor activities were recorded in 5-min bins using VitalView (Minimitter, Inc., Bend, OR, USA). After 1 week of baseline recording and habituating to the apparatus, animals were released from EP into either LP or SP for 4 weeks. The first two groups ($n = 6$/group) were moved to either LP or SP using the same protocol as that used in the ICC portion of the study, by delaying or advancing the dark onset. The third group ($n = 6$) was also moved to SP but through delaying the light onset. The duration of the active phase, stability of entrainment and phase angle for activity onset and offset were examined in each condition. No significant difference was observed between the two SP groups and the results were pooled together. Stability of entrainment was calculated as the standard deviation of the daily activity onset or offset time. Phase angles were calculated as the activity onset or offset time relative to the light onset or offset time, respectively. The quantification was based on data obtained during the last week of the recording and was analyzed using one-way ANOVA followed by Tukey post hoc comparisons.

Behavioral analysis of depression-like responses

Animals were singly housed in plexiglass cages (47 × 25 × 20 cm) and were assigned into two groups ($n = 8$/group). The first group remained in EP and the other was put into SP by advancing the dark onset (the same procedure as that used for our analysis of PER1). After 4 weeks, the two tests described below were performed to assess depression-like behaviors. There was a 3-day interval between the two tests.

Saccharin solution preference (SSP) test. Sweet-solution preferences are commonly used to detect anhedonia in animal models of depression (Willner et al., 1987; Papp et al., 1991). Grass rats were supplied with a bottle of 1.0% saccharin (Sigma, MI, USA) dissolved in tap water over 4 days, in addition to the regular supply of water and food. The saccharin concentration was selected based on previous experiments with diurnal sand rats and diurnal grass rats (Ashkenazy et al., 2009; Ashkenazy-Frolinger et al., 2010; Fonken et al., 2012). The amount of saccharin solution and water intake was measured every 24 h. SSP was calculated daily as a ratio of saccharin solution relative to total liquid (i.e. saccharin solution + water) consumed. The first 24 h of the exposure was used

as the training period for the animals to associate the location of the bottle and the taste, and the SSP over this period was defined as the baseline. There was no difference in the baseline preference between the two groups (EP: 55 ± 8%; SP: 54 ± 6%, *t*-test: $t_{14} = 0.16$, $p = 0.88$). If the sweet taste is a hedonic cue, an increase in the SSP in the following days is expected. Therefore, changes in the SSP for each of the subsequent testing days over the baseline were calculated for each animal, and were analyzed by two-way ANOVA followed by post hoc Tukey's test.

Forced swim test (FST). This test was performed as described in previous studies using mice (Porsolt et al., 1978) or grass rats (Ashkenazy-Frolinger et al., 2010; Fonken et al., 2012). The test involves two exposures, a pre-test and a test, to a cylindrical pool (35.5 cm tall × 30.5 cm diameter) filled with 25 cm of water maintained at 28–30 °C. Water in the tank was changed after each animal. For the pre-test training section, each grass rat was placed in the water tank for 10 min. Twenty-four hours later the animal was placed in the water again and videotaped for a 5-min test session. The time that animals spent immobile was scored from the videotapes by individuals blind to the experimental conditions of each animal. All the tests were performed between ZT6 and ZT10. The immobility was scored when the grass rats were passively floating without movements other than those necessary for keeping the head above water. Climbing was scored when the animals showed upward movements with their forepaws touching the wall of the water tank. Swimming was defined when the animals showed horizontal movements other than passive floating (Porsolt et al., 1978). The results were analyzed with unpaired *t*-test.

RESULTS

Photoperiodic effects on the expression of PER1 in the SCN

Daily rhythms of PER1 expression were observed in the SCN of animals housed in both LP and EP, with the highest staining seen around the time of dark onset (Fig. 1A). The number of PER1-ir nuclei was compared between the LP and EP groups (Fig. 1B). A two-way ANOVA revealed a significant effect of time and the interaction between time and photoperiod (photoperiod factor, $F_{1,46} = 0.03$, $p = 0.85$; time factor, $F_{5,46} = 14.2$, $p < 0.01$; interaction, $F_{5,46} = 4.43$, $p < 0.01$). The daily expression profile of PER1 was further analyzed in the LP and SP groups. In LP (Fig. 1B, left panel), one-way ANOVA revealed a significant effect of time ($F_{5,23} = 16.76$, $p < 0.01$), with a higher level of PER1 expression observed from ZT2 to 14 compared to the other time points (post hoc Tukey test, $p < 0.05$). In SP (Fig. 1B, right panel), the effect of time was also significant (one-way ANOVA, $F_{5,23} = 16.76$, $p < 0.01$), with the peak expression of PER1 found at ZT10 and 14 (post hoc Tukey test, $p < 0.05$).

Photoperiodic effects on the expression of PER2 in the SCN

Daily rhythms of PER2 expression were also observed in the SCN of animals housed in LP and EP (Fig. 2A). The number of PER2-ir nuclei was compared between the LP and EP groups (Fig. 2B). A two-way ANOVA revealed a significant effect of time and the interaction between time and photoperiod (photoperiod factor, $F_{1,46} = 0.44$, $p = 0.51$; time factor, $F_{5,46} = 13.38$, $p < 0.01$; interaction, $F_{5,46} = 19.55.43$, $p < 0.01$). The daily expression profile of PER2 was further analyzed in the LP and SP groups. In LP (Fig. 2B, left panel), one-way ANOVA revealed a significant effect of time ($F_{5,23} = 10.9$, $p < 0.01$), with a higher level of PER2 expression observed from ZT6 to 14 compared to the other time points (post hoc Tukey test, $p < 0.05$). In SP (Fig. 1B, right panel), the effect of time was also significant (one-way ANOVA, $F_{5,23} = 26.05$, $p < 0.01$), with the peak expression of PER2 found at ZT14 and 18 (post hoc Tukey test, $p < 0.05$).

Photoperiodic effects on daily rhythms in locomotor activity

Data on activity patterns of animals initially housed in EP and then exposed to either LP or SP are presented in Fig. 3. The animals showed clear diurnal patterns in their locomotor activity in all housing conditions. Following the transition from EP to LP (Fig. 3A), the animals showed slightly delayed activity onsets and an extended active phase. SP was produced using two different protocols: advancing the dark onset (SP/AD, Fig. 3B) or delaying the light onset (SP/DL, Fig. 3C). Following the advancement of dark onset, the animals gradually adjusted to the new LD cycle by advancing their behavioral rhythms until stable entrainment occurred approximately 2 weeks later. When day-length was shortened via a delay of the light onset (SP/DL, Fig. 3C), no apparent changes were observed in the daily activity pattern. Following the re-entrainment of daily rhythms to the new LD cycle, no significant difference was observed in the parameters examined between the SP/AD and SP/DL groups. Therefore, the results from the two groups were pooled together for further analysis. Quantitative analysis revealed a significant effect of photoperiod on the duration of active phase (Fig. 3D, one-way ANOVA, $F_{2,26} = 9.78$, $p < 0.01$). The active duration was higher in LP compared to that in EP and SP ($p < 0.05$, post hoc Tukey test). However, there was no significant difference in the active duration between the EP and the SP groups (Fig. 3D, $p > 0.05$, post hoc Tukey test). The stability of entrainment was analyzed for animals in each group. A significant effect of photoperiod was observed for both the onset time (Fig. 3E, one-way ANOVA, $F_{2,26} = 5.76$, $p < 0.01$) and offset time (Fig. 3F, one-way ANOVA, $F_{2,26} = 6.17$, $p < 0.01$). Post-hoc comparisons revealed that the onset time was less stable for the SP group compared to EP and LP groups (Fig. 3E, $p < 0.05$); while the offset time was more stable for the LP group (Fig. 3F, $p < 0.05$) without significant difference between the EP and SP groups (Fig. 3F, $p > 0.05$). A significant effect of photoperiod was also observed for the entrainment phase angles (Fig. 3G, one-way ANOVA, onset: $F_{2,26} = 48.38$, $p < 0.01$; offset: $F_{2,26} = 18.32$, $p < 0.01$). The SP group showed the largest phase angles while the LP group had the smallest phase angles of entrainment ($p < 0.01$, post hoc Tukey test).

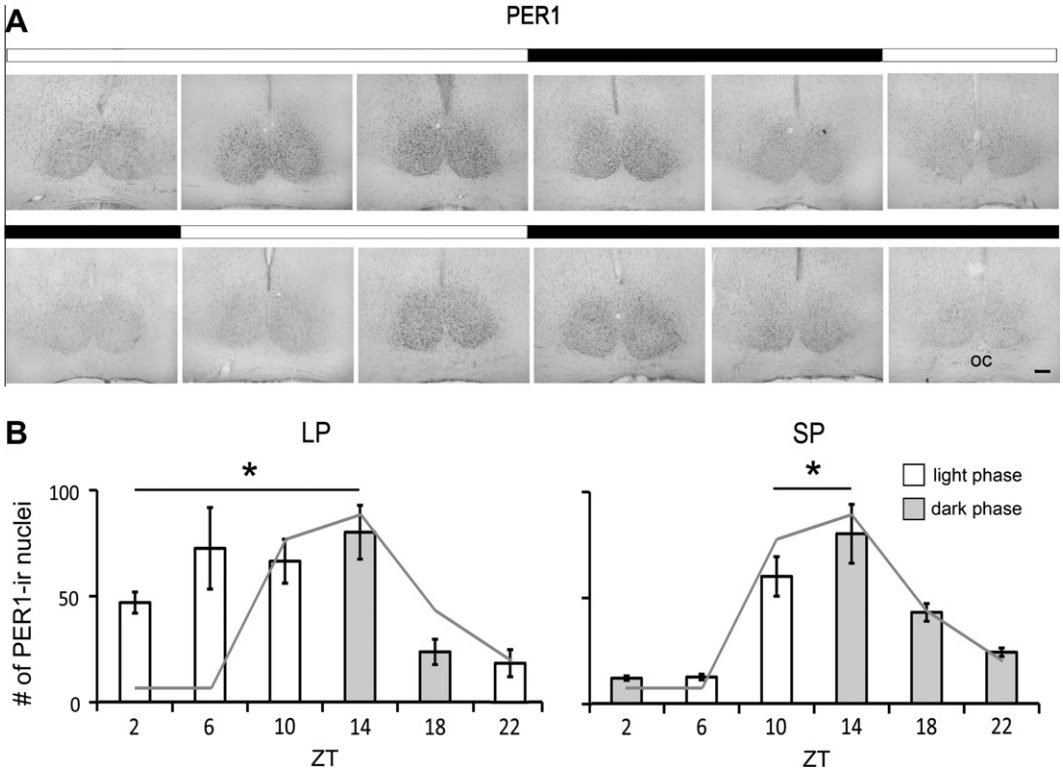

Fig. 1. PER1 expression in the SCN of grass rats in long- (LP, 16:8 h LD) or short-photoperiods (SP, 8:16 h LD). (A) Representative photomicrographs depicting PER1 expression in the SCN of grass rats housed in LP or SP. (B) Quantitative analysis showing the number of PER1-ir nuclei in the SCN of animals in LP (left panel) or SP (right panel). Data are presented as mean ± SEM, $n = 4$. The gray lines superimposed to the histograms show the daily profile of PER1-ir in the SCN of animals in EP. The data are re-plotted with permission using data previously published in Ramanathan et al. (2006). $^{*}p < 0.05$. Scale bar = 100 μm. oc, optic chiasm.

Depression-like behaviors induced by short photoperiods

To determine if the SP condition in the present study altered the affective state, the animals housed in SP and EP were first examined using the SSP test (Fig. 4A). The baseline SSP in the first 24 h of the exposure was not different between the EP and SP groups (EP: 55 ± 8%; SP: 54 ± 6%, *t*-test: $t_{14} = 0.16$, $p = 0.88$). Over the following 3 days of exposure, there was a marginally significant effect of photoperiod on the SSP, with diminished levels in the SP compared to the EP groups (Fig. 4A, $F_{1,42} = 3.82$, $p = 0.057$); this variable was not affected by day of testing ($F_{2,42} = 0.36$, $p = 0.70$) or by an interaction ($F_{2,42} = 0.06$; $p = 0.94$). The results suggest that the animals in the SP group did not develop a ''liking'' to the sweet taste as much as those in the EP group, indicating signs of anhedonia in the SP animals. The animals were also tested in the FST (Fig. 4B). There was a significant difference in the time of immobility and climbing between the EP and SP groups (*t*-test, $p < 0.05$). No difference was observed in the time spent swimming (*t*-test, $p > 0.05$).

DISCUSSION

The present study assessed the photoperiodic responses of the circadian system and depression-like behaviors in a diurnal non-seasonally breeding species, the unstriped Nile grass rat, *A. niloticus*. The results revealed that in long days, the diurnal grass rats show well-entrained behavioral rhythms and broadened peak durations of the PER1 and PER2 rhythm within the SCN, as expected from previous findings in nocturnal rodents and diurnal sheep (Nuesslein-Hildesheim et al., 2000; Lincoln et al., 2002; Sumova et al., 2004; VanderLeest et al., 2007). However, the grass rats did not respond effectively to a change from an equatorial photoperiod to short-days with respect to either the duration of their active phase in behavior or the elevated phase of PER1 or PER2 expression within the SCN. Furthermore, the grass rats housed in SP showed increased depressive responses compared to those in EP.

Light or photoperiod information is conveyed to the SCN through direct and indirect retinal projections (Moore, 1973). In habitats where the timing for lights on and off changes daily over the seasons, the circadian clock of organisms has to integrate photoperiodic information in order to maintain entrainment to the LD cycle (Pittendrigh and Takamura, 1989). Accumulating evidence on metabolic rate, electrical activity and clock gene expressions indicates that the day-length information is encoded by the SCN (Schwartz et al., 2001; Hofman, 2004; Sumova et al., 2004; Johnston, 2005; Meijer et al., 2007, 2010). To explore the responses of the SCN to photoperiodic changes in grass rats, the expression of PER1 and PER2, the

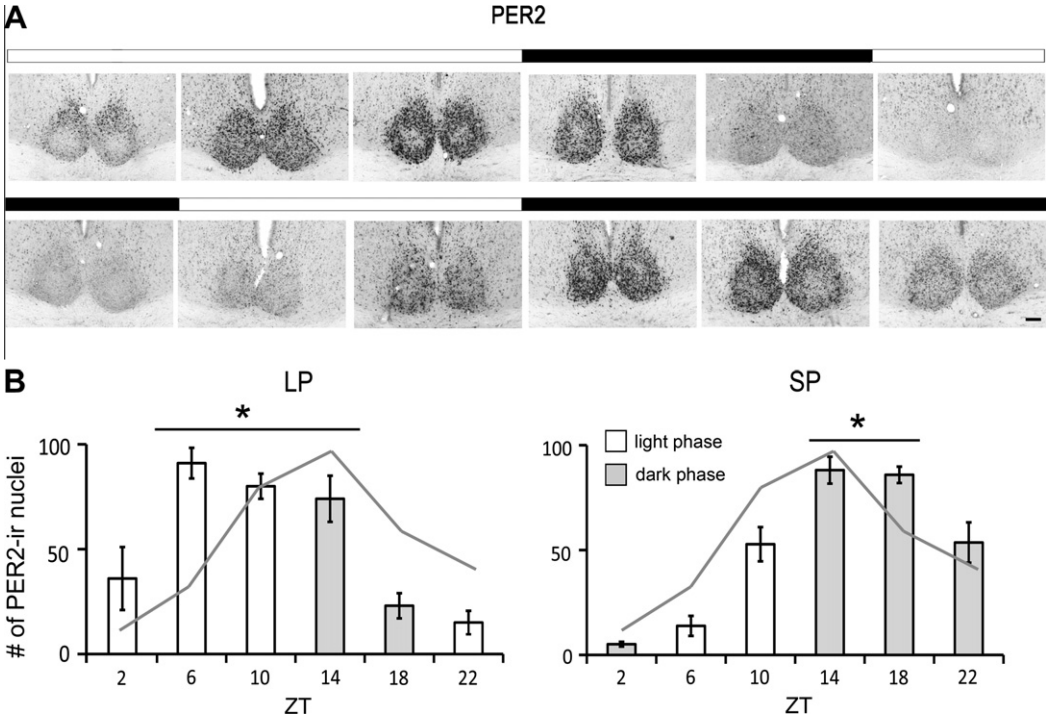

Fig. 2. PER2 expression in the SCN of grass rats in long- (LP, 16:8 h LD) or short-photoperiods (SP, 8:16 h LD). (A) Representative photomicrographs depicting PER2 expression in the SCN of grass rats housed in LP or SP. (B) Quantitative analysis showing the number of PER2-ir nuclei in the SCN of animals in LP (left panel) or SP (right panel). Data are presented as mean ± SEM, $n = 4$. The gray lines superimposed to the histograms show the daily profile of PER2-ir in the SCN of animals in EP. The data are re-plotted with permission using data previously published in Ramanathan et al. (2006). $^{*}p < 0.05$. Scale bar = 100 μm.

protein product of two core clock genes (Per1, Per2) were analyzed (Figs. 1 and 2). The results revealed time-of-day-dependent patterns of PER1 and PER2 in both long- and short-photoperiods. The daily rhythm of PER1 and PER2 in the SCN of grass rats under 12:12 h LD cycle (EP) has been characterized in a previous study (Ramanathan et al., 2006) and the data are re-plotted as line graphs in Figs. 1 and 2 to ease visual comparison. For PER1, an expansion in the peak duration was observed in LP (Fig. 1B, left panel), while the peak expression in SP was observed at ZT10 and 14, the same as that seen in EP, without showing apparent compression in the peak duration (Fig. 1B, right panel). The expression of PER2 under EP was high at ZT10 to 18, with a 40% reduction at ZT18 compared to ZT14 (Ramanathan et al., 2006). In LP, the number of PER2-ir nuclei reached the peak at ZT6 and remained the highest level until ZT14 before declining at ZT18, indicating an expansion in the elevated phase (Fig. 2B, left panel). In SP, the expression of PER2 started to increase at ZT10, was highest at ZT14 and 18 and declined at ZT22 (Fig. 2B, right panel). There was a delay in the peak level expression of PER2 in SP compared to that in EP, but the duration of the peak phase seemed to be similar to that in EP. Although the expression of PER1 and PER2 are generally synchronized under EP (Field et al., 2000; Ramanathan et al., 2006), slightly different responses of these proteins to photoperiodic changes have been observed previously (Nuesslein-Hildesheim et al., 2000). In Siberian hamsters housed under LP or SP as in the present study, the peak duration in LP was longer for PER1 compared to PER2 (15 vs 9 h), and there was a delay in the raising phase of PER2 compared to PER1 in SP (Nuesslein-Hildesheim et al., 2000). Consistently, different responses in the peak duration and phase between PER1 and PER2 were also observed in diurnal grass rats (present study). In addition to Per1 and Per2, numerous other core clock genes i.e. Cry1, Cry2, Clock, Bmal1, CK1ε, Rev-erbα, etc. have been identified (Reppert and Weaver, 2001; Ko and Takahashi, 2006). These genes and their protein products constitute the molecular clock through an interlocked transcriptional/translational feedback loop (Hastings et al., 2003; Okamura, 2004; Ko and Takahashi, 2006). While the expression of a single component in this inter-locked feedback loop could serve as an indicator for the overall circadian oscillation of the molecular clock (Yamazaki et al., 2000; Yamaguchi et al., 2001; Yoo et al., 2004), analyzing other clock genes and their protein products in addition to PER1 and PER2 could reveal a more completed picture of the photoperiodic responses of the circadian oscillations within the SCN. It should also be noted that the temporal resolution of sampling (4 h) of the present study put some constraints for detecting small differences between patterns of PER expression in the SP and EP groups. Even with these caveats, the results described here clearly show that PER proteins, especially PER1, a key element of the molecular oscillator within the SCN of grass rats is considerably

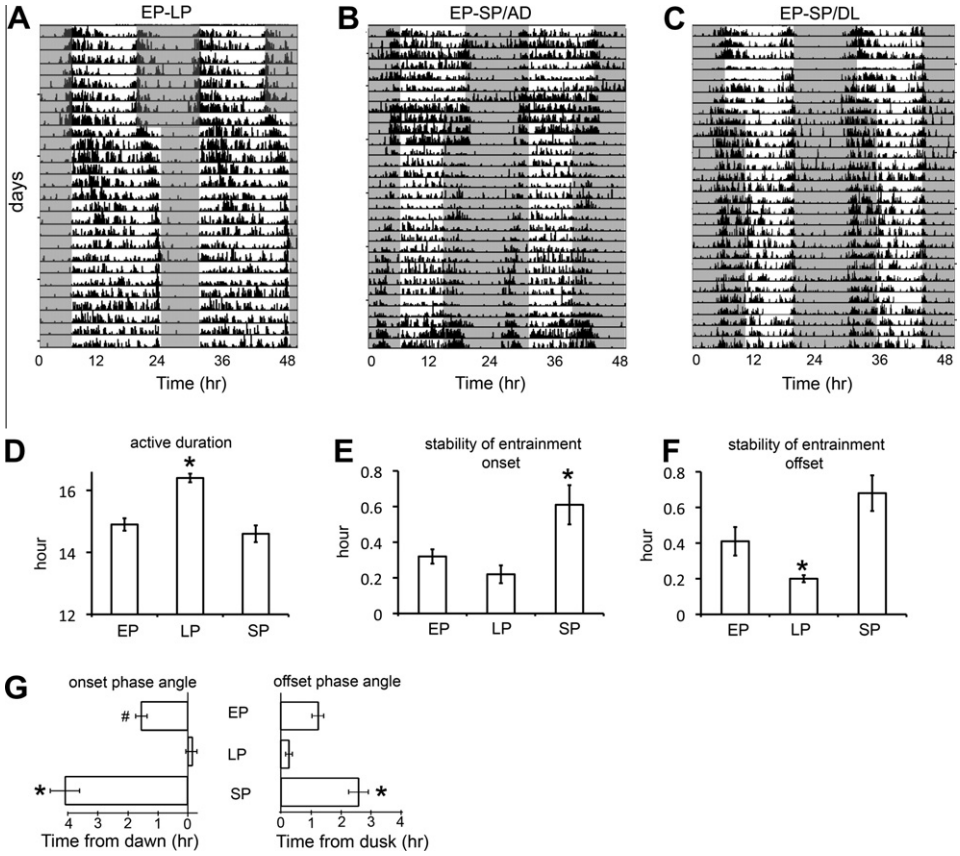

Fig. 3. Behavioral responses of grass rats to photoperiodic changes. (A–C) Representative double-plotted actograms of three grass rats that were housed initially in equatorial photoperiods (EP,12:12 h light/dark) and then to long photoperiods (LP 16:8 h light/dark, A), to short photoperiods by advancing dark onset (SP/AD, 8:16 h light/dark, B) or to short photoperiods by delaying light onset (SP/DL, 8:16 h light/dark, C). The bar graphs show the duration of active phase (D), the stability of entrainment for activity onset (E) and offset (F), the entrainment phase angle for activity onset and offset (G) of animals under each photoperiod. Gray shadow indicates the dark phase. Data are presented as mean ± SEM, $n = 18$ for EP, $n = 6$ for LP and $n = 12$ for SP, respectively. $^{*,\#}p < 0.05$.

less responsive when day-length gets shorter than when it gets longer.

The asymmetrical responses of the circadian system to LP and SP were also reflected on the rhythms of locomotor activity (Fig. 3). In contrast to the animals in LP that showed an extended daily active phase, those in SP did not show any compression in their active phase compared to animals in EP (Fig. 3A–D). The expansion and compression of the active phase in short and long photoperiods, respectively, has been well documented in nocturnal species (Nuesslein-Hildesheim et al., 2000; Lincoln et al., 2002; Refinetti, 2004; Sumova et al., 2004; VanderLeest et al., 2007). In general, diurnal species show opposite responses and expand their active phase in long days and compress it in short days (Sulzman et al., 1982; Challet et al., 2002; Lincoln et al., 2002; Lahmam et al., 2008). In a previous study in which grass rats were given access to running-wheels, the duration of the active phase also showed an incomplete compression (Refinetti, 2004). The grass rats are equatorial and their circadian system has been shaped by the 12:12 LD cycle in their natural environment. It is likely that there is a ‘‘floor effect’’ in their photoperiodic responses of daily rhythms, such that the duration of general activity in the grass rats cannot be further compressed when day-length changed from 12 to 8 h. In contrast, when day-length changed from 12 to 16 h, the active duration expanded to the entire light phase within a few days. The results seem to suggest that the circadian system in grass rats is less adaptive to SP compared to LP. It is noteworthy that when the animals were transferred from EP to SP, different protocols produced different behavioral responses (Fig. 3B, C). When the change occurred via an advance in dark onset there was a gradual shift in the times of both activity onset and offset, whereas when the light onset was delayed the daily behavioral rhythms were not affected. The results are consistent with the findings of Dr. Refinett’s study (Refinetti, 2004), and suggest that non-parametric entrainment by phase delaying light exposure around light offset is critical for this species. The parameters related to the entrainment of daily rhythms were also analyzed (Fig. 3E–G). The results show that in laboratory conditions, the daily rhythm of this equatorial species is best entrained in LP as indicated by the stability (precise onset and offset time) and the phase angle of entrainment. On the other hand, the entrainment in SP is less stable especially with the activity onset time, which is more variable or less precise compared to that in EP and LP conditions

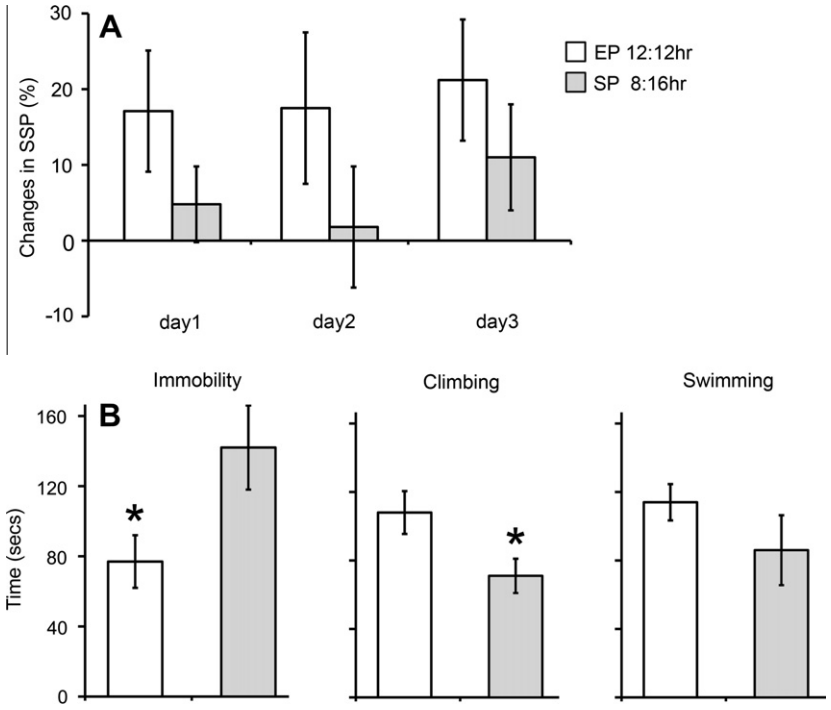

Fig. 4. Short day-length induces depression-like behavioral responses in grass rats. (A) Changes in the sweet solution preference (SSP) in animals housed in SP and EP. (B) Duration for immobility, climbing and swimming behaviors during FST. Data are presented as mean ± SEM (n = 8). $^{*}p < 0.05$.

(Fig. 3E). The SP group also showed larger phase angles compared to the other groups, indicating the longer duration of activities into the dark phase (Fig. 3G).

The results of PER1 expression in the SCN and daily locomotor activities collectively suggest that the circadian system in the grass rats is less effective in making adaptive changes to short days compared to long days. We then hypothesized that the inadequate response of the circadian system to short days could be one of the mechanisms underlying depressive behavior. It has been previously reported that, in grass rats, a 5:19 h LD cycle induced increased anhedonic responses and increased immobility time in forced swimming test compared to those housed in EP (Ashkenazy-Frolinger et al., 2010). Nonetheless, whether an 8:16 h LD could produce similar effects was unknown, and therefore, was tested in the present study. Due to the high variability between individual animals, the effect of day-length on sweet preference was only marginally significant (Fig. 4A, $p = 0.057$). The FST revealed significant differences in immobility and climbing times between the EP and SP groups (Fig. 4B). The results collectively confirmed the depression-like behavioral responses for animals housed under 8:16 h LD condition (Fig. 4). These typical depression-like behaviors associated with short days observed in grass rats mirror the depression symptoms seen in human SAD patients (Rosenthal et al., 1984), suggesting a great potential for the grass rats as an animal model for SAD (Workman and Nelson, 2011). Although many hypotheses focusing on the chronobiological mechanisms have been proposed to explain the etiology of SAD, how seasonal photoperiodic changes affect the brain clock in humans and more specifically in SAD patients remains a mystery. The present study examined the responses of the circadian system and depression-like behaviors following the same photoperiodic manipulation. Our results show that following 4 weeks in SP, the grass rats developed depression-like behaviors compared to those in EP. A question that needs to be addressed is *why* the short-day animals show increased depression-like behaviors compared to the controls in EP. In other words, what are the roles played by the circadian system or the SCN in SAD? One hypothesis is that the depressive behaviors are caused by mechanisms independent from the SCN or circadian system (LeGates et al., 2012). Alternatively, although not mutually exclusive, the depression could be derived from a mismatch between the internal timing generated by the SCN and the environment. One of the advantages of having an internal clock is that the organisms can predict and prepare for the changes in the environment (Pittendrigh, 1960). In short days when the day-length information is not effectively integrated into the daily time-keeping function of the SCN, the animals would anticipate dawn and dusk at a time prior to the actual dawn and later than the actual dusk. Although the underlying neural mechanisms are yet to be discovered, it has been shown that when the internal clock is running at a time in conflict with the environment, it can lead to many health consequences including mood changes (Abbott, 2003; Hastings et al., 2003; Haus and Smolensky, 2006).

In summary, using the equatorial diurnal grass rats *A. niloticus*, the present study showed that the circadian system of the grass rats is less responsive to short photoperiods compared to long photoperiods, and the animals in short days develop depression-like behaviors. The neuropathology of SAD is not well understood, but likely involves many factors beyond the circadian system (Levitan, 2007). Developing an adequate animal model will help to provide insights into the neurological mechanisms underlying this condition (Flaisher-Grinberg et al., 2011; Workman and Nelson, 2011; Kronfeld-Schor and Einat, 2012).

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REFERENCES

Abbott A (2003) Restless nights, listless days. Nature 425:896–898.

Ashkenazy T, Einat H, Kronfeld-Schor N (2009) We are in the dark here: induction of depression- and anxiety-like behaviours in the diurnal fat sand rat, by short daylight or melatonin injections. Int J Neuropsychopharmacol 12:83–93.

Ashkenazy-Frolinger T, Kronfeld-Schor N, Juetten J, Einat H (2010) It is darkness and not light: depression-like behaviors of diurnal unstriped Nile grass rats maintained under a short photoperiod schedule. J Neurosci Methods 186:165–170.

Blanchong JA, McElhinny TL, Mahoney MM, Smale L (1999) Nocturnal and diurnal rhythms in the unstriped Nile rat, *Arvicanthis niloticus*. J Biol Rhythms 14:364–377.

Challet E, Pitrosky B, Sicard B, Malan A, Pevet P (2002) Circadian organization in a diurnal rodent, *Arvicanthis ansorgei* Thomas 1910: chronotypes, responses to constant lighting conditions, and photoperiodic changes. J Biol Rhythms 17:52–64.

Field MD, Maywood ES, O'Brien J, Weaver DR, Reppert S, Hastings MH (2000) Analysis of clock proteins in mouse SCN demonstrates phylogenic divergence of the circadian clockwork and resetting mechanisms. Neuron 25:437–447.

Flaisher-Grinberg S, Gampetro DR, Kronfeld-Schor N, Einat H (2011) Inconsistent effects of photoperiod manipulations in tests for affective-like changes in mice: implications for the selection of appropriate model animals. Behav Pharmacol 22:23–30.

Fonken LK, Kitsmiller E, Smale L, Nelson RJ (2012) Dim nighttime light impairs cognition and provokes depressive-like responses in a diurnal rodent. J Biol Rhythms 27:319–327.

Goldman BD (2001) Mammalian photoperiodic system: formal properties and neuroendocrine mechanisms of photoperiodic time measurement. J Biol Rhythms 16:283–301.

Hastings MH, Reddy AB, Maywood ES (2003) A clockwork web: circadian timing in brain and periphery, in health and disease. Nat Rev Neurosci 4:649–661.

Haus E, Smolensky M (2006) Biological clocks and shift work: circadian dysregulation and potential long-term effects. Cancer Causes Control 17:489–500.

Hofman MA (2004) The brain's calendar: neural mechanisms of seasonal timing. Biol Rev Camb Philos Soc 79:61–77.

Howland RH (2009) An overview of seasonal affective disorder and its treatment options. Phys Sportsmed 37:104–115.

Johnston JD (2005) Measuring seasonal time within the circadian system: regulation of the suprachiasmatic nuclei by photoperiod. J Neuroendocrinol 17:459–465.

Klein DC, Moore RY, Reppert SM, editors. Suprachiasmatic nucleus. The mind's clock. New York: Oxford University Press.

Ko CH, Takahashi JS (2006) Molecular components of the mammalian circadian clock. Hum Mol Genet 15:R271–R277. Spec. No. 2.

Kronfeld-Schor N, Einat H (2012) Circadian rhythms and depression: human psychopathology and animal models. Neuropharmacology 62:101–114.

Lahmam M, El M'rabet A, Ouarour A, Pevet P, Challet E, Vuillez P (2008) Daily behavioral rhythmicity and organization of the suprachiasmatic nuclei in the diurnal rodent, *Lemniscomys barbarus*. Chronobiol Int 25:882–904.

LeGates TA, Altimus CM, Wang H, Lee HK, Yang S, Zhao H, Kirkwood A, Weber ET, Hattar S (2012) Aberrant light directly impairs mood and learning through melanopsin-expressing neurons. Nature 491:594–598.

Levitan RD (2007) The chronobiology and neurobiology of winter seasonal affective disorder. Dialogues Clin Neurosci 9:315–324.

Lincoln G, Messager S, Andersson H, Hazlerigg D (2002) Temporal expression of seven clock genes in the suprachiasmatic nucleus and the pars tuberalis of the sheep: evidence for an internal coincidence timer. Proc Natl Acad Sci U S A 99:13890–13895.

McElhinny TL, Smale L, Holekamp KE (1997) Patterns of body temperature, activity, and reproductive behavior in a tropical murid rodent, *Arvicanthis niloticus*. Physiol Behav 62:91–96.

Meijer JH, Michel S, Vanderleest HT, Rohling JH (2010) Daily and seasonal adaptation of the circadian clock requires plasticity of the SCN neuronal network. Eur J Neurosci 32:2143–2151.

Meijer JH, Michel S, Vansteensel MJ (2007) Processing of daily and seasonal light information in the mammalian circadian clock. Gen Comp Endocrinol 152:159–164.

Moore RY (1973) Retinohypothalamic projection in mammals: a comparative study. Brain Res 49:403–409.

Nuesslein-Hildesheim B, O'Brien JA, Ebling FJ, Maywood ES, Hastings MH (2000) The circadian cycle of mPER clock gene products in the suprachiasmatic nucleus of the Siberian hamster encodes both daily and seasonal time. Eur J Neurosci 12:2856–2864.

Okamura H (2004) Clock genes in cell clocks: roles, actions, and mysteries. J Biol Rhythms 19:388–399.

Papp M, Willner P, Muscat R (1991) An animal model of anhedonia: attenuation of sucrose consumption and place preference conditioning by chronic unpredictable mild stress. Psychopharmacology (Berl) 104:255–259.

Pittendrigh CS (1960) Circadian rhythms and the circadian organization of living things. In: Chovnick A, editor. Cold Spring Harbor symp quant biol XXV: biological clocks. New York: Cold Spring Harbor. p. 159–184.

Pittendrigh CS (1993) Temporal organization: reflections of a Darwinian clock-watcher. Annu Rev Physiol 55:17–54.

Pittendrigh CS, Takamura T (1989) Latitudinal clines in the properties of a circadian pacemaker. J Biol Rhythms 4:217–235.

Porsolt RD, Anton G, Blavet N, Jalfre M (1978) Behavioural despair in rats: a new model sensitive to antidepressant treatments. Eur J Pharmacol 47:379–391.

Ramanathan C, Nunez AA, Martinez GS, Schwartz MD, Smale L (2006) 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 1073–1074:348–358.

Refinetti R (2004) Daily activity patterns of a nocturnal and a diurnal rodent in a seminatural environment. Physiol Behav 82:285–294.

Reppert SM, Weaver DR (2001) Molecular analysis of mammalian circadian rhythms. Annu Rev Physiol 63:647–676.

Rosenthal NE, Sack DA, Gillin JC, Lewy AJ, Goodwin FK, Davenport Y, Mueller PS, Newsome DA, Wehr TA (1984) Seasonal affective disorder. A description of the syndrome and preliminary findings with light therapy. Arch Gen Psychiatry 41:72–80.

Rusak B, Morin LP (1976) Testicular responses to photoperiod are blocked by lesions of the suprachiasmatic nuclei in golden hamsters. Biol Reprod 15:366–374.

Schwartz WJ, de la Iglesia HO, Zlomanczuk P, Illnerova H (2001) Encoding le quattro stagioni within the mammalian brain: photoperiodic orchestration through the suprachiasmatic nucleus. J Biol Rhythms 16:302–311.

Sulzman FM, Fuller CA, Moore-Ede MC (1982) Circadian entrainment of the squirrel monkey by extreme photoperiods: interactions between the phasic and tonic effects of light. Physiol Behav 29:637–641.

Sumova A, Bendova Z, Sladek M, Kovacikova Z, Illnerova H (2004) Seasonal molecular timekeeping within the rat circadian clock. Physiol Res 53(Suppl. 1):S167–S176.

VanderLeest HT, Houben T, Michel S, Deboer T, Albus H, Vansteensel MJ, Block GD, Meijer JH (2007) Seasonal encoding by the circadian pacemaker of the SCN. Curr Biol 17:468–473.

Willner P, Towell A, Sampson D, Sophokleous S, Muscat R (1987) Reduction of sucrose preference by chronic unpredictable mild stress, and its restoration by a tricyclic antidepressant. Psychopharmacology (Berl) 93:358–364.

Workman JL, Nelson RJ (2011) Potential animal models of seasonal affective disorder. Neurosci Biobehav Rev 35:669–679.

Yamaguchi S, Kobayashi M, Mitsui S, Ishida Y, van der Horst GT, Suzuki M, Shibata S, Okamura H (2001) View of a mouse clock gene ticking. Nature 409:684.

Yamazaki S, Numano R, Abe M, Hida A, Takahashi R, Ueda M, Block GD, Sakaki Y, Menaker M, Tei H (2000) Resetting central and peripheral circadian oscillators in transgenic rats. Science 288:682–685.

Yan L, Silver R (2004) Resetting the brain clock: time course and localization of mPER1 and mPER2 protein expression in suprachiasmatic nuclei during phase shifts. Eur J Neurosci 19:1105–1109.

Yan L, Leach G, Adidharma W, Kott J (2011) A diurnal rodent model for SAD. In: Neuroscience meeting planner. Washington, DC: Society for Neuroscience.

Yoo SH, Yamazaki S, Lowrey PL, Shimomura K, Ko CH, Buhr ED, Siepka SM, Hong HK, Oh WJ, Yoo OJ, Menaker M, Takahashi JS (2004) PERIOD2::LUCIFERASE real-time reporting of circadian dynamics reveals persistent circadian oscillations in mouse peripheral tissues. Proc Natl Acad Sci U S A 101:5339–5346.

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