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ORIGINAL ARTICLE

Differential susceptibility of BALB/c, C57BL/6N, and CF1 mice to photoperiod changes

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Objective: Circadian disturbances common to modern lifestyles have been associated with mood disorders. Animal models that mimic such rhythm disturbances are useful in translational research to explore factors contributing to depressive disorders. This study aimed to verify the susceptibility of BALB/c, C57BL/6N, and CF1 mice to photoperiod changes.
Methods: Thermochron iButtons implanted in the mouse abdomen were used to characterize temperature rhythms. Mice were maintained under a 12:12 h light-dark (LD) cycle for 15 days, followed by a 10:10 h LD cycle for 10 days. Cosinor analysis, Rayleigh *z* test, periodograms, and Fourier analysis were used to analyze rhythm parameters. Paired Student's *t* test was used to compare temperature amplitude, period, and power of the first harmonic between normal and shortened cycles.
Results: The shortened LD cycle significantly changed temperature acrophases and rhythm amplitude in all mouse strains, but only BALB/c showed altered period.
Conclusion: These findings suggest that BALB/c, the preferred strain for stress-induced models of depression, should also be favored for exploring the relationship between circadian rhythms and mood. Temperature rhythm proved to be a useful parameter for characterizing rhythm disruption in mice. Although disruption of temperature rhythm has been successfully documented in untethered mice, an evaluation of desynchronization of other rhythms is warranted.

Keywords: Circadian dysregulation; mood disorders; core body temperature rhythms; mice

Introduction

A significant number of biological processes other than the sleep-wake cycle follow circadian rhythms. Because central and peripheral oscillators (found in the suprachiasmatic nucleus in mammals and in organs such as the spleen and lungs, respectively) differentially adapt to environmental changes, there can be desynchronization of rhythms.[1,2] Changes in rhythmicity, such as those induced by jet lag or shift work, have been shown to result in or contribute to disorganization of metabolic and endocrine rhythms,[3] increased susceptibility to gastrointestinal disorders,[4] obesity and diabetes,[5] decreased fertility and increased risk for some cancers.[6] Evidence that circadian desynchronization is associated with a variety of health issues, along with the recently identified changes in clock genes and their transcriptional regulators,[7] underscores the importance of further understanding human biological rhythms and the effects of desynchronization on specific disorders. Of particular interest to this study are the connections between disturbances in circadian rhythms and sleep and neuropsychiatric disorders.[8-10]

Translational research calls for animal models that employ known etiological and risk factors that can result in measurable behavioral and/or physiological changes, preferably mimicking those of the human disease of interest.[11] Mice have been extensively used in behavioral research both to predict psychoactive properties of molecular entities in research and development of drug discovery programs and to understand the pathophysiology of central nervous system (CNS) disorders.[12,13] Despite differences in endogenous periods among species, rodent models are suitable for studying the effects of rhythm disruption on behavior and physiology.[2,14,15]

Body temperature can be influenced by external and internal signals.[16] Because core temperature rhythm is less susceptible to change than the sleep-wake cycle, it is often used as a 'marker rhythm', providing a benchmark against which other rhythms can be tested for (de)synchronization. Aiming to incorporate circadian rhythm changes into experimental models of mood disorders, the present study was set up to compare the susceptibility

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of commonly used mouse strains (CF1, BALB/c, and C57BL/6N) to changes in photoperiod assessed by continuous body core temperature recordings.

Methods

Animals

Experiments were performed with 2-month-old male BALB/c, C57BL/6N, and CF1 mice (n=5/strain) obtained from the Fundação Estadual de Produção e Pesquisa em Saúde (FEPPS, Porto Alegre, RS, Brazil). Mice were housed in the animal facility of our institution under controlled conditions of temperature (22±1°C) and light intensity (12:12 h light-dark [LD] cycle) and allowed free access to food (Nuvilab CR1; Nuvital, Curitiba, PR, Brazil) and water for two weeks before the experiments. All procedures were carried out according to institutional policies on animal use in research. The study was approved by the Ethics Committee of the institution (protocol no. 22308).

iButtons

Thermochron iButtons (DS1921H-F5#; Dallas Semiconductor, Dallas, TX, USA) were used to record and store time and temperature data. Each iButton weighs approximately 3.3 g and has a diameter of 17.5 mm. For the purposes of this study, iButtons were programmed to record core body temperature every 30 min for 29 consecutive days.

Surgery

An iButton was implanted into the abdominal cavity of each mouse. The mice were anesthetized with intraperitoneal ketamine/xylazine (Cetamin®/Xilazin®; Syntec, Cotia, SP, Brazil) at a dose of 100/10 mg/kg body weight for CF1 strains and 70/7 mg/kg body weight for BALB/c and C57BL/6N strains. The skin of the abdomen was shaved, and the area was cleaned with 70% alcohol. A longitudinal incision of approximately 2 cm was made along the midline, and the iButton, previously cleaned with 70% alcohol, was inserted into the abdominal cavity. The incision was closed with 4-0 nylon sutures. Animals were kept warm until they recovered from anesthesia. Tramadol (Tramal®, Hipolabor, Sabará, MG, Brazil) was administered subcutaneously (1 mg/kg) immediately after surgery and the day after surgery. The mice were housed individually in cages after surgery until the end of the experiment, when they were euthanized by cervical dislocation and their iButtons removed.

Photoperiod manipulation

After surgery, all mice were maintained under a 12:12 h LD cycle for 19 days (the first 4 days of recording were considered recovery time, and the data were discarded), followed by a 10:10 h LD cycle for 10 days.

Locomotion

Locomotion was assessed between 2 p.m. and 4 p.m. the day before surgery and 10 days after surgery using activity cages (45 × 25 × 20 cm; Albarsch Electronic Systems, Porto Alegre, RS, Brazil) equipped with four parallel photocells. The number of crossings was recorded for 15 min, with the first 5 min regarded as exploratory activity and the final 10 min as locomotor activity.[17]

Statistical analysis

Cosinor analysis was used to evaluate temperature rhythm parameters (amplitude and acrophases) in each group of mice. The Rayleigh *z* test was used to analyze the acrophase of each group using individual vectors to determine the average vector for each group. Periodograms were used to identify the period of statistically significant oscillations. Fourier analysis was used to determine the power of the first harmonic. A chronobiology software (El Temps, Prof. Antoni Díez Noguera©, University of Barcelona, Barcelona, CA, Spain) was used for rhythm analysis.

Mean differences in temperature rhythm amplitude and period during exposure to 12:12 and 10:10 h LD cycles and in locomotion before and after surgery were tested by paired Student's *t* tests. Analysis of variance (ANOVA), followed by Student-Newman-Keuls (SNK) test, was used to compare group differences. Data are expressed as mean ± standard error of mean (SEM). Statistical significance was set at $p < 0.05$ for a two-tailed hypothesis. Data were analyzed using GraphPad Prism version 5.0 for Windows and SPSS version 19.0.

Results

Figure 1 shows the raw temperature data obtained during 12:12 and 10:10 h LD cycles for each strain group.

As shown in Figure 2, the shortened 10:10 h LD cycle induced a clear delay in acrophases and a significant decrease in amplitude of the temperature rhythm in all three mouse strains (BALB/c: $t_4 = 8.05$, $p < 0.001$; C57BL/6N: $t_4 = 15.64$, $p < 0.001$; and CF1: $t_4 = 4.89$, $p < 0.01$). The results of the ANOVA followed by SNK showed that BALB/c mice had significantly higher amplitudes in both normal and shortened cycles than the other two strains (12:12 h LD: $F_{2,12} = 6.37$, $p < 0.05$; 10:10 h LD: $F_{2,12} = 29.07$, $p < 0.01$).

Individual periodograms (Figure 3a) showed strong peaks at 1,440 min (or 24 h) when mice were under the 12:12 h LD cycle. Under the 10:10 h LD cycle, the main peaks were weakened, and new peaks appeared. Fourier analysis (Figure 3b) revealed significant changes in the power of the first harmonic in all strains (BALB/c: $t_4 = 6.53$, $p < 0.01$; C57BL/6N: $t_4 = 13.88$, $p < 0.001$; and CF1: $t_4 = 4.0$, $p < 0.05$). When normal and shortened cycles were compared by *t* tests, BALB/c was the only strain with a statistically significant change in period (BALB/c: $t_4 = 12.37$, $p < 0.001$; C57BL/6N: $t_4 = 1.55$,

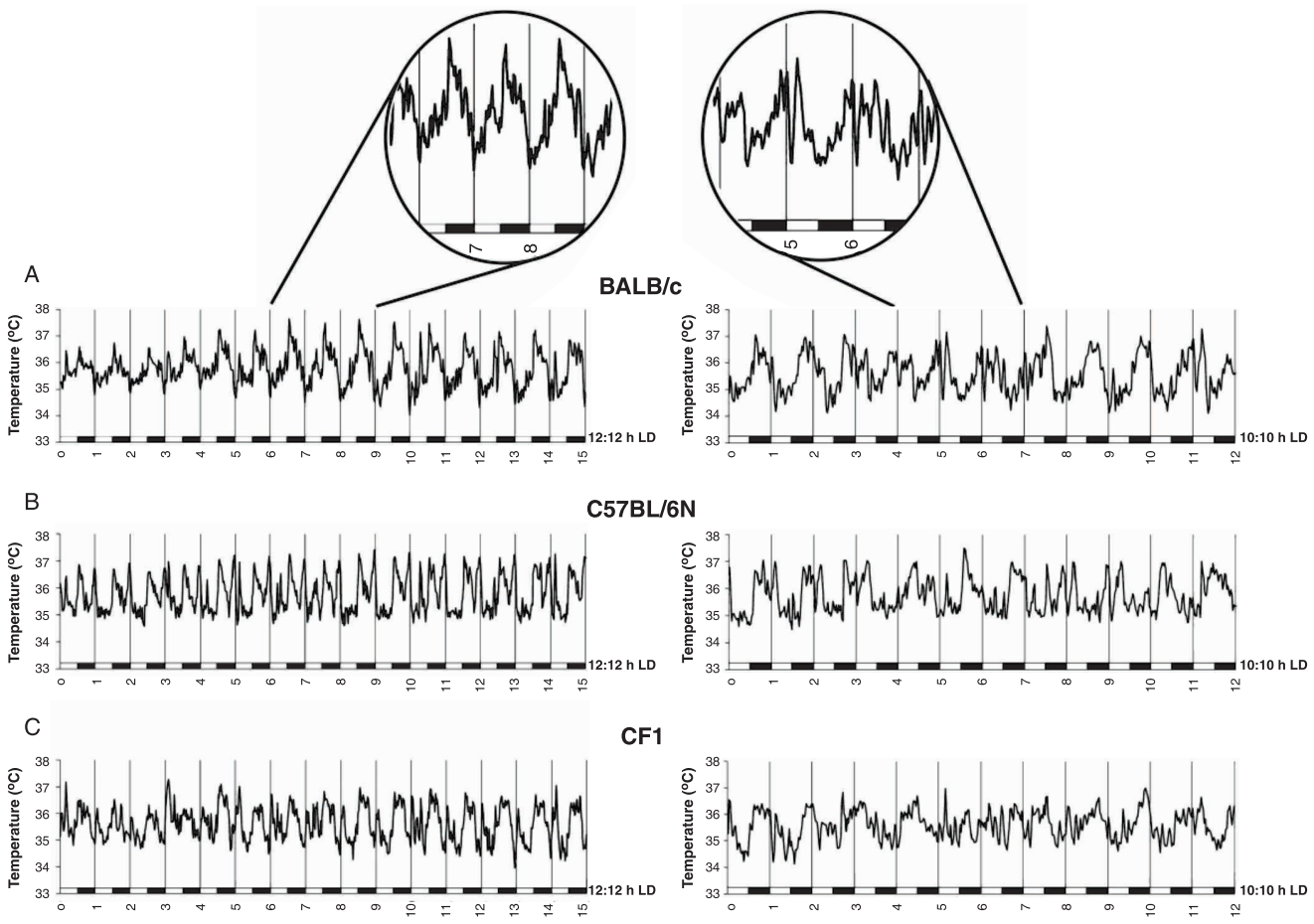

Figure 1 Temperature rhythms. Raw temperature data obtained from BALB/c (A), C57BL/6N (B), and CF1 (C) mice under 12:12 and 10:10 h light-dark (LD) cycles (n=5/strain). Black bars below each graph indicate the dark phase (lights off at 8 p.m.); numbers refer to LD cycles. Cycles are repetitive and homogeneous in 12:12 h LD (left side) and disrupted in 10:10 h LD (right side), as highlighted in the magnified view on the top.

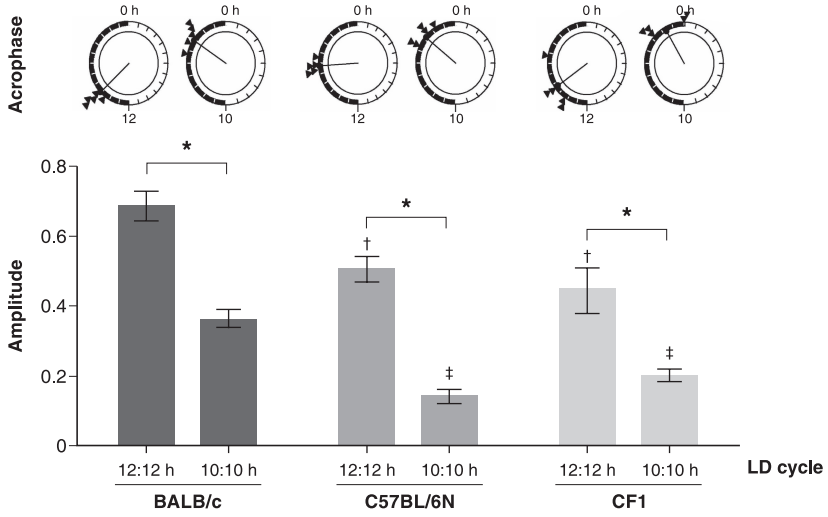

Figure 2 Acrophase and amplitude. Temperature rhythm acrophase and amplitude data obtained from BALB/c, C57BL/6N, and CF1 mice under 12:12 and 10:10 h light-dark (LD) cycles (n=5/strain). Acrophase: Rayleigh test; 0 h represents the time when the lights were turned on. Amplitude: mean ± standard error of mean (SEM). * $p < 0.01$, paired *t* test; † $p < 0.05$ compared with BALB/c 12:12 h; ‡ $p < 0.05$ compared with BALB/c 10:10 h, ANOVA/SNK.

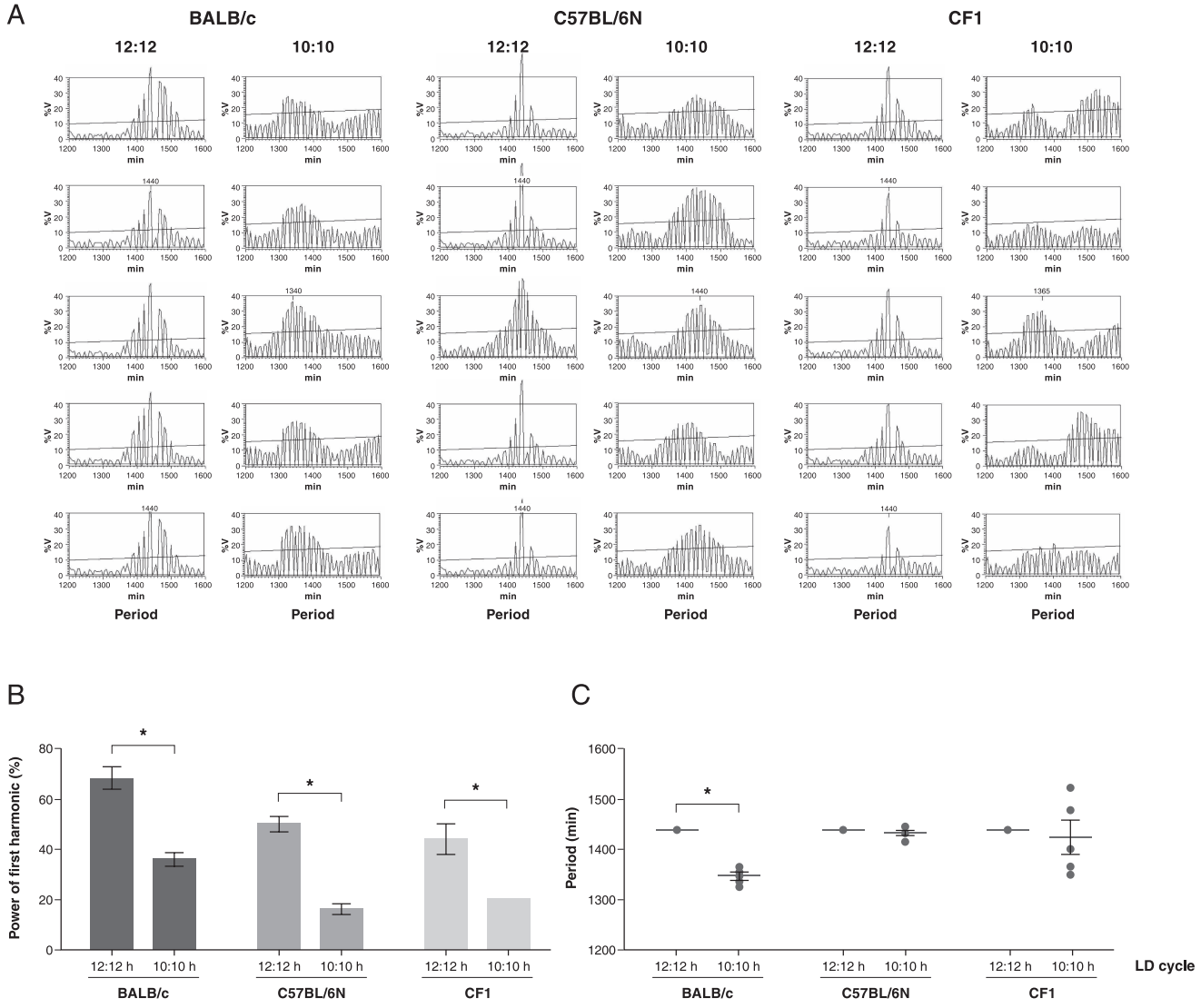

Figure 3 Periodograms. Temperature rhythms in BALB/c, C57BL/6N, and CF1 mice under 12:12 and 10:10 h light-dark (LD) cycles: individual periodograms (A), power of the first harmonic (B), and period (C) (n=5/strain). Power of first harmonic and period: mean ± standard error of mean (SEM). * $p < 0.05$ compared with 12:12 h, paired *t* test.

$p > 0.05$; and CF1: $t_4 = 0.47$, $p > 0.05$) (Figure 3c). As expected, the greatest variability of data occurred in the CF1 strain.

Discussion

This study showed that BALB/c, C57BL/6N, and CF1 mouse strains were susceptible to photoperiod changes. There was a delay in temperature rhythm acrophases in all three strains. As expected, acrophase data obtained from inbred BALB/c and C57BL/6N mice were more homogeneous than those obtained from outbred CF1 mice. Changes in the LD cycle reduced the robustness of temperature rhythms, with every mouse strain showing reduced amplitude under the short 10:10 LD cycle. In agreement with Connolly & Lynch, the highest amplitude was found in BALB/c mice,[18] which continued to demonstrate higher amplitude after the LD changes in our study. This study is relevant to the use of mouse models in the study of rhythm disruption because it shows that BALB/c mice are particularly sensitive to photoperiod changes. These mice showed the most pronounced shift delay, being the only strain in which the period of temperature rhythm was significantly altered, while also showing homogeneous temperature amplitude and acrophase before and after the shortened photoperiod.

BALB/c mice have been shown to be particularly sensitive to stress, exhibiting enhanced depression- and anxiety-related behaviors.[19] Suggesting higher anxiety levels, the BALB/c strain has shown higher plasma corticosterone in response to stress[20,21] and less exploratory behavior in new environments.[22] In addition, the development of social aversion after social defeat stress has been found predominantly in BALB/c mice.[23]

BALB/c mice, but not C57BL/6 mice, have exhibited deficits in spatial working memory and shifts of attention following infant maternal separation.[24] Unpredictable chronic mild stress has induced coat deterioration and decreased grooming behavior in BALB/c mice but not Swiss mice.[25] It is therefore not surprising that BALB/c is usually the strain of choice for studies in which stress plays a central role, such as studies on depression and/or antidepressant effects. Linking stress with rhythmicity, Takahashi et al. reported that BALB/c mice but not C57BL/6 mice showed changes in the rhythmic pattern of corticosterone and insulin secretion in response to repeated stress, as well as alterations in the circadian expression of liver clock genes.[26] Our study suggests that the BALB/c strain is highly sensitive to changes in rhythm even in the absence of stress, and further research would be desirable to determine whether changes in rhythm by themselves would lead to stress- and/or depression-related behavior.

The relevance of circadian rhythms to mood disorders,[10,27] the intertwined effects of desynchronization and stress,[28] and the antidepressant-like effects of melatonin[29] and agomelatin[30] reported in stress-induced rodent models of depression underscore the relevance of modeling rhythm disruption as a risk factor in mouse models of depression. It would be of interest to incorporate rhythm changes into translationally relevant rodent models,[11] given that changes in sleep (e.g., delayed onset, non-restful sleep, early morning awakening, and daytime fatigue) are among the most characteristic symptoms of depression.[31] Temperature rhythm itself is a robust parameter,[32] which, when combined with activity rhythms, may prove useful for characterizing desynchronization. Thermochron iButtons, data loggers that record time and temperature, have been able to successfully monitor core body temperature in rats,[33] and, in the present study, this approach was applicable to mice. No significant changes were observed in locomotion before and after iButtons were implanted, which is particularly relevant to the accurate interpretation of data from behavioral models. When compared with the "gold standard" (telemetry systems for continuous temperature monitoring in free-moving laboratory rodents), iButtons avoid the high initial setup cost.[34] Although circadian disruption of body core temperature has been successfully documented in untethered mice, a complete evaluation of desynchronization of other well-established rhythms is warranted.

This study showed that BALB/c was the mouse strain most sensitive to rhythm disruption. In addition to the high susceptibility to both stress and photoperiod changes found in BALB/c mice, the use of this strain to model an association between rhythm disruption and depression would be supported by the large body of relevant data available, including responses to different antidepressants in both behavioral and neurochemical correlates.[25,35,36]

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Disclosure

The authors report no conflicts of interest.

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