Physiology & Behavior 179 (2017) 200–207

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/physbeh

Duration and timing of daily light exposure influence the rapid shifting of BALB/cJ mouse circadian locomotor rhythms

Thomas J. Vajtay, Jeremy J. St. Thomas, Tyrus E. Takacs, Eric G. McGann, E. Todd Weber*

Department of Biology, Rider University, Lawrenceville, NJ 08648, USA

ARTICLE INFO

Keywords:
Photic entrainment
Masking
Circadian
BALB/cJ mice
Jetlag
Photoperiod

ABSTRACT

Photic entrainment of the murine circadian system can typically be explained with a discrete model in which light exposures near dusk and dawn can either advance or delay free-running rhythms to match the external light cycle period. In most mouse strains, the magnitude of those phase shifts is limited to several hours per day; however, the BALB/cJ mouse can re-entrain to large (6–8 hour) phase advances of the light/dark cycle. In this study, we demonstrate that the circadian responses of BALB/cJ mice are dependent on duration as well as timing of light exposure, with significantly larger phase shifts resulting from > 6-hour light exposures, yet loss of entrainment to photoperiods of < 2–3 hours per day or to skeleton photoperiods. Intermittent light exposures of the same total duration but distributed differentially over the same period of time as that of a 6-hour phase advance of the light cycle yielded phase shifts of different magnitudes depending on the pattern of exposure. Both negative and positive masking responses to light and darkness, respectively, were exaggerated in BALB/cJ mice under a T7 light cycle, but were not responsible for their rapid re-entrainment to chronic phase shifting of the light dark cycle. These results collectively suggest that the innately jetlag-resistant BALB/cJ mouse circadian system provides an alternative murine model in which to elucidate the limitations of photic entrainment observed in other commonly used strains of mice.

1. Introduction

Entrainment of the mammalian circadian system to the photic environment is achieved primarily through retinal projections to the hypothalamic suprachiasmatic nuclei (SCN), which serve to coordinate circadian rhythmicity in behavioral, cognitive and physiological processes [1]. Responses of the circadian system to light are dependent on the circadian phase of exposure, with early nighttime exposure typically yielding delays and later nighttime exposure yielding advances in the phase of circadian rhythms. In most mammalian animal models, the magnitude of light-induced phase shifts increases with light intensity or duration, but the response is saturable [2]. Maximal phase shifts can be achieved with relatively brief exposures (5–30 min) to moderately bright light, with a magnitude sufficient to adjust wild-type circadian rhythms to maintain entrainment to 24-hour light-dark cycles. Thus, entrainment of non-24-hour circadian rhythms to 24-hour photoperiods can typically be explained by discrete effects of retinal input to the suprachiasmatic pacemaker occurring near light-dark transitions. In this model, the magnitude of maximal shifts possible through discrete responses thereby determines the limits of entrainment.

Studies of circadian systems assessed by observation of overt behavioral or physiological rhythms (*e.g.*, running wheels, general locomotor activity, feeding, drinking, body temperature) are often confounded by “masking” in which light exposure suppresses these behaviors in nocturnal organisms (negative masking) or darkness at normally lit times promotes these behaviors (positive masking), without affecting the period or phase of their underlying pacemaker [3]. These expressions or suppressions of behavior can thereby “mask” the true phase of circadian rhythmicity otherwise evident in onsets or offsets of the behavioral measure.

In most strains of laboratory mice, maximal light-induced advances in the phase of circadian behavioral rhythms on the order of 1–2 h per day are typical, although larger responses are possible with pharmacological intervention [4–7] or genetic manipulation of the SCN [8–10]. In 2009, our group published a study describing re-entrainment of locomotor rhythms within the first day following 6- and 8-hour phase shifts of the light-dark cycle in BALB/cJ but not C57BL/6 J mice [11]. In this study, we document the contributions of light intensity and duration, photoperiod, and masking to these exaggerated responses in BALB/cJ mice, and demonstrate their ability to maintain entrainment to chronically advancing light-dark cycles. We propose that the BALB/cJ mouse may serve as a comparative model with which to elucidate the

* Corresponding author at: Department of Biology, Rider University, 2083 Lawrenceville Road, Lawrenceville, NJ 08648, USA.
E-mail address: tweber@rider.edu (E.T. Weber).

http://dx.doi.org/10.1016/j.physbeh.2017.06.010
Received 4 May 2017; Received in revised form 6 June 2017; Accepted 20 June 2017
Available online 20 June 2017

limitations of photic regulation observed in many other traditional mammalian models.

2. Methods

2.1. General methods

All experimental and animal care procedures were performed with the approval of the Rider University Institutional Animal Care and Use Committee and in accordance with the NIH Guidelines for the Care and Use of Animals in Research. For all experiments, age- and gender-matched cohorts of C57BL/6J and BALB/cJ mice, 5–6 weeks of age, were introduced to individual cages (46 × 25 × 15 cm), each equipped with a running wheel, with food and water provided *ad libitum*. All mice used in experiments were acquired directly or bred in-house for 1–2 generations from Jackson Laboratories stock (Bar Harbor, ME). Mice were housed under white light from Daylight™ (General Electric) fluorescent bulbs, of 250–400 lx intensity at cage bedding level unless stated otherwise. Behavior was quantified from wheel rotations recorded *via* magnetic switches reporting to a computer continuously running Clocklab circadian data collection software (Actimetrics, Inc. Evanston, IL), and analyzed using Clocklab analysis software.

For the quantification of changes to phase and period of rhythms, onsets of activity of were defined as bouts of activity persisting for at least 1 h following 3 h of inactivity. Period of activity rhythms was determined by correlation lines drawn through 5–7 consecutive onsets of activity. Phase shifts on the day following treatments were calculated as the difference between the onset of activity extrapolated from a correlation line through 5–7 onsets of stable post-treatment activity and the onset of activity predicted by extrapolation of pre-treatment onsets of activity. Re-entrainment of rhythms was considered complete when rhythms had re-established a similar phase relationship to the onset of darkness in the light cycle as that prior to the treatment, persisting for at least 3 consecutive cycles.

2.2. Phase response curve for 3-hour light pulses

Following 2 weeks of entrainment to a 12:12 LD cycle, mice (BALB/cJ and C57BL/6J, n = 7–8 males/group) were released into constant darkness (DD). Free-running mice were then subjected to multiple Aschoff type 1 phase shifts [12]. Once every 14 days, lights in housing chambers were turned on for 3 h for a total of 8 randomly timed light exposures per mouse. The circadian time of the midpoint of each light exposure was determined for each individual mouse by extrapolating its previous 5 onsets of activity to the day of treatment, with predicted onset of activity defined as CT12 for that subject. Phase shifts for each strain of mice were averaged for each 3-hour bin across the 24-hour cycle, resulting in 5–9 measures per time point.

2.3. Effects of light intensity on phase shifts

Mice (male C57BL/6J and BALB/cJ, n = 10/strain) were entrained for 14 days to a 12:12LD cycle at 25 lx intensity at cage bottom. Mice were subjected to a 6-h advance of the light-dark cycle for a duration of 21 days. Mice were then re-entrained for 20 days under 250 lx light and subjected to another 6-hour phase advance for a duration of 21 days. Rate of re-entrainment was quantified as the number of cycles required to re-establish a similar phase relationship of wheel running to the LD cycle for at least three consecutive cycles.

2.4. Entrainment to a skeleton light-dark cycle

Mice (male C57BL/6J, n = 8 and BALB/cJ, n = 11) were entrained to a 12:12 LD cycle for two weeks. The LD cycle was then converted into a skeleton light-dark cycle consisting of two one-hour light exposures retaining the initial onset and offset of the previous light cycle, for a duration of 5 weeks. Rhythms were considered entrained if they maintained a 24-hour periodicity with a stable phase relationship to the skeleton light cycle.

2.5. Effect of photoperiod on locomotor entrainment

Mice (male C57BL/6J and BALB/cJ, n = 5/strain) housed individually in cages with running wheels were entrained to a 12:12LD cycle for two weeks. Photoperiod was then sequentially decreased to 6 h of light for 3 weeks, to 4 h of light for 2 weeks, to 3 h of light for 2 weeks, to 2 h of light for 2 weeks, to 1 h of light for 2 weeks, followed by release to constant darkness for 1 week.

2.6. Effect of light duration on phase shifts

Mice (male C57BL/6J and BALB/cJ, n = 6–8/strain) entrained to 12:12 LD were subjected to Aschoff type 2 phase advances of the onset of the light-dark cycle [12]. On the day of treatment, light onset was advanced by 6 h, whereas the offset of light was varied to produce a singular light exposure of 6, 9, 12, 15 or 18 hour duration, followed by release into constant darkness for 7–10 days. Phase of the free-running rhythm following treatment was extrapolated from regression of 5 subsequent onsets of activity. Phase shifts were quantified as the difference between phases of the entrained (pre-treatment) rhythm and the post-treatment rhythm extrapolated to the day of treatment. Mice were subjected to each of the five light exposures after being re-entrained to a 12:12 light-dark cycle for at least 2 weeks prior to each manipulation, defined by at least five consecutive days with activity onsets concomitant with dark onset and consistent duration of the active period. Data were subjected to two-way ANOVA (strain x light treatment) followed by Tukey post-hoc analysis when appropriate.

2.7. Effects of intermittent light exposure during phase shifts

Mice (male C57BL/6J and BALB/cJ, n = 10/strain) were entrained to a 12:12 LD cycle prior to an Aschoff type II phase shift comprised of one of three light paradigms in which 6 h of total light exposure were distributed intermittently across the subsequent 12 h beginning at mid-subjective nighttime on the first cycle of constant darkness, followed by release to constant darkness. The paradigms were (A) 3 h of light + 6 h of dark + 3 h of light (3:6:3); (B) 1 h of light + 3 h of dark + 4 h of light + 3 h of dark + 1 h of light (1:3:4:3:1); or (C) 1.5 h of light + 2 h of dark + 1.5 h of light + 2 h of dark + 1.5 h of light + 2 h of dark + 1.5 h of light (1.5:2:1.5:2:1.5:2:1.5). Each manipulation was a 6-hour advance of initial light onset and final light offset. A fourth group of mice received 12 h of light exposure beginning at mid-subjective nighttime, as previously reported. A fifth group of mice received 6 consecutive hours of advanced light exposure beginning at mid-subjective nighttime. Phase shifts were calculated as the difference between phases of the entrained (pre-treatment) rhythm and the post-treatment rhythm extrapolated back to the day of treatment. Data were subjected to two-way ANOVA (strain × light treatment) followed by Tukey post-hoc analysis when appropriate.

2.8. Effects of chronically shifting light-dark cycles on locomotor entrainment

BALB/cJ and C57BL/6J mice (n = 5 males & 5 females/group) housed individually in cages equipped with running wheels monitored continuously by Clocklab were entrained to a 12:12 LD cycle for 3 weeks, then subjected to a chronically shifting light-dark cycle in which the phase of the photoperiod was advanced by 4 h every 3 days (hereafter referred to as "4 × 3") for 60 days, followed by release to constant darkness for 14 days.

A separate set of BALB/cJ and C57BL/6J mice (n = 15/strain) housed individually in cages with running wheels were entrained to a