Plasticity of Circadian Behavior and the Suprachiasmatic Nucleus following Exposure to Non-24-Hour Light Cycles

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Abstract Period aftereffects are a form of behavioral plasticity in which the free-running period of circadian behavior undergoes experience-dependent changes. It is unclear whether this plasticity is age dependent and whether the changes in behavioral period relate to changes in the SCN or the retina, 2 known circadian pacemakers in mammals. To determine whether these changes vary with age, *Per1-luc* transgenic mice (in which the luciferase gene is driven by the *Period1* promoter) of different ages were exposed to short (10 h light: 10 h dark, T20) or long (14 h light: 14 h dark, T28) light cycles (T cycles). Recordings of running-wheel activity in constant darkness (DD) revealed that the intrinsic periods of T20 mice were significantly shorter than of T28 mice at all ages. Aftereffects following the shorter light cycle were significantly smaller in mice older than 3 months, corresponding with a decreased ability to entrain to T20. Age did not diminish entrainment or aftereffects in the 28-h light schedule. The behavioral period of pups born in DD depended on the T cycle experienced in utero, showing maternal transference of aftereffects. Recordings of *Per1-luc* activity from the isolated SCN in vitro revealed that the SCN of young mice expressed aftereffects, but the periods of behavior and SCN were negatively correlated. Enucleation in DD had no effect on behavioral aftereffects, indicating the eyes are not required for aftereffects expression. These data show that circadian aftereffects are an age-dependent form of plasticity mediated by stable changes in the SCN and, importantly, extra-SCN tissues.

Key words SCN, oscillator, aftereffects, T cycle, Period gene, mPer1, entrainment, aging

The endogenous period of daily locomotor behavior is a characteristic of circadian biology that is dependent on a class of at least eight genes (reviewed in Van Gelder et al., 2003), leading to species- and strain-specific free-running periods (Schwartz and Zimmerman, 1990; Shimomura et al., 2001). However, circadian period is plastic—depending on prior lighting conditions; chronic inputs, including treatments such as lithium; and periodic interruptions to an animal's daily schedule (Pittendrigh and Daan, 1976a; Reebs and St-Coeur, 1994). These changes in period, which can persist for months in constant conditions,

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JOURNAL OF BIOLOGICAL RHYTHMS, Vol. 19 No. 3, June 2004 198-207
DOI: 10.1177/0748730404264156
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have been collectively referred to as period "aftereffects." An example of this phenomenon is seen with exposure to non-24-h light cycles (T cycles), which leads to long-term changes in the free-running period of locomotor activity in rodents (Pittendrigh and Daan, 1976a). The physiological basis for this plasticity in circadian behavior has not been investigated. While other forms of behavioral plasticity tend to decline with age (Leblanc et al., 1996; Schoenbaum et al., 2002; Brainard and Doupe, 2002; Rosenzweig et al., 2003), the effects of aging on aftereffects are unknown. In this study, we address whether aging affects the expression of these behavioral aftereffects.

In some mammalian species, variance in behavioral period with age or genotype has been associated with changes in the master circadian pacemaker, the suprachiasmatic nucleus of the hypothalamus (SCN). For example, the shortening of behavioral period in rats with age is accompanied by a shortening in the period of gene expression rhythms intrinsic to the SCN (Yamazaki et al., 2002c). SCN firing rate rhythms in vitro and in vivo reflect the genotype-specific behavioral period of the animal (Welsh et al., 1995; Liu et al., 1997; Herzog et al., 1998; Honma et al., 1998; Yamazaki et al., 1998; Albus et al., 2002; Nakamura et al., 2002), and transplanting the SCN from *tau*-mutant hamsters into SCN-lesioned, arrhythmic hamsters restores behavioral rhythmicity with the period of the donor (Ralph et al., 1990). In the present study, we tested whether behavioral aftereffects following exposure to long- or short-period light cycles correlate with changes in the endogenous period of SCN rhythmicity in vitro.

Transgenic mice (expressing the firefly luciferase gene via the *Period1* promoter, *Per1-luc*) of different ages were exposed to light:dark (LD) cycles either longer or shorter than 24 h and then maintained in constant darkness (DD). We found that, similar to other forms of behavioral plasticity, aftereffects of wheel-running activity were greatest in younger animals. We also found that pups exposed to T cycles in utero and born in DD showed aftereffects later in life. Among young adult mice, the period of the *Per1-luc* rhythm in the SCN differed between groups exposed to long and short T cycles. Surprisingly, the isolated SCN of young animals ran shorter following a long T cycle—a trend also seen in older animals. Because of the unexpected inverse relationship between the periods of behavior and explanted SCN, we hypothesized that aftereffects require input from extra-SCN tissues. Because the eyes influence the free-running rhythms in behavior (Yamazaki et al., 2002a) and in the SCN (Lee et al., 2003) and contain an endogenous circadian pacemaker (Tosini and Menaker, 1998; Tosini and Fukuhara, 2003), we tested whether the eyes are necessary for aftereffects. We found that enucleation had no effect on the expression of aftereffects. However, these results indicate that aftereffects involve long-lasting period changes in the SCN and, likely, other tissues.

MATERIALS AND METHODS

Assessment of Locomotor Period

Per1-luc transgenic mice (n = 79), in which expression of firefly luciferase is driven by a 6.7-kb genomic fragment of the mouse *Period1* promoter (C57Bl/6J; Herzog et al., 2004), and wild-type mice (C57BL/6NCrlBR, n = 44; Charles River, Wilmington, MA) were housed individually in the Hilltop animal facility (Washington University). Running wheel revolutions were logged every 6 min (Clocklab, Actimetrics, Evanston, IL) while mice were exposed to light schedules of defined periods (T cycles) or constant darkness (DD). For age-based analysis of aftereffects expression, we grouped mice based on their age at the onset of the T cycle: E0 (embryonic day 0), young (20-90 days postnatal), middle age (4-8 months postnatal), and old (8-16 months postnatal). E0 mice were conceived within the first 4 days of the T cycle and born in DD. All others were exposed to a 12:12 LD cycle (T24) for at least 1 week before the period of the light cycle was gradually changed, replicating the paradigm of Pittendrigh and Daan (1976a). The duration of both the light and dark phases of each cycle was increased or decreased by 5 min per day so that total cycle length changed by 10 min until, after approximately 3 weeks, the cycle length was 20 h (T20) or 28 h (T28). The gradual change in the light cycle enhances the likelihood that animals remain entrained. Upon reaching T20 or T28, the animals were immediately released into DD. E0 pups, born in DD following the light cycle, were housed individually upon weaning 3 weeks after birth. The free-running period of each mouse was then estimated from consecutive 7-day epochs in DD chi-squared periodogram analysis (Clocklab). Nearly identical periods were found by linear regression fits through daily activity onsets, differing on average by

0.3%. The maximum period difference for any given animal between the 2 methods was 4.4%.

Assessment of SCN Period

Following behavioral assessment, all *Per1-luc* mice were sacrificed for recordings of SCN rhythmicity. Briefly, we sacrificed mice in DD and rapidly removed their eyes and then their brains. The times of sacrifice were randomly distributed across the circadian cycle in all groups. Coronal sections (300 μm thickness) were made with a vibroslicer, and the paired SCN were cultured on membrane inserts (Millicell-CM, Millipore, Bedford, MA) in 1 mL of medium (Dulbecco's modified Eagle's medium, Sigma, St. Louis, MO) supplemented with 10 mM HEPES (Sigma), 2% B27, 25 U/mL penicillin, 25 μg/mL streptomycin, 2.2 mg/mL $NaHCO_3$, 4 mM L-glutamine, and 0.1 mM beetle luciferin (Promega, Madison, WI). Unless noted, medium ingredients were purchased from Invitrogen (Carlsbad, CA). Each culture was sealed in a Petri dish and maintained at 36 °C in darkness. Bioluminescence was collected in counts per minute for 7 to 10 days without a medium change using a photomultiplier tube (HC135-11MOD, Hamamatsu Corp., Shizouka, Japan).

Photon counts from each culture were detrended with a 3-h running average as described previously (Abe et al., 2002). The period of *Per1-luc* activity (recorded from 24 to 168 h in vitro) was assessed for each SCN culture using chi-squared periodogram (Clocklab) and Fast-Fourier transform nonlinear least squares analysis (Herzog et al., 1997). The period estimates for a given SCN from the 2 methods differed by a maximum of 2.9%. Periods are reported in this paper from the 41 of 79 SCN cultures that expressed significant circadian periodicity according to the chi-squared period analysis ($p < 0.05$). There were no differences in the proportion of rhythmic SCN between experimental groups. Nonrhythmic SCN cultures tended to have lower luminescence, so a plausible explanation is that low counts above background luminescence limited detection of rhythmicity in some cultures.

Surgical Removal of the Eyes

Enucleations were performed on wild-type mice (age 20 days to 3 months) 1 to 2 weeks after release into DD following exposure to T20 or T28 cycles. Young mice only were used for these experiments, based on their ability to entrain to T cycles. Briefly, mice were anesthetized with intraperitoneal ketamine (50 mg/kg) and medetomidine (1 mg/kg) prior to surgery. The area around the eye was then swabbed with 0.5% chlorohexidine, and both eyes were removed under infrared illumination. The orbits were packed with Gelfoam (Pharmacia, Kalamazoo, MI), and the mice were given intraperitoneal atipamezole (1 mg/kg) to accelerate recovery. Control animals were treated identically, but their eyes were left intact. Locomotor activity was recorded in DD for 6 to 7 weeks, and period estimates were made from successive 7-day epochs.

Statistical Analysis

Differences in the average period of wheel-running activity between T20 and T28 animals were determined using a 2-tailed Student's *t*-test. Comparisons between the average period in these 2 groups were made for assessment of aftereffects expression. Differences in the average period of wheel-running activity between young, middle-age, and old mice were analyzed for each T cycle by 1-way analysis of variance (ANOVA, Origin Software, OriginLab Corp., Northampton, MA). To determine whether aftereffects varied significantly with time in DD, a 2-way ANOVA compared the periods within each age category with respect to the number of weeks in DD and T cycle. The effects of T cycles on the behavioral period of E0 mice were tested by 2-tailed Student's *t*-test and were not compared with other age groups because they were measured only after 3 weeks in DD, when the animals were old enough to run on wheels. Differences in average period of bioluminescence activity between T20 and T28 SCN were determined by 1-way ANOVA. Correlations between the behavioral and SCN period of individual animals were made within each age group. For these correlations, estimates of behavioral period were made from the 7 days prior to sacrifice.

To assess the effects of eye removal on the persistence of aftereffects, we calculated the weekly change in free-running period relative to that of the first week in DD for each animal. The average weekly changes in period of enucleated and sham-operated mice were then compared for each T cycle treatment in DD by 2-way ANOVA.

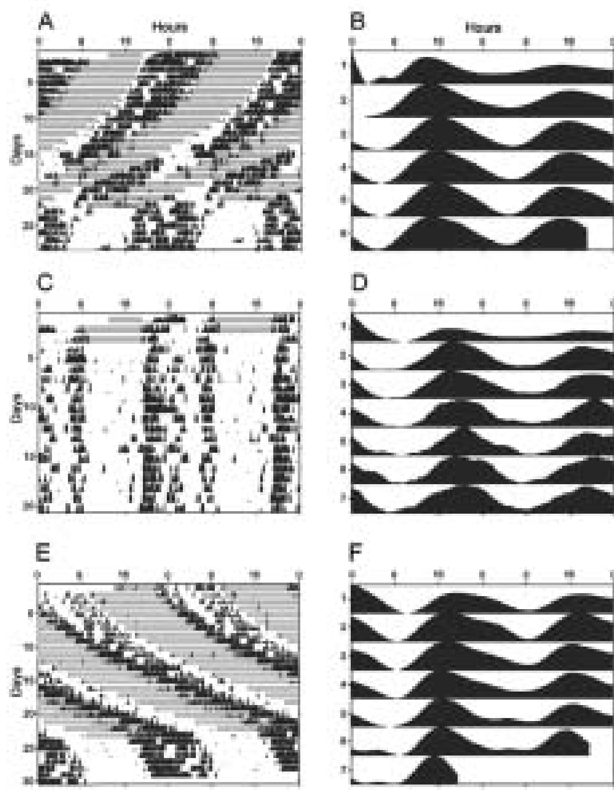

Figure 1. Aftereffects on rhythms in locomotor behavior and SCN gene expression. Wheel-running activity of representative *Per1-luc* transgenic mice exposed to a 20-h (A, T20), 24-h (C, T24), or 28-h (E, T28) light:dark cycle and the subsequent recording of the *Per1*-driven bioluminescence from their cultured SCN (B, D, F). Data are double-plotted as actograms in which each line shows wheel revolutions in 6-min bins over 2 days, normalized to the daily peak. Grey bars (A, C, and E) indicate times when lights were on. Free-running locomotor activity in constant darkness (DD) began on day 23 for A, day 4 for C, and day 24 for E and ended when the animals were sacrificed for in vitro SCN recording. Behavioral or *Per1-luc* periods were A = 23.7 h, B = 24.6 h, C = 23.9 h, D = 24.4 h, E = 24.8 h, and F = 23.2 h.

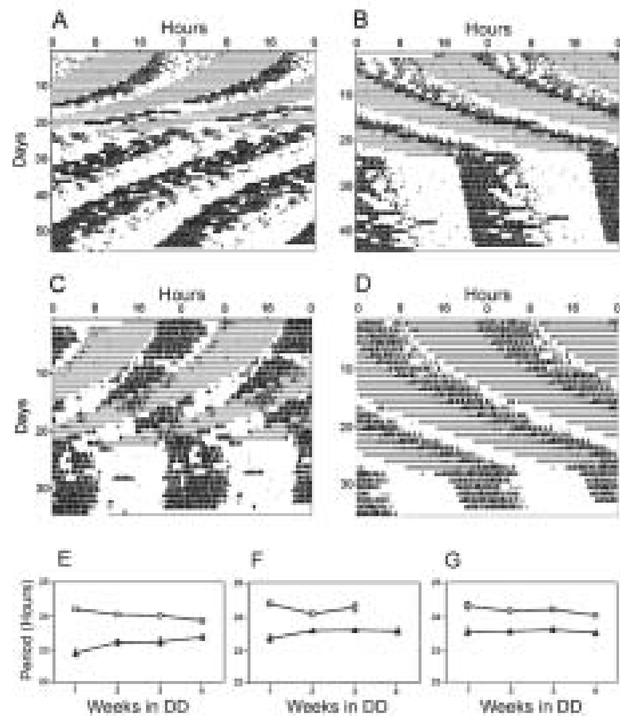

Figure 2. Persistence and age dependence of aftereffects. Young mice (20 days to 3 months of age at the start of the T cycle) expressed behavioral aftereffects in response to T20 (A) and T28 (B) light cycles that lasted for at least 3 weeks in constant darkness (E). Their free-running period was shortened following T20 (filled triangles) relative to the period following T28 (open squares). Old mice (8-12 months) failed to entrain to T20 (C) but entrained to T28 (D). Middle-age mice (4-8 months) and old mice also showed long-lasting aftereffects (F and G, respectively). Grey bars in actograms indicate times when lights were on. Error bars in E to G show SEM.

RESULTS

Entrainment to Short T Cycles Is Age Dependent

All mice younger than 3 months of age entrained to light cycles of changing length (T20, $n = 9$ of 9; T28, $n = 15$ of 15; Figs. 1 and 2). Consequently, their phase angles of entrainment depended on the period of the light cycle so that, on the first cycle in DD following their light cycle, T20 mice became active 1.7 ± 0.2 h (mean ± SEM) after light offset, and T28 became active 5.7 ± 0.4 h before light offset. Middle-age (4-8 months) and older animals (over 8 months) also entrained to T28 ($n = 8$ of 8; Fig. 2D), but no mice older than 4 months remained entrained during T20 ($n = 0$ of 6 middle-age mice and 0 of 5 old mice; Fig. 2C). Only some mice entrained to T20 at 3 months of age ($n = 2$ of 7 young mice), indicating that 3 months may be an upper age limit for entrainment to this T cycle. Failure to entrain was defined by a lack of consistent phase angle between the daily onsets of light and locomotor activity. These results are consistent with previous reports that photic entrainment is impaired in older mice (Benloucif et al., 1997; Valentinuzzi et al., 1997).

Short T Aftereffects Are Greatest in Younger Animals

T cycles induced long-lasting changes in the circadian period of running-wheel activity of mice in all age groups. The free-running periods of T20- and T28-

treated adults differed during the first 3 weeks in DD but did not change significantly during that time (2-way ANOVA, $p < 0.01$ for the effect of T cycles in all age groups, $p > 0.75$ for the effect of time in DD in all age groups; only young mice showed a significant interaction between the treatment and time in DD [Fig. 2E], $p = 0.04$).

The magnitude of aftereffects following T20 depended on age. Young mice expressed shorter periods than middle-age and older mice during the first week in DD following the 20-h light cycle (Fig. 3; 1-way ANOVA, $p < 0.05$, $F = 3.48$). This relationship between age and period following T20 lasted for at least 4 weeks in DD ($p < 0.05$ for weeks 1-4). However, there was no effect of age on the period of T28 ($p = 0.30$) or T24 mice ($p = 0.41$). The smaller aftereffects seen in older mice were likely related to their failure to remain entrained to the T20 cycle. In support of this idea, young mice that entrained to the short light cycle (n = 11 of 16) showed significantly greater aftereffects than those that did not entrain ($p = 0.002$, 2-tailed Student's *t*-test).

Aftereffects Can Be Maternally Transferred

Importantly, locomotor periods also differed between animals conceived during a T20 or T28 light cycle and born in DD (Fig. 4; $p = 0.03$, Student's *t*-test; T20—23.6 ± 0.1 h, $n = 16$; T28—23.9 ± 0.1 h, $n = 10$). Aftereffects can thus be induced either by direct exposure to a non-24-h light cycle or by maternal (in utero or postnatal) effects.

The SCN Expresses Aftereffects In Vitro

To assess whether the plasticity seen in circadian behavior is reflected in the period of the SCN, we recorded *Per1-luc* rhythms from the isolated SCN of a subset of mice after recording their locomotor activity (Fig. 1B,D,F). Surprisingly, the period of the SCN did not increase with the period of locomotor activity (Figs. 1 and 3C). The average period of the SCN taken from young, T20 mice (23.8 ± 0.1 h, $n = 10$) was significantly longer than that of T28 mice (Fig. 3B; 23.5 ± 0.1 h, $n = 13$; $p = 0.04$, 2-tailed Student's *t*-test). Furthermore, the periods of locomotor and SCN rhythms of individual young mice were significantly and negatively correlated ($r = -0.69$, $p = 0.002$; Fig. 3C). A similar trend was seen in the SCN of E0 (Fig. 4; $n = 1$ in T20, 3 in T28), middle-age, and older mice, but this did not reach significance (Fig. 3; $n = 3$ in T20 and 3 in T28, $p = 0.57$ for

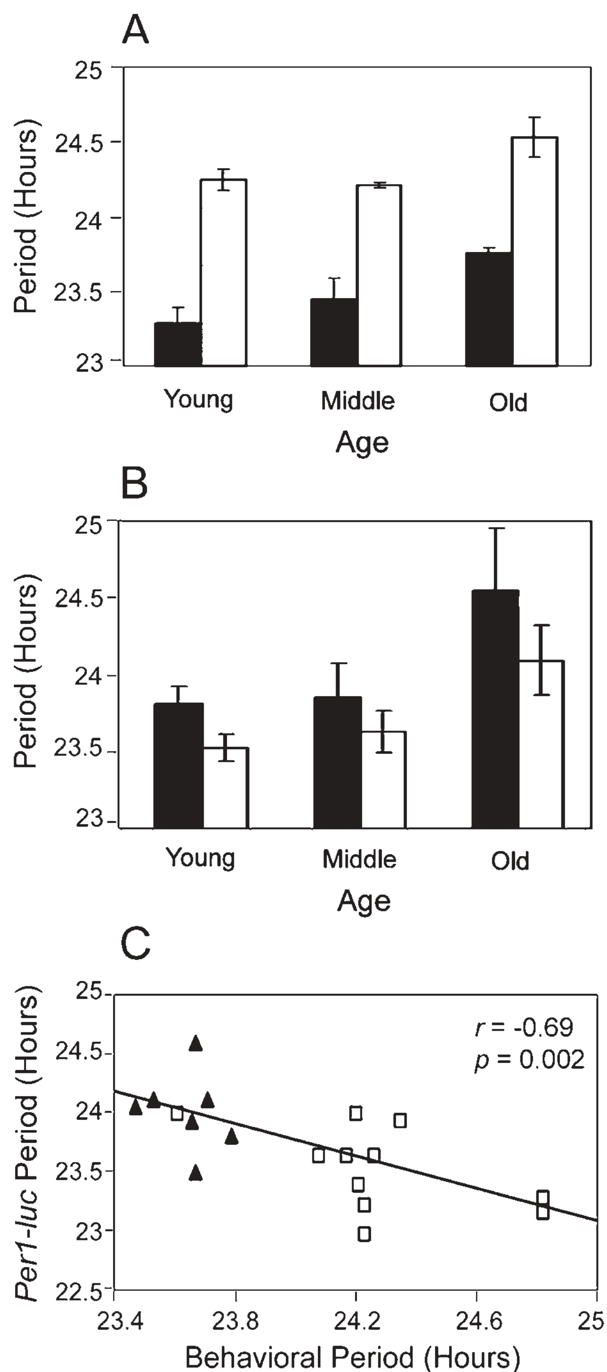

Figure 3. The large effects of T cycles on behavioral period are not seen on *Per1-luc* rhythms in the isolated SCN. All age groups showed significantly shorter behavioral periods in the first week in DD following T20 (filled bars) when compared with mice exposed to T28 (open bars) light cycles (A). In contrast, the SCN of young mice expressed significantly longer period rhythms in *Per1*-driven bioluminescence following T20 than in T28 (B). This trend did not reach significance for the SCN of other age groups. For both A and B, data were pooled from 2 experiments and included mice regardless of their ability to entrain to the light schedule. Error bars show SEM. Behavioral period was significantly and negatively correlated with the period of *Per1-luc* rhythms in individual young mice (C, $r = -0.69$, $p = 0.002$, T20 = filled triangles, T28 = open squares).

Table 1. Mean Period ± SEM (n) for Behavioral and *Per1-luc* Rhythms Recorded from the SCN of T20 and T28 Mice

	Behavior		Per1-luc	
Age	*T20*	*T28*	*T20*	*T28*
E0	23.6 ± 0.1 (16)	23.9 ± 0.1 (10)*	28.9 ± 0.0 (1)	25.1 ± 0.4 (3)
Young	23.2 ± 0.1 (16)	24.3 ± 0.1 (18)*	23.8 ± 0.1 (10)	23.5 ± 0.1 (13)*
Middle age	23.4 ± 0.2 (6)	24.2 ± 0.02 (3)*	23.9 ± 0.2 (3)	23.6 ± 0.1 (3)
Old	23.8 ± 0.04 (5)	24.5 ± 0.1 (5)*	24.5 ± 0.9 (3)	24.1 ± 0.2 (5)

*Significant difference between T20 and T28 groups ($p < 0.05$).

middle-age mice; $n = 3$ in T20 and 5 in T28, $p = 0.60$ for older mice). Due to the relatively small number of SCN slices measured in these age groups, little can be concluded about the relationships between their behavioral and SCN periods. However, the period of *Per1-luc* rhythms in the SCN of young animals depended on the prior light history. In contrast to previous studies finding a positive correlation between the effects of genetics or aging on behavioral and SCN rhythms, T cycles appeared to induce opposing period changes in locomotor and *Per1*-driven SCN rhythms.

Behavioral Aftereffects Are Not Sustained by the Eyes

The dissociation between SCN and behavioral rhythms suggests that behavioral aftereffects may not be mediated by changes in the SCN alone. Because the eyes influence the free-running period of locomotion in rodents (Yamazaki et al., 2002a; Wee et al., 2002) and express T cycle–dependent aftereffects in mollusks (Page et al., 1997), we hypothesized that the eyes are involved in the maintenance of behavioral aftereffects. Wild-type mice (age 20 days to 3 months) were entrained to T20 and T28 cycles with the same paradigm used for *Per1-luc* mice. Younger mice were chosen for this experiment based on their ability to entrain to both T cycles; all mice remained entrained for the duration of their respective light cycle. After T20 or T28 was reached, the animals were kept in these T cycles an additional week prior to release into DD. After 1 to 2 weeks in DD, mice underwent bilateral enucleation or a sham operation. At the time of surgery, enucleated and sham-operated mice from the same light cycle expressed similar free-running periods (2-tailed Student's t-test; T20—$n = 11$ enucleated, 22.7 ± 0.1 h, $n = 10$ sham, 23.1 ± 0.1 h, $p = 0.14$; T28—$n = 10$ enucleated, 24.2 ± 0.02 h, $n = 14$ sham, 24.1 ± 0.1 h, $p = 0.96$). Mice with and without eyes continued to show aftereffects, so that their periods changed similarly over the 6 to 7 weeks in DD (Fig. 5; 2-way ANOVAs for T20 and T28 on the change in period—effect of enucleation, $p > 0.1$, and of week in DD, $p < 0.05$; no significant treatment-by-week interaction). Thus, the persistence of behavioral aftereffects was not dependent on the presence of the eyes.

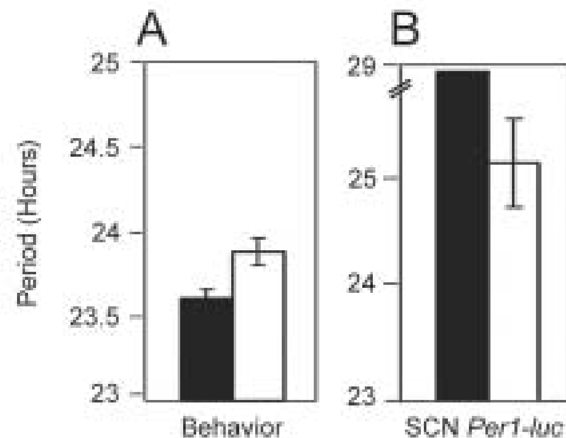

Figure 4. Maternal influences can induce aftereffects. (A) E0 mice conceived in a 20-h light cycle and born in DD (filled bars) expressed significantly shorter behavioral periods once weaned than mice conceived in a 28-h light schedule and born in DD (open bars). (B) *Per1-luc* rhythms of the SCN from E0 mice, similar to the SCN of other age groups, tended to express longer periods when taken from animals exposed to the shorter light cycle.

DISCUSSION

Long-lasting changes in the endogenous circadian period of a wide variety of organisms have been described following exposure to non-24-h light cycles (Pittendrigh and Daan, 1976a; Stephan, 1983; Page et al., 1997, 2001). We found that these period aftereffects, like other forms of behavioral plasticity, were more robust in younger animals. The ability to change intrinsic period appears related to the ability to entrain. This was especially clear in the 20-h light cycle

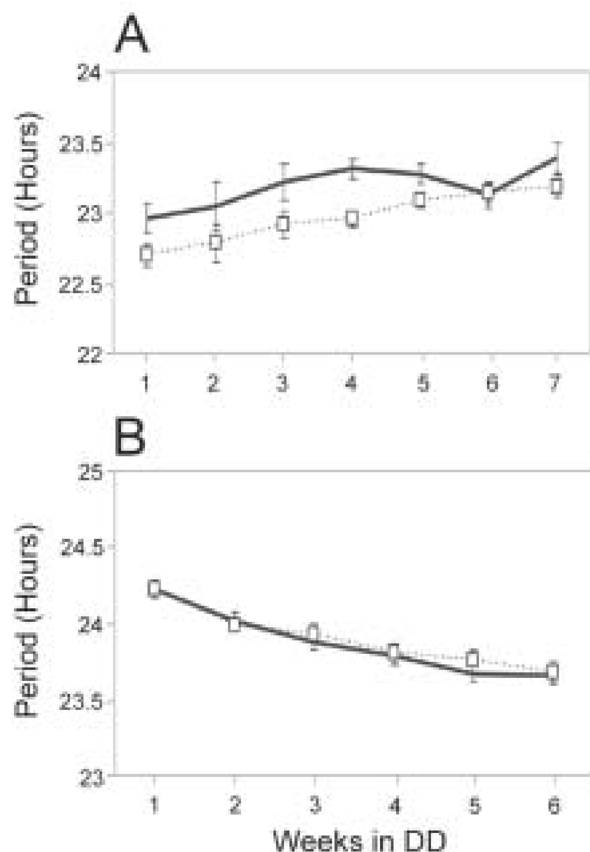

Figure 5. Enucleation has no effect on the persistence of aftereffects. The week-to-week change in period following exposure to T20 (A) and T28 (B) was similar in intact mice (solid line) and mice enucleated 1 to 2 weeks following release into DD (open squares, dotted line).

in which most young mice, but none older than 3 months, synchronized their daily activity to the light cycle. Those mice unable to advance their daily schedules with the light schedule showed smaller aftereffects. Thus, aging appears to diminish the ability of mice to phase advance, which prevents their entrainment to short T cycles and expression of extremely short endogenous periods. Although aging has been reported to have variable effects on circadian period (Davis and Viswanathan, 1998), diminished photic entrainment has been consistently found (Kolker et al., 2003). Deficits in photic entrainment with age have been attributed mainly to reduced retinal or SCN sensitivity to light (Zhang et al., 1998). The present results indicate that aftereffects can be induced in mice from birth to 1.5 years of age, with mice younger than 3 months showing the greatest plasticity (periods from 22.2 to 24.9 h vs. 23.1 to 24.2 h in middle-age mice and 23.7 to 25.1 h in old mice) because they entrain to a wider range of T cycles than older mice.

Mice that experienced the light cycle exclusively in utero also showed period aftereffects. Because the retinohypothalamic tract, required for direct photic entrainment, innervates its targets after birth (Muller and Torrealba, 1998; Speh and Moore, 1993), the effects of the T cycle on these mice must involve maternal signals. These signals likely require the maternal SCN (Reppert and Schwartz, 1983, 1986) and could include prenatal or postnatal humoral, temperature, or social cues from the mother (Davis and Gorski, 1985; Davis and Mannion, 1988; Weaver and Reppert, 1989; Duffield and Ebling, 1998; Viswanathan, 1999; Yamazaki et al., 2002b; Herzog and Huckfeldt, 2003; Ohta et al., 2003). Cross-fostering experiments could reveal whether maternal signaling of environmental period occurs in utero or after birth. Thus, while entrainment to non-24-h cycles is critical to induce aftereffects, the timing cues can be photic or maternal, and the induction can occur early in development.

Recordings from the isolated SCN showed that the prior light cycle induced long-lasting changes in the rhythmicity of *Per1* gene expression. It is not clear whether the change in period is intrinsic to single cells or results from interactions between SCN cells. Critically, although the sample size was small for other age groups, the periods of SCN *Per1-luc* and behavior were negatively correlated in young animals. This is the first demonstration of a condition that induces opposing changes in the periods of locomotor and SCN activity. These surprising results may indicate that extra-SCN tissues determine the behavioral period in T cycles, that the in vitro SCN is relieved of some input that drives its period in vivo, or that *Per1-luc* does not reflect the state of the SCN pacemaker driving behavioral rhythms in T cycles.

Limited evidence suggests that the SCN alone does not drive aftereffects expression. It has been demonstrated that replacing the SCN of an aftereffects-expressing animal with that of a non-T cycle-exposed donor does not abolish aftereffects (Matsumoto et al., 1996). Furthermore, when rats are rapidly transferred to short T cycles, they show rhythms in locomotor activity with 2 periods (Campuzano et al., 1998). One component entrains to the ambient T cycle, while the second component free runs with a period that is negatively correlated with the length of the T cycle. The SCN may be the pacemaker that failed to entrain in these animals. A second, unidentified circadian pacemaker would then be postulated to entrain to T cycles

and drive behavioral rhythms. Alternatively, the period of the SCN may be driven in vivo by tonic or rhythmic input from another part of the brain or body to determine the behavioral period. Isolating the SCN in vitro from this drive would be postulated to allow the SCN to express its intrinsic period that opposes the period change in vivo. Importantly, there is a precedent for this. The period expressed by the isolated snail eye has been shown to depend on both the prior T cycle and whether the eye is coupled to the brain (Page et al., 1997). Finally, the expression of *Per1-luc* from the ensemble of SCN cells may not reveal the activity of pacemaking cells within the SCN under all conditions, as was recently suggested for the SCN following shifts in the light schedule (Vansteensel et al., 2003). It may be, for example, that *Per1* is not part of the pacemaker that drives behavioral activity or that only a subset of SCN cells is changed by the T cycle to drive behavioral aftereffects so that bioluminescence from the *Per1-luc* transgene masks the state of the underlying pacemaker.

Each of these possibilities represents a major revision to the conventional model of the whole SCN as the master circadian pacemaker driving mammalian behavior (Ralph et al., 1990). Because there is extensive evidence for extra-SCN circadian oscillators (reviewed in Herzog and Tosini, 2001; Schibler et al., 2003) and recent evidence for retinal modulation of SCN rhythmicity (Lee et al., 2003), we tested whether the eyes could play a role in behavioral aftereffects. Enucleation did not disturb the long-lasting changes in locomotor period, indicating that the eyes are not required for aftereffects. Because the SCN receives a variety of neural inputs, it could be that multiple brain areas interact with the SCN to regulate plasticity of locomotor rhythms. It will be important, therefore, to determine whether other tissues show aftereffects and the direction of their period change.

Plasticity of the circadian system also includes changes in the duration of daily activity following exposure to long or short days (i.e., photoperiodism). In rodents, photoperiodic effects have been shown to change the waveform of free-running locomotor activity (Pittendrigh and Daan, 1976b) and electrical activity intrinsic to the SCN (Mrugala et al., 2000; Schaap et al., 2003). Thus, the SCN shows waveform changes that parallel behavioral aftereffects following exposure to different photoperiods. In contrast, *Per1* expression in the SCN does not parallel T cycle–induced changes in behavioral period. It is not clear whether the differences between aftereffects due to changes in cycle length or photoperiod represent fundamentally different forms of plasticity. It will be important to determine the effects of photoperiod on gene expression intrinsic to the SCN and the effects of T cycles on firing rate patterns of the in vivo SCN.

Although organisms do not normally experience non-24-h light cycles, the plasticity they show in response to such cycles likely reflects normal modulation of the circadian clockworks. The present results show that the intrinsic period of the SCN depends on the light history of the animal and that the behavioral period is regulated at an early age by photic and nonphotic inputs that influence the SCN and other components of the circadian system.

ACKNOWLEDGMENTS

We are grateful to Drs Daniel Granados-Fuentes and Ute Abraham for helpful discussions. The authors would like to thank Dan Piatchek and Jack Diani of the Washington University Hilltop animal facility, Tina Gegen, James Bogenpohl, and Laura M. Prolo for their expert technical assistance and animal care. This work was supported by the National Science Foundation (S.J.A.) and the National Institutes of Health (grants MH63104 and MH62517).

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