Circadian timekeeping in BALB/c and C57BL/6 inbred mouse strains

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Circadian rhythms of locomotion (wheel-running activity) in 12 inbred mouse strains were recorded for interstrain differences in \( \tau_{\text{DD}} \), the endogenous (free-running) period of the circadian pacemaker measured in constant environmental darkness. The results indicate that 1 or more genetic loci influence the value of \( \tau_{\text{DD}} \) and a large (50 min) difference in mean \( \tau_{\text{DD}} \) between 2 of the strains, BALB/cByJ and C57BL/6J, allowed further characterization of the origins and inheritance of the polymorphic expression of this circadian pacemaker property. The interstrain difference in mean \( \tau_{\text{DD}} \) was associated with an interstrain difference in light-induced shifts of the phase of the free-running locomotor rhythm; the BALB/c strain (with the shorter mean \( \tau_{\text{DD}} \)) displayed relatively fewer advance phase shifts. Neither the history of previous light exposure, albinism, nor elevated circulating testosterone levels could account for the interstrain difference in mean \( \tau_{\text{DD}} \). The value of \( \tau_{\text{DD}} \) based on the circadian rhythm of drinking activity (with the running wheel removed) was longer than that based on locomotion; this discrepancy was significantly greater and more variable in BALB/c than in C57BL/6 mice, though the interstrain difference in mean \( \tau_{\text{DD}} \) could not be attributed entirely to this effect. Reciprocal F, hybrids of BALB/c \( \times \) C57BL/6 matings revealed dominance of the C57BL/6 genotype, no sex linkage, and a significant (but small) maternal effect. Examination of CB6 recombinant inbred strains provided no support for the hypothesis of monogenic inheritance. Further study of inherited differences in the BALB/c and C57BL/6 strains may be a useful noninvasive experimental approach for investigation of the neurobiological substrates of circadian rhythmicity.

Daily rhythms of the biological activities in plants and animals are well-cataloged, universal phenomena. Ordinarily, environmental light synchronizes (entrains) the period and phase of these rhythms to the natural day–night cycle; but even in the absence of periodic environmental timing cues, many rhythms continue to oscillate (free run) with approximate 24-hr (circadian) periods. The persistence and properties of such environmentally independent, self-sustaining rhythms suggest the existence of an innate timekeeping mechanism, that is, a “biological clock” (Aschoff, 1981). Discrete neural sites that contain such clocks have now been identified in the nervous systems of a number of organisms. In mammals, such a pacemaker has been localized to the suprachiasmatic nuclei (SCN) of the anterior hypothalamus (for review, see Meijer and Rietveld, 1989): (1) lesions of the SCN result in a breakdown of the entrainment of genetic variation of circadian pacemaker properties. The techniques of induced mutagenesis and modern molecular genetics have yielded remarkable results identifying genetic loci that affect pacemaker behavior in Drosophila and Neurospora (for review, see Hall and Rosbash, 1987). “Clock mutations” in rodents are also known: there are congenitally anophthalmic rats (Richter, 1971; Ibuka, 1987) and mice (Scheuch et al., 1982; Gattermann et al., 1987) that do not entrain to light–dark (LD) cycles, mice with hypogenesis of the SCN that show disorganized circadian locomotor rhythmicity (Scheuch et al., 1982; Noguchi et al., 1986), mice that do not synthesize pineal melatonin (Ebihara et al., 1987), and golden hamsters with a mutation that shortens their pacemaker’s free-running circadian period (Ralph and Menaker, 1988). Natural genetic differences (polymorphisms) manifested by inbred strains of rodents also suggest that genetic background affects circadian rhythmicity (e.g., Ebihara et al., 1978; Oliverio and Malorni, 1979; Possidente and Hegenmann, 1980; Connolly and Lynch, 1981; Peleg et al., 1982; Büttner and Wollnik, 1984; Beau, 1988), but more work is needed in this area. Some of these studies were conducted in the presence of LD cycles, so free-running circadian rhythmicity...
Materials and Methods

Animals. Male mice, 4-6 weeks old at time of delivery, were obtained from the Jackson Laboratory, Bar Harbor, ME, or, in some instances, males and females were mated in our facility. Animals were individually housed in clear polycarbonate cages (19 \times 10.5 \times 8\) cm) within well-ventilated, light-proof environmental compartments (12 cages/compartment, 4 shelves of 3 cages each) isolated in an animal facility with temperature thermostatically controlled. Strains were not routinely segregated by shelf or compartment. Plastic cage bottoms were cut out and replaced by stainless-steel wire mesh (Penco Cage Products, Boston, MA), so that the cages could be suspended over trays containing wood shavings and charcoal pellets. Trays were cleaned and food hoppers and water bottles refilled once every 3 weeks without handling the animals. Light was provided by 15-W cool-white fluorescent tubes mounted above the shelves; intensity varied within the cages but was on the order of 300-400 lux at the midcage level. No light was present during darkness. When necessary, a single 15-W safe light with a dark red (series 2) filter was used to allow for routine care; mice were exposed to approximately 30 lux maximally and <1 lux usually.

Behavioral assays. Locomotor activity was monitored in mice when they were from 8 to 12 weeks old. A microswitch on the outside of each cage was activated by the rotation of a 5° metal rolling wheel. The number of switch closures per 15-min interval was automatically recorded and stored on hard disk by an IBM computer-based data-acquisition system (Dataquest, Mini-Mitter, Sunriver, OR). After log transformation, the number of wheel revolutions per 15-min interval was plotted for each mouse as an actogram; that is, activity over the course of each 24-hr period was plotted horizontally from left to right, with succeeding days stacked vertically from top to bottom. "Double-plotted" actograms repeated this format for 48-hr periods, that is, day n was followed by day n + 1 horizontally, succeeded by day n + 1 and n + 2 on the next line, then by day n + 2 and n + 3, and so on.

Drinking activity was monitored in similarly aged animals housed in the same cages but with the running wheels removed. Leads from false, raised cage floors constructed from 20-gauge galvanized-steel wire cloth (Hub Wire Cloth, Everett, MA) and from the metal spouts of drinking tubes were wired to lick sensors so that each tube contact was recorded and stored by the computer. After log transformation, the number of licks per 15-min interval was plotted in actogram format.

All mice were entrained in a 12-hr:12-hr LD cycle for 10-14 before exposure to constant darkness for an additional 2-4 weeks. \(rDD\) was calculated from the slope of a visually fit line through successive daily activity onsets (Pittendrigh and Daan, 1976a) and reported as mean + SEM. Each of the experiments further characterizing the BALB/c and C57BL/6 strains was conducted using new sets of purchased mice; the 2 strains or treatments to be compared were run concurrently in the same cabinets.

Results

Table 1 shows mean values of \(rDD\) for locomotor rhythms in the 12 inbred mouse strains we surveyed. The range of values was large (near 1 hr, from 22.94 to 23.93 hr), with a grand mean \(rDD\) of 23.53 hr for all the strains combined. Variability within the strains was not homogeneous across the 12 strains \(p < 0.05,\) Levene’s test for variances; \(F = 2.07; df = (11,60).\) An analysis of variance (ANOVA) comparing the variability between strains to the variability within strains (Welch approximation for unequal variances) showed that the 12 strain means were not homogeneous \(p < 0.0001;\) \(F = 26.62; df = (11,19).\) These tests indicate that the interstrain variation of \(rDD\) was significantly greater than its intrastrain variation.

We chose the BALB/cByJ and C57BL/6J strains to further investigate these interstrain differences. Their mean \(rDD\) difference of 0.83 hr (nearly 50 min) \(p < 0.001,\) pairwise t test with Bonferroni correction) was easily seen on actograms with the naked eye (Fig. 1) and represented nearly the full range of short and long \(rDD\)'s that we measured. Additional advantages to the choice of these 2 strains were their ready availability and low expense, the existence of recombinant inbred (RI) strains (see below), and their widespread use in a variety of biomedical research applications (Foster et al., 1982).

Light-induced phase shifts of locomotor rhythms in BALB/c and C57BL/6 strains

Despite the gross interstrain difference in mean \(rDD\) onset of locomotor activity in both strains began at or near the onset of darkness when animals were maintained in a 12-hr:12-hr LD cycle (Fig. 1). In some of the BALB/c mice, this observed phase of activity onset was not the phase predicted by backward extrapolation along the slope of onsets during the subsequent free run in constant darkness (e.g., Fig. 1, left), suggesting that earlier activity onset might be "masked" by the light phase of the LD cycle. This was not a consistent finding, however, and activity onset after lights out in other BALB/c mice appeared to reflect true entrainment. Because the phase relationship of an entrained circadian rhythm to the LD cycle is a function of both \(T\) and \(rDD,\) we investigated some of the potential origins of their inherited difference in \(rDD,\) and began to analyze the mode of inheritance of this difference. The goal of our experiments was to provide a foundation of basic information to encourage future physiological, pharmacological, and morphological studies of the circadian systems of these 2 widely utilized mouse strains.
Table 1. τ\textsubscript{DD} of inbred mouse strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>n</th>
<th>Mean τ\textsubscript{DD} ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>129/P\textsuperscript{o}</td>
<td>6</td>
<td>23.93 ± 0.07</td>
</tr>
<tr>
<td>RF/J\textsuperscript{o}</td>
<td>6</td>
<td>23.92 ± 0.06</td>
</tr>
<tr>
<td>C57BL/6/J</td>
<td>10</td>
<td>23.77 ± 0.02</td>
</tr>
<tr>
<td>SWR/J\textsuperscript{o}</td>
<td>3</td>
<td>23.70 ± 0.02</td>
</tr>
<tr>
<td>SEC/1ReJ</td>
<td>8</td>
<td>23.59 ± 0.04</td>
</tr>
<tr>
<td>AKR/J\textsuperscript{o}</td>
<td>5</td>
<td>23.52 ± 0.04</td>
</tr>
<tr>
<td>DBA/J\textsuperscript{o}</td>
<td>3</td>
<td>23.46 ± 0.05</td>
</tr>
<tr>
<td>C57BL/10J</td>
<td>5</td>
<td>23.43 ± 0.01</td>
</tr>
<tr>
<td>C57L/J</td>
<td>4</td>
<td>23.42 ± 0.13</td>
</tr>
<tr>
<td>A/J\textsuperscript{o}</td>
<td>10</td>
<td>23.37 ± 0.07</td>
</tr>
<tr>
<td>B10.D2(8N)/Sn</td>
<td>4</td>
<td>23.34 ± 0.15</td>
</tr>
<tr>
<td>BALB/c\textsuperscript{o}</td>
<td>8</td>
<td>22.94 ± 0.06</td>
</tr>
</tbody>
</table>

\textsuperscript{o} Albino strains.

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The value of τ is influenced by the parameters of the preceding entrainment schedule, that is, the LD cycle’s period and photoperiod, and by the history of previous light pulses (Pittendrigh and Daan, 1976a). Although BALB/c and C57BL/6 mice were both entrained to the same 12-hr:12-hr LD cycle for 10–14 d before τ\textsubscript{DD} was measured, we could not be certain how the animals were treated before they were shipped to us. In order to determine whether unexpected aftereffects of previous light exposure might account for the interstrain difference in mean τ\textsubscript{DD}, BALB/c and C57BL/6 mice were raised in darkness from conception through birth, weaning, and behavioral testing. Male and female mice of each strain were housed together until a vaginal plug was observed; impregnated females (2 BALB/c and 3 C57BL/6) were maintained in constant darkness through pregnancy, parturition, and lactation; and pups from all litters were weaned in darkness at 4 weeks and placed in running-wheel cages at 5–6 weeks. Mean values of τ\textsubscript{DD} for locomotor rhythms in these BALB/c and C57BL/6 mice were 22.98 ± 0.06 hr (n = 8) and 23.77 ± 0.04 hr (n = 11), respectively (Fig. 4); these values were clearly no different (p > 0.6 by ANOVA) from the values listed in Table 1 for mice that were previously entrained to an LD cycle before τ\textsubscript{DD} was measured.

The value of τ for locomotor rhythmicity is reported to be longer in castrated male mice than in sham-operated controls (Daan et al., 1975); this effect is reversed by testosterone replacement. Interestingly, plasma testosterone levels in BALB/c mice are reported to be twice the levels in C57BL/6 mice (Batty, 1978; Melanitou et al., 1987). In order to determine whether this interstrain endocrine difference might account for the shortened mean τ\textsubscript{DD} in the BALB/c strain relative to the C57BL/6 strain, male BALB/c mice underwent orchietomy or sham operation. Animals were first maintained for 3 weeks in constant darkness, then placed in a 12-hr:12-hr LD cycle for 7 d before surgery, and, after an additional 7 postoperative d in the LD cycle, they were reexposed to constant darkness. So that every

![Figure 1](image-url)
Figure 2. Light-induced phase shifts of free-running locomotor rhythms in BALB/c (left) and C57BL/6 (right) mice. Light pulses (100–200 lux for 15 min) were applied on the days (open arrowheads) and times (asterisks) indicated. Pulses presented during inactivity (subjective day) had little or no effect (shown are ct 9 for BALB/c, ct 5 for C57BL/6). Pulses near activity onset (early subjective night) caused phase delays (shown is ct 14 for both strains), whereas pulses near activity offset (late subjective night) caused phase advances in C57BL/6 mice only (shown are ct 24 for BALB/c and ct 1 for C57BL/6).
animal served as its own control, Δτ for each mouse was calculated by subtracting each animal's preoperative τDD from its postoperative τDD. For most mice, Δτ had a positive value (i.e., castration lengthened τDD by some amount); in 1 extraordinary animal, it increased from 22.72 to 23.50 hr (a Δτ of 0.78 hr, nearly 47 min; Fig. 5). However, the mean Δτ for the entire population of castrates was modest (+0.19 ± 0.10 hr, n = 8) and not significantly different from the mean Δτ for the population of sham-operated controls (+0.04 ± 0.06 hr, n = 6; p = 0.27 by ANOVA; F = 1.35, df = (1,12)).

The value of τ is also modified by environmental influences; the use of a running wheel appears to shorten τ for activity rhythms in hamsters (Pratt and Goldman, 1986) and rats (Yamada et al., 1988). In order to determine whether an interstrain difference in the running wheel's τ-shortening effect might account for the interstrain difference in mean τDD that we measured, another circadian rhythm (drinking activity with the running wheel removed) was recorded in BALB/c and C57BL/6 mice. In 1 group of mice, locomotor activity was first recorded as usual in constant darkness, running wheels were then removed and the cages equipped for measuring drinking activity while the animals were maintained for 1–2 weeks in a 12-hr:12-hr LD cycle, then drinking activity was recorded in constant darkness. The order of testing was reversed in a second group of mice. So that every animal served as its own control, Δτ for each mouse was calculated by subtracting each animal's τDD for the locomotor rhythm from its τDD for the drinking rhythm. For most mice, whatever the order of testing, Δτ had a positive value; that is, τDD (drinking) was longer than τDD (locomotion) (Fig. 6). This discrepancy appeared more marked, with much greater variability, in BALB/c mice; the range of Δτ values was 0.51 hr (nearly 31 min) in BALB/c mice but only 0.11 hr (less than 7 min) in C57BL/6 mice. The most extreme case was a BALB/c mouse with a τDD (locomotion) of 23.02 hr but a τDD (drinking) of 23.54

Figure 4. τDD for locomotor rhythms in BALB/c and C57BL/6 mice after 10–14-d entrainment to 12-hr:12-hr LD cycle (from Table 1) and in mice raised in darkness from conception through birth, weaning, and behavioral testing. See Results for details.

Figure 5. Δτ (τDD after surgery − τDD before surgery) for individual BALB/c mice. See Results for details.
A -to.40

There was no overall effect of sex on mean $\tau_{DD}$ [$p = 0.52$ by $\text{ANOVA; } F = 5.67$, $df = (1,14)$].

On the mode of inheritance of the interstrain difference in mean $\tau_{DD}$

Table 2 shows mean values of $\tau_{DD}$ for locomotor rhythms in the reciprocal $F_1$ hybrids of BALB/c and C57BL/6. All the values for the $F_s$ grossly resembled the C57BL/6 genotype. There was no overall effect of sex on mean $\tau_{DD}$ [$p = 0.52$ by ANOVA; $F = 0.43$, $df = (1,30)$].

Although it is likely that multiple genetic loci mediate the many interstrain differences in mean $\tau_{DD}$ listed in Table 1, a single locus may be responsible for the difference observed between any 2 strains (e.g., BALB/c and C57BL/6). One strategy to begin to assess this possibility makes use of RI strains (Bailey, 1981). These strains are derived by crossing 2 already existing inbred strains (the progenitor strains), then returning to brother x sister inbreeding from the $F_1$ generation onwards. The genome of each established RI strain is a replicable, homogeneous mixture of the 2 progenitor genomes. When a trait that differs between the BALB/c and C57BL/6 strains is a replicable, homozygous mixture $\text{ANOVA; } F = 3.39$).

Although it is likely that multiple genetic loci mediate the many interstrain differences in mean $\tau_{DD}$ listed in Table 1, a single locus may be responsible for the difference observed between any 2 strains (e.g., BALB/c and C57BL/6). One strategy to begin to assess this possibility makes use of RI strains (Bailey, 1981). These strains are derived by crossing 2 already existing inbred strains (the progenitor strains), then returning to brother $\times$ sister inbreeding from the $F_1$ generation onwards. The genome of each established RI strain is a replicable, homogeneous mixture of the 2 progenitor genomes. When a trait that differs between 2 of the progenitors (e.g., $\tau_{DD}$) is determined by a single genetic locus, an RI strain-distribution pattern emerges: one of the progenitors and approximately $\frac{1}{2}$ of the RI strains show one phenotype, while the second progenitor and the rest of the RI strains show the other phenotype. A polygenic mode of inheritance does not show this bimodal strain distribution pattern. Table 3 shows mean values of $\tau_{DD}$ for locomotor rhythms in the 7 CXB RI strains. Although these means were all relatively long (ranging from 23.59 to 23.87 hr) and superficially resembled the mean $\tau_{DD}$ in the C57BL/6 progenitor strain, they were not a homogeneous group [$p < 0.0001$ by ANOVA; $F = 8.87$, $df = (6,63)$].

Discussion

The murine circadian timekeeping mechanism functions as an innate clock, its development genetically programmed independently of the environment (Davis and Menaker, 1981). The availability of inbred mouse strains that display robust, non-pathological differences in the properties of their circadian systems allows an assessment of the genetic contributions to clock function. Inbred strains are derived from multiple generations of brother $\times$ sister matings; individuals within a given strain are homozygous and genetically identical. Therefore, differences within strains suggest environmental influences or errors of measurement, whereas differences between strains imply genetic variation at loci affecting the trait of interest. Our data indicate that 1 or more genetic loci influence the value of $\tau$, as others have previously concluded (Ebihara et al., 1978; Possidente and Hegmann, 1980, 1982; Buttner and Wollnik, 1984; Ralph and Menaker, 1988; Peleg et al., 1989). The mean $\tau_{DD}$ difference between the BALB/c and C57BL/6 strains of almost 1 hr is a large difference, considering that $\tau$ cannot deviate too far from 24 hr for the pacemaker to remain circadian. Indeed, even the harsh treatment of feeding mice heavy water elicits a $\tau$ change of little more than 1.5 hr (Daan and Pittendrigh, 1976b).

Table 4 shows previously published $\tau$'s for some of the inbred strains we surveyed, even though the studies differ in the substrains and recording techniques used, the measured $\tau$'s in BALB/c and C57BL/6 mice in these reports also appear relatively short and long, respectively. On the other hand, Olivierio and associates reported that BALB/c mice do not show a "clear-cut" circadian locomotor rhythm in constant darkness (quoted in Kempf et al., 1982). Rosenwasser (1990) has critiqued technical aspects of this work, and his examination of 5 BALB/c male mice instead disclosed activity rhythms that were present but markedly unstable. There were abrupt changes of $\tau$, disintegration of activity into ultradian periodicities, and apparent

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**Table 3. $\tau_{DD}$ of recombinant inbred strains**

<table>
<thead>
<tr>
<th>Strain</th>
<th>n</th>
<th>Mean $\tau_{DD}$ ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>CXBD/By</td>
<td>10</td>
<td>23.78 ± 0.03</td>
</tr>
<tr>
<td>CXBE/By</td>
<td>12</td>
<td>23.69 ± 0.03</td>
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<tr>
<td>CXBG/By*</td>
<td>10</td>
<td>23.87 ± 0.03</td>
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<tr>
<td>CXBH/By</td>
<td>10</td>
<td>23.62 ± 0.03</td>
</tr>
<tr>
<td>CXBH/By</td>
<td>12</td>
<td>23.73 ± 0.01</td>
</tr>
<tr>
<td>CXBJ/By</td>
<td>6</td>
<td>23.78 ± 0.04</td>
</tr>
<tr>
<td>CXXR/By</td>
<td>10</td>
<td>21.59 ± 0.04</td>
</tr>
</tbody>
</table>

* Albino strains.

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**Table 2. $\tau_{DD}$ of reciprocal $F_1$ hybrids**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Sex</th>
<th>n</th>
<th>Mean $\tau_{DD}$ ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>CByB6F$_1$</td>
<td>F</td>
<td>8</td>
<td>23.72 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>8</td>
<td>23.63 ± 0.05</td>
</tr>
<tr>
<td>B6CByF$_1$</td>
<td>F</td>
<td>8</td>
<td>23.80 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>8</td>
<td>23.84 ± 0.03</td>
</tr>
</tbody>
</table>

* F, female; M, male.
arhythmicity. We did not find such gross lability in our records; some of our BALB/c mice did not run well (most commonly by not running at all), but this was also true for mice of other strains (e.g., SEC/1ReJ). Although we did not study our animals for as long as Rosenwasser did (approximately 240 d), the circadian disorganization he observed appeared unrelated to the length of time that the mice spent in free-running conditions. Because his subjects were obtained from a private breeding stock originally derived from the Jackson Laboratory’s BALB/cJ strain, it is possible that genetic “drift” has led to differences between his BALB/c mice and ours. (We have now recorded locomotor rhythms in 6 BALB/cJ mice purchased from the Jackson Laboratory; 1 animal did not run, but the others expressed robust rhythms with a mean $t_{DD}$ of 23.12 ± 0.19 hr.) Another difference was the continuous presence of dim red light (25–50 lux) during Rosenwasser’s experiments; in addition, his mice cohabited with C57BL/6 females for intervals of 4–5 days while the study was in progress.

Our observation that the onset of locomotor activity in BALB/c and C57BL/6 mice adopted a similar phase relationship to the LD cycle implies that an interstrain difference in PRC shape must compensate for the interstrain difference in mean $t_{DD}$. On the basis of entrainment theory, we expected that either the value of $D$ in the BALB/c strain would be greater than its value in the C57BL/6 strain or (as in fact we found) the value of $A$ was greater. Our results on $r$ and PRC shape are in complete agreement with the work of Daan and Pittendrigh (1976a). These workers showed that $D - A$ has a positive value in the mouse (but a negative one in the hamster) and that the value of $D - A$ is larger (more positive) if $r$ is shorter. A decrease in $A$ was responsible for this effect in the mouse, whereas an increase in $D$ also occurred in the hamster (see also Puchalski and Lynch, 1988, for preferential changes of $D$ in hamsters). Thus, our data indicate that the correlation between $r$ and PRC shape holds not only for comparisons between species and between individuals within a species (Daan and Pittendrigh, 1976a), but also for comparisons between 2 inbred strains of a species.

Our next set of experiments on the strains investigated the possible influence of factors that are known to modify the value of $r$ in rodents. One such factor is the history of previous light exposure (Pittendrigh and Daan, 1976a). Although such aftereffects of light only temporarily influence $r$ in pigmented mice (Davis and Menaker, 1981), but not in cockroaches: see Barrett and Page, 1989), we were concerned that the albinism of BALB/c mice might have altered their light perception in some way (Possidente et al., 1982). Nonetheless, our data indicate that the interstrain difference in mean $t_{DD}$ remained unchanged even in mice that were never exposed to environmental light. Paretically, our survey (Table 1) revealed no obvious relationship between albinism and mean $t_{DD}$. 129/J, RF/J, SWR/J, AKR/J, A/J, and BALB/cByJ are all albino strains.

Our data also indicate that the interstrain difference in mean $t_{DD}$ did not depend on the higher circulating levels of testosterone found in BALB/c mice (Batty, 1978; Melanitou et al., 1987). Castrated male mice have been reported to show longer $r$’s than sham-operated animals (Daan et al., 1975), but this finding was partially due to a postsurgical shortening of $r$ in the shams, which orchitectomy prevented. Castrated male hamsters do not show longer $r$’s (Morin and Cummings, 1981; Davis et al., 1983). Additional evidence that circulating testosterone levels alone did not account for interstrain mean $t_{DD}$ differences comes from our survey (Table 1); while testosterone concentrations in BALB/c mice are twice those in C57BL/6 mice (Batty, 1978), testosterone concentrations in 129/J and RF/J mice (with the longest mean $t_{DD}$’s we measured) are reported to be approximately 8 times higher (Melanitou et al., 1987). Thyroxine concentrations are also higher in BALB/c than in C57BL mice (7.4 ± 0.5 $\mu$g/ml vs 5.2 ± 0.4 $\mu$g/ml; Stewart et al., 1978), but this difference is unlikely to be important in the determination of $r$ (Morin, 1988).

Finally, we recorded circadian rhythms of drinking activity in the 2 strains because there have been reports that access to a running wheel yields measurements of $r$ shorter than measurements based on other activity rhythms recorded in the same animals housed in cages without wheels [e.g., rhythms of burrow emergence (Pratt and Goldman, 1986), drinking (Yamada et al., 1986), and movement in the cage (Yamada et al., 1986)]. Because the act of running in a wheel seems to affect circadian timekeeping in rodents (for review, see Mrosovsky et al., 1989), we were concerned that the relatively short mean $t_{DD}$ in BALB/c mice might have been due in some way to their “hyperemotionality” (Thompson, 1953) and possibly greater activity (Lassalle and Le Pape, 1978; Beau, 1986) in the running wheel than C57BL/6 mice. However, because the mean $t_{DD}$’s in BALB/c mice was 0.20 hr (12 min) longer than the mean $t_{DD}$ in C57BL/6 mice (Possidente et al., 1982), the 50-min interstrain difference in mean $t_{DD}$ cannot be attributed entirely to a differential effect of the running wheel on the BALB/c strain. Nevertheless, the putative “feedback” of an expressed circadian rhythm to its pacemaker merits further study; the BALB/c strain may be a useful model for investigation of the running wheel’s apparent ability to accelerate the pacemaker’s endogenous oscillation. In BALB/c mice, the discrepancy between the measurement of $t_{DD}$ based on drinking and that based on locomotion varied widely among individual mice (from 0.6 to 31 min); in individual C57BL/6 mice, the measured $t_{DD}$ for drinking and locomotion were in no case different by much more than 5 min. Although our results were no different if we reversed the order of testing of the 2 circadian rhythms, our next task will be to assess more systematically spontaneous changes in $t_{DD}$ over time by long-term recordings in BALB/c mice (Rosenwasser, 1990). We must also address the possibility that

<table>
<thead>
<tr>
<th>Str</th>
<th>$t_{DD}$</th>
<th>$r$</th>
<th>Reference</th>
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<tbody>
<tr>
<td>C57BL/6</td>
<td>23.77</td>
<td>23.59</td>
<td>Ebihara et al., 1978</td>
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<td>Possidente and Stephan, 1988</td>
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<td>Ebihara et al., 1988b</td>
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<td></td>
<td>23.52</td>
<td></td>
<td>Abe et al., 1989</td>
</tr>
<tr>
<td>AKR/J</td>
<td>23.52</td>
<td>23.5</td>
<td>Possidente et al., 1982</td>
</tr>
<tr>
<td>DBA/2J</td>
<td>23.46</td>
<td>23.31</td>
<td>Possidente and Stephan, 1988</td>
</tr>
<tr>
<td>C57BL/10</td>
<td>23.43</td>
<td>23.47</td>
<td>Ebihara et al., 1978</td>
</tr>
<tr>
<td></td>
<td>23.9</td>
<td></td>
<td>Possidente et al., 1982</td>
</tr>
<tr>
<td></td>
<td>23.5</td>
<td></td>
<td>Welsh et al., 1986</td>
</tr>
<tr>
<td></td>
<td>23.30</td>
<td></td>
<td>Possidente and Stephan, 1988</td>
</tr>
<tr>
<td>A/J</td>
<td>23.37</td>
<td>22.9</td>
<td>Possidente et al., 1982</td>
</tr>
<tr>
<td>BALB/c</td>
<td>22.94</td>
<td>23.4</td>
<td>Haus et al., 1967</td>
</tr>
<tr>
<td></td>
<td>22.7</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>22.90</td>
<td></td>
<td>Possidente and Stephan, 1988</td>
</tr>
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*Values taken from the present study.
*Values taken from previous studies.
*Value of $r$ obtained in blinded mice.
*Value of $r$ obtained in mice maintained in constant dim red light.
drinking and locomotor rhythms express different \( \tau_{\text{fre}} \) simul-
taneously, that is, that the rhythms might be internally desynchron-
zied in individual BALB/c mice. Further work using a different type of cage and recording setup will be required to resolve this issue; however, drinking and locomotor rhythms are normally synchronized in rodents (Shibuya et al., 1980), and we are aware of no precedent for internal desynchronization of circadian rhythms in mice (Haus et al., 1967; Richardson et al., 1985; Welsh et al., 1985).

Further work will also be required to determine the mode of inheritance of the interstrain difference in mean \( \tau_{\text{fre}} \). Examination of reciprocal F1 hybrids will reveal dominance effects, sex linkage, and maternal influences transmitted to progeny in utero and during postnatal development. Although the C57BL/6 genotype was dominant in our cross, it is important to note that an allele dominant in a given hybrid combination may be recessive in another (e.g., in the cross between the C57BL/6 and DBA/2J strains; Possidente and Stephan, 1988). As previously reported in mice (Possidente and Stephan, 1988), we found no effect of sex on the mean value of \( \tau_{\text{fre}} \). On the other hand, we did find a statistically significant maternal influence, though its amount (0.14 hr) was small (i.e., on the order of 8 min). Possidente and Stephan (1988) noted a suggestive (but statistically insignificant) maternal effect on the value of \( \tau_{\text{fre}} \) in the reciprocal F1s of a cross between the C57BL/6 and DBA/2J strains, but they found no effect when C57BL/10Sn and BALB/c mice were crossed. Similarly, crosses between wild-type hamsters and those homozygous and heterozygous for the tau mutation produce offspring with altered \( \tau_{\text{fre}} \)s that are unaffected by their mother’s phenotype (Ralph and Menaker, 1988). Furthermore, crosses between inbred strains that differ in parameters describing their expressed circadian rhythms entrained to the LD cycle (e.g., phase and amplitude) yield reciprocal F1 hybrids that do not differ in these parameters (e.g., LD-entrained rhythms of glyceraldehyde-3-phosphate dehydrogenase activity of the thymus in C57BL/6 × A/J mice: Peleg et al., 1982; body temperature in C57BL/6 × C3H/2Hbg mice: Connolly and Lynch, 1983; locomotion in LEW/Ztm × ACI/Ztm rats: Wollnik et al., 1987; locomotion in C57BL/6 × BALB/c mice: Beau, 1988). Of interest, in 2 species of wild mice (Mus hoehuiga and Acomys cahirinus), the role of the mothers in organizing their pups’ circadian rhythmicity differs and may depend on whether the species is altricial or precocious in its development (Viswanathan and Chandrashekar, 1985; Weaver and Reppert, 1987). Considering the magnitude of the effect we found, it will be important to record circadian drinking rhythms in our CByB6 and B6Cby reciprocal F1 hybrids.

A similar RI strain-distribution pattern has been reported for the variation in size of the HincII and PstI restriction fragments, which hybridize to probes for 2 closely linked regions (the Fis-1 and Int-2 sites, respectively) on mouse chromosome 7 (Silver and Buckler, 1986). Importantly, our analysis using a multiple range test suggests that the recombinant strains are statistically separable into more than 1 phenotypic group. Thus, though the small number of available CXB RI strains limits the confidence with which conclusions can be made, our data provide no support for the hypothesis that the interstrain difference in mean \( \tau_{\text{fre}} \) is monogenically inherited. Beau (1988) reached a similar conclusion for the inheritance of several parameters describing LD-entrained locomotor rhythmicity in these strains.

In any case, whatever their genetic basis, inbred strain differences offer a promising noninvasive experimental tool for investigation of the neurobiological substrates of circadian rhythmicity (see, e.g., Hotz et al., 1987; Abe et al., 1989). Of particular interest have been recent studies exploring the response of the circadian systems of C57BL/6 and SK/Nga mice to agents that affect GABA neurotransmission (Ebihara et al., 1988a,b). GABA is the most plentiful substance identified in SCN axons and boutons (van den Pol and Goos, 1986) and is believed to modulate light-induced phase shifts of rodent circadian rhythms in a phase-dependent manner (i.e., the regulation of phase delays and phase advances appears to be regulated differently; Ralph and Menaker, 1989). It is noteworthy that several indices of GABA activity in the BALB/c and C57BL/6 strains show large differences: whole-brain glutamic acid decarboxylase activity is highest in BALB/c and lowest in C57BL/6 mice (of 7 strains tested; Tunnicliff et al., 1973), whole-brain \(^3\)H diazepam binding is lowest in BALB/c and nearly the highest in C57BL/6 mice (of 4 strains tested; Robertson, 1979), and an exploratory behavioral response to diazepam is lowest in BALB/c and second-highest in C57BL/6 mice (of 5 strains tested; Crawley and Davis, 1982). Therefore, pharmacological studies using BALB/c and C57BL/6 mice may help to illuminate the circadian functions of GABA and to elucidate its possible role in determining \( \tau \), PRC shape, and wheel-running behavior.

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