Different Effects of Intensity and Duration of Locomotor Activity on Circadian Period

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Abstract An outstanding unresolved issue in chronobiology is how the level of locomotor activity influences length of the free-running, endogenous circadian period (τ). To address this issue, the authors studied a novel model, 4 replicate lines of laboratory house mice (*Mus domesticus*) that had been selectively bred for high wheel-running activity (S) and their 4 unselected control (C) lines. Previous work indicates that S mice run approximately twice as many revolutions/day and exhibit an altered dopaminergic function as compared with C mice. The authors report that S mice have a τ shorter by about 0.5 h as compared with C mice. The difference in τ was significant both under constant light (control lines: τ = 25.5 h; selected: τ = 24.9 h) and under constant dark (control lines: 23.7 h; selected: 23.4 h). Moreover, the difference remained statistically significant even when the effects of running speed and time spent running were controlled in ANCOVA. Thus, something more fundamental than just intensity or duration of wheel-running activity per se must underlie the difference in τ between the S and C lines. However, despite significant difference in total wheel-running activity between females and males, τ did not differ between the sexes. Similarly, among individuals within lines, τ was not correlated with wheel-running activity measured as total revolutions per day. Instead, τ tended to decrease with average running speed but increase with time spent running. Finally, within individuals, an increase in time spent running resulted in decreased τ in the next few days, but changes in running speed had no statistically significant effect. The distinctions between effects of duration versus intensity of an activity, as well as between the among- versus within-individual correlations, are critical to understanding the relation between locomotor activity and pace of the circadian clock.

Key words artificial selection, dopamine, hyperactivity, Lomb-Scargle periodogram, *Mus domesticus*, sleep disorder, *tau*, wheel running

In a milestone paper, Aschoff (1960) suggested that the length of a free-running circadian rhythm, τ, should decrease with increasing "level of excitement" (e.g., level of locomotor activity). This hypothesis has been addressed in several studies applying a variety of approaches, and although many supported it (e.g., Daan et al., 1975; Edgar et al., 1991; Yamada et al., 1988, 1990; Shioiri et al., 1991; Oklejewicz et al., 1997; Hofstetter et al., 1999; Mistlberger and Holmes, 2000; Isobe and Nishino, 2001; Lewandowski and Usarek,

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2002), others were equivocal or even showed an opposite trend (e.g., Aschoff et al., 1973; Bult et al., 1993; Mrosovsky, 1999; Joshi, 1999; Deboer and Tobler, 2000). Conflicting results were reported even from experiments performed on the same species in the same laboratory (Mrosovsky, 1999, on hamsters).

One reason for the discrepancies could be an inconsistent or inadequate definition of the "level of activity." Differences in total locomotor activity among individuals or among treatment groups may arise from differences in the amount of time active, the average intensity of activity, or both. However, in all the studies cited above, "activity level" was measured as total activity, such as number of wheel revolutions averaged across 1 or several days, and the sources of individual or experimental group variation in the activity level were usually not discussed (but see Deboer and Tobler, 2000). For example, Lewandowski and Usarek (2002) showed that a bilateral lesion of the intergeniculate leaflet in mice results in both decreased locomotor activity and longer τ, as predicted by Aschoff's (1960) hypothesis. Although the authors reported only an overall activity level (wheel revolutions/day), inspection of a sample actogram indicates that running speed, rather than time spent on the activity, decreased after the lesion (Lewandowski and Usarek, 2002, Fig. 2). Conversely, Bult et al. (1993) found that mice from lines selected for low nest-building behavior had a higher total activity (wheel revolutions/day) but also had a longer τ compared to mice from control and high-selected lines—a result opposite to Aschoff's hypothesis. However, an inspection of a sample actogram suggests that in that experiment, the difference in overall activity resulted primarily from difference in time spent running, rather than running speed (Bult et al., 1993, Fig. 2). Perhaps the conflicting results are observed because circadian clock is oppositely associated with different components (speed vs. duration) of overall activity level.

Another difficulty with experimental tests of Aschoff's (1960) hypothesis is that a treatment applied to change the activity level can by itself change periodicity through an independent mechanism. This is especially a concern for light manipulation experiments but also for other treatments, such as food availability, access to running wheels, or even the size of the wheels, which are applied to cause higher or lower activity (Edgar et al., 1991; Yamada et al., 1988, 1990; Mrosovsky, 1999; Deboer and Tobler, 2000). Similarly, elegant surgical experiments that showed both a decreased locomotor activity and a prolonged τ after lesions of the intergeniculate leaflet (Lewandowski and Usarek, 2002) or ventral tegmental area (Isobe and Nishino, 2001) do not provide a definitive argument for a functional link between the traits because independent regulatory circuits may—theoretically—be located in the same organs. One solution to the problems is to explore inherent, genetically based variation in τ and locomotor activity.

Recent chronobiological research has focused on mutations of large effect, and some studies have shown the expected negative relation between τ and activity levels (Oklejewicz et al., 1997; Hofstetter et al., 1999). However, a genom-wide survey for quantitative trait loci, based on crosses of inbred mouse strains, failed to reveal loci related to both overall activity level and length of the circadian period (Shimomura et al., 2001). An alternative approach, applied here, is to identify genetic correlations among traits by use of an artificial selection experiment (Bult et al., 1993; Joshi, 1999; Garland, 2003).

In this study, we test Aschoff's (1960) hypothesis using a novel animal model: mice selectively bred for high voluntary wheel running (Swallow et al., 1998; Koteja et al., 1999; Girard et al., 2001; Rhodes et al., 2001; Garland, 2003; Rhodes and Garland, 2003). A three-way factorial experiment, in which males and females from the selected (S) and control (C) lines were tested under constant light (LL) or constant dark (DD), also allowed us to test for differences between the sexes and for interaction between genetic and environmental effects. Because wheel-running activity was recorded continuously with 1-min resolution, this design allows insight into the sources (speed vs. duration) of differences in total activity between selection groups, light regimens, sexes, and among individuals within the groups. Consequently, we are able to disentangle effects on τ of intensity versus duration of locomotor activity. Finally, we test whether within-individual temporal changes in the level of locomotor activity are associated with changes in τ.

MATERIAL AND METHOD

Subjects

Outbred, genetically polymorphic Hsd:ICR mice (Hauschka and Mirand, 1973; Rice and O'Brien, 1980; Carter et al., 1999) were used to establish 8 separate

lines (Swallow et al., 1998). In 4 lines, mice were selected for high wheel-running activity (S), measured as total daily wheel revolutions, while in the other 4 lines, the mice were randomly bred (C, control; sib-matings were disallowed in all lines). Voluntary wheel running was measured on Wahman-type activity wheels (1.12 m circumference = 0.36 m diameter; Lafayette Instruments, Indiana). In the S lines, the highest running male and female from each family were chosen as breeders; in the 4 C lines, breeders were chosen randomly from each family. Water and food (Harlan Teklad Laboratory Rodent Diet [W] 8604) were available ad libitum, photoperiod was set a constant 12:12-h light:dark cycle, and room temperature was controlled at approximately 23 °C. Details of the selection protocol and husbandry are given in our earlier work (Swallow et al., 1998).

For this experiment, we sampled 12 males and 12 females from each of the 8 lines (192 individuals) of generation 16 of the selection experiment. In C lines, we took randomly 1 (occasionally 2) individuals of each sex from each available family. In S lines, the highest running male and female from each family were used as breeders and were not available for this experiment. To account for the bias, we excluded the lowest running individuals from each family and chose randomly from the remaining individuals of each family.

Protocol

Two to 5 weeks after the regular 6-day wheel-running measurements (the selection criterion), the 192 individuals were weighed and placed in the cages with wheels connected to a recording system, which counted the number of wheel revolutions in 1-min intervals. Mice from each line were equally assigned to two rooms. The assignment was random but with the provision that not more than 1 male and 1 female from the same family were placed in the same room. Therefore, each line was represented by 6 males and 6 females in each room. During the first 7 days of the experiment, the usual 12:12-h L:D photoperiod was maintained in both rooms. Then the lights were left on permanently in room 1 (LL; ~400 lux, measured above cages) and off permanently in room 2 (DD; dim red lights were left on, to allow occasional cage maintenance; ~2 lux), and wheel activity was recorded during the next 30 days. The rooms were entered quietly only by one person once every few days for a few minutes, at irregular hours. The exception was day 14 of the constant-light condition (DD or LL), when both rooms were entered for about 1.5 h to change the animal cages and provide fresh food and water (wheels were not changed). Computer recordings of wheel activity were interrupted every few days for about 0.5 h to enable data transfer.

Research presented here was described in Animal Research Protocol No. A-48-9700-L00101-3-04-96, approved by the Institutional Animal Care and Use Committee of the College of Letters and Science, University of Wisconsin–Madison.

Data Analyses

The Lomb-Scargle periodogram analysis program (LSPER; Ruf, 1999; see also Van Dongen et al., 1999) was used to find the length of the free-running circadian period (τ). The method can effectively handle missing records and has been shown to be more accurate and less sensitive to deviations from sinusoidal activity pattern than the χ^2 periodogram (Ruf, 1999). The analysis was performed for periods ranging from 18 to 30 h with a 1-min increment (corresponding to recording intervals).

Analyses were performed two ways. First, τ was estimated from wheel-running activity recorded during the last 15 days of the experiment (i.e., days 15-30 of LL or DD). Second, for each individual, five estimates of τ were obtained from five consecutive 3-day segments of the same 15 days as above. The estimates of τ, based on only 3 days, bear a large random error, but this is compensated by a large number of data points. An important advantage of calculating five separate values for each individual is that it allows for an estimate of within-individual variation of the length of the circadian period (i.e., provides information about consistency of the circadian rhythm). Because standard deviation (SD) usually tends to increase with a trait mean value, we used a dimensionless coefficient of variation (τ_{cv} = 100 • SD/average τ obtained in five 3-day intervals) as a measure of consistency (actually, inconsistency) of the length of the circadian period.

One wheel was malfunctioning, 1 individual died of unknown causes, and 1 became arrhythmic, so it was not possible to estimate τ. Thus, data were available for 189 individuals. In three cages, wheels got blocked in the last few days, so the estimates were based on shorter intervals (total of 10 or 12 days), and only three or four rather than five 3-day intervals were suitable for analysis in these individuals. Thus, a total

of 940 three-day intervals were available for analysis of within-individual variation.

We used a cross-nested ANCOVA to test simultaneously the fixed effects of selection, sex, and light regimen (LL vs. DD); the random effect of replicate lines nested within the selection groups; and all possible interactions between the factors. Statistical significance of the effects was tested with appropriate denominator mean squares (MS) and degrees of freedom (df) according to Sokal and Rohlf (1981) (e.g., for the effect of selection—with 1 and 6 df and MS among replicate lines as an error term). Type III sums of squares were used for all tests. In all the analyses, wheel "resistance," measured as a number of free wheel rotations after the wheel has been accelerated to a constant speed, was included as a covariate (although it appeared not to affect significantly any variable analyzed). The dependent variables in the analyses were body mass, total number of wheel revolutions per day (total activity), per day number of 1-min intervals with any wheel activity (time spent active), number of wheel revolutions per active interval (average running speed measured), maximum number of wheel revolutions per interval (maximum running speed), circadian free-running period (τ), and within-individual coefficient of variation of the circadian period (τ_{cv}).

To test for the presence of a correlation (among individuals within experimental groups) between wheel-running activity and circadian rhythm, we used similar ANCOVA models with the total number of wheel revolutions per day or with the number of active intervals and running speed as additional covariates. Thus, all the coefficients of correlation (r) presented in the results are for partial correlation and were tested with appropriate df (corrected for number of cofactors and covariates in the model).

Finally, to test for the correlations at the within-individual level, we used separate values from five consecutive 3-day records and included individuals as categories (nested within Line × Sex × Light interaction) in the ANCOVA models (thus, the model had two levels of nesting). To remove the effect of a possible systematic, linear trend in τ across the five consecutive records, the model also included the trial number as a covariate. Significance of trends across the five 3-day records was also tested with a repeated-measures ANOVA and orthogonal polynomial contrasts.

All significance levels reported are for two-tailed tests, and $p < 0.05$ was used as the criterion for statistical significance in all tests. Analyses were performed with SYSTAT 10 for Windows (SPSS, Inc.).

RESULTS

Body Mass

Males were larger than females ($p < 0.001$), and initial mass was greater in the C mice than in the S mice ($p = 0.027$; mean ± SD: C females, 27.2 ± 2.56 g; S females, 25.5 ± 2.53 g; C males, 35.2 ± 3.15 g; S males, 32.0 ± 2.74 g). However, the S mice gained slightly more mass during the experiment, so that at the end of experiment, body mass did not differ significantly between the selection groups ($p = 0.077$; C females, 28.2 ± 2.79 g; S females, 26.5 ± 2.64 g; C males, 36.0 ± 2.86 g; S males, 33.4 ± 3.51 g). Preliminary analyses showed that body mass had no effect on circadian period ($p > 0.4$) either when included as a single covariate or in combination with wheel-running traits. Thus, body mass was not included in the final analyses presented below.

Wheel-Running Activity

As expected, S mice ran more revolutions/day than did C mice ($p = 0.002$), mice under DD ran more than those under LL ($p = 0.002$), and females ran more than males ($p = 0.002$; Fig. 1). No interaction between the factors was significant, which indicates that the effects were largely additive. Average running speed (total revolutions divided by the number of 1-min intervals with any revolutions) in the S lines was nearly twice as high as in the C lines, while the increase in time spent running (number of the intervals with any revolutions) was small and not statistically significant (Fig. 1). The maximum spontaneous running speed during any 1-min interval was also higher in the S lines. On the other hand, the difference between light conditions resulted entirely from time spent running, whereas the average and maximum running speeds were the same in LL and DD. Finally, both components of total activity contributed to the difference between sexes: females ran faster and spent more time running than did males (Fig. 1).

The number of active intervals and running speed were correlated among individuals within the Line × Sex × Light groups ($r = 0.220$, $p = 0.006$) and also across the five 3-day intervals within individuals ($r = 0.299$, $p < 0.001$). Therefore, to assess independent effects of

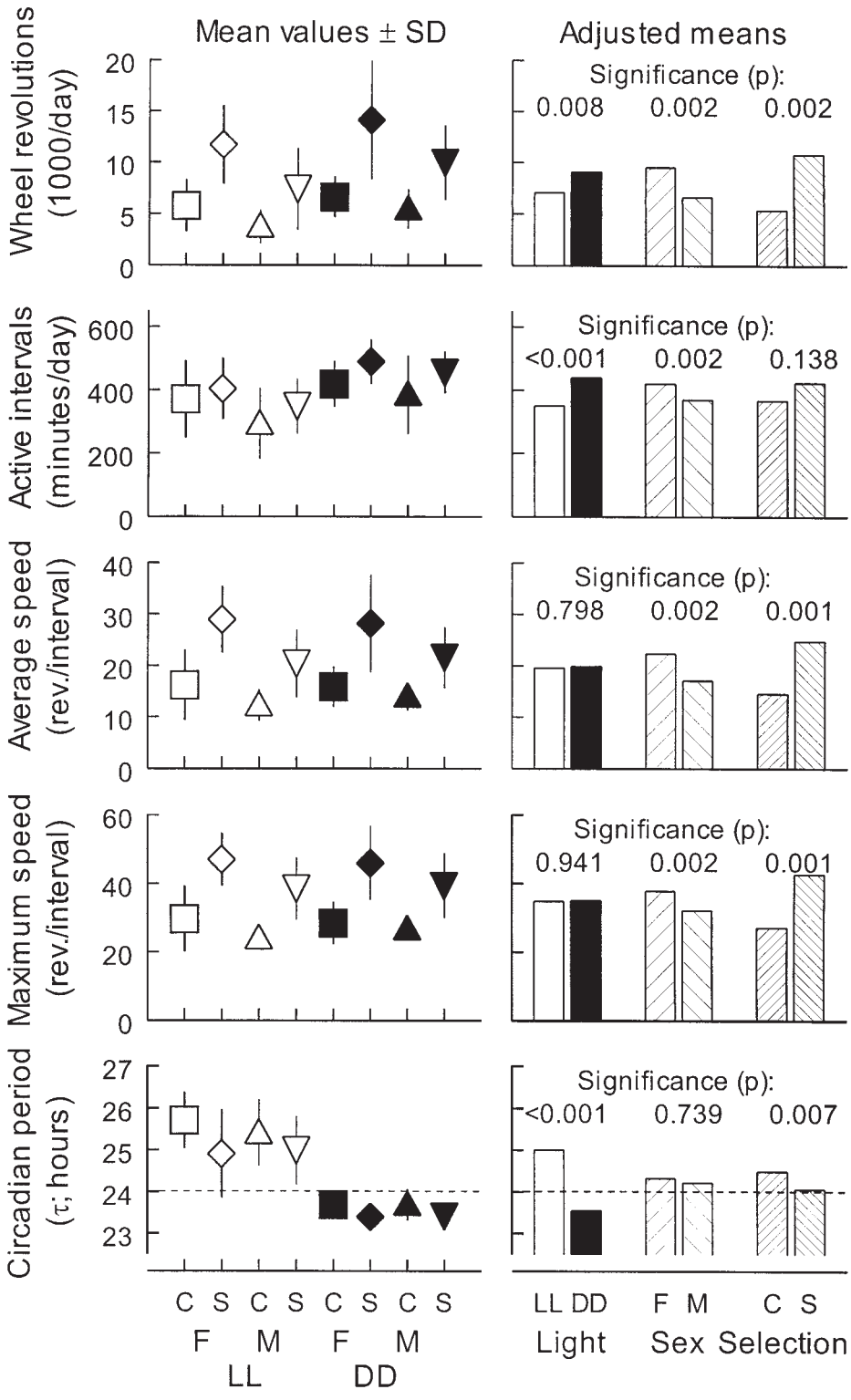

Figure 1. Summary statistics (left panel) and effects of experimental factors (right panel) on wheel-running traits and circadian rhythm in mice from 8 groups representing combinations of three factors: light condition (LL, constant light; DD, constant dark), sex (F, female; M, male), and selection history (C, control; S, selectively bred for high wheel running). The three factors were additive—no interaction effect was statistically significant. Variation among replicate lines, nested within selection groups, was significant for the total number of wheel revolutions, number of active intervals, and running speed (all $p < 0.001$) but not for circadian period ($p = 0.100$). The adjusted means are least square means from the ANCOVA model (with wheel resistance as a covariate; see Method).

the two components of locomotor activity on the length or consistency of circadian rhythm, running speed and time spent running were included simultaneously in the statistical models.

Length of Circadian Period (τ)

Circadian period calculated from 15-day records of wheel running was highly correlated with an average τ estimated independently in five 3-day segments of the same record (partial correlation among individuals within experimental groups: $r = 0.896$, $p < 0.001$), and means of the two estimates did not differ (paired t test: $p = 0.160$). Thus, although the estimates based on 3-day records certainly bear a large random error, they are not biased and are good predictors of the estimates obtained from longer and more typical lengths of time. In the main analysis, we decided to use τ values obtained from the entire 15-day record because this conforms to usual practice in chronobiological research. However, the estimates based on 3-day segments were used to assess consistency of the circadian period (within-individual CV) and within-individual correlations between τ and locomotor activity.

Circadian period was significantly shorter in S mice compared to C mice ($p = 0.007$; Fig. 1). An analysis of covariance indicated that the difference remained significant ($p = 0.046$), even when the effects of running speed and time spent running were controlled. Although the difference tended to be greater under LL (0.66 h, or 2.6% of average τ at LL) than under DD (0.30 h, or 1.3%), the interaction between selection history and light condition was not statistically significant ($p = 0.145$). τ was about 1.6 h shorter under DD than under LL ($p < 0.001$; Fig. 1). However, it did not differ between sexes ($p = 0.739$), and the effects of selection or light level on τ were not conditioned by sex (no interactions were significant).

Among individuals within the experimental groups, τ was not correlated with the number of wheel revolutions run per day (partial $r = 0.023$, $p = 0.777$). As expected from the comparison of S and C mice, τ tended to be negatively correlated with average running speed ($r = –0.128$, $p = 0.112$), and the trend was stronger, yet still not clearly significant, for the maximum running speed ($r = –0.151$, $p = 0.060$). In sharp contrast, τ and time spent running were positively correlated ($r = +0.192$, $p = 0.016$). The relations between running activity and τ did not differ significantly between experimental groups or between sexes (no interactions between the effects of groups and the covariates were significant).

Finally, at the level of within-individual variation across five 3-day intervals, the pattern of correlations between τ and the level of activity differed between sexes. Time spent running and τ, measured in the same 3-day trial, were correlated positively in females ($r = 0.111$, $p = 0.031$) but negatively in males ($r = –0.138$, $p = 0.008$), so that for pooled sexes, there was virtually no correlation ($r = 0.003$, $p = 0.937$). In both sexes, τ was correlated positively with time spent on locomotor activity in the preceding 3-day interval and negatively with time in the following 3-day interval, but the strength of the correlations differed between sexes. If an individual spent more than its usual time on running activity, it would have a relatively shorter circadian period in the next interval (females: $r = –0.196$, $p = 0.001$; males: $r = –0.064$, $p = 0.291$; pooled: $r = –0.137$, $p = 0.001$). On the other hand, a shorter than usual τ was followed by a few days with a relatively lower number of 1-min intervals with wheel-running activity (females: $r = 0.059$, $p = 0.324$; males: $r = 0.167$, $p = 0.005$; pooled: $r = 0.102$, $p = 0.016$). Running speed was correlated with τ only in males and only for the values measured in the same 3-day interval ($r = 0.134$, $p = 0.010$; note that the within-individual correlation had an opposite direction to that observed at the level of variation among individuals).

Consistency (τ_{cv}) of the Circadian Rhythm

Repeated-measures ANOVA indicated that running activity (both speed and number of 1-min active intervals) decreased across the five consecutive 3-day intervals (linear trend in an analysis of orthogonal polynomial contrasts; $p < 0.001$). However, the 3-day estimates of τ did not change significantly across the five 3-day intervals (repeated-measures ANOVA; $p = 0.788$). The difference between the highest and the lowest τ (averaged across all individuals for a 3-day interval) was only 3.3 min. This result indicates that the selected 15 days provide a valid basis for estimating τ.

Consistency of τ estimates, measured as the within-individual coefficient of variation of τ based on five 3-day records (τ_{cv}), tended to be higher (i.e., τ_{cv} values were lower) in the groups with higher activity, but the pattern was complicated by significant interaction terms (Fig. 2). A clear difference was found only between the DD and LL condition ($p = 0.002$). Among

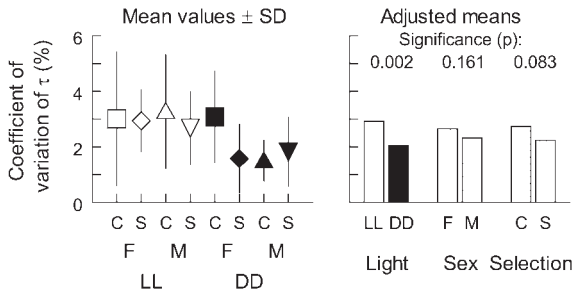

Figure 2. Summary statistics (left panel) and effects of experimental factors on (right panel) the consistency of circadian rhythm (τ_{cv} = 100 • within-individual SD_τ/τ) in mice from 8 groups representing combinations of three factors: light condition (LL, constant light; DD, constant dark), sex (F, female; M, male), and selection history (C, control; S, selectively bred for high wheel running). Two interactions between main factors were significant (Light × Sex, $p = 0.008$; Light × Sex × Selection, $p = 0.001$). Variation among replicate lines, nested within selection groups, was not significant ($p = 0.416$).

individuals, τ_{cv} tended to decrease with the total number of wheel revolutions ($r = -0.137$, $p = 0.087$). The correlation was clear for average running speed ($r = -0.251$, $p = 0.002$), but it was not significant for the number of 1-min active intervals (actually, the trend was in the opposite direction: $r = 0.103$, $p = 0.202$). Thus, consistency of the circadian rhythm increased with the average intensity of the activity but not with time spent on the activity.

DISCUSSION

Overview

Results showed that the relationship between the level of locomotor activity and the length of the circadian period in mice is more complicated than a simple negative correlation postulated in the classical paper by Aschoff (1960). Specifically, the sign of the relation may depend on whether one considers intensity (average speed) or duration (time spent running) as the indicator of locomotor activity and whether one considers correlations at the level of among- or within-individual variation. Moreover, within individuals, the sign of the relationship may depend on sex. Thus, no general relationship may exist, and this perspective may facilitate future studies of the relationship between level of activity and τ.

Speed versus Duration of Running

The pattern of differences in wheel-running activity between the selection groups was similar to that observed in measurements performed under 12:12 LD light conditions, reported for other generations of the selection experiment (e.g., Swallow et al., 1998; Koteja et al., 1999; Girard et al., 2001; Rhodes et al., 2001; Garland, 2003; Rhodes and Garland, 2003). The response to selection involved a near doubling of average running speed, while the increase in time spent running was small (especially for females; Fig. 1). A count of wheel revolutions during a 1-min period may not be a good indicator of actual running speed if running bouts are short relative to this period (Eikelboom, 2001). However, the maximum recorded running speeds during any 1-min interval were also higher in the S mice (Fig. 1; see also Koteja and Garland, 2001), and direct measurements of instantaneous running speeds from videotape analysis also showed that S mice run faster than C mice (Girard et al., 2001).

On the other hand, the difference between LL and DD groups resulted entirely from increased time spent running under DD. Again, as reported in our earlier work, both running speed and time spent running contributed to the difference in running activity between sexes (Fig. 1).

The comparisons of τ in the selection and the light regimen groups appear to corroborate Aschoff's (1960) hypothesis that τ is negatively correlated with the level of locomotor activity. In mice from the lines selected for high wheel-running activity, τ was about 0.5 h shorter than in the mice from control lines (Fig. 1). The correlated response to selection demonstrates the presence of a negative additive genetic correlation between the amount of wheel running and τ. Such a correlation is likely caused by pleiotropic actions of genes and suggests a shared regulatory mechanism (Garland, 2003). A nonsignificant interaction between the effects of selection and photoperiod indicates that the genetic correlation is not strongly affected by an important environmental factor, which by itself has a large effect on both wheel running and τ. As expected for nocturnal rodents (Aschoff, 1960), a higher locomotor activity under DD than under LL was accompanied by a shorter free-running period (Fig. 1).

On the other hand, despite a large difference in locomotor activity between females and males, their free-running period was virtually the same (Fig. 1). Similarly, despite a significant correlated response to selection, τ was not correlated with the number of

wheel revolutions run per day at the level of variation among individuals (residuals from nested ANCOVA models). Instead, τ tended to be negatively correlated with running speed but positively correlated with time spent running. Thus, fast-running individuals have a fast-running clock, but mice that run frequently have a slow one. To our knowledge, such an opposite correlation between τ and running speed versus duration is a novel observation. The contradictory effects of running speed and duration would appear to explain why the overall activity was not correlated with τ and why τ did not differ between the sexes.

It is also worth noting that consistency of the activity rhythm also increases (i.e., within-individual coefficient of variation τ_{cv} decreases) with an average intensity but not with duration of the activity of an individual. Thus, fast-running individuals have not only faster but also more precise clocks (Fig. 2).

Feedback to Pacemaker

The hypothesis that locomotor activity feeds back to the circadian pacemaker has important biomedical implications. Changes in the endogenous circadian rhythm have been implied as a possible cause of chronic insomnia and age-related sleep disorders (Billiard et al., 1996; Uchiyama et al., 2000; Zisapel, 2001). If locomotor activity was not merely correlated with τ but actually affected the endogenous pacemaker, then physical exercise could be used to induce changes in the length of the circadian rhythm and hence could be applied as a therapeutic tool in treating sleep disorders (Dijk et al., 1995; Baehr et al., 1999), as it has been applied to ameliorate the effects of time phase shifts (Mrosovsky and Salmon, 1987; Redlin and Mrosovsky, 1997; Baehr et al., 1999).

The existence of an among-individual correlation between the amount of locomotor activity and τ, or of a joint response of activity level and τ to an experimental manipulation, is widely cited as evidence of feedback from locomotor activity to the circadian pacemaker (e.g., Edgar et al., 1991; Weisgerber et al., 1997; Mrosovsky, 1999; Oklejewicz et al., 1997; Deboer and Tobler, 2000). However, such observations, including our results discussed above, do not provide strong support for the hypothesis. If locomotor activity and τ are genetically correlated, as they evidently are in our mice, then some individuals will be more active and have a short τ, whereas others will exhibit the converse (unless effects of environmental variation obscure this relation). This does not guarantee, however, that an individual's τ will change if it changes its activity level. Similarly, such environmental factors as light level or wheel access may induce changes in both τ and activity level, but this does not guarantee that the circadian clock of an individual maintained in a particular environment will react to spontaneous changes of its activity in a similar way (Mrosovsky, 1999). For example, Deboer and Tobler (2000) have found that mice given access to large wheels were more active and had a shorter τ than mice given access to small wheels (as predicted from Aschoff's 1960 hypothesis). However, mice from both of the experimental groups decreased locomotor activity across four consecutive 10-day trials, and their τ also got shorter rather than longer—as one might expect from the between-groups comparison (Deboer and Tobler, 2000, Fig. 3). Evidently, the correlation between activity level and τ revealed by the comparison between the experimental groups could not be used to correctly predict within-individual temporal changes of τ.

Controlled experiments testing the hypothesis of a feedback from activity to the circadian pacemaker may be feasible only in humans (if at all, given possible psychological effects of experimental instructions) because it is difficult to induce changes in an animal's activity pattern without changing some aspect of its environment, which may affect τ through other mechanisms. Remarkably, studies on humans have not shown unequivocal evidence of such feedback (Dijk et al., 1995; Baehr et al., 1999). One means of investigating the potential for feedback between activity and the circadian pacemaker is to exploit spontaneous temporal variation of activity in individuals maintained in a constant environment (although this approach may not lead to clear conclusions because the temporal variation could be a reaction to subtle environmental changes not fully controlled in the experiment). Thus, a third step in our data analysis was to examine correlations between within-individual variation in activity pattern among five consecutive 3-day intervals.

Within individuals, running speed had generally little effect on τ, and the relation between time spent on locomotor activity and τ depended on sex: in females, τ was longer in the trials with more running, whereas the converse was true in males. However, in both sexes, τ was correlated in the same direction with time spent on locomotor activity in the previous and next intervals. If an individual spent more than its

usual amount of time on running activity, then it had a relatively shorter circadian period in the next few days (correlation significant in females). On the other hand, a shorter than usual τ was followed by an interval of lower locomotor activity, which eventually would lead to a longer τ in the more distant future (correlation significant in males). It may be worth noting that τ was based here on short, 3-day records, so the estimates bore a large random error. Thus, the functional relation between the traits is probably stronger than suggested by strength of the observed correlations.

With the data at hand, we cannot propose a clear explanation for the difference between sexes. It may be hypothesized that factors behind the temporal variation in running activity could differ between sexes (e.g., in females, it might be associated with estrous cycle; but see Weinert, 1996) or that time delay of the effect of locomotor activity on τ differs between sexes. At any rate, the results suggest that locomotor activity indeed feeds back to the circadian pacemaker. However, the results also reveal a stabilizing negative feedback mechanism, which acts to minimize fluctuations in the activity pattern. If the latter occurs in humans, then it does not bode well for attempts to use exercise as a therapeutic tool in treating disorders related to abnormal τ. Even if locomotor activity therapy resulted in shortening τ, it might also result in reduced motivation for continued activity.

Neurobiology

Importantly, the difference in τ between the S and C lines remained statistically significant, even when the effects of running speed and time spent running were controlled in ANCOVA; thus, something more fundamental than just intensity or amount of wheel-running activity per se must underlie the difference in τ. It is also worth nothing that, as compared with C mice, mice from the S lines are more active in the wheels even when the wheels are locked to prevent rotation (Koteja et al., 1999); that they may be hyperactive in cages that lack wheels (Rhodes et al., 2001); and that their wheel-running bouts are shorter and more frequent (Girard et al., 2001).

Earlier studies of rats and mice had implied serotonin as a mediator in a feedback mechanism from locomotor activity to the main circadian pacemaker in the SCN (e.g., Shioiri et al., 1991; but see Mistlberger et al., 1998). However, separate studies on our mice indicated no significant difference in the number of Fos-positive cells in the SCN (Rhodes, 2002) or the number of arginine-vasopressin (AVP) neurons in the SCN (Hochstetler et al., 2003) between the S and C lines, and there was no evidence for a relationship between the number of AVP cells and total daily activity or characteristics of the circadian activity rhythm (Hochstetler et al., 2003). On the other hand, pharmacological studies implicated altered dopaminergic function, rather than altered serotonergic function, in the S mice (Rhodes et al., 2001; Rhodes and Garland, 2003). In light of these studies, the present results are consistent with recent evidence of involvement of a dopaminergic system in the ventral tegmental area (VTA) in regulating both wheel-running activity and circadian period in rats: lesion of VTA resulted in both decreased locomotor activity and longer τ (Isobe and Nishino, 2001).

Conclusions

Two major conclusions emerge from our results. First, strikingly little attention has been given to the distinction between the effects of duration versus intensity of locomotor activity on the structure of the circadian rhythm. Ignoring the distinction could contribute to notoriously conflicting results (Mrosovsky, 1999) and hinder efforts to detect genetic loci that underlie variation in circadian activity patterns (Shimomura et al., 2001): the search would be more fruitful if running speed and time were analyzed as separate traits, rather than as an overall activity level. Second, it is important to distinguish between among- and within-individual effects of locomotor activity on τ. Mice from lines that have been selectively bred for high levels of wheel running, which occurs mainly by increased running speed, do have a fast-running circadian clock. However, on a day-to-day basis, voluntary changes of average running speed by an individual have little effect on the pace of its clock. On the other hand, individuals that generally spend more time in locomotor activity tend to have a slow clock, but a voluntary increase of time spent running by an individual speeds up, rather than slows down, the endogenous pacemaker. These results are consistent with the hypothesis that circadian period is influenced by more than one regulatory mechanism (Daan et al., 2001; Isobe and Nishino, 2001; Lewandowski and Usarek, 2002) and suggest that the systems may interact with the level of locomotor activity in different ways.

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