Research Articles

Strain differences in the pattern and intensity of wheel running activity in laboratory rats

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Summary. Wheel running activity rhythms of three inbred rat strains, ACI/Ztm, BH/Ztm, and LEW/Ztm, were compared in order to evaluate the effect of genetic differences on circadian rhythm parameters. Significant strain differences were found in the general pattern of the activity rhythms and their characteristic periodicities as well as in the amount and duration of wheel running activity and the timing of activity onsets and offsets. The results suggest that genetic differences exist in the coupling of the multiple circadian oscillators that generate the overall pattern of wheel running activity.
Key words. Wheel running activity; ultradian and circadian rhythms; inbred rat strains; genetic differences; multi-oscillatory system.

Circadian rhythms in mammals are regulated by an endogenous multi-oscillatory pacemaker system located in or close to the suprachiasmatic nucleus in the hypothalamus [1, 2]. Circadian rhythms are influenced by environmental factors such as light and temperature [3] as well as by internal factors such as hormones [4] and pharmacological agents [5]. However, the effects of these factors are minor compared to the effects of genetically determined properties [6, 7].

In simpler organisms such as *Drosophila* [8] and *Neurospora* [9], several single gene mutations are known today that have an influence on certain characteristics of circadian rhythms. The only single-gene mutation in mammals that has been analyzed so far is a recently-described short period mutation in hamsters [10]. Nevertheless, genetic studies based on inbred strain comparisons have been performed for some time. For example, significant differences between strains of mice have been observed in the rhythms of food and water consumption [11], body temperature [12, 13], and wheel running activity [14].

Similar studies in rats have identified genetically determined differences in the pattern of locomotor activity [15, 16]. However, in all of these studies overall locomotor activity was measured with Animex or Animex-like systems that did not allow the discrimination of different kinds of locomotor and feeding-related activities. Furthermore, these studies provided no reliable information about the intensity of activity, either because animals were recorded as a group [16] or because activity was expressed only as a percentage of the 24-h mean value [15].

The present study examined the effect of genetically based differences on the rhythmic pattern of wheel running activity in three commonly available inbred strains of laboratory rats, ACI, BH, and LEW. It characterized the general pattern of activity based on two methods of period analysis and quantified it with respect to duration and intensity of wheel running activity as well as to timing and precision of activity onsets and offsets.

Materials and methods

Animals and housing: Male rats of the inbred strains ACI/Ztm (agouti; n = 10), BH/Ztm (black hooded; n = 9), and LEW/Ztm (albino; n = 10), originally obtained from the central animal laboratory at the Hanover Medical School (FRG), were bred and raised in our laboratory under controlled environmental conditions (12:12 h light-dark cycle, lights on at 07.00, room temperature 22 ± 1 °C). At 60–70 days of age, the animals were placed in individual cages (Makrolon Type IV, 35 × 55 × 10 cm) equipped with a running wheel (diameter 35 cm, width 10 cm). The animals were housed in a sound-attenuated colony room used exclusively for running wheel experiments. Wheel running activity was monitored under entrainment to a 12:12 h light-dark cycle (12:12 LD) for a total of 9 weeks. All animals had free access to the running wheel, food and water were supplied ad libitum. The animal room was entered 2–3 times a week at random times during the day to check the water bottles and food supply. The cages were cleaned every 3 weeks.

Data collection and analysis: The axle of each running wheel was equipped with three magnetic reed switches so that one complete wheel revolution resulted in three impulses. These were read on-line by a microcomputer (Apple II+) and stored on a disk every 5 min. All subsequent calculations were based on these 5-min counts.

For the purpose of plotting the data as activity records over 24 h, every two 5-min counts were combined into a 10-min count (fig. 1 a–f). The number of counts per 10-min interval is graphically represented as a bin. The maximum height of the bin indicates more than 100 counts (i.e., an average of 10 counts/min), while a blank space indicates fewer than 10 counts (i.e., an average of 1 count/min).

The 'chi square periodogram' [17] and 'harmonic spectral analysis' methods [18, 19] were used to test for the presence of characteristic frequencies in the data. These two ap-

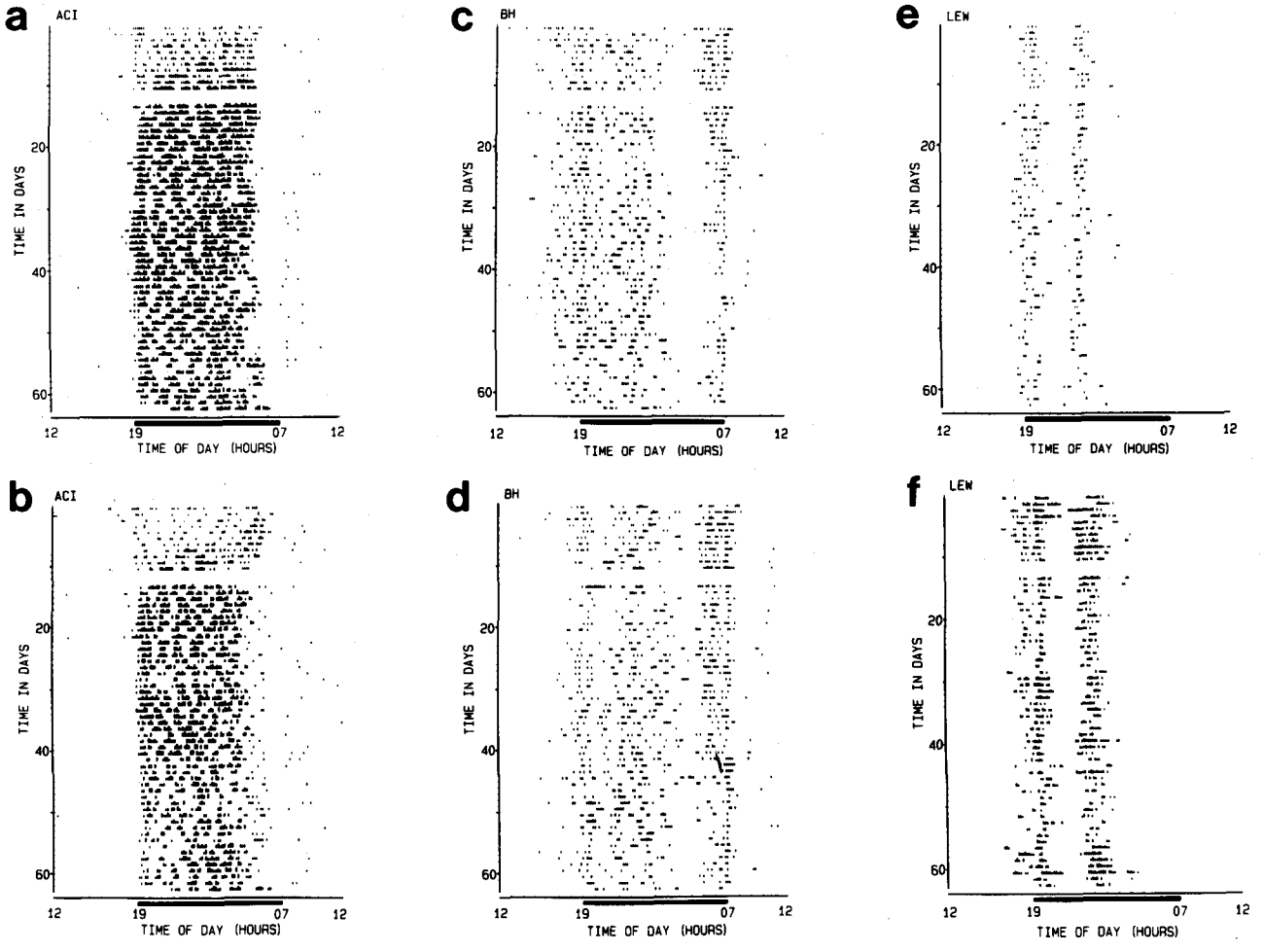

Figure 1. Characteristic records of wheel running activity for ACI/Ztm (*a, b*), BH/Ztm (*c, d*), and LEW/Ztm (*e, f*) rats maintained under a light-dark cycle of 12 h light and 12 h dark. Numbers on the vertical axes denote days of experiment, numbers and bars on the horizontal axes denote daytime hours and light-dark schedule, respectively. Each 24-h period consists of 144 10-min bins, with the height of each bin representing the number of impulses per 10-min interval (10–100 imp./bin). Blank lines indicate days with data missing due to power failure or system errors.

proaches are based on different statistical models, and the results of both methods were always compared to verify the reliability of the analyses. Both techniques and their application to circadian and ultradian rhythms have been described in greater detail elsewhere[20] and have been tested with artificial data of different periods, amplitude, and waveforms in the presence of different levels of noise. These tests confirmed that in the harmonic spectral analysis the amplitudes of spectral estimates are strongly correlated to the true amplitude of the rhythm, whereas in the periodogram, the amplitude of significant peaks is not correlated with the true amplitude of the rhythm but depends on the signal-to-noise ratio.

Additional parameters calculated from the activity recordings include: (1) amount of wheel running activity given in impulses/day: total number of counts over a 24-h period; (2) duration of wheel running activity given in min/day: total length of all 5-min intervals within 24 h containing more than 5 impulses; (3) onset and offset of the two major activity bouts determined by visual inspection of the event records. The day was excluded if determination was not possible. For each animal, at least 42 days' data were available for further analysis. Differences between strains were assessed using standard procedures of variance analysis (one-way ANOVA). Post hoc comparisons were made using Tukey's multiple t-test. Pearson's r was used for expressing correlations.

Inbred strain comparisons provide a convenient method of estimating genetic effects. Since individuals of the same strain are genetically identical, differences within strains must reflect environmental effects or errors of measurement. Differences between strains, however, reflect both environmental and genetic effects. Therefore, standard procedures of covariance analysis were used to compare strain means, and to obtain components of additive genetic variance and covariance (see Hegmann and Possidente[21] for details). These components allowed the estimation of heritability and genetic correlations for parameters of running wheel activity. Heritabilities were estimated as $1/2\ V_{BS}/(1/2\ V_{BS} + V_{WS})$, where V_{BS} and V_{WS}

represent the components of variance between and within strains, respectively. The genetic correlations were estimated as $COV_{BS}(xy))/(V_{BS}(x) \cdot V_{BS}(y))$, where $COV_{BS}(xy)$ represents the component of covariance between strains. Environmental correlations were calculated following the same procedure but using within-strain variance components.

Results

Wheel running activity pattern: Characteristic activity recordings for ACI, BH, and LEW rats under LD 12:12 entrainment are shown in figure 1 a–f. The daily pattern of wheel running activity demonstrated remarkable strain differences. Strain ACI exhibited the strongest activity rhythm, characterized by a high activity level, clear onsets of activity, and distinct differences between activity and rest time. Strain BH, on the other hand, exhibited a rather weak activity rhythm with a lower activity level, blurred onsets and offsets of activity, and expanded times of activity. The activity pattern of strain LEW was characterized by two rather short activity bouts during the first half of the dark period. Activity stopped almost 6 h before lights-on.

Figure 2 summarizes the average daily activity pattern of each strain. Strain ACI had a clear unimodal activity pattern, whereas strain BH had a more bimodal activity pattern with two activity bouts about 12 h apart from each other. The activity pattern of the LEW strain was characterized by two very short activity bouts about 4–5 h apart during the first half of the dark period.

Period analysis: Harmonic spectral analysis (fig. 3) and the chi square periodogram (fig. 4) detected characteristic frequencies that were different for the three strains. The activity pattern of strain ACI showed only one rhythmic component with a period of 24 h, whereas the activity patterns of strains BH and LEW were characterized by two additional components of 12 and 6 h, and 4 and 4.8 h, respectively. ANOVA of spectral estimates of the harmonic spectral analysis revealed significant strain differences in the amplitude of the following periods: 24 h ($F(2, 26) = 30.99$; $p \leq 0.01$), 12 h ($F(2, 26) = 3.58$;

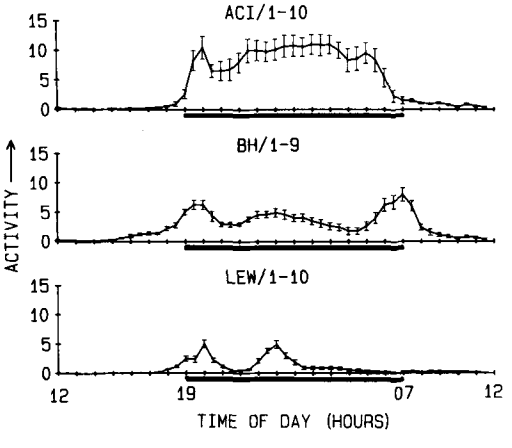

Figure 2. Average distribution of wheel running activity of ACI/Ztm (upper panel, n = 10), BH/Ztm (middle panel, n = 9), and LEW/Ztm rats (lower panel, n = 10) during entrainment to a 12:12 LD cycle. 30-min mean values are plotted over time, with standard deviations between animals shown as vertical lines.

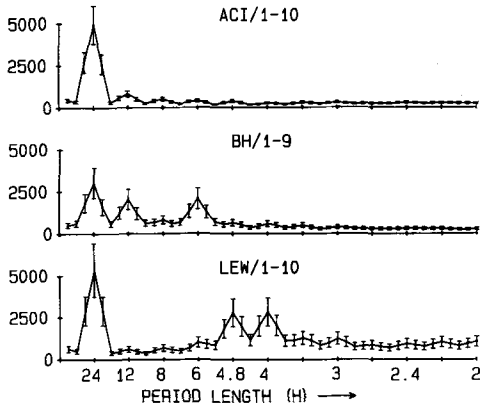

Figure 3. Harmonic spectral analysis of wheel running activity for strains ACI/Ztm (upper panel, n = 10), BH/Ztm (middle panel, n = 9), and LEW/Ztm (lower panel, n = 10). Power spectra of individual animals were pooled for each strain. The 95% confidence limits of spectral estimates are plotted as vertical lines.

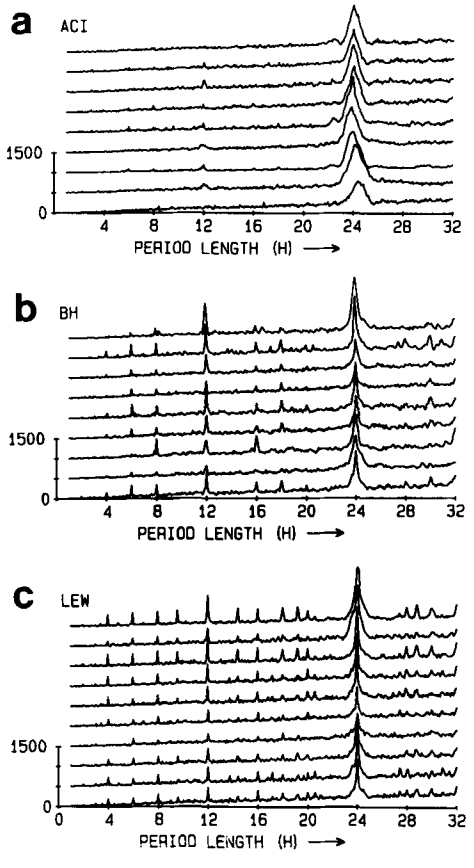

Figure 4. Chi square periodogram analysis of wheel running activity for individual animals of strains ACI/Ztm (a), BH/Ztm (b), and LEW/Ztm (c). Notice that rhythms in the ultradian range produce additional peaks at periods representing multiples of the original period, e.g., an ultradian rhythm of 4 h will produce additional peaks at 8, 12, 16, and 20 h. Hence, multiple peaks must be interpreted as the result of only the largest common submultiple of all peaks.

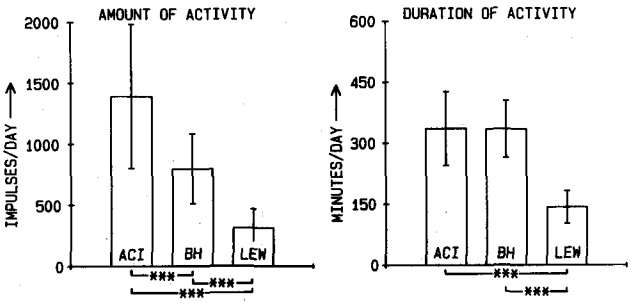

Figure 5. Parameters of wheel running activity for strains ACI/Ztm (n = 10), BH/Ztm (n = 9), and LEW/Ztm (n = 10). Histograms show strain means ± standard deviations between animals. Significant strain differences are indicated by asterisks (***$p \leq 0.01$). Left: Amount of activity given in impulses/day and defined as the total number of impulses over a 24-h period. Right: Duration of activity given in minutes/day and defined as the total length of all 5-min intervals within a 24-h period containing more than 5 impulses.

$p \leq 0.05$), 6 h ($F(2, 26) = 12.15$; $p \leq 0.01$), 4.8 h ($F(2, 26) = 28.29$; $p \leq 0.01$), and 4 h ($F(2, 26) = 41.09$; $p \leq 0.01$).

Amount and duration of activity: Figure 5 summarizes the differences between the three strains with respect to the amount and duration of wheel running activity. One-way ANOVA revealed significant strain effects for both amount ($F(2, 27) = 19.62$, $p \leq 0.001$) and duration of activity ($F(2, 27) = 25.86$, $p \leq 0.001$). The amount of activity was lowest for the LEW strain (314 ± 154 imp./day), about twice as high for the BH strain (799 ± 271 imp./day), and highest for the ACI strain (1393 ± 591 imp./day). The duration of activity was not significantly different between ACI (336 ± 91 min/day) and BH (334 ± 66 min/day), but was significantly shorter for the LEW strain (143 ± 40 min/day).

Activity onset and offset: Figure 6 depicts the timing of activity onsets and offsets relative to the LD cycle. Significant strain differences were found for activity onset ($F(2, 24) = 33.21$; $p \leq 0.001$) with BH (120 ± 42 min) starting activity earlier than ACI (15 ± 24 min) and LEW (25 ± 17 min), as well as for activity offset ($F(2, 24) = 140.46$; $p \leq 0.001$) with LEW (360 ± 60 min) ending activity earlier than ACI (30 ± 66 min) and BH (−50 ± 35 min). The precision of the wheel running

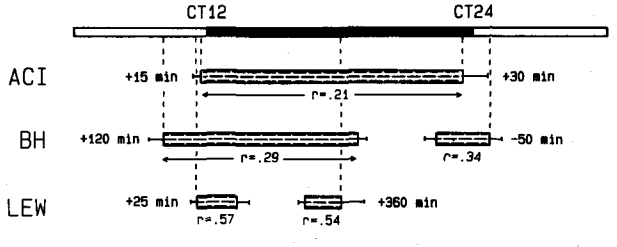

Figure 6. Timing of activity onset and offset with respect to the 12:12 LD cycle. Onsets and offsets of the main activity bouts were determined by visual inspection of activity records of each individual day. Phases are expressed as differences in min between onset/offset of activity and onset/offset of the dark period. Phase is positive when activity onset/offset preceded the light change and negative when activity onset/offset followed the light change. Pearson's r was used for correlations.

Heritability and estimates of genetic and environmental correlations for amount, duration, onset and offset of wheel running activity

	Heritabilities	
Amount	0.78	
Duration	0.77	
Onset	0.89	
Offset	0.97	
	Genetic correlation	Environmental correlation
Amount and duration	0.84	0.88
Amount and onset	0.28	−0.28
Amount and offset	0.74	0.01
Duration and onset	−0.32	−0.20
Duration and offset	0.04	0.98
Onset and offset	−0.47	−0.23

rhythm, as expressed by the standard error of activity onsets, differed across strains with ACI (± 46 min) showing the smallest variation compared to LEW (± 51 min) and BH (± 72 min). This difference, however, did not prove significant ($F(2, 24) = 2.03$; n.s.).

Correlation analysis was used to evaluate the strengh of coupling between onset and offset of individual activity bouts. For ACI rats, the correlation between onset and offset was small ($r = 0.21$) but still significant, indicating that a spontaneous delay or advance of the activity onset was usually followed by a similar delay or advance of the activity offset. For BH as well as for LEW rats, correlations between onset and offset of each activity bout were also fairly high, while no significant correlations were found between the two activity components. These results suggest a strong determination of the length of each activity component but only a weak coupling between the two components.

Heritability and estimates of genetic and environmental correlation: The table summarizes the heritability estimates and coefficients of genetic and environmental correlation calculated for various parameters. Since the heritability estimates for amount, duration, onset and offset of activity are fairly high, these traits are obviously genetically determined and would change in response to selection. The coefficients of genetic and environmental correlation (0.48 and 0.88, resp.) found for amount vs duration of activity are almost identical and rather high. This means that the intensity of activity (i.e. running speed), as expressed by the ratio of amount over duration, is more or less the same for all three strains. High genetic correlations were also found for amount vs offset and duration vs offset, suggesting that these parameters could not be altered independently from each other by selection. In contrast, rather small genetic correlations were found for amount vs onset and duration vs onset. Changes in amount and duration of activity are, therefore, more likely to be accompanied by changes in activity offset than by changes in onset.

Discussion

This study revealed distinct strain differences in the pattern of wheel running activity of commonly available

inbred strains of laboratory rats. ACI rats had a unimodal activity pattern characterized by a clear activity onset, a greater intensity of activity, and distinct differences between activity and rest time. Period analysis detected only one rhythmic component with a period of 24 h. A unimodal activity pattern like that is typical for most laboratory rats, although for individual animals, bi- or multimodal activity patterns have also been described [22]. BH rats had a bimodal activity pattern with blurred onsets and offsets of activity and longer activity time. Period analysis detected a weaker 24-h rhythm, and additional components of 12 and 6 h periods. Finally, LEW rats had two rather short activity bouts during the first half of the dark period. Period analysis detected rhythmic components with periods of 24, 4.8 and 4 h.

For the strains ACI and BH, the general pattern of wheel running activity was similar to that previously observed for overall locomotor activity [15, 16]. The wheel running activity pattern of the LEW strain, however, differed from previous recordings of the same strain [15, 20, 23, 24] in that it showed two rather than three or four activity bouts during the dark period. It is not clear yet whether this difference reflects a slow modification in the genetic background of the LEW strain or whether it is due to different technical setups. Unfortunately, some of the recordings of LEW and other inbred rats that have been performed over the last 10 years used different techniques for measuring locomotor activity. The first studies on LEW rats used an Animex-like system [15, 20, 23] that measures overall activity inside the animal cage and, therefore, does not allow discrimination between different kinds of locomotor and feeding-related activities. When recorded with this setup LEW rats showed a multimodal activity pattern, with three activity bouts during the dark period and an additional small peak after lights-on. However, the elapsed time between these peaks was similar to that found in the present study, and period analysis revealed rhythmic components with periods of 4 and 4.8 h. In subsequent studies of LEW rats using Wahman running wheel cages, two different activity patterns were found [24]. Most of the animals exhibited only two activity bouts during the dark period, but there were always a few animals with three distinct activity bouts. Regardless of the number of activity bouts, period analysis of these activity patterns always detected rhythmic components of 4 and 4.8 h periods.

A comparison of activity measurements over the last 10 years revealed a gradual change of the activity pattern of LEW rats, resulting in the loss of the third activity bout. It seems that this change is not merely an artifact due to different measurement methods, for other inbred strains were measured using the same methods and did not show similar changes. However, in order to prove that there is indeed a change in the genetic background of the LEW strain it would be necessary to repeat the measurements of overall locomotor activity with animals of the present LEW strain.

All parameters of wheel running activity measured in the present study (amount, duration, onset, offset) had substantial broad-sense heritability, indicating that these traits would respond rapidly to selection. It is important to note, however, that heritability values estimated from inbred-strain comparisons are not necessarily valid for randomly bred populations. This is especially true when, as in the present study, the strains compared were rather extreme examples [21]. The results of this and other studies [11–15] demonstrate nonetheless the genetical determination of circadian rhythm parameters in mammals.

The neurophysiological or neurochemical basis for the observed strain differences is unknown. Using the same inbred strains as in the present study, Lemmer et al. [16] investigated concentration and turnover of dopamine in the striatum and of norepinephrine in the hypothalamus/midbrain. They found no correlation between activity level and either of the above parameters. However, significant strain differences were found for pineal size and melatonin-forming capacity [25]. LEW rats and other albino strains had the most active pineals. Moderately active pineals were found in two hooded rat strains. E3/Han and BDE/Han. The smallest and least active pineals were found in the fully pigmented BN/Han and DA/Han rats. It seems unlikely that the observed strain differences in the present study are simply due to the different pigmentations, because previous investigations of other inbred rats had found a typical unimodal activity pattern in both pigmented and albino strains [15].

The present study found a strong correlation between the intensity of wheel running activity and the coherence of the 24-h rhythm. Considering the recently demonstrated feedback effect of activity on the circadian system [26, 27], it is interesting to note that the level of activity is lowest in the strain LEW which has an unusual activity pattern and highest in the strain ACI which has a normal activity pattern. It may well be that the unusual activity patterns of strains BH and LEW result from a reduction of feedback that is due to their low activity levels. The theory of an activity-induced modulation of the rhythmic pattern is further supported by observations that female LEW rats show profound changes in their activity pattern during the estrous cycle [28]. The day of estrus is always characterized by a high activity level and a unimodal activity pattern, while days following ovulation show a bi- or trimodal activity pattern. It is obvious that this theory needs further investigation. For example, effects of pharmacological agents on the activity levels of LEW and BH rats could be studied.

There are two lines of evidence that suggest that a distinct coupling of multiple circadian oscillators is the cause of the unusual activity patterns of the BH and LEW strains. First, when tested using different methods of period analysis, such as harmonic spectral analysis [18, 19] and the chi square periodogram [17], the activity pattern of each strain showed characteristic periodicities. Beside the general 24-h rhythm, the BH strain had two additional compo-

nents of 12 and 6 h, and the LEW strain had components of 4 and 4.8 h. The fact that these additional periodic components are subharmonics of the 24-h rhythm can be explained with either an ultradian modulation of the activity pattern or with a unique phase coupling of multiple circadian oscillators[1]. The activity pattern of the LEW rats cannot be a true ultradian rhythm like, for example, the feeding and running-wheel rhythms of voles, because, unlike the ultradian rhythm of voles[29], the LEW activity pattern does not persist after complete SCN lesions[24].

The second line of evidence for the multi-oscillatory nature of the activity pattern is based on the statistical analysis of activity bouts in BH and LEW rats. This revealed a strong correlation between onset and offset of each of the two activity components but no significant correlation between the two bouts. For a true ultradian rhythm, however, the correlation between the two bouts should have been of the same magnitude as the correlation between onset and offset within each bout. Visual inspection of individual activity records such as figure 1 e easily verifies that the timing of individual activity components is more or less independent.

Experimental results obtained over the past 15 years strongly suggest that the circadian system of mammals consists of multiple circadian oscillators coordinated by both hierarchical and non-hierarchical coupling relationships[1]. Although some of the major neural components of the mammalian circadian system seem to have been identified, it is not clear how this complex multi-oscillatory system is represented in the nervous system. The present study suggests that genetically fixed differences result in changes in the coupling of those circadian oscillators generating the overall pattern of wheel running activity rhythms of BH and LEW rats. Therefore, these inbred strains can provide a powerful tool for studying the neuronal mechanisms and physiological organization of circadian rhythms in mammals.

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1 Rosenwasser, A. M., and Adler, N. T., Neurosci. Biobehav. Rev. *10* (1986) 431.
2 Meijer, J. H., and Rietveld, W. J., Physiol. Rev. *69* (1989) 671.
3 Aschoff, J., Z. Tierpsychologie *49* (1979) 225.
4 Turek, F. W., and Gwinner, E., in: Vertebrate Circadian Systems, p. 173. Eds J. Aschoff, S. Daan and G. A. Groos. Springer Verlag, Berlin 1982.
5 Turek, F. W., Trends pharmac. Sci. *8* (1987) 212.
6 Konopka, R. J., in: Handbook of Behavioral Neurobiology: Biological Rhythms, p. 173. Ed. J. Aschoff. Plenum Press, New York 1981.
7 Wollnik, F., Lab. Anim. *23* (1989) 107.
8 Konopka, R. J., A. Rev. Genet. *21* (1987) 227.
9 Feldman, J. F., A. Rev. Plant Physiol. *33* (1982) 583.
10 Ralph, M. R., and Menaker, M., Science *241* (1988) 1225.
11 Possidente, B., and Hegmann, J. P., J. comp. Physiol. *139* (1980) 121.
12 Conolly, M. S., and Lynch, C. B., Physiol. Behav. *27* (1981) 1045.
13 Conolly, M. S., and Lynch, C. B., Behav. Genet. *13* (1983) 491.
14 Ebihara, S., Tsuji, K., and Kondo, K., Physiol. Behav. *20* (1978) 795.
15 Büttner, D., and Wollnik, F., Behav. Genet. *14* (1984) 137.
16 Lemmer, B., Casparl-Irving, G., and Weimer, R., Pharmac. Biochem. Behav. *15* (1981) 173.
17 Sokolove, P. G., and Bushell, W. N., J. theor. Biol. *72* (1978) 131.
18 Halberg, F., and Panofsky, H., Exp. Med. Surg. *19* (1961) 285.
19 Panofsky, H., and Halberg, F., Exp. Med. Surg. *19* (1961) 322.
20 Wollnik, F., and Döhler, K.-D., Physiol. Behav. *38* (1986) 229.
21 Hegmann, J. P., and Possidente, B., Behav. Genet. *11* (1981) 103.
22 Stephan, F. K., Physiol. Behav. *30* (1983) 451.
23 Wollnik, F., Naturwissenschaften *72* (1985) 158.
24 Wollnik, F., and Turek, F. W., Am. J. Physiol. *256* (1989) R1027.
25 Vollrath, L., Huesgen, A., Seidel, A., Manz, B., and Pollow, K., Z. Versuchstierkd. *32* (1989) 57.
26 Mrosovsky, N., Reebs, S. G., Honrado, G. I., and Salmon, P. A., Experientia *45* (1989) 696.
27 Turek, F. W., J. biol. Rhythm *4* (1989) 135.
28 Wollnik, F., and Turek, F. W., Physiol. Behav. *43* (1988) 389.
29 Gerkema, M. P., Groos, G. A., and Daan, S., J. biol. Rhythm *5* (1990) 81.

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