Interstrain differences in activity pattern, pineal function, and SCN melatonin receptor density of rats

Gabriela Klante, Karin Secci, Mireille Masson-Pévet, Paul Pévet, Berthe Vivien-Roels, Stephan Steinlechner, and Franziska Wollnik. Interstrain differences in activity pattern, pineal function, and SCN melatonin receptor density of rats. Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R1078–R1086, 1999.—We investigated the possibility that strain-dependent differences in the diurnal pattern of wheel running activity rhythms are also reflected in the melatonin profiles. The inbred rats strains ACI/Ztm, BH/Ztm, and LEW/Ztm. LEW were examined for diurnal [12:12-h light-dark (LD)] wheel running activity, urinary 6-sulphatoxymelatonin (aMT6s) excretion, melatonin concentrations of plasma and pineal glands, and melatonin receptor density in the suprachiasmatic nuclei (SCN). ACI rats displayed unimodal activity patterns with a high level of activity, whereas BH and LEW rats showed multimodal activity patterns with ultradian components and reduced activity levels. In contrast, the individual daily profiles of aMT6s excretion and mean melatonin synthesis followed a unimodal time pattern in all three strains, suggesting that different output pathways of the SCN are responsible for the temporal organization of locomotor activity and pineal melatonin synthesis. In addition, melatonin synthesis at night and SCN melatonin receptor density at day were significantly higher in BH and LEW rats than in ACI rats. These results support the hypothesis of a long-term stimulating effect of melatonin on its own receptor density in the SCN.

6-sulphatoxymelatonin; pineal gland; suprachiasmatic nuclei; rat strains; wheel running activity; ACI/Ztm; BH/Ztm; LEW/Ztm

The mammalian circadian pacemaker is located in the suprachiasmatic nuclei (SCN) of the hypothalamus (27), generating endogenous rhythms in a wide variety of physiological and behavioral functions, including locomotor activity (48) and melatonin synthesis in the pineal gland (38). Under natural conditions, circadian rhythms are entrained or adjusted to the 24-h period by so-called zeitgebers such as the light-dark (LD) cycle. It has been suggested that circadian rhythms of locomotor activity and pineal melatonin synthesis are driven by the same pacemaker system, because they show characteristic similarities in their phase-shifting response to light pulses (15) and in their entrainment to non-24-h lighting cycles (28). The SCN regulate the rhythmic synthesis and release of melatonin in the pineal gland by a multisynaptic pathway via the paraventricular nucleus of the hypothalamus, the intermediolateral cell columns of the spinal cord, the superior cervical ganglion, and postganglionic adrenergic innervation (37). Although the efferent projection from the SCN to the pineal gland is well characterized, the neuronal pathway mediating circadian rhythms of locomotor activity has not yet been identified. It may not even be a necessary precondition, because SCN transplantation experiments in hamsters have demonstrated that a diffusible signal is sufficient to restore circadian activity rhythms in the SCN-ablated hosts (46), whereas reestablishment of melatonin rhythms has never been observed (30).

The SCN not only drive the circadian rhythms of locomotor activity and melatonin synthesis, but are also a target for feedback effects of both activity (40) and melatonin (2, 5, 6, 8, 43). For example, it has been demonstrated that spontaneous activity or some kind of arousal can alter the period or induce phase shifts in the free-running rhythm in hamsters (39, 40) and mice (14), whereas in rats only weak feedback effects of a daily treadmill schedule on circadian activity rhythm were described (36). Furthermore, changes in rhythmic melatonin synthesis or administration of exogenous melatonin lead to altered locomotor activity rhythms in rats and hamsters (2, 6, 8, 9, 25, 32), although different circadian responses to exogenous melatonin occur depending on species, strain, age, and method of administration (43). The mammalian SCN contain melatonin receptors (12, 44), and melatonin given in vitro can decrease the metabolic activity (7) and phase shift the neuronal firing rate of the SCN (35). Melatonin injections in vivo accelerate the reentrainment of activity rhythms (2), synchronize disrupted components of a circadian rhythm under constant light (6), and affect SCN melatonin receptor density (16). Therefore, locomotor activity and melatonin synthesis may not only be controlled by the same pacemaker system, but they may also affect each other by feedback effects mediated through the SCN.

Comparisons of selected lines or inbred strains of rats have already been used as a suitable experimental approach for further elucidation of the interaction of components of the circadian system (4, 10, 11, 13, 55) and the pineal gland (24, 53). For example, rats of the inbred strains ACI/Ztm, BH/Ztm, and LEW/Ztm exhibit...
genetically determined differences in circadian rhythm of wheel running activity, with characteristic differences in rhythm amplitude and in the absence (ACI) and presence (BH, LEW) of significant ultradian components (55). Fragmentation of the activity phase into discrete bouts suggests control by individual circadian pacemakers that are part of a coupled multisellar system (45). The aim of the present study was to investigate the characteristics of diurnal melatonin synthesis in these inbred strains of rats as well as the relationship between melatonin patterns and differences in wheel running activity rhythms. Individual melatonin rhythms in activity-registered rats were determined by measurement of 6-sulphatoxymelatonin (aMT6s), the main catabolic product that is excreted into urine (1) and is known to reflect the pattern of the pineal melatonin rhythm both qualitatively and quantitatively but is time delayed by 1–2 h as a result of catabolism (3, 42, 49). The aMT6s data of these three inbred rat strains were validated by direct measurements of plasma and pineal melatonin concentrations in an additional experiment. Furthermore, melatonin receptor density in the SCN was analyzed to look for possible strain-dependent differences in the feedback effect of melatonin on the circadian pacemaker.

MATERIALS AND METHODS

Animals and Housing

Rats of the inbred strains ACI/Ztm, BH/Ztm, and LEW/Ztm were bred and raised in our laboratory under controlled environmental conditions (22 ± 1°C room temperature, 55 ± 5% relative humidity, 12:12-h LD cycle, lights on at 0700, 500 lx at cage level). Food (Altromin 1320) and tap water were available ad libitum.

Experiment I

Monitoring of wheel running activity. Six male rats of each strain, 15 wk old, were used for this study. The animals were individually housed in Macrolon cages (type IV; Becker, Castrop-Rauxel, Germany) equipped with a running wheel (diameter 35 cm, width 10 cm). Activity recordings started 4 wk after transfer to a reversed LD cycle (12:12 h, lights on at 2200) and continued for 4 wk, except on days 14 and 21, when the animals were transferred for 48 h to metabolic cages. Wheel rotations were detected by a magnetic reed switch (Hamlin) mounted on the wheel axle, so that one complete wheel rotation resulted in one impulse. The number of impulses per 5-min interval was recorded continuously and registered by a personal computer. Subsequent calculations of mean daily activity and 24-h profiles were based on days 1–13, 17–20, and 24–28 of the registration period, omitting only the two 48-h periods of urine sampling and the first day immediately after the transfer from the metabolic cages back to the wheel running cages. Calculations of \( \chi^2 \) periodogram analysis (47) were performed only for the first 13-day segment preceding the transfer to the metabolic cages.

Urine collection. For urine collection, the animals were singly housed in metabolic cages (diameter 19.5 cm) for an adaptation period of 24 h and a sampling interval of 24 h. Urine was sampled from each animal twice within 21 days. During sampling the animals were deprived of food but provided with water ad libitum. Each metabolic chamber was equipped with a special funnel system, which separated urine from feces. In an automated setup, the funnel and connecting tubes (silicone, inner diameter 1 mm) were washed every hour with 1 ml distilled water from a peristaltic pump (pump 22, Harvard Apparatus, South Natick, MA). Urine samples, including water, were transported by a peristaltic pump (type XV, Altesa, Sweden) and collected automatically in fractions of 1 h (Collector MM 10; Neolab, Heidelberg, Germany). The volume of the urine samples was determined to an accuracy of 0.1 ml, and all samples were frozen at −20°C for later analysis.

RIA for aMT6s measurements. All urine fractions were centrifuged at 2,500 g for 10 min (Megafuge 1.0 R; Heraeus Sepatech, Osterode, Germany) to remove solid material and contamination. We analyzed 0.1–20 µl of the supernatant, depending on binding (B), so that 20–80% B/B0 was guaranteed in the RIA. All urine samples were assayed in duplicate, using \( ^{125} \)I-labeled aMT6s tracer and antibody from Stock- grand (Guildford, UK), according to the procedure of Aldous and Arendt (1). The standard curve (triplicate) was performed in buffer solution. A prior test revealed that adding 20 µl daytime urine to the standard solutions did not interfere with binding behavior. All samples were counted in a gamma counter (Compugamma CS 1282; LKB Wallac, Turku, Finland) for 2 min, and aMT6s amounts were calculated by the counter's built-in software (RIA Calc; Pharmacia, Wallac Oy, Turku, Finland). The intra-assay coefficient of variation was 11.7%, calculated from 10 samples of 10 pg aMT6s. The interassay coefficient of variation was 14.6%, calculated from 10 RIA. In each urine sample the concentration of aMT6s was related to the concentration of creatinine (26) using Jaffe's test (Merck, Darmstadt, Germany). The 24-h profiles of urinary aMT6s excretion were determined twice for each rat and averaged afterward. Total daily aMT6s excretion was calculated by using the mean daily creatinine excretion rate of each strain as a reference.

Experiment II

Collection of plasma and pineal glands. Forty-eight male rats of each strain, 8–20 wk of age, were used for this study. Six animals of each strain were killed by decapitation at various times throughout a 24-h cycle (times indicated in Figs. 3 and 4), during which times a dim red light source (<1 lx) was used during the dark period. Blood was collected and treated with 10 µl/ml blood of 10% Titriplex III (Merck) to obtain plasma after centrifugation at 1,500 g for 10 min (Megafuge). The pineal glands were quickly removed and immediately frozen on dry ice. All samples were stored at −80°C until further processing.

RIA for melatonin measurements. Pineal glands were homogenized in 500 µl tricine buffer (Sigma) containing 9.9% sodium chloride and 0.1% gelatin. After centrifugation at 1,500 g for 5 min, aliquots of 100 µl of the supernatant were assayed in duplicate. Plasma samples were extracted using dichloromethane. The dichloromethane phase was evaporated in a speed-vaporizer at 4°C under nitrogen, and the residue was reconstituted in tricine buffer solution. From the plasma extract aliquots of 200 µl were assayed in duplicate. The average efficiency of extraction was 87 ± 2%. Standards (Sigma) were extracted in triplicate using the same procedure as for the plasma samples. The RIA was performed using a rabbit antiserum (R19540) at a final dilution of 1/200,000, provided by INRA (Nouzilly, France), and the radioligand \( ^{125} \)I-labeled 2-melatonin prepared in the laboratory with 10,000 cpm/tube. Cross reactivity of the antiserum has been reported earlier (51). The assay was validated by controlling the parallelism between serial dilutions of pineal supernatant or dichloromethane plasma extracts and the standard
activity pattern with ultradian components of 4 and 6 h period (Fig. 2). LEW rats also displayed a multimodal
3, 4, and 6 h in addition to the most significant 24-h
analyses detected significant ultradian components of
in relation to the dark phase (Fig. 1). Periodogram
offsets of activity and an expanded duration of activity
is shown in Fig. 2). On the other hand, BH rats showed
not the most significant 24-h
leptin (LEF) treated with vehicle or leptin and transgenic to
aMT6s profiles

Statistics
Daily profiles of wheel running activity, aMT6s excretion, and plasma and pineal melatonin levels were analyzed by
two-way ANOVA (Statistica, Statsoft) using the Fisher's least
significant difference test or Tukey's honest significant differ-
tence test. For unequal sample sizes, Sjostvolv/ Stoline's test
was used. Pearson's r was calculated for correlation analysis.
Furthermore, estimates of broad-sense heritability and ge-
netic correlation were calculated according to the procedure
described by Hegmann and Possidente (20).

RESULTS

Experiment I: Individual Actograms
and aMT6s Profiles
The rats used in this study exhibited strain-specific locomotor activity patterns that were in good agreement with previous findings in these three strains (55, 56). ACI rats had a unimodal activity pattern characterized by a gradual increase of wheel running activity after onset of darkness, reaching maximal values in the second half of the dark phase (Fig. 1). Periodogram analysis revealed only one strong rhythmic component with a period of exactly 24 h (a representative actogram together with the respective periodogram of one ACI rat is shown in Fig. 2). On the other hand, BH rats showed a rather weak activity rhythm with blurred onsets and offsets of activity and an expanded duration of activity in relation to the dark phase (Fig. 1). Periodogram analyses detected significant ultradian components of 3, 4, and 6 h in addition to the most significant 24-h period (Fig. 2). LEW rats also displayed a multimodal activity pattern with ultradian components of 4 and 6 h and an extended activity phase similar to the BH rats. All three strains showed an additional short activity peak at the onset of light. The maximum activity levels of LEW rats were as high as those of ACI rats, but were interrupted by short periods of rest. Thus two thirds of the nighttime points were significantly different between ACI and LEW rats, and half of them were significantly different between BH and LEW (Fig. 1; P < 0.05). ANOVA revealed significant strain differences [F(2, 14) = 4.85, P < 0.025] with respect to the number of wheel rotations per day. The mean number of rotations per day was highest in ACI rats and lowest in BH rats. This resulted in a moderate estimate of broad-sense heritability, indicating that this parameter is genetically determined (Table 1).

Individual 24-h profiles of urinary aMT6s excretion were determined twice for each animal. They were highly consistent (r = 0.922, P < 0.001) and were therefore pooled for further analysis. All three strains showed pronounced unimodal diurnal rhythms of mean urinary aMT6s excretion (Fig. 3), which could also be observed in the individual 24-h profiles (not shown). aMT6s levels of all rat strains began to rise 2–3 h after onset of darkness, reached highest values 2–3 h later,
and remained elevated until the end of the dark phase. At the end of the dark period, aMT6s levels declined steadily, reaching daytime values below 10 ng/mg creatinine 2–5 h after lights on. Amplitudes of the mean aMT6s rhythms in BH and LEW rats were about twice as high as in ACI rats. Thus most nighttime aMT6s values of ACI rats were significantly different from those of the BH and LEW strains, but no differences were found between BH and LEW rats (P < 0.05). Linear regression analysis of daily amount of activity versus daily aMT6s excretion of all strains detected a significant negative correlation (r = −0.628, P = 0.007). However, this relationship did not prove significant within each strain (rACI = 0.017, P = 0.974; rBH = −0.455, P = 0.441; rLEW = 0.376, P = 0.463).

The 24-h integrals of total aMT6s excretion showed significant differences [F(2,14) = 12.21, P < 0.0009] between the strains (Table 1). Estimates of broad-sense heritability for total daily aMT6s excretion were rather high (Table 1).

Table 1. Strain differences in wheel running activity and urinary aMT6s excretion and estimates of broad-sense heritability

<table>
<thead>
<tr>
<th>Strain</th>
<th>n</th>
<th>Activity, Wheel Rotations/day</th>
<th>Total aMT6s Excretion, ng/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACI</td>
<td>6</td>
<td>896 ± 114</td>
<td>60 ± 2</td>
</tr>
<tr>
<td>BH</td>
<td>5</td>
<td>367 ± 70*</td>
<td>239 ± 7*</td>
</tr>
<tr>
<td>LEW</td>
<td>6</td>
<td>695 ± 145†‡</td>
<td>303 ± 9*</td>
</tr>
<tr>
<td>Heritability</td>
<td></td>
<td>0.491 ± 0.133</td>
<td>0.738 ± 0.093</td>
</tr>
</tbody>
</table>

Values represent means ± SE. Statistical tests are described in MATERIALS AND METHODS. aMT6s, 6-sulphatoxymelatonin; ACI, ACI/Ztm; BH, BH/Ztm; LEW, LEW/Ztm. *P < 0.01 vs. ACI; †P < 0.01 vs. BH; ‡P < 0.05 vs. ACI.

Experiment II: Daily Profiles of Melatonin and SCN Melatonin Receptor Density

Pineal and plasma melatonin concentrations revealed clear 24-h rhythms in all three strains (Figs. 4 and 5). Melatonin concentrations began to rise with onset of darkness and reached their highest values within the following 4 h. Nighttime values remained stable over the dark phase and started to decline at the
beginning of the light phase. Thus melatonin levels displayed a unimodal pattern rather similar to the daily rhythm of aMT6s excretion. The temporal patterns of plasma melatonin content resembled those of pineal melatonin but showed higher variations in their nighttime values (Figs. 4 and 5). The 24-h profiles of plasma melatonin and pineal melatonin differed from each other at various time points. For example, the plasma concentrations in LEW rats were highest when their pineal melatonin contents showed a slight depletion phase. Furthermore, ACI rats showed a decline in plasma melatonin in the second half of the dark phase, which was not obvious in the pattern of pineal melatonin. Therefore, linear correlation between those two parameters was strong in BH rats (r = 0.578, P < 0.0001) but rather weak in ACI (r = 0.411, P < 0.005) and LEW rats (r = 0.377, P < 0.011). Compared with ACI rats, BH and LEW rats exhibited pineal melatonin rhythms with three- and fivefold amplitudes and plasma melatonin rhythms with two- and threefold amplitudes, respectively. Thus most nighttime values of BH and LEW rats were significantly different from the corresponding values of ACI rats (P < 0.05).

Strain differences in the amount of melatonin synthesis were more obvious at the level of daily production rates or 24-h integrals (Table 2). Daily melatonin synthesis was four and six times higher in BH and LEW rats, respectively, than in ACI rats, resulting in highly significant strain differences [F(2,15) = 198.8, P < 0.0001]. In contrast, ANOVA of the 24-h integrals of plasma melatonin revealed only a barely significant strain difference [F(2,15) = 5.34, P < 0.018], with LEW rats showing only ~30% higher plasma melatonin concentration than ACI and BH rats. Estimates of broad-sense heritability were rather high for daily pineal melatonin synthesis, but moderate for daily plasma melatonin levels (Table 2).

In vitro autoradiography revealed marked strain differences of specific [125I]-labeled 2-melatonin binding in the SCN of animals that were killed in the middle of the light period (Fig. 6). A statistically higher density of melatonin binding sites was observed in the SCN of the LEW rats (P < 0.05) compared with the two other strains. Indeed, the maximal melatonin binding was similar in the ACI and BH rats (Bmax = 7.4 ± 0.6 and 7.8 ± 0.5 fmol/mg protein, respectively) but higher in the LEW rats (Bmax = 10.6 ± 0.3 fmol/mg protein). The SCN receptor affinity to [125I]-labeled 2-melatonin, as revealed by the Kd, was highest in the BH rats (Kd = 106 ± 15 pM) followed by LEW and ACI rats (Kd = 10.2 ± 0.3 pM on November 7, 2017 http://ajpregu.physiology.org/ Downloaded from

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**Fig. 3.** Twenty-four-hour profiles of 6-sulphatoxymelatonin (aMT6s) excretion in ACI, BH, and LEW rats. Values are 1-h means ± SE (n = 6, 6, and 5 rats for ACI, LEW, and BH, respectively). Black bar indicates dark period. *,+ P < 0.05 vs. ACI and BH, respectively.

**Fig. 4.** Twenty-four-hour profiles of melatonin concentration in pineal gland in ACI, BH, and LEW rats (means ± SE, n = 6). Black bar indicates dark period. *,+ P < 0.05 vs. ACI and BH, respectively.
Saturation data indicated the presence of a single high-affinity receptor site for melatonin in all three strains ($r_{ACI} = 0.994$, $r_{BH} = 0.996$, $r_{LEW} = 0.999$).

**DISCUSSION**

Inbred strains of small rodents with genetically determined differences in their activity rhythms provide a powerful tool for investigating the temporal organization of the circadian system (10, 11, 13, 55, 56). In the present study, rats of the inbred strains ACI, BH, and LEW, with characteristic differences in amplitude and temporal organization of diurnal wheel running activity rhythms (55), were analyzed for comparable strain differences in another output parameter of the circadian system: the qualitative and quantitative pattern of melatonin secretion.

The results of the present study confirmed previous findings that aMT6s is a suitable parameter for monitoring melatonin rhythms of individual animals (3, 49). The aMT6s excretion rates of all rat strains nicely reflected the temporal pattern of pineal and plasma melatonin concentrations both quantitatively and qualitatively, with, however, a time lag of about 2 hours in all three strains as a result of melatonin catabolism. Therefore, strain-dependent differences in catabolic capacity for the conversion of melatonin to its metabolite can be excluded. The two aMT6s 24-h cycles analyzed for each rat proved to be highly consistent; i.e., aMT6s excretion patterns were very stable within individuals. A similar low cycle-to-cycle variability of aMT6s has also been reported in humans (31).

In Syrian hamsters, a close relationship between temporal patterns and phase shift responses of pineal melatonin and wheel running activity rhythms has been demonstrated (15, 28), which suggests that both parameters are controlled by the same rhythm-generating system. We therefore expected to find strain-dependent differences in the melatonin rhythm as well. However, the diurnal patterns of aMT6s excretion and melatonin concentration were similar in all three strains, and they all displayed a unimodal pattern. This suggests a considerable divergence in the temporal organization of the two output parameters of the circadian system. However, the finding of separate temporal patterns in locomotor activity and melatonin synthesis does not come as a complete surprise for the following reasons. First, none of the numerous studies on melatonin secretion has ever reported the occurrence of several discrete melatonin peaks during an undisturbed dark phase. Second, uncoupling or divergence of different circadian rhythms or components is quite common. For example, in continuous bright light...
rats become arrhythmic with respect to locomotor activity and melatonin production, whereas neural oscillations within the SCN persist (21). When mammals are maintained in moderate constant light, the activity rhythm exhibits an endogenous period dependent on light intensity, whereas the melatonin rhythm is completely suppressed (2). Third, SCN transplants can restore the circadian rhythm of locomotor activity most probably by diffusible signals (46), no reestablishment of pineal function has ever been observed (30). The present findings, therefore, support the hypothesis that the circadian rhythms of locomotor activity and melatonin synthesis are controlled by different output pathways from the SCN.

The efferent projection from the SCN to the pineal gland is well characterized and ends in a sympathetic innervation of the pineal gland regulating the melatonin synthesis (37). On the other hand, the neural pathway mediating circadian rhythms of locomotor activity has not yet been identified, and there is no reason to exclude the possibility that ultradian modulations of the activity patterns are a result of physiological or neuronal modifications of the output pathways.

The rat strains clearly differed in the amplitudes of aMT6s and melatonin rhythms. ACI rats, which showed the highest level of activity, had the lowest amounts of melatonin. In contrast, BH and LEW rats, which had low levels of activity, displayed two- to fourfold higher melatonin amplitudes. In studies on Wistar rats (12:12-h LD cycle), maximal aMT6s rhythm amplitudes of ~60 and 25 ng/h were found by Brown et al. (3) and Kennaway and Rowe (23), respectively. Our findings in BH and LEW rats are consistent with Brown’s data, whereas the aMT6s measurements of the ACI rats are similar to Kennaway’s measurements. Therefore, we cannot identify one strain that shows “normal” melatonin values. In general, the amount of melatonin produced during the night correlates positively with the light intensity present during the photophase (22, 33). This raises the possibility that the highest levels of pineal melatonin were found in LEW rats, because the albinotic LEW strain perceives light more intensively than the pigmented ACI and BH strains. This hypothesis is partly supported by a direct comparison of eight different rat strains demonstrating that most pigmented strains show smaller-sized pineal glands with lower melatonin concentration than albino strains (24, 54). However, maximal melatonin content measured in the albinotic LEW/Han rats was only 700 ± 29 pg/pineal, which corresponds to the melatonin concentration found in the present study for the pigmented ACI/Ztm rats (751 ± 124 pg/pineal), whereas our albinotic breeding stock LEW/Ztm showed far higher melatonin levels of 3,691 ± 358 pg/pineal. The assumption of a pigmentation-dependent melatonin production is also in conflict with a recent study on Wistar rats, which demonstrated that pigmented or hooded Wistar rats had similar aMT6s rhythm amplitudes but started aMT6s excretion ~2 h earlier than albino Wistar rats (23). The pigmented Wistar rats produced even more melatonin during the night. Thus it seems unlikely that the observed strain differences in pineal function were simply a result of the different eye pigmentation of the rats.

In the present study, regression analyses of melatonin synthesis and aMT6s excretion versus wheel running activity revealed a strong negative correlation between strains but not within strains. This suggests that the levels of melatonin synthesis and locomotor activity are not causally related but are strain-dependent and most probably genetically coupled characteristics of the circadian system. It is now well accepted that exogenous melatonin has an entraining and phase-shifting effect on the circadian system (2, 5, 8, 25, 32, 43), but there is only limited evidence for a physiological effect of melatonin on the level of activity. For example, systemic administration of melatonin at pharmacological doses reduces locomotor activity via a decrease of brain serotonin release (9). Such an inhibitory effect of melatonin on locomotor activity would at least partly explain the low level of overall activity accompanied by high melatonin concentrations in BH and LEW rats. However, this hypothesis needs to be verified by additional studies comparing the effects of pinealectomy and/or constantly high melatonin administration on activity levels among the strains.

A recent study of the three inbred rat strains ACI, BH, and LEW demonstrated strain-dependent differences in the density of arginine vasopressin (AVP) neurons of the SCN (56). The number of AVP neurons was significantly higher in ACI rats, which have a strong unimodal activity pattern, than in BH and LEW rats, which have dissociated multimodal activity patterns. Such a positive correlation between the number of AVP neurons and the strength of circadian activity rhythms has also been demonstrated in selected lines of mice (4). These data suggest that AVP neurons may be part of the SCN output pathways controlling circadian rhythms of locomotor activity. Furthermore, they raise the question of a causal relationship between the number or density of AVP neurons, melatonin secretion from the pineal gland, and dissociation of circadian activity patterns.

It is assumed that melatonin can affect the coupling of oscillatory units within the SCN (8), inasmuch as melatonin can accelerate the reentrainment of activity rhythms and synchronize disrupted components of the circadian system in constant light (6). In accordance with our working hypothesis, the bimodal and multimodal activity patterns of BH and LEW rats, respectively, could be the result of a desynchronization of multiple circadian oscillators caused by the reduction or complete loss of melatonin feedback on the SCN. However, the present findings of rather high melatonin secretion levels in BH and LEW rats do not support this hypothesis, because one would expect the two strains with the ultradian activity patterns to have a rather low level of melatonin. A possible explanation for this obvious contradiction would be the partial or complete insensi-
tivity of the SCN to the feedback effect of melatonin. Therefore, we also measured the density of melatonin receptors in the SCN of all three strains in the middle of the photophase, when receptor density should be elevated (18, 19, 29, 34). However, melatonin receptor density was higher in LEW rats, which showed higher melatonin content, than in ACI rats with low levels of melatonin. Thus the present results of high melatonin levels and receptor density do not support our previous assumption that the bimodal and multimodal activity patterns of BH and LEW rats are caused by a reduction or loss of melatonin feedback on the SCN.

An additional noteworthy result of the present study is that the strain difference in SCN melatonin receptor density supports previous findings that melatonin seems to affect the density of its own receptors in the SCN (16–18). However, other studies have reported conflicting results. On one hand, there is evidence for an inverse relationship between melatonin levels and receptor density. For example, elevated melatonin concentrations at night were accompanied by increased melatonin receptor density (18). Furthermore, enhanced melatonin concentrations induced by stress reduced specific melatonin binding in the SCN of the rats (50). These short-term effects of melatonin are most likely regulated by a mechanism of downregulation. On the other hand, there is evidence that long-term effects of melatonin can stimulate the density of its receptors in the SCN. For example, long-term pinealectomy decreased melatonin binding sites (17). Furthermore, maternal melatonin seems to stimulate the synthesis of melatonin receptors in the SCN of newborn rats, because receptor density was ~20% lower in pups born to pinealectomized dams (57). This stimulating effect of melatonin on its own receptor density in the SCN is in accordance with our results in the LEW rats, where high levels of melatonin were accompanied by a high melatonin receptor density.

The present study demonstrated pronounced strain differences in activity rhythms, pineal function, and melatonin receptor density in the SCN. ACI rats displayed unimodal locomotor activity patterns with a high amplitude of activity, but low levels of melatonin secretion and melatonin receptor density in the SCN. In contrast, LEW rats showed dissociated activity patterns with a reduced amplitude of overall activity but rather high levels of melatonin and melatonin receptor density in the SCN. The inbred rat strains ACI, BH, and LEW may therefore prove to be useful models to further investigate the complex interaction between the circadian control of pineal function and feedback effects of melatonin on the SCN as well as on other circadian output parameters, such as locomotor activity.

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