Aging alters resynchronization of the circadian system in rats after a shift of the light-dark cycle

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Summary. Four days following an 8-h advance of the light-dark cycle, the circadian rhythms in the pineal N-acetyltransferase activity and melatonin content reappeared in 7-week-old rats, but were still abolished in 24-month-old animals.
Key words. Aging; rat; circadian system; entrainment; N-acetyltransferase; melatonin.

With aging, the amplitude of a circadian rhythm may decrease, and a rhythm may even disappear[1]. A circadian pacemaker can itself change with age[2,3]; namely, its free-running period may alter. Older people possibly resynchronize more slowly to a new time after a phase shift[4]. In old rats, the locomotor rhythm may[5] or may not[6] take a longer time to re-entrain after a reversal of the light-dark (LD) cycle. The idea that the ability to adjust to schedule changes decreases with aging has been put forth many times in the literature[7,8], but without conclusive evidence. The lack of data on the rate of re-entrainment of the circadian system in aging mammals prompted us to study this question.

For our studies, we chose the rhythm of change in N-acetyltransferase (NAT, EC 2.3.1.87) activity in the rat pineal gland which drives the rhythm of melatonin production[9,10], and the rhythm of pineal melatonin concentration itself. Similarly to other overt daily rhythms[11], the NAT rhythm is controlled by a pacemaker located in the suprachiasmatic nucleus of the hypothalamus[12] and may thus serve as a model circadian rhythm for following entrainment of the pacemaker[13]. In young adult rats, after an 8-h advance of a LD cycle, i.e., after a simulated eastward transition over 8 time zones, the NAT rhythm is completely abolished during the first three cycles following the advance shift[14]. It reappears during the fourth cycle only; however, its pattern is still compressed considerably. Only during the fifth cycle does the waveform of the NAT rhythm begin to resemble the pre-shift pattern. In the present study, we subjected young and old rats to an 8-h advance of the LD cycle and we followed the NAT and melatonin rhythms during the fourth cycle after the shift, to find out whether the rhythms reappeared during the threshold cycle even in old animals.

Materials and methods

We used 7-week-old 'young' and 24-month-old 'old' male Wistar rats housed under artificial lighting regime of 12 h of light and 12 h of darkness per day (LD 12:12) and at a temperature of 23 ± 2 °C. The animals had free access to water and commercial food pellets. Light provided by overhead 40 W Tesla fluorescent tubes was automatically turned on at 06.00 h and off at 18.00 h. In order to advance the LD cycle by 8 h, one dark period was shortened by 8 h and thereafter the light and dark period alternated again regularly (fig., C). 40 young and 40 old rats were killed before the advance shift, i.e., in the original LD schedule (night − 1), and 44 young and 44 old rats were killed during the fourth cycle after the shift (night + 4). The first groups of rats were killed in the light, just 10 min before the onset of the darkness, in order to determine "daytime" NAT and melatonin levels which are always low, i.e. baseline, in the light. Thereafter, rats were killed at 1–2-h intervals throughout the whole dark period. The last groups of rats were killed 30 min after the morning light onset. When rats were to be killed in darkness, they were exposed prior to decapitation to a very faint red light for less than 1 min.

Within 48 h after decapitation, a single pineal was homogenized in 94 µl of 0.33 mM [1 − ^{14}C] acetyl-CoA (sp. act. 37 MBq/mmol) containing 0.1 M sodium phosphate buffer, pH 6.8. 75 µl of the homogenate were immediately mixed with 25 µl of 40 mM tryptamine containing phosphate buffer and NAT activity was determined by a modification[15] of the method of Deguchi and Axelrod[16]. Units of NAT activity were defined as nmol N-acetyltryptamine formed in 1 h per 1 mg of pineal tissue. The remaining 19 µl of the homogenate were diluted with 581 µl of 0.05 M phosphate buffer, pH 7, and stored at −20 °C for melatonin determination. The diluted homogenate was extracted with 1 ml of methylene chloride. The organic phase was washed twice with 0.2 ml of 0.1 M NaOH and evaporated to dryness.

Melatonin was estimated in the dry residue in duplicate samples by a radioimmunoassay[17]. The melatonin concentration was expressed as ng per 1 mg of pineal tissue. The limit of detection of the assay was 6 pg/tube. [1 − ^{14}C]-acetyl-CoA (2.07 GBq/mmol) and [^{3}H] melatonin (3.15 TBq/mmol) were purchased from the Biochemical Centre, Amersham, U.K. The melatonin antiserum, batch 704-189, was kindly provided by Dr. J. Arendt via Guildhay Antisera, Department of Biochemistry, University of Surrey. Data were analyzed using a one-way analysis of variance. The t-test with Bonferroni probabilities (BMDP Statistical Software, University of California, Los Angeles) was used for the post hoc comparison, with $\alpha = 0.05$ required for signifi-

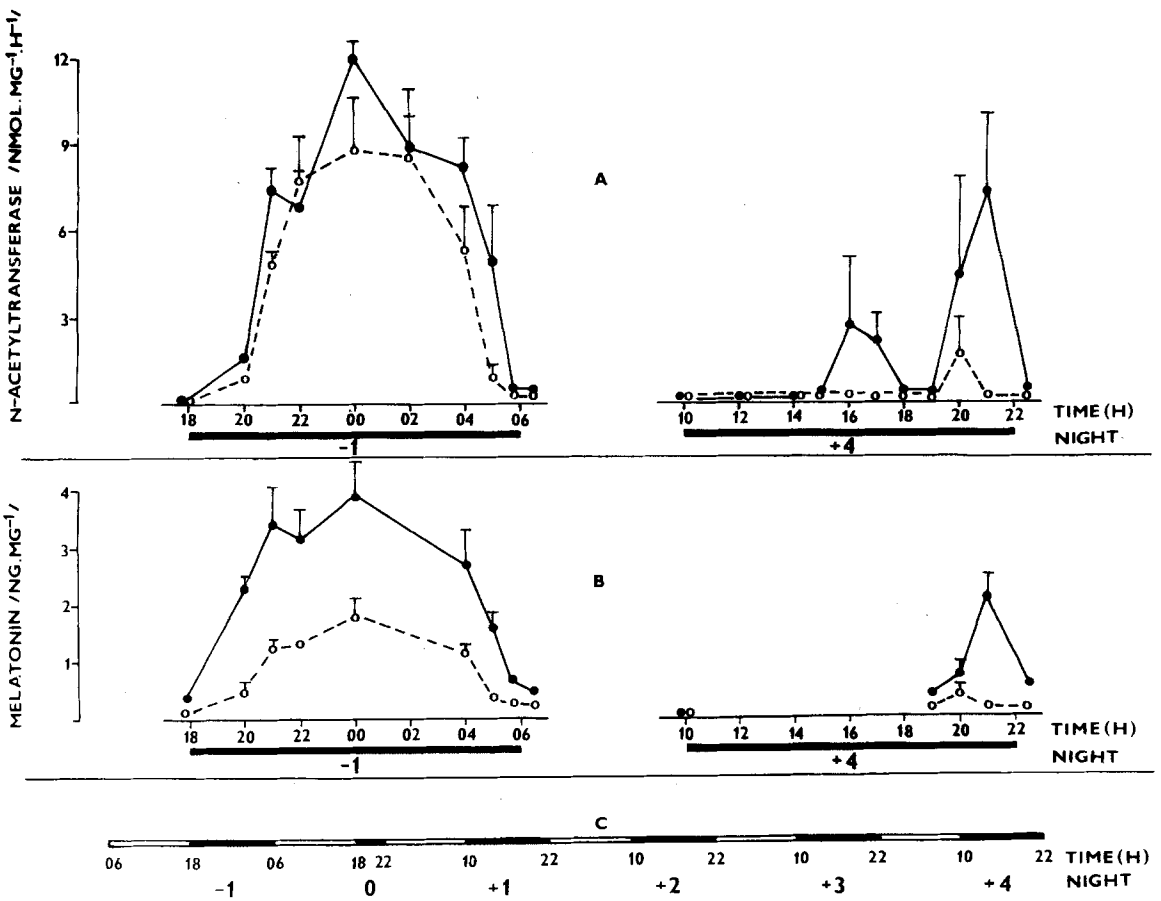
cance. Heterogeneity of variance was reduced by log transformation of the data.

Results and discussion

In the entrained state, the NAT rhythm in the old rats was similar to that in the young rats (fig., A). Neither the daytime levels at the end of the light period nor the nighttime ones around the middle of the dark period differed significantly between the two groups. The same did not hold true for the rhythm in melatonin concentration (fig., B). The pooled nighttime values at 21.00 h, 22.00 h and 23.00 h were significantly higher in the young rats than in the old ones (3.48 ± 0.31 ng/mg vs 1.48 ± 0.13 ng/mg, $p < 0.001$) and so were the baseline daytime values at 17.50 h (0.41 ± 0.07 ng/mg vs 0.12 ± 0.01 ng/mg, $p < 0.001$). In both young and old rats, the NAT activity increased significantly above the daytime value at 20.00 h ($p < 0.001$ and $p < 0.05$, respectively) and so did melatonin concentration ($p < 0.001$ and $p < 0.001$, respectively). However, the morning decline occurred about 45 min earlier in the old rats than in the young ones; in the old rats, the NAT activity and melatonin concentration had already decreased significantly from the high nighttime values at 05.00 h ($p < 0.01$ and $p < 0.001$, respectively), while in the young rats the decrease was significant only at 05.45 h ($p < 0.001$ and $p < 0.001$, respectively).

After the 8-h advance shift of the LD cycle, the NAT rhythm reappeared during the dark period of the fourth cycle in the young rats only. The activity increased significantly above the baseline value at 21.00 h ($p < 0.001$), though in individual rats a high nighttime activity appeared earlier. Similarly, melatonin concentration increased significantly above daytime values at 21.00 h ($p < 0.001$). In the old rats, the pineal rhythms were still abolished. Neither NAT activity, nor the melatonin concentration at the end of the dark period, increased significantly above daytime baseline values. A nocturnal rise in NAT and melatonin occurred in just one rat.

The finding of lower pineal melatonin concentrations during the day, and in almost all cases throughout the whole night in the old rats in comparison with the young ones, amplifies an earlier report on lower melatonin lev-



The effect of an 8-h advance of the LD cycle on the N-acetyltransferase rhythm (A) and melatonin rhythm (B) in young (full circles) and old (open circles) rats. C indicates a schedule of advancing the LD cycle by shortening of one dark period. Rats adapted to LD 12:12 were either killed during the night before the advance shift (night −1) or during night + 4 after the shift. Lines under the abcissa indicate periods of darkness. Data are expressed as means + SEM of 4 animals. Where SEM lines are omitted, the SEM are encompassed by the area of the symbol. The data on melatonin at 02 h during night −1 and at 12 h, 14 h, 15 h, 16 h, 17 h and 18 h during night + 4 are missing due to overturning of the samples.

els in aged rats determined at two time-points during the night [18]. Reduction of the melatonin rhythm amplitude during aging does not occur only in rats, but in other species as well, e.g. in Syrian [19] and Djungarian [20] hamsters, in gerbils [19] and even in humans [21], and is in accordance with a decline of amplitude of other rhythms [1]. As the activity of NAT, which forms the melatonin precursor N-acetyl-serotonin, does not change with age, the reduction in melatonin levels may be due to the reported decline of another enzyme of the melatonin forming pathway during aging, i.e. of hydroxyindole-o-methyltransferase (HIOMT) [22]. This suggestion is in accordance with an earlier proposal that the NAT rhythm drives the melatonin rhythm but the maximum melatonin production may depend on HIOMT [10].

As during the fourth cycle after the 8-h advance shift the NAT and melatonin rhythm were expressed in the young, but still abolished in the old rats, the re-entrainment of both rhythms in the aged animals apparently proceeded at a slower rate. It appears that after travel over the longitudes, the rhythm in serum melatonin also adjusts more rapidly in younger than in older humans [23]. The slower rate of adjustment during aging may be due to a change in the entrainment or in the output pathway or to a change in the clock itself [2, 3]. Our data are in favor of the conclusion that re-entrainment of the circadian system proceeds more slowly in aged mammals. As melatonin itself can entrain the circadian system [24] and accelerate its re-entrainment [25, 26], low melatonin levels and a slow rate of re-entrainment of the melatonin rhythm in old individuals may further slow down the course of circadian rhythm re-entrainment in aged mammals.

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Calculation of drug-melanin binding energy using molecular modeling

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Summary. Conformational analysis and molecular graphics are used to model a representative melanin structure to estimate a chemical's in vitro affinity for melanin. The modelling data fit to a simple linear equation relative to a logarithmic transformation of the experimentally-derived binding data (r = 0.901). The goodness of fit, as evidenced by the F-statistic, $F_{(1,14)} = 60.09$ (p = 0.000002), for the regression indicates that this technique gives an accurate representation of the interaction of these chemicals with melanin in vitro.
Key words. Conformational analysis; molecular modelling; melanin.

Knowledge of the interactions of compounds with biological macromolecules is critical to the design of new drug candidates and to the prediction of adverse reactions for those and other compounds. However, while the binding parameters for some systems (e.g., stable proteins, DNA) are readily accessible through in vitro binding assays, it is frequently necessary to examine binding in systems which are not amenable to such direct