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Age-related disruptions in circadian timing: evidence for "split" activity rhythms in the SAMP8

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Abstract

In the senescence-accelerated mouse of the P8 line (SAMP8), age-related changes in circadian timing include a decrease in amplitude of the rhythm, a slower rate of re-entrainment following a phase advance, and a longer free-running period (τ). The present study extends previous research by investigating possible split activity rhythms in the SAMP8. Running wheel activity was examined in 2-, 7-, and 12-month SAMP8. Consistent with previous research, rhythms of older SAMP8 were decreased in amplitude and showed high levels of activity during the light phase of the light-dark cycle. Contrary to previous reports, lengthening of τ in constant darkness was not observed. Additionally, activity rhythms were "split" in older SAMP8, demonstrated by 1) the appearance of a secondary peak in the periodogram at approximately half the value of τ and 2) the perceptual classification of actograms by naïve observers. This result differs from previous studies in hamsters demonstrating an age-related decrease in the incidence of rhythm splitting. Overall, the present findings provide further evidence for age-related disruption of circadian timing in SAMP8.

Keywords: Circadian rhythms; Aging; Senescence-accelerated mouse; SAMP8; Split activity rhythms

1. Introduction

Age-related disruptions of circadian rhythms are observed in many species, including humans, and are characterized by both changes in behavior and physiology [5,18, 22,50]. Behavioral disturbances include fragmented sleep-wake patterns, weak coupling with environmental rhythms, high levels of nighttime activity, and reduced daytime cognitive performance [52,53]. In mammals, dysfunction of the circadian clock located in the suprachiasmatic nucleus (SCN) of the hypothalamus may be responsible for disruptions in circadian timing [34,40,49]. Evidence for this functional breakdown in the SCN with age derives from 1) a reduction in the number of cells and volume of the SCN [6,37,43], 2) a reduction in total and rhythmic expression of mRNA for vasoactive intestinal polypeptide in SCN [19, 21], and 3) demonstrations that age-related rhythm fragmentation can be reversed by transplantation of fetal SCN cells [49].

The senescence-accelerated mouse line (SAM) is a model of accelerated aging that offers one approach to studying effects of age on circadian timing [45,47]. Two major lines of SAM have been developed with one line prone to the early onset of age-related pathologies (SAMP) and the other line resistant to accelerated aging and exhibiting a normal rate of aging (SAMR). Each of these lines has been further divided into sub-lines that have different types of pathology. Much research on SAM lines has been conducted on the P8 sub-line of the SAMP (SAMP8).

Two areas in which SAMP8 demonstrate accelerated aging are learning/memory [12,23,24,57] and circadian timing [44]. With respect to circadian timing, SAMP8 show various age-related changes in their near 24-h locomotor activity rhythms, including a reduction in the amplitude of the rhythm, a slower rate of re-entrainment following a phase advance, and a lengthened free-running period in constant dark conditions (τ_{DD}) [1,23,24,38,41,44]. These age-related changes in circadian timing in SAMP8 are characteristic of those frequently considered part of the normal aging process in rodents [8,31,54–56], although some rodents such as hamster, rat and deer-mice show a shortening of their free-running period with age rather than a lengthening [25,29,36,51]. Some aspects of activity rhythms in SAMP8 appear to be unusual. SAMP8 produce an unusu-

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ally high level of locomotor activity, with a large proportion of that activity occurring during daytime when nocturnal rodents are normally inactive [23,24,38]. Disruptions of this sort are reminiscent of various rhythm disorders associated with human aging. Patients with senile dementia in particular, show excessive levels of nighttime activity, in relation to healthy elderly of the same age [39,53]. For this reason, the SAMP8 may be useful for investigating disruptions in circadian timing associated with senile dementia and aging [23].

One specific type of rhythm disruption that occurs in many rodents is rhythm splitting [15]. Rhythm splitting is a phenomenon that occurs when a single daily bout of locomotor activity dissociates into two periodic components. Given enough time, the two components can become locked in anti-phase, resulting in a split circadian rhythm with two peaks of activity 180° apart. Splitting commonly occurs in young adult hamsters maintained in constant light conditions (LL) [30] and is known to occur in variety of other mammals and birds [2,14,16,28]. Splitting in LL conditions can also be induced by selectively timed dark pulses [4], as well as entrained in LD [3]. Effects of age on rhythm splitting have been examined in hamsters [10,25], but not in other animals. In the hamster, the incidence of spontaneous rhythm splitting in constant light and splitting induced by dark pulses decreased with age.

Two pieces of indirect evidence suggest that split-like activity rhythms may occur in SAMP8. First, a large proportion of activity during the light phase tends to occur primarily during the first three to four hours of the light period [23]. This activity in addition to the normal activity observed during the dark phase is consistent with a split rhythm. Second, the spontaneous motor activity data reported by Miyamoto (Fig. 5) appears to show a split-like activity rhythm in the 8 and 12-month old SAMP8 that is not observed in younger subjects, although the author does not comment on this observation [23]. These results are suggestive of an increased incidence of split-like activity rhythms in aged SAMP8, a conclusion opposite the findings in hamsters [10,25], which show a decreased incidence of split rhythms. Thus, the present study was conducted to establish 1) whether circadian rhythm are split in the SAMP8 as hinted at by some previous data, 2) whether the incidence of split rhythms is increased or decreased with age, and 3) the age at which split rhythms start to appear.

2. Method

2.1. Animals

Twenty-nine naïve, male SAMP8, ages two-months (n = 10), seven months (n = 9), and twelve months (n = 10) were subjects for the study. The mice were bred and raised in the animal research facility at Bowling Green State University. Two to four same-sex littermates were housed together, with food and water available ad libitum. Lights in the animal rooms were on a 12 h light/12 h dark cycle (lights on at 7:30 AM).

2.2. Apparatus

Wheel-running activity was monitored using four Wahmann running wheels (110 cm circumference, 7.5 cm width) located in a room separate from the animal colony room. The room was illuminated at 165 lux during the light phase. A cage (22.5 × 12.5 × 12.5 cm) was attached to each wheel, containing food and water. The mice were able to freely move between the cage and the wheel. An IBM compatible computer with custom software recorded the activity of each wheel by time-stamping each wheel revolution. A wheel revolution occurred whenever a magnet attached to the wheel's axis of rotation made contact with an electronic switch sending a signal to the computer.

2.3. Procedure

Wheel-running rhythms were monitored for 16 days. For the first 6 days, the colony room's 12:12 light/dark (LD) cycle was maintained in order to determine a baseline measure of the entrained wheel-running rhythms (τ_{LD}). For the last 10 days, the mice were kept in constant darkness (DD) in order to estimate each animal's free-running period of wheel-running activity (τ_{DD}).

2.4. Data analysis

Overall activity was measured by summing (or "binning") the number of wheel revolutions in successive 6-min intervals over a 24-h period for the sixteen days of the experiment. To consider possible age-related changes in the proportion of activity in the light phase, P (activity|lights on), the amount of activity for the first 12 h of each day (light phase) was summed and then divided by the total daily activity for the 6 days of LD.

To assess activity rhythms, double-plotted actograms showing the amount of wheel running over the course of the experiment were constructed. Estimates of τ_{LD} and τ_{DD} were obtained by constructing chi-square periodograms from binned activity of the first six days under LD conditions and the next ten days under DD conditions, respectively. The reader is referred to Sokolove and Bushell (1978) for an excellent discussion of the chi-square periodogram and its use in the analysis of circadian rhythms [42]. For the chi-square periodogram, a Q_p statistic was calculated for hourly periods between 8 and 30 h using a bin size of 6 min. The Q_p statistic represents the degree to which each period is present in the data, after accounting for differences due to chance. In that sense, Q_p is similar to a chi-square statistic. In accordance with previous research, the period associated with the largest value of Q_p provides an estimate of τ, the true circadian period [35].

To assess the presence of split circadian rhythms, ten naïve observers were asked to classify the double-plotted actograms as either having four equally-spaced bands (corresponding to four phase-locked bouts of activity in a 48-h period) or two equally-spaced bands (corresponding to two phase-locked bouts of activity in a 48-h period). Observers were supplied with ideal representations of 2-banded (Fig. 4a) and 4-banded (Fig. 4c) double-plotted actograms to serve as guides. No information was provided to the observers about the subjects or purpose of the classification of the pictures; observers were only instructed to make simple perceptual judgments about the number of bands, ignoring both the width and darkness of the bands. They were presented each actogram and asked to rate it on a six-point scale: 1—definitely two bands, 2—very likely two bands, 3—maybe two bands, 4—maybe four bands, 5—very likely four bands, 6 definitely four bands. The set of actograms were presented to observers twice, each time in a random order. The first set of ratings was treated as practice, with the second set of ratings used for the analysis.

The statistical results reported below were conducted using SPSS for Windows (Version 9.0, SPSS, Inc., Chicago, IL); Analysis of variance (ANOVA) was used to assess differences in overall activity, daytime activity, proportional activity, free-running circadian period, and the Q_p statistic. For the ANOVA on proportional activity, an arc sine transformation was first applied to the data in order to correct for a violation in the assumption of normality. All post-hoc comparisons between the three age groups were performed using Tukey's HSD with an alpha level of 0.01.

3. Results

Overall, young SAMP8 were more active than older SAMP8. For the 2-month and 7-month groups, the amount of run-wheel activity tended to increase over the course of the first six days, and then stabilize after day six (Fig. 1). For the 12-month group, the amount of run-wheel activity varied from day to day, but did not shown a general trend to increase over the course of the experiment. Confirming these observations, the analysis of variance (ANOVA) on mean daily activity (with day as a within-subject factor and age as a between-subjects factor) demonstrated a significant main effect of day, [$F(15,390) = 11.40$, $P < 0.001$], a significant main effect of age [$F(2,26) = 58.05$, $P < 0.001$] and a significant interaction between day and age [$F(30,390) = 2.58$, $P < 0.001$]. Post-hoc analyses of overall activity revealed significant differences between all three groups (2-months–7-months, $P < 0.001$; 2-months–12-months, $P < 0.001$; 7-months–12-months, $P < 0.001$). One possible explanation for the increase in activity over the first six days that was observed with the 2-month and 7-month groups may be related to the SAMP8 learning to run in the wheels, as none of the mice had prior experience with running wheels.

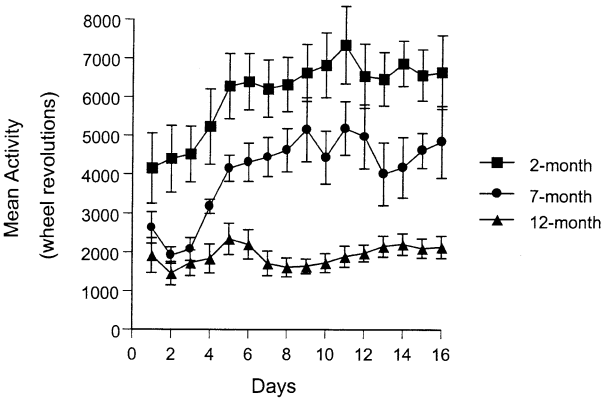

Fig. 1. Time course of daily activity for 2-, 7-, and 12-month SAMP8. Activity was assessed by running wheels and is expressed as the number of wheel revolutions. Overall activity increased as a function of days and decreased with age. Increases in activity across days occurred for the 2- and 7-month groups, but not the 12-month old group. During days 1–6, SAMP8 were in a 12:12 h light-dark cycle. During days 7–16, mice were in a constant dark condition.

Double-plotted actograms showed that all SAMP8 were entrained to the 12:12 LD cycle. Using chi-square periodograms constructed for the LD phase, mean τ_{LD} values were within 0.1 h of 24 h for all ages; the margin of error expected for a 6-min bin size. In DD, all three ages of SAMP8 had free-running circadian periods that were significantly less than 24 h (Fig. 2). Mean estimates of τ_{DD} were 23.56 ± 0.06, 23.58 ± 0.05, and 23.69 ± 0.06 h for the 2-month, 7-month, and 12-month SAMP8, respectively (mean ± standard error). Individual estimates of τ_{DD} for each mouse are listed in Table 1. Although the data suggest a lengthening of τ_{DD} with age, a one-way ANOVA indicated that this difference was not significant [$F(2,26) = 1.71$, ns]. Overall, estimates of τ_{DD} ranged between 23.2 and 24.0 h.

Overall, the 7-month old and 12-month old SAMP8 tended to spend proportionally more time running during the light phase than did the 2-month old SAMP8 (Fig. 3b). Age-related changes between 2- and 7-months in the proportional daytime activity reflected both a decrease in overall activity and an increase in daytime activity (see Fig. 3a). An ANOVA on the arc-sine transformed P(activity|lights on) confirmed significant main effects of age and day [age: $F(2,26) = 22.78$, $P < 0.001$; day: $F(5,130) = 7.15$, $P < 0.001$], but no interaction between these two factors [$F(10,130) = 1.967$, ns]. Post-hoc comparisons between the three groups using Tukey's HSD confirmed that these age-related changes in proportional daytime activity were primarily between 2-months and 7-months ($P < 0.001$), with the proportions of daytime activity for the 7-month and 12-month SAMP8 similar ($P > 0.75$). A second ANOVA was performed on overall daytime activity to confirm that the differences in proportional activity were partially due to an increase in daytime activity, not just a decrease in nighttime activity. As expected, the ANOVA showed significant

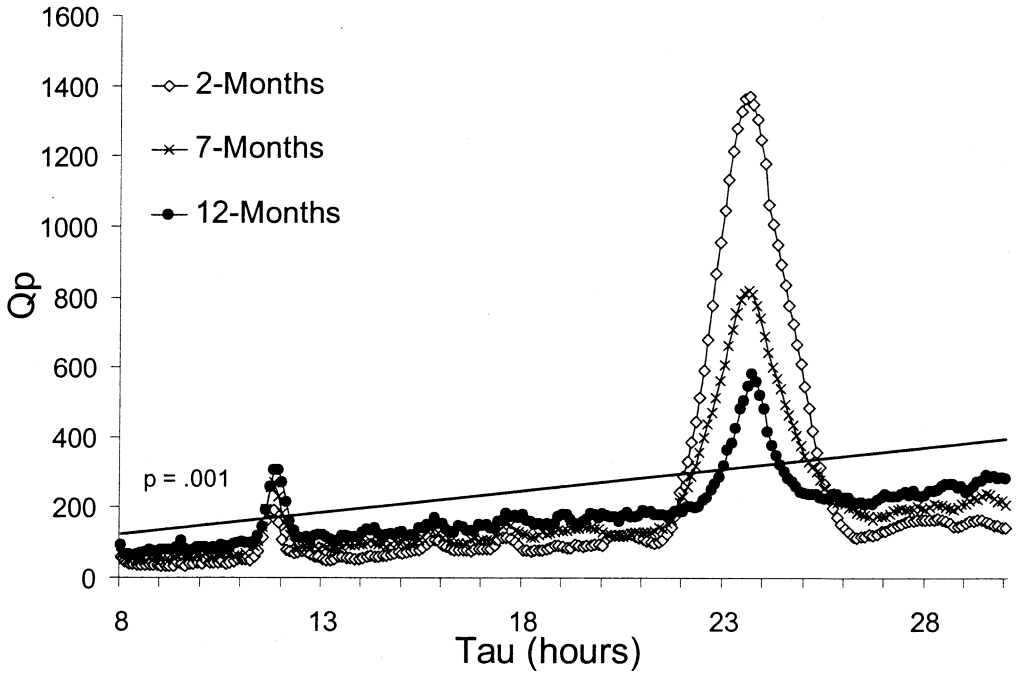

Fig. 2. Chi-square periodograms during constant dark conditions for the 2-, 7-, and 12 month SAMP8. Analysis of the periodogram uses a Q_p statistic that represents the degree to which each period is present in the data, after accounting for differences due to chance. Hourly periods between 8 and 30 h were examined using a bin size of 6 min. Free-running period (τ), which was not significantly different between ages, can be seen as peaks in the periodograms near 24-hours. The mean estimates of τ for the 2-, 7-, and 12-month SAMP8 were 23.56, 23.58, and 23.69 h, respectively.

main effects of day [$F(5,130) = 3.48$, $P < 0.01$], and age [$F(2,26) = 5.85$, $P < 0.001$] and a significant interaction between these two factors [$F(10,130) = 2.24$, $P < 0.05$]. Post-hoc comparisons between the three groups using Tukey's HSD showed that the increase in daytime activity from 2- to 7-months was significant ($P < 0.05$), as well as the decrease in activity from 7- to 12-months ($P < 0.01$).

Closer inspection of the actograms revealed that the large proportion of daytime activity that was found with the 7-month SAMP8 tended to be concentrated in the first few hours after lights on (see Fig. 4d). This finding is similar to that reported by Miyamoto (1997) [23], but contrasts with the typical pattern of locomotor activity observed with the 2-month old SAMP8 (see Fig. 4b). These observations suggested the possibility of split activity rhythms in some of the older SAMP8. In order to assess the presence and incidence of split activity rhythms in the three age groups, naïve observers were asked to rate actograms as consisting of two or four equally-spaced bands, corresponding to the "unsplit" and "split" conditions, respectively; see method sections for details of the rating procedure. Based on the ratings, naïve observers judged the 7 month and 12-month SAMP8 to be more split than the 2-month mice. For example, the mean ratings for the actograms of the 2-month old and 7-month old SAMP8 shown in Figs. 4b and 4d were 1.5 and 5.0, respectively. Overall, 66% and 50% of the 7- and 12-month SAMP8 were classified as split: (receiving mean ratings greater than 3.5), whereas 0% of the 2-month SAMP8 were similarly classified as split. A one-way ANOVA on the mean ratings demonstrated a main effect of age [$F(2,26) = 10.6$, $P < 0.001$]. Post-hoc comparisons between the three groups using Tukey's HSD revealed that the 7-month and 12-month old SAMP8 were more "split" than the 2-month old SAMP8 ($P < 0.01$), but that there were no significant differences in the perceptual classification of the two older age groups ($P > 0.65$).

Evidence of split activity rhythms was also provided by the appearance of a strong secondary peak in the perio-

Table 1
Individaul estimates of τ_{DD} for the 2-month, 7-month, and 12-month old SAMP8. Each estimate was obtained by determining the time of the peak Qp statistic in the Chi-square periodograms

2 Month		7 Month		12 Month	
Mouse	Tau (Hrs.)	Mouse	Tau (Hrs.)	Mouse	Tau (Hrs.)
y21	23.4	m11	23.8	o22	23.7
y23	23.2	m12	23.5	o23	23.8
y24	23.4	m13	23.7	o24	24.0
y31	23.6	m21	23.4	o31	23.7
y33	23.6	m23	23.5	o33	23.7
y34	23.8	m24	23.4	o34	23.7
y41	23.6	m32	23.7	o41	23.7
y42	23.6	m42	23.6	o44	23.8
y43	23.8	m44	23.6	o52	23.4
y44	23.6			o53	23.4
Mean	23.56	Mean	23.58	Mean	23.69
S.E.M.	0.06	S.E.M.	0.05	S.E.M.	0.06

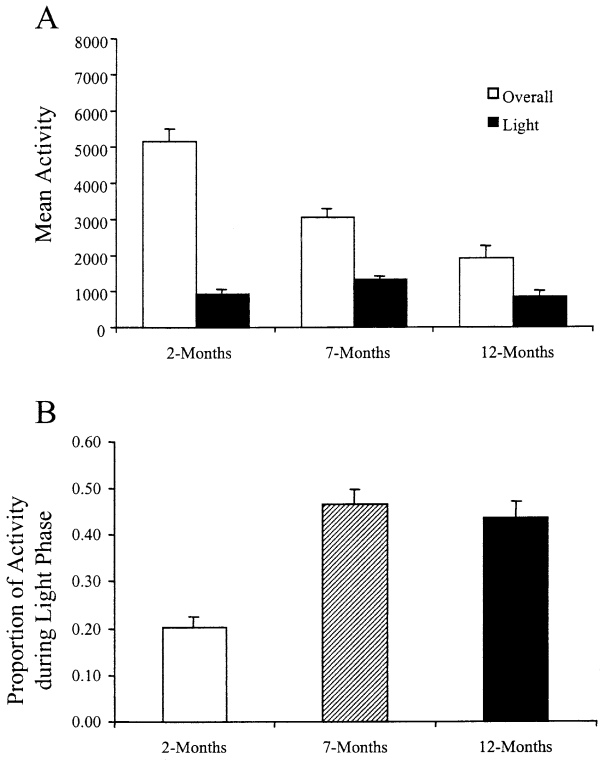

Fig. 3. Overall activity, daytime activity, and proportional daytime activity of the 2-, 7-, and 12-month SAMP8 during 12:12 light-dark conditions. (A) Overall activity was greatest for the 2-month group and decreased with age. In contrast, daytime activity increased from 2-months to 7-months, but then decreased for 7- to 12-months. (B) Proportional daytime activity was much higher for the 7- and 12-month groups than for the 2-month group. Total activity was assessed as the mean number of wheel revolutions in a 24-h period. Daytime activity was assessed as the mean number of wheel revolution for the 12 h when the lights were on. Dividing the daytime activity by the total activity assessed the proportion of activity that occurred during the daytime.

dograms of the 7-month and 12-month old mice in DD. The secondary peak (Q_{p12}) occurred at approximately half of the estimated $\tau_{DD,}$ and its amplitude was significantly correlated with the observer ratings of individual subjects; higher Q_{p12} values were associated with higher mean ratings, $r = 0.44$, $P < 0.01$. To further assess whether the incidence of split rhythms changed with age, we focused on the Q_p statistic for the secondary peak of the periodograms that occurred at approximately 12 h (Q_{p12}). A one-way ANOVA revealed a significant main effect of age, $F(2,26) = 3.35$, $P < 0.05$, with larger Q_{p12} values observed with older SAMP8. Post-hoc analyses of Q_{p12} using Tukey's HSD revealed significant differences between the 2-month old group and the two older age groups ($P < 0.01$), but no difference between the 7-month and 12-month old groups. The Q_{p12} metric also tended to be associated with a simultaneous decrease in the amplitude of the primary peak near 24 h. The overall strength of this correlation, however, was only marginally significant ($r = -0.27$, $P = 0.08$), and did not seem to suggest a correlated occurrence within age groups. Thus, changes in the amplitude of Q_{p24} and Q_{p12} may measure independent age-related phenomena: one related to a decrease in the amplitude of the rhythm with age and the other related to increased incidence of split activity rhythms.

4. Discussion

The present study examined age-related changes in circadian rhythms of SAMP8. Rhythms were assessed at 2-, 7-, and 12-month of age using run-wheel activity. Consistent with previous research, we observed an age-related decrease in the amount of wheel-running activity [1], an age-related decrease in the overall amplitude of τ_{DD} [1] and an age-related increase in the proportion of daytime activity [23]. However, we failed to replicate a lengthening of τ_{DD} in aged SAMP8 [1]. Most of the observed differences were between the 2- and 7-month old age groups. With the exception of rhythm amplitude, the activity rhythms of the 7- and 12-month old animals were quite similar.

The main focus of this study was to establish whether circadian rhythms are split in the SAMP8 as hinted at by some previous data, whether the incidence of split rhythms is increased or decreased with age, and the age at which split rhythms start to appear. The results from this study confirmed split activity rhythms in SAMP8, as evidenced by 1) the perceptua1 classification of the set of double plotted actograms by naïve observers, and 2) the appearance of a strong secondary peak in the periodograms at approximately half the value of τ_{DD}. Splitting was found to emerge at 7-months of age, with the incidence of splitting roughly constant between the 7- and 12-month age groups.

Split activity rhythms have been observed previously in various types of animals, but most research on this topic has involved hamsters [2,14,16,28,30]. For hamsters, which are nocturnal like mice, split activity rhythms emerge spontaneously under conditions of constant light [25,30]. Age reduces the incidence of spontaneous and dark-pulse induced splitting of the circadian rhythm in hamsters [10,25]. Results of the present study found the opposite, i.e. increased incidence of split rhythms in aged SAMP8 as compared to young SAMP8. Discrepancies between our findings and those in previous studies may be due to several factors including species differences of the subjects (mouse vs. hamster) and constant condition (dark vs. light).

Another possible reason for the difference is that the split activity rhythms observed with the SAMP8 may not represent the same type of splitting typically observed with hamsters. Rhythm splitting in hamsters is evidenced by an initial transitory phase where the two components of the rhythm initial free-run with distinct periods before becoming "locked" in anti-phase. With the SAMP8, no such transitory period was evident, leaving at least two potential explanations of the split activity rhythms: [1] there are two independent periodic components underlying the split rhythms, each with approximate 24-h rhythms and running in anti-phase or [2] there is a single component with an

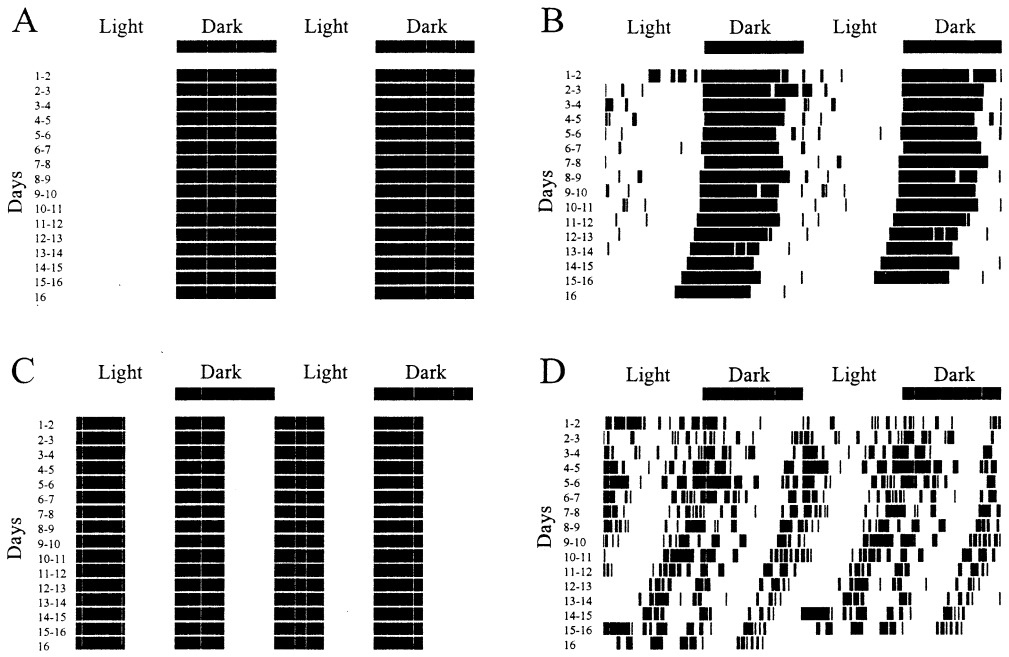

Fig. 4. Double plotted actograms of a mouse with an unsplit rhythm and a mouse with a split rhythm. During days 1–6, mice were subjected to a 12:12 h light-dark cycle. During days 7–16, mice were located in a constant dark environment. Naïve human observers were asked to make perceptual judgments of actograms and rate actograms as consisting of two or four bands. An ideal actogram with 2 equally spaced bouts of activity (A) and another with four equally spaced bouts of activity (C) were given to observers as guides. These ideal actograms represented unsplit (two bands) and split (four bands) rhythms, although observers were not given any interpretations of the actograms. The double-plotted actogram from a 2-month SAMP8 (B) with an unsplit activity rhythm shows a high amount of activity during each of the two dark phases. The fact that the onset of activity starts at approximately the same time during days 1–6 demonstrates that mice were entrained to the light-dark cycle. The gradual phase-advance during days 7–16 shows that the free-running period was less than 24 h. The double plotted actogram from a 7-month SAMP8 (D) shows, in contrast, four distinct bouts of activity rather than two. The four bouts of activity were approximately equally spaced demonstrating a split activity rhythm with the two components approximately 12 h apart. This actogram also illustrates the high degree of fragmentation that was observed in the 7- and 12-month SAMP8. The activity bands generally are synchronized with the lights on and lights off during days 1–6 demonstrating entrainment with the 12:12 light-dark cycle. Similar to the 2-month mice, the activity bouts gradually phase-advance during days 7–16 showing that this mouse had a free-running period less than 24 h.

approximate 12-h rhythm. If age-related changes in the circadian system of the SAMP8 lead to a *split condition*, then we may have simply missed any transitory phase because of the ages examined in this study. Indeed, the 7- and 12-month SAMP8 that were classified as "split" appeared to show split-like rhythms during the first six days of LD in addition to during DD, making it difficult to behaviorally distinguish these two possibilities within the present framework. We therefore refer to "split" rhythms in quotations to reflect the possibility that we may be describe a phenomena that is different from that described for hamsters. Nonetheless, the present data are at least consistent with a two-component explanation.

In the two-component explanation of splitting, there are two mutually coupled oscillators with two stable modes of entrainment [30]. The two oscillators are referred to as the E and M oscillators, respectively, with the E oscillator controlling the onset of activity in the evening and the M oscillator controlling the offset of activity in the morning. Normally, the E and M oscillators (each with slightly different periods) are mutually entrained with the M oscillator lagging the E oscillator by several hours. Under the right conditions, detuning of the oscillators occurs, and the E and M oscillators split, first free-running independently, before re-entraining with a 180° phase relationship and a slightly shorter composite free-running period (τ).

For hamsters, there is some physiological evidence to suggest that the two putative oscillators in the coupled-oscillator model may be functionally localized to the left and right hemispheres of the SCN. First, unilateral SCN lesions in split hamsters abolish the splitting, and produce a single bout of locomotion [7]. Second, cyclic expression of the *Per1* gene in the SCN is asymmetrically expressed in split hamsters, but not in un-split hamsters [9]. Of potential interest, then, in trying to distinguish the two-component and one-component explanations of "split" rhythms in the SAMP8 is whether for SAMP8, there are age-related differences in the cyclical expression of *Per1* in the SCN of the SAMP8. Asymmetrical expression of Per1 in the SAMP8 for split animals, but not for unsplit animals would support a two-oscillator explanation of splitting in the SAMP8.

A second issue that arises in this study is whether the reported age-related disruptions of circadian rhythms are accelerated in SAMP8 in comparison to a resistant line of SAM, such as SAMR1. Because the focus of the present study was the phenomenon of splitting in SAMP8 only, it

is not presently know whether the reported age-related increases in split activity rhythms are specific to the SAMP8 or may be a more general finding in SAM mouse lines or even other strains or species. A number of age-related brain and behavioral changes have been documented for several SAM lines that have some bearing on this question [11,12, 13,17,20,24,26,27,45,46,47,48,57]. Based on these studies, it is a matter of debate whether behavioral disturbances observed in the SAMP8 circadian rhythm are due to accelerating aging of the SCN clock. Few differences between SAMP8 and SAMR1 strains have been reported for the SCN [38]. Neither overall size of the SCN, nor the number of arginine-vasopressin (AVP) containing neurons in the SCN, varied significantly between 10-month old SAMP8 and SAMR1. Overall, these findings suggest age-related changes in circadian timing may not be accelerated in SAMP8 compared to SAMR1, although further work is needed to directly address this question.

One discrepancy between the present study and previous research is that we did not find a significant lengthening of free running τ with age, as has been previously reported. At least three possible reasons may account for the failure to replicate the age-related changes of $\tau_{DD.}$ First, the effect of age on τ_{DD} may be small. Indeed, a trend was observed for a lengthening of τ_{DD} with age, but this observation was not statistically significant. Previous studies in SAMP8 show a relatively small effect of age on $\tau_{DD,}$ on the order of 0.3 h [1]. Second, age-related alterations in free running τ may only appear after an extended time in constant conditions. In one study reporting age-related changes in $\tau_{DD,}$ differences only appeared after several months in constant darkness [8]. Finally, according to a two-oscillator explanation of "splitting," free running τ in the split condition should be shorter than in the unsplit condition, which given the age-related increase in splitting observed with the SAMP8 might partially counteract an independent age effect that lengthened τ. An exploratory t test between the τ values for mice classified as "split" and "unsplit" did not show a significant difference ($P > 0.3$), although this is perhaps not surprising given the small n and variability of τ. To carefully address this possibility would require a longitudinal, rather than a cross-sectional design, with each mouse serving as it's own control (monitored both in an unsplit and split condition).

In summary, the present study found evidence for increased "split" activity of the circadian rhythms of older SAMP8. The "splitting" occurred between 2- and 7-months of age with no further changes between 7- and 12 months of age other than overall activity/rhythm amplitude. This result contrasts with those in hamsters that have found a decreased incidence of splitting with age. Additionally, the present study replicates most of the findings of previous studies on age-related changes in the circadian rhythms of SAMP8. The types of disruptions in circadian rhythm that we and others have found for SAMP8 are similar to those reported in aged humans and patients with senile dementia [39,50]. Normal aged and senile subjects have reduced amplitudes of rhythms of a number of physiological and behavioral measures (see reference [50] for a review) and increased fragmentation of sleep patterns [32,33]. Although we did not measure sleep in the present study, the actograms of the aged SAMP8 were clearly fragmented in the normally inactive (light) time of the day, suggesting a disrupted sleep. Aged humans, like our aged SAMP8, have higher levels of activity during the normally inactive period [52]. In conclusion, the present findings provide further support for the use of the SAMP8 as a model of circadian rhythm disruptions associated with normal aging and senile dementia in humans.

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