ELSEVIER

Neurobiology of Aging 25 (2004) 111–123

NEUROBIOLOGY OF AGING

www.elsevier.com/locate/neuaging

Circadian rhythms in SAMP8: a longitudinal study of the effects of age and experience

Kevin C.H. Pang*, Jonathan P. Miller, J. Devin McAuley

Department of Psychology, J.P. Scott Center for Neuroscience, Mind, and Behavior, Bowling Green State University, Bowling Green, OH 43403, USA

Received 13 August 2002; received in revised form 6 January 2003; accepted 7 February 2003

Abstract

Age-related effects on circadian rhythms include reductions of rhythm amplitude, alterations in re-entrainment, and increased fragmentation. Currently, the pattern of these changes across an individuals' lifespan is unknown. The present study used a cross-sequential experimental design to determine the pattern of circadian rhythm changes, identify predictors of later circadian rhythm disruption, and assess the effect of prior run-wheel experience on circadian rhythms. Run-wheel activity was assessed in senescence-accelerated mice (SAMP8) at 2, 7, and 12 months of age. Age-related changes included decrease of run-wheel activity, decrease in circadian rhythm amplitude, increase in proportion of light activity, and increase in split activity rhythms. Proportion of light activity at 2 months was a good predictor of circadian rhythm disruption at 7 months. Run-wheel experience increased overall activity and decreased proportion of light activity, but did not alter rhythm amplitude or period. These results demonstrate that aging produces several patterns of circadian rhythm changes, describe predictive measures of future rhythm disruptions, and suggest an intervention to reduce circadian rhythm disruptions.

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

1. Introduction

Age-related disruptions of circadian rhythms are a common occurrence in many species. In elderly humans, rhythm disturbances include altered sleep–wake patterns, weak coupling with environmental rhythms, reduced daytime cognitive performance, and increased nighttime activity [27,29,31]. Similar behavioral changes occur in aged animals [25,33]. These changes in circadian rhythms may, at least in part, contribute to the cognitive and physical impairments observed in aged individuals [1].

The senescence accelerated mouse line (SAM) developed by Takeda and associates is one of several animal models of aging [23,24]. Mice of this line were selectively bred for differences in a senescence score [9], lifespan and pathologic phenotypes [22]. Currently, sub-lines of SAM differ in the rate of aging for various phenotypes. The P8 sub-line (SAMP8) has been investigated for its early onset of age-related disruptions of learning, memory and circadian rhythms [22]. The SAMP8 are frequently compared to the SAMR1, a sub-line that was selected for resistance to age-related disruptions.

In SAMP8, age-related changes in circadian rhythms include a reduction in amplitude and increased fragmentation [2,10–12,18,20,22]. These age-related changes are characteristic of those observed in the aging process of other animals, including humans [4,15,30,32,33]. Furthermore, these changes of circadian rhythms are accelerated in SAMP8 as compared to SAMR1 [2,11,12,18]. With the SAMP8, a lengthened free-running period has also been reported, but the evidence for this is mixed [2,10,18]. An age-induced lengthening of free-running period differs from that observed with some other animals. For instance, rodents such as hamster, rat and deer–mice show a shortening of their free-running period with age rather than a lengthening [13,14,17,26].

Some aspects of activity rhythms in SAMP8 appear to be unusual regardless of age. Recently, we reported that some older SAMP8 produce split activity rhythms [10]. This splitting of activity rhythms resulted in a secondary peak of activity at approximately 12 h intervals and decreased the amplitude of the primary peak at approximately 24 h. SAMP8 also show a faster rate of re-entrainment following a phase advance, an overall higher level of locomotor

* Corresponding author. Tel.: +1-419-372-6013; fax: +1-419-372-6013.
E-mail address: kpang@bgnet.bgsu.edu (K.C.H. Pang).

0197-4580/$ – see front matter © 2003 Elsevier Science Inc. All rights reserved.
doi:10.1016/S0197-4580(03)00029-0

activity, and a higher proportion of activity during the normally inactive light phase when compared to age-matched SAMR1 [11,12,18]. Disruptions of this sort in the normal pattern of rodent locomotor activity are reminiscent of various rhythm disorders associated with human aging. For example, patients with senile dementia in particular, show excessive levels of nighttime activity, in relation to healthy elderly of the same age [19,29].

To date, most studies of the effects of aging on circadian rhythms have involved cross-sectional experimental designs. Although cross-sectional designs are useful for investigating the effects of age of a population of animals, they are not designed to assess patterns of change that occur across the lifespan of an individual. Longitudinal designs, thus, make it possible to determine whether or not the aging process produces consistent alterations in circadian rhythms, which might be masked by between-group variability in a cross-sectional design. Additionally, longitudinal assessments may help to identify measures in young subjects that predict the early onset of circadian rhythm disruptions. These predictive measures may be useful for developing early interventions that are designed to prevent or delay age-related disruptions of circadian rhythms.

The few longitudinal animal studies that have examined age-related changes of circadian rhythms have used hamsters [5,7,13]. In these studies, general activity levels and the incidence of rhythm splitting decreased with age. These studies differed in their findings with respect to free-running period, with two studies reporting no change [5,7] and one concluding a shortening of the free-running period with age [13]. To our knowledge, longitudinal studies using species other than the hamster have not been reported. Such studies, however, are necessary to determine the generality of the age-related effects observed in hamsters.

The SAMP8 provides an excellent animal model for a longitudinal assessment of circadian rhythms because these mice age rapidly, requiring a relatively short study length, and demonstrate clear changes in circadian rhythm measures that are characteristic of those frequently considered part of the normal aging process in animals, including humans [2,10,18]. The present study investigated the circadian rhythms of wheel-running activity in SAMP8 using a cross-sequential design. A cross-sequential design combines both cross-sectional and longitudinal components. Circadian rhythms were initially assessed in three groups of naïve mice that were ages 2, 7, and 12 months. The mice initially tested at 2 months were then re-tested at 7 and 12 months, and the mice initially tested at 7 months were then re-tested at 12 months, forming the longitudinal component of the study. This design permitted both an assessment of circadian rhythm changes across the lifespan of the SAMP8 and an assessment of the impact of run-wheel experience early in life on circadian rhythm disruptions later in life. The effect of run-wheel experience was examined primarily by comparing the circadian rhythms of the naïve 12-month-old SAMP8 with those that had been tested previously once (at 7 months) or twice (at 2 and 7 months).

2. Method

2.1. Animals

Twenty-five male SAMP8 were subjects for the study; eight were tested at 2, 7, and 12 months of age, six were tested at 7 and 12 months of age, and eleven were tested at only 12 months of age. 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 12:12 h light–dark cycle (lights on at 7:30 a.m.). All procedures followed the NIH guidelines for handling and caring for animals and were approved by the Bowling Green State University Animal Care & Use Committee.

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 cm × 12.5 cm × 12.5 cm) was attached to each wheel, containing food and water. Mice were able to move freely between the cage and the wheel. An IBM compatible computer with custom designed software recorded the activity of each wheel by recording the time of each wheel revolution. A magnet attached to the wheel's axis of rotation closed a switch, signaling to the computer that a wheel revolution occurred.

2.3. Procedure

During testing, wheel-running activity was monitored for 16 days. For the first 6 days, mice were monitored on a 12:12 h light–dark (LD) cycle to determine a baseline measure of the entrained wheel-running rhythm (τ_{LD}). The onset and offset of light during LD coincided with the light–dark cycle of the animal colony room. For the remaining 10 days, mice were kept in constant darkness (DD) to estimate the period of their free-running rhythms (τ_{DD}).

2.4. Data analysis

Eight SAMP8 were examined at 2, 7, and 12 months of age and comprise the subject pool for analysis of age-related changes in circadian rhythms. To examine the effects of run-wheel experience on circadian rhythms, 12-month SAMP8 were examined after 0 (tested at 12 months of age), 1 (tested at 7 and 12 months) or 2 (tested at 2, 7, and 12 months) prior experiences in the running wheels.

Overall daily activity was assessed by obtaining the number of wheel revolutions in a 24-h period for the sixteen days of the study. The amount of activity during the light phase (light activity) and dark phase (dark activity) of the light–dark cycle was also determined. To consider possible age-related changes in the proportion of light activity, the number of wheel revolutions during the light phase was divided by the total number of wheel revolutions for the each of the 6 days of LD.

To assess activity rhythms, double-plotted actograms showing the pattern 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 the "binned" activity (6 min bins) of the first 6 days under LD conditions and the subsequent ten days under DD conditions, respectively [21]. A Q_P statistic was calculated for hourly periods between 8 and 30 h. The Q_P statistic represents the degree to which each period is present in the data, after accounting for differences due to chance. As with previous research, the period associated with the largest value of Q_P provides an estimate of τ, the true circadian period [16].

To determine whether an animal's circadian rhythm was split, 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 two-banded and 4-banded double-plotted actograms to serve as guides. No information was provided to the observers about the graphs or the purpose of their classification; observers were only instructed to make simple perceptual judgments about 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 was presented to observers twice, each time in a random order with one set presented before the other set. The first set of ratings was treated as practice, with the second set of ratings used for the analysis. In a previous study, ratings by naïve observers correlated with the appearance of a secondary peak in the chi-square periodograms; both measures providing independent confirmation of split circadian rhythms [10].

The statistical analyses were conducted using SPSS for Windows (Version 10.1, SPSS, Inc., Chicago, IL). Analysis of variance (ANOVA) was used to assess differences in overall activity, light activity, proportion of light activity, τ_{LD}, τ_{DD}, and Q_P. For the ANOVA on proportion of light activity, an arc sine transformation was first applied to the data in order to correct for possible violation of the assumption of normality. Post hoc analyses of effects of age (a within-subjects variable) involved paired contrasts between data obtained at 2 and 7 months, 7 and 12 months, and 2 and 12 months. All pair-wise post hoc analyses of effects of run-wheel experience (a between-subjects variable) used Tukey's HSD correction. All statistical tests were evaluated with $\alpha = 0.05$.

Correlations matrices were constructed for light activity, dark activity, free-running activity during constant dark conditions, proportion of light activity, observer ratings of actograms, τ_{DD}, maximum Q_P near 24 h (Q_{P24}), and maximum Q_P near 12 h (Q_{P12}) at 2, 7, and 12 months in order to determine the measures at 2 months that best predict rhythm disruptions later in life. Correlations were calculated using Excel 2000 (Microsoft Corp., Redmond, WA). Based on eight subjects and $\alpha = 0.05$ (two-tailed), the critical value of Pearson's r used to determine statistical significance was 0.7067.

3. Results

3.1. Effect of age

3.1.1. General activity

For the eight mice tested at all three time points (2, 7, and 12 months), the overall run-wheel activity was influenced by age and session day. An age (2, 7, and 12 months) × days (1–16) repeated measures ANOVA was performed on the number of daily wheel revolutions. An age-related decline in overall run-wheel activity was observed, as demonstrated by a main effect of age, $F(2, 12) = 32.3$, $P < 0.001$ (Fig. 1). Post hoc analyses revealed that activity at 2, 7, and 12 months were all significantly different from each other. Run-wheel activity was highest at 2 months, intermediate at 7 months and lowest at 12 months.

Activity varied across the 16-day experiment. After an initial decrease in activity from day 1 to day 2 (presumably because of the relative novelty of being in the run-wheel on day 1), the amount of run-wheel activity increased until approximately day 6 and then remained relatively stable for the remaining days (Fig. 1). A main effect of day was observed, $F(15, 90) = 10.5$, $P < 0.001$, as was an age × day interaction, $F(30, 180) = 1.84$, $P < 0.01$. Changes in activity across days were present at 2 and 7 months, but not at 12 months (Fig. 1). At 12 months, the amount of run-wheel activity did not show a consistent trend over the course of the experiment.

Consistent with our previous cross-sectional study, the proportion of activity that occurred during the normally inactive light phase (a measure of rhythm disruption) increased with age as observed by a main effect of age, $F(2, 14) = 9.0$, $P < 0.01$ and an age × day interaction, $F(10, 70) = 1.98$, $P < 0.05$ (Fig. 2). Post hoc analyses revealed proportionally more light activity at 7 and 12 months compared to 2 months. No differences were observed between 7 and 12 months. In contrast to our previous cross-sectional study, the absolute amount of light activity was similar at 2, 7, and 12 months, $F(2, 14) = 0.35$, $P > 0.7$, suggesting that the age-related changes in proportion of light activity was due

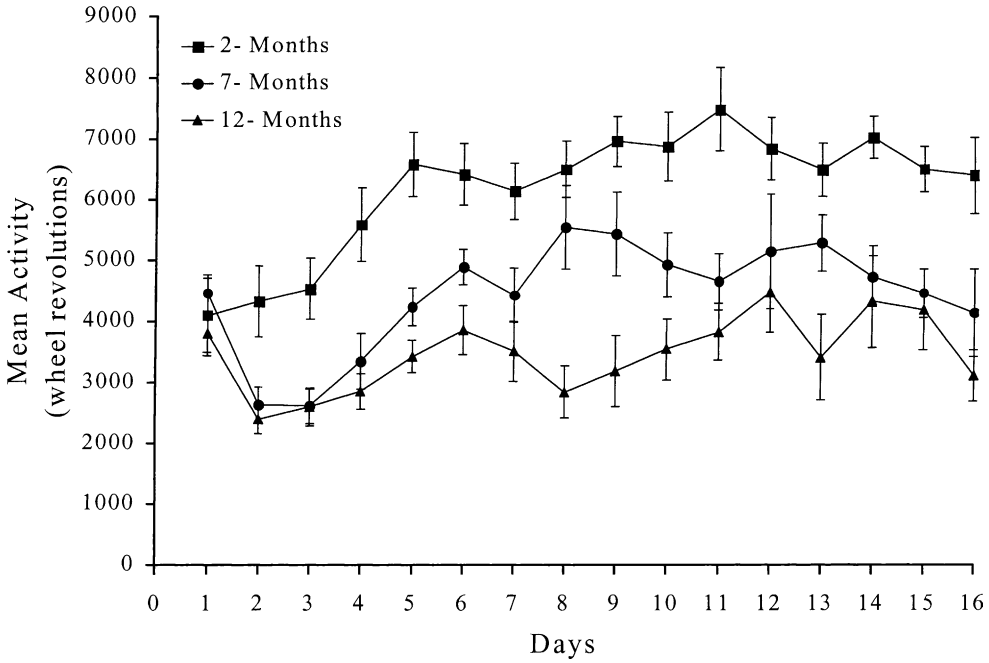


Fig. 1. Time course of daily activity for SAMP8 tested at all three ages (2, 7, and 12 months). Activity is expressed as the number of revolutions of a run-wheel. Overall activity increased as a function of days and decreased with age. Activity increased across days at 2 and 7 months, but not at 12 months. 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.

to a reduction in overall daily activity and not to increased light activity.

3.1.2. Circadian rhythms

Visual inspection of the actograms revealed different age-related activity patterns. In some mice, wheel-running rhythms were relatively intact at 12 months (Fig. 3A), while rhythms were fragmented at 12 months in other mice (Fig. 3B). Rhythm fragmentation took on two forms: (1) general disruption of activity patterns without a noticeable rhythm and (2) split rhythms consisting of activity around the subjective day–night transition and another bout of activity around the subjective night–day transition. It was interesting to note that during the DD condition (days 7–16) some mice progressed from a stable rhythm at 2 months (Fig. 3A and B) to generally disrupted activity rhythms at 7 months (Fig. 3A and B) back to a more stable rhythm at 12 months that was similar to the rhythm at 2 months (Fig. 3A) or was split (Fig. 3B). Other mice showed a more uniform decline in rhythmicity across the lifespan (not shown).

Effects of age on entrained and free-running circadian rhythm amplitude and period were examined by constructing chi-square periodograms and through visual inspection of the double-plotted actograms. Mean τ_{LD} values at 2, 7, and 12 months (based on periodograms constructed for LD only) were 23.95 ± 0.03, 23.95 ± 0.04 and 23.90 ± 0.05 (mean $\pm$ standard error). The differences were within the margin of error expected for a 6-min bin size and were not significantly different from one another, $F(2, 14) = 0.538$, $P > 0.5$. During DD conditions, free-running circadian periods were generally less than 24 h (Fig. 4). Mean values of τ_{DD} at 2, 7, and 12 months were 23.54 ± 0.07, 23.64 ± 0.09, and $23.68 \pm .10$. No consistent age differences were observed for τ_{DD}, $F(2, 14) = 0.708$, $P > 0.5$. However, individual estimates of τ_{DD} were variable, especially at 12 months, with the range almost doubling from 0.5 at 2 months to 0.9 at 12 months.

Although the period of circadian rhythms did not change with age, a consistent reduction of the circadian rhythm amplitude during DD conditions was observed. Mean values of Q_{P24} (the maximum value of Q_P for periods near 24 h) at 2, 7, and 12 months of age were 1413 ± 141, 791 ± 78, and 768 ± 100 (mean $\pm$ standard error). Q_{P24} decreased significantly with age, $F(2, 14) = 10.85$, $P < 0.001$ (Fig. 4). Post hoc analyses determined that Q_{P24} was similar at 7 and 12 months with both being lower than at 2 months.

The incidence of split activity rhythms at 2, 7, and 12 months was assessed using two independent measures: naïve observer ratings of actograms and amplitude of Q_{P12} (the maximum value of Q_P for periods around 12 h). Naïve observers were asked to rate the actograms as having two or four equally-spaced bands, corresponding to the "unsplit" and "split" conditions, respectively. Ratings of actograms were significantly different across ages, $F(2, 12) = 12.1$, $P < 0.001$. Post hoc analyses found that ratings of actograms obtained at 2 months were significantly lower than ratings at 7 and 12 months, while ratings at 7 and 12 months were not significantly different. This pattern supports the view that the incidence of rhythm splitting increases between 2 and 7 months and then remains relatively constant between 7 and

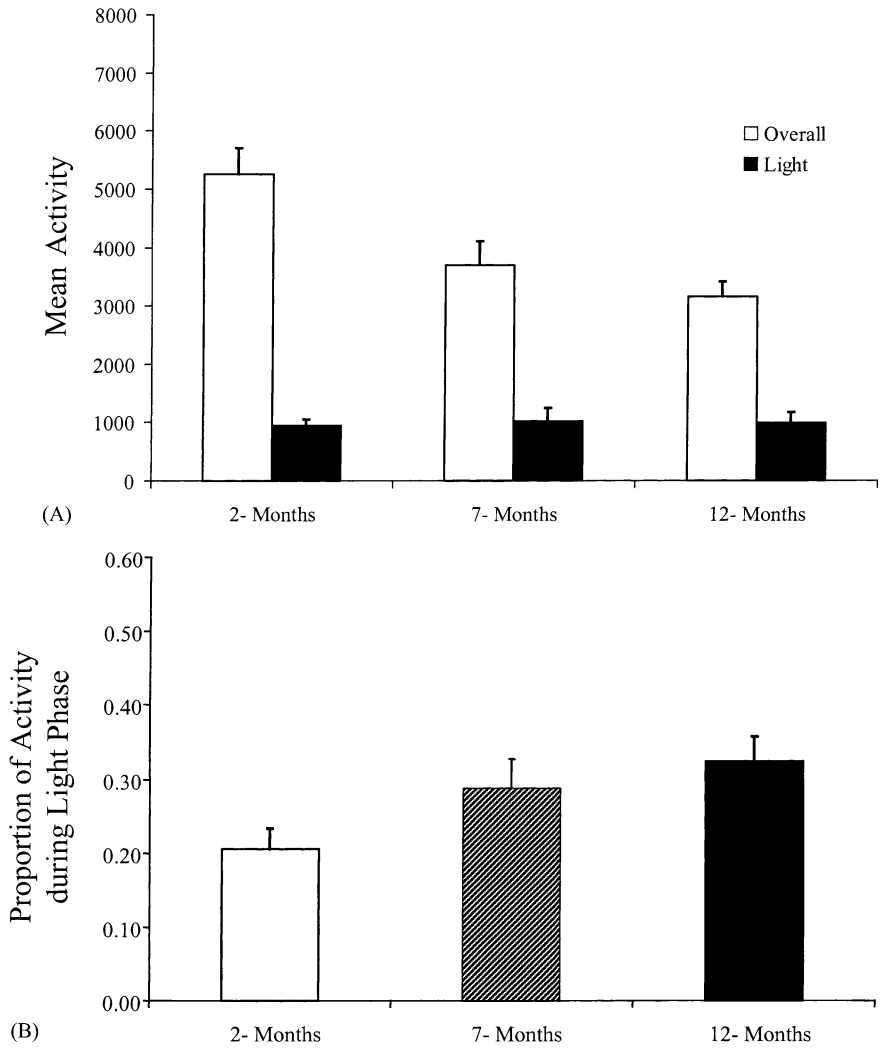


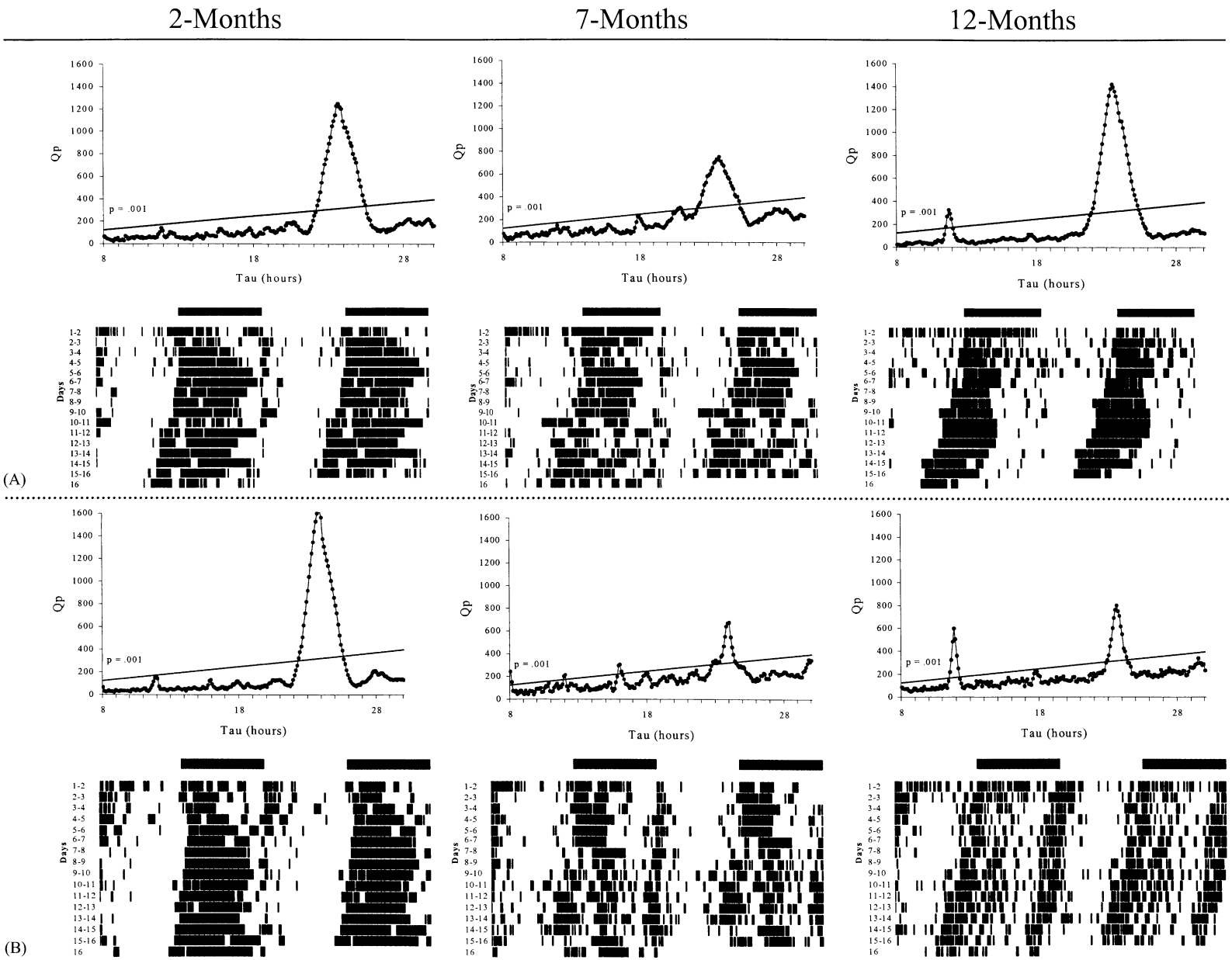
Fig. 2. Overall activity and activity during the light phase at 2, 7, and 12 months. Data were obtained during 12:12 h light–dark conditions. Overall run-wheel activity decreased with age, but the *amount* of activity during the light phase did not change (A). *Proportion* of activity during the light phase increased with age (B), primarily reflecting the decrease in overall activity. Overall activity was assessed as the mean number of wheel revolutions in a 24-h period; activity during the light phase was the mean number of wheel revolution for the 12 h when the lights were on. Activity during the light phase divided by the overall activity was used to determine the proportion of activity during the light phase.

12 months. Perhaps the most striking aspect of the rating data was the difference in the range of values at 2, 7, and 12 months. Mean ratings for individual mice ranged from 1.0 to 2.8 at 2 months, from 1.7 to 4.7 at 7 months, and from 1.4 to 5.0 at 12 months. None of the actograms obtained at 2 months were classified as "split" (rating ≥ 3.5), whereas 28.6 and 42.9% of the actograms from the same animals were classified as split at 7 and 12 months, respectively.

Evidence that aging increased the incidence of split activity rhythms was also provided by the appearance of a strong secondary peak in the periodograms during DD. The secondary peak occurred at approximately half the estimated τ_{DD}, and the amplitude of the secondary peak (Q_{P12}) was significantly enhanced with increasing age, $F(2, 14) = 3.96$, $P < 0.05$ (Fig. 4). Post hoc analyses revealed similar Q_{P12} at 2 and 7 months, while Q_{P12} at 2 and 12 months were significantly different and were marginally different at 7 and 12 months ($P = 0.056$). In fact, Q_{P12} was significantly correlated with the observer ratings of actograms; higher Q_{P12} values were associated with higher mean ratings, $r = 0.63$, $P < 0.001$.

3.2. Predictors of circadian disruption

Given the disruptions in circadian rhythm at 7 and 12 months, the longitudinal component of the experimental design allowed identification of measures at 2 months that predict circadian rhythm at 7 or 12 months (Table 1). The following measures were examined: proportion of light activity, observer ratings of actograms, maximum Q_P near 24 h (Q_{P24}), light activity, dark activity, free-running activity during constant dark conditions, and τ_{DD}. Overall, the


2-Months
7-Months
12-Months
(A)
(B)
Qp
Tau (hours)
p = .001
Days

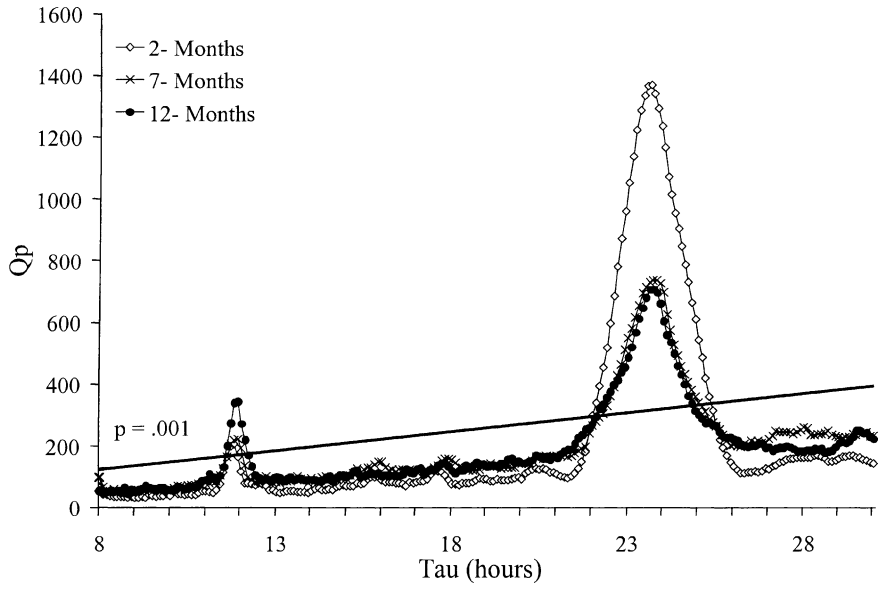


Fig. 4. Average chi-square periodograms for the eight SAMP8 mice that were examined at 2, 7, and 12 months. Periodograms were constructed from data obtained during constant dark conditions. Each point represents the mean Q_P value of all eight SAMP8 mice. The maximum Q_P was near 24 h (23.56, 23.58, and 23.69 h for 2, 7, and 12 months, respectively). The maximal Q_P decreased with age, the largest decline occurring between 2 and 7 months. A secondary Q_P peak near 12 h increased with age. This represents an increase of rhythm splitting that occurred with age. The slowly rising straight line represents the $P = 0.001$ statistical level.

Table 1
Correlation of circadian rhythm and activity measures at different ages

	2 months						
	Proportion of light activity	Observer ratings	Q_{P24}	Light activity	Dark activity	Free-running activity	τ_{DD}
7 months							
Proportion of light activity	0.338	0.409	−0.270	0.270	−0.288	−0.385	0.481
Observer ratings	0.742*	0.694	−0.588	0.802*	−0.681	−0.768*	0.071
Q_{P24}	−0.723*	−0.495	0.286	−0.632	0.836**	0.486	−0.161
Light activity	0.038	0.325	−0.381	0.038	0.062	−0.195	0.212
Dark activity	−0.563	−0.300	0.140	−0.425	0.560	0.469	−0.799*
Free-running activity	−0.929**	−0.464	0.298	−0.804*	0.966**	0.690	−0.597
τ_{DD}	0.641	−0.053	−0.509	0.728*	−0.458	−0.385	0.370
12 months							
Proportion of light activity	0.895**	0.306	−0.683	0.948**	−0.745*	−0.595	0.479
Observer ratings	0.535	0.247	−0.414	0.662	−0.372	−0.460	0.234
Q_{P24}	0.257	−0.102	−0.172	0.157	−0.285	−0.077	0.252
Light activity	0.184	0.072	−0.397	0.310	−0.015	0.067	0.047
Dark activity	−0.910**	−0.321	0.636	−0.921**	0.838**	0.566	−0.359
Free-running activity	0.028	0.270	−0.311	0.002	−0.092	−0.205	−0.408
τ_{DD}	−0.174	0.126	−0.340	0.053	0.338	−0.109	−0.590

* Correlation is significant at the 0.05 level (two-tailed).
** Correlation is significant at the 0.01 level (two-tailed).

Fig. 3. Representative chi-square periodograms and double plotted actograms for two SAMP8 mice. 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 (DD). Periodograms (top of A and B) were constructed from data obtained during DD. Analysis of the periodogram used 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. The mean estimates of free-running period (τ_{DD}) for 2-, 7-, and 12-month SAMP8 were 23.56, 23.58, and 23.69 h, respectively, and were not significantly different across ages. Actograms representing a mouse with unsplit rhythm (A) and a mouse with a split rhythm at 12 months (B) are shown (bottom of A and B). The double-plotted actograms at 2 months show unsplit activity rhythms with high amounts 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. Solid bars above the actograms represent the dark phase for days 1–6. The gradual phase-advance during days 7–16 shows that the free-running period was less than 24 h. At 7 months, both mice show disruption of the circadian rhythm during DD. At 12 months, one mouse had unsplit rhythms (i.e. two bands, A), while the other mouse had split activity rhythms (i.e. four bands, A).

best predictor for future circadian rhythm disruption was proportion of light activity at 2 months. Proportion of light activity at 2 months was positively correlated with observer ratings of actograms ($r = +0.742$), and negatively correlated with Q_{P24} ($r = -0.723$) and free-running activity ($r = -0.929$) at 7 months of age. The positive correlation with observer ratings and negative correlation with Q_{P24} suggest that proportion of light activity at 2 months may be a good predictor of circadian rhythm disruption at 7 months. It is noteworthy that proportion of light activity at 2 months was not significantly correlated with observer ratings or Q_{P24} at 2 months, suggesting that ratings of actograms and Q_{P24} are not necessarily tightly correlated with proportion of light activity. Proportion of light activity at 2 months was also positively correlated with proportion of light activity ($r = +0.895$) and negatively correlated with amount of dark activity ($r = -0.910$) at 12 months.

Free-running activity at 2 months was negatively correlated with observer ratings of actograms at 7 months ($r = -0.768$). This correlation might be expected because free-running activity is inversely related to proportion of light activity. In addition, proportion of light is intimately related to both amount of light activity and amount of dark activity. These two measures were therefore examined for possible correlations. Amount of light activity at 2 months was positively correlated with observer ratings of actograms ($r = +0.802$) and τ_{DD} ($r = +0.728$), and negatively correlated with free-running activity ($r = -0.804$) at 7 months. Amount of light activity at 2 months was also positively correlated with proportion of light activity ($r = +0.948$) and negatively correlated with amount of dark activity ($r = -0.921$) at 12 months. Amount of dark activity at 2 months was positively correlated with Q_{P24} ($r = +0.836$) and free-running activity ($r = +0.966$) at 7 months, and negatively correlated with proportion of light activity at 12 months ($r = -0.745$). Thus, amount of activity during both light and dark phases predicted circadian rhythm disruptions later in life with the best predictions for 7 months of age. Furthermore, high light activity at 2 months predicted *greater* circadian rhythm disruptions later in life, whereas high dark activity predicted *less* circadian rhythm disruptions in later life.

3.3. Effects of run-wheel experience

3.3.1. General activity

In order to examine the effect that run-wheel experience might have on our results, we examined three groups of 12-month SAMP8 differing in the amount of prior run-wheel experience (0 previous exposures, 1 previous exposures, and 2 previous exposures). A mixed design ANOVA was performed on overall activity with experience as a between group factor and days (1–16) as a within subject factor. Previous experience in the run-wheel increased the overall amount of run-wheel activity, as demonstrated by a significant main effect of run-wheel experience, $F(2, 21) = 14.29$, $P < 0.001$ (Fig. 5). Post hoc analyses found that overall activity was least for mice without any previous experience, while activity was similar for mice with 1 and 2 prior

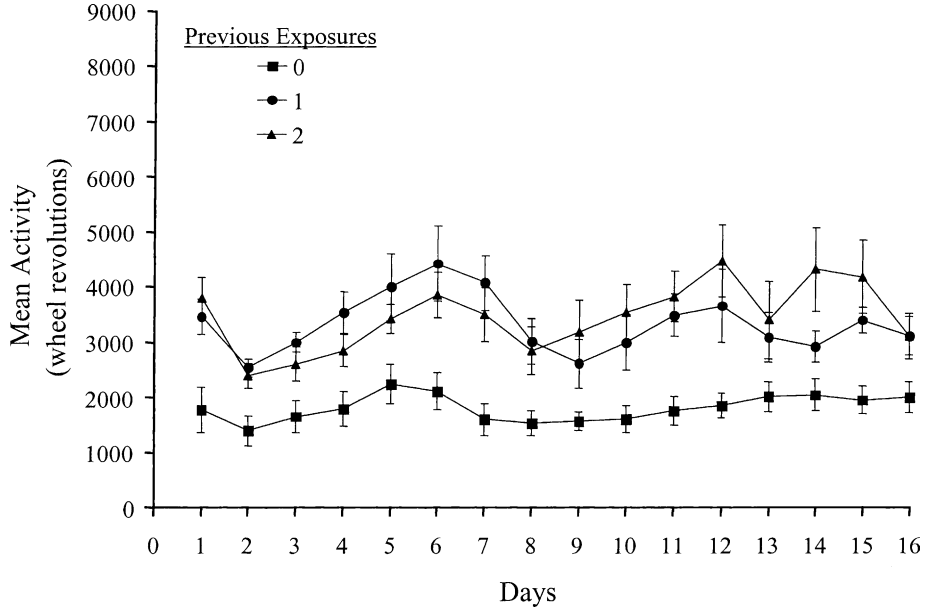


Fig. 5. Daily activity as a function of days and number of previous exposures to the run-wheel. To determine whether experience in the run-wheel influenced overall activity, the number of daily wheel revolutions were examined in 12-month SAMP8 with different 0, 1 or 2 previous experiences. Previous exposures to the run-wheel (1 or 2 prior exposures) enhanced the level of activity compared to naïve mice. The activity of experienced 12-month mice approached the activity levels of 2-month mice (see Fig. 1). Note that the 12-month SAMP8 group in Fig. 1 is identical to the 12-month SAMP8 with two previous experiences in this figure.

experiences in the run-wheel. Overall activity also varied across the session day, as noted by a significant main effect of day, $F(15, 315) = 4.67$, $P < 0.01$. Finally, previous experience interacted significantly with day, $F(30, 315) = 1.52$, $P < 0.05$. The two main effects were still significant when the Greenhouse-Geiser procedure was used to correct for a violation in the assumption of sphericity, but the interaction was not significant.

Previous run-wheel experience also served to influence the overall proportion of light activity, $F(1, 22) = 157.81$, $P < 0.001$ (Fig. 6). The mean proportion of light activity for mice with 0, 1, and 2 previous exposures was 0.40 ± 0.04, 0.24 ± 0.05, and 0.29 ± 0.04, respectively. Post hoc analyses revealed that the proportion of light activity was higher for inexperienced mice than experienced mice, while no differences were observed between mice with 1 and 2 prior exposures to the run-wheel.

3.3.2. Circadian rhythms

Although previous run-wheel experience increased overall activity and decreased the proportion of light activity, prior experience did not alter the estimated amplitude or period of the free-running rhythms (Fig. 7). Separate one-way ANOVAs showed no effect of previous run-wheel experience on either Q_{P24}, $F(2, 22) = 2.02$, $P > 0.15$, or τ_{DD}, $F(2, 22) = .932$, $P > 0.4$. There was also no evidence that

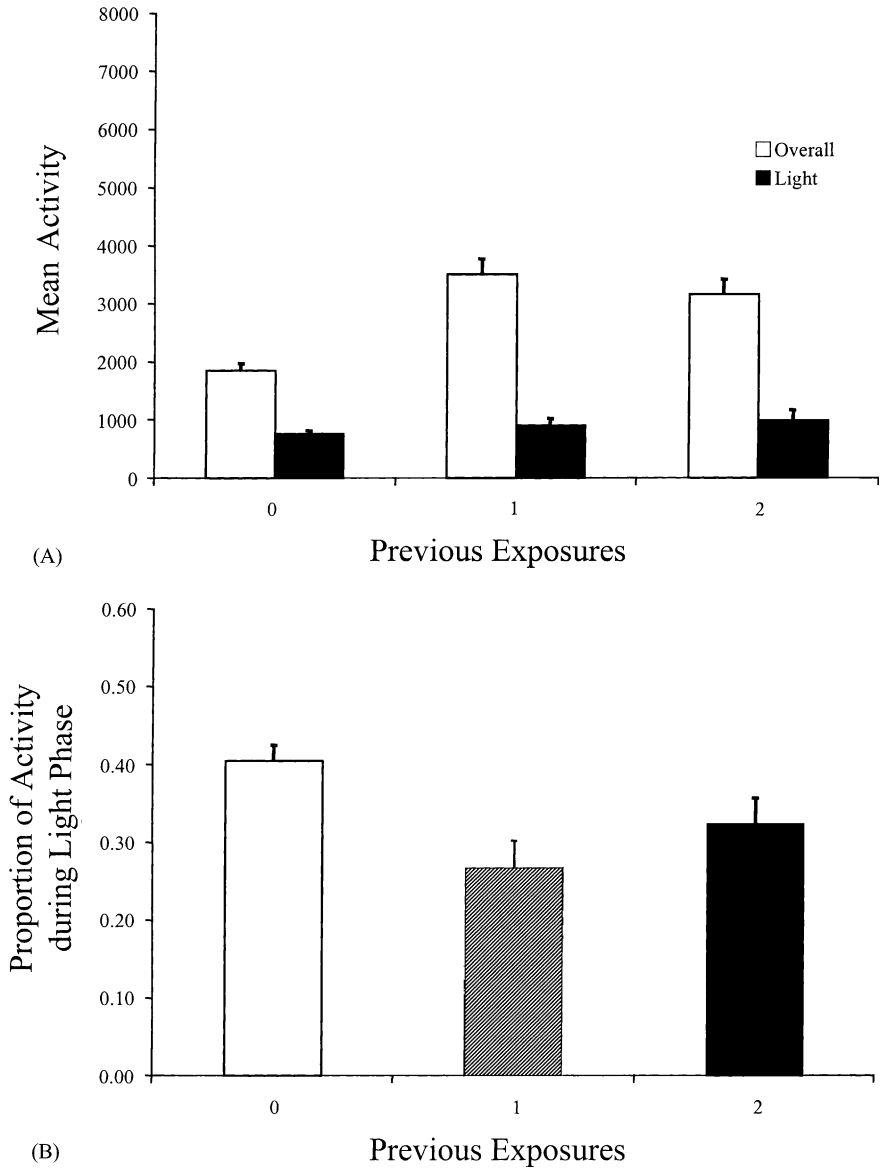


Fig. 6. Overall daily activity and activity during the light phase for 12-month SAMP8 with different amounts of run-wheel experience. Overall daily activity increased (A) and the proportion of activity during the light phase decreased (B) with increasing experience in the run-wheel apparatus. A single previous exposure to the run-wheel was as effective as two exposures in enhancing overall activity and reducing the proportion of activity during the light phase. The results suggest that prior experience to the run-wheel makes aged SAMP8 behave more like young mice with respect to activity measures.

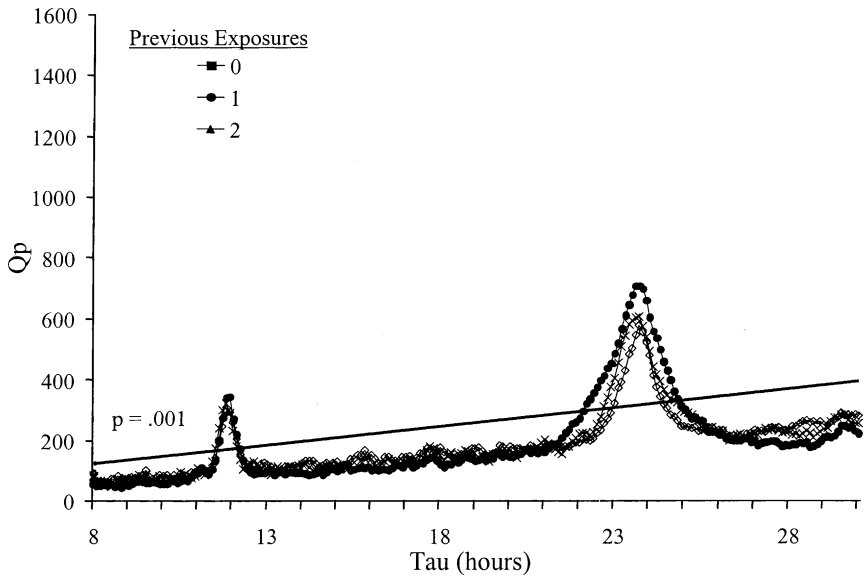


Fig. 7. Average chi-square periodograms for 12-month SAMP8 mice with varying amounts of run-wheel experience. Periodograms were constructed from data obtained from constant dark conditions. Previous experience in the run-wheel did not alter τ_{DD}, maximum amplitude of Q_P near 24 h, or the amplitude of Q_P near 12 h. These results support the view that prior experience does not alter circadian rhythms.

run-wheel experience influenced the incidence of split activity rhythms as measured by Q_{P12}, $F(2, 22) = .598$, $P > 0.5$, or the observer ratings of actograms, $F(2, 22) = .275$, $P > 0.5$.

4. Discussion

Disruptions in circadian rhythms are commonly found with the elderly, especially in individuals afflicted with dementia. Previous studies involving the P8 line of the SAM model have been valuable for advancing our understanding of circadian rhythm disturbances associated with aging and dementia [2,10,18]. These previous studies of SAMP8 used cross-sectional experimental designs. However, the rapid aging of the SAMP8 makes it an ideal candidate for longitudinal assessments of circadian rhythms. The present study examined the circadian rhythms of the SAMP8 at three points in their lifespan (2, 7, and 12 months). Consistent disruptions in activity rhythms were observed by 7 months of age.

The present study found (1) a large age-related decrease in overall run-wheel activity and rhythm amplitude, (2) an increase in the proportion of activity that occurred during the normally inactive light phase, (3) the appearance of split activity rhythms in some of the older SAMP8, and (4) no effect of age of the free-running period of the SAMP8, even though the rhythms of the aged SAMP8 were clearly disrupted in other ways.

An age-related decrease in rhythm amplitude and increased incidence of rhythm fragmentation, as measured by proportion of light activity, are characteristic of those frequently considered part of the normal aging process in rodents [4,8,13,15,25,30,32,33], and the same pattern of results has been a consistent finding in studies of aged SAMP8 [2,10,18]. In contrast, the evidence for changes in free-running period with age is less consistent. Some rodents such as hamster, rat and deer-mice show a shortening of their free-running period with age [13,14,17,26] although other studies using an longitudinal experimental design found no age-related change in free-running period in hamsters [5,7]. Other animals such as mice demonstrate a lengthening of free-running period with age [15,25]. In SAMP8, Asai et al. [2] have previously reported a lengthening of τ_{DD} with age, whereas a previous study found no significant effect of age on free-running period [10]. This latter study is consistent with the present longitudinal assessment of the SAMP8.

Our failure to replicate the lengthening of free-running period reported by Asai et al. [2] is reminiscent of the inconsistencies observed for hamsters [5,7,13]. Several explanations have occurred to us. First, effects of aging on free-running period may simply be weak and therefore hard to detect statistically. For the one previous study that indicated a statistically reliable lengthening of τ_{DD} with age, the difference between the 2- and 12-month-old groups was only about 0.3 h [2]. Weak effects of age on τ_{DD} may be due to variability across the population of SAMP8, or may be related to inconsistent effects of age between individuals in that population. In the latter case, τ_{DD} might lengthen in some mice and shorten in other mice, producing no net average change for the sample. The latter explanation is supported to a degree by the present study because the variability of τ_{DD} across subjects increased with age. Future studies

with a larger number of subjects will be necessary to determine whether there may perhaps be a bimodal distribution of τ_{DD} in aged animals. A second possibility is that age-related alterations of τ_{DD} may only appear after an extended time in constant conditions. In one study, free-running period of the hamster lengthened for the first 20 weeks in constant dim light conditions [5]. After this time, free-running period remained stable. Although the hamsters did not show an age-related alteration of free-running period in that study [5], it is possible that we may have observed a more consistent lengthening of τ_{DD} in the present study if the SAMP8 were continuously monitored in constant conditions for a longer period of time.

One of the aims of this study was to describe the changes in circadian rhythms as individual subjects age. The results demonstrate that a range of rhythm changes can be observed across the lifespan. Activity rhythms remained relatively intact across the lifespan for some SAMP8, whereas in others, activity rhythms became increasingly disrupted with age. Two distinct patterns of rhythm disturbance were observed by 7 months of age. Some of the animals appeared to be arrhythmic, whereas others appeared to transition to a split activity pattern with two distinct bouts of activity separated by approximately 12 h.

The increased incidence of split activity rhythms, with the corresponding increase in a 12-h rhythm, may partly explain the reduction of the amplitude of the near 24-h rhythm. Split circadian rhythms were verified by visual inspection of actograms and the appearance of a secondary peak in the periodogram around 12 h. The split rhythms in the present study have the appearance of rhythm splitting in hamsters maintained in constant light conditions although some differences were noted. In particular, the increased incidence of split rhythms in aged SAMP8 contrasts with the reduced probability of rhythm splitting in aged hamsters [8,13]. Significant differences in the species and conditions inducing split rhythms (constant light versus constant dark) may be responsible for the discrepancy. Alternatively, the split rhythms that we report here in SAMP8 and those observed in hamsters may be fundamentally different. For instance, rhythm splitting in hamsters is accompanied by a decoupling of the activity of the left and right suprachiasmatic nuclei so that each hemisphere oscillates about 12 h out of phase [6]. Future studies will be required to determine whether a similar decoupling of the suprachiasmatic nucleus occurs in aged SAMP8 with split activity rhythms.

An exciting finding of the present study was that several measures at 2 months predicted circadian rhythm disruption or lack of disruption at 7 and 12 months. Proportion of light activity at 2 months was highly correlated with various measures of circadian rhythm disruption at 7 months, even though the proportion of light activity was not correlated with these same measures at 2 months. Similarly, the proportion of light activity was not a good predictor of rhythm disturbances at 12 months, possibly due to the overall high incidence of rhythm disturbances at this age resulting in a floor effect. The amount of light and dark activities at 2 months also predicted circadian rhythm at 7 and 12 months, with increased light activity at 2 months correlated with *increased* rhythm disruption and increased dark activity at 2 months correlated with *decreased* rhythm disruption later in life. The high correlation for 7-month measures of rhythm disruption suggests that proportion of light activity and amount of light activity at 2 months may be good behavioral predictors of those individuals that will have an early-onset of circadian rhythm disturbance. In contrast, the amount of dark activity at 2 months may be a good behavioral predictor of those individuals that will continue to have strong circadian rhythms throughout their lives. Thus, these behavior predictors may be used to identify those individuals that may be helped by intervention treatments (i.e. exercise) to prevent or delay the early onset of rhythm disturbances.

In the present study, the age-related increase in the relative proportion of light activity was not due to a change in the absolute amount of activity during the light phase. Rather, age-related changes in the proportion of light activity tended to reflect a constant amount of activity in the light phase at all ages with decreases in overall run-wheel activity at the 7 and 12 months ages. Others have reported that SAMP8 have unusually high proportions of light activity when compared to other mouse strains such as the SAMR1 [11,18]. Assuming the predictive value of proportion of light activity holds across species as it does across age, one might speculate that aged SAMR1 should have less disruption of circadian rhythms than aged SAMP8. This appears to be the case when examining the actograms of Sanchez-Barcelo et al. (see Fig. 2 in [18]). More longitudinal studies in SAMP8 and other animal models will be necessary to evaluate potential behavioral predictors of circadian rhythm disturbances.

A final discussion point concerns the combined cross-sectional and longitudinal analysis of the effect of prior run-wheel exposure on activity rhythms. Previous cross-sectional studies could not address this issue because animals in each age-group were naïve at the time of testing. The present investigation considered the effects of previous run-wheel experience on circadian rhythms by comparing 12-month SAMP8 mice with 0, 1 and 2 previous exposures to the running wheel for a 16-day duration. Prior experience influenced both overall activity and proportion of light activity, making older SAMP8 with run-wheel experience appear more like young SAMP8. However, the effects of previous run-wheel experience on rhythm amplitude or period in constant dark conditions did not reach significance. Run-wheel activity, as short as 8 days, alters the expression of genes involved in the maintenance and growth of neurons, providing a possible neurobiological explanation for the effects of run-wheel experience [3,7].

The reduced proportion of light activity that was found with increased running-wheel experience is similar in some respects to previous reports of the effects of exercises on rest-activity rhythms in elderly humans. In elderly humans,

long-term fitness training reduced the fragmentation of rest-activity rhythms [28]. In fact, fragmentation was negatively correlated with fitness level as measured by max VO_2. It is possible that overall fitness level reduces the incidence of circadian rhythm fragmentation in both humans and mice. Thus, exercise combined with early detection of future circadian rhythm disturbance (i.e. proportion of light activity) may be used to prevent or delay the onset of disorders associated with disrupted circadian rhythms.

Overall, the present findings help to clarify and extend previous research on effects of aging on the circadian rhythms of the SAMP8. In summary, the present study found age-associated changes in circadian rhythms and activity in SAMP8 mice using a cross-sequential experimental design. Activity levels were increased, circadian rhythm amplitude was decreased and an increased incidence of splitting was observed with age. Although the general trend was from intact circadian activity rhythms to more disrupted rhythms, the actual transitions from one state to the other varied greatly from individual to individual. Furthermore, proportion of light activity at 2 months was highly correlated with various measures of circadian rhythm disruption at 7 months, suggesting a possible use for detection of early-onset rhythm disruption. Finally, changes in activity levels, but not circadian rhythm, were influenced by number of run-wheel experiences. The results from this longitudinal experiment support and extend the results from previous cross-sectional studies that disruptions of circadian rhythms are observed at 7 and 12 months of age in SAMP8 mice.

Acknowledgments

We are grateful for the assistance that Eric Beck provided on this study.

References

[1] Antoniadis EA, Ko CH, Ralph MR, McDonald RJ. Circadian rhythms, aging and memory. Behav Brain Res 2000;111:25–37.
[2] Asai M, Ikeda M, Akiyama M, Oshima I, Shibata S. Administration of melatonin in drinking water promotes the phase advance of light–dark cycle in senescence-accelerated mice, SAMR1 but not SAMP8. Brain Res 2000;76:220–4.
[3] Cotman CW, Berchtold NC. Exercise: a behavioral intervention to enhance brain health and plasticity. Trends Neurosci 2002;25:295–301.
[4] Davis FC, Menaker M. Hamsters through time's window: temporal structure of hamster locomotor rhythmicity. Am J Physiol 1980;239:R149–55.
[5] Davis FC, Viswanathan N. Stability of circadian timing with age in Syrian hamsters. Am J Physiol 1998;275:R960–8.
[6] de la Iglesia HO, Meyer J, Carpino Jr A, Schwartz WJ. Antiphase oscillation of the left and right suprachiasmatic nuclei. Science 2000;290:799–801.
[7] Duffy JF, Viswanathan N, Davis FC. Free-running circadian period does not shorten with age in female Syrian hamsters. Neurosci Lett 1999;271:77–80.
[8] Duncan MJ, Deveraux AW. Age-related changes in circadian responses to dark pulses. Am J Physiol Regul Integr Comp Physiol 2000;279:R586–90.
[9] Hosokawa M. Grading score system: a method of evaluation of the degree of senescence in senescence-accelerated mouse (SAM). In: Takeda T, editor. The SAM Model of Senescence. Amsterdam: Elsevier; 1994. p. 23–8.
[10] McAuley JD, Miller JP, Beck E, Nagy ZM, Pang KCH. Age-related disruptions in circadian timing: evidence for "split" activity rhythms in the SAMP8. Neurobiol Aging 2002;23:625–32.
[11] Miyamoto M. Characteristics of age-related behavioral changes in senescence-accelerated mouse SAMP8 and SAMP10. Exp Gerontol 1997;32:139–48.
[12] Miyamoto M, Kiyota Y, Yamazaki N, Nagaoka A, Matsuo T, Nagawa Y, et al. Age-related changes in learning and memory in the senescence-accelerated mouse (SAM). Physiol Behav 1986;38:399–406.
[13] Morin LP. Age-related changes in hamster circadian period, entrainment, and rhythm splitting. J Biol Rhythms 1988;3:237–48.
[14] Pittendrigh CS, Daan S. Circadian oscillations in rodents: a systematic increase of their frequency with age. Science 1974;186:548–50.
[15] Possidente B, McEldowney S, Pabon A. Aging lengthens circadian period for wheel-running activity in C57BL mice. Physiol Behav 1995;57:575–9.
[16] Refinetti R. Laboratory instrumentation and computing: comparison of six methods for the determination of the period of circadian rhythms. Physiol Behav 1993;54:869–75.
[17] Rietveld WJ, Boon ME, Korving J, van Schravendijk K. Circadian rhythms in elderly rats. Cycle Res 1985;16:154.
[18] Sanchez-Barcelo EJ, Megias M, Verduga R, Crespo D. Differences between the circadian system of two strains of senescence-accelerated mice (SAM). Physiol Behav 1997;62:1225–9.
[19] Satlin A, Teicher MH, Lieberman HR, Baldessarini RJ, Volicer L, Rheaume Y. Circadian locomotor activity rhythms in Alzheimer's disease. Neuropsychopharmacology 1991;5:115–26.
[20] Shibata S, Asai M, Oshima I, Ikeda M, Yoshioka T. Melatonin normalizes the re-entrainment of senescence accelerated mice (SAM) to a new light–dark cycle. In: Olcese J, editor. Melatonin after four decades. New York: Kluwer Academic/Plenum Press; 2000. p. 261–70.
[21] Sokolove PG, Bushell WN. The chi square periodogram: its utility for analysis of circadian rhythms. J Theor Biol 1978;72:131–60.
[22] Takeda T. Senescence-accelerated mouse (SAM): a biogerontological resource in aging research. Neurobiol Aging 1999;20:105–10.
[23] Takeda T, Hosokawa M, Higuchi K. Senescence-accelerated mouse (SAM): a novel murine model of accelerated senescence. J Am Geriatr Soc 1991;39:911–9.
[24] Takeda T, Hosokawa M, Takeshita S, Irino M, Higuchi K, Matsushita T, et al. A new murine model of accelerated senescence. Mech Ageing Dev 1981;17:183–94.
[25] Valentinuzzi VS, Scarbrough K, Takahashi JS, Turek FW. Effects of aging on the circadian rhythm of wheel-running activity in C57BL/6 mice. Am J Physiol 1997;273:R1957–64.
[26] van Gool WA, Witting W, Mirmiran M. Age-related changes in circadian sleep-wakefulness rhythms in male rats isolated from time cues. Brain Res 1987;413:384–7.
[27] van Someren EJ, Hagebeuk EE, Lijzenga C, Scheltens P, de Rooij SE, Jonker C, et al. Circadian rest-activity rhythm disturbances in Alzheimer's disease. Biol Psychiatry 1996;40:259–70.
[28] Van Someren EJ, Lijzenga C, Mirmiran M, Swaab DF. Long-term fitness training improves the circadian rest-activity rhythm in healthy elderly males. J Biol Rhythms 1997;12:146–56.
[29] van Someren EJ, Mirmiran M, Swaab DF. Non-pharmacological treatment of sleep and wake disturbances in aging and Alzheimer's disease: chronobiological perspectives. Behav Brain Res 1993;57:235–53.

[30] Wax TM. Runwheel activity patterns of mature-young and senescent mice: the effects of constant lighting conditions. Journal of Gerontology 1975;30:22–7.
[31] Weitzman ED, Moline ML, Czeisler CA, Zimmerman JC. Chronobiology of aging: temperature, sleep-wake rhythms and entrainment. Neurobiol Aging 1982;3:299–309.
[32] Welsh DK, Richardson GS, Dement WC. Effect of age on the circadian pattern of sleep and wakefulness in the mouse. J Gerontol 1986;41:579–86.
[33] Witting W, Mirmiran M, Bos NP, Swaab DF. The effect of old age on the free-running period of circadian rhythms in rat. Chronobiol Int 1994;11:103–12.