Quantitative analysis of the age-related fragmentation of hamster 24-h activity rhythms

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Quantitative analysis of the age-related fragmentation of hamster 24-h activity rhythms. Am. J. Physiol. 273 (Regulatory Integrative Comp. Physiol. 42): R2132–R2137, 1997.—The continuous monitoring of spontaneous locomotor activity has emerged as one of the most widely used metrics in rodent circadian research. This behavioral measure is also extremely useful for the description of the effects of aging on circadian rhythms. The present study describes the successful use of a log-survivorship approach to identify discrete bouts of hamster wheel-running activity and provides a detailed description of the age-related fragmentation in the 24-h profile of this behavioral variable. In addition, stepwise discriminant analysis identified the most important quantitative measures for distinguishing between the individual patterns of wheel-running activity of young (3 mo) and old (17–18 mo) golden hamsters. The results suggest that this method of bout analysis can be a valuable tool for the study of genetic, developmental, neurochemical, physiological, and environmental factors involved in the temporal control of rodent locomotor behavior.

Materials and methods

Animals and laboratory conditions. Young male golden hamsters (Mesocricetus auratus) were purchased from Charles River Lakeview (Newfield, NJ; Lak:LVG/SYR) and arrived in the laboratory at 8–10 wk of age. Retired male breeders were obtained from the same vendor at 9 mo of age and kept in an aging colony (University of Wisconsin-Parkside) for 8–9 mo before their arrival at Northwestern University [the average life span of golden hamsters under laboratory conditions is ~2 yr (7)]. All hamsters were housed in individual running-wheel cages (wheel diameter = 17 cm) inside ventilated light-proof boxes (6 cages per box) under a 14:10-h light-dark cycle (white fluorescent light, 20–90 lx at cage top). The ambient temperature was maintained at 21 ± 1°C, and food and water were available at all times. The wheel-running activity of the animals was monitored continuously with an on-line data acquisition system (Stanford Software Systems, Stanford, CA), and the number of wheel revolutions was quantified in 1-min bins. All hamsters were allowed a 10-day period of adaptation to the laboratory conditions before the beginning of the experiment.

Log-survivorship analysis. The quantitative analysis of the activity pattern in golden hamsters was based on a well-established technique for the identification of discrete clusters in continuous sequences of behavioral events, known as log-survivorship analysis (18). Briefly, when wheel-running events are randomly distributed in time, the intervals between them follow a negative exponential distribution and the plot of the resulting log-survivor function appears as a straight line. However, if the wheel-running behavior occurs...
in temporal clusters (activity bouts), brief periods of inactivity (within-bout intervals) are relatively common, whereas longer between-bout intervals become more rare. This gives rise to a concave log-survivor function, which allows the definition of a minimal between-bout time criterion (bout criterion interval).

Wheel-running activity records, collected over a 7-day period from a reference group of 20 young animals, were used in the log-survivorship analysis. The resulting function confirmed the presence of discrete clusters of wheel-running behavior in hamster activity records and was used to select a 15-min bout criterion interval (Fig. 1). The use of the same log-survivorship method with wheel-running data from old hamsters produced comparable results, indicating the lack of considerable age-related changes in the magnitude of the bout criterion interval (data not shown).

A subsequent analysis of all activity bouts in the reference data set that were identified according to this criterion revealed the presence of occasional artifacts in the recorded sequences. These low-intensity events were associated with the recording of some "false-positive" wheel revolutions due to nonspecific behaviors, such as grooming, stretching, nest building, food hoarding, or general cage activity. Therefore, an additional bout detection criterion, which requires a peak running-wheel intensity of at least four revolutions in any 1-min period of the potential activity bout, was introduced to remove such nonspecific noise artifacts from further analysis.

Characterization of the 24-h pattern of wheel-running activity. The 24-h patterns of wheel-running activity, recorded over a 7-day period in a group of 20 old hamsters, were characterized and compared with those of an additional 20 young animals that were not part of the prior log-survivorship analysis. A computerized algorithm, based on the bout detection criteria established earlier in the study, was used to identify the activity bouts in all of these records. Each bout was further characterized by several parameters, including time of onset and offset, total duration, amount of time with and without wheel-running activity, total number of wheel revolutions (bout size), as well as average and peak 1-min wheel-running intensity. If the time of bout onset or offset occurred during the lights-off period, the bout was defined as a scotophase episode. If both bout onset and offset occurred during the time of lights on, the bout was considered to be a photophase event.

The bout parameters of each hamster were used to generate a set of variables reflecting quantitative characteristics of its activity pattern during each day of monitoring. Individual results were averaged across the 7-day recording period to obtain a single set of estimates for each animal. One-way analysis of variance was used to compare these quantitative characteristics of individual activity patterns between the two age groups. All parameters throughout the text are reported as means ± SE, and statistical significance implies \( P < 0.05 \) unless otherwise specified.

In addition, stepwise discriminant analysis was used to identify which of the variables in the above set are most important for distinguishing between the 24-h patterns of locomotor activity in young and old animals and to develop a scoring procedure for hamster wheel-running records based on the presence of bout characteristics, which are similar to those that occur spontaneously during senescence. Wilk's lambda was used as a criterion for variable selection, with probabilities of \( F \) set at 0.05 for entry and 0.10 for exclusion. Because the performance of a discriminant function is better in the sample from which it is derived than in a different sample from the same population, the leave-one-out method was used to obtain an improved estimate of the true performance of this approach. This involved sequentially leaving out a single set of individual values, calculating the corresponding discriminant function on the basis of the remaining data, and then classifying the left-out case.
RESULTS

Seven-day records illustrating the qualitative changes in the daily patterns of hamster wheel-running activity with advanced age are shown in Fig. 2. The ability to detect and characterize activity bouts in the data of all young and old experimental animals made possible a number of initial descriptive comparisons. Thus clear differences in the frequency distribution of the identified bouts by size, duration, and timing were detected between the two age groups (Fig. 3).

The set of measures, derived from the characteristics of individual bouts to reflect the average 24-h pattern of wheel-running activity of each animal, provided valuable tools for the quantitative assessment of age-related fragmentation in hamster locomotor behavior (Table 1). The statistical comparison of these measures between the young and old group of animals revealed the presence of significant differences in 15 of the 18 variables (Table 1). In contrast, the time of onset of the first bout, the time of offset of the last bout, and the 1-min peak intensity during the dark period remained rather similar in both age groups.

Figure 4 shows the results of a discriminant analysis, in which the simultaneous use of the variables with
Table 1. Daily pattern characteristics of wheel-running activity in young and old hamsters

<table>
<thead>
<tr>
<th>Variable</th>
<th>Young</th>
<th>Old</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light period</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of bouts</td>
<td>0.3±0.1</td>
<td>0.6±0.1</td>
<td>0.025</td>
</tr>
<tr>
<td>Longest bout, min</td>
<td>4.6±1.0</td>
<td>8.5±1.6</td>
<td>0.041</td>
</tr>
<tr>
<td>Largest bout, rev</td>
<td>21±5</td>
<td>85±27</td>
<td>0.023</td>
</tr>
<tr>
<td>Peak intensity, rpm</td>
<td>7.9±1.6</td>
<td>16.2±3.1</td>
<td>0.022</td>
</tr>
<tr>
<td>Mean intensity, rpm</td>
<td>3.4±0.7</td>
<td>7.9±1.5</td>
<td>0.010</td>
</tr>
<tr>
<td>Total bout time, min</td>
<td>1.8±0.5</td>
<td>4.2±0.9</td>
<td>0.025</td>
</tr>
<tr>
<td>Total activity time, min</td>
<td>1.2±0.4</td>
<td>3.2±0.7</td>
<td>0.017</td>
</tr>
<tr>
<td>Total activity, rev</td>
<td>9.3</td>
<td>45±14</td>
<td>0.017</td>
</tr>
</tbody>
</table>

| Dark period |       |     |       |
| Number of bouts | 2.7±0.1 | 4.1±0.3 | <0.001 |
| Longest bout, min | 248±11 | 121±15 | <0.001 |
| Largest bout, rev | 3.118±715 | 3246±588 | <0.001 |
| Peak intensity, rpm | 66.2±2.6 | 62.7±2.2 | 0.310 (NS) |
| Mean intensity, rpm | 32.8±2.0 | 24.4±2.2 | 0.007 |
| Total bout time, min | 310±11 | 204±17 | <0.001 |
| Total activity time, min | 256±9 | 159±14 | <0.001 |
| Total activity, rev | 10.141±714 | 5.096±672 | <0.001 |
| First bout onset, ZT | 12.2±0.1 | 12.3±0.1 | 0.644 (NS) |
| Last bout offset, ZT | 20.6±0.2 | 20.2±0.2 | 0.064 (NS) |

Values are means ± SE; n = 20 animals in each group. Rev, revolutions; rpm, revolutions/min; ZT, zeitgeber time (by convention, ZT 12 is the time of lights off in nocturnal rodents).

significant age-related differences from Table 1 produced a clear distinction between the patterns of wheel-running activity in 19 of the 20 hamsters (95%) in each age group. A subsequent stepwise discriminant analysis identified two variables, which were most important for distinguishing between the 24-h patterns of this locomotor behavior in young and old animals. Thus a discriminant function, based on the individual number of bouts during the light period and the longest bout duration during the night, correctly classified 92.5% of the hamsters in both age groups. The use of the leave-one-out method and the same two variables to predict the group membership of each animal, assuming no prior knowledge of its age, provided an estimate of 90% for the true performance of this discriminant approach under similar experimental conditions (correct assignment of 18 of 20 of the hamsters in each age group).

DISCUSSION

The results of this study indicate that log-survivorship analysis can be successfully used for the identification of discrete clusters of wheel-running activity (bouts) in golden hamsters (Fig. 1). Additional studies with other species in our laboratory (including Djungarian hamsters and several inbred mouse strains) support the general applicability of the log-survivorship approach for the quantitative characterization of this rodent behavior (Ref. 22 and unpublished data). Because various factors, such as species and strain background, environmental stimuli, laboratory conditions, and experimental interventions, can modify the pattern of wheel-running activity, it is important to verify and (if necessary) adjust the selected bout identification criteria in each specific set of circumstances.

The ability to detect and characterize discrete bouts of hamster wheel-running activity provided valuable new measures for the quantitative analysis of the age-related changes in this behavior. Consistent with previous qualitative descriptions (2, 5, 11, 20), the results of this study indicate the presence of considerable fragmentation in the diurnal rhythm of locomotor activity with advanced age (Fig. 2). Thus the wheel-running patterns of the old hamsters were characterized by a significantly increased number of activity bouts with shorter duration, smaller size, and more variable timing (Table 1). Interestingly, whereas fragmentation of the locomotor activity rhythm is more pronounced in old hamsters under a light-dark cycle, during exposure to constant light old hamsters show less “splitting” of the activity rhythm into two 12-h components (8). Thus rhythm fragmentation and splitting may have separate physiological bases. The age-related fragmentation of the activity-rest cycle is in good agreement with the reported deterioration of the sleep-wake cycle in old rodents (23, 27). The availability of methods for monitoring and analysis of 24-h patterns of rodent sleep (15, 23, 27) and locomotor activity (present study) will allow a more detailed description of their temporal and quantitative relationships.

Whether the observed fragmentation of the 24-h rhythm of locomotor activity is a consequence of age-related changes in the circadian system, its output pathways, or other nonspecific processes in the senescent organism remains an issue of considerable interest (20). Despite the significant reduction in the amount and duration of nocturnal activity in the aged animals compared with those in the young group, all old hamsters exhibited clear 24-h rhythms of wheel-running behavior (Fig. 2). In addition, the similarity in the timing of onset and offset, respectively, of the first and the last nocturnal bout in the two age groups suggests the lack of significant age-related differences in the overt entrainment of the monitored activity rhythms to the external light-dark cycle. However, it has been reported that age-related changes in entrainment be-

![Discriminant Score](image-url)
come apparent only under very dim light-dark cycles (30), and the presence of some masking effects of light on the diurnal profiles of locomotor activity in this study cannot be excluded. As pointed out by Witting et al. (28), the effects of age on the expression of circadian rhythmicity may be influenced by a variety of environmental factors.

The finding of increased daytime activity in the presence of an external light-dark cycle emerged as an important feature of the 24-h pattern of wheel-running activity in the old hamsters (Table 1). Indeed, the number of bouts during the light portion of the daily cycle was one of the two variables that were most important for distinguishing between the 24-h activity patterns of young and old animals. These results support the potential existence of age-related changes in the circadian timing system and/or deficits at the level of the central circadian pacemaker (16, 20). The development of methods for quantitative analysis of the 24-h patterns of wheel-running activity of old hamsters and in animals with spontaneous or experimentally induced lesions of the circadian system is particularly important for the future collection of valuable comparative data (12, 19, 25). Similarly, because central monoaminergic deficits may contribute to some of the age-related changes in overt circadian rhythmicity (9, 10), it would be of interest to compare the patterns of locomotor activity in animals with intact and genetically or pharmacologically altered monoaminergic neurotransmission (4, 29).

The hypothesis that an age-related decay of circadian organization may be part of the physiological deterioration that limits life span (13) represents another exciting area in the field of circadian biology and aging. The high predictive power of the reported discriminant analysis (Fig. 4) indicates that quantitative measures of fragmentation in the 24-h pattern of wheel-running activity may be used as functional markers of aging. Most importantly, this approach allows the future exploration of several intriguing questions regarding the potential relationship between the degree of disruption of the diurnal rhythm of locomotor activity and the long-term functional status, morbidity, and longevity of the organism. The current availability of rodent models carrying single circadian gene mutations (14, 24) has expanded the scope of research on the significance of temporal factors for the “successful” aging of the organism. However, the characteristic patterns of daily locomotor activity associated with these mutations have received little attention and await detailed quantitative description. The progress in the field of suprachiasmatic nucleus transplantation in aged rodents (5) offers another promising application for the proposed quantitative approach.

In summary, the results of the present study suggest that the described method for bout analysis of rodent wheel-running activity can provide valuable information in a variety of experimental paradigms, using genetic, pharmacological, neurophysiological, and behavioral approaches to dissect the time structure of mammalian locomotor behavior.

Perspectives

The circadian rhythm of locomotor activity has been, and will no doubt continue to be, the most well-studied “output rhythm” regulated by the central circadian clock in many vertebrate species, particularly in rodents. Characterization of the activity rhythm has often been limited to a few variables such as activity onset time, duration of the active period, the freerunning period, and rhythm amplitude. However, quite often following a particular perturbation or during long-duration studies one sees a variety of other changes in the activity rhythm, which are often vaguely defined with terms such as “lack of precision,” “fragmentation,” “sloppy,” etc. In this paper we describe how a log-survivorship approach can be used to identify discrete bouts of hamster wheel-running activity and how this information can be used to quantify the degree to which rhythms are fragmented or sloppy. Although we have applied this quantitative approach for analyzing the age-related fragmentation of the activity rhythm, this approach can also be used for quantifying how genetic, developmental, physiological, pharmacological, and environmental factors may influence locomotor activity, as well as other circadian rhythms in which the expression of rhythmicity occurs in discrete clusters of a measurable variable. We are particularly interested in using this approach for determining the potential predictive value of age-related changes in locomotor activity (and other rhythms, e.g., sleep-wake cycle) for the health and longevity of the organism.

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