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LIFESPAN DAILY LOCOMOTOR ACTIVITY RHYTHMS IN A MOUSE MODEL OF AMYLOID-INDUCED NEUROPATHOLOGY

Michael R. Gorman[1] and Steven Yellon[2]

[1]Department of Psychology and Center for Chronobiology, University of California, San Diego, La Jolla, California, USA
[2]Department of Physiology, Center for Perinatal Biology. Loma Linda University, School of Medicine, Loma Linda, California, USA

Using a rodent model for neuropathology induced by human amyloid precursor protein, the present study tested the hypothesis that 24 h rest/activity rhythms deteriorate with age. A lifespan of rest/activity patterns was studied in transgenic Tg2576 mice and wild-type controls. Classic indices of circadian timekeeping, including onsets, offsets, and the duration of nighttime activity, were stable throughout the 96-week study. Analyses of ultradian bout activity revealed significant genotype and age-related changes in the duration and intensity of activity bouts, as well as amplitude of the 24 h rhythm. Tg2576 mice had more total activity counts, fewer bouts/24 h, more counts/bout, and longer bout time than wild-type controls. Amyloid deposits and plaques were solely found in specific cortex regions in aged postmortem Tg2576 mice, but were not evident in the hypothalamus or suprachiasmatic nucleus; this neuropathology was absent from brains of wild-type controls. These findings suggest that amyloidosis of the Tg2576 mouse exerts little influence on timing of locomotor activity in the circadian domain but significantly alters the temporal structure of ultradian activity. (Author correspondence: syellon@llu.edu)

Keywords Suprachiasmatic nucleus; Tg2576; Circadian; Alzheimer's disease; Aging; Ultradian; Amyloid plaques

INTRODUCTION

The rest/activity cycle is among the most commonly studied rhythms in humans and rodents. As humans age, marked changes in the rest/activity rhythm and sleep may result in cognitive deficits and potentially

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Address correspondence to Dr. Steven M. Yellon, PhD Department of Physiology, Center for Perinatal Biology, Loma Linda University, School of Medicine, Loma Linda, CA 92350; Tel: 909-558-4325; Fax: 909-558-4029; E-mail: syellon@llu.edu

contribute to physiological and psychological impairment (Oosterman et al., 2009). Alzheimer's disease (AD) is a pathological progressive impairment of cognitive function that is associated with age and, in specific regions of the cortex, characterized by amyloid-containing plaques and neurofibrillary tangles (Duyckaerts et al., 2009; Selkoe, 2001). Daytime sleep and nighttime bouts of activity are common disruptions of the 24 h rest/activity rhythm in AD patients (Klaffke & Staedt, 2006; Lee et al., 2007; Martin et al., 2000; van Someren et al., 1999; Witting et al., 1990). AD patients also exhibit "sundowning," an increased incidence of agitation, confusion, and/or restlessness in late afternoon or evening, that is associated with reduced synchrony of 24 h temperature and activity rhythms (Mahlberg et al., 2004; Volicer et al., 2001). Moreover, AD patients demonstrate increased nocturnal activity and significant phase-delays or irregularities in rhythms of core-body temperature, activity, and melatonin compared with patients with frontal temporal depression or age-matched controls (Satlin et al., 1991; Skene & Swaab, 2003). These and other well-documented sleep and circadian rhythm disorders are major management considerations for institutionalization as AD progresses (Hebert et al., 2001).

The mechanism that regulates physiological and behavioral circadian rhythms, including the rest/activity cycle, is recognized to be internally generated by a neural clock in the suprachiasmatic nucleus (SCN) of the anterior hypothalamus (Duguay & Cermakian, 2009). In AD patients, postmortem structural changes are reported in the SCN compared to adult or age-matched controls (Liu et al., 2000; Stopa et al., 1999; Swaab et al., 1985; Wu et al., 2007). Neuropathology and evidence of diffuse amyloid beta deposits (not plaques) are present in the hypothalamus of aged AD patients, but their presence is apparently unrelated to the duration of symptoms of dementia (van de Nes et al., 1998). These findings complement other evidence that the structure or function of the SCN or its output pathways may be impaired in AD patients in ways that differ from those associated with normal aging (Goudsmit et al., 1990; Harper et al., 2005; Skene & Swaab, 2003; Standaert et al., 1991).

In rodents, aging is also associated with changes in locomotor activity rhythms and expression of immediate early genes in the SCN (Benloucif et al., 1997; Scarbrough et al., 1997). Age-related alterations in clock-controlled activities are likely to reflect changes in both circadian inputs and pacemaker function. In addition to diminished amplitude of the activity rhythm, fragmentation of nocturnal activity and daytime bouts of activity are correlated with age (Penev et al., 1997; Valentinuzzi et al., 1997). Valentinuzzi et al. (1997) recognized two distinctive domains in the pattern of locomotor activity: (1) predictable daily fluctuations in activity as reflected by parameters of the circadian waveform, and (2) the

temporal structure of activity related to length and intensity of bouts throughout the day.

The first objective of the present investigation was to assess whether age differentially affects lifespan rest/activity cycles with respect to standard measures of period (τ) and phase, as well as structure of 24 h fluctuations in bouts of activity. In addition, a second objective was to determine whether age-related changes in circadian timekeeping are associated with neuropathology in a transgenic mouse model for AD that expresses a mutant form of amyloid precursor protein. A variety of transgenic mice that overexpress amyloid precursor protein have age-related alterations in activity rhythms, including sundowning-like increases in activity (Ambree et al., 2006; Huitron-Resendiz et al., 2002; Vloeberghs et al., 2004). In Tg2576 mutant mice with the APP695 double mutation (K670N, M671L), total locomotor activity and open arm maze entries are increased compared to that in wild-type controls (Gil-Bea et al., 2007). In aged Tg2576 mice, Wisor et al. (2005) found longer circadian τs of the wheel-running rhythm in constant darkness, but no differences in total wheel running activity with entrainment to a light/dark cycle compared to age-matched controls. Absence of significant changes in wheel running activity with respect to age or amyloidosis in this study may reflect limitations in the cross-sectional design, or descriptive analyses of relatively short recording intervals of 2 to 7 days, or a consequence of wheel activity as a voluntary form of exercise (Yuede et al., 2009).

In the present study, circadian and ultradian activity endpoints were passively assessed to test the hypothesis that characteristics of the 24 h rest/activity cycle deteriorate with aging and with age-associated neuropathology in Tg2576 mice. Lifespan differences in the amount and structural patterns of activity between wild-type and Tg2576 mice support the conclusion that the clock-control of rest/activity rhythms change with age, but occur without deposition in the SCN of amyloid.

METHODS

Male Tg2576 mice maintained on a B6SJLF1 background and their wild-type littermate controls were purchased from Taconic laboratories (#001349, Germantown, New York, USA; n = 5–6/group). Tg2576 mice express a mutant form of human amyloid precursor protein (APP_{SWE}) in the brain, which results in age-related increases in pathophysiologic changes that are correlated with amyloid plaque deposits and gliosis and behavioral alterations that are typical of some, but not all aspects of AD (Hsiao et al., 1996; Irizarry et al., 1997; Kawarabayashi et al., 2001; Lee et al., 2009). Males were used to eliminate confounding considerations related to reproductive cycles that complicate rhythm analyses. The experimental timeline began when mice arrived at 10 weeks of age and

FIGURE 1 Timeline of experiment with respect to age of mice. Upon arrival at 10 weeks of age, mice were initially quarantined (Quar) then individually housed in cages for continuous passive monitoring of activity at 17 weeks of age. Open arrow indicates approximate onset of amyloidosis based upon previous studies (Hsiao et al., 1996; Irizarry et al., 1997; Ognibene et al., 2005). Numbers indicate two-week durations when locomotor activity was statistically analyzed (n = 4–6 mice/genotype). *Mice transfer to constant dark at 46–47 weeks of age to assess free-run period (τ). X indicates termination of study at 96 weeks.

were maintained in cages with corncob bedding on a 12 h light:12 h dark cycle (i.e., LD12:12); food and water were provided ad libitum (Figure 1). Fluorescent bulbs generated a light intensity of ~100 lux in each light phase. Dim green LEDs above each cage lid were constantly illuminated and thus produced ~0.1 lux throughout each dark phase. From17 weeks of age, mice were housed individually in polypropylene caging (27 × 16 × 13 cm). Mice remained on LD12:12 throughout except for 11 days beginning at 46 weeks of age. During that time, the bright lights were extinguished, and the circadian rhythms were allowed to free-run in constant dim illumination for measurement of the circadian τ. To verify that any observed individual variation in rhythmicity was intrinsic to the mice and not to any uncontrolled variations between recording stations, mice in the last two weeks of the experiment were moved to adjacent recording stations. Rhythm patterns were unequivocally associated with the animals and not the recording devices (data not shown).

Mice were sacrificed at 96 weeks of age, which corresponds to ~60% cumulative survival for both TG2576 and wild-type controls, according to data provided by the supplier, and when frailty and welfare became a health concern. Mice were euthanized with pentobarbital, a tail biopsy taken to confirmed genotype (run #122503, Mendelworks now Embark Sciences, Austin, Texas, USA), and brains immersion-fixed in 4% paraformaldehyde. Brains were post-fixed overnight then stored in 20% sucrose at −20°C. Brains were washed overnight in phosphate buffered saline (PBS), immersed in 100% ethanol for several days, then paraffin embedded, sectioned through the preoptic and hypothalamic areas at 10 μm, and processed with antibodies by immunohistochemistry to identify amyloid beta 1–40 and 1–42 antibodies as previously described (Zelcer et al., 2007). All experimental procedures were approved by the University of California San Diego (UCSD) Institutional Animal Care and Use Committee (IACUC) and conform to international ethical standards for care and use of animals in scientific research (Portaluppi et al., 2008).

Circadian and Ultradian Analyses

Locomotor activity was monitored with a passive infrared motion detector mounted inside the filter-top lid of each cage. Movement across 3 of 27 zones of detection triggered an electric relay that was compiled in 6 min bins by Dataquest III hardware and software (Mini-mitter, Bend, Oregon, USA). Data files were subsequently imported for analysis into Excel and ClockLab (Actimetrics, Evanston, Illinois, USA). Three types of activity analyses were performed.

Bout Structure of Activity

ClockLab software was used to evaluate the ultradian structure of activity profiles with respect to the number of bouts/day, average bout length, and the number of counts/bout. An activity bout was defined as a span during which activity never was <3 counts/min for >30 min. Activity bouts without regard to circadian timing were assessed over six intervals of 20 days, spaced $\sim$12 weeks apart. In addition, novelty-induced activity was assessed by summing activity counts over the 3 h interval immediately following a cage change, which occurred weekly $\sim$2–3 h into the light phase. For all other analyses, data on the day of cage changes were excluded.

Circadian Measures of Activity

The same spans of data were used for the second analysis, which characterized the periodic 24 h fluctuation in activity. Values in each 6 min bin were averaged across the individual 20-day spans to generate a 24 h histogram from which the following characteristics of the 24 h activity profile were derived. *Activity Onset* was identified as the first time-point from 6 h before lights-off where activity counts exceeded the 24 h mean and was sustained above average over three of the six following bins. *Activity Offset* was the latest point with three of the previous six bins also above the 24 h mean. *Activity Duration* was the time between activity onset and activity offset. *Daytime Activity* was the percentage of total activity occurring during the light hours. The same epochs of data were subjected to chi-squared periodogram analysis for determination of the Statistical Power at 24 h. Statistical Power (Q_p) is the maximum value of the chi-square periodogram and provides a measure of rhythm robustness (Refinetti, 2006; Sokolove & Bushell, 1978). It is based on the amount of variance that can be attributed to a rhythmic process of a given τ and is independent of the scaling (amplitude) of the rhythm. *Free-running period* of the circadian rhythm in locomotor activity was determined using the chi-squared periodogram analysis in ClockLab. To

verify this Clocklab analyses, individual records of each mouse obtained over the 11 days of continuous dim illumination (started on week 46) were inspected, and eye-fitted lines were manually drawn through activity onsets to estimate the free-running circadian τ.

Lifespan Analysis of Activity

The broad patterns of longitudinal change in activity patterns were assessed in individual mice. Activity data collected over ~40-day spans during different stages of the lifespan were collapsed into 1 h bins to produce a 24 h histogram. The first interval, from 16–23 weeks of age, was considered a young adult baseline, as animals at that time would be fully acclimated to the facility but not yet experiencing expected neuropathology and behavioral deficits that are generally minimal at that age (Kawarabayashi et al., 2001). The hourly activity over each mouse's lifespan was determined to be increased or decreased relative to this baseline.

Statistical Analyses

Longitudinal activity data were assessed with repeated-measures analysis of variance (ANOVA, Statview 5.0, SAS Institute, Cary, North Carolina, USA), with genotype as a between-subjects factor. ANOVAs were run on the data from weeks 19–76 during which all animals were represented (Figure 1 assessments). Before the final assessment interval (weeks 88–90), one wild-type mouse died, while another ceased activity. Thus, week 88 mean data do not include these two animals. Values for this final assessment interval are plotted in all figures for illustrative purposes but are not included in the statistical analyses.

RESULTS

Rhythms in locomotor activity varied with respect to both age and genotype. In the circadian domain, significantly greater Statistical Power, a measure of rhythm robustness, was observed in the Tg2576 versus wild-type mice (repeated-measures ANOVA $F_{1,9} = 10.4$, $p < 0.05$; Figure 2). No significant effect of genotype was found for Daytime Activity, Activity Onset, Activity Offset, Activity Duration (data not shown). Statistical Power also decreased significantly with age ($F_{5,45} = 6.3$, $p < 0.001$), whereas Daytime Activity and Activity Offset significantly increased with age ($F_{5,45} = 3.4$, 2.6, respectively; $p < 0.05$). In each of these analyses, there was no significant genotype x age interaction. The free-running circadian τ was assessed at 46–47 weeks of age, and no significant differences were found between groups (wild-type: 24.27 ± 0.10 h versus Tg2576: 24.06 ± 0.06 h; $F_{5,45} = 1.5$, $p > 0.10$). Near the end of the study,

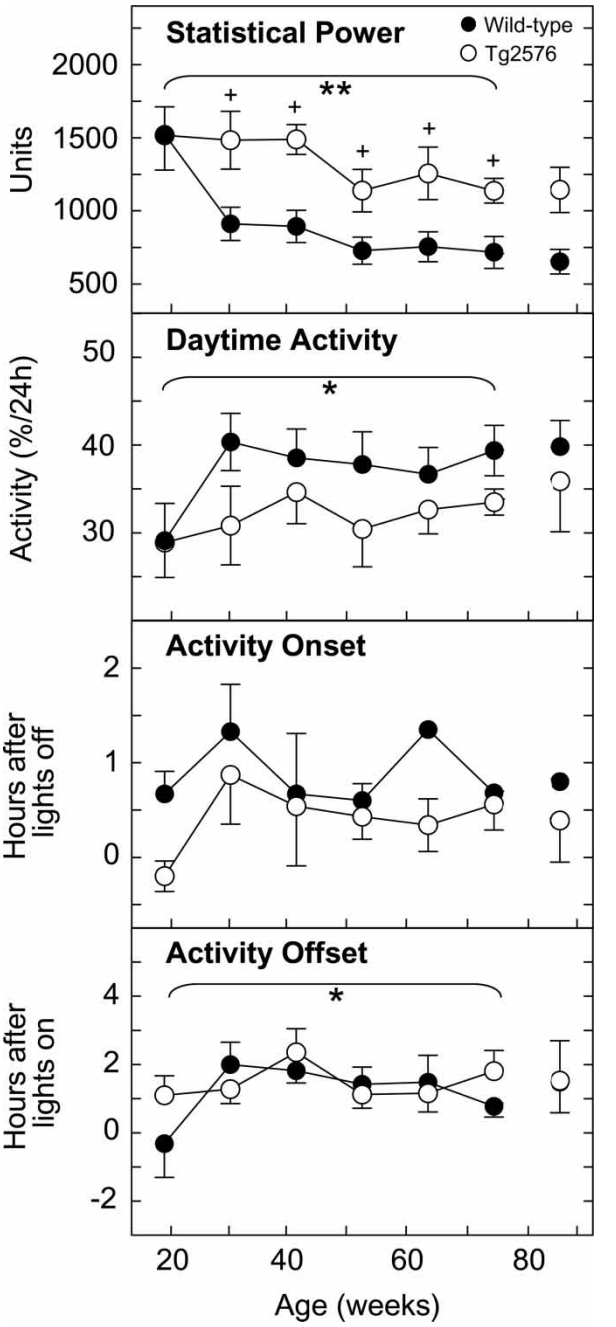

FIGURE 2 Parameters of circadian rhythms with age in wild-type and Tg2576 mice (mean ± SEM; n = 6 closed symbols or n = 5 open symbols, respectively). The " $^{+}$ " symbol indicates $p < 0.05$ versus same age WT control; a significant main effect of genotype in the repeated-measures ANOVA was found only for Statistical Power. The horizontal brackets indicate a statistically significant main effect of age over weeks 19–76 of age (repeated-measures ANOVA; $^{*}p < 0.05$; $^{**}p < 0.01$). Data at 88–90 weeks of age are presented, but were not included in the statistical analyses due to reduced numbers of mice/group (details in Statistical Analyses).

one Tg2576 mouse failed to entrain to the light cycle beginning at 80 weeks of age and sustained a free-running τ of ~24.2 h (data not shown).

Assessments of ultradian bout activity parameters likewise revealed important differences between genotypes and age. Tg2576 mice exhibited trends towards more total activity counts and fewer bouts/24 h than wild-type controls ($F_{1,9} = 4.8$, 4.0, respectively; $p < 0.10$; Figure 3). Activity bouts of Tg2576 mice contained more counts/bout and were longer in duration than those by wild-type mice ($F_{1,9} = 11.3$, 23.4, respectively; $p < 0.01$). As mice aged, the total number of counts, counts/bout, and bout length decreased, whereas the number of bouts/24 h increased ($F_{5,45} = 9.5$, 5.2, 7.1, respectively; $p < 0.001$). Activity induced by the novelty of a cage change was greater for Tg2576 mice than wild-type mice ($F_{1,9} = 6.2$; $p < 0.05$), and this response decreased with age ($F_{5,45} = 2.4$; $p < 0.05$). In each of these analyses, there was also no significant genotype x age interaction.

Average activity profiles calculated over the 40-day durations beginning at 60 weeks of age illustrate the greater amplitude of the daily activity rhythms of Tg2572 mice versus wild-type controls. The between-subject comparisons are characterized by substantial inter-individual variation, even within genotypes, as evident in individual plots of these profiles (Figure 4). Analysis of 40-day-long monitoring intervals permitted precise estimation of individual circadian waveforms with very small standard errors at any given timepoint. Further, comparison of individual activity records of representative mice close to the beginning and end of the study reveal that these marked individual differences were stable features of the locomotor activity rhythms (Figure 5). Although all mice were clearly nocturnal, some were characteristically active throughout the night, whereas others showed spans of nocturnal inactivity (Tg#77 vs. Tg#72). There were also marked individual differences in the presence and robustness of activity after lights-on (e.g., WT#79 vs. Tg#72). Stability of the individual differences is evident by comparing the top and bottom panels of the actograms, each of which represents three months of data beginning about one year apart.

Despite stable rhythm signatures over time, activity rhythms did change with aging, again with substantial individual differences in how they did so even within the same genotype. As represented by the selected individual plots, decreases (black) and increases (white) in activity in 1 h bins over the 24 h day/night cycle are compared to the young adult baseline at weeks 17–23 of age (Figure 6). In one Tg2576 mouse (#76), activity levels decreased from baseline during most of the dark phase and increased in much of the light phase, i.e., a general flattening of the nocturnal rhythm. In a second Tg2576 mouse (#72), nighttime activity decreased during the first few hours, whereas the remainder of the night showed increases that were subsequently reversed except

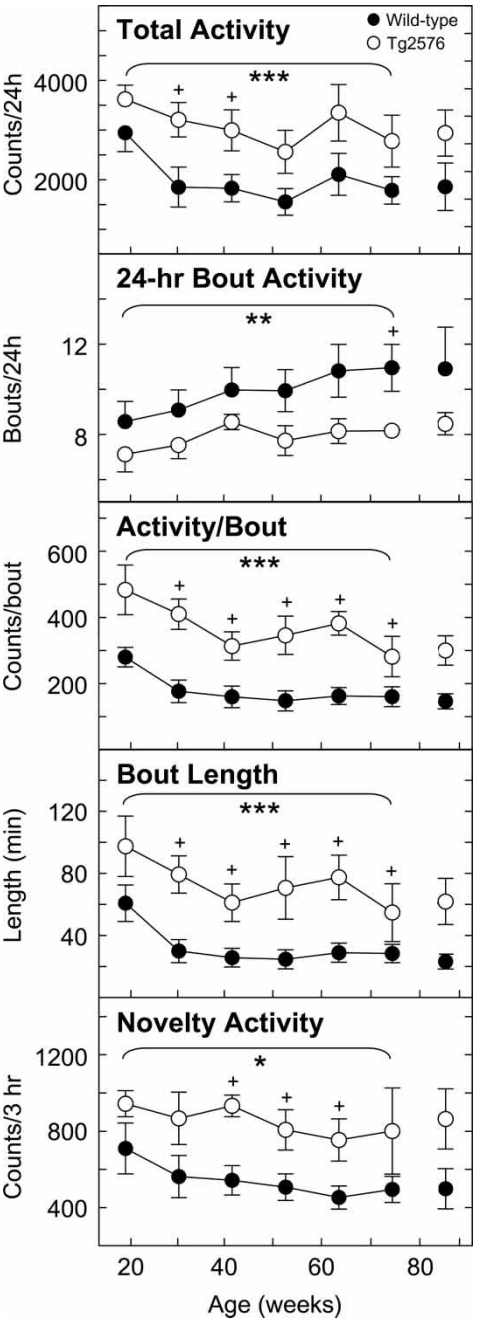

FIGURE 3 Mean ± SEM of various measures of ultradian activity for bouts and novelty induced by the cage change each week in wild-type controls (closed symbols, n = 6) and Tg2576 mice (open symbols, n = 5). The " $^{+}$ " symbol indicates $p < 0.05$ versus same age WT controls following a significant main effect of genotype in the repeated-measures ANOVA. The horizontal brackets indicate a statistically significant main effect of age over weeks 19–76 of age (repeated-measures ANOVA; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$). Data at 88–90 weeks of age were not included in the statistical analyses due to reduced numbers of mice/group (details in Statistical Analyses).

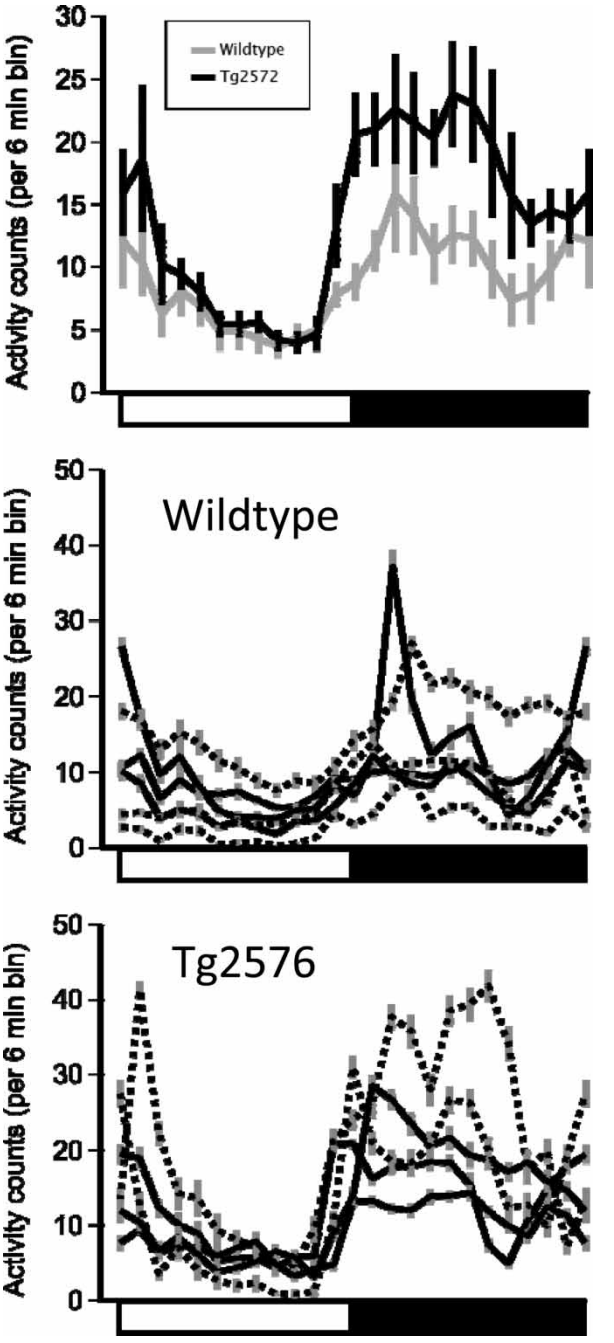

FIGURE 4 Mean ± SEM (between-subject) daily waveforms in wildtype and Tg2576 mice at 60 weeks of age (top panel) and mean ± SEM (within-subject) daily waveforms of activity in individual Tg2576 (middle panel) and wild-type (lower panel) animals over the same interval. Each plot consists of 24 data points, representing the average activity level during each clock hour calculated over 41 days beginning at 54 weeks of age. The light/dark cycle is depicted below the abscissa as the open/dark bar.

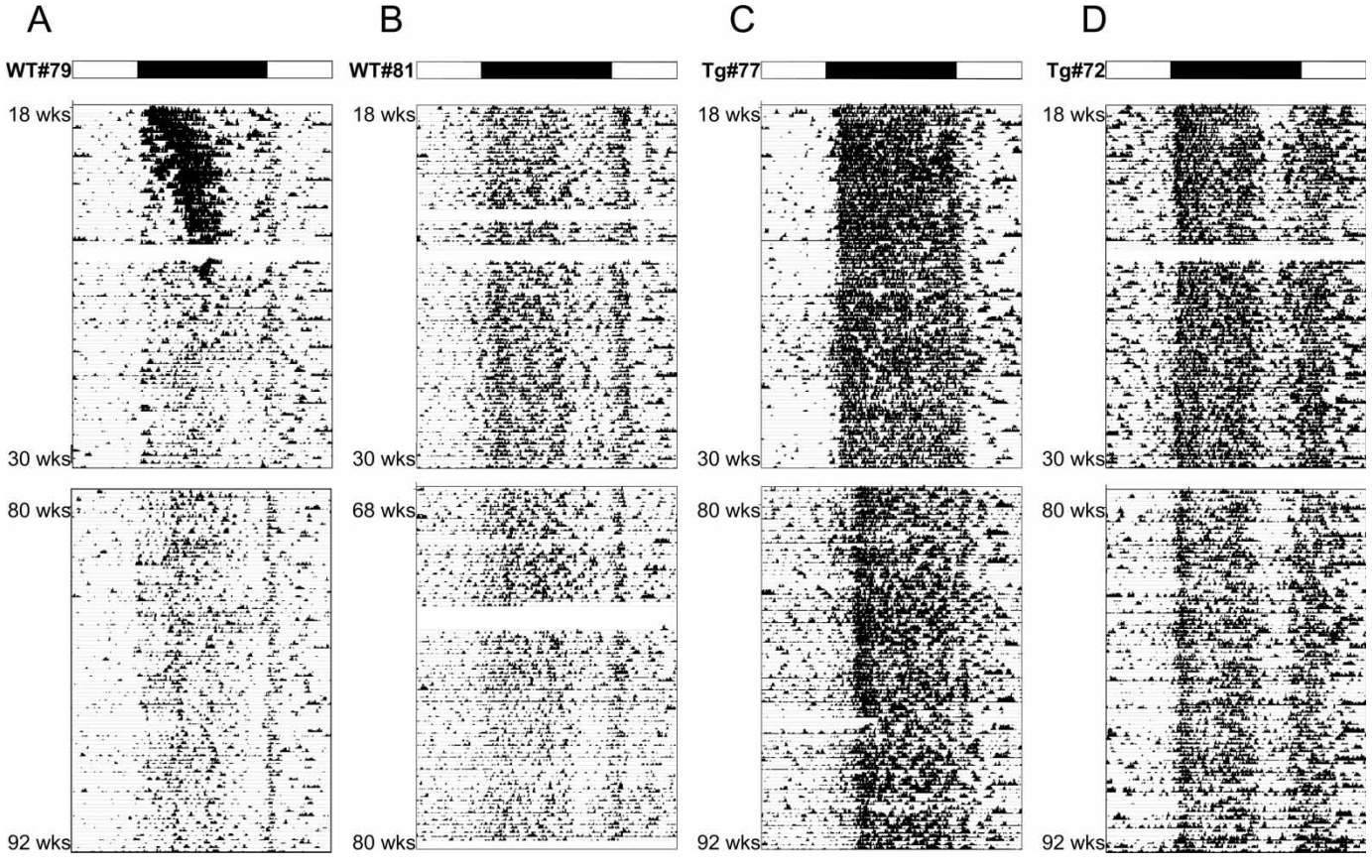

FIGURE 5 Representative passive locomotor activity records of two wild-type (#79, #81) and two Tg2576 (#77, #72) mice near the beginning and end of the study. Age in weeks is specified. Actograms are scaled identically from 0–6 counts/min. Light/dark cycle is indicated in bar above each actogram.

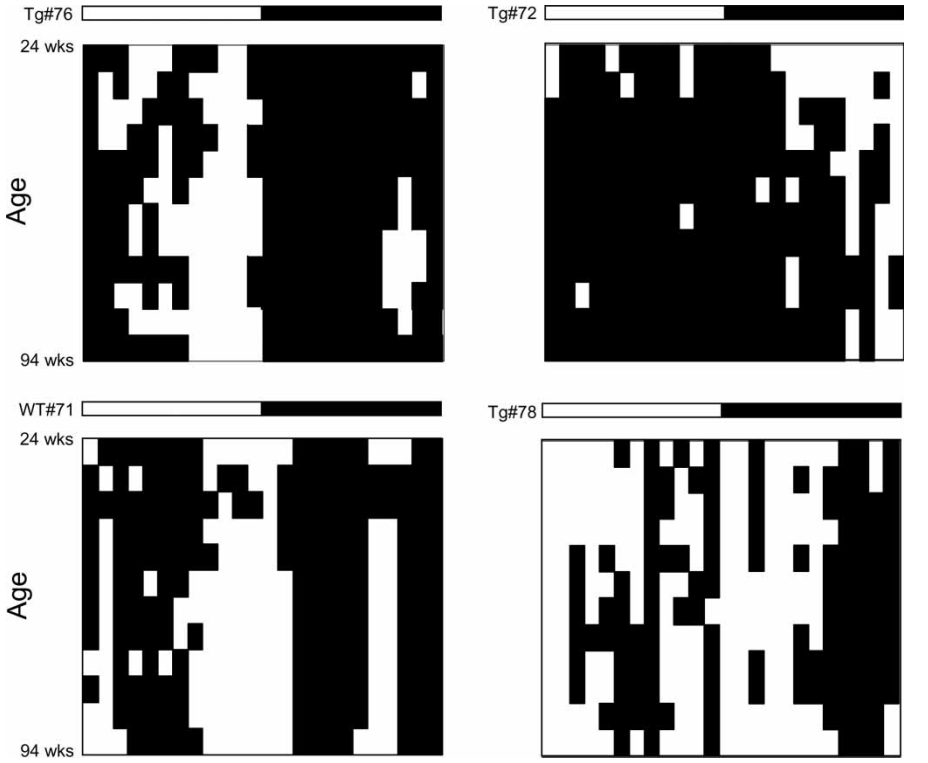

FIGURE 6 Plot of individual circadian change across the lifespan in three Tg2576 mice (#76, #72, and #78) and one wild-type control (#71). The light/dark cycle is depicted in the open and dark bars above each panel with each box representing a 1 h × 6 week timeframe. Black or white indicates a decrease or increase, respectively, in the total activity counts relative to each individual's young adult baseline (17–23 weeks of age). Details of this lifespan analysis of circadian activity are described in Methods.

around the lights-on transition. The overall patterns of day/night change in these two mice were almost mirror images. Wild-type mouse #71, which resembles Tg2576 mouse #76 in its pattern of change, illustrates that these individual differences are not particular to the genotype. For the final mouse illustrated (Tg2576 mouse #78), intervals of increase versus decrease were more balanced. Activity increases predominated in the first half of both the dark and light phases, whereas decreases were concentrated late in the latter half of the night. Although differences in activity between individuals were pronounced, no mouse exhibited a random pattern of increase or decrease across the day/night cycle.

Evidence of amyloidosis was apparent in cortical regions of the brain of Tg2576 mice, but not wild-type controls. Amyloid plaques were abundant in the many regions of the brain of transgenic mice, including the cortex, caudate, and hippocampus, but absent in the brains of aged wild-type controls (Figure 7, top panels). In the SCN, specifically, as well as other areas

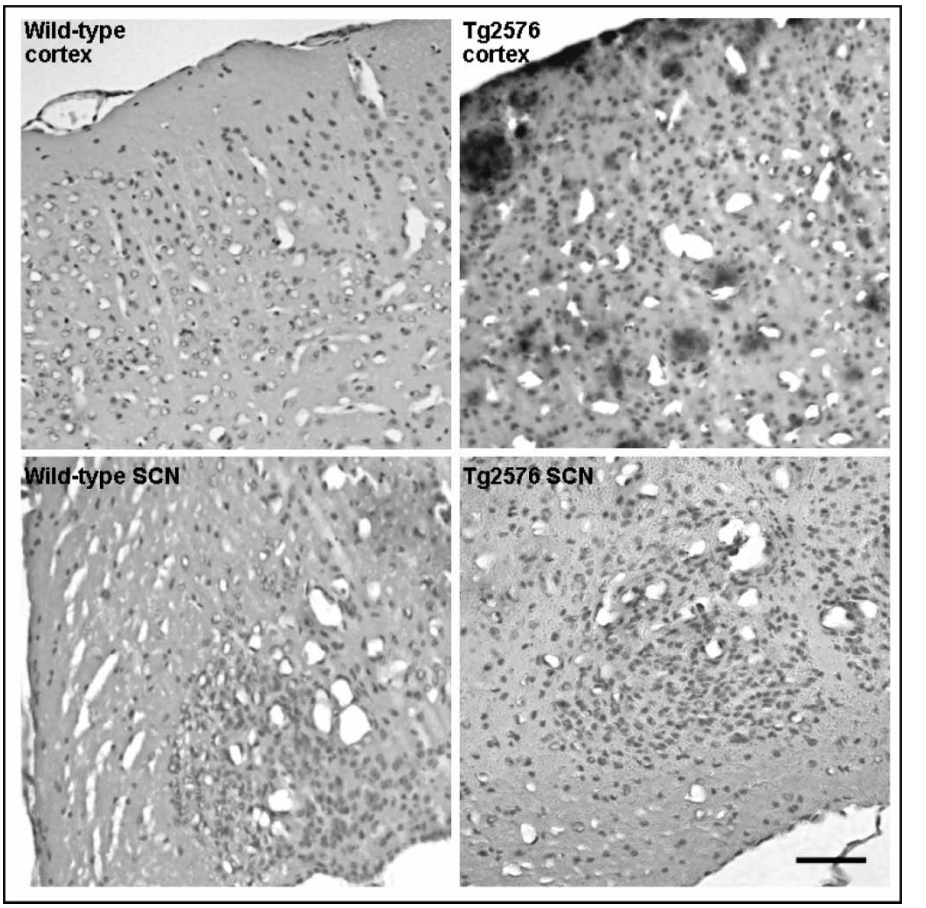

FIGURE 7 Photomicrographs of cortex (top) and suprachiasmatic nucleus (bottom) of the hypothalamus from an aged wild-type control or Tg2576 mouse at 96 weeks of age. The presence of amyloid beta stain is indicated by dark blotches of plaque in cortex from the Tg2576 mouse. Scale bar = 50 μm.

of the hypothalamus, no evidence of amyloid beta stain in aged wild-type or Tg2576 mice was found (Figure 7, bottom panels). Neither fine fibrils of amyloid within a circumscribed area, as described in SCN of AD patients (van de Nes et al., 1998) nor an amyloid core, characteristic of the plaques in the cortex of Tg2576 mice, were present. Thus, distribution of amyloid beta was specifically found in characteristic areas of the cortex (frontal and temporal) and hippocampus in aged Tg2576 mice, but not present in these areas or other regions of the brain of aged wild-type controls.

DISCUSSION

The present study was designed to assess activity patterns in mice with age and in age-related amyloid-induced neuropathology. Amyloidosis

in aged Tg2576 mice markedly increases in cortical brain regions in Tg2576 mice between 7 and 12 months of age (Hsiao et al., 1996; Irizarry et al., 1997) and is associated with deterioration of cognitive performance (Zhuo et al., 2008). Similarly, in the present study, numerous amyloid plaques were present in characteristic cortical brain centers in all Tg2576 mice at the conclusion of the study. Deposits of these predominant forms of amyloid beta (1-40 and 1-42) were not found in the SCN or surrounding hypothalamus, consistent with a previous postmortem report on human AD patients (van de Nes et al., 1998). Whether stereological analyses might suggest neuropathology in the SCN in the present or other studies is uncertain, but there was no suggestion of detectable changes in the classical indices of circadian pacemaker functions here or after microinjections of amyloid beta into the SCN of hamsters (Furio et al., 2002). These findings also do not exclude the possibility that other forms of amyloid might contribute to altered functions by clock-related neurons or other aspects of neuropathology in the Tg2576 mouse, a model that reflects some, but not all aspects of AD (Irizarry et al., 1997; Lewis et al., 2001; Morrissette et al., 2008). Nevertheless, the findings suggest that circadian pacemaker function can be sustained with age and with age-related increased amyloid load in cortical brain regions in a mouse model for neuropathology.

Daily rhythms in activity, physiological processes, sleep, or other behaviors, reflect the interaction of the circadian pacemaker with downstream elements, which themselves can be influenced by internal and external variables. Assessments of timekeeping by the endogenous clock often rely upon indices of suprachiasmatic nucleus function that include the free-running τ, as well as locomotor activity onset and offset. The τ of circadian rhythmicity in constant conditions is considered to be among the most direct assessments of the circadian clock and, in the present study, this measure along with other indices of SCN function did not differ between wild-type and Tg2576 mice at 46–47 weeks of age. By contrast, Wisor et al. (2005) found that the circadian τ of the wheel-running rhythm was $\sim$0.25 h longer in Tg2576 mice than in littermate controls. However, the τ of the wheel-running rhythm in Tg2576 mice was the same as that in the present study, which passively monitored activity ($\sim$24.1 h). The shorter τ of activity in wild-type controls ($\sim$23.8 vs. 24.2 h in the present study) accounted for the statistical difference compared to Tg2576 mice in Wisor et al. (2005). Since the presence of a running wheel shortens the free-running τ by $\sim$0.5 h in mice (Edgar et al., 1991a, 1991b), these findings suggest that the wheel running may differentially affect this aspect of pacemaker timekeeping in wild-type and transgenic mutant mice. Moreover, activity onset, another classic measure of entrainment of the circadian rest/activity cycle, did not differ between groups in the present study, which analyzed 6 min bins of activity over 480 h

intervals across a lifespan of daily monitoring. This corroborates, with substantially more resolution, a previous report that evaluated wheel-running activity in 3 h bins over a 72 h duration (Wisor et al., 2005). Together, the findings support the conclusion that important aspects of circadian timekeeping are sustained with age and irrespective of the amyloid-depositing genotype.

Whereas indices of circadian pacemaker function are not demonstrably altered in Tg2576 mice, other measures of locomotor activity did depend upon genotype. The amount of activity over the 24 h, the Statistical Power of the activity rhythm, and bouts of activity throughout the day were significantly altered over the lifespan of Tg2576 mice compared to wild-type controls. More bouts and greater activity/bout occurred in Tg2576 mice compared to controls. This finding across age is consistent with a previous report of increased locomotor activity in adult Tg2576 mice (Gil-Bea et al., 2007) and AD patients (Satlin et al., 1991; Skene & Swaab, 2003) and suggests that amyloidosis in brain regions downstream from the pacemaker, i.e., outside the SCN, may counter declines with age in overall activity. The results also highlight the importance of considering individual differences in assessments of age effects on activity and suggest that amount of activity/bout, i.e., vigilance, as well as duration of bouts may be critical characteristics associated with amyloidosis in specific brain regions.

In addition, novelty activity induced by a cage change was significantly greater in transgenic mice than wild-type controls. Transgenic mice that express a variety of mutant forms of amyloid beta spend more time in the open arms and have a higher number of entries in the open arms of the plus-maze than wild-type controls (Lalonde et al., 2003; Ognibene et al., 2005). These anxiolytic-like behaviors parallel the increase in disinhibition that reflects spatial memory deficits reported by others and where an increase in the average amount of exploratory activity was found in Tg2576 compared to wild-type controls. Responses to novelty appear to be construct-specific, as activity was decreased in mice with amyloidosis in the 2 h after mice were transferred to cages for circadian monitoring (Vloeberghs et al., 2004).

Beyond activity levels, the temporal organization of daily activity was altered in transgenic mice. Bouts of activity in these mice lasted longer, and, as a result, individual bouts contained more total counts with fewer bouts each day. Although changes in bout structure with age have been noted in wild-type mice (Valentinuzzi et al., 1997), such measures of the local timing of activity have received much less empirical attention than standard circadian assessments (Davis & Menaker, 1980; Penev et al., 1997; Valentinuzzi et al., 1997). In the primate mouse lemur, the locomotor rhythm amplitude declines and daytime bouts of activity increase with age—analogous to observations of age-associated fragmentation and attenuation of activity rhythms in humans (Aujard et al., 2006). The classical circadian pacemaker

determines the onset and offset of nocturnal activity, while other timing mechanisms—circadian, ultradian, or homeostatic—dictate the structure of local activity bouts (Davis & Menaker, 1980). Much more work is required to determine the mechanistic basis for changes in the ultradian structure of rest/activity cycles with aging, as well as with age-associated neuropathology and cognitive impairments in humans (Nagels et al., 2006). Dynamic changes in production or clearance of amyloid may also be a consideration for age-related changes in ultradian activity patterns (Batemen et al., 2007). Long-term studies of rest/activity patterns in aging humans could confirm the value of bout analyses as diagnostic of neuropathology.

The significant findings of this study were restricted to the main effects of age and of genotype. The lack of any significant age × genotype interactions indicates that there was no evidence that the aging process differed by genotype. This is consistent with the evidence in the present study that longevity was not compromised in Tg2576 mice compared to that of wild-type controls. Tests of statistical interactions are typically less strongly powered than are main effects and, for example, a much larger sample size could provide statistical support for a differential aging pattern (e.g., Tg2576 mice at 40 weeks of age had the same number of bouts and counts per day as wild-type mice at 20 weeks). Regardless, the data do not suggest that any aspect of behavioral aging was accelerated in Tg2576 mice as might have been expected with progression of an age-dependent neuropathology.

Emphasis has been placed here on individual differences at a given point in time (Figure 4) and in the patterns of change across the lifespan (Figure 6) without regard to genotype. A relatively small sample size precludes statistical characterization of early individual differences as predictors of subsequent activity as in one of the few longitudinal studies of aging and activity rhythms (Pang et al., 2004). Instead, our purpose here is to draw attention to and illustrate the idea that aging produces changes in activity rhythm waveforms that are particular to individuals (Figure 4) beyond those that are common to groups (Figures 2–4), an observation that is possible only with longitudinal analysis of activity rhythms. Besides amplitude, τ, and specific markers of phase (e.g., activity onset and offset) of the activity rhythm, there is a recognizable consistency in waveform shape of the activity rhythm of individuals (i.e., rhythm signatures). Additionally, with aging, these rhythms change gradually and according to different patterns between individuals. No animal, however, showed either randomly distributed or across-the-board increases or decreases in activity. Changes in activity patterning (e.g., a decrease in activity in the middle of the active phase or particular to late night) that occur outside of the classic domain of circadian analyses could conceivably be equally or more relevant for successful human aging than are canonical measures of the circadian pacemaker. The judicious use of inbred, outbred, and hybrid

mouse strains should facilitate an understanding of individual differences in activity patterning, as well as the circadian aging process in humans (Irizarry et al., 2001; Morrissette et al., 2008; Tankersley et al., 2002).

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REFERENCES

Ambree O, Touma C, Gortz N, Keyvani K, Paulus W, Palme R, Sachser N. (2006). Activity changes and marked stereotypic behavior precede Abeta pathology in TgCRND8 Alzheimer mice. *Neurobiol. Aging* 27:955–964.

Aujard F, Cayetanot F, Bentivoglio M, Perret M. (2006). Age-related effects on the biological clock and its behavioral output in a primate. *Chronobiol. Int.* 23:451–460.

Bateman RJ, Wen S, Morris JC, Holtzman DM. (2007). Fluctuations of CSF amyloid-β levels Implications for a diagnostic and therapeutic biomarker *Neurology* 68:666–669.

Benloucif S, Masana MI, Dubocovich ML. (1997). Light-induced phase shifts of circadian activity rhythms and immediate early gene expression in the suprachiasmatic nucleus are attenuated in old C3H/HeN mice. *Brain Res.* 747:34–42.

Davis FC, Menaker M. (1980). Hamsters through time's window: Temporal structure of hamster locomotor rhythmicity. *Am. J. Physiol.* 239:R149–R155.

Duguay D, Cermakian N. (2009). The crosstalk between physiology and clock proteins. *Chronobiol. Int.* 26:1479–1513.

Duyckaerts C, Delatour B, Potier MC. (2009). Classification and basic pathology of Alzheimer disease. *Acta Neuropathol.* 118:5–36.

Edgar DM, Kilduff TS, Martin CE, Dement WC. (1991a). Influence of running wheel activity on free-running sleep/wake and drinking circadian rhythms in mice. *Physiol. Behav.* 50:373–378.

Edgar DM, Martin CE, Dement WC. (1991b). Activity feedback to the mammalian circadian pacemaker: Influence on observed measures of rhythm period length. *J. Biol. Rhythms* 6:185–199.

Furio AM, Cutrera RA, Castillo TV, Perez LS, Riccio P, Caccuri RL, Brusco LL, Cardinali DP. (2002). Effect of melatonin on changes in locomotor activity rhythm of Syrian hamsters injected with beta amyloid peptide 25–35 in the suprachiasmatic nuclei. *Cell. Mol. Neurobiol.* 22:699.

Gil-Bea FJ, Aisa B, Schliebs R, Ramirez MJ. (2007). Increase of locomotor activity underlying the behavioral disinhibition in tg2576 mice. *Behav. Neurosci.* 121:340–344.

Goudsmit E, Hofman MA, Fliers E, Swaab DF. (1990). The supraoptic and paraventricular nuclei of the human hypothalamus in relation to sex, age and Alzheimer's disease. *Neurobiol. Aging* 11:529–536.

Harper DG, Volicer L, Stopa EG, McKee AC, Nitta M, Satlin A. (2005). Disturbance of endogenous circadian rhythm in aging and Alzheimer disease. *Am. J. Geriatr. Psychiatry* 13:359–368.

Hebert R, Dubois MF, Wolfson C, Chambers L, Cohen C. (2001). Factors associated with long-term institutionalization of older people with dementia: Data from the Canadian Study of Health and Aging. *J. Gerontol. A Biol. Sci. Med. Sci.* 56:M693–M699.

Hsiao K, Chapman P, Nilsen S, Eckman C, Harigaya Y, Younkin S. Yang F, Cole G. (1996). Correlative memory deficits, Abeta elevation, and amyloid plaques in transgenic mice. *Science* 274:99–102.

Huitron-Resendiz S, Sanchez-Alavez M, Gallegos R, Berg G, Crawford E, Giacchino JL, Games D, Henriksen SJ, Criado JR. (2002). Age-independent and age-related deficits in visuospatial learning, sleep-wake states, thermoregulation and motor activity in PDAPP mice. *Brain Res.* 928:126–137.

Irizarry MC, McNamara M, Fedorchak K, Hsiao K, Hyman BT. (1997). APPSw transgenic mice develop age-related A beta deposits and neuropil abnormalities, but no neuronal loss in CA1. *J. Neuropathol. Exp. Neurol.* 56:965–973.

Irizarry MC, Locascio JJ, Hyman BT. (2001). Beta-site APP cleaving enzyme mRNA expression in APP transgenic mice: Anatomical overlap with transgene expression and static levels with aging. *Am. J. Pathol.* 158:173–177.

Kawarabayashi T, Younkin LH, Saido TC, Shoji M, Ashe KH, Younkin SG. (2001). Age-dependent changes in brain, CSF, and plasma amyloid (beta) protein in the Tg2576 transgenic mouse model of Alzheimer's disease. *J. Neurosci.* 21:372–381.

Klaffke S, Staedt J. (2006). Sundowning and circadian rhythm disorders in dementia. *Acta Neurol. Belg.* 106:168–175.

Lalonde R, Lewis TL, Strazielle C, Kim H, Fukuchi K. (2003). Transgenic mice expressing the betaAPP695SWE mutation: Effects on exploratory activity, anxiety, and motor coordination. *Brain Res.* 977:38–45.

Lee JH, Bliwise DL, Ansari FP, Goldstein FC, Cellar JS, Lah JJ, Levey AI. (2007). Daytime sleepiness and functional impairment in Alzheimer disease. *Am. J. Geriatr. Psychiatry* 15:620–626.

Lee KW, Kim JB, Seo JS, Kim TK, Im JY, Baek IS, Kim KS, Lee JK, Han PL. (2009). Behavioral stress accelerates plaque pathogenesis in the brain of Tg2576 mice via generation of metabolic oxidative stress. *J. Neurochem.* 108:165–175.

Lewis J, Dickson DW, Lin WL, Chisholm L, Corral A, Jones G, Yen SH, Sahara N, Skipper L, Yager D, Eckman C, Hardy J, Hutton M, McGowan E. (2001). Enhanced neurofibrillary degeneration in transgenic mice expressing mutant tau and APP. *Science* 293:1487–1491.

Liu RY, Zhou JN, Hoogendijk WJ, van Heerikhuize J, Kamphorst W, Unmehopa UA, Hofman MA, Swaab DF. (2000). Decreased vasopressin gene expression in the biological clock of Alzheimer disease patients with and without depression. *J. Neuropathol. Exp. Neurol.* 59:314–322.

Mahlberg R, Kunz D, Sutej I, Kuhl KP, Hellweg R. (2004). Melatonin treatment of day-night rhythm disturbances and sundowning in Alzheimer disease: An open-label pilot study using actigraphy. *J. Clin. Psychopharmacol.* 24:456–459.

Martin J, Marler M, Shochat T, Ancoli-Israel S. (2000). Circadian rhythms of agitation in institutionalized patients with Alzheimer's disease. *Chronobiol. Int.* 17:405–418.

Morrissette DA, Parachikova A, Green KN, Laferla FM. (2008). Relevance of transgenic mouse models to human Alzheimer disease. *J. Biol. Chem.* 284:6033–6037.

Nagels G, Engelborghs S, Vloeberghs E, Van Dam D, Pickut BA, De Deyn PP. (2006). Actigraphic measurement of agitated behaviour in dementia. *Int. J. Geriatr. Psychiatry* 21:388–393.

Ognibene E, Middei S, Daniele S, Adriani W, Ghirardi O, Caprioli A, Laviola G. (2005). Aspects of spatial memory and behavioral disinhibition in Tg2576 transgenic mice as a model of Alzheimer's disease. *Behav. Brain Res.* 156:225–232.

Oosterman JM, van Someren EJ, Vogels RL, Van Hartens B, Scherder EJ. (2009). Fragmentation of the rest-activity rhythm correlates with age-related cognitive deficits. *J. Sleep Res.* 18:129–135.

Pang KC, Miller JP, McAuley JD. (2004). Circadian rhythms in SAMP8: A longitudinal study of the effects of age and experience. *Neurobiol. Aging* 25:111.

Penev PD, Zee PC, Turek FW. (1997). Quantitative analysis of the age-related fragmentation of hamster 24-h activity rhythms. *Am. J. Physiol.* 273:R2132–R2137.

Portaluppi F, Touitou Y, Smolensky MH. (2008). Ethical and methodological standards for laboratory and medical biological rhythm research. *Chronobiol. Int.* 25:999–1016.

Refinetti R. (2006). *Circadian physiology*. Boca Raton, FL: CRC Press/Taylor & Francis Group.

Satlin A, Teicher MH, Lieberman HR, Baldessarini RJ, Volicer L, Rheaume Y. (1991). Circadian locomotor activity rhythms in Alzheimer's disease. *Neuropsychopharmacology* 5:115–126.

Scarbrough K, Losee-Olson S, Wallen EP, Turek FW. (1997). Aging and photoperiod affect entrainment and quantitative aspects of locomotor behavior in Syrian hamsters. *Am. J. Physiol.* 272:R1219–R1225.

Selkoe DJ. (2001). Alzheimer's disease: Genes, proteins, and therapy. *Physiol. Rev.* 81:741–766.

Skene DJ, Swaab DF. (2003). Melatonin rhythmicity: Effect of age and Alzheimer's disease. *Exp. Gerontol.* 38:199–206.

Sokolove PG, Bushell WN. (1978). The chi square periodogram: Its utility for analysis of circadian rhythms. *J. Theor. Biol.* 72:131–160.

Standaert DG, Lee VM, Greenberg BD, Lowery DE, Trojanowski JQ. (1991). Molecular features of hypothalamic plaques in Alzheimer's disease. *Am. J. Pathol.* 139:681–691.

Stopa EG, Volicer L, Kuo-Leblanc V, Harper D, Lathi D, Tate B, Satlin A. (1999). Pathologic evaluation of the human suprachiasmatic nucleus in severe dementia. *J. Neuropathol. Exp. Neurol.* 58:29–39.

Swaab DF, Fliers E, Partiman TS. (1985). The suprachiasmatic nucleus of the human brain in relation to sex, age and senile dementia. *Brain Res.* 342:37–44.

Tankersley CG, Irizarry R, Flanders S, Rabold R. (2002). Circadian rhythm variation in activity, body temperature, and heart rate between C3H/HeJ and C57BL/6J inbred strains. *J. Appl. Physiol.* 92:870–877.

Valentinuzzi VS, Scarbrough K, Takahashi JS, Turek FW. (1997). Effects of aging on the circadian rhythm of wheel-running activity in C57BL/6 mice. *Am. J. Physiol.* 273:R1957–R1964.

van de Nes JA, Kamphorst W, Ravid R, Swaab DF. (1998). Comparison of beta-protein/A4 deposits and Alz-50-stained cytoskeletal changes in the hypothalamus and adjoining areas of Alzheimer's disease patients: Amorphic plaques and cytoskeletal changes occur independently. *Acta Neuropathol.* 96:129–138.

van Someren EJ, Swaab DF, Colenda CC, Cohen W, McCall WV, Rosenquist PB. (1999). Bright light therapy: Improved sensitivity to its effects on rest-activity rhythms in Alzheimer patients by application of nonparametric methods. *Chronobiol. Int.* 16:505–518.

Vloeberghs E, Van Dam D, Engelborghs S, Nagels G, Staufenbiel M, De Deyn P. (2004). Altered circadian locomotor activity in APP23 mice: A model for BPSD disturbances. *Eur. J. Neurosci.* 20:2757–2766.

Volicer L, Harper DG, Manning BC, Goldstein R, Satlin A. (2001). Sundowning and circadian rhythms in Alzheimer's disease. *Am. J. Psychiatry* 158:704–711.

Wisor JP, Edgar DM, Yesavage J, Ryan HS, McCormick CM, Lapustea N, Murphy GM. (2005). Sleep and circadian abnormalities in a transgenic mouse model of Alzheimer's disease: A role for cholinergic transmission. *Neuroscience* 131:375–385.

Witting W, Kwa IH, Eikelenboom P, Mirmiran M, Swaab DF. (1990). Alterations in the circadian rest-activity rhythm in aging and Alzheimer's disease. *Biol. Psychiatry* 27:563–572.

Wu YH, Zhou JN, van Heerikhuize J, Jockers R, Swaab DF. (2007). Decreased MT1 melatonin receptor expression in the suprachiasmatic nucleus in aging and Alzheimer's disease. *Neurobiol. Aging* 28:1239–1247.

Yuede CM, Zimmerman SD, Dong H, Kling MJ, Bero AW, Holtzman DM, Timson BF, Csernansky JG. (2009). Effects of voluntary and forced exercise on plaque deposition, hippocampal volume, and behavior in the Tg2576 mouse model of Alzheimer's disease. *Neurobiol. Dis.* 35:426–432.

Zelcer N, Khanlou N, Clare R, Jiang Q, Reed-Geaghan EG, Landreth GE, Vinters HV, Tontonoz P. (2007). Attenuation of neuroinflammation and Alzheimer's disease pathology by liver x receptors. *Proc. Natl. Acad. Sci. USA* 104:10601–10606.

Zhuo JM, Prakasam A, Murray ME, Zhang, HY, Baxter MG, Sambamurti K, Nicolle MM. (2008). An increase in Abeta42 in the prefrontal cortex is associated with a reversal-learning impairment in Alzheimer's disease model Tg2576 APPsw mice. *Curr. Alzheimer Res.* 5:385–391.