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Behavioral and SCN neurophysiological disruption in the Tg-SwDI mouse model of Alzheimer's disease

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Abstract

Disruption of circadian rhythms is commonly reported in individuals with Alzheimer's disease (AD). Neurons in the primary circadian pacemaker, the suprachiasmatic nucleus (SCN), exhibit daily rhythms in spontaneous neuronal activity which are important for maintaining circadian behavioral rhythms. Disruption of SCN neuronal activity has been reported in animal models of other neurodegenerative disorders; however, the effect of AD on SCN neurophysiology remains unknown. In this study we examined circadian behavioral and electrophysiological changes in a mouse model of AD, using male mice from the Tg-SwDI line which expresses human amyloid precursor protein with the familial Swedish (K670N/M671L), Dutch (E693Q), Iowa (D694N) mutations. The free-running period of wheel-running behavior was significantly shorter in Tg-SwDI mice compared to wild-type (WT) controls at all ages examined (3, 6, and 10 months). At the SCN level, the day/night difference in spike rate was significantly dampened in 6–8 month-old Tg-SwDI mice, with decreased AP firing during the day and an increase in neuronal activity at night. The dampening of SCN excitability rhythms in Tg-SwDI mice was not associated with changes in input resistance, resting membrane potential, or action potential afterhyperpolarization amplitude; however, SCN neurons from Tg-SwDI mice had significantly reduced A-type potassium current (I_A) during the day compared to WT cells. Taken together, these results provide the first evidence of SCN neurophysiological disruption in a mouse model of AD, and highlight I_A as a potential target for AD treatment strategies in the future.

Keywords

Alzheimer's disease; suprachiasmatic nucleus; circadian rhythms; A-type potassium current; electrophysiology

INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative form of dementia associated with elevated levels of the amyloid β ($A\beta$) peptide and the formation of pathological $A\beta$

plaques and neurofibrillary tangles (Selkoe, 1999). The level of circulating A β , which is also found in the cerebrospinal fluid of (CSF) of healthy individuals, is tightly coupled to sleep/wake cycles and is molecular clock-dependent (Kress et al., 2018), with A β levels increasing throughout the activity phase of humans and rodents (Kang et al., 2009). Furthermore, prolonged sleep deprivation has been found to potentiate A β plaque formation in multiple mouse models of AD (Kang et al., 2009; Roh et al., 2012). Patients with AD often exhibit “sundowning syndrome,” a constellation of symptoms including late afternoon/evening delirium, hyperactivity, restlessness, confusion, and aggression, along with misaligned core body temperature and activity rhythms (Volicer et al., 2001; Khachiyants et al., 2011). These symptoms suggest a dysregulated circadian network, which regulates sleep/wake timing and allows anticipation of and preparation for daily recurring environmental events. Indeed, circadian rhythm disruption has been demonstrated in numerous mouse models of AD (Sterniczuk et al., 2010; Stranahan, 2012; Coogan et al., 2013; Oyegbami et al., 2017).

In mammals, the primary circadian pacemaker is located in the suprachiasmatic nucleus (SCN) of the hypothalamus. SCN neurons exhibit daily rhythms in spontaneous action potential (AP) firing which are critical for robust and consolidated circadian behavior (Schwartz et al., 1987). Loss or dampening of SCN neuronal activity rhythms has been described in animal models of other neurodegenerative disorders (Kudo et al., 2011a; Kudo et al., 2011b) and aging (Nakamura et al., 2011; Farajnia et al., 2012); however, changes in SCN neurophysiology in AD models have not yet been examined. Therefore, in the present study, we sought to determine whether changes in SCN neuronal excitability are associated with circadian behavioral disruption in a mouse model of AD and to identify the ionic mechanism driving these neurophysiological changes.

MATERIALS AND METHODS

Animals

Male mice expressing the human amyloid precursor protein with the familial Swedish (K670N/M671L), Dutch (E693Q), Iowa (D694N) homozygous mutations (Tg-SwDI; (Davis et al., 2004); commercially available from Mutant Mouse Research and Resource Center JAX or MMRRRC; Stock #034843), congenic on a C57-BL6/J background were bred within the University of Alabama at Birmingham (UAB) animal colony and compared to age-matched C57-BL6/J wild-type (WT) mice (generated within the colony or purchased from Jackson Laboratories, Bar Harbor, ME) for all experiments. Female mice were not used to avoid the potential effect of estrous on circadian behavior (Leise and Harrington, 2011) and A-type potassium current (I_A; (Pielecka-Fortuna et al., 2011)). Animals were housed in a 12:12 light/dark cycle (LD) or constant dark (DD) with food and water *ad libitum* in accordance with UAB IACUC guidelines and the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978). All animals were euthanized with cervical dislocation and rapid decapitation. For loose-patch experiments, mice were individually housed in cages with running wheels for at least three weeks in DD and sacrificed and enucleated in the dark with the aid of night-vision goggles at circadian time (CT; where CT 12 is defined as the onset of activity) 4 or 16. For whole-

cell recordings, mice were group-housed in LD and sacrificed between zeitgeber time (ZT; where ZT 0 equals time of lights-on) 1–2 for day recordings or 11–12 for night recordings.

Behavior

Mice were individually housed with running wheels starting at 3 months or 6 months of age. The same animals were used for the 6 and 10 month old behavioral measurements, and the animals were re-entrained to LD for at least 1 month prior to beginning behavioral measurements at 10 months of age. Wheel-running activity was recorded and analyzed using ClockLab software (Actimetrics, Wilmette, IL) as described previously (Paul et al., 2012). Behavior was analyzed across 10 days of activity after the mice had been in DD for 7–10 days. The free-running period was determined by chi-squared periodogram analysis. Activity onset was fit by eye and activity offset was defined as the last point at which the activity in three of the previous six bins exceeded the mean activity level.

Electrophysiology

Fresh, coronal brain slices containing the SCN were prepared from Tg-SwDI and WT mice (6–8 months) using previously published methods (as in (Paul et al., 2012)). Loose-patch recordings were performed between CT 6–8 or CT 18–20. All whole-cell recordings were performed from ZT 4–8 or ZT 14–18. Resting membrane potential and input resistance were measured in current clamp mode (as in (Paul et al., 2016)). Persistent sodium current (I_{NaP}) was measured using a slow depolarizing voltage ramp (–100mV to +10mV, 59mV/s) and analyzed using previously described methods (as in (Paul et al., 2016)). A-type potassium current (I_A) was measured in the presence of TEA (1 mM, in place of 1 mM NaCl), bicuculline (20 μ M), tetrodotoxin (0.5 μ M) and CdCl₂ (25 μ M) using progressive depolarizing voltage steps from –40 mV to 40 mV (at 5 mV steps) from a holding potential of –90 mV and again from a holding potential of –30 mV (to inactivate I_A). I_A was isolated by subtracting the cell response to the second step protocol from that of the first step protocol using offline subtraction. I_A recordings were limited to cells in the dorsomedial SCN which have been previously shown to exhibit circadian changes in I_A (Itri et al., 2010).

Data analysis and availability. All statistics were calculated using SPSS 22 software. Data were analyzed with independent samples t-tests, two-way ANOVAs and mixed-design two-way ANOVAs. In the event that assumptions of normality and homogenous variances were not met, a nonparametric Kruskal-Wallis test was used instead. Significance was ascribed at $p < 0.05$. The data that support the findings of this study are available from the corresponding author upon reasonable request.

RESULTS

Circadian behavior is altered in Tg-SwDI as early as 3-months of age

To determine whether circadian behavior was disrupted in Tg-SwDI, as well as examine behavioral changes across disease progression, we monitored wheel-running behavior of Tg-SwDI and WT mice at 3, 6 and 10 months of age. In LD, both Tg-SwDI and WT mice demonstrated clear entrainment to the light cycle at all ages (Fig. 1A), with the majority of activity taking place during the dark phase (Table 1). At 3 months, Tg-SwDI mice exhibited

significantly higher activity compared to WT mice while in LD (independent samples t-test, $t_{11} = 3.086$, $p < 0.01$; Table 1), but this difference was lost when the animals were placed in DD (Fig. 1C; Table 2). At 10 months, the same increase in activity was seen in Tg-SwDI in LD (two-way mixed design ANOVA, significant genotype X age interaction, Table 3; Fisher's LSD post-hoc, $p < 0.05$) and in DD ($p < 0.005$; Table 3, Fig. 1C). There were no other significant differences found between genotypes in LD behavior (Table 1).

When placed in DD, several differences in circadian behavior were revealed (Fig. 1; Table 2). Most notably, the free-running period (FRP) of Tg-SwDI mice was significantly shorter than that of WT mice at all observed ages (3 mo.: independent samples t-test, $t_{11} = 2.208$, $p < 0.05$; 6 and 10 mo.: two-way mixed design ANOVA, main effect of genotype $p < 0.001$; Table 3; Fig. 1B). This difference was greatest at 6 months, when the FRP of Tg-SwDI activity was ~22 minutes shorter than in WT mice. Although the amplitude/strength of the behavioral rhythms did not differ between the two genotypes at any age as measured by the chi-squared periodogram power or number of activity bouts per day (Tables 2, 3), the organization of activity differed between the two genotypes. The interdaily variability of activity offset was significantly increased in Tg-SwDI mice across all ages (3 mo.: independent samples t-test, $t_{11} = 2.331$, $p < 0.05$; 6 and 10 mo.: main effect of genotype, $p < 0.001$ 10 mo., $t_{9.7} = 4.179$, $p < 0.005$; Fig. 1E), while there was decreased interdaily variability in the activity onset in Tg-SwDI 6 and 10 months of age (Fig. 1D; main effect of genotype, $p = 0.05$; Tables 2 and 3).

SCN neurophysiological rhythms are altered in Tg-SwDI

To examine whether SCN neuronal activity rhythms were also disrupted in a model of AD, we next conducted loose-patch electrophysiological recordings during the subjective day (CT 6–8) or subjective night (CT 18–20) in Tg-SwDI and WT mice that were housed in DD for at least 3 weeks. Both WT and Tg-SwDI neurons displayed the typical day/night differences in spontaneous firing rate (SFR), with higher activity during the day and lower activity at night (Fig. 2; two-way ANOVA, main effect of time, $F_{1,216} = 99.325$, $p < 0.001$). However the rhythm in SFR was dampened in Tg-SwDI mice (genotype×time interaction, $F_{1,216} = 13.385$, $p < 0.001$). This was especially prevalent during the subjective day, when Tg-SwDI neurons were firing at a rate nearly 2 Hz slower than WT neurons ($p < 0.001$).

We next sought to examine altered membrane properties in Tg-SwDI neurons that could explain this change in SCN neuronal activity. To do this, we conducted whole-cell current clamp recordings during the day (ZT 4–8) or night (ZT 14–18) in SCN neurons from Tg-SwDI and WT (Fig. 3). Surprisingly, Tg-SwDI neurons continued to show day/night differences in resting membrane potential (RMP; Fig. 3C) and input resistance (Rinput; Fig. 3D) similar to that seen in WT neurons (two-way ANOVA, main effect of time: RMP, $F_{1,59} = 4.102$, $p < 0.05$; Rinput, $F_{1,59} = 19.186$, $p < 0.001$; Fig. 3C,D) with day-night variation in SFR similar to that observed with loose patch (Fig. 2). Furthermore, the daily variation in the action potential after-hyperpolarization amplitude was present in both genotypes (two-way ANOVA, main effect of time, $F_{1,49} = 10.091$, $p < 0.005$; Fig. 3E), and there did not appear to be any other differences in the average action potential waveforms of each genotype (Fig. 3F).

We have recently reported that the persistent sodium current (I_{NaP}), a major contributor to the excitatory drive in SCN neurons (Pennartz et al., 1997; Jackson et al., 2004; Kononenko et al., 2004), is rhythmic within the SCN, and this rhythm contributes to the daily change in SCN neuronal activity independent of changes in RMP (Paul et al., 2016). Therefore, we next sought to examine I_{NaP} in Tg-SwDI and WT neurons during the day and the night using a slow depolarizing voltage ramp (as in Paul, et al., 2015; Fig. 4). Consistent with our previous findings, peak I_{NaP} magnitude was increased during the day, when SCN cells are more active (two-way ANOVA, main effect of time, $F_{1,84} = 5.246$, $p < 0.05$), but, surprisingly, the magnitude of I_{NaP} in Tg-SwDI neurons was not different from that of WT cells at either time (Fig. 4A).

A-type potassium current (I_A) is another current that exhibits a daily change in magnitude in SCN neurons and contributes to SCN excitability rhythms with greater current during the day (Itri et al., 2010; Farajnia et al., 2012). In SCN neurons and neocortical interneurons, I_A allows continuous spontaneous firing without spike adaptation (Itri et al., 2010; Williams and Hablitz, 2015). Moreover, recent reports have shown reduced I_A in hippocampal neurons from a different mouse model of AD and in neurons treated with A β 42 (Hall et al., 2015; Scala et al., 2015). Thus, we next sought to determine whether I_A is altered in SCN neurons of Tg-SwDI. To do this, we performed voltage-clamp recordings in SCN neurons from Tg-SwDI and WT mice during the day and night. To isolate I_A , cells were exposed to two sets of progressive depolarizing voltage steps (see Methods) first from a holding potential of -90 mV and then from a holding potential of -30 mV to inactivate I_A channels. The difference between the responses to these two stimuli was then used to determine the peak outward current at each step. As expected, the WT neurons had greater I_A during the day than at night (Fig. 5A,D). This day/night difference was not seen in Tg-SwDI neurons (Fig. 5B,D). A comparison of I_A at the highest step, normalized to cell capacitance, revealed that I_A was significantly increased during the day in WT neurons (Kruskal-Wallis, $H(3) = 19.025$, $p < 0.001$; post hoc asymptotic significant, $p < 0.005$; Fig. 5C) but not in Tg-SwDI neurons ($p = 0.627$; Fig. 5C). In fact, during the day, normalized I_A was significantly reduced in Tg-SwDI neurons compared to WT I_A ($p < 0.001$).

DISCUSSION

Circadian rhythm disruption is one of the most widely reported, non-cognitive symptoms found in Alzheimer's disease, but the underlying cause of these circadian changes is still poorly understood. Similar circadian decline has also been associated with normal aging and aging-related disorders, such as Huntington's and Parkinson's diseases (Kudo et al., 2011a; Kudo et al., 2011b; Nakamura et al., 2011; Farajnia et al., 2012). Moreover, mouse models of these disorders have demonstrated that decline in the circadian system can be found in the SCN at the membrane level before changes in the molecular clock can be found (Kudo et al., 2011a; Kudo et al., 2011b; Nakamura et al., 2011). These reports have led to the proposal that the neurophysiological output of the SCN may be the "weak-link" of the circadian system in aging and aging-related disorders (Colwell, 2011). In the present study, we provide the first evidence that disruption of rhythmic SCN neuronal activity is also present in a mouse model of AD which exhibits altered circadian behavior. Additionally, we found suppressed daytime I_A to be underlying these electrophysiological deficits.

Our first major finding was that Tg-SwDI mice exhibit a significantly shortened FRP as early as 3 months of age. This result is consistent with previous studies which also found shortened FRPs in other AD mouse models (triple transgenic and APP/PS1) early in the disease progression (Sterniczuk et al., 2010; Oyegbami et al., 2017). Together, these findings suggest that circadian changes have the potential to serve as an early warning for individuals in the early stages of AD. Measuring FRP in human patients can be difficult due to the need for constant conditions; however, chronotype could be used as an alternative measure since it has been shown to correlate with FRP (Duffy et al., 2001).

Although many studies have reported circadian behavioral deficits in various mouse models of AD, evidence that this decline occurs at the level of the master circadian pacemaker, the SCN, has been limited. In fact, no evidence of A β plaque formation in the SCN has been reported (van de Nes et al., 1998; Stopa et al., 1999; Gorman and Yellon, 2010), consistent with our unpublished observations. Given the numerous reports of physiological changes occurring other brain areas of AD mice prior to plaque formation or neuronal degeneration (Hsia et al., 1999; Jacobsen et al., 2006; Tomiyama et al., 2010), we hypothesized that alterations in membrane excitability in SCN neurons could explain changes in behavioral rhythms in the absence of gross pathology. Our finding that SCN neuronal excitability rhythms are altered in AD provides novel evidence that the circadian system is can be directly impacted in Tg-SwDI as young as 6 months of age. Considering the proximity of the SCN to the third ventricle, and thus access to elevated A β circulating in the CSF (Reiter et al., 2014), it is possible that circulating A β in the CSF can directly act on SCN neurons. Numerous reports have shown that circadian behavior degrades in the process of normal aging (Valentinuzzi et al., 1997; Dijk and Duffy, 1999). Likewise, the electrophysiological output of the SCN declines with aging (Nakamura et al., 2011; Farajnia et al., 2012). Thus, age can be a major confounding factor when studying the effect of neurodegenerative disorders on circadian rhythms because many animal models do not develop pathology until reaching an advanced age. To avoid this, we used selected the Tg-SwDI AD model because this model begins developing A β pathology and spatial learning deficits as early as 3 months (Xu et al., 2007).

The dampening of SCN rhythms in TgSwDI mice was predominantly driven by decreased neuronal activity during the day. Furthermore, our data show that reduction in I_A during the day was underlying these changes in spontaneous SCN activity. Interestingly, decreased daytime neuronal excitability and A-type current have also been reported in SCN neurons of WT mice near the end of their lifespan (~2yr; (Farajnia et al., 2012)). Reduction of I_A has also been reported in dentate granule cells from the J20 AD mouse model and in large ventrolateral neurons of sleep deprived flies overexpressing A β Arctic (Hall et al., 2015; Tabuchi et al., 2015). However, in both of these cases decreased I_A was associated with hyperexcitability in those cells, whereas I_A appears to have the opposite effect in the SCN. Our finding that reduced I_A has an inhibitory effect on neuronal activity is consistent with previous research in the SCN showing that pharmacological inhibition of I_A with 4-AP during the day suppresses SCN spontaneous spiking, mimicking the phenotype of SCN activity in old mice as well as Tg-SwDI mice (Farajnia et al., 2012). In other brain regions, 4-AP in neocortical fast-spiking basket cells improves spike accommodation, increasing the inter-spike interval during repetitive spiking (Williams and Hablitz, 2015). The inter-

regional differences in the role of I_A in the maintenance and rate of action potential firing and how it is altered in neurodegeneration are important areas of future research.

Although multiple studies have described the rhythm in I_A in the SCN (Itri et al., 2010; Farajnia et al., 2012) and shown its importance for circadian behavior (Granados-Fuentes et al., 2015), the specific mechanism driving its rhythmicity has remained elusive. Importantly, multiple A-type channel sub-units (Kv4.1 and Kv4.2) did not show day/night rhythms in transcript expression or protein levels when examined at ZT 6 and ZT 14 (Itri et al., 2010). Interestingly, mice lacking expression of Kv4.2 sub-units exhibit shortened free running periods in circadian behavior similar to that found in Tg-SwDI mice (Granados-Fuentes et al., 2012). In other brain regions, phosphorylation of Kv4.2 at Ser-616 has been shown to regulate I_A density (Hu et al., 2006; Scala et al., 2015). Moreover, recent work has demonstrated that A β 42-induced suppression of I_A in hippocampal neurons is associated with an increase in Kv4.2 phosphorylation at this site (Scala et al., 2015). Future work will be needed to determine if post-translational modifications of A-type potassium channels are responsible for regulating daily rhythms in I_A in the SCN. Moreover, it is important to note that the present studies were conducted in male mice, and thus, future studies should determine whether sex impacts day-night differences in SCN physiology. This knowledge could provide insight into the causes of circadian decline in aging and aging-related disorders as well as provide a direction towards new treatment strategies to restore proper rhythmicity in various diseases.

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Figure 1. Period of circadian behavioral rhythms is shortened in Tg-SwDI mice at all examined ages

(a) Representative double plotted actograms of wheel-running activity for WT (left) and Tg-SwDI mice (right) at 3, 6 and 10 months of age beginning in LD and then released into DD. (b-e) Quantification of behavioral rhythms in DD at each age. Bar graphs indicate means $\pm$ SEM of free-running period (b), average activity (c), interdaily variability of activity onset (d) and offset (e). Symbols denote significant difference between genotypes at one age (* p < 0.05) or significant main effect of genotype for 6 and 10 months animals (# p < 0.05 or ### p < 0.001. n = 6–7 mice per genotype, per age.

Figure 2. Neuronal activity rhythm is impaired in Tg-SwDI SCN neurons

(a) Means $\pm$ SEM spontaneous action potential frequencies of SCN neurons during the subject day and subjective night from WT and Tg-SwDI mice housed in DD. *** $p < 0.001$; $n = 45\text{--}76$ cells, 2–3 slices per group. (b) Representative cell-attached loose-patch traces (5 s) from each group in (a).

Figure 3. Passive and active membrane properties of WT and Tg-SwDI neurons
 (a) Representative traces (5 s) of current clamp recordings from WT or Tg-SwDI neurons during the day or early-night. (b-d) Means $\pm$ SEM of **spontaneous action potential frequency** (b), resting membrane potential (c) and input resistance (d) of cells represented in (a). $n = 11-21$ cells, 3-4 slices per group. (e-f) Mean $\pm$ SEM of afterhyperpolarization amplitude (e) and average action potential waveforms (f) of the spontaneously active neurons from (a-d). $n = 9-18$ cells per group. Main effect of time, $p < 0.05$ for (c) and $p < 0.005$ for (d-e).

Figure 4. Persistent sodium current rhythm remains intact in Tg-SwDI neurons

Means $\pm$ SEM of peak inward current (a) and averaged normalized response (b-c) to slow depolarizing voltage ramp (-100 to $+10$ mV; 59 mV/s) from WT or Tg-SwDI cells during the day (b) or night (c). $n = 16$ – 29 cells, 3 – 4 slices per group. Main effect of time, $p < 0.05$ for (a).

Figure 5. Day/night difference in I_A is lost in dorsal SCN of Tg-SwDI mice
 (a-b) I-V plots (mean $\pm$ SEM) of I_A from WT (black) and Tg-SwDI (gray) neurons measured during the mid-day (a) or early night (b) using of progressive depolarizing voltage steps (-40mV to $+40\text{mV}$, 5mV steps) from a holding potential of first -90mV and then -30mV . I_A was isolated by subtracting the current response to the second protocol from the response to the first protocol. (c) Mean $\pm$ SEM of normalized peak I_A (at $+40\text{mV}$) of cells in (a-b). ** $p < 0.005$, $n = 25-39$ cells, 3-4 slices per group. (d) Representative traces showing the subtracted current response to $+40\text{mV}$ voltage step from neurons in (a-c).

Table 1

Summary of circadian behavioral parameters for Tg-SwDI mice in LD.

	WT	Tg-SwDI
<i>3 months</i>	<i>n</i> = 7	<i>n</i> = 6
Activity (rev/min)	16.1 ± 1.3	21.6 ± 1.2
Light Activity (rev/day)	718 ± 157	1583 ± 962
Dark Activity (rev/day)	22510 ± 1908	29563 ± 1008
Total Activity (rev/day)	23227 ± 1896	31146 ± 1665
% Lights on Activity (%)	3.2 ± 0.7	4.6 ± 2.5
Phase Angle (hr)	0.10 ± 0.06	0.53 ± 0.03
Activity Onset Error (hr)	0.18 ± 0.05	0.18 ± 0.08
Activity Offset Error (hr)	0.9 ± 0.22	0.84 ± 0.12
<i>6 months</i>	<i>n</i> = 7	<i>n</i> = 7
Activity (rev/min)	13.7 ± 1.2	11.2 ± 1.7
Light Activity (rev/day)	2401 ± 497	1536 ± 320
Dark Activity (rev/day)	17291 ± 1882	14654 ± 2340
Total Activity (rev/day)	19693 ± 1798	16191 ± 2492
% Lights on Activity (%)	13.2 ± 3.2	10.2 ± 2.2
Phase Angle (hr)	0.25 ± 0.19	-0.11 ± 0.14
Activity Onset Error (hr)	0.37 ± 0.09	0.33 ± 0.09
Activity Offset Error (hr)	1.02 ± 0.22	1.09 ± 0.34
<i>10 months</i>	<i>n</i> = 7	<i>n</i> = 7
Activity (rev/min)	8.2 ± 1.4	13.9 ± 1.5
Light Activity (rev/day)	1058 ± 381	793 ± 216
Dark Activity (rev/day)	10757 ± 1924	19184 ± 2221
Total Activity (rev/day)	11815 ± 2050	19976 ± 2149
% Lights on Activity (%)	11.1 ± 3.9	4.5 ± 1.3
Phase Angle (hr)	0.81 ± 0.39	0.09 ± 0.11
Activity Onset Error (hr)	0.48 ± 0.11	0.16 ± 0.11
Activity Offset Error (hr)	1.4 ± 0.34	1.41 ± 0.28

Table 2

Summary of circadian behavioral parameters for Tg-SwDI mice in DD.

	WT	Tg-SwDI
<i>3 months</i>	<i>n = 7</i>	<i>n = 6</i>
Tau (h)	23.84 ± 0.03	23.68 ± 0.07
Power	1457 ± 120	1430 ± 135
Activity (rev/min)	17.4 ± 2.2	21.5 ± 3.0
Alpha length (h)	11.9 ± 1.1	14.3 ± 1.2
Fragmentation (bouts/day)	3.0 ± 0.4	3.6 ± 0.4
Avg. bout length (min)	230.8 ± 35.5	182.6 ± 25.6
<i>6 months</i>	<i>n = 7</i>	<i>n = 7</i>
Tau (h)	23.91 ± 0.04	23.54 ± 0.05
Power	1322 ± 127	1276 ± 110
Activity (rev/min)	12.1 ± 1.6	13.3 ± 1.9
Alpha length (h)	13.0 ± 0.6	13.0 ± 0.5
Fragmentation (bouts/day)	3.9 ± 0.4	4.5 ± 0.3
Avg. bout length (min)	146.1 ± 20.7	120.7 ± 14.2
<i>10 months</i>	<i>n = 7</i>	<i>n = 7</i>
Tau (h)	23.89 ± 0.03	23.57 ± 0.06
Power	872 ± 83	1177 ± 145
Activity (rev/min)	7.2 ± 1.3	14.3 ± 1.3
Alpha length (h)	13.6 ± 0.2	13.8 ± 1.2
Fragmentation (bouts/day)	5.5 ± 0.4	4.4 ± 0.5
Avg. bout length (min)	74.7 ± 12	141.8 ± 20.1

Table 3

Summary of ANOVA results for circadian behavioral parameters for 6 & 10 month old Tg-SwDI mice.

	Main effect genotype <i>F (P)</i>	Main effect age <i>F (P)</i>	Interaction <i>F (P)</i>
<i>LD</i>			
Activity	0.62 (0.45)	9.94 (0.008)	80.73 (<0.001)
Light Activity	1.62 (0.23)	14.79 (0.002)	1.22 (0.29)
Dark Activity	0.99 (0.34)	2.92 (0.11)	88.90 (<0.001)
Total Activity	0.62 (0.45)	9.94 (0.008)	80.75 (<0.001)
% Lights on Activity	1.68 (0.22)	6.08 (0.03)	1.22 (0.29)
Phase Angle	3.41 (0.09)	5.47 (0.04)	1.24 (0.29)
Activity Onset Error	3.15 (0.10)	0.10 (0.76)	1.87 (0.20)
Activity Offset Error	0.01 (0.92)	2.63 (0.13)	0.02 (0.89)
<i>DD</i>			
Tau	56.66 (<0.001)	0.00 (1.00)	0.39 (0.54)
Power	0.91 (0.36)	7.86 (0.02)	3.22 (0.10)
Activity	4.79 (0.05)	3.37 (0.09)	7.35 (0.02)
Alpha length	0.01 (0.93)	1.23 (0.39)	0.03 (0.87)
Fragmentation	0.46 (0.51)	3.92 (0.07)	5.70 (0.03)
Avg. bout length	1.2 (0.29)	2.77 (0.12)	9.36 (0.01)
Activity Onset Error	4.76 (0.05)	2.43 (0.15)	1.15 (0.31)
Activity Offset Error	34.31 (<0.001)	1.44 (0.25)	0.19 (0.67)