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Circadian rhythm disturbances in Alzheimer's disease: insights from plaque-free and plaque-burdened stages in $APP_{SWE}/PS1_{dE9}$ mice

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Abstract

Background Disruptions in circadian rhythms are commonly observed in patients with Alzheimer's disease (AD) and could potentially accelerate the progression of the condition. However, the relationship between circadian rhythm disruptions and AD development, as well as the mechanisms involved, remain poorly understood.

Methods This study investigated the circadian behavior, rhythmic gene expression in multiple brain regions, and its correlation with sleep architecture of AD mice at two disease stages: plaque-free stage (2-month-old) and plaque-burdened stage (10-month-old) as compared to age-matched wild-type (WT) mice.

Results Two-month-old AD mice already displayed alteration in the activity patterns compared to WT mice, showing increased activity during the light phase and decreased activity during the dark phase, and the change in the activity pattern of 10-month-old AD mice was more significant. Further, electroencephalogram (EEG) examination showed increased wakefulness and reduced non-rapid eye movement (NREM) sleep in 2- and 10-month-old AD mice. In addition, we documented a significant change in circadian core clock genes in the suprachiasmatic nucleus (SCN), hippocampus, and cortex of 2- and 10-month-old AD mice. Correlation analyses demonstrated the close relationship between circadian clock gene expression level and specific sleep-wake parameters, especially within the SCN and hippocampus.

Conclusions These findings revealed that circadian rhythm disturbances in AD mice preceded Aβ deposition. The circadian rhythm disturbances observed in the early AD might be attributed to the abnormal expression of core clock genes in the brain regions involved in circadian rhythm regulation.

Keywords Alzheimer's disease, Circadian rhythm, Sleep-wake cycle, Clock gene, Suprachiasmatic nucleus

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Introduction

Alzheimer's disease (AD) is a significant neurodegenerative condition that leads to gradual cognitive decline and represents the most common form of dementia among the elderly [1]. AD is pathologically marked by the presence of neurofibrillary tangles, senile plaques, and the loss of neurons within the brain [2]. In addition to progressive cognition and memory decline, many AD patients exhibit other symptoms, such as circadian disruption [3, 4]. Circadian rhythm can be observed through behavioral patterns such as sleep-wake cycles and rest-activity rhythms [5]. In AD patients, disruptions in circadian rhythm often manifest as fragmented sleep, heightened wakefulness, and reduced daytime activity [6–8]. Importantly, circadian rhythm disturbances tend to emerge before cognitive decline, starting as early as the pre-clinical or asymptomatic phases of AD progression [9–12]. Furthermore, core body temperature cycles, motor activity patterns, and pineal melatonin rhythms represent manifestations of circadian rhythms, all of which are altered in AD [13, 14]. Circadian disruption in AD may create a self-reinforcing feedback loop that promotes pathologies such as amyloid deposition, oxidative stress, and cell death, which further disrupt circadian rhythms [12, 15, 16].

Circadian behaviors are primarily regulated by the suprachiasmatic nucleus (SCN) of the hypothalamus, which serves as the body's "master clock" [17, 18]. In patients with AD, a dysfunctional or degenerating SCN can result in diminished circadian synchronization, increased fragmentation, and diminished amplitude [19, 20]. The regulation of circadian rhythms relies on the dynamic activity of clock genes within the SCN and other peripheral tissues. These genes are essential for generating circadian rhythms through transcriptional-translational feedback loops, which drive the rhythmic fluctuations of mRNA and protein expression in multiple tissues [21]. These clock genes, which include Bmal1 and Clock, play a critical role in generating circadian rhythms by participating in transcriptional-translational feedback loops that produce rhythmic fluctuations in mRNA and protein levels across multiple tissues. *Bmal1* and *Clock* act as transcription factors, activating the expression of their repressors—*Per1*, *Per2*, *Cry1*, *Cry2*, and Nr1d1. This results in a 24-hour cycle of gene expression that aligns with daily light cues [22]. Consequently, the assessment of clock gene oscillations in the SCN and other organs may serve as a biological indicator of circadian function.

The SCN acts as the central pacemaker for circadian rhythms; however, other brain regions and peripheral tissues also maintain their own circadian cycles [23]. The roles of clock genes within the SCN and outside the SCN are thought to differ, reflecting the unique functions of specific brain regions [24]. The primary cerebral regions impacted by AD pathology are the cortex and the hippocampus. Memory processes are associated with molecular circadian oscillations in the hippocampus [25, 26]. However, the association between circadian disruptions in AD and changes in clock gene expression within the SCN or other regions has remained scarcely investigated.

To comprehend the mechanisms that contribute to abnormal circadian rhythms in motor activity and sleep in AD, and to formulate strategies for their mitigation, appropriate animal models are frequently used, including $APP_{SWE}/PS1_{dE9}$ transgenic mice (AD mice). Our previous study demonstrated that the AD mice at the pre-plaque stage (3 and 4 months of age) displayed distinct profiles of sleep architecture and sleep electroencephalogram (EEG) [27]. In this study, we conducted a detailed analysis of circadian rhythm behaviors in AD mice at two distinct stages of disease progression: the plaque-free stage and the plaque-burdened stage. We not only examined the circadian behavior of AD mice, but also investigated the expression of clock genes both in the SCN and extra-SCN regions (hippocampus and cortex) of AD mice, and explored their relationship with sleep-wake characteristics.

Method

In this study, we investigated the circadian behavior (wheel-running rhythm and sleep-wake cycle) of AD mice during disease progression, clock genes expression in multiple brain regions, neuropathology, and their correlation with sleep architecture. A schematic representation of the experimental design is provided in Figure S1.

Animals

AD mouse model was obtained from the Jackson Laboratory (B6C3-Tg ($A\beta PP_{swe}$, $PSEN1_{dE9}$)85Dbo/Mmjax, Bar Harbor, MA, USA). AD mice and wild-type littermates (WT mice) were kept under standard conditions (12–12 h light-dark cycle, lights on at 8:00 AM, denoted by Zeitgeber Time (ZT) 0, room temperature 22 ± 1 °C, and relative humidity 50 ± 10%). The AD and WT mice at 2 and 10 months of age were randomly sacrificed at ZT2 (10:00), 8 (16:00), 14 (22:00) and 20 (04:00). All experimental procedures and animal care protocols were approved by the Institutional Animal Care Committee at Dalian Medical University and adhered to the Laboratory Animal Care Guidelines.

Circadian wheel-running behavior

To study circadian behavior, mice were individually housed in separate cages (35.3 × 23.5 × 20 cm) equipped with running wheels (12.7 cm in diameter, Lafayette Instrument Model 80820) and provided with free access to food and water. The running wheels were linked to a computer via a signal acquisition system, allowing

real-time monitoring of motor activity through Vital View software, which recorded data continuously over 24 h.

EEG monitoring wake and sleep

We implanted brain electrodes (Pinnacle Technology Inc., Lawrence, USA) in mice following the manufacturer's recommended procedure. Mice were anesthetized with continuous isoflurane inhalation (R500IE, RWD, Shenzhen, China) and positioned in a stereotaxic apparatus with the skull exposed. Electrode placement was at 1.0 mm anterior to bregma and 1.5 mm lateral on each side, and 1.0 mm anterior to the posterior fontanelle with the same lateral placement. After drilling skull openings, electrodes were carefully inserted and secured, and two electromyography (EMG) electrodes were placed into the trapezius muscles bilaterally. The electrodes were fixed to the skull with tissue glue and dental cement. All mice received an intraperitoneal injection of penicillin to prevent infection, followed by a one-week recovery period. Continuous 24-hour EEG recordings were then performed using Sirenia EEG acquisition software (Pinnacle Technology Inc., Lawrence, USA), and data were analyzed with Sirenia Sleep 2.1.1. The frequency bands analyzed included delta (0.1–4 Hz), theta (5.1–9 Hz), alpha (9.1–12 Hz), and beta (12–20 Hz).

Tissue extraction and immunofluorescent staining

Mice were deeply anesthetized with isoflurane (RWD Life Science, China) and perfused through the vascular system with cold 0.1 M phosphate buffer and 4% paraformaldehyde. After dehydration, tissue sections were prepared using a cryostat (CM-1950 S, Leica, Germany) and incubated overnight at 4 °C with primary antibodies. Subsequently, tissues were incubated for 1 h with secondary antibodies. Details of the antibodies used are listed in Table S2. Ten sections per animal, with three randomly selected fields per section at the same reference position, were photographed, and results were quantified using ImageJ software.

Realtime quantitative polymerase chain reaction (RT-qPCR)

The mRNA expression level was measured by RT-qPCR. Brain tissue samples were collected and combined with 1 ml of Trizol and a fifth of the volume of chloroform. The mixture was allowed to stand for 5 min before being centrifuged at 12,000 g for 15 min. The supernatant was removed, and half the volume of isopropanol was added to the remaining solution. After mixing and standing for 10 min, the mixture was centrifuged again at 12,000 g for 10 min. The supernatant was discarded, and the pellet was washed twice with 75% ethanol. Once dried, the pellet was resuspended in RNase-free water, and the RNA concentration of the samples was measured. Reverse transcription was performed following the instructions provided in the Hifair III 1st Strand cDNA Synthesis SuperMix for qPCR (YEASEN, 11141es60, China) manual. The transcription products were diluted five times with RNase-free water and used for qPCR reactions following the instructions provided in the Taq SYBR Green qPCR Premix (YUGONG, EG20117M, China) manual. The reactions were conducted on an Applied Biosystems 7500 Real-Time PCR System. The sequences of the primers used are provided in Table S1. The housekeeping gene GAPDH was used as an internal reference, and relative expression levels were calculated using the 2-ΔΔCt method.

Statistics

Statistical analyses were performed using Prism 8.0 software. The Student's t-test was used for comparisons between two groups, whereas multiple group comparisons were analyzed using two-way analysis of variance (ANOVA) followed by Sidak's post-hoc test. Rhythmicity in gene expression was evaluated with Metacycle in RStudio (version 1.1.419) [28]. Genes were considered rhythmically expression when p-values<0.05. Furthermore, Spearman's correlation test was used to evaluate the relationship between core clock gene expression and key electrophysiological outcomes, with p-values < 0.05 regarded as statistically significant.

Results

Neuropathology in different brain regions of AD mice

First, we measured the Aβ pathological change and p-tau Thr231 level in the cortex and hippocampus of AD mice at 2-month-old and 10-month-old (Fig. 1). There was no plaque deposited in 2-month-old AD mice (Fig. 1A). However, there were many plaques in the hippocampus and cortex of 10-month-old AD mice (Fig. 1A and a). The p-Tau Thr231 level in the cortex of 10-month-old AD mice was significantly higher than that of 2-month-old AD mice (age: $F(1,12) = 9.104$, $P < 0.05$; Fig. 1B-b and C-c). There was no Aβ immunostaining was detected in the SCN region of 2 and 10-month-old AD mice and thus the result was not presented here. The p-Tau Thr231 levels in the SCN of AD mice were significantly elevated compared to WT mice (genotype: $F(1,12) = 16.13$, $P < 0.01$; Fig. 1D-d).

Alteration of voluntary wheel-running activity in the AD mice

The current study initially examined circadian rhythms in wheel-running activity among AD mice at 2 and 10 months of age. Figure 2A illustrates the circadian pattern of wheel-running activity. As nocturnal animals, mice typically display low activity levels during the light phase and increased activity during the dark phase. We

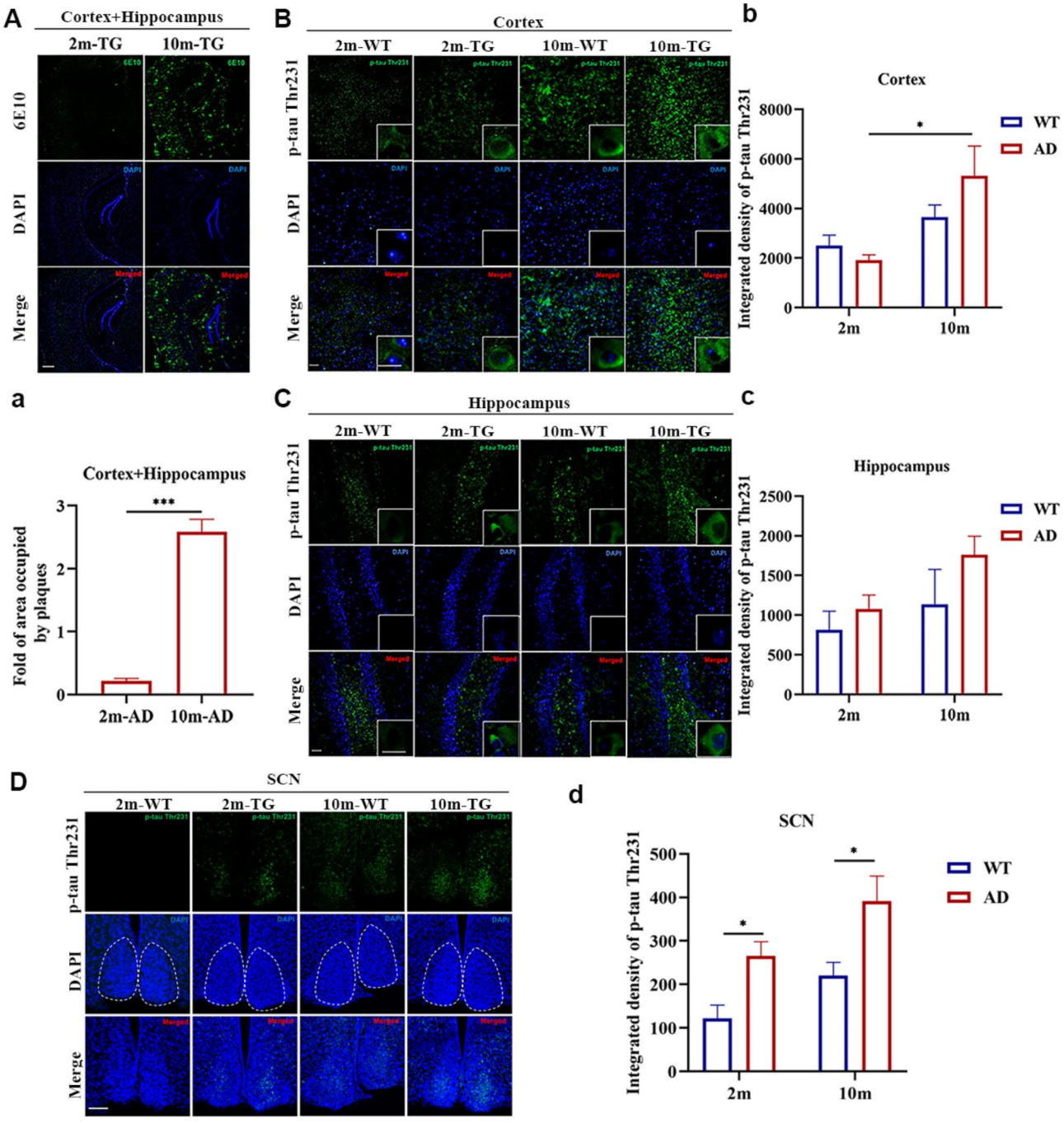

Fig. 1 Immunofluorescent staining of Aβ plaque and p-Tau Thr231 in the SCN, cortex and hippocampus of 2 and 10-month-old mice. The staining of Aβ plaque in the cortex and hippocampus of AD mice, scale bar: 100 μm (**A**). The area occupied by plaques in the hippocampus and cortex of AD mice (**a**). Data were the mean ± SEM values, $n = 4$–6 mice in each group. ***$p < 0.001$, by Student's t-test. The p-Tau Thr231 staining in the mouse cortex and hippocampus, left scale bar: 50 μm, right scale bar: 20 μm (**B-C**). The p-Tau Thr231 staining in the SCN of mouse, scale bar: 50 μm (**D**). Integrated density of p-Tau Thr231 staining in the SCN, cortex and hippocampus were analyzed (**b-d**), $n = 4$ mice in each group. Data were the mean ± SEM values. *$P < 0.05$, by two-way ANOVA with Sidak's multiple comparisons test

found a decreased overall run-wheel activity in the AD mice (Fig. 2A), which can lead to a lower amplitude in the circadian sleep distribution and increased sleep fragmentation. In addition, APP/PS1 mice exhibited reduced activity at 10 months, likely reflecting disease progression. In 2-month-old AD mice, the activity pattern deviated from that of WT mice, with higher activity noted during the light phase and reduced activity during the dark phase (Fig. 2B). Specifically, the total distance covered per hour of wheel running was significantly

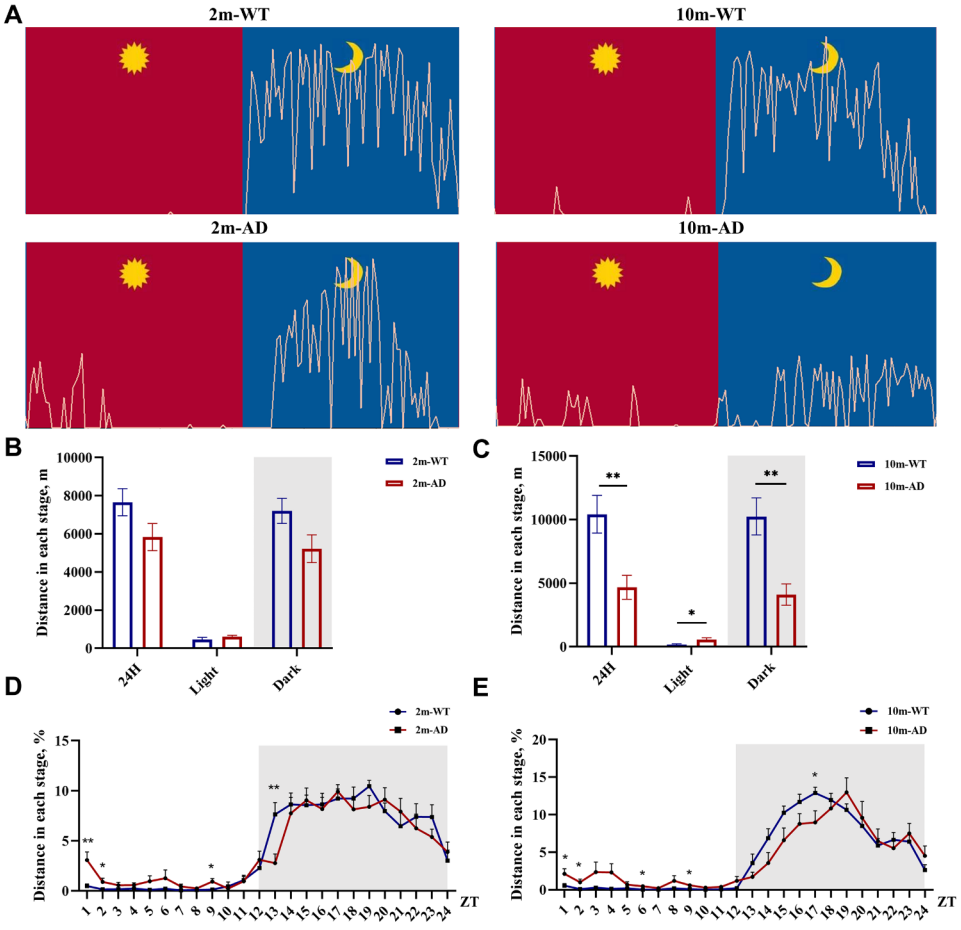

Fig. 2 Circadian wheel-running behavior in WT and AD mice at 2 and 10 months of age. Mice wheel-running behavior chart (**A**). Wheel-running distance during 24 h, light phase, and dark phase of WT and AD mice at 2 (**B**) and 10 (**C**) months of age. Time course of distance percentages in AD and WT mice at 2 (**D**) and 10 (**E**) months of age. Data were presented as mean ± SEM and analyzed by using Student's t-test, $n = 13–15$ mice in each group, $^{*}P<0.05$, $^{**}P<0.01$

elevated during the light phases ZT1-2 and ZT9 but decreased during the dark phase at ZT13 compared to age-matched WT mice (Fig. 2D). For 10-month-old AD mice, these changes in activity were even more pronounced (Fig. 2C). The total distance run per hour was notably higher during light phases ZT1-2, ZT6, and ZT9, while activity declined during the dark phase at ZT17 compared to WT mice (Fig. 2E). This disrupted pattern parallels observations in AD patients, who often experience diminished daytime activity and increased restlessness in the evening [29].

Sleep disruptions and altered sleep architecture in the AD mice

The sleep-wake cycle is a common biological process regulated by circadian rhythms; when disrupted, it can contribute to the development of AD [30]. EEG/EMG electrophysiological monitoring is commonly used as the gold standard for sleep/wake study since it can accurately distinguish between various sleep states. Therefore, we analyzed sleep architecture in AD mice by recording EEG/EMG. Figures 3A and 4A presents representative EEG/EMG traces and hypnogram of AD and WT mice in 24-hour. We analyzed the percentage of time spent in three distinct states (Wake, non-rapid eye movement (NREM) sleep, and rapid eye movement (REM) sleep) at per hour intervals over 24 h (Figs. 3B-D and 4B-D) as well as the light (rest state) and dark phases (activity state) separately (Figs. 3E-G and 4E-G). Significant differences in wake time were observed between AD and WT mice, with the AD mice exhibiting increased wakefulness in the whole light-dark cycle and particularly during the dark phase (Figs. 3E and 4E). This increase in wakefulness was accompanied by a reduction in NREM sleep. NREM sleep time was significantly decreased in AD mice during both the full 24-hour period and the dark phase, regardless of age (2 or 10 months; Figs. 3F and 4F). In addition, a significant reduction in NREM sleep was also observed during the light phase in 2-month-old AD mice compared to age-matched WT controls (Fig. 3F). The AD mice revealed a uniform trend towards similarly reduced

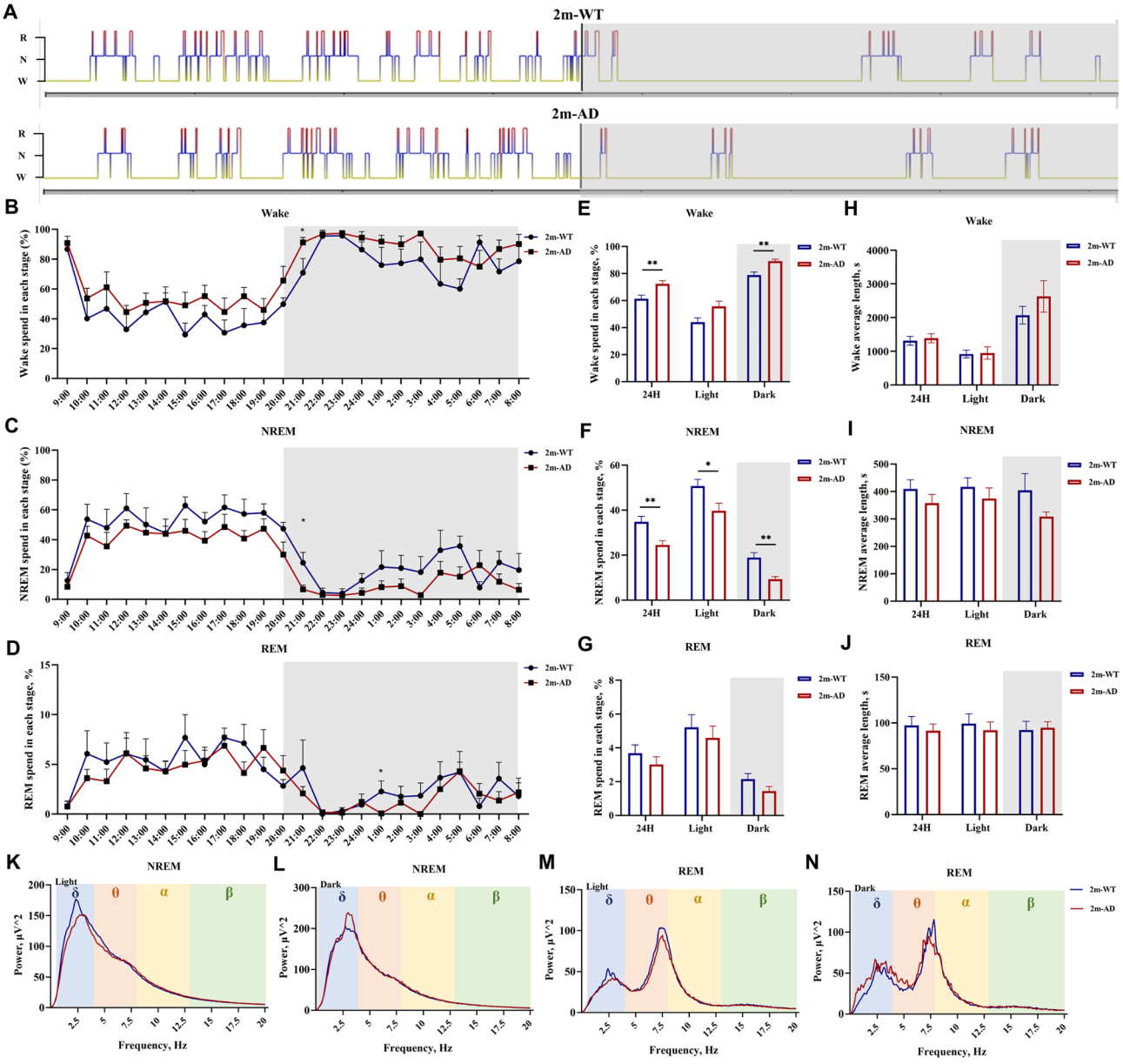

Fig. 3 Sleep-wake profiles of WT and AD mice at 2 months of age. Twenty-four-hour representative hypnogram recording of WT and AD mice at 2 months of age (**A**). Time course of wakefulness (**B**), NREM sleep (**C**), and REM sleep (**D**) percentages in the WT and AD mice at 2 months of age. Time spent of wakefulness (**E**), NREM sleep (**F**), and REM sleep (**G**) during 24 h, light phase, and dark phase of WT and AD mice at 2 months of age. Average length of wakefulness (**H**), NREM sleep (**I**), and REM sleep (**J**) in WT and AD mice at 2 months of age. Data were the mean ± SEM values, $n = 6$–8 mice in each group. $^{*}p < 0.05$, $^{**}p < 0.01$, by Student's t-test. EEG power spectrum of NREM sleep (**K-L**) and REM sleep (**M-N**) during light phase and dark phase in WT and AD mice at 2 months of age

REM sleep. However, the reduction was not statistically significant (Figs. 3G and 4G).

Sleep quality in mice was assessed by measuring the average duration of each stage (wake, NREM sleep, and REM sleep bout durations). Analysis of 24-hour recordings, along with light and dark phases, showed no significant differences in average wake duration or average REM sleep duration between WT and AD mice at both 2 and 10 months of age (Figs. 3H and J and 4H and J). However, average length of NREM sleep was reduced in AD mice at 10 months age (Fig. 4I), indicating increased sleep fragmentation. EEG power spectrum analysis revealed that altered EEG power of both NREM and REM sleep in 2 and 10-month-old AD mice. NREM delta power was slightly reduced in AD mice compared with that in WT mice in the light period, accompanied by shifts in the power spectra toward higher frequencies (Figs. 3K and 4K). During the dark phase, NREM delta

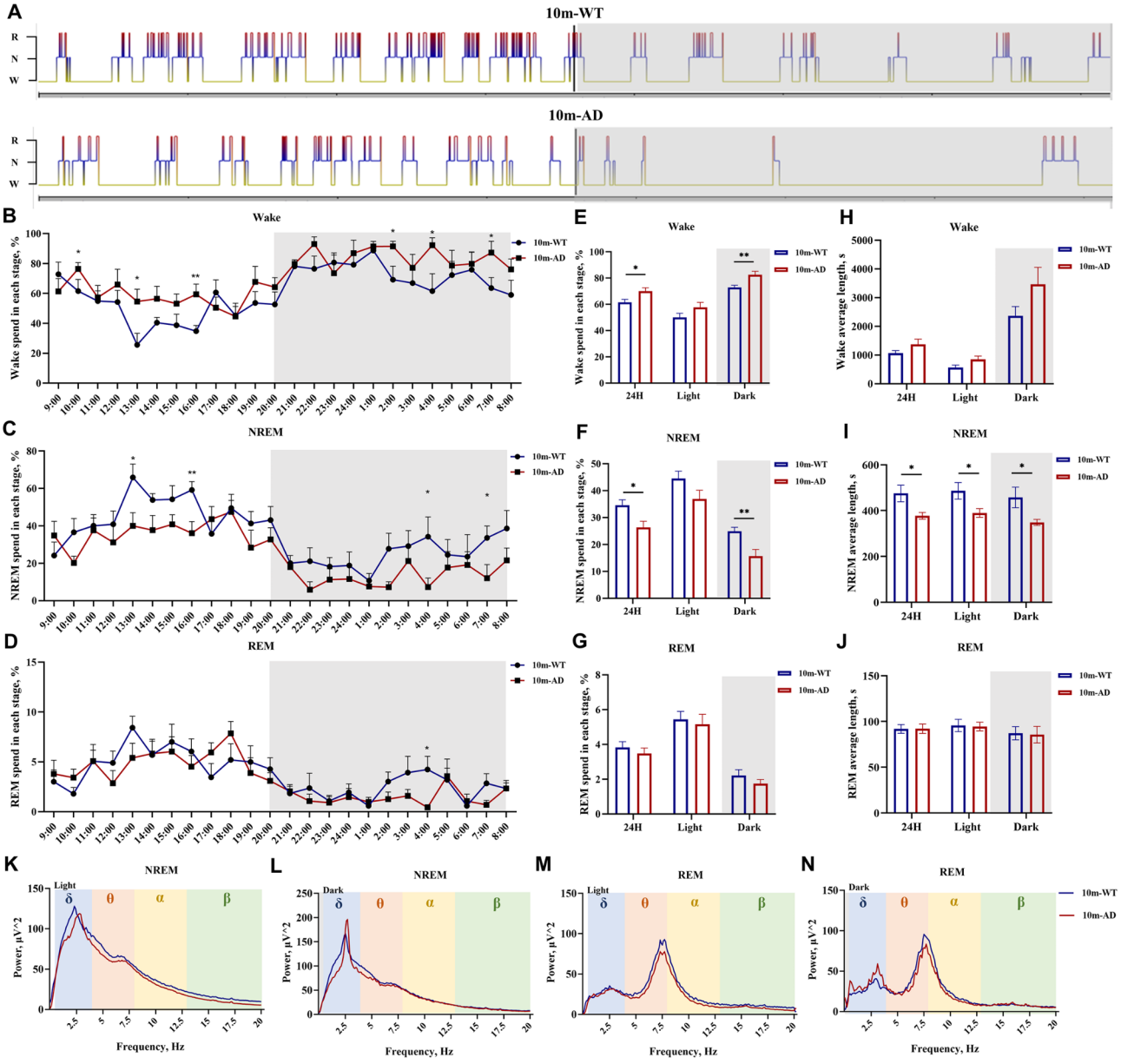

Fig. 4 Sleep-wake profiles of WT and AD mice at 10 months of age. 24-hour representative hypnogram recording of WT and AD mice at 10 months of age (**A**). Time course of wakefulness (**B**), NREM sleep (**C**), and REM sleep (**D**) percentages in the WT and AD mice at 10 months of age. Time spent of wakefulness (**E**), NREM sleep (**F**), and REM sleep (**G**) during 24 h, light phase, and dark phase of WT and AD mice at 10 months of age. Average length of wakefulness (**H**), NREM sleep (**I**), and REM sleep (**J**) in the WT and AD mice at 10 months of age. Data were the mean ± SEM values, $n = 7–8$ mice in each group. *$p < 0.05$, **$p < 0.01$, by Student's t-test. EEG power spectrum of NREM sleep (**K-L**) and REM sleep (**M-N**) during light phase and dark phase in the WT and AD mice at 10 months of age

power showed a slight increase (Figs. 3L and 4L), while REM power was marginally lower in AD mice compared to WT mice across the theta frequency range during both light and dark phases (Figs. 3M-N and 4M-N).

Circadian dysregulation of core clock genes in the SCN region of AD mice

Circadian genes are essential regulators of the sleep-wake cycle. In mammals, the SCN functions as the primary circadian regulator, governing behavioral rhythms and synchronizing peripheral clocks across various organs, including the liver, kidneys, and heart [31, 32]. The expression of core clock genes at the mRNA level exhibits rhythmic fluctuations over time. We examined the mRNA expression of core clock genes (*Clock*, *Bmal1*, *Dec1*, *Dec2*, *Cry1*, *Cry2*, *Rora*, *Rorb*, *Per1*, *Per2*, *Dbp*, and *Nr1d1*) every 6 h at four time points: ZT2, ZT8, ZT14, and ZT20 following the onset of the light phase.

We compared the rhythmic expression patterns of these genes in AD mice and WT mice. Our findings revealed that significant disruptions in the circadian oscillations of clock gene expression within the SCN of AD mice. In 2-month-old WT mice, all core clock genes exhibited clear circadian rhythmicity with rhythmic p-values < 0.05 (Fig. 5A, Table S3). However, by 10 months of age, the rhythmic expression of *Clock* mRNA was lost (Fig. 5B, Table S3). In AD mice, the genes *Rorb* and *Bmal1* did not display significant circadian rhythms at 2 months old (Fig. 5A, Table S3), but at 10 months old, the expression of *Per2*, *Bmal1*, *Nr1d1*, *Cry2*, *Rora*, *Dec1* genes lost rhythm (Fig. 5B, Table S3). Interestingly, at 2 months, APP/PS1 mice exhibited circadian gene expression differences compared to age-matched WT mice: decreased *Dec1* and *Per2* with increased *Rora* at ZT2; elevated *Nr1d1* at ZT8; higher *Per2* and lower *Nr1d1* at ZT14; and increased *Rorb* with reduced *Cry1* at ZT20 (Fig. 5A), suggesting early dysregulation in circadian transcriptional machinery.

Circadian dysregulation of core clock genes in the hippocampus and cortex of AD mice

The hippocampus and cortex are recognized as the regions implicated in the earliest stages of AD pathology. Memory consolidation and retrieval are partly regulated by a local circadian clock within the hippocampus [33, 34]. For our molecular studies on circadian disruption, we examined tissue from both the hippocampus and cortex, uncovering impairments in several key components. In 2-month-old WT mice, we observed clear circadian rhythms for *Bmal1*, *Dec1*, *Dec2*, *Cry1*, *Cry2*, *Rora*, *Rorb* and *Per2* genes in the hippocampus (Fig. 6A, Table S4). Circadian mRNA expression of the core clock genes, *Bmal1*, *Dbp*, *Dec1*, and *Dec2* were obviously disrupted in 2-month-old AD mice (Fig. 6A, Table S4). In both WT and AD mice, a greater number of clock genes exhibited a loss of rhythmicity with age. The rhythm expression of *Rorb*, *Nr1d1*, *Cry1*, *Dbp*, and *Dec1* was preserved in the hippocampus of 10-month-old WT mice (Fig. 6B, Table S4). Whereas, *Per2*, *Bmal1*, *Nr1d1*, *Cry1*, and *Dbp* retained rhythm in the hippocampus of 10-month-old AD mice (Fig. 6B, Table S4). Rhythm analysis indicated that the expression of *Per1*, *Per2*, *Cry1*, *Dec1*, and *Dec2* in the cortex of 2-month-old WT mice exhibited rhythmicity, whereas the expressions of *Per1*, *Per2*, *Rorb*, *Nr1d1*, and *Cry1* in the cortex of 2-month-old AD mice were rhythmicity (Fig. 7A, Table S5). The expression rhythms of *Clock*, *Cry1*, and *Dbp* were maintained in 10-month-old WT mice (Fig. 7B, Table S5). Only *Dbp* mRNA expression exhibited a rhythmic pattern in 10-month-old AD mice (Fig. 7B, Table S5). In addition, APP/PS1 mice also exhibited circadian gene expression differences in the hippocampus and cortex compared to age-matched WT mice. To further understand circadian disruptions, we measured the diurnal expression of core clock genes across multiple brain regions over a 24-hour cycle. The findings indicate that AD mice experience significant dysregulation of diurnal gene expression across various brain areas, including those central to circadian regulation.

Correlation between sleep architecture and circadian clock gene expression in multiple brain regions

The correlation analyses were performed across all mice (WT and APP/PS1 combined) in order to identify general relationships between clock gene expression and sleep architecture. First, for the SCN, there was a significant positive correlation between the mRNA expressions of *Clock*, *Cry1*, *Rorb*, *Per1*, *Per2* and the proportion of waking time, as well as the average duration of waking episodes (Fig. 8A and D). The mRNA expressions of *Clock*, *Rorb*, *Per1*, *Per2* showed a significant negative correlation with the proportion of NREM sleep, but not the average duration of NREM sleep episodes (Fig. 8B and E). Additionally, the expression of *Clock* and *Rorb* was negatively correlated with the proportion of REM sleep and average duration of REM sleep episodes (Fig. 8C and F). And there was a significant negative correlation with the expression of *Per1* and proportion of REM sleep, and between *Rora* and average duration of REM sleep episodes (Fig. 8C and F). For hippocampus Spearman's correlation showed that the *Clock*, *Cry2*, *Per1*, *Per2*, *Rora*, *Rorb* were positively correlated with the average duration of waking episodes and were negatively correlated with proportion of NREM sleep (Fig. 9B and D). The expressions of *Clock*, *Cry2*, *Rora*, and *Rorb* revealed positive correlation with the proportion of waking time, however, possessing negative correlation with the proportion of REM sleep (Fig. 9A and C). In addition, the expression of *Per2* was positively and significantly related with the proportion of waking time and *Per1* demonstrated negative and significant correlation with the average duration of REM sleep episodes (Fig. 9A and F). In the cortex, only the expression of *Nr1d1* was positively correlated with the proportion of waking time and the average duration of waking episodes and negatively correlated with proportion of NREM and REM sleep, as well as average duration of REM sleep episodes (Fig. 9G-L). The link between circadian rhythms and sleep architecture extends beyond the SCN to peripheral tissues, such as the hippocampus, where circadian clock gene expression has been associated with electrophysiological outcomes. These observations help explain our findings, suggesting that the altered sleep architecture observed in the AD mice may be related to disrupted circadian rhythms in clock gene expression.

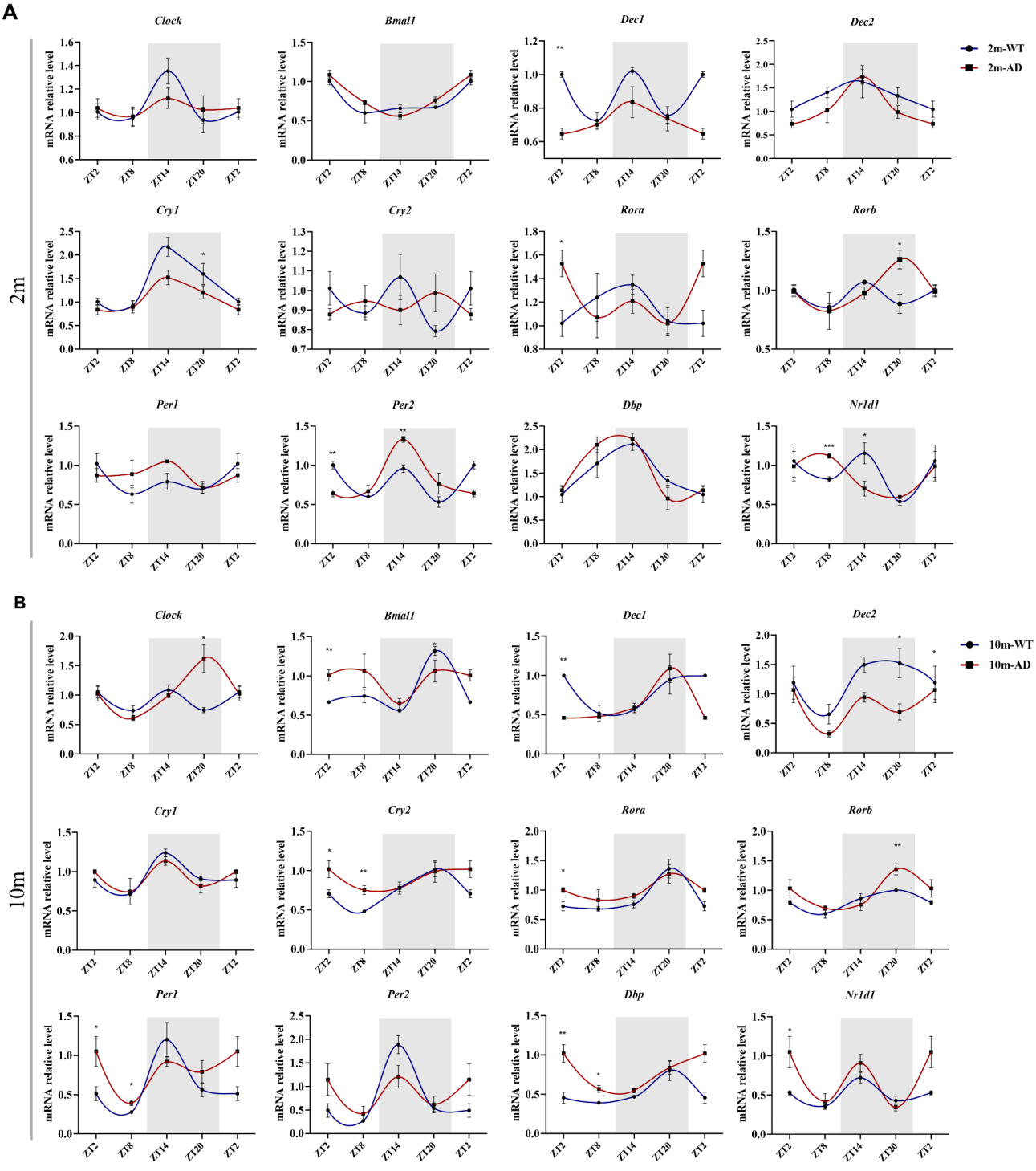

Fig. 5 Clock gene expression rhythms in the SCN region of WT and AD mice. The mRNA levels of clock genes (*Clock*, *Bmal1*, *Dec1*, *Dec2*, *Cry1*, *Cry2*, *Rora*, *Rorb*, *Per1*, *Per2*, *Dbp*, *Nr1d1*) in the SCN region of WT and AD mice at 2 months of age (**A**). The mRNA levels of clock genes (*Clock*, *Bmal1*, *Dec1*, *Dec2*, *Cry1*, *Cry2*, *Rora*, *Rorb*, *Per1*, *Per2*, *Dbp*, *Nr1d1*) in the SCN of WT and AD mice at 10 months of age (**B**). The white box indicates the light phase, while the gray box indicates the dark phase. The asterisks indicate significant differences between WT and AD mice at each time point. Data were presented as mean ± SEM and analyzed by using Student's t-test, $n = 4$ mice in each group, *$P<0.05$, **$P<0.01$, ***$P<0.001$

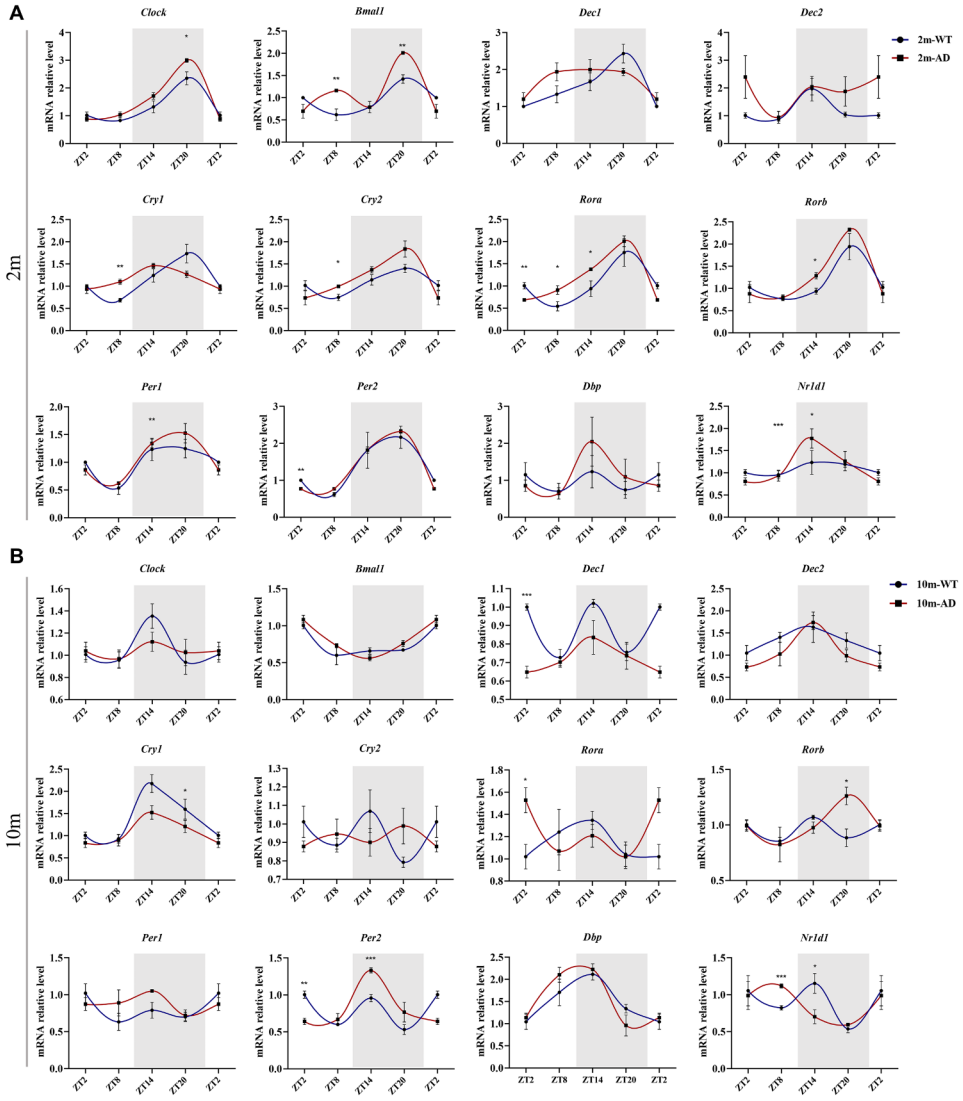

Fig. 6 Clock gene expression rhythms in the hippocampus of WT and AD mice. The mRNA levels of clock genes (Clock, Bmal1, Dec1, Dec2, Cry1, Cry2, Rora, Rorb, Per1, Per2, Dbp, Nr1d1) in the hippocampus of WT and AD mice at 2 months of age (**A**). The mRNA levels of clock genes (Clock, Bmal1, Dec1, Dec2, Cry1, Cry2, Rora, Rorb, Per1, Per2, Dbp, Nr1d1) in the hippocampus of WT and AD mice at 10 months of age (**B**). The white box indicates the light phase, while the gray box indicates the dark phase. The asterisks indicate significant differences between WT and AD mice at each time point. Data were presented as mean ± SEM and analyzed by using Student's t-test, $n = 4$ mice in each group, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$

Discussion

The current study is consistent with our previous research showing the alteration of sleep architecture in the AD mice [27]. The most notable finding is the alteration of circadian rhythms in the AD mice as early as 2 months of age (plaque-free stage). The study results further indicate that circadian rhythm disruption in the AD mice is evident through irregular sleep-wake cycles, increased sleep fragmentation, reduced locomotor activity, a higher ratio of daytime activity to total activity, and a lower ratio of nocturnal activity to total activity.

Our results reveal disruptions in the circadian rhythms of voluntary wheel-running activity in the AD mice, which may exacerbate during the plaque-burdened phase. The AD mice exhibit increased daytime activity and decreased night activity, and such patterns are commonly observed in human AD patients, who often experience "sundowning" behaviors and reduced sleep at night [35]. Activity rhythms alterations have also been observed in people with mild cognitive impairment [36, 37]. Our data document the disturbances in wheel-running activity prior to plaque formation in the AD mice. Epidemiological study reports disrupted circadian rhythms in mild cognitive impairment adults, as measured by actigraphy, constitute a significant risk factor for AD onset [36]. Moreover, several longitudinal studies spanning 5 to 41 years have reported greater cognitive decline and a heightened risk of dementia among

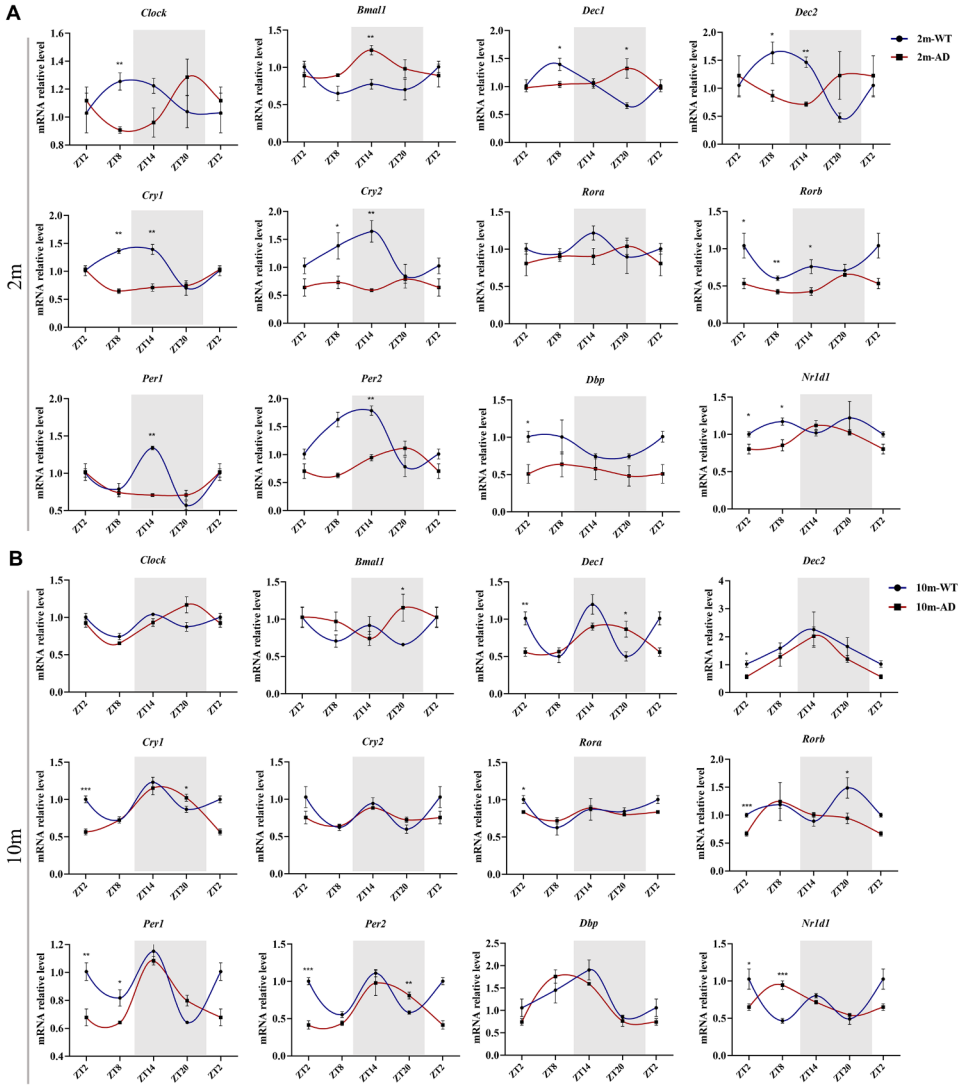

Fig. 7 Clock gene expression rhythms in the cortex of WT and AD mice. The mRNA levels of clock genes (Clock, Bmal1, Dec1, Dec2, Cry1, Cry2, Rora, Rorb, Per1, Per2, Dbp, Nr1d1) in the cortex of WT and AD mice at 2 months of age (**A**). The mRNA levels of clock genes (Clock, Bmal1, Dec1, Dec2, Cry1, Cry2, Rora, Rorb, Per1, Per2, Dbp, Nr1d1) in the cortex of WT and AD mice at 10 months of age (**B**). The white box indicates the light phase, while the gray box indicates the dark phase. The asterisks indicate significant differences between WT and AD mice at each time point. Data were presented as mean ± SEM and analyzed by using Student's t-test, $n = 4$ mice in each group, *$P<0.05$, **$P<0.01$, ***$P<0.001$

individuals with circadian disruptions compared to those without such disruption [36–38]. In addition, 10-month-old WT mice showed increased running distance compared to 2-month-old WT mice. In contrast, APP/PS1 mice exhibited reduced activity at 10 months, likely reflecting disease progression linked to Aβ pathology [39]. This contrast underscores the divergence between normal aging and AD-related circadian. These findings indicate that disruptions in circadian-regulated activity are not only symptomatic but may actively contribute to disease pathophysiology, emphasizing the importance of circadian rhythm regulation in AD.

The sleep-wake cycle is the most recognized indicator of circadian system [40]. In addition to changes in activity rhythms, we observed significant alterations in the sleep structure of the AD mice, particularly during the dark phase. The AD mice showed increased wakefulness and a corresponding decrease in NREM sleep, which are consistent with findings in AD patients [10, 41]. Notably, our findings reveal a significant reduction in the average length of NREM sleep in the AD mice at 10-month-old (plaque-burdened stage), suggesting an inability to maintain continuous NREM sleep. This disruption likely diminishes the cognitive benefits typically associated with this sleep stage, particularly in memory consolidation, potentially impacting cognitive behaviors [42]. Current research highlights concerns that disrupted

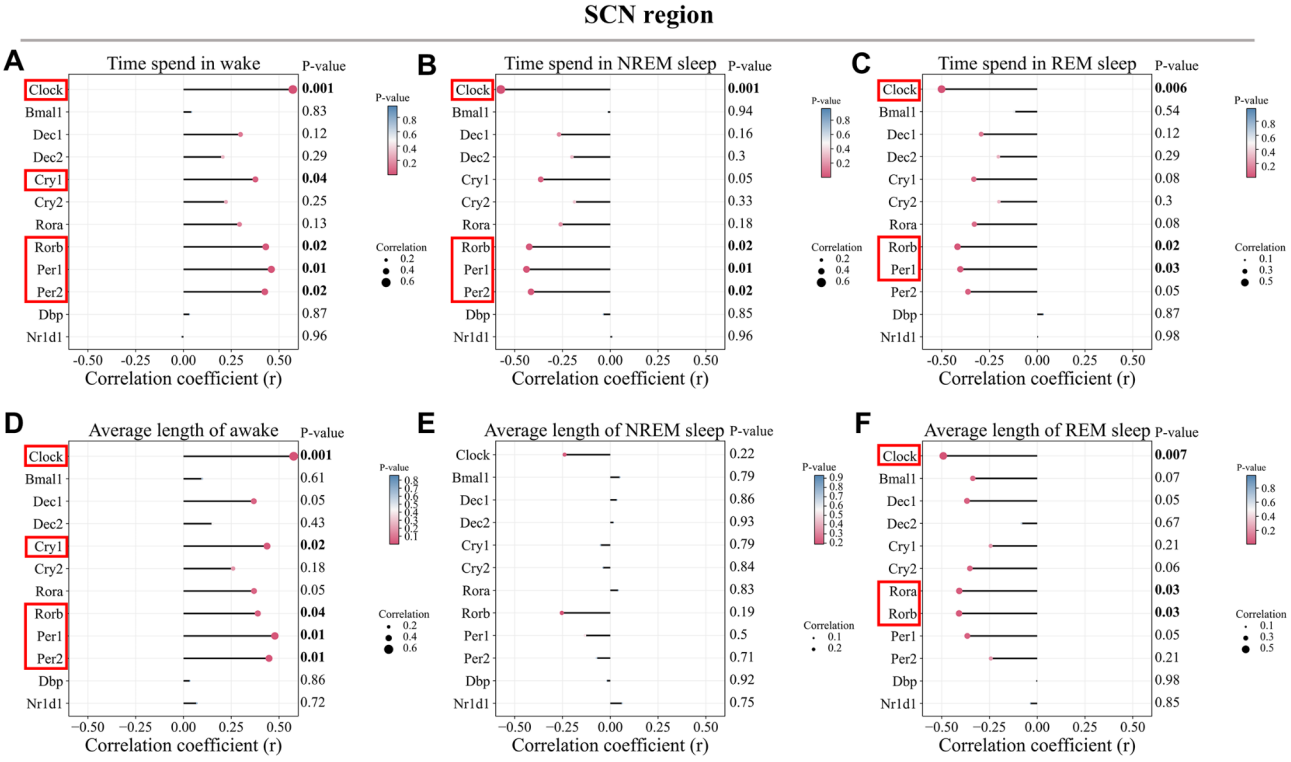

Fig. 8 The relationship between sleep architecture and core clock gene expression in the SCN region. The size of the dot indicates the strength of the association between the clock genes and the sleep structure; a larger dot indicates a stronger correlation. The dot's color represents the *p*-value; red boxes indicate significant correlations

sleep continuity can hinder memory consolidation and negatively influence working memory [43].

Clock genes are expressed throughout the central nervous system, forming a distributed circadian network with varying levels of autonomy and dependence on the SCN [44]. Our molecular analyses revealed disrupted expression of core circadian clock genes within the SCN, hippocampus, and cortex, which are crucial regions for circadian regulation and cognitive function. The altered expression of key genes, including *Clock*, *Bmal1*, *Per1*, and *Per2* in the SCN suggests that the central circadian rhythm generator is compromised in AD mice, which could propagate dysregulated circadian signals to peripheral clocks in the hippocampus and cortex. The hippocampus and cortex, as primary regions for Aβ deposition, are also key areas that reflect changes in circadian rhythm. Dysregulation of hippocampal and cortical clock genes may have direct consequences on memory consolidation and sleep-wake cycle control. In 3×Tg-AD mice, the rhythmic expression of core clock genes like *Bmal1*, *Clock*, *Per*, and *Cry* in the hippocampus is notably disrupted [45]. Our study similarly identified circadian rhythm disturbances in these core clock genes within the hippocampus and cortex of AD mice. This loss of rhythmicity aligns with prior research demonstrating dysregulated clock gene expression in patients with AD as well as in AD mouse models [46, 47].

The role of circadian gene dysfunction in sleep regulation remains uncertain, particularly concerning whether these effects are mediated directly by the SCN, given that circadian genes are expressed throughout the brain, not solely in the SCN. Thus, the specific contributions of the SCN and circadian genes to sleep regulation are still not fully understood. Early studies involving SCN lesions in rats demonstrated that damaging the SCN disrupts the sleep-wake rhythm and alters sleep distribution. Mutations in core circadian clock genes in both mice and humans are associated with irregular sleep patterns, including reduced sleep duration, phase shifts, and fragmented sleep-wake cycles [48, 49]. Additionally, sleep deprivation can affect clock gene expression, highlighting the interrelation of these systems. Wakefulness and NREM sleep show a significant correlation with hippocampal atrophy. Our correlation analyses underscore the link between circadian clock gene expression and specific sleep-wake parameters, particularly in the SCN and hippocampus. For example, *Clock*, *Cry1*, and *Per2* expression in the SCN correlated with increased wakefulness and reduced NREM sleep, while hippocampal clock genes expressions correlated with wake duration and REM sleep proportions. Aβ accumulation

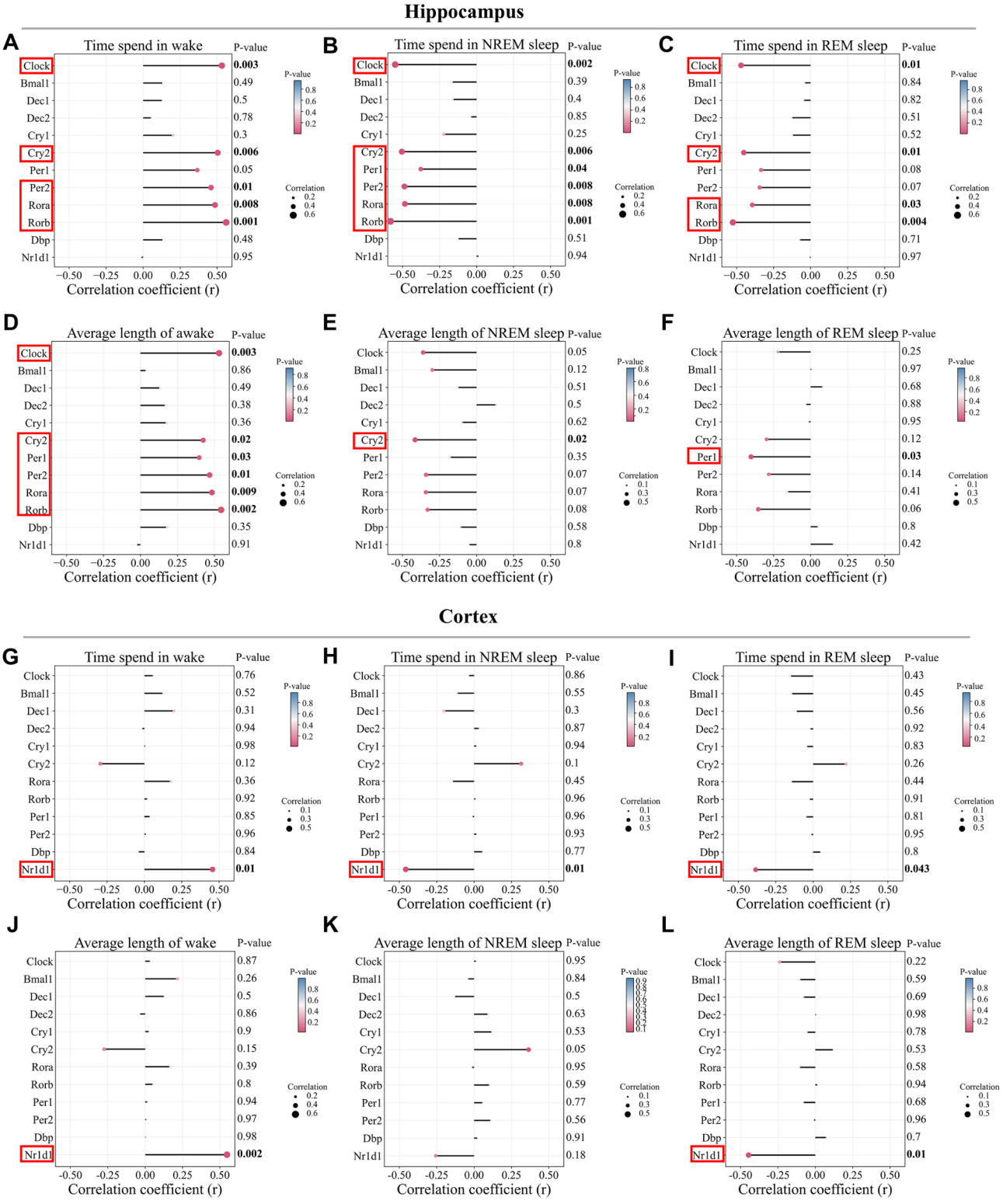

Fig. 9 The relationship between sleep architecture and core clock gene expression in the hippocampus and cortex. The size of the dot indicates the strength of the association between the clock genes and the sleep structure; a larger dot indicates a stronger correlation. The dot's color represents the *p*-value; red boxes indicate significant correlations

in the hippocampus could disrupt glutamatergic signaling to the SCN, weakening circadian output [50], while SCN dysfunction may reduce hippocampal neurogenesis, accelerating cognitive decline [51]. In addition, our previous study demonstrated that circadian disruption exacerbates Aβ deposition [52], while Whittaker et al. showed that restoring clock function improves cognition in AD models [53]. These findings suggest that disruptions in molecular circadian regulation are intricately connected to sleep disturbances in AD, with dysfunction in both the SCN and hippocampus contributing to these effects.

Significantly, the detection of p-Tau Thr231 in the SCN at 2 months. There is evidence that soluble Aβ pathology can induce secondary tau phosphorylation at specific epitopes, including Thr231 [54]. We focused on p-Tau Thr231 due to its association with early axonal dysfunction and circadian disruption in preclinical models [55]. Baril et al. have revealed higher plasma p-Tau Thr231 were associated with unstable sleep-wake cycles [55]. The SCN exhibits high metabolic and synaptic activity due to its role as the central circadian pacemaker [20]. This renders it susceptible to oxidative stress and mitochondrial dysfunction, which may accelerate tau phosphorylation [56]. In addition, impaired clock gene expression in the SCN may further exacerbate tau pathology by weakening protein degradation pathways [57]. The early dysregulation of core clock genes in the SCN and hippocampus of 2-month-old APP/PS1 mice—prior to amyloid plaque deposition—suggests that soluble Aβ oligomers likely activate kinases like GSK-3β, destabilizing circadian transcriptional machinery and promoting tau phosphorylation.

It is worth noting that constant darkness conditions are widely used to unmask endogenous circadian rhythms by eliminating external light cues [58]. However, our experimental design intentionally maintained a 12-hour light/dark cycle to model the real-world circadian disruptions experienced by AD patients, who remain exposed to environmental light despite their deteriorating biological rhythms. This approach allowed us to evaluate circadian dysfunction in a clinically relevant context, where external Zeitgebers (e.g., light) may fail to entrain a weakened central clock. However, we acknowledge that constant darkness experiments could further clarify whether the observed circadian rhythm disturbances in APP/PS1 mice stem from impaired rhythm generation (SCN-intrinsic defects) or defective photic entrainment. Future studies under constant darkness conditions would help dissect these mechanisms and are an important direction for our ongoing work.

Limitations

This study provides valuable insights into circadian rhythm disruptions and their relation to sleep architecture in the AD mouse model. However, several limitations should be acknowledged. First, while we examined circadian and sleep disruptions at two disease stages, the study does not account for other age groups, limiting generalizability to different AD progression stages. Additionally, this study uses the AD mouse model, which primarily models amyloid pathology without fully capturing the complexity of human AD pathology, such as tauopathy and neuroinflammation, potentially impacting the applicability of findings to human disease. Finally, the observational design restricts causal interpretations regarding whether circadian rhythm disruptions directly contribute to AD progression or are secondary consequences of neurodegeneration.

Conclusions

In summary, our findings demonstrate that circadian rhythm disturbances, including disrupted activity patterns, altered sleep-wake cycles and irregular clock gene expression, emerge early in AD and exacerbate with disease progression. AD mice exhibit certain characteristics of circadian rhythms disorders like those observed in AD patients, such as heightened activity and reduced sleep during the light phase, and decreased activity during the dark (active) phase. Furthermore, our research points to a link between abnormal clock gene expression in the regions such as SCN, hippocampus, and cortex, and disrupted sleep architecture, emphasizing the potential of targeting clock gene regulation to restore sleep and circadian rhythms, potentially delaying disease onset and progression. Future research should investigate therapeutic strategies aimed at circadian rhythm restoration to assess their impact on cognitive outcomes and disease trajectory in AD.

Abbreviations

AD	Alzheimer's disease
SCN	Suprachiasmatic nucleus
AD mice	$APP_{SWE}/PS1_{dE9}$ transgenic mice
EEG	Electroencephalogram
EMG	Electromyography
WT mice	Wild-type littermates
ZT	Zeitgeber Time
RT-qPCR	Realtime quantitative polymerase chain reaction
ANOVA	Analysis of variance
NREM sleep	Non-rapid eye movement sleep
REM sleep	Rapid eye movement sleep

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13195-025-01724-8.

Supplementary Material 1

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Not applicable.

Author contributions
HY conducted the experimental work, performed data analysis, and drafted the original manuscript. NL assisted with experimental work, manuscript editing, and revisions. LT and YH contributed to the experimental work. CC and SL provided manuscript edits. WL supervised the project, conceptualized the research, and contributed to manuscript editing and revisions. All authors have reviewed and approved the final manuscript and agree to be accountable for its content.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
The Institutional Animal Care Committee at Dalian Medical University approved the protocol.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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