scientific reports

Check for updates

OPEN

Optogenetic targeting of cortical astrocytes selectively improves NREM sleep in an Alzheimer's disease mouse model

Qiuchen Zhao[1,3], Shinya Yokomizo[1,3], Stephen J. Perle[1], Yee Fun Lee[1], Heng Zhou[1], Morgan R. Miller[1], Hanyan Li[1], Dmitry Gerashchenko[2], Stephen N. Gomperts[1], Brian J. Bacskai[1] & Ksenia V. Kastanenka[1]✉

Alzheimer's disease (AD) is a progressive neurodegenerative condition marked by memory impairments and distinct histopathological features such as amyloid-beta (Aβ) accumulations. Alzheimer's patients experience sleep disturbances at early stages of the disease. APPswe/PS1dE9 (APP) mice exhibit sleep disruptions, including reductions in non-rapid eye movement (NREM) sleep, that contribute to their disease progression. In addition, astrocytic calcium transients associated with a sleep-dependent brain rhythm, slow oscillations prevalent during NREM sleep, are disrupted in APP mice. However, at present it is unclear whether restoration of circuit function by targeting astrocytic activity could improve sleep in APP mice. To that end, APP mice expressing channelrhodopsin-2 (ChR2) targeted to astrocytes underwent optogenetic stimulation at the slow oscillation frequency. Optogenetic stimulation of astrocytes significantly increased NREM sleep duration but not duration of rapid eye movement (REM) sleep. Optogenetic treatment increased delta power and reduced sleep fragmentation in APP mice. Thus, optogenetic activation of astrocytes increased sleep quantity and improved sleep quality in an AD mouse model. Astrocytic activity provides a novel therapeutic avenue to pursue for enhancing sleep and slowing AD progression.

Keywords Optogenetics, Astrocyte, EEG, Sleep, NREM, REM, Wake, Alzheimer's disease, Slow waves, Slow oscillations, Delta power, Sleep fragmentation

Abbreviations

AAV	Adeno-associated virus
Aβ	Amyloid β
AD	Alzheimer's disease
APP	APPswe/PS1dE9
ChR2	ChannelRhodopsin-2
EEG	Electroencephalography
EMG	Electromyography
GPCRs	G-protein coupled receptors
IACUC	Institutional Animal Care and Use Committee
NREM	Non-rapid eye movement
REM	Rapid eye movement
SWA	Slow-wave activity

[1]Department of Neurology, MassGeneral Institute of Neurodegenerative Diseases, Massachusetts General Hospital, Harvard Medical School, Charlestown, MA 02129, USA. [2]Department of Psychiatry, Harvard Medical School and Veterans Affairs Boston Healthcare System, West Roxbury, MA 02132, USA. [3]Qiuchen Zhao and Shinya Yokomizo contributed equally to this work. ✉email: kkastanenka@mgh.harvard.edu

Alzheimer's disease (AD) is a complex neurodegenerative condition that primarily affects cognitive functions, initially presenting as short-term memory disturbances[1,2]. As the disease advances, long-term memory disruptions, executive function impairments, and neuropsychiatric symptoms become increasingly evident. AD is marked by the accumulation of amyloid-beta (Aβ) in the extracellular space and presence of reactive astrocytes at early stages of disease prior to cognitive decline[1–3]. Studies indicate that changes in cerebrospinal fluid Aβ42 levels can be detected more than a decade before cognitive symptoms manifest[4].

Sleep plays a critical role in overall health, influencing various aspects of well-being, from cognitive function to memory consolidation[5]. Also, sleep disturbances occur early during Alzheimer's progression[6,7]. Deficits in sleep, specifically deep non-rapid eye movement (NREM) sleep, are common in Alzheimer's patients during early and preclinical stages[8,9]. NREM sleep is characterized by high-amplitude, synchronized slow-wave activity (SWA) propagating throughout the cortex[8,10]. Slow oscillations (< 1 Hz) are part of SWA and are impaired at early stages of Alzheimer's disease. NREM sleep and SWA are vital for memory consolidation during sleep and are associated with cognitive function[8,11]. Disruptions in NREM sleep and SWA in cognitively healthy individuals are associated with high risk of developing Alzheimer's disease[9,12]. Furthermore, Alzheimer's patients exhibit sleep fragmentation[13]. Overall, Alzheimer's patients experience deficits in sleep quantity and quality that could contribute to their disease progression and memory impairments.

Sleep facilitates clearance of Aβ, a toxic protein that accumulates in the brains of Alzheimer's patients. Brain's clearance system is especially active during deep NREM sleep[14–17]. Sleep disruptions or the loss of NREM sleep specifically can blunt clearing capability, enabling Aβ to accumulate in the brain and increase AD risk[5,18,19]. Yet a recent study reported that brain clearance had been inhibited not potentiated during sleep, suggesting that future studies are needed to fully elucidate the relationship between sleep and brain clearance[20].

The astrocyte is a ubiquitous glial cell type found in the brain. These cells play a crucial role in the regulation of sleep, in addition to their traditional roles in supporting neuronal function, maintaining the blood-brain barrier, and regulating neurotransmitter levels[21–24]. Astrocytes are integral components of tripartite synapses, interacting with both pre- and post-synaptic neuronal structures. These glial cells have the potential to modulate synaptic transmission through release of gliotransmitters[25]. Astrocytes participate in sleep-dependent brain rhythms[21,26]. Neuronal activity is reflected in the rise in intracellular calcium levels within astrocytes[26,27]. Astrocytes exhibit calcium transients that are synchronized with slow oscillations in neurons[28]. We found that astrocytic calcium transients associated with slow waves are disrupted in APPswe/PS1dE9 (APP) mice, a model of amyloidosis[29]. Astrocytes can be activated through optogenetic stimulation[29–31]. Optogenetic activation of astrocytes at the frequency of slow waves restores SWA. Chronic activation reduces amyloid deposition, prevents neuronal calcium elevations, and improves memory performance in APP mice[29]. However, the role of astrocytes in sleep impairments associated with AD is unclear. Here we determined the degree to which restoration of circuit function by targeting astrocytic activity could improve sleep in APP mice.

APP mice exhibit sleep disruptions, spending less time in deep NREM sleep and more time awake at 6 months[17]. Here, channelrhodopsin-2 (ChR2)-expressing cortical astrocytes were optogenetically activated at the frequency of slow oscillations in 8-month-old APP mice. Mice underwent sleep monitoring using a fully implantable electroencephalography (EEG) /electromyography (EMG) telemetry system. Optogenetic stimulation restored NREM sleep duration without significantly affecting the duration of rapid eye movement (REM) sleep or locomotion. Additionally, we saw an increase in delta power and a decrease in sleep fragmentation during NREM sleep, indicating enhanced sleep quality. These effects were specific to the optogenetic stimulation of ChR2-expressing astrocytes, since control mice treated with light stimulation of mCherry in the absence of ChR2 failed to show sleep improvements.

Methods

Animals

This study adhered to the guidelines and protocols set by the Institutional Animal Care and Use Committee (IACUC, protocol number 2012N000085) and the National Institutes of Health Guidelines for the Ethical Treatment of Laboratory Animals. We utilized the transgenic mouse line APPswe/PS1dE9 (APP), which simultaneously overexpresses the Swedish mutation of the human amyloid precursor protein and the deltaE9 mutation in human presenilin 1[32] (sourced from The Jackson Laboratory, stock number 034829). The study included male and female mice. Animals were 8 months of age at study onset. The animals were sex-segregated and housed in groups of no more than four per cage. Mice were housed individually following surgical interventions. Mice were maintained in microdialysis cages during chronic optogenetic treatment periods. All mice had unlimited access to food and water. The animals lived in a controlled environment, with a consistent 12-hour light/12-hour dark cycle (lights on from 07:00 to 19:00 o'clock) and were maintained in a pathogen-free setting. The study is reported in accordance with ARRIVE guidelines.

Stereotactic adeno-associated virus (AAV) infusions and cannula implantation

The methodology for intracortical viral injections followed previously established protocols[29]. Briefly, mice were anesthetized using 4% isoflurane. The mouse's head was positioned in the stereotaxic device. Once securely fastened, mice were exposed to 1.5% isoflurane via the nasal probe throughout the surgery. After sterilizing the scalp area, a midline incision was made on the skin. After creating the burr hole through the skull using a Micro Drill (Harvard Apparatus), viral injections were administered into the left anterior cortex using the coordinates AP + 1 mm, ML + 1.5 mm, DV -0.9 mm, at a rate of 0.1 µl/min (Fig. 1A). A 1-ml Hamilton syringe was used for injecting one of two viral solutions: either 1.5 µl of AAV8-GFAP104-ChR2-mCherry with a titer of 2.5×10^{12} virus molecules/mL or 1.5 µl of AAV8-GFAP104-mCherry with a titer of 2.7×10^{12} virus molecules/mL. The injected virus was given 10 min to disperse before the needle was withdrawn from the brain. Following the injection, the incision was sutured. Mice were placed on a heating pad, free of anesthesia, until they regained full alertness and

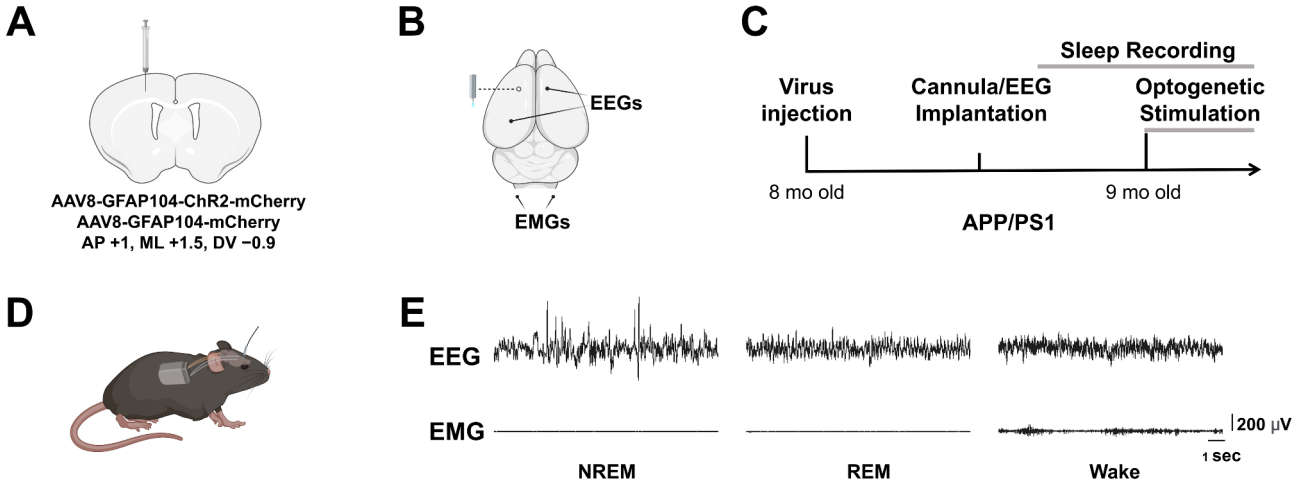

Fig. 1. Schematic description of the experimental sleep recording and optogenetic stimulation setup. (**A**) Viral injection strategy to target astrocytes with AAV8-GFAP104-ChR2-mCherry (ChR2-mCherry) or AAV8-GFAP104-mCherry (mCherry). (**B**) Schematic view showing placement of EEG/EMG implant. Placement of EEG, EMG electrodes, and fiber-optic cannula on the skull. EMG electrodes were placed within the nuchal musculature. (**C**) Experimental design. Following AAV infusion and cannula installation, sleep recordings were acquired prior and during continuous optogenetic stimulation (400 ms, 0.6 Hz, 24 h/day). (**D**) Diagram showing placement of EEG/EMG implant. The processed signal was transferred wirelessly to acquisition software. (**E**) Representative EEG and EMG traces during NREM, REM, and Wake states.

mobility. Mice were given at least three weeks of rest before starting experimental protocols to ensure adequate recovery and viral integration. Successful viral integration was confirmed in all mice postmortem following in vivo experiments. A 400 µm light-guide cannula from Doric Lenses was carefully positioned and affixed above the cortex over the viral expression site under isoflurane anesthesia. C&B Metabond (Parkell) was applied to cement two small screws placed at the anterior and posterior edges of the surgical area, thereby securing the cannula. Meloxicam (5 mg/kg) and acetaminophen (300 mg/100 mL) were administered as post-operative analgesics for 3 days.

Optogenetic stimulation protocol

A recovery period of at least 10 days after the viral expression and cannula installation was allowed before starting optogenetic stimulation. Following recovery, neurons within the anterior cortex were subjected to optogenetic control. Laser pulses of 473 nm blue light (Opto Engine), each lasting 400 milliseconds with an intensity of 5 to 7 mW, were emitted at 0.6 Hz (the frequency of endogenous slow oscillations) through a fiberoptic cable connected to each cannula (Doric Lenses). Light treatment was administered for one day (24 h starting at 07:00 o'clock). During the period of optogenetic treatment, each mouse was housed in its microdialysis cage (provided by Harvard Apparatus, Holliston, MA), with unrestricted access to food and water. Control APP mice expressing mCherry lacking ChR2 underwent the same light treatment as ChR2-expressing animals.

Sleep recordings

Sleep-wake cycle monitoring was conducted in mice using previously established methods[17]. Briefly, mice were implanted with HD-X02 wireless telemetry devices, offering a 0.1 to 200 Hz bandwidth for electroencephalography (EEG) and electromyography (EMG) recordings (DSI Harvard Bioscience, Minneapolis, MN) under isoflurane anesthesia (3% for induction and 1.5% for maintenance). EEG/EMG telemetry was implanted simultaneously with canula installation. An experienced experimenter implanted the devices subcutaneously using sterile techniques. After exposing and cleaning the skull, two stainless steel screws (US Micro Screw, Seattle, WA), acting as cortical electrodes, were inserted through the skull to contact the dura mater. One screw was positioned 1 mm anterior to the bregma and 1 mm lateral to the sagittal suture. The other screw was placed 3 mm posterior to the bregma and 3 mm contralateral to the sagittal suture. EMG electrodes were secured to the neck muscles. Meloxicam (5 mg/kg) and acetaminophen (300 mg/100 mL) were administered as post-operative analgesics for 3 days. A recovery and habituation period of 10 days was allowed before starting data collection. Following recovery, individual cages containing singly housed mice were positioned on PhysioTel receiver plates (model RPC-1). The plates facilitated the real-time transmission of data from the wireless implants to a computer through the MX2 data exchange matrix and Dataquest ART software. Data collection was managed by Ponemah Software v6.50 (DSI Harvard Bioscience, Minneapolis, MN). The physiological parameters, including single-channel EEG and EMG, were sampled at a rate of 500 Hz, while activity counts were sampled at 50 Hz. Sleep was monitored in the same animals before and during optogenetic stimulation. After the experiments, mice were euthanized by carbon dioxide inhalation.

Sleep data analyses

Sleep scoring was conducted using NeuroScore Software v3.3 from DSI Harvard Bioscience, Minneapolis, MN. Raw telemetry data, including EEG, EMG, and locomotor activity readings were imported into NeuroScore from Ponemah. These signals were then bandpass filtered (EEG at 0.5–100 Hz and EMG at 10–100 Hz) and assessed in 10-second intervals. Stages were classified as Wake, rapid eye movement (REM) sleep, or non-REM (NREM) sleep, utilizing the NeuroScore Mouse Sleep scoring module. Frequencies within the delta band ranged from 0.5 to 4 Hz, and the theta band frequencies used for scoring ranged from 6 to 9 Hz. Wakefulness was characterized by a mix of variable high-frequency EEG signals alongside increased EMG activity. NREM sleep was defined by low-frequency, high-amplitude EEG signals and reduced EMG activity. In contrast, REM sleep was characterized by a predominance of theta frequencies in the EEG (with a theta/delta ratio greater than 3) and decreased EMG activity. Sleep was scored in 10-second intervals. At least two successive 10-second intervals of the same sleep state defined a continuous sleep segment. For spectral analysis, EEG signals underwent a fast Fourier transform using a Hanning Window in NeuroScore, which was applied to all epochs free from artifacts without overlapping. This facilitated direct comparisons of EEG power spectra by assessing relative band power, i.e. the ratio of specific band power to the total power, and power ratios, i.e. ratios between power in different bands. Regarding telemetry locomotion activity data, the maximum activity count per 10-second epoch was determined, and the cumulative activity counts per hour and the total activity within 12–24 h were derived.

Statistics

Statistical analyses were performed in GraphPad 8.0. Data were expressed as mean ± SEM. Datasets were tested for normality (Shapiro-Wilk normality test, D'Agostino & Pearson omnibus normality test, or Kolmogorov-Smirnov test), after which appropriate statistical tests were used (t-test or ANOVA for normally distributed data, Mann-Whitney or Kruskal-Wallis test followed by Dunn's multiple comparison test for nonparametric data). Paired t-tests were used when comparing mice before and during light treatment. For datasets comparing two conditions, $p < 0.05$ was considered significant.

Results

Study design for optogenetic stimulation of astrocytes during sleep recordings in freely moving mice

APPswe/PS1dE9 (APP) mice exhibit sleep disruptions early in the disease progression[33,34]. Our recently published study reported sleep disruptions, including deficits in NREM sleep and increases in Wake, in 6-month-old APP mice[17]. Astrocytes play an active role in modulating sleep-dependent brain rhythms, including slow oscillations. The disruptions in astrocytic calcium transients at the frequency of slow oscillations are associated with slow wave impairments[29]. Thus, we conducted experiments to determine the degree to which activation of astrocytes could alleviate sleep impairments in APP mice. To that end, viral vectors encoding channelrhodopsin-2 (ChR2)-mCherry or mCherry lacking ChR2 were delivered to cortical astrocytes of 8-month-old APP mice. Thus, APP mice expressing ChR2 were termed APP-ChR2, and APP mice lacking ChR2 were termed APP-mCherry. Their sleep was monitored prior to (APP-ChR2, APP-mCherry) and during light stimulation (APP-ChR2-opto, APP-mCherry-opto). The viral vectors were strategically targeted to astrocytes through the GFAP promoter and administered into the left hemisphere (Fig. 1A). Two weeks later, mice received cannula implantation on top of the cortex over the viral expression site for delivery of blue 473 nm light to ChR2-expressing astrocytes. We employed an untethered fully implantable electroencephalography (EEG) /electromyography (EMG) telemetry system for comprehensive sleep-wake monitoring. EEG/EMG telemetry was implanted simultaneously with cannula installation (Fig. 1B,C). The use of implanted EEG telemetry permitted long-term assessments of freely mobile mice in their home cages, enabling us to define the EEG signature associated with optogenetic stimulation (Fig. 1D). Mice exhibited NREM and REM sleep as well as Wake states while maintained individually in their home cages during baseline recordings in the absence of optogenetic stimulation (Fig. 1E). The time spent in these behavioral states was stable across the three days of baseline recordings (Supplemental Fig. 1), suggesting a stable baseline for testing purposes.

Optogenetic stimulation of astrocytes at the slow oscillation frequency increased NREM sleep in APP mice without disruptions of locomotor activity

EEG/EMG recordings were collected prior to and during optogenetic stimulation. As expected, APP-ChR2 mice were nocturnal, spending considerable amounts of time sleeping during the light phase. Also, APP-ChR2 mice took naps during the dark phase (Fig. 2A,C-F). Optogenetic stimulation of ChR2 (APP-ChR2-opto) led to a significant increase in the duration of NREM sleep compared to their pre-stimulation state (APP-ChR2) when assessed during the 24-hour period (11.83 ± 3.65 for APP-ChR2 vs. 16.53 ± 3.98 for APP-ChR2-opto: $P < 0.0001$), the 12-hour light (17.20 ± 6.81 for APP-ChR2 vs. 22.89 ± 6.38 for APP-ChR2-opto: $P = 0.0005$) and the dark periods (6.45 ± 1.80 for APP-ChR2 vs. 10.16 ± 2.29 for APP-ChR2-opto: $P = 0.0219$) (Fig. 2B,C). Optogenetic stimulation resulted in decreased wakefulness when assessed during the 24-hour period (44.94 ± 3.45 for APP-ChR2 vs. 40.61 ± 3.55 for APP-ChR2-opto: $P < 0.0001$), the 12-hour light (37.91 ± 6.47 for APP-ChR2 vs. 33.13 ± 5.98 for APP-ChR2-opto: $P = 0.0016$) and the dark periods (51.97 ± 2.32 for APP-ChR2 vs. 40.48 ± 1.91 for APP-ChR2-opto: $P = 0.0148$) (Fig. 2C), while the REM sleep duration remained comparable to that prior to stimulation when assessed during the 24-hour period (3.24 ± 1.22 for APP-ChR2 vs. 2.87 ± 1.45 for APP-ChR2-opto: $P = 0.1651$), the 12-hour light (4.86 ± 1.59 for APP-ChR2 vs. 4.11 ± 1.81 for APP-ChR2-opto: $P = 0.0506$) and the dark periods (1.61 ± 1.23 for APP-ChR2 vs. 1.63 ± 1.41 for APP-ChR2-opto: $P = 0.9141$) (Fig. 2C).

The inverse relationship between locomotion and sleep is well-established, as locomotor activity and sleep are tightly intertwined, influencing each other in various ways[35]. Here, we also investigated the locomotion before and during stimulation. Locomotor activity was detected based on the animal's movement (i.e., the abdominal

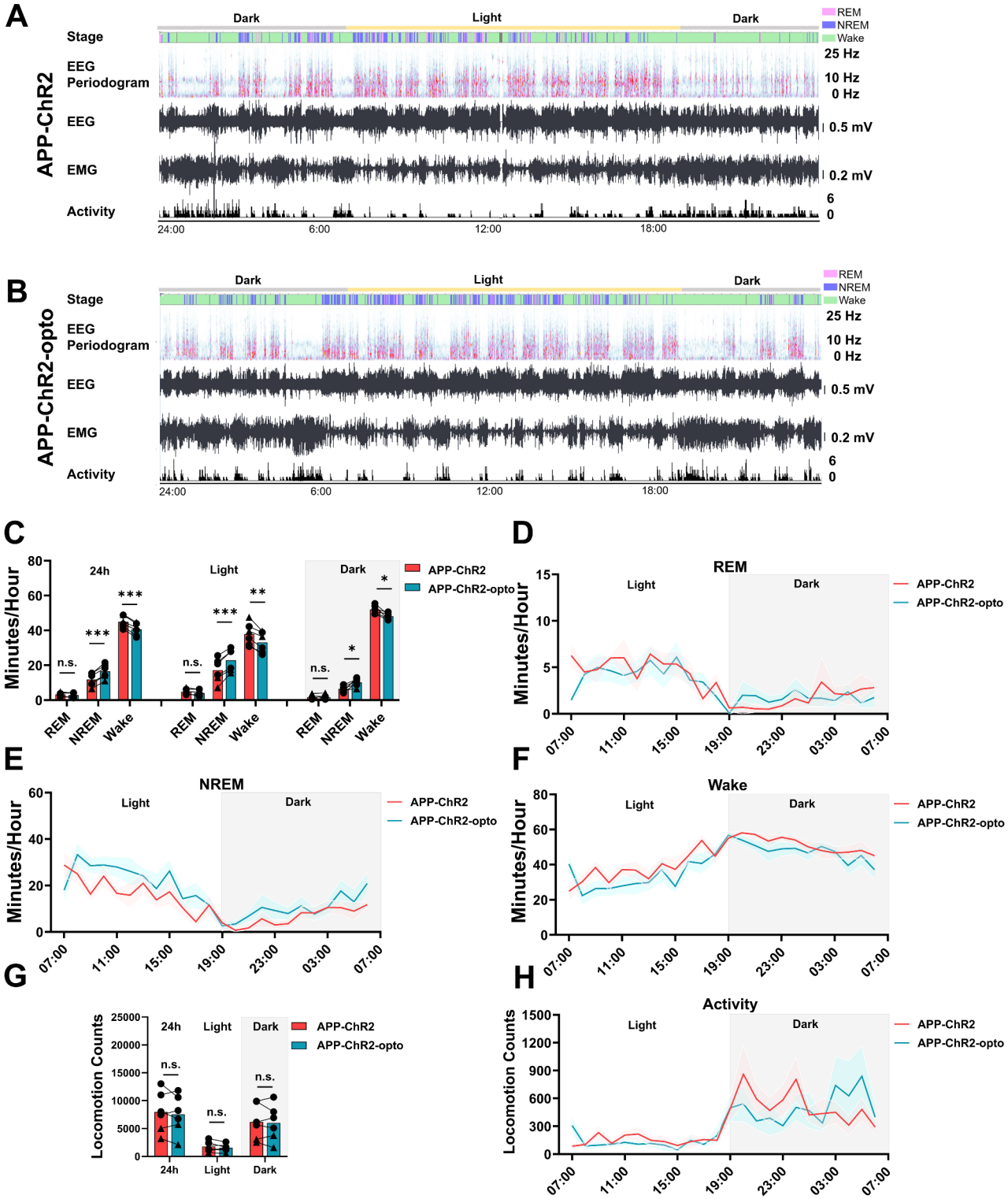

Fig. 2. Optogenetic stimulation of astrocytes increased NREM sleep in APP mice. (**A**, **B**) Overall 24-hour sleep pattern and sleep architecture of an APP-ChR2 mouse before (**A**) and during (**B**) optogenetic stimulation (APP-ChR2-opto). (**C**) Average time spent in each sleep-wake cycle stage (NREM, REM and Wake) during 24-hour, 12-hour dark phase and 12-hour light phase in APP-ChR2 mice before and during optogenetic stimulation. (**D**-**F**) Time course of the changes in REM (**D**), NREM (**E**) and Wake states (**F**) in APP-ChR2 mice before and during optogenetic stimulation. (**G**) Counts of locomotor activity in APP-ChR2 mice before and during optogenetic stimulation. (**H**) Time course of locomotor activity in APP-ChR2 mice before and during optogenetic stimulation. All data are expressed as mean ± standard error. Data points represented by filled circles indicate measurements obtained from male mice, while filled triangles denote data from female mice. Paired t-tests were used. The number of mice examined: APP-ChR2 = 6 mice. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$. n.s., not significant.

telemetry) across the DSI receiver positioned under the cage. Despite the increased sleep duration, optogenetic stimulation of ChR2 targeting astrocytes (APP-ChR2-opto) failed to significantly alter locomotion counts across the 24-hour period, or the 12-hour light and dark periods (Fig. 2G,H).

Overall, our data demonstrate that optogenetic stimulation of astrocytes resulted in considerable increases in NREM sleep duration accompanied by significant reductions in wakefulness. Interestingly, the duration of REM sleep remained comparable to that prior to stimulation despite alterations in NREM sleep and wakefulness. Locomotion did not change significantly, despite the observed increases in sleep duration. Thus, optogenetic treatment increased NREM sleep and decreased Wake.

Optogenetic stimulation of astrocytes increased delta power and improved sleep fragmentation in APP mice

When compared to WT mice, APP mice spent more time awake and less time in NREM sleep[17]. APP mice also exhibited lower delta power and increased sleep fragmentation[17,34,36]. Based on this, we tested whether optogenetic stimulation of astrocytes affected EEG power and sleep fragmentation in APP mice. With the sleep states determined, state-dependent power spectral analysis was conducted. The normalized power spectra for NREM, REM, and Wake for the 24-hour and 12-hour light and dark phases were compared between the two groups. To examine which frequency bands were affected, the power spectra were binned into delta (0.5–4 Hz), theta (4–8 Hz), alpha (8–12 Hz), sigma (12–16 Hz), and beta (16–24 Hz) frequency bands.

Delta (0.5–4 Hz) power during NREM sleep was increased in response to stimulation relative to the baseline in 24-hour (1.97 ± 0.18 for APP-ChR2 vs. 2.04 ± 0.15 for APP-ChR2-opto: $P = 0.0111$) and 12-hour dark phases (1.99 ± 0.21 for APP-ChR2 vs. 2.07 ± 0.23 for APP-ChR2-opto: $P = 0.0066$) (Figs. 2A,B and 3A,C-E). Increases in delta were accompanied by decreases in alpha (8–12 Hz) power in 24-hour (0.82 ± 0.07 for APP-ChR2 vs. 0.78 ± 0.05 for APP-ChR2-opto: $P = 0.0111$) and 12-hour dark phases (0.80 ± 0.08 for APP-ChR2 vs. 0.76 ± 0.07 for APP-ChR2-opto: $P = 0.0018$) (Figs. 2A,B and 3A and C-E). Optogenetic stimulation did not reveal significant power differences during REM sleep (Fig. 3B,F-H). Additionally, optogenetic stimulation effectively mitigated sleep fragmentation, evidenced by prolonged NREM bout lengths throughout the recording period, while maintaining the overall bout numbers (Fig. 3I,J).

Altogether, we showed that optogenetic stimulation of astrocytes improved delta power and decreased alpha, coincident with improving sleep quality by reducing sleep fragmentation.

Light stimulation of mCherry lacking ChR2 failed to significantly alter sleep in APP mice

To control for light toxicity, sleep recordings were acquired in APP mice expressing mCherry and lacking ChR2. Light stimulation of mCherry in the absence of ChR2 failed to induce significant alterations in sleep architecture among APP mice (Fig. 4A,B). Specifically, there were no discernible differences in the durations of NREM, REM sleep, or wakefulness before and during light stimulation during both light and dark phases (Fig. 4C-F). Locomotion was not significantly affected by light stimulation either (Fig. 4G,H). Additionally, Fourier transform analysis revealed comparable power spectral density plots during NREM and REM sleep (Fig. 5A-H). There was no substantial change in sleep fragmentation (Fig. 5I,J). Light stimulation of mCherry failed to affect sleep. Thus, light stimulation of ChR2 resulting in optogenetic activation of astrocytes was necessary for the increase in NREM sleep.

Altogether, our data suggest that optogenetic stimulation of ChR2 targeting astrocytes improved sleep quantity and quality.

Discussion

The amyloid cascade hypothesis implicates Aβ as the initiator of a pathological pathway leading to AD. Growing evidence supports a direct relationship between Aβ and sleep disruptions[2,6]. Sleep impairments, which are prevalent during preclinical stages, are thought to contribute to AD onset[7]. Increasing Aβ pathology is thought to further disrupt sleep by causing sleep fragmentation, reducing NREM sleep while increasing wakefulness[4,9,18,37,38]. Since disruptions of sleep and sleep-dependent brain rhythms actively contribute to Alzheimer's progression, enhancing NREM sleep and SWA might slow AD progression[17,29,39,40]. Indeed, optogenetic stimulation of neurons and astrocytes that increased SWA slowed pathophysiology associated with AD and rescued memory impairments in APP mice[17,29]. Here, we determined the degree to which optogenetic stimulation targeting astrocytes improved sleep in APP mice.

This study investigated the effects of optogenetic stimulation of astrocytes on sleep patterns in APP mice, a widely used mouse model of amyloidosis[32]. Prior investigations revealed sleep disturbances in APP mice. These disturbances included reduced NREM sleep and increased wakefulness[17,33,34]. Thus, we monitored sleep/wake using an EEG/EMG telemetry system in 8-month-old APP mice that were treated with optogenetic stimulation of astrocytes. Cortical astrocytes were targeted with viral vectors to express ChR2-mCherry or a control, mCherry without ChR2. Our findings showed that optogenetic stimulation significantly increased the duration of NREM sleep but not REM sleep. Additionally, we observed an increase in delta power during NREM sleep. Higher delta power indicated more profound and restorative sleep[41]. To elucidate the role of astrocytes in sleep disturbances associated with AD, further investigation is required to determine whether the effects of optogenetic stimulation targeting astrocytes are specific to the amyloidosis mouse model. Previous studies reported that astrocyte-specific optogenetic stimulation of C57BL/6J mice had increased durations of REM and NREM sleep[24], consistent with our results. We also recently reported that optogenetic targeting of astrocytes at slow wave frequency restored slow oscillation power with no significant effect on slow oscillation frequency in APP mice[29]. We have sought to determine underlying biological mechanisms to account for the observed changes.

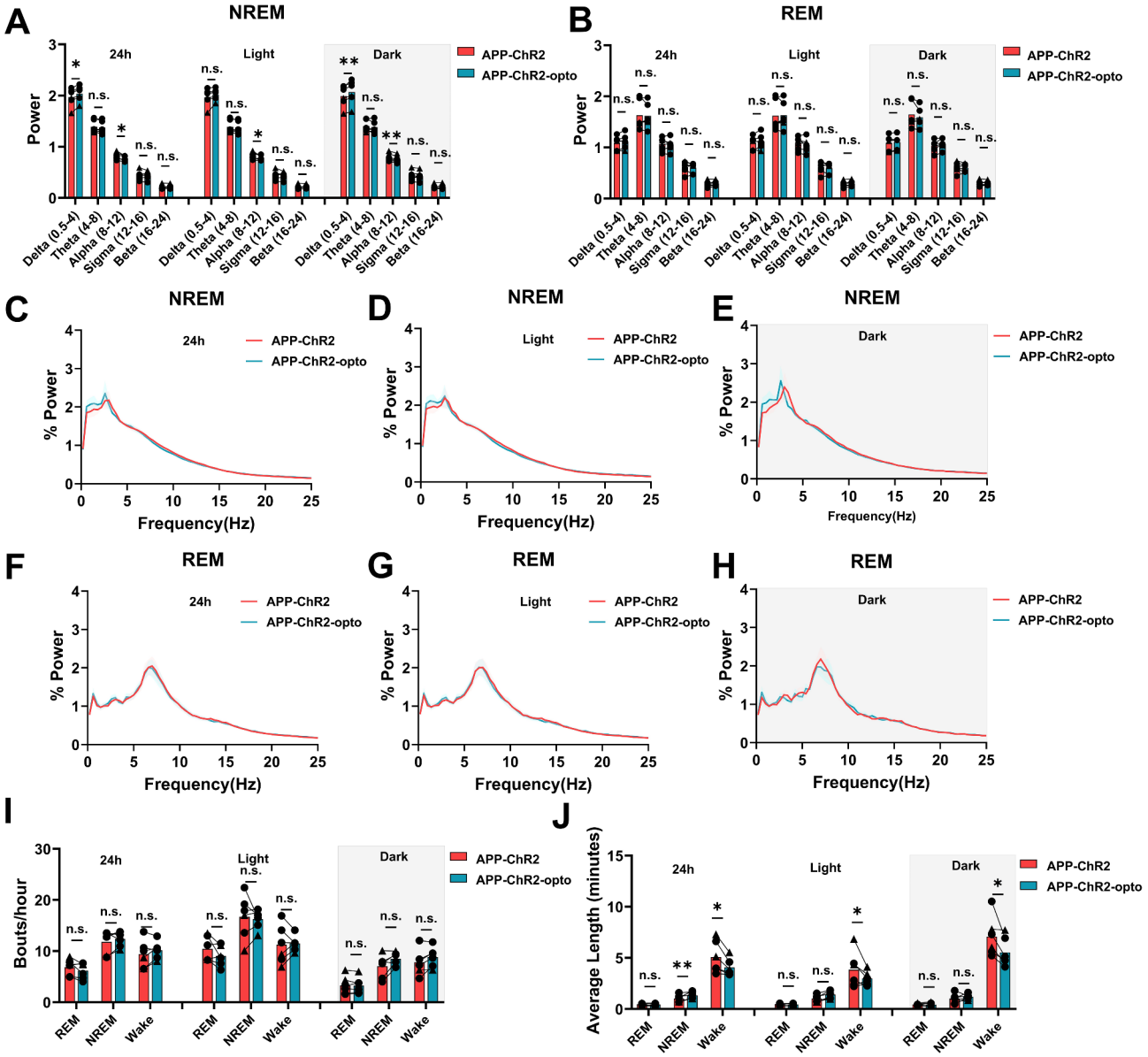

Fig. 3. Optogenetic stimulation of astrocytes increased delta power during sleep and promoted sleep integrity in APP mice. (**A**, **B**) The average EEG power density in the delta (0.5–4 Hz), theta (4–8 Hz), alpha (8–12 Hz), sigma (12–16 Hz), and beta (16–24 Hz) bands during NREM and REM sleep in the 24-hour, 12-hour light phase and 12-hour dark phase in APP-ChR2 mice before and during optogenetic stimulation (APP-ChR2-opto). (**C**-**H**) Relative power spectral density of NREM and REM sleep during 24-hour, 12-hour light phase, and 12-hour dark phase in APP-ChR2 mice before and during optogenetic stimulation. (**I**, **J**) Average bout count (**I**) and length (**J**) in each sleep-wake cycle stage (NREM, REM, and Wake) during the 24-hour, 12-hour dark phase, and 12-hour light phase in APP-ChR2 mice before and during optogenetic stimulation. All data are expressed as means ± standard error. Data points represented by filled circles indicate measurements obtained from male mice, while filled triangles denote data from female mice. Paired t-tests were used. The number of mice examined: APP-ChR2 = 6 mice. $^{*}P < 0.05$ and $^{**}P < 0.01$. n.s., not significant.

Sleep fragmentation, characterized by frequent awakenings and interruptions of the sleep cycle, is a well-recognized risk factor for AD[13,42,43]. Aβ leads to sleep fragmentation[36,44]. Sleep fragmentation further exacerbates amyloid pathology by increasing Aβ levels[44,45]. Thus, sleep fragmentation is not just an AD symptom but also actively contributes to the disease's progression[36,46]. Sleep fragmentation can negatively impact memory formation by interfering with the neural processes essential for memory consolidation, changing the patterns of brain activity during sleep, and elevating stress levels. These disruptions can ultimately hinder the encoding and retrieval of memories[46]. In our study, optogenetic activation of astrocytes effectively improved sleep by reducing sleep fragmentation, evidenced by prolonged NREM bout lengths, while maintaining the overall bout numbers. Our finding that optogenetic activation of astrocytes reduces sleep fragmentation is potentially clinically

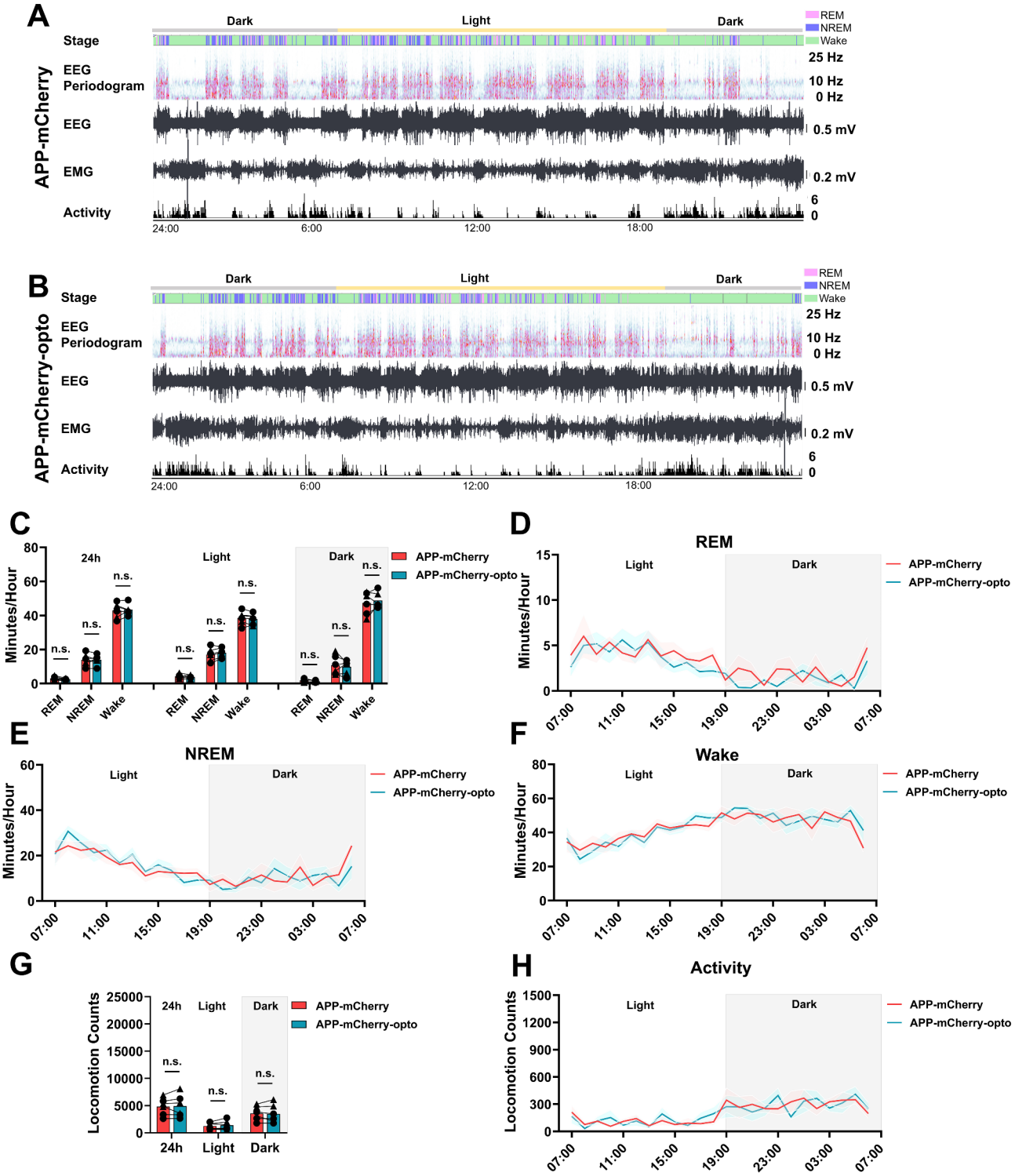

Fig. 4. Light stimulation of mCherry in the absence of ChR2 did not significantly affect NREM sleep in APP mice. (**A**, **B**) Overall 24-hour sleep pattern and sleep architecture of APP-mCherry mice before (**A**) and during (**B**) light stimulation (APP-mCherry-opto). (**C**) Average time spent in each sleep-wake cycle stage (NREM, REM, and Wake) during the 24-hour, 12-hour dark phase and 12-hour light phase in APP-mCherry mice before and during light stimulation. (**D**-**F**) Time course of the changes in REM (**D**), NREM (**E**), and Wake states (**F**) in APP-mCherry mice before and during light stimulation. (**G**) Counts of the locomotor activity in APP-mCherry mice before and during light stimulation. (**H**) Time course of the locomotor activity in APP-mCherry mice before and during light stimulation. All data are expressed as mean ± standard error. Data points represented by filled circles indicate measurements obtained from male mice, while filled triangles denote data from female mice. Paired t-tests were used. The number of mice examined: APP-mCherry = 6 mice. n.s., not significant.

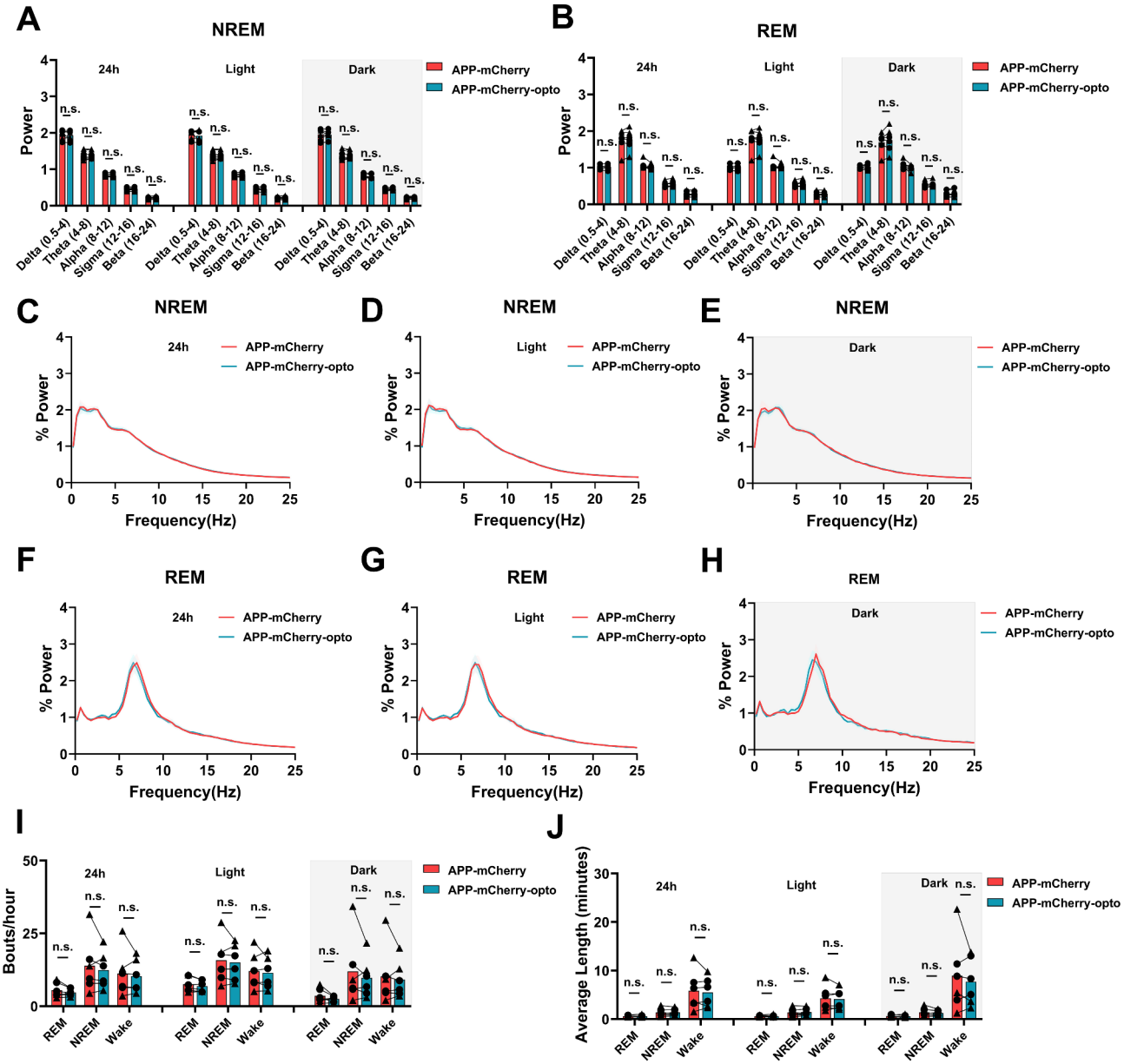

Fig. 5. Light stimulation of mCherry in the absence of ChR2 did not significantly affect power during sleep and sleep integrity in APP mice. (**A**, **B**) The average EEG power density in the delta (0.5–4 Hz), theta (4–8 Hz), alpha (8–12 Hz), sigma (12–16 Hz), and beta (16–24 Hz) bands during NREM and REM sleep in 24-hour, 12-hour light phase, and 12-hour dark phase in APP-mCherry mice before and during light stimulation (APP-mCherry-opto). (**C**-**H**) Relative power spectral density of NREM and REM sleep during 24-hour, 12-hour light phase, and 12-hour dark phase in APP-mCherry mice before and during light stimulation. (**I**, **J**) Average bout count (**I**) and length (**J**) in each sleep-wake cycle stage (NREM, REM, and Wake) during 24-hour, 12-hour light phase, and 12-hour dark phase in APP-mCherry mice before and during light stimulation. All data are expressed as means ± standard error. Data points represented by filled circles indicate measurements from male mice, while filled triangles denote data from female mice. Paired t-tests were used. The number of mice examined: APP-mCherry = 6 mice. n.s., not significant.

important since sleep fragmentation is a risk factor for AD and a condition that accelerates neuropathological changes in AD mouse models. Importantly, none of these effects on sleep were observed in the control group treated with light stimulation of mCherry lacking ChR2. This underscores the fact that brain rhythm rescue as a result of optogenetic stimulation of astrocytes led to beneficial effects on sleep. These results are consistent with our previous findings and other literature[17,24].

Astrocytes are essential components of the tripartite synapse, which includes the pre- and postsynaptic neuronal processes, as well as the astrocytic process[25]. Astrocytes can release gliotransmitters in ways that depend on calcium levels. Their capacity to release gliotransmitters is crucial for their function in integrating

information within neuronal networks[22]. Astrocytic calcium signals vary noticeably throughout the sleep-wake cycle, showing reductions during sleep phases compared to periods of wakefulness. The genetic removal of a critical pathway for astrocytic calcium signaling resulted in disrupted NREM sleep[26]. The majority of astrocytic calcium is thought to result from upstream activation of G-protein coupled receptors (GPCRs)[47]. Enhancements of in vivo astrocytic Gi-GPCR signaling increased delta power without altering sleep duration. Conversely, reducing Gq-GPCR signaling resulted in longer sleep durations without altering sleep depth in mice[23]. Other investigations into the role of astrocyte activation during changes in cortical states have spotlighted the critical roles of calcium waves in ion channel signaling[48,49].

Astrocytes can become dysfunctional, potentially exacerbating disease progression as part of AD. Astrocytes undergo changes in morphology and function, leading to impaired synaptic support, altered neuroinflammatory responses, and compromised Aβ clearance[2,50]. These reactive astrocytes contribute to the progression of AD pathology and exacerbate cognitive decline[50]. Furthermore, disruptions in astrocytic calcium transients were reported due to dysregulations in astrocytic calcium homeostasis in AD models[29,51]. We reported that astrocytes exhibited aberrant calcium transients associated with alterations in slow oscillations in APP mice[29]. Optogenetic targeting of cortical astrocytes restored slow oscillations possibly through the restoration of astrocytic calcium transients associated with slow waves. Restoration of circuit function resulting in slow wave rescue alleviated sleep impairments as we saw in the current study. Another study reported that optogenetic stimulation of astrocytes in the posterior hypothalamus increased sleep in C57BL/6J mice[24]. Thus, further investigations are necessary to clarify the underlying mechanisms between astrocyte stimulation and sleep regulation.

Locomotor activity is often used as a proxy for sleep assessments in mice, since locomotion is inversely related to sleep[52]. As our data showed that optogenetic stimulation of astrocytes did not affect locomotor behavior, the increases in sleep time were not due to decreased or impaired mobility. Mice exhibit periods of quiet wakefulness during which they are relatively inactive but not fully asleep. These quiet wakefulness periods would be indistinguishable from sleep based solely on locomotor activity[53,54]. In addition, mice are known to engage in nesting behavior, particularly during rest periods. They may spend significant amounts of time inside their nests without exhibiting locomotor activity, which could be mistakenly interpreted as sleep[53,54]. Thus, EEG/EMG measures provide a more sensitive readout of behavioral states since it provides distinction between quiet wakefulness and sleep.

Astrocytes are abundant throughout the brain. One limitation of our study is the optogenetic activation of astrocytes in a single brain region, anterior cortex, left hemisphere. It is unclear whether optogenetic activation of astrocytes in other parts of the cortex or the deeper brain structures, such as thalamus, would elicit slow waves and rescue sleep. Future studies are needed to test these possibilities.

We previously reported that optogenetic stimulation of GABAergic interneurons in the cortex at a similar location in anterior cortex could rescue sleep impairments in APP mice[17]. Thus, it is possible that astrocytic rescue of sleep impairments could be mediated through recruitment of GABAergic interneurons.

In summary, our study demonstrate that optogenetic activation of astrocytes enhanced NREM sleep quantity and quality in APP mice. This may contribute to the capacity for astrocytic activation to reduce amyloid burden in APP mice[29]. Since sleep impairments contribute to AD progression, modulating astrocytic activity provides a novel therapeutic avenue to pursue for enhancing sleep and slowing AD progression.

Data availability

All data supporting the conclusions of this article are included within the article and in additional files provided.

Received: 24 June 2024; Accepted: 13 September 2024
Published online: 04 October 2024

References

1. Jack, C. R. et al. NIA-AA research framework: Toward a biological definition of Alzheimer's disease. *Alzheimers Dement.***14**, 535–562 (2018).
2. Knopman, D. S. et al. Alzheimer disease. *Nat. Rev. Dis. Primers***7**, 33 (2021).
3. Sarlus, H. & Heneka, M. T. Microglia in Alzheimer's disease. *J. Clin. Invest.***127**, 3240–3249 (2017).
4. Hardy, J. & Selkoe, D. J. The amyloid hypothesis of Alzheimer's disease: Progress and problems on the road to therapeutics. *Science***297**, 353–356 (2002).
5. Liew, S. C. & Aung, T. Sleep deprivation and its association with diseases—a review. *Sleep. Med.***77**, 192–204 (2021).
6. Musiek, E. S. & Holtzman, D. M. Mechanisms linking circadian clocks, sleep, and neurodegeneration. *Science***354**, 1004–1008 (2016).
7. Ju, Y. E. S. et al. Sleep quality and preclinical Alzheimer disease. *JAMA Neurol.***70**, 587–593 (2013).
8. Zavecz, Z. et al. NREM sleep as a novel protective cognitive reserve factor in the face of Alzheimer's disease pathology. *BMC Med.***21**, 156 (2023).
9. Mander, B. A. et al. β-Amyloid disrupts human NREM slow waves and related hippocampus-dependent memory consolidation. *Nat. Neurosci.***18**, 1051–1057 (2015).
10. Gerashchenko, D., Wisor, J. P. & Kilduff, T. S. Sleep-active cells in the cerebral cortex and their role in slow-wave activity. *Sleep. Biol. Rhythms***9**, 71–77 (2011).
11. Wilckens, K. A., Ferrarelli, F., Walker, M. P. & Buysse, D. J. Slow-wave activity enhancement to improve cognition. *Trends Neurosci.***41**, 470–482 (2018).
12. Lucey, B. P. & Holtzman, D. M. How amyloid, sleep and memory connect. *Nat. Neurosci.***18**, 933–934 (2015).
13. Lim, A. S. P., Kowgier, M., Yu, L., Buchman, A. S. & Bennett, D. A. Sleep fragmentation and the risk of Incident Alzheimer's Disease and Cognitive decline in older persons. *Sleep***36**, 1027–1032 (2013).
14. Xie, L. et al. Sleep drives metabolite clearance from the adult brain. *Science***342**, 373–377 (2013).
15. Kang, J. E. et al. Amyloid-beta dynamics are regulated by orexin and the sleep-wake cycle. *Science***326**, 1005–1007 (2009).
16. Iliff, J. J. et al. A paravascular pathway facilitates CSF flow through the brain parenchyma and the clearance of interstitial solutes, including amyloid β. *Sci. Transl Med.***4**, 147ra111 (2012).

17. Zhao, Q. et al. Sleep restoration by optogenetic targeting of GABAergic neurons reprograms microglia and ameliorates pathological phenotypes in an Alzheimer's disease model. *Mol. Neurodegener.***18**, 93 (2023).
18. Blattner, M. S. et al. Increased cerebrospinal fluid amyloid-β during sleep deprivation in healthy middle-aged adults is not due to stress or circadian disruption. *J. Alzheimers Dis.***75**, 471–482 (2020).
19. Shokri-Kojori, E. et al. β-Amyloid accumulation in the human brain after one night of sleep deprivation. *Proc. Natl. Acad. Sci. U. S. A.***115**, 4483–4488 (2018).
20. Miao, A. et al. Brain clearance is reduced during sleep and anesthesia. *Nat. Neurosci.***27**, 1046–1050 (2024).
21. Brancaccio, M., Patton, A. P., Chesham, J. E., Maywood, E. S. & Hastings, M. H. Astrocytes control circadian timekeeping in the suprachiasmatic nucleus via glutamatergic signaling. *Neuron***93**, 1420–1435e5 (2017).
22. Haydon, P. G. Astrocytes and the modulation of sleep. *Curr. Opin. Neurobiol.***44**, 28–33 (2017).
23. Vaidyanathan, T. V., Collard, M., Yokoyama, S., Reitman, M. E. & Poskanzer, K. E. Cortical astrocytes independently regulate sleep depth and duration via separate GPCR pathways. *Elife***10** (2021).
24. Pelluru, D., Konadhode, R. R., Bhat, N. R. & Shiromani, P. J. Optogenetic stimulation of astrocytes in the posterior hypothalamus increases sleep at night in C57BL/6J mice. *Eur. J. Neurosci.***43**, 1298–1306 (2016).
25. Perea, G., Navarrete, M. & Araque, A. Tripartite synapses: Astrocytes process and control synaptic information. *Trends Neurosci.***32**, 421–431 (2009).
26. Bojarskaite, L. et al. Astrocytic Ca2+ signaling is reduced during sleep and is involved in the regulation of slow wave sleep. *Nat. Commun.***11**, 3240 (2020).
27. Peng, W. et al. Regulation of sleep homeostasis mediator adenosine by basal forebrain glutamatergic neurons. *Science***369** (2020).
28. Szabó, Z. et al. Extensive astrocyte synchronization advances neuronal coupling in slow wave activity in vivo. *Sci. Rep.***7**, 6018 (2017).
29. Lee, Y. F. et al. Optogenetic targeting of astrocytes restores slow brain rhythm function and slows Alzheimer's disease pathology. *Sci. Rep.***13**, 13075 (2023).
30. Salmina, A. B. et al. Optogenetic and chemogenetic modulation of astroglial secretory phenotype. *Rev. Neurosci.***32**, 459–479 (2021).
31. Figueiredo, M. et al. Comparative analysis of optogenetic actuators in cultured astrocytes. *Cell. Calcium***56**, 208–214 (2014).
32. Jankowsky, J. L. et al. Mutant presenilins specifically elevate the levels of the 42 residue beta-amyloid peptide in vivo: Evidence for augmentation of a 42-specific gamma secretase. *Hum. Mol. Genet.***13**, 159–170 (2004).
33. Zhang, F. et al. Alteration in sleep architecture and electroencephalogram as an early sign of Alzheimer's disease preceding the disease pathology and cognitive decline. *Alzheimers Dement.***15**, 590–597 (2019).
34. Kent, B. A., Strittmatter, S. M. & Nygaard, H. B. Sleep and EEG power spectral analysis in three transgenic mouse models of Alzheimer's disease: APP/PS1, 3xTgAD, and Tg2576. *J. Alzheimers Dis.***64**, 1325–1336 (2018).
35. Storch, C., Höhne, A., Holsboer, F. & Ohl, F. Activity patterns as a correlate for sleep-wake behaviour in mice. *J. Neurosci. Methods***133**, 173–179 (2004).
36. Sethi, M. et al. Increased fragmentation of sleep-wake cycles in the 5XFAD mouse model of Alzheimer's disease. *Neuroscience***290**, 80–89 (2015).
37. Dufort-Gervais, J., Mongrain, V. & Brouillette, J. Bidirectional relationships between sleep and amyloid-beta in the hippocampus. *Neurobiol. Learn. Mem.***160**, 108–117 (2019).
38. Ettore, E. et al. Relationships between objectives sleep parameters and brain amyloid load in subjects at risk for Alzheimer's disease: The INSIGHT-preAD study. *Sleep***42**, zsz137 (2019).
39. Jagirdar, R. et al. Restoring activity in the thalamic reticular nucleus improves sleep architecture and reduces Aβ accumulation in mice. *Sci. Transl. Med.***13**, eabh4284 (2021).
40. Wang, J. et al. Thermoneutral temperature exposure enhances slow-wave sleep with a correlated improvement in amyloid pathology in a triple-transgenic mouse model of Alzheimer's disease. *Sleep***47** (2024).
41. Long, S. et al. Sleep quality and electroencephalogram delta power. *Front. Neurosci.***15**, 803507 (2021).
42. Li, P. et al. Circadian disturbances in Alzheimer's disease progression: A prospective observational cohort study of community-based older adults. *Lancet Healthy Longev.***1**, e96–e105 (2020).
43. Johnson, C. E., Duncan, M. J. & Murphy, M. P. Sex and sleep disruption as contributing factors in Alzheimer's disease. *J. Alzheimer's Dis.***97**, 31–74 (2024).
44. Duncan, M. J. et al. Chronic fragmentation of the daily sleep-wake rhythm increases amyloid-beta levels and neuroinflammation in the 3xTg-AD mouse model of Alzheimer's disease. *Neuroscience***481**, 111–122 (2022).
45. Minakawa, E. N. et al. Chronic sleep fragmentation exacerbates amyloid β deposition in Alzheimer's disease model mice. *Neurosci. Lett.***653**, 362–369 (2017).
46. Joiner, W. J. Neuroscience: Sleep fragmentation impairs memory formation. *Curr. Biol.***29**, R1181–R1184 (2019).
47. Agulhon, C. et al. What is the role of astrocyte calcium in neurophysiology? *Neuron***59**, 932–946 (2008).
48. Wang, F. et al. Distinct astrocytic modulatory roles in sensory transmission during sleep, wakefulness, and arousal states in freely moving mice. *Nat. Commun.***14**, 2186 (2023).
49. Reitman, M. E. et al. Norepinephrine links astrocytic activity to regulation of cortical state. *Nat. Neurosci.***26**, 579–593 (2023).
50. Selkoe, D. J. Early network dysfunction in Alzheimer's disease. *Science***365**, 540–541 (2019).
51. Kuchibhotla, K. V. et al. Abeta plaques lead to aberrant regulation of calcium homeostasis in vivo resulting in structural and functional disruption of neuronal networks. *Neuron***59**, 214–225 (2008).
52. Park, J. et al. Prediction of locomotor activity by infrared motion detector on sleep-wake state in mice. *Clin. Psychopharmacol. Neurosci.***19**, 303–312 (2021).
53. Rayan, A. et al. Sleep scoring in rodents: Criteria, automatic approaches and outstanding issues. *Eur. J. Neurosci.***59**, 526–553 (2024).
54. Rattenborg, N. C. et al. Sleep research goes wild: New methods and approaches to investigate the ecology, evolution and functions of sleep. *Philos. Trans. R Soc. Lond. B Biol. Sci.***372** (2017).

Acknowledgements

Illustrations were created with BioRender.com.

Author contributions

Conception and design of study (Q.Z, Y.L, K.K), mouse surgery (Q.Z), data analysis (Q.Z, S.P, M.M with help from H.Z, H.L), manuscript preparation (Q.Z, S.Y with help from K.K, D.G) and project supervision (S.G, D.G, B.B, K.K). All authors read and approved the final manuscript.

Funding
This work was supported by the BrightFocus Foundation Grants A2022028F and A2020833S; The Alzheimer's Association Grant AARG-18-52336; The Overseas research fellowship, Japan Society for the Promotion of Science 202360054; National Institutes of Health Grant R01AG066171.

Declarations

Ethics approval and consent to participate
This study has no human data. All animal studies are approved by Massachusetts General Hospital IACUC.

Consent for publication
All authors approve of this manuscript and consent to publication.

Competing interests
The authors declare no competing interests.

Additional information
Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-024-73082-8.

Correspondence and requests for materials should be addressed to K.V.K.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.