Functional restoration of REM sleep fragmentation, hippocampal oscillatory activity and altered cholinergic signaling at presymptomatic stages of AD

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Research Article

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Abstract

Background

Alzheimer’s disease (AD) is a severe neurodegenerative disorder that is hallmarked by the progressive accumulation of amyloid beta (Aβ) and tau proteins in the brain, leading to dementia. Sleep alterations occur at early stages of AD, before cognitive symptoms become apparent, which could play an important role in the pathophysiology and detection of AD. The current study aimed to evaluate how circadian rhythm, sleep macro-architecture and hippocampal oscillations are altered at pre-plaque and early-plaque stages of AD in TgF344-AD rats.

Methods

We performed 24-hour hippocampal electrophysiological measurements in TgF344-AD rats and wildtype littermates at pre- and early-plaque stages of AD, combined with histological analysis to evaluate histopathological alterations.

Results

We observed a differential impact of AD on rapid eye movement (REM) and non-REM (NREM) sleep. Circadian rhythmicity was intact and TgF344-AD rats did not show signs of NREM sleep fragmentation. We observed a significantly increased probability for shorter REM bouts, suggestive of REM sleep fragmentation, in TgF344-AD rats at the pre-plaque stage, which recovered at the early-plaque stage. In addition, we observed a significantly decreased theta-gamma coupling, a measure for task-related information ordering, at the pre-plaque stage, which was partially restored at the early-plaque stage. Moreover, theta-gamma coupling in the slow gamma range was significantly increased during the pre-plaque stage in TgF344-AD rats but returned to WT levels at the early-plaque stage. Interestingly, the partial compensation of hippocampal activity and REM sleep behavior coincided with an increased number of cholinergic synapses in the hippocampus during the early-plaque stage in TgF344-AD rats, suggestive of basal forebrain cholinergic compensation mechanisms.

Conclusion

The results from this study reveal early changes in sleep architecture and hippocampal function prior to Aβ plaque deposition in AD. In addition, the current findings highlight the important role of the cholinergic system to compensate for AD-related network alterations. Network disturbances and sleep alterations are known to drive disease progression. Modulation of cholinergic signaling in early, presymptomatic AD might be a promising therapeutic strategy to alter disease progression by restoring network function and sleep architecture.

1. Background
Alzheimer’s Disease (AD) is a progressive neurodegenerative disorder characterized by the accumulation of extracellular amyloid-beta (Aβ) plaques, intracellular tau-aggregation, synaptic dysfunction, and neuronal loss, resulting in cognitive dysfunction and eventually, dementia. Neuropathological changes underlying AD start 10–20 years before the onset of cognitive symptoms, with accumulation of Aβ being the first identifiable hallmark [1–3]. Studies have demonstrated that soluble Aβ species, which are the precursors of Aβ plaques, interfere with synaptic function before cognitive symptoms become apparent [4–10].

Brain regions and pathways important for sleep and wake regulation [11], such as the cholinergic system [12] and locus coeruleus [13], are affected early by Aβ and tau accumulation [2, 3, 14]. Recent studies have demonstrated that soluble Aβ interferes with sleep regulation inducing increased or decreased wakefulness depending on the oligomer species present in the brain [15, 16]. In addition, several studies have observed changes in sleep architecture and efficiency during asymptomatic stages of AD in humans [17] and animal models of AD [18, 19]. Under physiological circumstances the sleep-wake cycle modulates Aβ levels in the interstitial fluid and cerebrospinal fluid [20]. Neuronal activity increases the generation and release of Aβ [21], leading to increased extracellular Aβ levels during wakefulness. Conversely, Aβ is cleared from the interstitial fluid during sleep by the enhanced activity of the glymphatic system [22]. These observations suggest a feed-forward loop between sleep loss and Aβ accrual, wherein altered sleep architecture (eg. decreased total sleep time, decreased amount of NREM sleep and sleep fragmentation) results in increased extracellular Aβ levels and aggregation, which in turn further disrupts sleep, therefore, driving AD progression [18]. However, the neuropathological mechanisms underlying Aβ induced sleep disruptions remain elusive.

Sleep is imperative for hippocampus-dependent memory processes and changes in sleep architecture or the total amount of sleep leads to decreased cognitive performance [23]. Hippocampal neuronal activity underlies memory consolidation through specific, coordinated electrophysiological events such as sharp wave-ripples (SWR), slow oscillations and theta-gamma coupling. Several studies have observed alterations in hippocampal neuronal oscillations, which coincided with cognitive alterations, in different animal models of AD [24–28]. These phenomena rely heavily on the delicate interplay of GABA-ergic, glutamatergic and cholinergic neurotransmission. Perturbations of these neurotransmitter systems due to the early accumulation of soluble Aβ could disrupt these oscillatory events at pre-plaque stages of AD, and might prove to be a relevant biomarker, and therapeutic target for AD.

A recent study of our group demonstrated spatiotemporal alterations in brain network activity, suggestive of synaptic dysfunction, already at the pre-plaque stage in anesthetized TgF344-AD rats. Hence, we hypothesized that soluble Aβ-associated alterations in neurotransmitter signaling (cholinergic, glutamatergic and/or GABA-ergic) at pre-plaque and early-plaque stages of AD, disrupt sleep architecture and hippocampal oscillatory activity during sleep. Therefore, we have performed 24-hour hippocampal electrophysiological measurements in 4-month-old TgF344-AD rats, displaying soluble Aβ pathology in the absence of Aβ plaques, and 6-month-old TgF344-AD rats demonstrating hyperphosphorylated Tau accumulation in the locus coeruleus and hippocampal and cortical Aβ plaques. In addition, histological
analyses of GABA-ergic, glutamatergic and cholinergic synapses in the hippocampus was performed to further investigate possible disease mechanisms underlying the electrophysiological and behavioral alterations. Investigation of the synaptic mechanisms underlying sleep disturbances and hippocampal dysfunction during sleep, might offer novel insights and therapeutic targets during presymptomatic stages of AD.

2. Material and Methods

2.1. Experimental design, animals, and ethical statement

To evaluate hippocampal dysfunction and sleep disturbances during early stages of AD, cross-sectional electrophysiological experiments were performed. All procedures were in accordance with the guidelines approved by the European Ethics Committee (decree 2010/63/EU) and were approved by the Committee on Animal Care and Use at the University of Antwerp, Belgium (approval number: 2019-06). Electrophysiological experiments were performed in 4- and 6-month-old TgF344-AD male rats (N = 7/N = 5) and wildtype (WT) male littermates (N = 5/N = 5). Rats were group housed prior to the electrode implantation but housed separately afterwards. All animals were kept on a reversed, 12h light/dark cycle, with controlled temperature (20–24°C) and humidity (40–60%) conditions. Standard food and water were provided ad libitum and cage enrichment was provided to each animal. An additional 24 male rats were used for histological analysis of pathology and synaptic markers (6 WT and 6 TgF344-AD rats for each time point).

2.2. Chronic hippocampal electrophysiological measurements

2.2.1. Surgical procedure

Anesthesia was induced using 5% isoflurane (Isoflo®, Abbott Laboratories) (medical air 1L/min) and maintained using 2–3% isoflurane (at 1L/min) during the surgery. Animals were placed in a stereotaxic frame and a craniotomy was made above the right dorsal hippocampus (AP -3.00, ML 2.50). A 16-channel laminar electrode (E16 + R-100-S1-L6 NT, Atlas Neuro-engineering, Belgium) with internal reference was carefully lowered (DV 2.5–3.5 mm) into the dorsal hippocampus and the pointy tip feature of the electrode will penetrate the dura without damaging the dura. The exact depth of the recording sites was identified online by the layer-specific local field potentials (LFP) of the hippocampus. The craniotomy was sealed with a sterile silicon gel (Kwik-Cast, WPI). Stainless steel screws were drilled into the skull overlaying the olfactory bulb, frontal cortex, left hippocampus, and cerebellum, of which the latter served as ground electrode. The implant was covered in several layers of dental cement (Stoelting) and the wound was closed. Rats were treated with antibiotics until three days after the surgery (5mg/kg, Enrofloxacin (Baytril®, Bayer) (in drinking water) and analgesics were administered (0.05mg/kg Buprenorphine (Temgesic®, Indivior Europe Limited) subcutaneous during surgery, followed by 2 daily
injections of 5mg/kg Carprofen (Rimadil®, Pfizer) for two days after surgery. Rats were allowed to recover for at least 7 days prior to the LFP recordings.

### 2.2.2. Neurophysiological data acquisition

Prior to the recordings, animals were habituated for at least 24 hours to the ventilated, light-regulated recording chamber and recording setup. Using a wireless electrophysiology system (W2100 system, Multichannel systems, Germany), electrophysiological signals (LFPs, neuronal spiking activity, and EMG activity) were acquired for 24 hours while the animal was freely behaving or sleeping in its home cage. During these measurements, animals were maintained on the 12/12h reversed light/dark cycle and had ad libitum access to food and water.

### 2.3. Validation of electrode position

Anesthesia was induced with 5% isoflurane and was maintained at 2-2.5%. An electrical current (30µA, 3s) was applied via the electrode at the top, middle, and bottom channels to allow validation of the electrode position. Thereafter, the animals were euthanized via intravenous injection of 50mg/kg pentobarbital (Dolethal®, Vetoquinol, Belgium), followed by cardiac perfusion with ice-cold phosphate-buffered saline (PBS) and 4% Paraformaldehyde (Merck Millipore, Merck KGaA, Darmstadt, Germany). The brains were surgically removed and postfixed for 4–6 hours using 4% PFA. A sucrose gradient was applied (5%, 10%, and 20% Sucrose in 0.1M PBS), after which the brains were snap frozen using liquid nitrogen and stored at -80°C until further processing. The frozen brains of the animals were sliced into 12 µm thick coronal sections using a cryostat (Cryostar NX 70, Thermo Fisher Scientific). The sections were stained with Nissl staining (Cresyl Violet 0.1%, Sigma-Aldrich) and studied under the light microscope to validate the position of the electrodes for each animal.

Each layer of the hippocampus has distinct functions and therefore, distinct oscillatory activity. The focus of this study is on the different layers of the CA1 region. Therefore, histological electrode validation was performed. Based on the location of the tip of the electrode (Fig. 1), we could infer which channels were placed in the different layers of CA1. These channel locations were further validated by the distinct oscillatory patterns of the different hippocampal layers, such as occurrence of sharp wave-ripples and theta power across different hippocampal layers. This channel information was used in further analyses. For 2 WT animals, validation was not possible because the lesions weren't visible under the microscope and are not shown in the figure. However, a comparison of the distinct oscillatory patterns with other WT animals showed no differences. Therefore, they were still included in the data analysis.

**Figure 1: histological validation of electrode position.** A) Schematic overview of electrode position in 4-month-old wildtype (WT) animals. Each blue electrode represents track of the electrode of a different WT subject. B) Schematic overview of the electrode position in 4-month-old TgF344-AD (Tg) animals. Each orange electrode represents track of the electrode of a different Tg subject. The red insert shows exemplary light microscopy images of Nissl-stained brain slices showing the electrode trace in the hippocampus of a WT animal (A) and TgF344-AD rat (B). Exemplary images of the electrode validation
2.4. Analysis

2.4.1. Sleep architecture and fragmentation

An automated sleep scoring algorithm was used to determine vigilance states for each 10 second epoch based on the theta-delta ratio and EMG activity. Each 10 second epoch was labeled as wake, rapid eye movement sleep (REM) or non-rapid eye movement sleep (NREM). Next, the automatic sleep scoring was checked and adjusted through visual inspection by two independent investigators. The scores of the two investigators were combined to a single score per animal that was used in our further analysis of sleep architecture. The percentage time spent in each vigilance state was calculated in 3h time periods across the 24h recording and sleep bout lengths during the light (inactive) and dark (active) phase of the day were derived to evaluate sleep fragmentation. Mean sleep bout lengths during the active and inactive phase and over the entire 24h period were calculated for each subject. The individual sleep bout lengths were used in a second analysis to create a cumulative probability plot and Kolmogorov-Smirnov tests (FDR corrected p < 0.05) were used to evaluate differences in sleep fragmentation between genotypes.

2.4.2. Power analysis of hippocampal LFPs

For each channel on the electrode, power spectra were calculated for each vigilance state using a Fast Fourier Transform in Brainstorm [29] using the Welch’s method (window size 20s, 50% overlap). Power spectra were normalized to the sum of the power across all LFP frequencies (0.5–250 Hz) to minimize variation in amplitude due to differences in the exact placement of the electrode. Normalized power spectra were averaged across genotypes separately for each state. Next, power for each vigilance state was calculated for specific frequency bands of interest, i.e., delta (0.4-4Hz), theta (5-12Hz), slow gamma (30-45Hz), fast gamma (60-120Hz) and sharp wave-ripple (120-250Hz). The power across the three channels with the highest power at each frequency was averaged for each subject and compared between groups and ages.

2.4.3. Phase-Amplitude coupling (PAC)

The amplitude of the hippocampal gamma rhythm is modulated by the phase of theta, a phenomenon named phase-amplitude coupling (PAC). To evaluate the strength of the theta-gamma coupling, the modulation index (MI) was used, that after bandpass filtering uses the phase of the slow oscillation and the amplitude envelope of the fast oscillation, to create a complex vector of which the length represents the amplitude of each fast oscillation whereas the phase of the slow oscillation is represented by the angle. In the case of an absence of PAC, these vectors form a roughly uniform circular shape centered around zero, however, if there is modulation, then the amplitude at a certain phase is higher, which will create a bump in the polar plot. The MI represents the length of this mean vector length (for a detailed description of calculations we refer to [30, 31]). The analysis of the PAC was performed on one channel, which was located in the pyramidal layer of the CA1, based on histological electrode validation (described...
in section 2.3). First, for each subject, a comodulogram was calculated which demonstrates the MI for each pair of frequencies. The theta band (5-12Hz) and gamma band (30-120Hz) were divided into 1Hz bins and for each combination of theta and gamma frequencies the mean modulation index across each REM epoch was calculated and averaged across all REM epochs to create a subject-based comodulogram. Next, a group-averaged comodulogram was computed in Matlab which demonstrated for each group the main frequencies of modulation. The main frequency modes of modulation were examined and were used to perform a time-resolved PAC (tPAC) analysis. tPAC computes the MI between specific frequency bands estimated from the group-averaged comodulogram for each 1-second epoch. The advantage of this method is that it allows more reliable quantification of the strength of the PAC. The obtained MI were averaged across epochs for each subject and the mean was statistically compared between groups and ages.

### 2.4.4. Offline detection and analysis of sharp wave-ripples

All preprocessing steps were conducted using the Fieldtrip toolbox [32]. First, to detect ripples in the pyramidal layer of CA1, the wide-band signal was band-pass filtered between 120 and 250Hz using a 400th order Butterworth infinite response filter and was afterwards down-sampled to 1200 Hz. Filtered data was segmented based on the NREM epochs obtained in Neuroscore. The segmented data was z-scored, rectified and smoothed using a rectangular filter window with a length of 8ms, generating the ripple power signal [33]. SWR were identified in the channels which were placed in the pyramidal layer of the CA1, based on the electrode validation described in 2.3.1. If the power within the ripple band exceeded a threshold of 4 standard deviations from the mean. Events were expanded until the power fell below 2 standard deviations and events with a duration shorter than 30ms and/or a peak spectral frequency lower than 140 Hz were discarded. Subsequently, a thresholding algorithm was applied to detect sharp waves in the stratum radiatum. Signals in the channel that demonstrated strong sharp waves were band pass filtered between 0.5 and 20Hz using a 400th order Butterworth filter. Sharp wave (SW) events which lasted between 20ms and 400ms were detected when the power of the filtered signal exceeded the threshold of 2.5 standard deviations from the mean [34]. Ripples that co-occurred with SW were kept for further analysis. The power in the sharp wave-ripple (SWR) band, the peak spectral frequency and the duration of events were extracted and compared between groups and ages.

### 2.5. Histology

#### 2.5.1. Immunofluorescence staining

To evaluate AD alterations in synaptic markers, histological analyses were performed on cryosections as described in [35]. Briefly, brains were extracted, and hemispheres were separated. Next, left hemispheres were embedded in OCT-embedding medium for sectioning. At 0.4, 1.40- and 3.90-mm lateral from the midline, sagittal sections of 12 µm were made using a Leica CM1950 cryomicrotome (Leica BioSystems, Belgium), thaw-mounted on VWR Superfrost Plus micro slides (VWR, Leuven, Belgium) and dried for 2 hours at 37°C. All immunohistochemical incubations were carried out at RT. Sections were pre-incubated for 30 min in blocking buffer containing 1% Triton X-100 before an overnight incubation with the primary
antibodies (Table 1). For the detection of the immunoreactivity, the sections were incubated for 4 hours with the appropriate combination of fluorescent-conjugated secondary, followed by a nuclear counterstain using 4’,6-diamidino-2-phenylindole (DAPI, Sigma-Aldrich, Hoeilaart, Belgium) for 10 min at room temperature. Samples were mounted in Citifluor AF1 (EMS, Hathfield, USA). Respective single-labeling studies which resulted in comparable staining, were performed to rule out nonspecific findings resulting from the multiple-staining process. Negative staining controls were performed by substitution of non-immune sera for the primary or secondary antisera.

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2.5.2. Image acquisition

Confocal images of immunolabeled tissue sections were acquired with a Perkin Elmer Ultraview Vox dual spinning disk confocal microscope, mounted on a Nikon Ti body using a 40x Plan Apo objective (NA 0.95). Lasers with wavelength 405nm, 488nm, 561nm and 640nm were used in combination with a quadruple dichroic and 445/60–525/50–615/70–705/90nm emission filters. Detection was done on a Hamamatsu C9100-50 CMOS camera. Image acquisition was done using Volocity software. Regions of interest were localized based on the DAPI staining. Per animal, 3 images were acquired on 3 non-consecutive sections in 3 axial positions separated by a 2 µm spacing.

2.5.3. Image analysis

Image analysis of the synaptic excitatory/inhibitory ratio was done in FIJI image analysis software [36] as described in [35]. A macro script was written for FIJI image analysis software [1] to detect synaptic markers and measure their intensity and is available on github (https://github.com/DeVosLab/SynapseDetection) [37, 38]. After maximum projection of the Z-stacks, synaptic marker spots were enhanced using a single or multi-scale Laplace filter with user-defined kernel sizes. For each marker, the threshold settings were kept constant for both rat groups and age categories studied. A manually defined threshold per region was applied to segment the spots after which an additional max finding (and region growing) step was included to untangle clustered spots. Only spots that had a projected area within a specific range (0.20–3.00 µm) were retained. Images with more than 10000 spots were discarded to exclude over segmented images (N = 8, 5.56%).

2.6. Statistics

The statistical analysis of the data was performed using the JMP Pro software (Version 16, SAS Institute Inc., Cary, NC, 1989–2021). For the statistical analysis of the sleep architecture, circadian rhythmicity,
power across different frequency bands, SWR characteristics, and PAC, outlier detection was performed on group level for each age using principal component analysis (PCA). Measurements with a $T^2$ statistic index higher than the 95% confidence interval were excluded. A two-way ANOVA (genotype, age, genotype*age) per region was performed. In case of a significant genotype*age interaction, post-hoc Student’s t-tests were performed. A false discovery rate correction using the Benjamini-Hochberg procedure was applied to correct for multiple comparisons (FDR $p < 0.05$). The interaction term was removed no significant interaction was observed and a two-way ANOVA (genotype, age) was used to evaluate genotype or age effects.

Regarding the analysis of the synaptic markers, outlier detection was performed using the interquartile range (1.5x IQR) per genotype, age, and region on all individual images (3 per animal). Animals with less than 2 images were excluded and the remaining images were averaged for each animal. A second outlier detection was performed on the subject averages, using principal component analysis. Measurements with a $T^2$ statistics indices higher than the 95% confidence interval were excluded. A two-way ANOVA (genotype, age, genotype*age) per region was used to evaluate differences in vGLUT/vGAT ratio and the number of cholinergic synapses. In case of a significant genotype*age interaction, post-hoc FDR-corrected Student’s t-test (FDR $p < 0.05$) were performed. The interaction term was removed no significant interaction was observed and a two-way ANOVA (genotype, age) was used to evaluate genotype or age effects. Graphical representation of the data was obtained using GraphPad Prism (version 9.2.0 for Windows, GraphPad Software, San Diego, California USA, www.graphpad.com).

3. Results

Circadian rhythmicity during pre- and early-plaques stages of AD

Research has demonstrated that alterations in sleep rhythm precede cognitive alterations in AD [39, 40]. We investigated if alterations in circadian rhythm were present at pre-plaque (4M) and early-plaque (6M) stages in a rat model (TgF344-AD) that recapitulates the main hallmarks of AD such as Aß plaques, tau tangles and cognitive impairment. Overall circadian rhythmicity was intact at both pre- and early plaque stages of AD in TgF344-AD rats, where the total amount of sleep was higher in the inactive phase than in the active phase, similar to what was observed in wildtype (WT) rats (Fig. 2A). However, small alterations in circadian rhythmicity were observed in TgF344-AD rats, mainly during REM sleep. A significant age*genotype interaction was observed in the total time spent in REM sleep, where a significantly decreased amount of REM sleep was observed in the TgF344-AD rats at the pre-plaque stage with respect to WT littermates. Total REM time was significantly increased at the early-plaque stage in both groups (Fig. 2A, Suppl. Table 1, 2). Significant age effects were observed in the total time spent in REM and NREM during the inactive phase, where NREM sleep is increased and REM sleep is decreased at the early-plaque stage, irrespective of genotype (Fig. 2A, Suppl. Table 1). No significant age or genotype effects were observed in the percentage time spent in REM and NREM during the active phase (Fig. 2A, Suppl. Table 1).
Next, 24-hour acquisitions were divided into 3-hour epochs and the percentage time spent in each state across each epoch was plotted (Fig. 2B,C). Significant age*genotype interactions were observed during the inactive phase in NREM sleep (Fig. 2B, Suppl. Table 3). TgF344-AD rats showed an increased time spent in NREM between Z6-Z9 at the pre-plaque stage compared to age-matched WT rats, while the time spent in NREM between Z9-Z12 was significantly lower (Fig. 2B, Suppl. Table 4). When comparing the time spent in REM across 3-hour epochs, significant genotype effects were observed during the active period, demonstrating decreased time spent in REM between Z18- Z21, and an increased time spent in REM between Z21- Z24 in TgF344-AD rats (Fig. 2C, Suppl. Table 3). In addition, significant age effects were observed for two 3-hour epochs during the inactive phase (Z9-12, Z12-15), demonstrating decreased REM time in 6-month-old rats compared to 4-month-old animals irrespective of genotype. These results demonstrate subtle alterations in circadian rhythm were present at the pre- and early-plaque stages of AD in TgF344-AD rats.

Figure 2: Circadian rhythm in TgF344-AD rats. A) Percentage time spent in NREM and REM during the total 24-hour acquisition and during the active and inactive phases. B-C). Percentage time spent in NREM (B) or REM (C) during 3-hour epochs. On the x-axis, Zeitgeber time (Z) is represented. Between Z0 and Z12, lights are on, between Z12 and Z24, lights are off (Grey shading). Bars demonstrate the mean +/- SEM across animals within each group, whereas dots represent subject values. Dark shading on the x-axis represents the active phase, when lights are turned off. Abbreviations: WT = wildtype, Tg = TgF344-AD, Z = Zeitgeber. * = p < 0.05, ** = p < 0.01, *** = p < 0.001

Sleep fragmentation during NREM sleep

During aging, sleep architecture changes towards more awakenings and decreased bout length. Therefore, mean NREM bout length was evaluated. Age effects were observed across the whole 24-hour period as well as during the active and inactive period, demonstrating decreased NREM bout lengths at the early-plaque stage, irrespective of genotype (Fig. 3A, Suppl. Table 5). Next all the bout lengths of all subjects were combined in one cumulative probability plot, that demonstrated no significant differences in NREM bout length in TgF344-AD rats at pre- and early-plaque stages of AD ($p_{pre-plaque} = 0.1109$, $p_{early-plaque} = 0.4706$) (Fig. 3B, Suppl. Figure 1).

Figure 3: NREM Fragmentation in TgF344-AD rats. A) Mean NREM bout lengths during 24h (left), during the active period (lights off, middle) and inactive period (lights on, right). Bars represent the mean +/- SEM. ANOVA analysis was performed to test for statistical differences. B) Cumulative probability plots of sleep bout lengths for each group. The left panel shows the cumulative distribution of NREM bouts during the pre-plaque stage, while the right plot show the early-plaque stage. Kolmogorov-Smirnov tests (FDR $p < 0.05$) were performed to evaluate if distributions were significantly different. s = seconds, WT = wildtype, Tg = TgF344-AD, h = hour. ** = p < 0.01, *** = p < 0.001.

Alterations in oscillatory activity during NREM sleep
Most of the studies on sleep in AD have focused on alterations during NREM sleep. Aβ and hyperphosphorylated Tau have been known to interfere with synaptic function, hence inducing altered oscillatory activity during NREM sleep, which in turn is linked to cognitive symptoms of AD [8, 41]. When investigating the oscillatory power during NREM sleep in TgF344-AD rats, a significant age effect was observed in the delta band, demonstrating a decreased power at the early-plaque stage in both groups (Fig. 4A,B, Suppl. Table 6). In addition, significant age effects were detected in the fast gamma band, showing an increase in the power of these frequencies over time. However, no significant genotype effects were observed in the power across different frequency bands during NREM sleep.

During NREM sleep, SWR which are fast oscillatory events in the CA1 regions of the hippocampus, are considered a hallmark of memory replay and are therefore suggested to play a role in memory consolidation [42]. To investigate if SWR activity was already altered at pre-plaque and early-plaque stages of AD in the TgF344-AD rats, SWR were extracted (Fig. 4C, cfr. Materials and Methods) and several characteristics were examined. No significant genotype or age effects were observed for the power of the SWR (Fig. 4D, Suppl. Table 7). A significant age*genotype interaction was observed in the peak spectral frequency (PSF) of the SWR oscillations (Fig. 4E, Suppl. Table 7,8). Post-hoc analysis demonstrated a significant age effect in the TgF344-AD rats where the PSF decreased with age, an effect that was absent in the WT littermates. The duration of SWR has been associated with memory performance [43]. A significantly increased duration of SWR was observed in the TgF344-AD rats, irrespective of age (Fig. 4F, Suppl. Table 5). Histograms of the relative frequency for each duration of SWR demonstrates a skewed distribution (Fig. 4G). All SWR events were divided into short ripples (< 60 ms), medium ripples (60–100 ms) and long ripples (> 100 ms) [43]. Statistical analysis demonstrates a decreased fraction of short ripples, and an increased fraction of long ripples (Fig. 4H, Suppl. Table 9) in the TgF344-AD rats, demonstrating that the relative number of long duration ripples is increased at pre- and early-plaque stages of AD in the TgF344-AD rats (Fig. 4H).

Figure 4: Power and sharp wave-ripple activity during NREM sleep in TgF344-AD rats and wildtype littermates. A) Mean normalized power spectra during. Shading indicates SEM across the group. B) Averaged normalized power across distinct frequency bands of interest (+/- SEM). C) Illustrative sharp wave-ripple (SWR) of a wildtype (WT) and TgF344-AD (Tg) rat. Trace shows filtered data (120-250Hz) of the ripple, while the bottom time-frequency plot demonstrates the frequency and power of the ripple. D-F) Bar plots demonstrating the mean power (D), peak spectral frequency (E) and duration (F) of SWR. G-H) Histograms showing the group average relative frequency of each duration of SWR at 4 months (left) and 6 months (right). H) Bar plots showing the ratio of short ripples (left) and long ripples (right) vs all ripples. Bar plots show mean +/- SEM, whereas dots represent subject values. gen = genotype, WT = wildtype, Tg = TgF344-AD, HFO = high frequency oscillations, ms = millisecond, SWR = sharp wave-ripple, PSF = peak spectral frequency, Hz = Herz, V = Volt. * = p < 0.05, ** = p < 0.01

E/I imbalance in TgF344-AD rats
SWR events are induced by a delicate interaction between excitatory neurons and GABAergic interneurons that, if disrupted, can lead to pathological forms of activity which leads to memory impairments [24, 44–46]. Therefore, we aimed to evaluate if altered excitatory or inhibitory balance could be attributing to the altered SWR activity observed in TgF344-AD rats. Analysis of the excitatory/inhibitory balance (ratio between glutamatergic and GABA-ergic synapses) revealed significant age effects in the CA1 layer of the hippocampus demonstrating an increased excitation and/or decreased inhibition at the pre-plaque stage in both groups (Fig. 5B, Suppl. Table 10). Interestingly, a significantly decreased excitation and/or increased inhibition was observed in the dentate gyrus (DG) in the TgF344-AD rats irrespective of age (Fig. 5B, Suppl. Table 10).

Figure 5: Changes in E/I balance in TgF344-AD rats. A) Exemplary images of the glutamatergic (magenta) and GABA-ergic synapses (yellow) synapses in the CA1 layer of the hippocampus in wildtype littermates (WT) (left) and TgF344-AD rats (right) at 4 and 6 months of age. B Group-averaged vGLUT/vGAT ratio per region of interest. Bars represent the mean +/- SEM. ANOVA analysis was performed to test for statistical differences. DG = dentate gyrus, WT = wildtype, Tg = TgF344-AD, vGAT = vesicular GABA transporter, vGLUT = vesicular glutamate transporter, E/I = excitatory/inhibitory, gen = genotype. * = p < 0.05

REM sleep fragmentation

REM sleep is associated with local synaptic plasticity, consolidation of declarative memory and modulation of emotional memories [47–49]. Disruption of REM sleep has been associated with memory problems and emotional problems, such as increased anxiety, common symptoms observed in patients with AD [47, 49]. Therefore, we aimed to evaluate REM sleep disturbances at pre- and early-plaque stages of AD. Analysis of the mean REM bout length demonstrated age effects, where shorter REM bouts were observed at the early-plaque stage, when REM bout length was calculated over the entire 24h period and only during the active phase (Fig. 6A, Suppl. Table 11). Interestingly, when REM bout length was analyzed during the inactive phase, a significant age*genotype interaction was observed. Post-hoc analysis reveals a significant decrease in REM bout length in WT rats while aging, an effect that was absent in the TgF344-AD rats (Fig. 6A, Suppl. Table 12). When investigating the cumulative distribution of the REM bouts for each group separately, a significantly increased probability of shorter REM bouts was observed in TgF344-AD rats at the pre-plaque stage, but not at the early-plaque stage (Fig. 6, Suppl. Figure 2).

Figure 6: REM Fragmentation in TgF344-AD rats. A) Mean REM bout lengths during 24h (left), during the active period (lights off, middle) and inactive period (lights on, right). Bars represent the mean +/- SEM. ANOVA analysis was performed to test for statistical differences. B) Cumulative probability plots of sleep bout lengths for each group. The left panel shows the cumulative distribution of REM bouts during the pre-plaque stage, while the right plot shows the early-plaque stage. Kolmogorov-Smirnov tests (FDR p < 0.05) were performed to evaluate if distributions were significantly different. gen = genotype, WT = wildtype, Tg = TgF344-AD, s = seconds. * = p < 0.05, ** = p < 0.01

Alterations in oscillatory activity during REM sleep
Disruption of oscillatory activity during REM sleep has been observed at late stages of AD and has been proven to lead to memory deficits [50–52]. When investigating alterations in the power of hippocampal oscillations during REM sleep, a significant genotype effect was present in the fast gamma power, demonstrating a reduction in fast gamma oscillations in the TgF344-AD rats irrespective of age (Fig. 7A,B, Suppl. Table 13).

Phase-amplitude coupling (PAC) is an electrophysiological phenomenon where the amplitude of fast oscillations is modulated by the phase of slower oscillations. Theta-driven modulation of gamma oscillations during REM sleep has been implicated in memory processing and has been demonstrated to be an important mechanism of synaptic plasticity and synaptic homeostasis [51, 53]. Alterations in synaptic function could impair theta-gamma coupling, therefore we evaluated PAC during REM sleep. First, the main frequencies modulated by theta frequencies were analyzed using a comodulogram. Mean comodulograms were plotted which demonstrate modes of coupling between theta frequencies (6.5-9 Hz) and fast gamma in all groups (80–100 Hz) (Fig. 7C). Interestingly, strong coupling was observed between theta (6.5-9 Hz) and slow gamma oscillations (30–45 Hz) mainly in 4-month-old TgF344-AD rats.

Next, to evaluate the strength of the theta-gamma coupling, a time-resolved PAC analysis was performed (Fig. 7C,D, Suppl. Table 14). A significant age*genotype interaction was observed in the coupling between theta and slow gamma frequencies. Post-hoc analysis revealed increased coupling strength in TgF344-AD rats during the pre-plaque stage, which was restored to WT levels at the early-plaque stage (Fig. 7D, Suppl. Table 15). When evaluating the coupling between theta and fast gamma frequencies, a significantly decreased coupling strength was present in the TgF344-AD rats, irrespective of age (Fig. 7E, Suppl. Table 14). Moreover, a significant age effect was observed, demonstrating an increase in the strength of PAC in the 6-month-old rats, which is mainly driven by an increasing MI in the TgF344-AD rats over time, suggesting a partial recovery of PAC in TgF344-AD rats at the early-plaque stage.

Figure 7: Power and theta-gamma coupling in TgF344-AD rats during REM sleep. A) Mean normalized power spectra. Shading indicates SEM across the group. B) Averaged normalized power across distinct frequency bands of interest (+/- SEM). C) Comodulogram averaged across subjects demonstrating main frequencies of coupling. Color bar indicates the strength of the modulation index at different frequencies of theta (x-axis) and gamma (y-axis). D) Mean (+/- SEM) MI across subjects between theta-frequencies (6.5-9Hz) and slow gamma frequencies (30-45Hz). Dots represent subject MI values. E) Mean (+/- SEM) MI across subjects between theta frequencies (6.5-9HZ) and fast gamma frequencies 80-110Hz. Dots represent subject MI values. gen = genotype, Tg = TgF344-AD, WT = wildtype, MI = modulation index, Hz = Hertz. * = p < 0.05, *** = p < 0.001

Cholinergic synapses in the hippocampus

Cholinergic dysfunction has been implicated to occur at early stages of AD. REM sleep and PAC are heavily dependent on cholinergic signaling [12, 54–57]. To evaluate if the observed alterations in REM bout length and theta-gamma coupling could be attributed to alterations in cholinergic function,
histological analysis of cholinergic synapses in the hippocampus was performed (Fig. 8A). When statistically comparing the abundance of cholinergic synapses, significant genotype*age effects were observed in the DG and in the CA1 (Fig. 8A, Suppl. Table 16). Cholinergic synaptic density in the CA1 region and DG was paradoxically increased at 6-months of age in TgF344-AD rats compared to WT littermates. Moreover, a significant increase in cholinergic synapses was observed over time in the TgF344-AD rats in both hippocampal regions, which was absent in WT littermates (Fig. 8B, Suppl. Table 17).

**Figure 8: Increased number of cholinergic synapses in hippocampus during early-plaque stage.** A) Exemplary images of cholinergic synapses (grey) in the CA1 layer of the hippocampus in wildtype littermates (WT) (left) and TgF344-AD rats (right) at the pre-, and early-plaque stage of AD. B) Group-averaged numbers of cholinergic synapses per region of interest. Bars represent the mean +/- SEM. Dots represent subject MI values. ANOVA analysis was performed to test for statistical differences. DG = dentate gyrus, TG = TgF344-AD, gen = genotype. *** = p < 0.001

4. Discussion

The current study aimed to evaluate how circadian rhythm, sleep macro-architecture and sleep micro-architecture are altered at pre-plaque and early-plaque stages of AD in TgF344-AD rats. We observed no significant differences in percentages of time spent in NREM and REM, suggesting circadian rhythmicity is unaltered at early phases of AD in TgF344-AD rats. However, we did observe REM fragmentation demonstrated by a significantly decreased REM bout length in the TgF344-AD rats at the pre-plaque stage, but not the early-plaque stage. In addition, we observed micro-architectural changes in oscillatory activity during REM and NREM sleep. In TgF344-AD rats, a significantly decreased fast gamma power was observed during REM sleep. Moreover, a decreased PAC between theta and fast gamma oscillations was observed in the TgF344-AD rats at both ages. Interestingly, at 4 months of age in TgF344-AD rats, a significant increase in PAC was present between theta and slow gamma oscillations, which was absent at 6 months of age, suggesting functional compensation during the early-plaque stage in TgF344-AD rats. During NREM sleep we did not observe alterations in power of different oscillations. However, when investigating properties of SWR, we observed an increased ripple duration in TgF344-AD rats, mainly at 6 months of age, while the power of the oscillations and peak spectral frequency was not significantly different between genotypes. These alterations in oscillatory activity during sleep coincided with changes in synaptic density of GABA-ergic, glutamatergic and cholinergic synapses, where a decreased excitation and/or increased inhibition was observed in the dentate gyrus TgF344-AD rats at both ages. Interestingly, an increase of cholinergic synapses was observed only at 6 months of age in the hippocampus of TgF344-AD rats.

**Differential impact of behavioral state on hippocampal activity in AD**

A recent paper investigated how hippocampal oscillatory activity was affected at late stages of AD during REM sleep, NREM sleep and during awake behaviors. They observed that the impact of Aβ aggregates on
hippocampal oscillatory activity was different between NREM sleep and during wake an REM sleep as hyperactivity was observed during NREM sleep, while hypoactivity was observed during REM sleep and wake [58]. Similarly, we have observed that hippocampal oscillatory activity during explorative behavior and quiet wakefulness was differentially impacted in TgF344-AD rats during the pre-plaque stage. In that previous study we observed power and PAC alterations mainly during quiet wakefulness [59]. The current results where REM sleep was more severely impacted than NREM sleep further confirm this differential impact of Aβ on hippocampal activity during different behavioral states.

REM fragmentation

Research over the past decades demonstrated physiological changes in circadian rhythm and sleep architecture, such as earlier chronotype, reduced total sleep time and increased sleep fragmentation, emerge during the normal aging process [60, 61]. Approximately 60% of the people suffering from AD develop sleep disturbances similar to those reported in normal aging, but with a greater magnitude [40, 62, 63]. The current study revealed increased probability of shorter REM bouts at pre-plaque stage, which disappeared during the early-plaque stage, in the TgF344-AD rats, in the absence of decreased time spent in REM. Previous studies in 16-month-old TgF344-AD rats observed increased fragmentation of both REM and NREM, without differences in time spent in these vigilance states [64], suggesting that the alterations in sleep architecture worsen as the disease progresses in TgF344-AD rats, similar to what is observed in human AD [40, 62, 65]. In addition, several studies in other animal models of AD observed alterations in REM sleep macro-architecture [66–68]. Interestingly a study that involved patients with subjective cognitive decline, a self-perceived cognitive deterioration which is associated with an increased risk for developing AD, also observed decreased time spent in REM sleep [69]. In addition, a longitudinal follow-up study in people with patients who converted from mild cognitive impairment to AD-related dementia reported reduced REM sleep [70], demonstrating that reduced time spent in REM is associated with a higher risk of developing dementia [71]. Aforementioned results imply that alterations in REM sleep also occur early in human AD, often in the absence of NREM disturbances, suggesting that REM sleep alterations could be an early sign of AD.

The disease mechanisms underlying early changes in REM sleep in AD remain elusive and research is trying to unravel the role of different neurotransmitter systems in REM sleep disruptions in AD. One of the proposed pathological mechanisms is that (soluble) Aβ and tau pathology disrupt the neuronal function in brain regions important in the regulation of sleep [72, 73]. One obvious link is the early accumulation of hyperphosphorylated Tau in the locus coeruleus (LC), a region involved in the regulation of sleep-wake transitions. LC neurons are silent during REM sleep, which is critical for brain excitability and emotional regulation during sleep [13]. Early accumulation of hyperphosphorylated Tau in the LC, which is observed in the TgF344-AD rat model, might lead to insufficient LC silencing, inducing restless REM sleep. Restless REM sleep has been associated with impaired emotional adaptation during REM sleep, which could lead to increased anxiety [47], as is observed in TgF344-AD rats already at 5 months of age [74]. Moreover, the LC receives dense orexin innervation important in the regulation of REM sleep [75], where orexin has an excitatory effect on LC neurons. Studies have demonstrated that orexin levels are increased in MCI
patients with REM sleep disturbances, suggesting that insufficient LC silencing might be the result of increased orexin signaling in the brain of MCI patients [69, 76]. Acetylcholine originating from brainstem nuclei known as the REM-ON center is a key regulator of REM sleep [68, 72]. Recent studies in the Tg2576 mouse model of AD observed degeneration of pontine cholinergic nuclei, which coincided with impaired REM sleep [67]. During REM sleep, cortical and hippocampal ACh concentrations are very high, causing cortical activation mainly induced by the release of ACh from basal forebrain cholinergic neurons [12]. Degeneration of the basal forebrain cholinergic nuclei occurs early in AD and has been shown to be predictive of amyloid burden [77, 78]. Increasing ACh with cholinesterase inhibitors, a type of drug commonly used to delay disease progression in AD, is associated with increased amounts of REM sleep, which is in turn correlated with improvement of cognitive function in MCI patients [68, 79–81]. These results might indicate that at the pre-plaque stage in TgF344-AD rats, alterations in cholinergic signaling, caused by the accumulation of soluble AB, are contributing to REM sleep fragmentation.

Compensatory mechanisms at early-plaque stage

In the current study we observed mainly alterations in REM sleep architecture and oscillatory activity during REM sleep. The genotypic differences observed at the pre-plaque stage were larger than the differences at the early-plaque stage, where oscillatory activity and REM sleep architecture in TgF344-AD rats was partially recovered. Interestingly, we observed an increase in cholinergic synapses in the hippocampal regions, a mechanism postulated to be a sign of compensation for loss of cholinergic tone at earlier stages [82–84]. Similar increases in cholinergic synapses have been observed at pre-plaque stages in APP/PS1 mice and MCI patients [82, 85]. This increase in cholinergic synapses is a phenomenon that is commonly observed after glutamatergic lesioning of the medial entorhinal cortex, resulting in a loss of glutamatergic input to the hippocampus [86, 87]. This glutamatergic denervation of the hippocampus is known to induce hyperexcitability in several hippocampal regions, including the DG and CA1 layer [87]. Recent studies have demonstrated that the increase in cholinergic innervation compensates for network hyperactivity, which has been associated with recovery of spatial memory [86, 88].

The REM-associated alterations observed in this study are strongly modulated by the cholinergic system [11, 89, 90], strengthening the assumption that compensatory mechanisms are present at 6 months of age in TgF344-AD rats. Similar to the observations in the current study, research has demonstrated that alterations in theta-gamma coupling could be reversed by administration of cholinesterase inhibitors in aged APP/PS1 mice [58]. In contrast, alterations in oscillatory activity during NREM sleep, during which ACh modulation is virtually absent, became worse over time in the TgF344-AD rats. This suggests that the cholinergic sprouting might be an important early compensation mechanism in AD which slows down disease progression, by restoring the network imbalance [82, 86]. However, the increased activity of the cholinergic system at presymptomatic stages of AD might be contributing to the vulnerability of this system [91].

5. Conclusion
The results from the current study reveal alterations in REM sleep architecture (eg. REM fragmentation) and hippocampal oscillations (eg. decreased gamma power, decreased theta-gamma coupling) at the pre-plaque stage, thus in the absence of Aβ plaques. REM bout length, hippocampal gamma power and theta-gamma coupling were partially recovered during the early-plaque stage. This functional recovery coincided with increases in the number of cholinergic synapses, suggesting a role of cholinergic signaling in the restoration of hippocampal activity and REM sleep behavior during the early-plaque stage of AD. These findings highlight the important role of the cholinergic system to compensate for network disturbances during very early stages of AD. Network disturbances and sleep alterations are known to drive disease progression. Modulation of cholinergic signaling in early, presymptomatic AD might be a promising therapeutic strategy to alter disease progression by restoring (hippocampal) network function and sleep architecture.

**Abbreviations**
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>AD</td>
<td>Alzheimer's Disease</td>
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<tr>
<td>Aβ</td>
<td>Amyloid-beta</td>
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<td>ANOVA</td>
<td>analysis of variance</td>
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<td>AP</td>
<td>Anterior-posterior</td>
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<td>CA</td>
<td>Cornu Ammonis</td>
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<td>Dentate Gyrus</td>
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<td>Dorsal-ventral</td>
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<td>EMG</td>
<td>electromyography</td>
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<td>E/I</td>
<td>excitatory/inhibitory</td>
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<td>FDR</td>
<td>False discovery rate</td>
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<td>Hertz</td>
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<td>LFP</td>
<td>local field potential</td>
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<td>LC</td>
<td>Locus Coeruleus</td>
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<td>Medial-lateral</td>
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<td>mm</td>
<td>millimeter</td>
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<td>MI</td>
<td>Modulation index</td>
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<td>NREM</td>
<td>non rapid eye movement</td>
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<td>PFA</td>
<td>Paraformaldehyde</td>
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<td>PSF</td>
<td>Peak spectral frequency</td>
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<td>PAC</td>
<td>Phase-amplitude coupling</td>
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<tr>
<td>Pyr</td>
<td>pyramidal layer</td>
</tr>
<tr>
<td>REM</td>
<td>Rapid eye movement</td>
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<tr>
<td>SW</td>
<td>Sharp wave</td>
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<td>SWR</td>
<td>Sharp wave-ripples</td>
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<td>Tg</td>
<td>TgF344-AD</td>
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<tr>
<td>tPAC</td>
<td>timeresolved phase-amplitude coupling</td>
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<td>WT</td>
<td>wildtype</td>
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<td>Z</td>
<td>Zeitgeber</td>
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Declarations

Ethics approval and consent to participate

All procedures were in accordance with the guidelines approved by the European Ethics Committee (decree 2010/63/EU) and were approved by the Committee on Animal Care and Use at the University of Antwerp, Belgium (approval number: 2019-06).

Consent for publication

Not applicable.

Data availability statement

The electrophysiological datasets generated during the current study are publicly available in the OpenNeuro repository (DOI: ..., ).

Competing interests

The authors declare that they have no competing interests

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Author’s contributions

MvdB, MV, and GAK designed the study. SM and MvdB purchased and maintained the TgF344-AD rat colony of which the animals were used in this study. GK, MvdB and JVA optimized the electrophysiological experiments. MvdB performed the acquisition of the electrophysiology data. MvdB, LH, MHA, GAK, and MV optimized and performed the electrophysiological and behavioral data analysis and interpretation. DT and MvdB performed the histological validation of the electrode position, under supervision of GAK. IP, WDV, and MvdB were involved in designing the histological experiments. IP optimized and performed the immunofluorescent stainings and imaging. IP, WDV, and MVe designed and implemented the analysis methods for the immunofluorescent stainings. MvdB performed the histological analyses and statistical analyses of the histological data. MvdB, GAK, and MV wrote the manuscript. AvL, GAK, and MV supervised the study and supported the study with equipment and materials. All co-authors provided comments and intellectual input that led to the final version of the manuscript. The authors read and approved the final manuscript.
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**Figures**

A  
**4M WT**

B  
**4M Tg**

C  
**6M WT**

**Pyr**

**CA1**

**DG**

**CA3**

**6M Tg**
**Figure 1**

**Histological validation of electrode position.** A) Schematic overview of electrode position in 4-month-old wildtype (WT) animals. Each blue electrode represents track of the electrode of a different WT subject. B) Schematic overview of the electrode position in 4-month-old TgF344-AD (Tg) animals. Each orange electrode represents track of the electrode of a different Tg subject. The red insert shows exemplary light microscopy images of Nissl-stained brain slices showing the electrode trace in the hippocampus of a WT animal (A) and TgF344-AD rat (B). Exemplary images of the electrode validation (C) where the arrow points towards the pyramidal layer (Pyr) of the CA1 region of the hippocampus. DG = Dentate Gyrus
Figure 2

**Circadian rhythm in TgF344-AD rats.** A) Percentage time spent in NREM and REM during the total 24-hour acquisition and during the active and inactive phases. B-C) Percentage time spent in NREM (B) or REM (C) during 3-hour epochs. On the x-axis, Zeitgeber time (Z) is represented. Between Z0 and Z12, lights are on, between Z12 and Z24, lights are off (Grey shading). Bars demonstrate the mean +/- SEM across animals within each group, whereas dots represent subject values. Dark shading on the x-axis represents
the active phase, when lights are turned off. Abbreviations: WT = wildtype, Tg = TgF344-AD, Z = Zeitgeber.
* = p <0.05, ** = p <0.01, *** = p <0.001

Figure 3

**NREM Fragmentation in TgF344-AD rats.** A) Mean NREM bout lengths during 24h (left), during the active period (lights off, middle) and inactive period (lights on, right). Bars represent the mean +/- SEM. ANOVA analysis was performed to test for statistical differences. B) Cumulative probability plots of sleep bout lengths for each group. The left panel shows the cumulative distribution of NREM bouts during the pre-plaque stage, while the right plot show the early-plaque stage. Kolmogorov-Smirnov tests (FDR p<0.05) were performed to evaluate if distributions were significantly different. s = seconds, WT = wildtype, Tg = TgF344-AD, h = hour. ** = p<0.01, *** = p<0.001.
Figure 4

Power and sharp wave-ripple activity during NREM sleep in TgF344-AD rats and wildtype littermates. A) Mean normalized power spectra during. Shading indicates SEM across the group. B) Averaged normalized power across distinct frequency bands of interest (+/- SEM). C) Illustrative sharp wave-ripple (SWR) of a wildtype (WT) and TgF344-AD (Tg) rat. Trace shows filtered data (120-250Hz) of the ripple, while the bottom time-frequency plot demonstrates the frequency and power of the ripple. D-F) Bar plots demonstrating the mean power (D), peak spectral frequency (E) and duration (F) of SWR. G-H) Histograms showing the group average relative frequency of each duration of SWR at 4 months (left) and 6 months (right). H) Bar plots showing the ratio of short ripples (left) and long ripples (right) vs all ripples. Bar plots show mean +/- SEM, whereas dots represent subject values. gen = genotype, WT = wildtype, Tg = TgF344-AD, HFO = high frequency oscillations, ms = millisecond, SWR = sharp wave-ripple, PSF = peak spectral frequency, Hz = Herz, V = Volt. * = p<0.05, ** = p<0.01
Figure 5

Changes in E/I balance in TgF344-AD rats. A) Exemplary images of the glutamatergic (magenta) and GABA-ergic synapses (yellow) synapses in the CA1 layer of the hippocampus in wildtype littermates (WT) (left) and TgF344-AD rats (right) at 4 and 6 months of age. B Group-averaged vGLUT/vGAT ratio per region of interest. Bars represent the mean +/- SEM. ANOVA analysis was performed to test for statistical differences. DG = dentate gyrus, WT = wildtype, Tg = TgF344-AD, vGAT = vesicular GABA transporter, vGLUT = vesicular glutamate transporter, E/I = excitatory/inhibitory, gen = genotype. * = p<0.05
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**Supplementary Files**

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- SupplementaryInformation.docx