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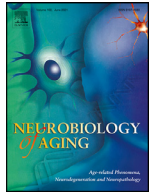
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# Neuronal hyperexcitability in the Tg2576 mouse model of Alzheimer's disease – the influence of sleep and noradrenergic transmission

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## ABSTRACT

The link between Alzheimer's disease (AD) and network hypersynchrony – manifesting as epileptic activities – received considerable attention in the past decade. However, several questions remain unanswered as to its mechanistic underpinnings. Therefore, our objectives were (1) to better characterise epileptic events in the Tg2576 mouse model throughout the sleep-wake cycle and disease progression *via* electrophysiological recordings and (2) to explore the involvement of noradrenergic transmission in this pathological hypersynchrony. Over and above confirming the previously described early presence and predominance of epileptic events during rapid-eye-movement (REM) sleep, we also show that these events do not worsen with age and are highly phase-locked to the section of the theta cycle during REM sleep where hippocampal pyramidal cells reach their highest firing probability. Finally, we reveal an antiepileptic mechanism of noradrenergic transmission *via*  $\alpha$ 1-adrenoreceptors that could explain the intriguing distribution of epileptic events over the sleep-wake cycle in this model, with potential therapeutic implications in the treatment of the epileptic events occurring in many AD patients.

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## 1. Introduction

During the last decade, increasing attention has been paid to the long-dismissed link between network hypersynchrony and Alzheimer's disease (AD) (Lam & Noebels, 2020). It is now agreed upon that AD patients tend to develop seizures and exhibit sub-clinical epileptiform events on their electroencephalogram (EEG)

compared to age-matched healthy individuals (Horváth et al., 2018; Lam et al., 2020; Vossel et al., 2016). Intriguingly, seizures can be detected before the onset of memory deficits in AD patients and even in amnesic Mild Cognitive Impairment (aMCI) that often progresses to AD (Amatniek et al., 2006). Moreover, studies with aMCI patients who had epilepsy showed symptoms of cognitive decline on average 6.8 years earlier than patients with aMCI who did not have epilepsy (Vossel et al., 2013). On the other hand, treatment with low doses of the antiseizure medication levetiracetam has been shown to reduce epileptic activity and to improve cognitive performance in aMCI patients (Bakker et al., 2012, 2015) as well as in AD patients in the early-to-moderate stages of the disease (Vossel et al., 2021). This is all the more intriguing since AD patients with comorbid epilepsy seem to follow a steeper course of cognitive decline than AD patients without epilepsy (Haoudy et al., 2022; Horvath et al., 2021; Vöglein et al., 2020; Vossel et al., 2016). Altogether, these results suggest that network hypersynchrony and epilepsy might contribute to the cognitive dysfunctions observed in AD and aMCI.

**Abbreviations:** AD, Alzheimer's Disease; aMCI, Amnesic Mild Cognitive Impairment; APP, Amyloid Precursor Protein; AR, adrenoreceptor; AWK, Wakefulness; EA, Epileptic Activities; EEG, Electroencephalogram; LC, Locus Coeruleus; NA, Noradrenaline; REM sleep, Rapid Eye Movement Sleep; RM, Repeated Measures; SWS, Slow Wave Sleep; M, mean.

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# Equal contributions

Although clinical studies have considerably increased on this topic in the past few years (Brunetti et al., 2020; Horváth et al., 2018; Lam et al., 2020; Vossel et al., 2016), such results remain scarce and often controversial. However, the abundant studies in different mouse models of AD coherently show network hypersynchrony leading to an epileptic phenotype consistently manifesting as epileptic activities (EA) across models and, occasionally, as seizures (see Kazim et al., 2021 for a review).

We have also documented this network hypersynchrony previously in the Tg2576 mouse model (Bezzina et al., 2015; Bezzina et al., 2015). The Tg2576 mouse model has a progressive evolution of the pathology, in particular cognitive decline. Indeed, these mice show subtle cognitive deficits at 3 months of age (Deacon et al., 2008; Hsiao et al., 1996; King & Arendash, 2002), then a significant memory decline appears at the age of 6 months and becomes more evident at 9–10 months of age (Jacobsen et al., 2006; Mouri et al., 2007; Westerman et al., 2002). Finally, severe memory deficits and senile plaque deposition throughout the neocortex settle in at approximately one year of age (Verret et al., 2013). Our previous observations revealed that the network hypersynchrony is already present in this model as early as 6 weeks of age, preceding cognitive deficits (Bezzina et al., 2015). However, the progression of the network hyperexcitability throughout the lifespan of these animals is only partially unveiled. Indeed, another study has suggested that epileptic activity (EA) might increase with ageing, hence with cognitive deficits, in Tg2576 mice. However, data is only available for up to 7 months of age (Kam et al., 2016). Therefore, the first aim of this study was to test if the frequency of epileptiform events progresses with age at the same pace as neurobiological and cognitive deficits in Tg2576 mice throughout their lifespan. For this purpose, we recorded the EEG of Tg2576 mice and their nontransgenic (NTg) littermates in different age groups: 1.5 months, 5.5–7 months and 8–10 months of age.

Moreover, in rodent models of AD, EA seems to occur primarily during rapid eye movement sleep (REM) (Gureviciene et al., 2019; Kam et al., 2016). Since REM sleep is necessary for memory consolidation (Boyce et al., 2016; Karabulut et al., 2019; Li et al., 2017) and synaptic downscaling (Grosmark et al., 2012; Watson & Buzsáki, 2015), EA occurring during REM-sleep could add insult to injury to the cognitive decline and especially to memory deficits. However, REM sleep predominant EA are somewhat surprising since the desynchronised nature of neuronal activity renders the occurrence of epileptic events highly improbable in epileptic patients without AD during REM sleep (Ng & Pavlova, 2013). Moreover, artificially triggering hippocampal theta rhythm, a predominant feature of REM sleep, prevents EA in rats (Miller, 1994). Therefore, the second objective of this work was to confirm that epilepsy in Tg2576 is exacerbated during REM sleep and to explore whether this particular phenotype evolves with ageing.

This Gordian knot of the factors behind such a surprising EA distribution over the sleep-wake cycle is still waiting to be cut. Indeed, an interneuronal dysfunction leading to unleashed excitation in a network already under siege by the excitatory impact of the accumulating  $A\beta$  (e.g., Busche et al., 2012; DeVos et al., 2013; Palop et al., 2007) could seem like an obvious explanation of network hypersynchrony, even so since such interneuronal dysfunctions have been described in different models of amyloidosis (Cattaud et al., 2018; Verret et al., 2012). However, such a unifactorial explanation only accounts for the mere presence of EA, not for their peculiar distribution over the sleep-wake cycle. Therefore, this study's third objective was to gain better insight into the underlying mechanisms of the previously observed rise of EA in REM sleep (Brown et al., 2018 in hAPP-J20 mice; Gureviciene et al., 2019 in APP/PS1 mice; Kam et al., 2016 in Tg2576 mice) via signal processing and pharmacological methods detailed below.

On the one hand, although the theta rhythm protects the brain from EA in mouse models of epilepsy or epileptic patients, it might have a completely different influence in the context of Alzheimer's disease. To tackle this issue, we examined whether EA occur during a specific phase of the theta cycle during REM sleep. In addition, since different cell types fire on different phases of the theta rhythm (Klausberger et al., 2003), this analysis could help identify the cell types plausibly implicated in EA.

On the other hand, the early dysfunction of neurotransmitter homeostasis may also contribute to the imbalance between excitation and inhibition. The dynamics of these molecules throughout the sleep-wake cycle might offer more precise insights into the REM sleep-related EA occurrence. One such possibility was suggested in a study by Kam et al. (2016). They hypothesised that cholinergic hyperactivity in the early stages of the disease – which occurs before the well-documented decline of cholinergic transmission – might contribute to the epileptic phenotype of Tg2576 mice. In their study, the blockade of cholinergic transmission through muscarinic receptors by *atropine* administration led to decreased EA. However, atropine interferes with the sleep-wake cycle – most notably, it completely suppresses REM sleep. Another alluring pathway is that of noradrenergic transmission. Indeed, the dynamics of noradrenergic transmission inversely correlate with the increase of EA frequency in Tg2576 mice: the activity of noradrenergic neurons is maximal during wakefulness, gradually decreases during slow-wave sleep (SWS) to finally become quasi-null during REM sleep (Aston-Jones & Bloom, 1981; Hobson et al., 1975). Moreover, noradrenaline (NA) has an enhancing effect on GABAergic systems (Waterhouse et al., 1980) and was shown to suppress seizures via either separate activations of  $\alpha_{1A}$  and  $\alpha_{1D}$  adrenoceptor subtypes or via combined activation of the  $\alpha_2$  and  $\beta_2$  receptor subtypes (Clinkers et al., 2010). Therefore, it is plausible that a decreased noradrenergic tone can lead to decreased inhibitory activity, resulting in neuronal hyperexcitability and epileptic activity (Ross et al., 2015). We thus hypothesised that amyloid-induced imbalance between excitation and inhibition might be countered by a sustained noradrenergic tone during wakefulness, thus preventing epileptic activity. Therefore, our last aim was to test if noradrenergic antagonists could induce the occurrence of EA during wakefulness. For this purpose, a subset of Tg2576 mice and their nontransgenic (NTg) littermates had their EEG recorded after the injection of propranolol or prazosin, which respectively antagonise the  $\beta_{1/2}$  and the  $\alpha_1$  adrenoceptors.

## 2. Methods

### 2.1. Ethics statement

All experiments were performed in strict accordance with the policies of the European Union (86/609/EEC), the French National Committee of Ethics (87/848), and the local committee's recommendations (C 31-555-11, Direction départementale de la protection des populations) for the care and use of laboratory animals. The animal facility of the CRCA is fully accredited by the French Direction of Veterinary Services (C 31-555-11, Feb 9, 2011), and animal surgery and experimentation conducted in this study were authorised by the French Direction of Veterinary Services (#31-1155521, 2002; #20210, 2019). All efforts were made to improve the animals' welfare and minimise their suffering.

### 2.2. Mouse line

Experiments were performed on male mice from the transgenic line Tg2576 (Hsiao et al., 1996; Hsiao et al., 1995) from our in-house colony (Krezymon et al., 2013; Lassalle et al., 2008;

Verret et al., 2013), aged from 1.5 to 10 months of age. Tg2576 mice overexpress a double mutant form of human APP695 (Lys670-Asn, Met671-Leu [K670N, M671L]), driven by a hamster prion protein promoter. Tg2576 males were bred with C57B6/SJL F1 females (Charles River, L'Arbresle, France), and the offspring were genotyped for the hAPP transgene using DNA obtained from post-weaning tail biopsies. Mice were maintained on a 12 hours light/12 hours dark cycle with *ad libitum* access to food and water.

### 2.3. Implantation of EEG electrodes for the 24 hour-long recordings

Male Tg2576 mice and non-transgenic littermates of different age groups were implanted for EEG recordings (1.5 months: NTg: n = 15; Tg2576: n = 18; 5.5–7 months: NTg: n = 8; Tg2576: n = 15; 8–10 months: NTg: n = 15; Tg2576 n = 16). The EEG signal of 6 Tg2576 mice was of insufficient quality to be analyzed (1.5 months: n = 1; 5.5–7 months: n = 1; 8–10 months: n = 4). In order to decrease the number of animals used, 3 Tg2576 mice and 2 NTg animals implanted and recorded at 1.5 months of age were also recorded at 6 months.

For the surgical procedure, mice were anaesthetised with isoflurane (2%), and a local anaesthetic (lidocaine 5%) was injected subcutaneously under the scalp before its incision. A veterinary ophthalmic gel (Ocrygel, Laboratoire TVM, Lempdes, France) was applied to the eyes to avoid dryness. Then, the skull was drilled, and 2 silver electrodes (EEG) were placed, either unilaterally over the left parietal and frontal cortices or bilaterally over the parietal cortices. In the latter case, for some mice, a tungsten LFP intra-hippocampal replaced one of the parietal electrodes. A silver ground electrode was positioned through the occipital bone over the cerebellum and served as a reference. One EMG electrode was placed in the neck muscles. Electrodes were fixed to the skull with dental cement (Superbound + Duralay) and plugged into a miniature connector (PlasticsOne, Roanoke, NC, USA). Lidocaine was applied to the flesh before suturing the skin. The animals were recovering for at least a week before any experimental procedure.

### 2.4. 24 hour-long EEG recordings

For recordings, mice were connected with a 6-channel cable (PlasticsOne) and head-staged with a homemade tension follower. This cable was connected to a multichannel commutator (PlasticsOne) that allowed mice to move in their cage freely. EEG and EMG signals were amplified and band-pass filtered (for EEG: 0.3–1 kHz; for EMG: 3 Hz–20 kHz) using an AM system 3500 amplifier (AM system, Sequim, WA, USA) and sampled at 1kHz for EEG and 100 Hz for EMG signals (Power 1401 mk-II, CED, Cambridge, UK). In a few cases, the EEG was sampled at 200 Hz instead of 1kHz (Tg2576 = 4, NTg = 8). Experimental recordings lasted 24h and were preceded by at least 24 hours of habituation.

### 2.5. Sleep scoring

One investigator analysed the recordings offline using Spike 2 V7.11 software (Cambridge Electronic Design, Milton, UK). Vigilance states were scored manually by visual inspection of the EEG and EMG signals for consecutive 5-s epochs according to standard criteria (Franken et al., 1998) as follows: (1) wakefulness (high and variable EMG activity and a low-amplitude EEG signal), (2) SWS (high EEG amplitude, dominated by slow waves, low EMG - for at least 10 seconds for better comparability with other publications), and (3) REM sleep (low EEG amplitude, theta oscillations [4–12 Hz] and loss of muscle tone). The total duration of each state and the mean duration of each episode were determined using MATLAB scripts (MATLAB and Statistics Toolbox Release 2012b, The MathWorks,

Inc., Natick, Massachusetts, United States) as well as a script created in Spike 2 V7.11 software (Cambridge Electronic Design, Milton, UK) and were compared, for each vigilance state, between genotypes and ages using a repeated-measures 2-way ANOVA with Tukey posthoc analyses.

### 2.6. Detection of epileptiform abnormalities on EEG traces

EA were defined for this analysis as the occurrence of epileptic activities, defined as sharp (2–50 ms) positive or negative deflections with amplitudes exceeding twice the baseline EEG recording (Minkeviciene et al., 2009). Every deflection passing -0.55 mV was examined, and those matching both morphological and temporal criteria (2–50 ms) were considered epileptic events. As for spike trains where only one spike passed the threshold, the other spikes were also scored as separate epileptic activities. A vigilance state was assigned to each spike to calculate the total number of EA and the EA frequency (spikes/minute) per vigilance state. Each REM episode was separately analysed for spikes to establish whether EA frequency is stable or fluctuating throughout REM sleep. Then the link between episode length and EA frequency was analysed by fitting a nonlinear model over the REM sleep episode length and spike frequencies.

### 2.7. Spike-phase coherence analysis

Spike-containing segments were analysed in one-second-long bins for each transgenic animal using a homemade code operating in Matlab to determine whether there is a preference for spike occurrence during a specific phase of the theta oscillations. Spikes were centred on the middle of the segment, and the best-fitting frequency of theta oscillations (between 3 and 12 Hz) was determined before a fast Fourier transformation was applied. A Hilbert transform was then used on the resulting component corresponding to the dominant frequency to extract the phase of the theta oscillation at each data point of the segment. This allowed us to determine the oscillation angle at each time point where spikes occurred throughout the dataset. A Rayleigh test was applied to the data of each animal to test for the non-uniformity of circular statistics using the CircStat Matlab toolbox (Berens, 2009). Note that while data is represented in twenty bins, each 18° large for better readability, the statistical analysis was applied to unbinned data for more precision.

### 2.8. Pharmacology

To explore the proposed potential role of noradrenergic transmission systems in REM-specific network hypersynchrony (Brown et al., 2018), we aimed at hindering noradrenergic transmission through temporary receptor blockade during wakefulness to reproduce the conditions of low noradrenergic tone present during REM sleep. We compared the effect of 2 adrenoreceptor (AR) antagonists, the  $\beta_{1/2}$ -AR specific propranolol and the  $\alpha_1$ -AR antagonist prazosin, on the occurrence of EA during wakefulness (AWK) in Tg2576 mice between 2 and 6.5 months of age (NTg: n = 15; Tg2576: n = 22). Mice were implanted as described above. EEG recordings were performed on 4 consecutive days as follows (Suppl. Fig. 1.)

On the habituation day, mice were connected to the EEG for 11 hours. Throughout the following 3 days, mice were connected to the EEG each morning at 9 AM and were returned to their home cage at 5 PM. They were injected with a different pharmacological agent during each of these 3 days at 2 different doses (a small dose for the first injection and a large dose for the second, see details below). The first injection took place at least 90 minutes after the



beginning of the recording, whereas the second injection followed the first one by 2 hours. We did separate 2-Way Repeated Measures ANOVAs on each treatment (NaCl, Propranolol, Prazosin) and each vigilance state (within-subject factor: dose, between-subject factor: genotype).

Sodium chloride (0.9 %) was injected on the first experimental day. On the second day, propranolol hydrochloride was used. Finally, on day 3, Prazosin hydrochloride was administered (for details on drug preparation, see the paragraph below). One transgenic mouse and 2 nontransgenic littermates had to be excluded due to the bad quality of the signals impeding the analysis, while a third NTg mouse had to be excluded due to concerns over its wellbeing resulting from a problem during the intraperitoneal injection on the last experimental day. Following the recordings, EEGs were analysed using Spike 2 V7.11 software (Cambridge Electronic Design, Milton, UK) both for sleep scoring and for EA detection as described above. For each recording day, 3 sections of EEG recordings were analysed: (1) the 90 minutes preceding the first injection serving as a baseline and (2 and 3) the 90 minutes starting 20 minutes post-injection for both doses (Suppl. Fig. 1). Due to a mistake, one batch of mice only had 76.5 minutes of recording before the first NaCl injection recording instead of 90 minutes, while 4 Tg2576 mice did not undergo the experiment's last day (prazosin) for the same reason.

### 2.9. Drug preparation

The control solution was sodium chloride (0.9 %). Propranolol hydrochloride (Sigma Aldrich, St Louis, MO, USA) was dissolved in a sodium chloride 0.9 % solution to reach a 2 mg/ml concentration and was administered at 10 and 20 mg/kg for small and large doses, respectively. Prazosin hydrochloride (Sigma Aldrich, St Louis, MO, USA) was dissolved at 0.1 g/ml in a solution of 2-hydroxypropyl  $\beta$ -cyclodextrin (Sigma Aldrich, St Louis, MO, USA) that was administered at 0.5 and 1 mg/kg. Drugs were injected intraperitoneally in a volume of 0.05 ml/ 10 g for the first injection and 0.1 ml/ 10 g for the second one.

### 2.10. Statistical analysis

All statistical analyses of sleep-stage distribution and spike frequencies were performed using the JASP software (Version 0.13.1) (Love et al., 2019). For posthoc analyses, Holm/Tukey corrections were applied on posthoc tests used for parametric ANOVAs, whereas CONOVER posthoc tests with Bonferroni corrections were applied for Friedman tests. The phase-locking analysis was run via a home-built Matlab code (MATLAB and Statistics Toolbox Release 2020a, The MathWorks, Inc., Natick, MA, USA). Finally, the figures and the nonlinear fit were generated via the Prism 5 software (GraphPad Software Inc., La Jolla, CA, USA).

## 3. Results

### 3.1. Alterations of REM sleep and SWS in Tg2576 mice across the lifespan

We observed significant impacts on the time spent in each vigilance state over 24 hours of both genotype (SWS:  $F(1, 75) = 23.02$ ,  $p < 0.001$ ; REM sleep  $F(1, 75) = 49371$ ,  $p < 0.001$ ; wakefulness:  $F(1) = 28.46$ ,  $p < 0.001$ ) and age (SWS:  $F(2, 75) = 8.91$ ,  $p < 0.001$ , REM sleep:  $F(2, 75) = 7.97$ ,  $p = 0.001$ , wakefulness:  $F(2, 75) = 4.2$ ,  $p = 0.02$ ). There was a significant interaction between these factors for SWS ( $F(2, 75) = 3.79$ ,  $p = 0.027$ ) as well as a marginally significant interaction for wakefulness ( $F(2, 75) = 2.96$ ,  $p = 0.06$ ; Fig. 1A, Suppl. Table 1 for detailed statistics). This was due, on the

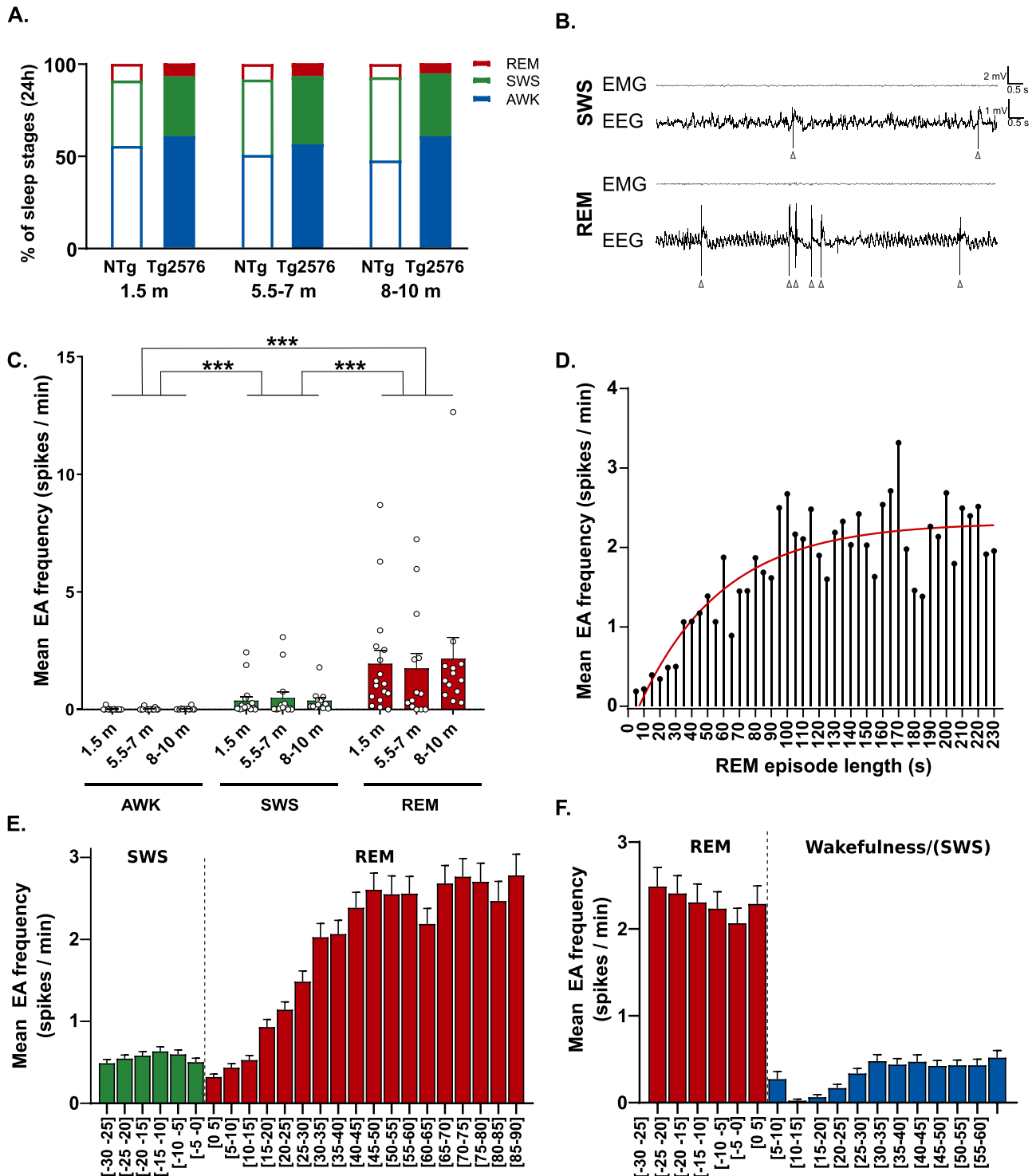
side of nontransgenic animals, to a gradual decrease of time spent awake throughout ageing (from  $55.2 \pm 5.1$  % at 1.5 months to  $47.3 \pm 8.8$  % at 8–10 months), mirrored by a concomitant gradual increase of SWS (from  $35.8 \pm 4.9$  % at 1.5 months to  $45.6 \pm 7.56$  % by 8–10 months), REM sleep duration also decreased (from  $8.9 \pm 1.8$  % at 1.5 months to  $7.1 \pm 1.6$  % by 8–10 months), a decrease also captured in Tg2576 mice, but with significantly lower percentages independent of age and as early as 1.5 months of age ( $7 \pm 1.7$  % at 1.5 months,  $5.3 \pm 1.8$  % by 8–10 months, see main genotype effect above). Moreover, for Tg2576 mice, no clear tendencies were observed for wakefulness ( $60.4 \pm 4.9$  % at 1.5 months and  $60.2 \pm 7.2$  % at 8–10 months) nor for SWS ( $32.6 \pm 4.5$  % at 1.5 months and  $34.5 \pm 6.1$  % months). However, these gradual changes for NTg mice and stagnating values for Tg2576 mice did not result in significant differences between genotypes for wakefulness and SWS until 8–10 months of age as revealed using Tukey posthoc tests (Tg2576 vs. NTg at 8–10 months:  $t(75) = 5.04$ ,  $p < 0.001$  for SWS and  $t(75) = -5.13$ ,  $p < 0.001$  for wakefulness, no significant differences at other ages). Altogether, these results suggest an alteration of REM sleep as early as 1.5 months for Tg2576 mice, while this decrease becomes perceptible much later (8–10 months) for nontransgenic animals. However, SWS quantity is not altered in the earliest stages of disease progression as a decrease becomes apparent only around 8–10 months of age for Tg2576 mice.

### 3.2. Epileptic events preferentially occur during REM sleep in Tg2576 mice and increase with REM sleep episode length

We restricted the spike analysis to Tg2576 animals because EA was observed almost exclusively in these mice (only 6 spikes were observed across all NTg animals recorded).

First of all, we observed that EA frequency (spikes/min) was highly dependent on the sleep-wake cycle (Fig. 1B and C. Friedman test:  $\chi^2(2) = 75.26$ ;  $p < 0.001$ , Suppl. Table 3). Specifically, post hoc tests revealed a gradual increase in EA from wakefulness to SWS ( $t(84) = 4.67$ ,  $p < 0.001$ ) and from SWS to REM ( $t(84) = 4$ ,  $p < 0.001$ , Fig. 1C). Our data thus confirm the observation of Kam et al. (2016) that EA primarily occurs during REM sleep in Tg2576 mice. However, in contrast to the same study, we did not find any age effect on EA occurrence in Tg2576 mice, as EA frequency is stable at least between 1.5 months and 8–10 months of age (Kruskal-Wallis tests: AWK:  $H(2) = 2.86$ ,  $p = 0.24$ ; SWS:  $H(2) = 2.92$ ,  $p = 0.88$ ; REM:  $H(2) = 1.31$ ,  $p = 0.52$ , Suppl. Table 2). Moreover, the EEG of a subset of 6 Tg2576 mice (4 of which were initially recorded at 8–10 months for the main analysis) was recorded at 13–15 months. In these mice, there is a tendency towards decreased EA frequency in the later stages of the disease (mean EA frequency/minute  $\pm$ SD =  $0.005 \pm 0.006$  during AWK;  $0.26 \pm 0.3$  during SWS and  $0.53 \pm 0.47$  during REM sleep). However, this might have resulted from higher mortality rates of mice with more frequent epileptic events. Therefore, given this potential bias and the small sample size, data from the 13–15 month-old animals were excluded from the main analyses.

The REM-sleep-related dramatic increase of EA is a robust result, and we tried to further understand its mechanical underpinnings. It has been suggested that REM sleep reorganises hippocampal excitability by decreasing it throughout REM sleep episodes (Grosmark et al., 2012). Therefore, we analysed spike frequencies throughout REM-sleep episodes to determine whether EA frequency evolves toward increase or decrease during the length of single REM episodes. Based on 3891 REM sleep episodes, our data seem to contradict the proposition about decreasing hippocampal excitability during REM sleep – at least in Tg2576 mice (Fig. 1D and E). Indeed, the data were well fitted by a nonlinear fit using an exponential plateau model (adjusted  $R^2 = 0.72$ , Suppl. Table 4,



**Fig. 1.** Epileptiform activities increase during REM sleep and with REM sleep episode length, independent of disease progression. (A) Changes in the sleep-wake cycle for Tg2576 mice and nontransgenic littermates (NTg) over the lifespan, analysed via 2-way ANOVAs. Genotype effect:  $p < 0.001$  for all 3 vigilance stages. Age effect:  $p < 0.001$  for SWS,  $p = 0.001$  for REM sleep and  $p = 0.02$  for wakefulness. Interaction:  $p = 0.03$  for SWS,  $p = 0.06$  for wakefulness, REM sleep: ns. Post hoc tests and further statistics are detailed in the text above and in Suppl. Table 1. (B) Example of interictal spikes during SWS and REM sleep in Tg2576 mice. (C) Spike frequency over the sleep-wake cycle in Tg2576 mice throughout the lifespan, analysed via a Repeated Measures ANOVA for the sleep effect and via a Kruskal-Wallis test for the age effect (ns). (D) Interictal spike frequency during REM sleep episodes of different lengths over 3891 REM sleep episodes of Tg2576 mice. Datapoints represent the mean of spike frequency per 5-second bins of REM sleep. (E) Spike frequency before REM sleep onset and following the first 90 seconds of REM sleep in Tg2576 mice. Dashed line marks REM onset (F) Spike frequency during the last 30 seconds of REM sleep and the first 60 seconds after REM sleep offset for episodes longer than 60 seconds in Tg2576 mice ( $n = 1506$  episodes). Dashed line marks REM offset, which is generally followed by wakefulness, or, in rare cases, SWS. Note that for panels D, E and F, the number of episodes per a given length decreases with duration as shorter durations are more frequent. Abbreviations: AWK, Awake; REM, Rapid-eye movement sleep; SWS, Slow-wave sleep. \*\*\*:  $p < 0.001$

which was better than the linear fit:  $R^2 = 0.54$ ;  $F(1,44) = 51.47$ ,  $p < 0.001$ ). It is also clear that the offset of REM sleep led to an instant drop rather than a gradual decrease in EA (Fig. 1F). Altogether, these data suggest that hippocampal excitability fails to decrease in Tg2576 mice across REM sleep episodes.

### 3.3. Epileptic activities are highly phase-locked to the theta cycle

To better understand how this peculiar EA is generated in Tg2576 mice, we dug deeper into analysing the spikes occurring during REM sleep. We looked at the timing of these spikes during theta oscillations, the dominant brain oscillation of REM sleep (Jouvet, 1967). To do so, we analysed the REM sleep episodes of all Tg2576 mice during this vigilance state and over the entire recording. Note that many mice presented spike trains; in these cases, only the first event of each spike was included in the analysis, which led to 5675 included epileptic events.

First of all, on a qualitative level, spikes brutally disrupted ongoing theta oscillations, sometimes for seconds and most of the time disrupting its rhythmicity (Fig. 2A for an illustration). Second, on a more quantitative level, analyses on individual animals revealed that every animal had spikes whose phase distribution was significantly locked to the trough and the first half of the ascending phase of the cortical theta oscillation ( $p < 0.001$  for the 33 mice which had at least 10 EA during REM sleep,  $p = 0.009$  and  $p = 0.004$  for one animal with 7 spikes and another with only 9, Fig. 2B). Actually, 73.41% of all spikes (pooled for all mice) appear between  $162^\circ$  and  $270^\circ$ . This angle range covers mainly the trough and the first half of the ascendant phase of the cortical theta cycle (Fig. 2C), which corresponds to the phase when pyramidal cells are most likely to fire action potentials (Klausberger et al., 2003).

### 3.4. Opposing effects of the $\alpha_1$ - and $\beta_2$ -AR blockade on spike frequency

Since we were interested in the effect of propranolol and prazosin on EA during wakefulness and to a lesser extent during SWS, we first verified that these treatments did not drastically reduce these 2 vigilance states in Tg2576 mice ( $n = 21$  age range: 2.3–6.5 months, Mean (M) = 4.9 months) or in NTg littermates ( $n = 13$  age range: 2.5–6.5 months, M = 4.9 months, Fig. 3A, Suppl. Table 5). As mentioned above, 1 batch ( $n = 4$ ) of Tg2576 mice did not undergo the last experimental day, but it did not significantly change the composition of the group age-wise for this last day ( $n = 17$ , age range identical, mean age = 4.6 months). In NTg animals, both propranolol and prazosin dose-dependently increased wakefulness (Mwake =  $64.5\% \pm 23.0\%$  of the recording time following high dose of propranolol and  $61.5\% \pm 22.5\%$  following the high dose of prazosin vs.  $29.6\% \pm 12.9\%$  after the large volume of NaCl on the control day) and concomitantly decreased SWS (MSWS =  $34.5\% \pm 21.8\%$  of the recording post-high dose of propranolol and  $36.5\% \pm 21.5\%$  after the high dose of prazosin vs.  $61.3\% \pm 10.1\%$  following the second NaCl injection on the control day) as well as REM sleep (MREM =  $0.96\% \pm 1.46\%$  post-high dose of propranolol and  $2.0\% \pm 2.7\%$  following the high dose of prazosin vs.  $9.1\% \pm 4.3\%$  after the large volume of NaCl). Tg2576 mice exhibited already high levels of wakefulness and low levels of SWS at baseline compared to NTg animals, therefore, these treatments had no significant effect on these 2 vigilance states (Suppl. Table 5). However, the treatments still decreased REM sleep in Tg2576 mice (REM =  $1.0\% \pm 1.67\%$  of the total recording time after the large dose of propranolol and  $2.5\% \pm 3.5\%$  following the same dose of prazosin vs.  $7.8\% \pm 4.4\%$  after the large volume of NaCl). Given these changes, the effect of the drugs on the sleep-wake cycle did not impede the analysis of epileptic events during wakefulness and SWS in Tg2576 mice.

The next step was to quantify the impact of these interventions on EA frequency. Since, given our starting hypothesis, we focused the spike-frequency analysis on wakeful periods, the sharp decrease in REM sleep did not affect the feasibility nor the power of the following analysis (Fig. 3B, Suppl. Table 5). This analysis was run only for Tg2576 mice since NTg littermates still did not exhibit any EA whatever the injection (*data not shown*). First of all, the frequency of EA during wakefulness was similar across experimental days in the pre-injection period, establishing a stable baseline (Friedman test for the 3 pre-injection periods,  $\chi^2(2) = 1.79$ ,  $p = 0.41$ ). The Friedman tests on the 3 experimental days (Repeated Measures factor: Dose) revealed no impact of the NaCl nor the propranolol injections on EA frequency during wakefulness ( $\chi^2(2) = 2.36$ ,  $p = 0.31$  and  $\chi^2(2) = 2.22$ ,  $p = 0.33$ , respectively). However, prazosin seemed to increase EA frequency in awake Tg2576 mice ( $\chi^2(2) = 12.12$ ,  $p = 0.002$ ). Post hoc tests revealed that this was due to a difference between the baseline and the large dose (Mean EA frequency per minute:  $0.06 \pm 0.09$  at baseline,  $0.29 \pm 0.26$  after the large dose;  $t(32) = 3.49$ ,  $p = 0.004$ ). A tendency towards a gradual increase of spiking activity from baseline to small dose and from small dose to large dose was visually observed but was potentially hindered by the Bonferroni corrections.

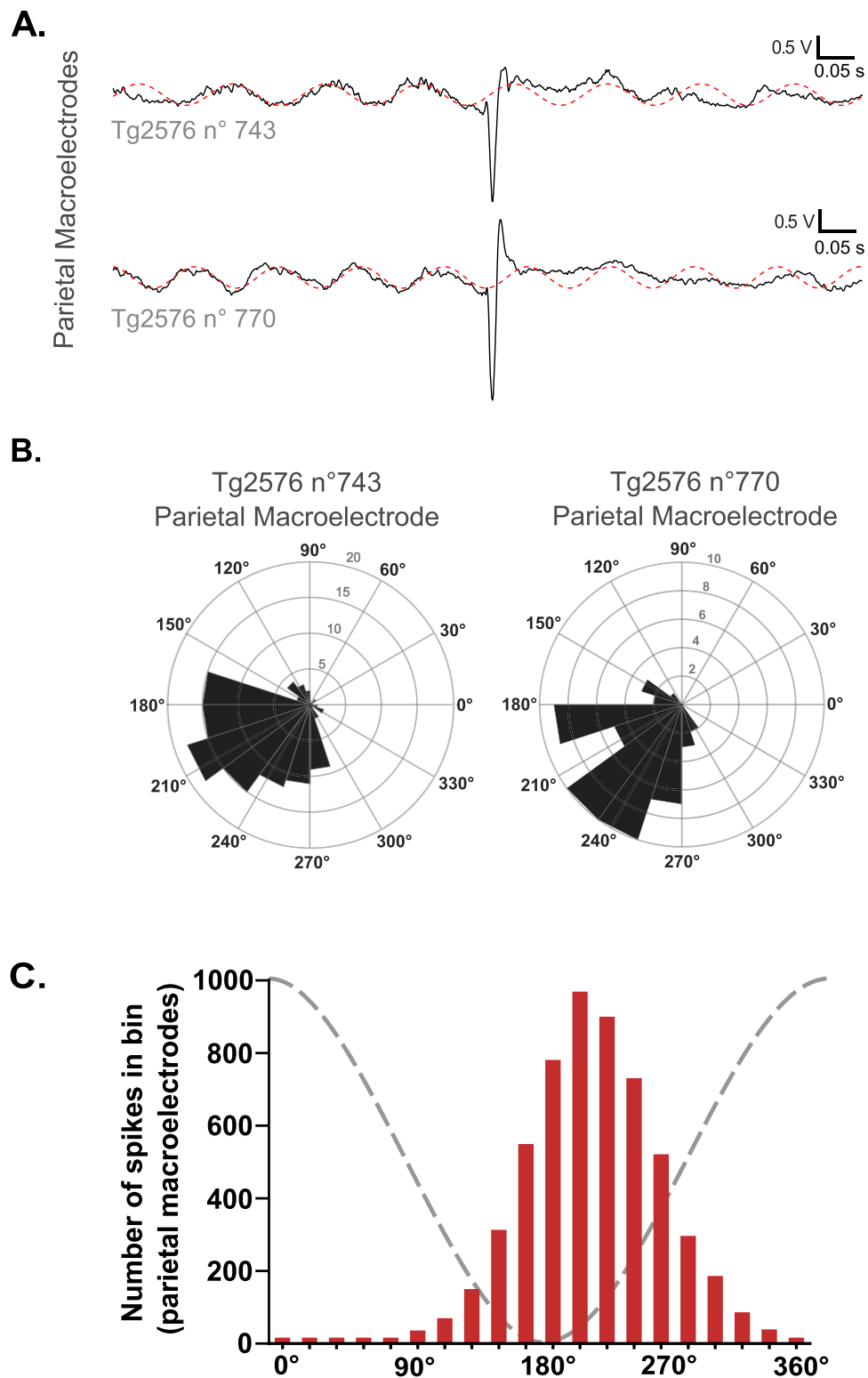
We expected the same results on EA frequency to reproduce at a more modest level during SWS when the concentration of NA is naturally lower and the baseline spike frequency is higher (Fig. 1C). Once again, we observed no difference between the baseline EA frequency across experimental days and indeed confirmed the proepileptic impact of prazosin (Mean EA frequency per minute:  $0.63 \pm 0.68$  at baseline,  $1.36 \pm 1.6$  after the large dose;  $\chi^2(2) = 6$ ,  $p = 0.05$ ) on EA frequency. Compatible with our hypothesis, this effect was more modest than during AWK, as post hoc tests revealed it to be more of a tendency between the baseline and the large dose ( $t(24) = 2.35$ ,  $p = 0.081$ ). Altogether, these results are consistent with those obtained for awake periods and point towards an antiepileptic effect of NA via  $\alpha_1$ -ARs.

Interestingly, propranolol significantly decreased EA frequency during SWS (Mean EA frequency per minute:  $0.92 \pm 1.45$  at baseline and  $0.41 \pm 0.92$  after the large dose, Friedman test:  $\chi^2(2) = 18.35$ ,  $p < 0.001$ ) (Fig. 3C; baseline vs. small dose:  $t(34) = 1.03$ ,  $p_{\text{bonf}} = 0.93$ ; baseline vs. large dose:  $t(34) = 4.12$ ,  $p_{\text{bonf}} < 0.001$ , small vs. large dose:  $t(34) = 3.09$ ,  $p_{\text{bonf}} = 0.01$ ). It is important to note that only animals with no missing data (that is, animals that slept during all 3 analysed intervals of a given day) were taken into account in the Friedman test leading to 16, 18 and 13 animals analysed on the 3 experimental days (NaCl, Propranolol, Prazosin), respectively. These results indicate an SWS-specific proepileptic effect of NA via  $\beta$ -ARs.

Altogether, these data support, on the one hand, the hypothesis that the drop in NA release at the beginning of sleep would be a trigger to the spike generation in Tg2576 mice and that noreadrenalin would play a protective role against it via  $\alpha_1$  adrenoreceptors during wakefulness. On the other hand, the apparent absence of the antiepileptic impact of propranolol during wakefulness that is evident during SWS might be explained by the age-related binding capacities of  $\alpha_1$  and  $\beta$  ARs (Klingner et al., 2003).

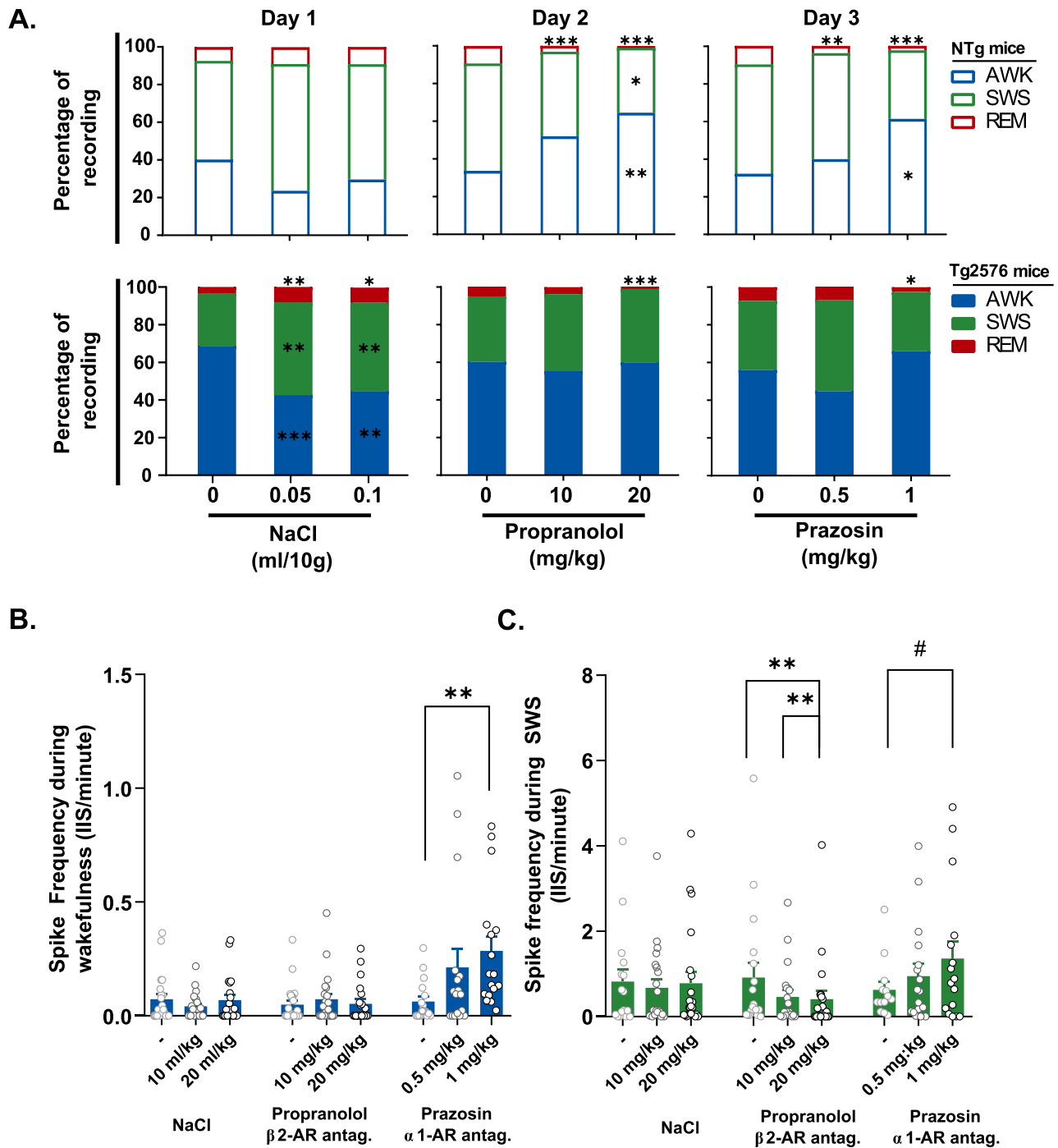
## 4. Discussion

Our work shows that Tg2576 mice present an altered sleep-wake cycle as early as 1.5 months. Wakefulness increases at the detriment of REM sleep and SWS by 8–10 months. As for spontaneous epileptic activities, we confirm that they are present as young as 1.5 months of age in this mouse model of AD; however, these abnormal activities are highly variable between individuals



**Fig. 2.** Epileptiform activities are phase-locked on theta activity. (A) Example of an interictal spike (continuous line) on parietal macroelectrodes. The dashed red line represents the perfect theta wave best fitting the EEG before the interictal spike and shows what the theta rhythm would look like for the given segment if it was stable after the spike. (B) Example of spiking activity from a Tg2576 mouse on the theta cycle on parietal macroelectrodes with 180° representing the trough. Spike numbers are represented by inner circles where each bin corresponds to 18°. (C) Spike-timing around the theta cycle (marked with a dashed gray line) recorded with parietal macroelectrodes during REM sleep for all recorded spikes from  $n = 38$  Tg2576 mice. Total number of spikes = 5675. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article).





**Fig. 3.** Epileptiform activities are modulated by noradrenergic transmission via  $\alpha$ 1 adrenergic receptors in Tg2576 mice. (A) Sleep-wake cycle modulation of propranolol ( $\beta$ 2-AR antagonist) and prazosin ( $\alpha$ 1 antagonist) in varying doses over 1.5 hour-long periods in Tg2576 mice ( $n = 21$ , age range: 2.3–6.5 months, Mean (M) = 4.9 months on the first two days) and NTg littermates ( $n = 12$ , age range: 2.5–6.5 months, M = 4.93 months). Four Tg2576 mice did not undergo the last experimental day, hence, in this group,  $n = 17$ , age range: 2.3–6.5 months, M = 4.56 months. Only statistical differences pertaining to dose or dose\*genotype (if applicable) effects are marked with asterisks, compared to the baseline of each day and vigilance stage (asterisks above some bars correspond to the REM sleep below). (B and C) Modulation of Propranolol ( $\beta$ 2-AR antagonist) and prazosin ( $\alpha$ 1 antagonist) in varying doses over 1.5 hour-long periods on epileptiform activity during wakefulness (B) and SWS (C) (only in Tg2576 mice). Darker circles indicate increasing doses of the given pharmacological treatment. \*:  $p < 0.05$ ; \*\*:  $p < 0.01$ ; \*\*\*:  $p < 0.001$ ; #:  $p < 0.1$ .

and do not aggravate with disease progression. Interestingly, these aberrant events are absent during quiet wakefulness and, preferentially, if not exclusively, appear during sleep, with a drastic increase during REM sleep. Moreover, we show that these epileptic events occur during REM sleep at the trough and the first half of the as-

ending phase of theta oscillations, suggesting abnormal pyramidal cell activity behind epileptic events. Intriguingly we also found that EA frequency increases with REM sleep episode length, indicating that hippocampal excitability levels may fail to decrease during REM sleep episodes, contrary to what was described by

Grosmark et al. (2012) under non-pathologic conditions. Furthermore, we showed that  $\alpha_1$  adrenoreceptor blockade during wakefulness and SWS increases the frequency of EA in adult Tg2576 mice. We propose that NA decreases neuronal hypersynchrony through its  $\alpha_1$  adrenoreceptors during wakefulness and that this protective effect is lost during REM sleep when noradrenergic neurons cease firing, giving rise to exacerbated epilepsy.

#### 4.1. Differential alteration of SWS and REM sleep in Tg2576 mice across the lifespan

We first showed that sleep architecture, especially REM sleep, is altered in Tg2576 mice as early as 1.5 months, while SWS disruption becomes apparent only later, at 8–10 months of age. Although this is discordant with a previous study by Wisor et al. (2005), it corroborates the data obtained by Zhang et al. (2005). Indeed, Wisor and colleagues found that the percentage of SWS and REM sleep were similar between Tg2576 mice and NTg littermates event at 8–15 months, while Zhang and collaborators reported that Tg2576 (bred on a B6C3 genetic background) exhibited a 30% decrease of time spent in REM sleep at 6 months of age, which is similar to our observations (22% decrease at 1.5 months of age, 27% at 8–10 months). In light of the present results, the Tg2576 mouse model seems to reproduce typical alterations of the sleep-wake cycle observed in AD patients, as well as in other mouse models of AD (e.g., PLB1 and 5XFAD mice; in Platt et al., 2011; Sethi et al., 2015, respectively), such as increased wakefulness and decreased sleep duration (Ju et al., 2014; Peter-Derex et al., 2015). The implication of epileptiform events in the decreased REM sleep quantity is yet to be explored. However, our qualitative observations suggest that contrary to individual spikes, which only momentarily disrupt theta oscillations without waking up the animals, spike trains seem to interrupt REM sleep episodes themselves (*data not shown*).

#### 4.2. Epileptic events increase with increasing sleep depth: a common ground for AD models?

Our results suggesting an increasing frequency of epileptic events from wakefulness to SWS and SWS to REM sleep are also coherent with previous findings in this model (Kam et al., 2016). However, comparison with other models is often complicated as many studies using EEG recordings to detect epileptic events did not analyse the sleep-wake cycle (for a detailed list of studies, see Kazim et al., 2021). From the few studies where sleep was also investigated, seizure activity was observed during REM sleep in the APdE9 model by Minkeviciene et al. (2009). In APP/PS1 models, a detailed analysis of different types of abnormal network activities was undertaken by Gureviciene et al. (2019). They barely found giant spikes (the ones detected in the present study) during active periods, but they noted that these EA occurred with the same probability during REM sleep and SWS. In hAPPJ20 mice, epileptic events were detected during reduced gamma oscillatory activity (Verret et al., 2012), which were later confirmed to correspond mostly to REM sleep (Brown et al., 2018). Albeit a direct verification is needed, our results might also be comparable to EA in the APP/TTA model, in which these activities are correlated with an absence of locomotor activity (Born et al., 2014). However, it is difficult to tell if these periods correspond to quiet wakefulness, SWS or even REM sleep, as the studies mentioned above used activity count instead of sleep EEG/EMG to determine locomotor activity levels.

It would suggest that REM sleep promotes hypersynchronous events in Tg2576 mice, which is quite unlikely given the seizure paucity reported in epileptic patients during REM sleep (Ng &

Pavlova, 2013). Indeed, REM sleep is characterised by EEG desynchronisation that would somewhat limit the emergence of hypersynchronous events. Moreover, theta oscillations that are prominent during this vigilance state can play an antiepileptic role as their artificial induction by the electrical or chemical stimulation of the medial septum in rodents was shown to stop seizures induced by the administration of a GABA receptor antagonist (Miller, 1994).

Altogether, these data suggest that epileptic events during REM sleep, however rare in non-AD related epilepsy, might represent a common feature in models of amyloidosis. Understanding this unique, AD-related epileptic phenotype may hold important information concerning AD pathophysiology.

#### 4.3. A unique epileptic phenotype

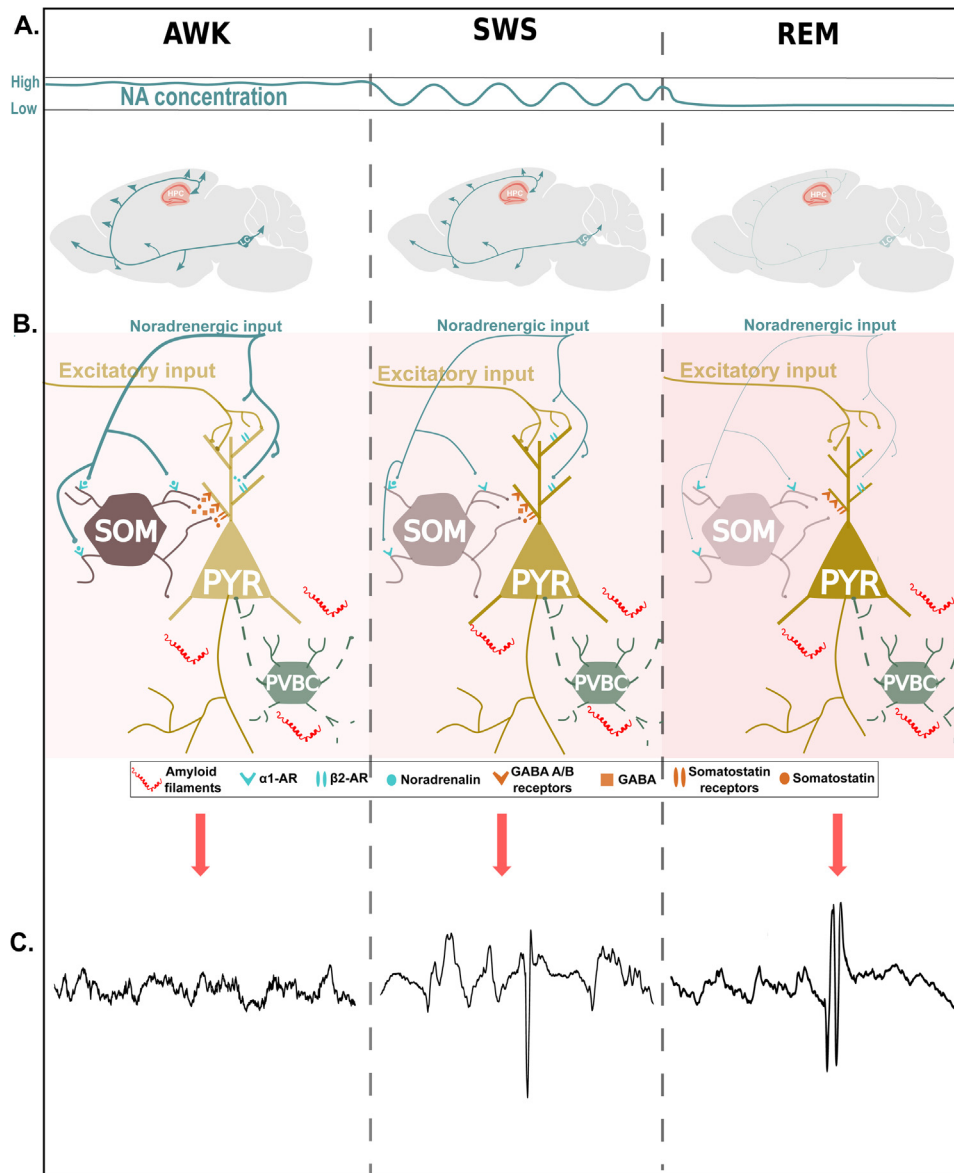
Our first, qualitative observation is that epileptic events strongly disrupt the theta cycle, which often takes several cycles to fall back to a normal rhythm. On a more quantitative level, we revealed a very strong phase-locking of EA to the trough and the first half of the ascending phase of the theta cycle. Therefore, it would seem that EA occurs when pyramidal cells are at their highest probability of firing (Klausberger et al., 2003), hinting at a potential faulty inhibition of pyramidal cell activity that might become uncontrolled and pathological. Such a failure of inhibitory interneurons has already been reported at early ages in different mouse models of amyloidosis (Cattaud et al., 2018; Hijazi et al., 2019; Krantic et al., 2012; Verret et al., 2012); more specifically at the level of parvalbumin positive interneurons that provide the most substantial perisomatic inhibition to pyramidal neurons that is essential for preventing aberrant activities (i.e., epileptic spikes, Gulyás & Freund, 2015). This hypothesis between faulty interneurons and epileptic events will require further testing in the future, but results in other AD models are already promising (Martinez-Losa et al., 2018; Verret et al., 2012).

Interestingly, several lines of research have demonstrated the importance of REM sleep and its predominant oscillations in memory consolidation-associated processes (e.g., Bohbot et al., 2017; Boyce et al., 2016). Recent evidence has shown that preventing theta oscillations during REM sleep without disrupting REM sleep itself impairs hippocampal-dependent memory (Boyce et al., 2016). Therefore, it is highly possible that the EA observed during REM sleep in Tg2576 mice, which interrupt theta oscillations, impair mnemonic processes. Our findings should stimulate research that, on the one hand, will carefully look for the existence of epileptic events during REM sleep in patients at early stages of AD (only examined so far in Lam et al., 2020) and, on the other hand, decipher the link between epileptic events and memory consolidation, both in rodent models and patients.

#### 4.4. Noradrenaline - a potential modulator of epileptic activity through $\alpha_1$ adrenoreceptors

Theta oscillations are also predominant during exploratory behaviours when no EA is observed. Therefore, other factors than those generating theta activity must come into play in the REM sleep predominant EA in Tg2576 mice. Indeed, we found that noradrenergic transmission, primarily through  $\alpha_1$ -ARs, could eventually counterbalance the neuronal hyperexcitability in Tg2576 mice (Fig. 4). Blocking  $\alpha_1$  AR receptors with prazosin led to an increased spike frequency during wakefulness and, to some extent, during SWS in Tg2576 mice, yet not inducing EA in their nontransgenic littermates. The implication of NA has been under suspicion for several reasons in AD-related epilepsy in mouse models.

First, the antiepileptic effect of NA has been suggested by previous publications describing higher seizure rates in NA-deficient



**Fig. 4.** Suggested model on the role of noradrenergic transmission via  $\alpha_1$ -ARs in epileptic activities in the Tg2576 mouse model. (A) Decrease of noradrenergic transmission from wakefulness to SWS (with infra-slow oscillations), and a quasi-silence of noradrenergic neurons during REM sleep that uncovers epileptic activities in Tg2576 mice (marked by flashes). (B) While under non-AD conditions, the antiepileptic effect of noradrenergic transmission from the Locus Coeruleus (LC) is not visible (as epileptic events are absent), in Tg2576 mice, its effect is potentially due to the excitation-inhibition balance being tipped towards pathological excitation due to  $A\beta$  accumulation and the known dysfunctions of the parvalbumin-positive interneurons. With the decrease of noradrenergic tone with increasing sleep depth (shown symbolically by decreasing width of noradrenergic axons), however, the antiepileptic effect of NA is lost, leading to epileptic events shown in panel (C): Examples of epileptic events during the different sleep stages (one-second-long examples). Red shades indicate increasing network hyperexcitability, which, contrary to non-pathological situations, does not decrease during REM sleep, further contributing to the epileptic phenotype (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article).

rodents (Szot et al., 1999) or that the decrease in seizure susceptibility observed after vagus nerve stimulation might be mediated by the increased NA concentration that follows the stimulation (Raedt et al., 2011; see Weinshenker & Szot, 2002 for a review). Indeed,  $\alpha_1$ -ARs are decreased in number both in epileptic rats (Nicoletti et al., 1986) and in the epileptic foci of epilepsy patients (Brière et al., 1986), which has been hypothesised to contribute to the onset of seizures (Brière et al., 1986), yet again hinting at an antiepileptic effect of NA. Finally, several anti-seizure medications have been shown to induce increased levels of NA, as was reviewed by Weinshenker and Szot (2002) and by Giorgi et al. (2004). Moreover, we observed a concomitant increase in EA frequency with increasing REM sleep episode length, which

is concordant with a gradual dissipation of residual NA in the brain during REM sleep episodes (Fig. 4A and B).

Secondly, the frequency of EAs in several AD mouse models is inversely mirroring NA concentrations: NA is constantly released during wakefulness, shows a decrease during SWS and quasi-absent during REM sleep, while EAs are the most frequent during REM and show decreasing probability from SWS to wakefulness – just as was confirmed in the current study. It is of note that recent findings have shown that NA release does not simply decrease during SWS but shows an infra-slow oscillatory pattern (~50 s). This pattern has been hypothesised not only to be a vigilance-promoting mechanism rendering the animal less vulnerable to potential dangers during SWS via micro-arousals around

the peak of these oscillations but was also shown to be driving sleep spindles essential for memory consolidation (Kjaerby et al., 2022; Osorio-Forero et al., 2021). It was also suggested previously that EAs during sleep might be a consequence of derailed memory consolidation-related oscillations, especially sharp-wave ripples that generally ride on the trough of sleep spindles (Halász et al., 2019). Given this hypothesis and that spindles were shown to be driven by the descending phase of the infra-slow NA oscillations in the work of Kjaerby et al. (2022), it is possible that EAs during SWS indeed occur during spindle-locked ripples in the Tg2576 model, when NA is dissipating and 10%–18% of hippocampal neurons and neurons of surrounding areas fire at the same time to generate a ripple (Watson & Buzsáki, 2015). It would be plausible in this case that the parvalbumin-positive interneurons, that are dysfunctional from an early age in AD mice (Cattaud et al., 2018; Corbett et al., 2013; Hijazi et al., 2019), could not keep up with such an increased excitatory tone. Even if other types of interneurons have been shown to try to compensate for the fallout of the parvalbumin-positive interneurons' perisomatic inhibition during EAs (see Gulyás & Freund, 2015), their weaker inhibitory impact could not always succeed in preventing EAs, especially when NA concentrations are relatively low. This hypothesis is also coherent with findings showing decreased sharp-wave ripple densities in the presence of epileptic events in mice (Gélinas et al., 2016) and with decreased spindle densities around the epileptic focus in patients (Schiller et al., 2022).

The third reason for the antiepileptic effect of NA is a finding from Hillman and collaborators (2005, 2007, 2009), who identified a subpopulation of GABAergic somatostatin positive interneurons in the CA1 activated by NA through  $\alpha_{1A}$  adrenoreceptors. Interestingly, in EA-like situations (i.e., excess glutamate release), these interneurons can efficiently decrease neuronal activity by inhibiting the postsynaptic pyramidal cells through GABA<sub>A</sub>, GABA<sub>B</sub> or somatostatin receptors (Hillman et al., 2009, Fig. 4B). Therefore, the already increased excitation-inhibition ratio due to A $\beta$ -filaments (and later, oligomers and plaques) and interneuron failure may be uncovered by the gradual decrease of NA concentration during sleep, especially during REM sleep (Fig. 4C).

The centre of noradrenergic innervation of the forebrain is the Locus Coeruleus (LC), a cluster of brainstem nuclei with far-reaching projections (for a review, see Osorio-Forero et al., 2022). In Tg2576 mice, this structure has been shown to degenerate between 6.5 and 8 months of age (Guérin et al., 2009), indicating that in the Tg2576 mice included in the pharmacology experiment (between 2.5 and 6.5 months of age), this structure was primarily intact. However, the degeneration of the LC after 6.5 months of age would imply that spike frequency increases afterwards, especially during wakefulness, which we surprisingly did not observe in our cohort of 8–10 month-old Tg2576 mice (Fig. 1C). However, we did observe a remarkably high interindividual difference in EA frequency across the lifespan, which grew even more prominent at 8–10 months of age (Fig. 1C). Even though it needs further investigation, it cannot be excluded that mice have a slight increase in spike frequency around this age which could be correlated to the rate of LC degeneration in a given individual. It is also a possibility that mice with degenerating LC experience a disinhibition and an increase in spike frequency which may lead to seizure onset and eventual death, leading to a biased dataset in which mice with relatively low baseline EA are over-represented. Finally, another plausible hypothesis is that the absence of increase in spike frequency with ageing is related to an increased and compensatory activity from remaining LC neurons. Such compensatory activity was observed in rodents following the induction of a limited loss of LC neurons via 5  $\mu$ g/ $\mu$ l of the neurotoxin 6-hydroxydopamine 3 weeks post-treatment (Szot et al., 2016). Indeed, extracellular NA concen-

trations do not seem to drop after LC lesions until at least 10% of LC neurons remains functional (for reviews, see Berridge et al., 2012; Van Egroo et al., 2022). Another argument in favour of compensatory activity of remaining LC neurons is from AD patients, where the level of NA is sometimes even higher in the cerebrospinal fluid and the plasma than in cognitively healthy older adults (see Mather, 2021).

Interestingly, Fitzgerald (2021) also posited the possibility of early LC hyperactivity on the pathophysiological path leading up to AD. This would lead to excess NA that could trigger early neuronal hyperactivity, as activation of  $\beta$ -ARs by NA has been shown to activate the amyloidogenic amyloid precursor protein (APP) cleavage pathway (Ni et al., 2006), increasing the rate of A $\beta_{1-42}$  deposition. This would be, on the one hand, coherent with the pro-epileptic effect of NA through  $\beta$ -ARs that we have observed in SWS and, on the other hand, with the known excitatory effect of noradrenergic transmission through presynaptic  $\beta$ -ARs in the hippocampus and the prefrontal cortex (Gereau & Conn, 1994; O'Dell et al., 2010).

Moreover, in AD patients, the LC is the first region where p-tau accumulates (Braak et al., 2011), even in MCI subjects (often converting to AD) (Grudzien et al., 2007). Tau protein accumulation is associated with neuronal hypoactivity (Ranasinghe et al., 2021), especially in its soluble form (Busche et al., 2019). Furthermore, tau proteins aim for excitatory neurons versus inhibitory ones (Fu et al., 2019), further tipping the balance toward hypoexcitability (for details, see Harris et al., 2020). This early Tau release may be a byproduct of the LC hyperactivity as tau production increases with increasing neuronal activity (Wu et al., 2016; Yamada et al., 2014), potentially via calcium-dependent pathways (Pooler et al., 2013). However, as phosphorylating tau near the microtubule domain renders the tau-Fyn-PSD95 interaction impossible, leading to the endocytosis of NMDA receptors and decreasing the excitotoxic effects of A $\beta$  (Ittner et al., 2010), it is plausible this early tau accumulation might be a compensatory mechanism against the aforementioned early LC hyperexcitability.

It remains to explain why epileptic events seem to be more present during wakefulness and SWS in AD patients compared to REM sleep, when EAs are scarce (Horváth et al., 2018; Lam et al., 2020; Vossel et al., 2016). Furthermore, many other questions remain to be answered on the clinical aspects of AD-related EAs, starting from their prevalence to their place on the timeline of disease progression (Romanelli et al., 1990; Vossel et al., 2016). Given the gradual degeneration of the LC, this latter question (early or late occurrence) may influence the state and, therefore, the potential involvement of noradrenergic transmission in this comorbidity. Finally, contrary to the controlled and limited number of factors intervening in mouse models, a vast panoply of mediating factors (such as APO-E genotype) come into play in AD patients that were recently reviewed and that could modify the distribution of epileptic events across the sleep-wake cycle and need to be investigated (B. Szabo et al., 2022; Harris et al., 2020).

Hence, the suggested antiepileptic effect of NA via  $\alpha_1$ -ARs in Tg2576 mice both during wakefulness and SWS could also be valid in AD patients but requires further investigations both on the clinical and the preclinical ends.

#### 4.5. Perspectives and limitations

One of the limitations of this study is the absence of sufficient recordings from mice with the more advanced stages of AD (i.e., after 10 months, here reported as observations but not included in the analysis due to the insufficient number of mice). However, given that survival rates drop with age, probably decimating more of the mice with more frequent EA or even seizures, including animals at those ages would introduce a bias towards decreased



epileptic event frequency. This is precisely what we observed in the few mice recorded at 13–15 months. In the future, longitudinal studies with the same mice recorded at different ages would be helpful to understand better the progression and the impact of epileptic activity in rodent models of amyloidosis, as well as in mouse models expressing Tau pathology or Tau pathology and amyloidosis concomitantly. This could be the first step toward potential clinical applications (i.e., adapted treatment plans) in the future aiming at harnessing potentially beneficial effects of NA.

## 5. Conclusion

Our study unveils an early disruption of REM sleep and a gradual decrease of sleep quantity across the lifespan in the Tg2576 mouse model. These results complement clinical studies consistently finding decreased sleep quality in the earliest, prodromal stages of AD. We also confirm the presence of epileptic events as early as 1.5 months in Tg2576 mice and unveil its dependence on the sleep-wake cycle, namely from wakefulness to SWS and a drastic increase in REM sleep, during which epileptic events occur at the phase of the theta oscillation when pyramidal neurons are at their maximal firing probability. Finally, we revealed that this apparent early hippocampal hyperexcitability could be counterbalanced by noradrenergic transmission through  $\alpha_1A$  adrenoceptors. Therefore, the delayed impairment of the LC in AD models compared to patients could at least partially explain why the distribution of epileptic events in patients differs from that observed in AD models. Potential therapeutic pathways should be explored in the future, considering the broad scope of the effects of different noradrenergic receptors. Finally, a more pronounced study of REM-sleep related epileptic events in AD patients on epilepsy and Alzheimer's disease should be undertaken.

## Disclosure statement

The authors have no actual or potential conflicts of interest.

## Verification

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## Author Contribution

Conceptualisation: LD; Experimental work: VC, ABS, CB, RD, FS, SG, CL.; Data Analysis: ABS, VC, CB. Writing and editing: ABS, VC, LVe, LD, CR, LVA. Preparation of figures and tables: ABS. Funding acquisition: LVe, CR, LD, LVA; Supervision: LD

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## Supplementary materials

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