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# A translational study of the link between Alzheimer's disease, sleep and epilepsy : prevalence, underlying factors and consequences on memory consolidation

Anna Szabo

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# THÈSE

**En vue de l'obtention du  
DOCTORAT DE L'UNIVERSITÉ DE TOULOUSE  
Délivré par l'Université Toulouse 3 - Paul Sabatier**

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**Présentée et soutenue par  
Anna SZABO**

Le 15 novembre 2022

**Etude translationnelle du lien entre la maladie d'Alzheimer, le  
sommeil et l'épilepsie - prévalence, facteurs sous-jacents et  
conséquences sur la consolidation mnésique**

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Ecole doctorale : **BSB - Biologie, Santé, Biotechnologies**

Spécialité : **NEUROSCIENCES**

Unité de recherche :  
**CRCA - Centre de Recherches sur la Cognition Animale**

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# Curriculum Vitae

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## Education

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**2018 – present: PhD studies** in Neuroscience at Université Paul Sabatier (France)

**2016-2018: MSc** - Biology-Health: specialization in Cellular Signalling and Integrative Neuroscience, Université Paris-Saclay, France

**2015-2016: MSc 1** in Human Movement Science, specialization in Sport Psychology, Motor Control and Sport Performance, Université Paris-Saclay, France

**2014-2015: BA (3<sup>rd</sup> year)** in Dance Research, Université Paris 8, France

**2013-2014:** Member of Illyés Sándor Institute for Advanced Studies - Eötvös Loránd University, Budapest, Hungary

**2012-2014: Psychology BA (1<sup>st</sup>-2<sup>nd</sup> year)** in Psychology at Eötvös Loránd University, Budapest, Hungary

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## Previous Research Experience

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**2018. 11. 01 – present: PhD** - *A translational study of epileptic activities in Alzheimer's disease – Prevalence, underlying factors and consequences on memory consolidations processes.*

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*Preclinical project:* Research Center for Animal Cognition (CRCA). **Supervisor:** Lionel Dahan, PhD

**2018: MSc internship (6 months)** at the Vision Institute (Paris, **Supervisor:** Angelo Arleo, PhD) and at the Swiss Federal Institute of Technology in Lausanne (EPFL, **Supervisor:** Ricardo Chavarriaga Lozano, PhD) on *The neural correlates of the age-related changes in the useful field of view.*

**2016: MSc Internship (2 months)** at the “Maison des Sciences de l’Homme - Paris Nord” on *The impact of dance classes on the sense of agency in Huntington's disease.* **Supervisor:** Iris Trinkler, PhD

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## List of Publications

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**B. Szabo, A.,** Cretin, B., Gerard, F., Curot, J., Barbeau, E., Pariente, J., Dahan, L.\*, Valton, L.\* (2022). Sleep: The Tip of The Iceberg in the Bidirectional Link Between Alzheimer's Disease and Epilepsy. *Frontiers in Neurology*. \*equal contributions. DOI: 10.3389/fneur.2022.836292

**B. Szabo, A.\*,** Cattaud, V.\*, Bezzina, C., Sayegh, F., Valton, L., Lopez, S., Lejards, C., Verret, L., Rampon, C., Dahan, L. Neuronal hyperexcitability in the Tg2576 mouse model of Alzheimer's disease – The influence of sleep and noradrenergic transmission (*in press in Neurobiology of Aging*) \*equal contributions.

**B. Szabo, A.,** Lopez, S., Lejards, C., Verret, L., Rampon, C., Dahan, L. Epilepsy, amyloid- $\beta$ , and dopamine receptors: no apparent pathogenic link *in vivo* in the Tg2576 mouse model of amyloidosis. (*in preparation*)

**B. Szabo, A.,** Curot, J., Barbeau, E. J., Gerard, F., Debs, R., Denuelle, M., Rulquin, F., Georges, C., Lemesle, B., Péran, P., Thalamas, C. Pariente, J., Valton, L.\*, Dahan, L.\* - Silent epileptic activities in Alzheimer's disease during sleep – prevalence, pathophysiological links and consequences on disease progression (*in preparation*).

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## Posters and Presentations

---

**B. Szabo, A.** - *Etude translationnelle du lien entre la maladie d'Alzheimer, le sommeil et l'épilepsie - prévalence, facteurs sous-jacents et conséquences sur la consolidation mnésique* – Présentation orale aux Journées Scientifiques de la Fédération des Centres Mémoire (21. Octobre 2022, Toulouse)

**B. Szabo A.,** Curot, J., Barbeau, E. J., Gerard, F, Debs, R., Denuelle, M., Rulquin, F., Georges, C., Lemesle, B., Péran, P., Pariente, J., Valton, L.\*, Dahan, L.\* - *Silent epileptic activities: the missing link between Alzheimer's disease, disrupted sleep and dysfunctional memory consolidation?* – Poster at the FENS Forum 2022 (9-13 July 2022, Paris, France)

**B. Szabo, A.** – *Maladie d'Alzheimer et épilepsie : des liens physiopathologiques ?* (AD and Epilepsy: What pathophysiological links?) Invited symposium talk at the Annual Meeting of French Neurologists (JNLF 2022, 12-15 April), Strasbourg, France



**B. Szabo A.\***, Cattaud, V.\*, Bezzina, C., Dard, R., Sayegh, F., Valton, L., Lopez, S., Lejards, C., Verret, L., Rampon, C., Dahan, L. - *Epileptiform events in Tg2576 mice do not worsen with age but with sleep depth: involvement of noradrenergic transmission*. Poster at AD/PD 2022, Barcelona, Spain.

**B. Szabo, A.** *Etude de l'activité épileptiforme dans la maladie d'Alzheimer*. Flash talk at the conference GDR Mémoire 2021, Vers, France

**Szabo, A.** (2014). *Role and movement quality imagery as a facilitator of flow for dancers*. Presentation at the *21st International Student Congress on Sport Sciences*, 10-12 February 2014, Budapest, Hungary

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## Published Abstracts

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**B. Szabo, A.**, Dahan, L., Curot, J., Pariente, J., Debs, R., Denuelle, M., & Valton, L. (2022). *Maladie d'Alzheimer et épilepsie: des liens physiopathologiques?* *Revue Neurologique*, 178, S152.

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## Teaching and Mentoring

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**2021/2022:** Master's internship supervision (5 months): Amel Bouloufa – A translational study of sleep microarchitecture dysfunction by epileptic activities in Alzheimer's disease

**2019/2020, 2020/2021, 2021/2022:** Teaching at Université Toulouse 2 - Jean Jaurès : Psychophysiology, Psychology BA - 1<sup>st</sup> year (98 hours in total)

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## Science communication

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**2015 – present:** Scientific Journalist for an online Psychology journal (~100 articles on Neuroscience, Psychology and Health, articles accessible (in Hungarian)): <https://pszichoforyou.hu/author/szaboa/>

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## Abstract

With more than 30 million people diagnosed worldwide, Alzheimer's disease (AD) is the most frequent neurodegenerative disorder. Previous research on murine models of AD highlighted the potential role of cerebral excitatory-inhibitory imbalance in the pathophysiology of AD. Clinical research has since reported that it may give rise to epileptic activities in as much as 50% of AD patients. However, these epileptic events may remain undetected due to their silent, mostly sleep-occurring nature.

Therefore, the first part of this work corresponded to a preclinical study in which we aimed at (I) characterizing epileptic activities over the sleep-wake cycle in the Tg2576 mouse model and (II) gaining better insight into the mechanistic underpinnings of these peculiar epileptic events. Tg2576 mice displayed frequent epileptic activities that were predominant during REM sleep and highly locked to the phase of the theta cycle corresponding to the maximal firing probability of pyramidal cells, hinting at a potential deficit of inhibitory activity leading to hyperexcitability – which is coherent with previous results in our laboratory. Secondly, this epileptic activity seems to be prevented during wakefulness - and partially during slow-wave sleep - by noradrenergic signalling via  $\alpha 1$  adrenoreceptors. In contrast, EA would be unmasked during REM sleep when noradrenergic cells cease firing. Given the early degeneration of the locus coeruleus – the primary source of noradrenaline in the brain – in AD patients, this could also have important clinical implications.

In the second part of this thesis, the objective was to explore the prevalence and the distribution of epileptic activities during sleep in a cohort of 30 AD patients compared to a group of age-matched, healthy controls and to understand the impact of these activities on cognitive decline and sleep-related memory consolidation. To this end, a full-night video-EEG combined with polysomnography was undertaken, together with a two-part neuropsychological test battery with pre-and post-sleep memory tests included. These measures were combined with brain imaging (MRI), genetic analyses (only for AD patients), and the evaluation of lifestyle-related factors to evaluate, with the strict minimum of biasing factors, the impact of epileptic activities on AD progression. Preliminary results with 25 participants per group are reported here. Our results hint at a risk ratio for EA occurrence five times higher in patients than controls. In contrast to our preclinical results, epileptic events are predominant during non-REM sleep and especially during deeper (N2 and N3) stages. We also report increased percentages of superficial sleep in patients at the detriment of deeper ones. Coherently, slight alterations of the sleep-microarchitecture, and namely, of sleep spindle quantities that are otherwise the most pronounced during N2 sleep, are observed in the patient group. Notably, the prevalence of sleep apnoea syndrome is unexpectedly high: whilst still higher in AD patients (63%) than in controls (50%), concerns at least half of the participants in both groups. While our sample size is currently insufficient to draw steady conclusions from our results, our preliminary models suggest a combined impact of sleep quantity, sleep apnoea, epileptic events and AD itself on memory consolidation, especially on episodic memory. We suggest that treating EAs, sleep apnoea and improving sleep quality could potentially slow down or lessen memory complaints in AD patients.

## Résumé de la thèse

Avec plus de 30 million de personnes touchées, la maladie d'Alzheimer (MA) est la démence la plus répandue dans le monde. Des recherches sur des modèles murins de la MA ont montré que le déséquilibre entre excitation et inhibition cérébrales provoque des activités épileptiques qui pourraient jouer un rôle la physiopathologie de cette maladie. Des études cliniques ont depuis montré que des activités épileptiques peuvent également être présentes jusqu'à chez 50% des patients atteints de MA, mais qu'elles peuvent passer inaperçues en raison de leur nature silencieuse et de leur survenue essentiellement nocturne.

Par conséquent, la première partie de ce travail correspond à une étude préclinique dans laquelle nous avons cherché à caractériser (I) ces activités épileptiques au cours du cycle veille-sommeil chez la souris Tg2576 (modèle de la MA) tout au long de la vie et (II) ses mécanismes sous-jacents. Nous avons observé des activités épileptiques uniquement pendant le sommeil, et particulièrement pendant le sommeil paradoxal (SP). De plus, ces activités épileptiques surviennent sur la phase du rythme thêta du SP où les cellules pyramidales ont le plus de probabilité de déclencher des potentiels d'action, ce qui suggère une sous-inhibition potentielle – conformément à des résultats antérieurs de notre laboratoire. Finalement, ces activités épileptiques semblent contrebalancées par la noradrénaline via les récepteurs de type  $\alpha 1$ -AR pendant l'éveil et en partie pendant le sommeil lent, et seraient démasquées durant le SP quand les cellules noradrénergiques cessent leur activité. Étant donné l'atteinte précoce des neurones noradrénergiques chez les patients atteints de MA, cela pourrait également avoir des implications cliniques importantes.

Dans la deuxième partie, les objectifs étaient de (I) caractériser la prévalence et la distribution des activités épileptiques au cours des différents stades du sommeil chez 30 patients atteints de la MA, en comparaison avec une population de contrôles appariés et de (II) comprendre leur impact sur le déclin cognitif et sur la consolidation mnésique. A cette fin les participants ont subi un examen vidéo-EEG avec détection des activités épileptiques et polysomnographie, un examen neuropsychologique, avec des tests de mémoire réalisés à la fois avant et après la nuit de sommeil. Ces mesures ont été complétées par une IRM cérébrale, une analyse génétique (chez les patients), et une évaluation des facteurs liés au mode de vie afin d'évaluer, avec le moins de biais possible, l'impact des activités épileptiques sur la MA.

Les résultats préliminaires avec 25 participants par groupe montrent un risque de survenue des activités épileptiques 5 fois plus élevé chez les patients. Contrairement aux souris, les patients présentent des activités épileptiques surtout pendant le sommeil lent, pendant les stades plus profonds. De plus, la proportion de sommeil superficiel chez les patients est augmentée au détriment du sommeil profond, avec des altérations mineures de la microarchitecture du sommeil, notamment une diminution du nombre des fuseaux du sommeil durant le stade N2. Par ailleurs, la prévalence du syndrome d'apnée du sommeil est étonnamment élevée dans notre échantillon : 50% chez les participants contrôles, et encore plus (63% ) chez les patients. Bien que la taille de notre échantillon soit actuellement insuffisante pour tirer des conclusions définitives de nos résultats préliminaires, ceux-ci suggèrent un impact composite de la quantité du sommeil, des anomalies respiratoires, des AEs et de la MA elle-même sur les déficits de la consolidation mnésique en particulier en mémoire épisodique. Ainsi, la recherche et le traitement des activités épileptiques et des apnées du sommeil ainsi que l'amélioration de la qualité du sommeil pourrait potentiellement ralentir le déclin des fonctions mnésiques chez les patients atteints de la MA.

## ABBREVIATIONS

A $\beta$	Amyloid $\beta$ peptide
ACh	Acetylcholine
AD	Alzheimer's Disease
AHI	Apnoea-Hypopnea Index
AICD	Amyloid precursor protein IntraCellular Domain
ALF	Accelerated Long-term Forgetting
(a)MCI	(Amnestic) Mild Cognitive Impairment
AMPA	$\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
APO- $\epsilon$	Apolipoprotein $\epsilon$
APP	Amyloid Protein Precursor
ASM	AntiSeizure Medication
BACE-1	Beta-site APP Cleaving Enzyme-1
BECTS	Benign rolandic Epilepsy with CentroTemporal Spikes
CA	Cornu Ammonis
CNS	Central Nervous System
CPAP	Continuous Positive Airway pressure
CRIq	Cognitive Reserve Inventory questionnaire
CSA	Central Sleep Apneas
CSF	CerebroSpinal Fluid
CSWS	Continuous Spike-Wave of Sleep
DA	Dopamine
dDpME	dorsal Deep MEsencephalic reticular nuclei
DG	Dentate Gyrus
EA	Epileptic Activities
EAS	Epileptic Amnestic Syndrome
EEG	ElectroencEphaloGram/ElectroEncephaloGraphy

EMG	Electromyogram
EOG	Electrooculogram
EPSP	Excitatory Post-Synaptic Current
FAD	Familial Alzheimer's Disease
(f)MRI	(functional) Magnetic Resonance Imagery
fNART	French National Adult Reading Test
FO	Foramen Ovale
(n)FSI	(non)-Fast-Spiking Interneuron
GABA	Gamma-Aminobutyric Acid
GSK-3 $\beta$	Glycogen synthase kinase 3
Hz	Hertz
IED	Interictal Epileptic Discharge
ILAE	International League Against Epilepsy
IPSP	Inhibitory Post-Synaptic Currents
IWG	International Working Group
LC	Locus Coeruleus
LDT	Laterodorsal Tegmental Nuclei
LOAD	Late-Onset Alzheimer's Disease
MAPK	Mitogen-Activated Protein Kinase
MMP	Matrix MetalloProtease
MMSE	Mini-Mental State Examination
MRI	Magnetic Resonance Imagery
MTL	Mesial Temporal Lobe
mTLE	Mesial Temporal Lobe Epilepsy
MSA	Mixed Sleep Apnea
NA	Noradrenaline
NFT	Neurofibrillary Tangle
NMDA	N-methyl-D-aspartate

NREM sleep	Non-Rapid Eye Movement sleep
OSA	Obstructive Sleep apnea
PDS	Paroxysmal Depolarization Shift
PNN	PeriNeural Net
PPT	Pedunculo-pontine Nucleus
PS	Presenilin
PV+	Parvalbumin-positive
PVBC	Parvalbumin-positive Basket Cells
REM sleep	Rapid Eye Movement sleep
sAPP $\alpha$	Soluble APP fragment
SAS	Sleep Apnoea Syndrome
SLD	Sublaterodorsal Nucleus
SO	Slow Oscillation
SOM	Somatostatin
SSS	Sporadic Sleep Spikes
SW-R	Sharp-Wave Ripple
TIRDA	Temporal Intermittent Rhythmic Delta Activity
V	Volt
VEEG-PSG	Video-EEG with Polysomnography
VIP	Vasoactive Intestinal Peptide
VIPAG	Ventrolateral PeriAquaductal Gray
WT	Wild Type

*“You dig deeper and it gets more and more complicated, and you get confused, and it's tricky and it's hard, but... It is beautiful.”*

*//Brian Cox//*

# **INTRODUCTION**

# **CHAPTER 1 – ALZHEIMER’S DISEASE**

## **1.1 The discovery and the characterization of the disease**

In 1906, at the 37th Conference of South-West German Psychiatrists in Tübingen, Dr Alois Alzheimer presented the case of a patient of his, Auguste Deter (often mentioned as Auguste D. in the literature), a middle-aged woman admitted at 51 years of age to the Frankfurt Psychiatric Hospital in November 1901. Upon her admission, the patient’s husband described the progressive decline of his wife’s mental state, starting with personality changes and paranoid ideations that later expanded to sleep disturbances, rapid memory decline and bursts of aggression or episodes of confusion (Hippius & Neundörfer, 2003). The transcripts from the notes of Dr Alzheimer from 1901 (translated by Maurer et al., 1997) reveal an extensive and advanced cognitive decline, as can be seen even from this short excerpt from November 29<sup>th</sup>, 1901:

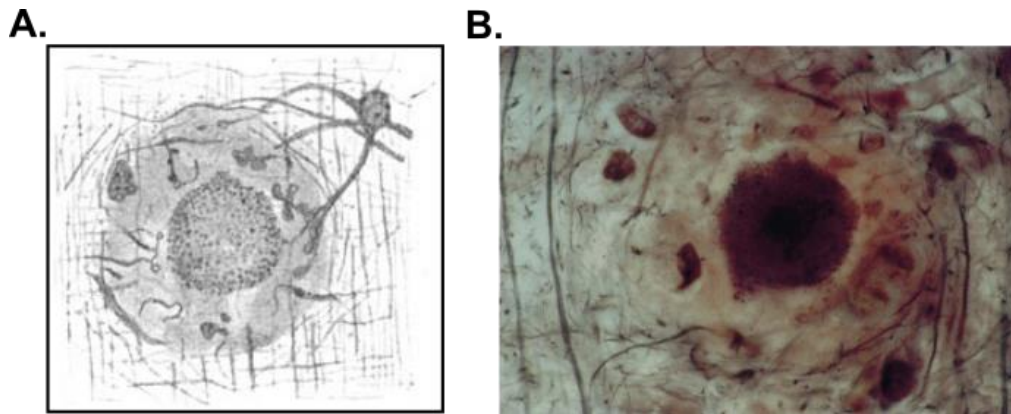
- *“What year is it?*
- *Eighteen hundred.*
- *Are you ill?*
- *Second month.*
- *What are the names of the patients? She answers quickly and correctly.*
- *What month is it now? The 11th.*
- *What is the name of the 11th month?*
- *The last one, if not the last one.”*

Dr Alzheimer could not follow the case of Frau Deter for long as he moved to Tübingen in 1902 to work with Emil Kraepelin, one of the most distinguished psychiatrists in Germany who later coined the term “Alzheimer’s disease” in the 8<sup>th</sup> edition of the Handbook of Psychiatry (Kraepelin, 1910; cited in Cipriani et al., 2011). However, upon the passing of Frau Deter in 1906 due to sepsis, the medical records, as well as brain samples, were sent to Dr Alzheimer to Munich, where Kraepelin’s lab, together with Dr Alzheimer, was moved in 1903 (Hippius & Neundörfer, 2003). Using cutting-edge staining methods of his era, he described the two types of aggregates that we still consider the main pathophysiological hallmarks and the roots of the progression of Alzheimer’s disease (AD): extracellular amyloid plaques (A $\beta$  plaques, **Figure**



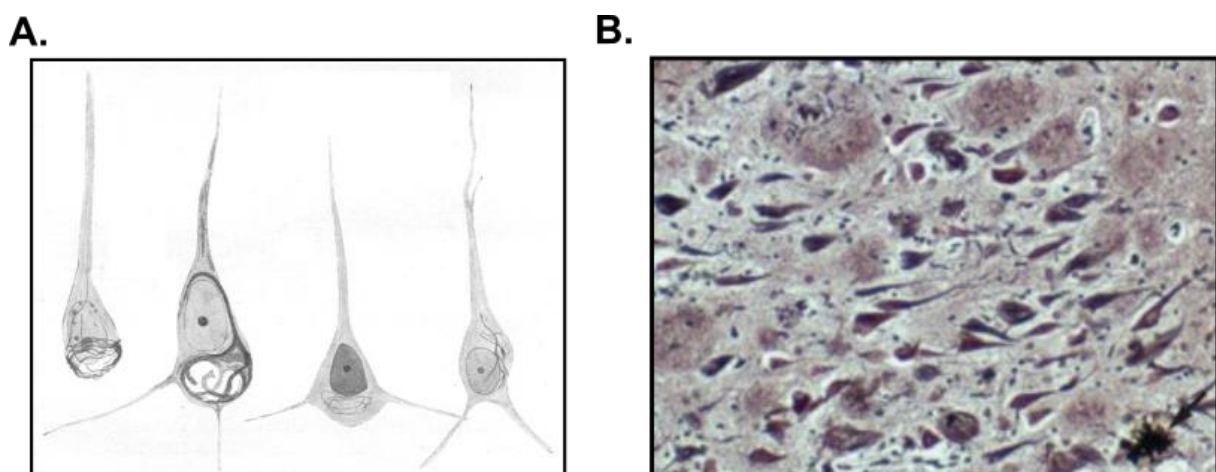
**1A, B)** and intracellular neurofibrillary tangles (NFT, **Figure 2A, B**). His description of those can be read in his publication of 1907 (Alzheimer, 1906; translated by Stelzmann et al., 1995):

On what we consider today as amyloid plaques, he noted: *“Distributed all over the cortex, but especially numerous in the upper layers, there are minute miliary foci which are caused by the deposition of a special substance in the cortex.”*



**Figure 1: Amyloid depositions in the brains of the first known cases of AD, as drawn by Alois Alzheimer and re-analyzed with Bielschowsky-staining almost a century later. (A)** Drawings of the Bielschowsky-stained tissue from sections of one of the latter cases (Johann F.) described by Dr Alzheimer, containing senile plaques but devoid of neurofibrillary tangles, published by Alois Alzheimer (1911). **(B)** Bielschowsky-stained tissue from Johann F’s brain, re-analyzed and presented by Graeber et al. in (1997).

When describing neurofibrillary tangles, he wrote: *“Inside of a cell which appears to be quite normal, one or several fibrils can be distinguished by their unique thickness and capacity for impregnation. Further examination shows many fibrils located next to each other which have been changed in the same way.”*



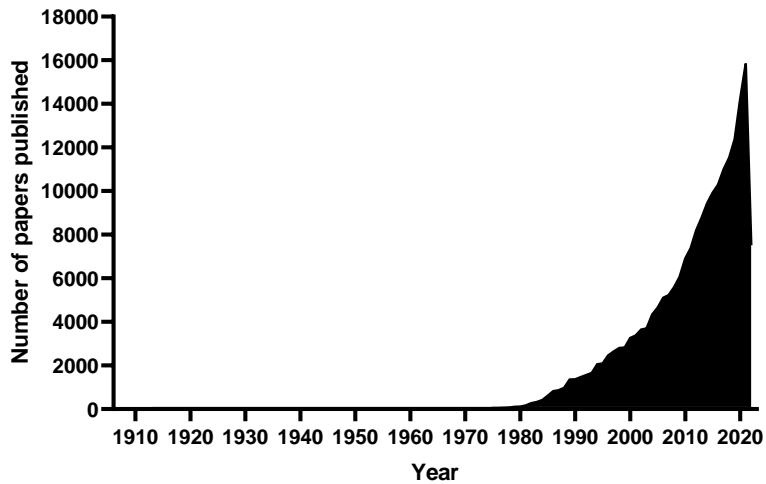
**Figure 2: Neurofibrillary tangles in the brain of the first known case of AD, as drawn by Alois Alzheimer and another example from a different sample using Bielschowsky-staining. (A)** Drawings of neurofibrillary tangles made by Alois Alzheimer, depicting the tangles he observed upon autopsy of the brain of Auguste D. using Bielschowsky staining **(B)** Silver staining evidencing neurofibrillary tangles in cortical tissue (black arrow) from Kumfor, Halliday and Piguet (2017)

These pieces of information were part of his presentation at the conference in Tübingen. Unfortunately, this presentation did not get much attention or comments, as the public was more eager to hear the following talk on compulsive masturbation (Cipriani et al., 2011). In defence of the audience, at that time, they could not have been sure which of the two presentations' topics would become a severe public health issue and one of the leading causes of death in the not-so-distant future. However, Dr Alzheimer's presentation could certainly not go unnoticed in our era as AD, the leading cause of dementia, has a colossal impact on society and the individuals who compose it. Approximately 43.8 million people are living with dementia around the globe (Nichols et al., 2019), of whom 70% are estimated to suffer from Alzheimer's disease (Reitz et al., 2011). Apart from the obvious personal and societal burden of this insidious condition, the weight it imposes on the economy is also exorbitant: the global cost of dementia was estimated at US \$818 billion in 2015 (Wimo et al., 2017), of which around 70% (\$572.6 billion) could be attributable to AD. Predictions state that the global cost of AD will reach 2 trillion US dollars by 2030, which equals approximately 3.3% of the global GDP in 2018 (as assessed by the OECD in 2019).

Therefore, it is not surprising that AD has been one of the leading research topics in Neuroscience over the past few decades. This statement is supported by a search on the US National Library of Medicine – National Institutes of Health<sup>1</sup> for the term “Alzheimer's disease” in June 2022 that returns an astonishing result of 185 521 publications that seem to follow a fast-growing rhythm (11 publications per year in 1970, 15 800 in 2020, **Figure 3**). These papers all contributed to a better understanding of the molecular mechanisms underlying the pathophysiology of AD and the genetic and lifestyle-related risk factors that can lead to its onset. However, even though more and more promising results are published about potential treatments in animal models (e.g. Mohajeri, 2002; Roberson et al., 2007a) and clinical trials of more than 200 compounds have been undertaken (for a review, see Hung & Fu, 2017) it seems that Alzheimer's disease has not met its pharmacological vanquisher. Therefore, depending on the patient's age at disease onset and several potential confounding factors discussed below, AD leads to death in approximately 3.5 to 9 years following diagnosis (Brookmeyer et al., 2002). So what did we learn from Dr Alzheimer's work, and what have we learnt about AD since 1906?

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<sup>1</sup> <https://pubmed.ncbi.nlm.nih.gov>



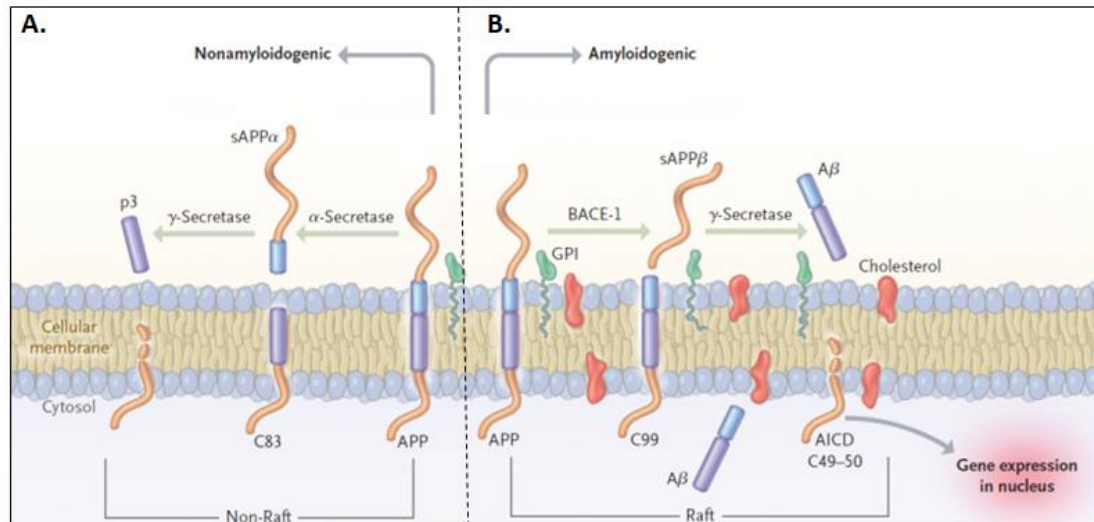
*Figure 3: The number of publications per year on the topic of Alzheimer’s disease, as returned by a search on the US National Library of Medicine – National Institutes of Health on June 9, 2022. The jaw-dropping acceleration of AD research is well-illustrated by the fact that while only 11 articles appeared in 1970 containing the term “Alzheimer’s disease”, an astonishing 15800 publications were published in 2020 alone.*

## 1.2. The road to diagnosis: biomarkers, cognitive decline and emerging views

### *1.2.1 Beta-amyloid*

Even though the cases described by Dr Alzheimer were rapidly progressing early onset cases, a century of clinical observations and scientific results showed that the main biomarkers of AD start to accumulate years or even decades before the first mild symptoms sneak into the patients’ everyday life. As mentioned above, Dr Alzheimer described two main types of aggregates in the brain of Auguste Deter that we consider even today as the main biomarkers of AD. The first of these belongs to the extracellular deposits called amyloid plaques. These were later shown to be composed of the peptide  $\beta$ -amyloid ( $A\beta$ ). This peptide is derived from the Amyloid Precursor Protein (APP) that can follow two distinct pathways, both consisting of successive cleavages by different secretases (**Figure 4**). Under physiological conditions leading to non-accumulation-prone fragments (*nonamyloidogenic pathway*, **Figure 4 A**), the first cleavage is undertaken by the  $\alpha$ -secretase enzyme, which leads to the generation of soluble APP fragment ( $sAPP\alpha$ ) as well as a C-terminal fragment of 83 amino acids. The latter is further cleaved by another enzyme called  $\gamma$ -secretase, resulting in a p3 fragment and the amyloid intracellular domain (or AICD, a nucleus-targeted fragment that acts as a transcriptional

activator intervening in the expression of  $\text{Ca}^{2+}$ -signaling-related genes (Leissring et al., 2002)). This pathway not only implies the generation of non-aggregating fragments but the sAPP $\alpha$  has been suggested to be solely responsible for the beneficial physiological functions of APP, such as in the regulation of circadian activity and exploratory behaviour as well as in spatial learning and long-term potentiation (Ring et al., 2007).

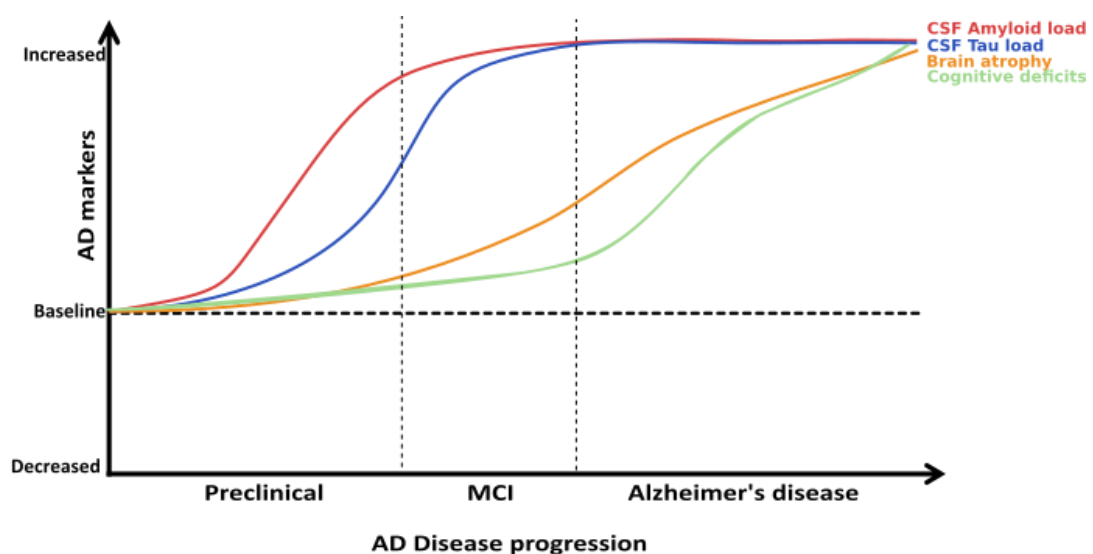


**Figure 4. Nonamyloidogenic and amyloidogenic cleavage of the APP protein.** (A) The successive cleavages of the APP protein by the  $\alpha$ -then  $\gamma$ -secretases lead to the generation of non-pathologic fragments: the extracellular sAPP $\alpha$  and p3, as well as the intracellular AICD domain. (B) The amyloidogenic pathway is also composed of two successive cleavages, one by the BACE-1 enzyme and another by a  $\gamma$ -secretase, leading to the generation of a nucleus-targeted AICD domain and an A $\beta$  fragment of 36-43 amino acids. Adapted from Querfurth and LaFerla (2010).

However, the resulting fragments of the amyloidogenic pathway (**Figure 4 B**) are undoubtedly different. Here, the first cleavage is done by the so-called BACE-1 enzyme ( $\beta$ -secretase beta-site amyloid precursor protein-cleaving enzyme 1), creating a sAPP $\beta$  fragment and a 99 amino acid-long C-terminal fragment. Once again, it is a  $\gamma$ -secretase that intervenes to further cleave the C-terminal fragment, but this time it results in the generation of an A $\beta$  fragment of varying length and an AICD. The most frequent A $\beta$  peptides generated via this pathway consist of 36 to 43 amino acids, but the two main forms associated with AD research are the less aggregation-prone A $\beta_{40}$  and the rapidly aggregating A $\beta_{42}$  (Querfurth & LaFerla, 2010). Interestingly, the length of the generated A $\beta$  fragment differs as the  $\gamma$ -secretase activity seems to contain several successive steps that progressively shorten the resulting fragment (in Selkoe & Hardy, 2016). In case of a dysfunction of the enzyme complex (such as in the genetic AD cases discussed below), these successive steps are halted before the completion of the process, leading to longer, more aggregation-prone cleavage products. Then, the aggregation-

prone A $\beta$  monomers first cluster into dimers, followed by larger oligomers and even more impressive protofibrils, forming fibrils that will further aggregate to form A $\beta$ -plaques (Hampel et al., 2021).

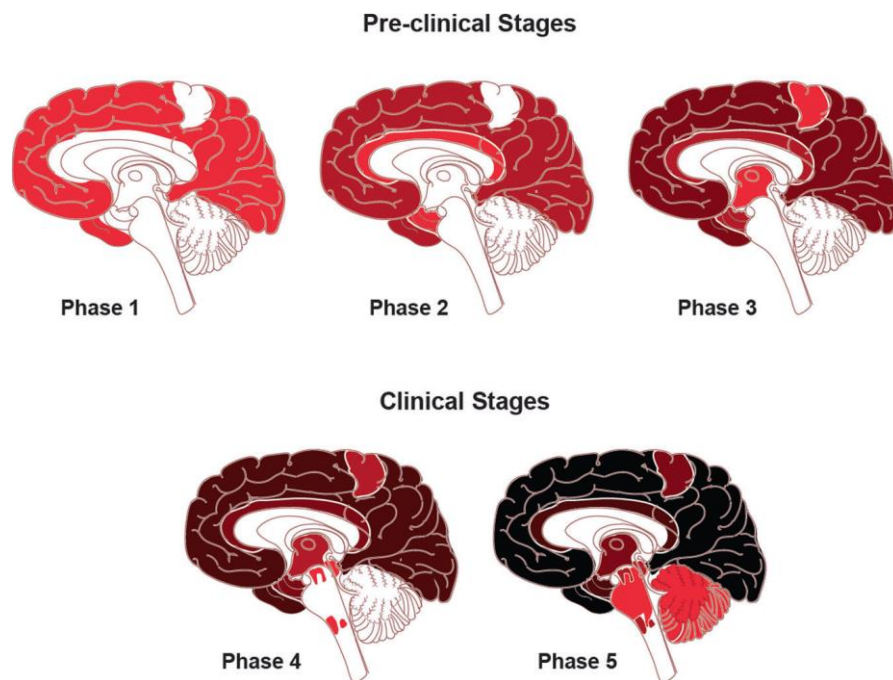
A $\beta$  accumulation has been in the spotlight of AD research as the first influential theory on its pathophysiological underpinnings, the so-called *Amyloid-cascade hypothesis*, posited that this is the central event from which AD derives (Hardy & Allsop, 1991; Selkoe, 1991). This theory stated that all other abnormalities in AD are the consequences of amyloid accumulation and strongly recommended designing pharmacological compounds that could put a stop to its accumulation. This theory was based on several pillars. First, even though most AD cases (>95%) are so-called sporadic ones with no clear-cut genetic mutation, the remaining fraction of patients are diagnosed with familial AD, which is generally associated with earlier onset and steeper cognitive decline. This form is due to mutations transmitted in an autosomal dominant manner and related to APP processing. Indeed, these mutations are either found on the gene coding for the APP protein itself (*APP*) (St George-Hyslop et al., 1987) or on the genes coding for Presenilin-1 and Presenilin-2 (*PS1*, *PS2*) that are the catalytic elements of the  $\gamma$ -secretase enzyme and are found on chromosomes 1 and 14, respectively (Levy-Lahad et al., 1995; St George-Hyslop et al., 1992). Furthermore, a missense mutation on the APP gene (A673T) has been shown to induce a reduction in APP cleavage by the BACE-1 enzyme (Jonsson et al., 2012), leading to less aggregation-prone APP cleavage products and an impressively decreased risk of AD (reviewed in Selkoe & Hardy, 2016).



**Figure 5. Proposed timeline of biomarker- and symptom progression of AD.** It is generally accepted that amyloid deposition largely precedes Tau accumulation and the subsequent neuronal death resulting in brain atrophy, and happens years or decades before the first clinical symptoms can be observed or perceived by the patients. Adapted from B. Szabo et al. (2022)



Moreover, AD is often observed in Down Syndrome, a pathology due to the trisomy of chromosome 21 on which the *APP* gene happens to be located, leading to abnormally increased A $\beta$  generation throughout the lifespan (Menéndez, 2005). Finally, A $\beta$  accumulation starts years or even decades before the first clinical symptoms and is considered the most upstream pathological process in AD. This accumulation is such that by the time the first clinical symptoms appear, and a diagnosis of either Mild Cognitive Impairment (MCI) or AD can be posited, the amyloid load measured in the cerebrospinal fluid (CSF) and used as a proxy of brain A $\beta$  accumulation in clinical settings has virtually reached its maximum (**Figure 5**). The spatial distribution and progression of this deposition are well defined. They are first restrained to the neocortex before expanding towards subcortical areas, eventually reaching the midbrain and, last, the cerebellum and the brainstem (**Figure 6**).



**Figure 6. Topographical progression of amyloid deposition in the brain**, from preclinical stages (upper row) to clinical ones (bottom row). While amyloid deposition is restricted to cortical regions in the earliest stages, it later spreads towards midbrain areas before reaching the brainstem and the cerebellum. Lighter shades indicate starting accumulation, and continued deposition in later phases in the same areas is illustrated by darker and darker shades of red (eventually black). Figure in Hampel et al. (2021), adapted from Thal et al. (2002).

Still, several concerns have arisen concerning the central role of amyloid deposition in AD over the past decades (Hardy, 2002; Selkoe & Hardy, 2016). The first one is the fact that even though hundreds of drugs were tested to target amyloid accumulation (targeting BACE-1 or  $\gamma$ -secretase activity, or immunotherapies removing aggregating forms of A $\beta$ ), these often fail to meet their clinical endpoints due to an absence of impact on the cognitive decline despite the

decrease in plaque load (see Hung & Fu, 2017; Roberson & Mucke, 2006 for reviews on these drugs). The only A $\beta$ -targeting drug approved by the US Food and Drug Administration agency, aducanumab (Sevigny et al., 2016), is currently the cradle of controversies (Howard & Liu, 2020) as the effect size of the differences observed were only minor and, according to a new analysis (Knopman et al., 2021), not forcedly due to biomarker changes. The other sore point of the amyloid-cascade hypothesis is that the quantity of amyloid plaques does not show a suitable correlation with the level of cognitive impairment (Hardy, 2002). However, recent data suggest that this does not mean that A $\beta$  is unrelated to mnesic complaints. Instead, it is being more and more accepted that it is not only the non-soluble plaques but the smaller, soluble forms of the peptide as well that are highly neurotoxic, and their quantities show a better correlation with cognitive decline (reviewed in Haass & Selkoe, 2007; but see McLean et al., 1999; and Mucke et al., 2000 for specific clinical and preclinical examples, respectively). Therefore, the amyloid-cascade hypothesis is still of significant interest as A $\beta$ , and its early accumulation seems to be the most common denominator across patients. However, the multifactorial nature (and potentially, origin) of AD should not be neglected.

### *1.2.2. Neurofibrillary tangles*

The second major type of aggregates, described by Dr Alzheimer and still considered as one of the biomarkers for the diagnosis of AD is that of the neurofibrillary tangles (NFTs). These intracellular inclusions are composed of the hyperphosphorylated form of the microtubule-associated protein Tau, as was shown by Kosik et al. (1986). Under physiological conditions, Tau confers stability to microtubules, which is essential for cell structure integrity. In this case, Tau is phosphorylated (to a non-pathological level) at specific sites by the glycogen synthase kinase 3 (GSK-3 $\beta$ ), the cyclin-dependent kinase (cdk5) or the mitogen-activated protein kinase (MAPK), amongst others (Querfurth & LaFerla, 2010). However, an increase in kinase activity or, on the contrary, a reduction of phosphatase activity can induce hyperphosphorylation, generally around regions flanking the microtubule binding domain (Querfurth & LaFerla, 2010). This leads to a loss of affinity of Tau for the microtubules. This means, first of all, a destabilization of the structural integrity of microtubules but, on top of that, this hyperphosphorylated, insoluble form clusters into paired-helical filaments that self-aggregate

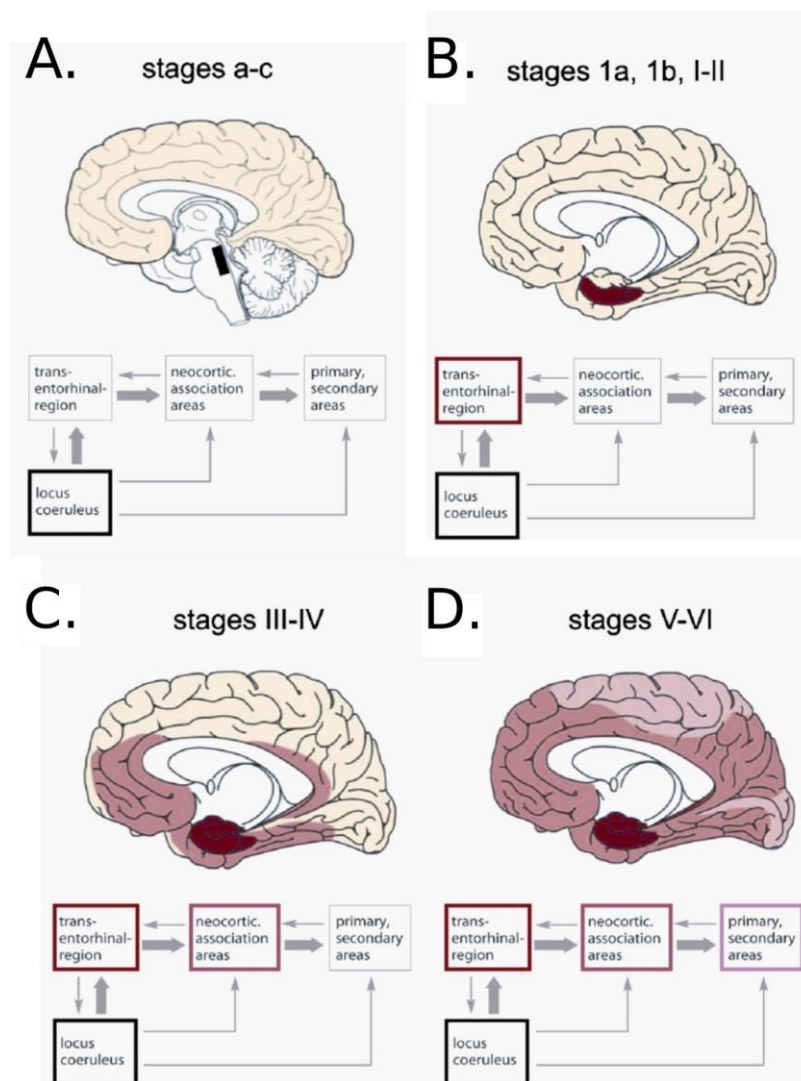
inside the pyramidal cells into neurofibrillary tangles. The importance of Tau has been highlighted, amongst many others, in an elegant preclinical study that showed that a reduction in endogenous Tau could rescue behavioural deficits and lead to dampened neurodegeneration even if A $\beta$  levels are left unchanged (Roberson et al., 2007), which opens the door to important potential therapeutic pathways. Tau pathology also largely precedes the onset of cognitive symptoms (**Figure 5**) and is indeed generally well-correlated with atrophy and cognitive decline (Arriagada et al., 1992), lending further support to future therapeutic implications involving Tau. It is of note, however, that the correlation between Tau pathology and cognition is the strongest when it is concomitantly present with amyloid pathology, suggesting a synergistic effect of the two types of aggregates and their oligomers (reviewed by Busche & Hyman, 2020). This is further underlined by the work of Ittner, Ke and collaborators (2010), revealing that Tau is required for the excitotoxic effects of A $\beta$  by its impacts on N-methyl-D-aspartate receptor (NMDAR) stability.

Despite their apparent synergistic effect, there seems to be an initial spatial decoupling between the two types of aggregates. Indeed, contrary to A $\beta$ , NFTs are first observed in the cluster of brainstem nuclei considered as the centre of noradrenergic innervation in the brain, the *locus coeruleus* (LC), followed by temporal-lobe structures, before spreading to associative areas and finally reaching primary and secondary cortical regions (**Figure 7**). However, as amyloid accelerates Tau seeding in the brain (He et al., 2018; Lodder et al., 2021; Vergara et al., 2019) even in mouse models only possessing mutations affecting amyloid deposition (Maia et al., 2013), Tau pathology eventually spreads to cortical areas where amyloid plaques are already present in large quantities. These deposits lead to cytotoxic effects that ultimately result in massive neuronal death explaining the progressive thinning of cortical areas and a general atrophy of even subcortical structures.

The implication of these two aggregates is undoubted, and it is no wonder they are part of the diagnostic criteria for AD. Along with an augmentation of tracer retention on amyloid positron emission tomography (PET) or with marked medial temporal lobe atrophy (or more posterior in the case of early-onset AD) on magnetic resonance imaging (MRI), the decreased A $\beta_{1-42}$  load with concomitant increased Tau levels in the cerebrospinal fluid (CSF) are considered the most direct AD biomarkers (Dubois et al., 2014). Indeed, given the free moving of proteins from the



brain towards the CSF, we can infer the level of brain amyloid/Tau from the quantities present in the CSF (Reitz et al., 2011).

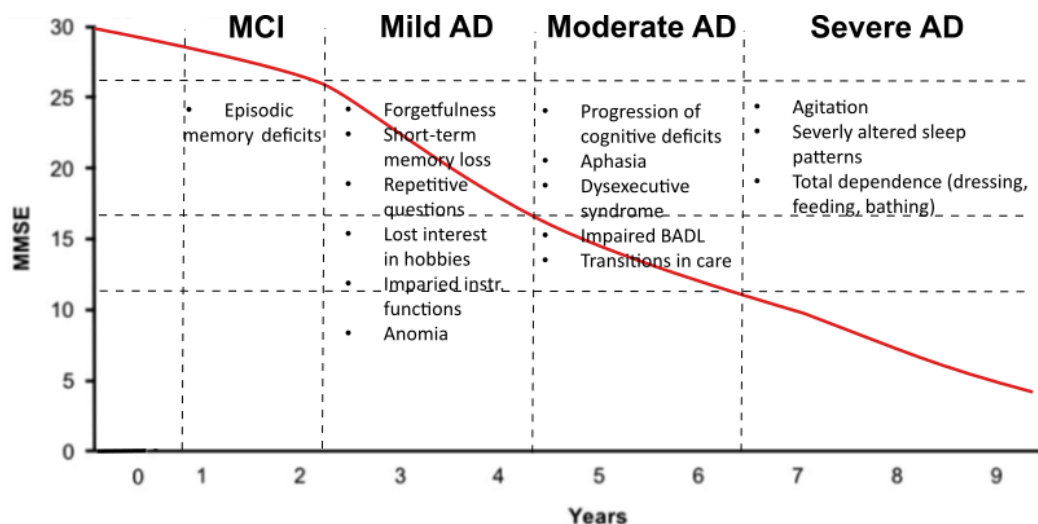


**Figure 7. Neurofibrillary tangle propagation in the brain in function of the currently used Braak staging.** NFTs first appear in the locus coeruleus during stages a-c (A), followed by temporal-lobe structures during stages 1 and I to II (B) before spreading to associative areas in stages III-IV (C) and finally reaching primary and secondary cortical regions in stages V-VI (D). Darker areas illustrate earlier deposition sites, while lighter shade indicates sites where Tau accumulation starts later. From Braak et al. (2011)

### 1.2.3. Cognitive decline

These biomarkers are combined with the cognitive symptoms, many of which overlay with the ones observed by Alois Alzheimer – even though the progression of most cases is generally slower than that of Auguste D, who had the familial form of the disease with a *PS-1* mutation (Müller et al., 2013). In sporadic cases, AD diagnosis is often preceded by a Mild Cognitive

Impairment (MCI) diagnosis if the subjective cognitive symptoms are perceived severe enough by the patient or by their family to seek medical help. Even though the initial change in cognition can affect one or several cognitive domains (executive functions, language, visuospatial perception), most MCI patients who will later progress towards AD report episodic memory deficits as a first symptom (Albert et al., 2011). During the mild stages of AD, this is followed by a progressive decline in cognitive functions, including general forgetfulness, loss of short-term memory, and a gradual start towards apathy. Instrumental functions (including verbal expression and comprehension capacity, visuospatial skills and debuting apraxia) also start their decline. Later on, during the moderate stages, these initial deficits further aggravate and are joined by aphasia and a dysexecutive syndrome (encompassing inhibition deficits, perseverative behaviours and decreased attention) while executing basic activities of daily living imposes more and more difficulties. In many cases, institutionalization occurs at this point, as patients require round-the-clock care and attention. In the severe stages, these symptoms are further aggravated, agitation sets in and sleep patterns are further disrupted – over and above the ones that will be discussed later relating to the earlier stages. In the severe stage, autonomy is no longer preserved, and patients depend on caregivers in every aspect of their lives (including dressing, bathing, etc., **Figure 8**). Over and above these symptoms, stages are often defined by the so-called MMSE-score (standing for Mini-Mental State Examination), a short tool for examining cognitive status with 30 items (Folstein et al., 1975). This tool contains items relating to spatial and temporal orientation, executive and instrumental functions, and short-term memory. A score of 26 or more is generally considered normal/pre-dementia, whereas a mild dementia diagnosis can be posited under that threshold. The moderate stage is considered to start around 17 points, while 10-11 is the hallmark of the severe stage (Feldman & Woodward, 2005).



**Figure 8. The clinical progression of Alzheimer's disease.** An example of typical AD progression in function of symptoms and MMSE scores. Over the course of the disease, patients go through the milder, pre-AD stage called MCI (mainly with episodic memory symptoms) and progress towards more and more important memory deficits as well as executive and instrumental function perturbations. In the severe stage, autonomy is no longer maintained. The time scale may vary from one patient to another. Figure modified after Feldman and Woodward (2005).

It is to be kept in mind that this staging and symptomatology is valid for typical, sporadic cases. As mentioned earlier, FAD (<5% of all AD cases) generally appears earlier and follows a steeper disease course. However, supposedly sporadic AD versions exist that are often early onset (< 65 years of age, accounting for around 6-14% of all AD cases) and present themselves with atypical symptomatology. The IWG-2 criteria, one of the standard diagnostic criteria of AD, distinguishes four atypical AD variants (Dubois et al., 2014). These all have the specificity of relatively spared memory functions and include: (1-2) two posterior variants characterized mainly by early and progressive visuospatial dysfunctions; (3) a logopenic variant defined by early word-retrieval difficulties and repetition of sentences; and (4) a frontal variant with predominant behavioural changes (apathy, disinhibition) or eventually with primary deficits in executive functions. On top of these, a Down syndrome-related variant is also classified as atypical AD, also characterized mainly by behavioural changes and executive function deficits in Down syndrome patients with a chromosome 21 triplication including the segment coding for APP. This symptomatology-based subtyping somewhat echoes the very recent results of Vogel and collaborators (2021). Indeed, based on Tau-PET scans from over 1600 participants, they described four different spatial deposition patterns of NFTs that were associated with specific clinical profiles. These included (1) a limbic-predominant variant with cognitive profiles mainly reproducing typical AD (memory deficits but relatively spared overall

cognition); (2) a mesial-temporal lobe (MTL) sparing variant with younger age of onset and more pronounced executive deficits (similar to the logopenic variant of the IWG-2 criteria); (3) a left temporal lobe-predominant tau deposition pattern for patients with decreased overall cognitive functioning and language scores but relatively spared memory (similar to the logopenic variant with the executive function deficit predominance in the IWG-2). Finally, (4) a posterior tau-deposition pattern was also distinguished that reproduced the visuospatial deficits of the IWG-2 posterior variant and was associated with a later onset. This again underlies the phenotypic variability in AD and the role of Tau in cognitive symptoms. Yet, such variability and differing disease progression rates suggest that there is more to AD than these two protein aggregates.

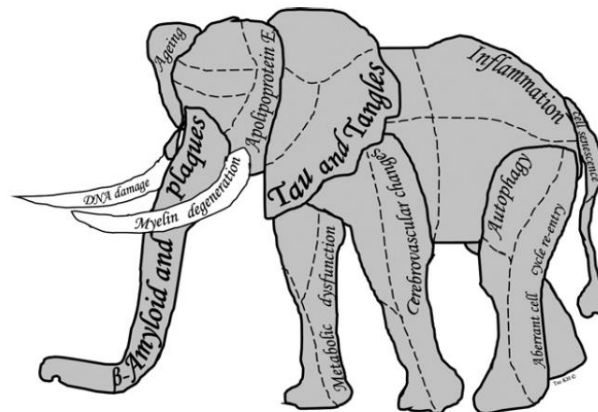
#### *1.2.4. Emerging views on the underlying factors of AD*

Over the years, many risk factors and interactions have been revealed to play a potential role in the onset of sporadic AD, leading to the impression that all roads lead to AD. Thankfully, a few away from it have also been uncovered and can be used as a base for the development of preventive methods. These latter ones include mainly lifestyle factors related to an antioxidant- and omega-3-rich diet, increased levels of aerobic fitness and higher educational levels (detailed in Reitz et al., 2011). As to the risk factors, many of them impact not forcefully the generation of amyloid peptides but rather the clearance mechanisms that would allow the brain to eliminate waste and potentially harmful proteins – such as aggregation-prone forms of Tau and A $\beta$ . This incorporates, amongst others, midlife hypertension, decreasing the integrity of the blood-brain barrier, deficits of the glymphatic system (Iliff et al., 2012) and type-2 diabetes, where insulin may impact amyloid clearance by competing for the insulin-degrading enzyme (Reitz et al., 2011). Hindered A $\beta$  clearance from the brain is also associated with one of the best-known genetic risk factors for developing sporadic AD: the presence of one or two copies of the gene allele coding for the apolipoprotein  $\epsilon$ 4 (APOE). The main role of Apolipoprotein-E in the central nervous system – just as on the periphery – is lipid transport: more specifically, in the central nervous system, it ensures cholesterol transport to neurons (see Belloy et al., 2019). Contrary to its other isoforms, the  $\epsilon$ 2 and  $\epsilon$ 3 alleles, the  $\epsilon$ 4 allele highly increases the risk of AD and decreases the age of its onset. The approximate increase

in AD risk with one  $\epsilon 4$  allele is around 2-4 fold, whereas being a homozygote for the  $\epsilon 4$  allele increases this risk by 8-12 fold. Furthermore, APOE- $\epsilon 4$  carriers are as much as around 12 years younger at disease onset than those without this variant (estimated in the review of Belloy et al., 2019). Even though many controversies remain concerning the mechanisms by which this allele exerts this effect, the most widely reproduced finding states that the  $\epsilon 4$  allele leads to highly increased amyloid deposition and potentially does so by hindering A $\beta$  clearance from the brain (reviewed extensively in Kim et al. (2009)).

However, over and above risk factors – be it genetic or lifestyle-related – that may increase amyloid deposition in the brain, we might consider other competing models that are not based on A $\beta$  yet could contribute to the onset or the progression of AD. Such was the work undertaken recently by Tse and Herrup (2017), who tried to recapitulate these models (**Figure 9**). Their work is based on the premise that amyloid accumulation does not always induce AD. Indeed, amyloid pathology is present in almost half of the population over 90 years without AD (Tse & Herrup, 2017). However, the common denominator of AD – apart from familial cases – is age. Therefore, according to one of the alternative models presented in the article mentioned above, it is also plausible that accumulating DNA damage in cells that occurs naturally with ageing would lead to the progressive breakdown of many signalling pathways and lead to progressive cognitive decline. As another alternative model, they also invoke the potential role of myelin degeneration that is gradually ongoing from the age of thirty and could explain the gradual (not necessarily) pathological slow decline of cognition starting from the peak cognitive performance of the third decade of life. Another main alternative model suggests an aberrant cell-cycle re-entry of neurons (almost exclusively post-mitotic cells having exited the cell cycle during development) potentially induced by DNA damage affecting the checkpoints proteins regulating the cell cycle. This would lead to neuronal death contributing to the brain atrophy and cognitive symptoms accompanying AD. These models – such as other ones that are not detailed here, including cerebrovascular changes, inflammation or metabolic dysfunction – may indeed be significant factors contributing to AD onset and maybe even potentially explaining the differential toxicity of amyloid burden across individuals. The takeaway message of the failures of recent drug trials and the review of Tse and Herrup is that even though A $\beta$  plaques and NFTs are the most visible parts of AD (that we could palpate easily with blindfolds on their “imagery elephant of AD”, **Figure 9**),

complementary pathways towards AD are still underexplored despite their potential impact for treatment.



**Figure 9. Suggested underlying mechanisms behind Alzheimer's disease.** In their 2017 paper, Tse and Herrup imagine AD as an elephant that researchers have been mapping with blindfolds on, leading to an overrepresentation of research and models around the most easily palpable parts, while other, less distinguishable factors have remained largely unexplored but could just as well influence AD onset. Figure from Tse & Herrup (2017)

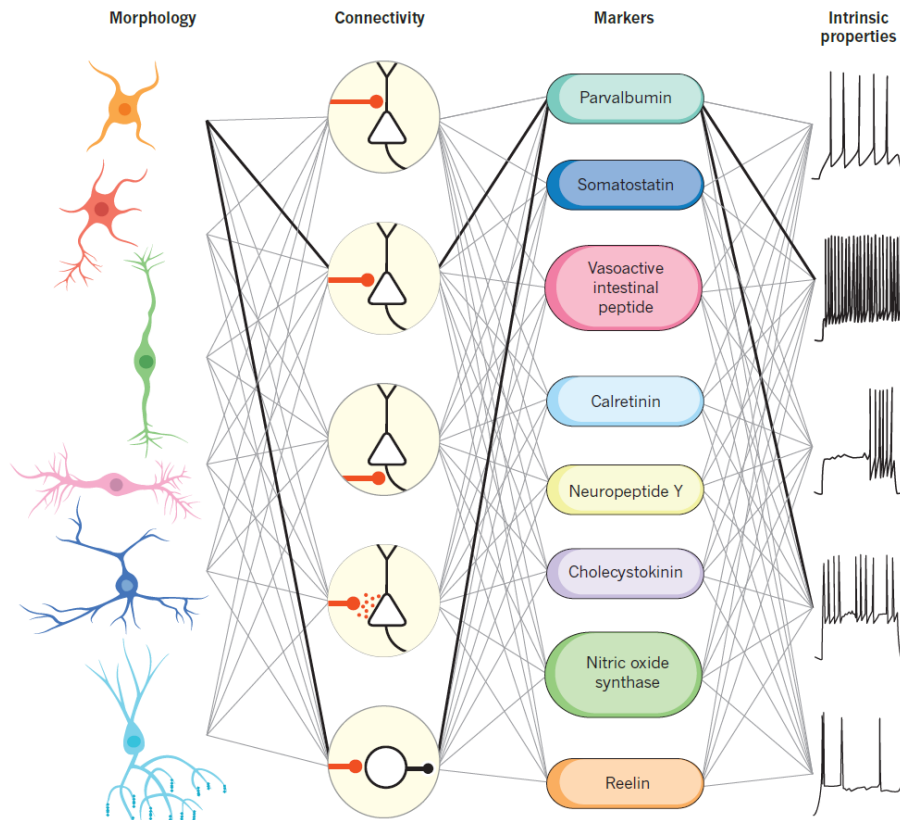
One such pathway that we could also draw on the elephant of Tse and Herrup recapitulating the alternative models, as well as the issue with current approaches to the search for a cure of AD (**Figure 9**), is neuronal hyperexcitability and hyperactivity eventually leading to epileptic activities – a potential mechanism that, as we will see, have been under our nose for more than a century.

## **CHAPTER II – EPILEPSY**

### 2. 1. Balancing neuronal activity

Neuronal activity is essentially a succession of electric impulses generated and propagated by some 86 billion neurons populating the human brain (Herculano-Houzel, 2012). This activity underlies our cognitive functions, thoughts and behaviour; however, only if it is strictly regulated and organized in order to avoid pathologic activities leading to dysfunctions of the system. This regulation is ensured by the precisely synchronized activity of excitatory and inhibitory neurons in the brain. Excitatory neurons represent over two third of all neurons (Bekkers, 2011) and are mostly pyramidal and granule cells. The most common excitatory neurotransmitter in the brain is glutamate, but other, more uncommon ones also contribute to the finely regulated neuronal activity, such as noradrenaline, acetylcholine, dopamine or histamine.

Contrary to this, interneurons that release mainly Gamma-aminobutyric acid (GABA), glycine, or less prevalent inhibitory neurotransmitters such as serotonin, act as the breaks that regulate excitatory activity. Despite representing only a minority of cells in the brain, interneurons are an astonishingly diverse group that we can divide into different subgroups. This diversity is such that the excitatory cells of the CA1 region of the hippocampus alone have been estimated to receive inhibitory input from 16 types of interneurons (Somogyi & Klausberger, 2005). However, in order to appreciate this interneuron diversity, these subtypes need to be differentiated based on a set of criteria such as morphology, molecular markers, connectivity patterns, and intrinsic firing properties (Kepecs & Fishell, 2014, **Figure 10**). Along the morphology axis, we can distinguish, amongst others, chandelier cells, arcade cells, basket cells, neurogliaform cells, horse-tail cells, Martinotti cells and Cajal-Retzius cells (DeFelipe et al., 2013). Connectivity-wise, interneurons can target different domains of the post-synaptic cells, including the axon-initial segment, dendritic domains or the soma itself. As seen in **Figure 10**, the number of markers expressed by the different interneurons is vast, and one subtype can express several of those at once.

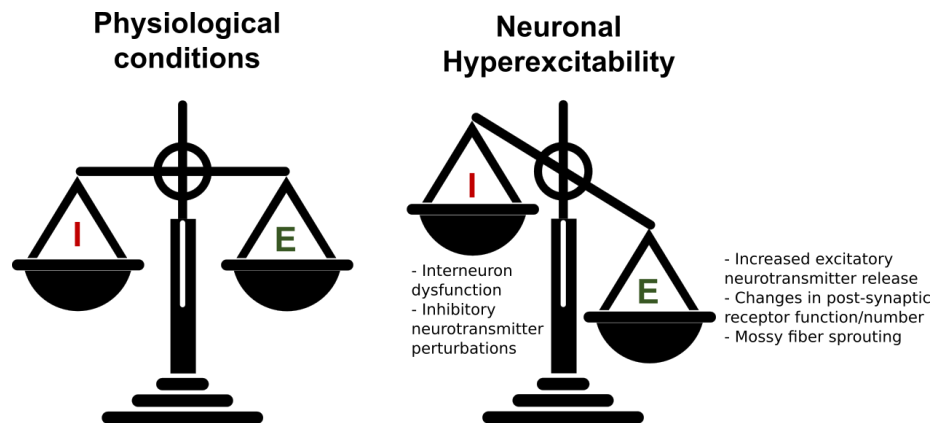


**Figure 10. Suggested dimensions to consider when differentiating interneuron types.** The wide range of interneuronal subtypes makes categorization quite challenging. However, they can be defined based on their main characteristics. This task can be achieved on four main axes: morphology, connectivity (where the interneuronal axon connects to the post-synaptic cell), markers expressed by the interneuron and their intrinsic firing properties. From Kepecs & Fishell (2014).

Amongst the most often mentioned types of interneurons in the field of Alzheimer’s disease, as we will see further down the line, are interneurons expressing Parvalbumin (PV) and, to some extent, Somatostatin (SOM) or the Vasoactive intestinal peptide (VIP). As per spiking properties, the most numerous ones are fast-spiking interneurons (FSI), but we also distinguish low threshold-spiking and non-fast-spiking interneurons. Inhibitory interneurons fulfil a range of functions – some of which will be discussed later – allowing them to control and time neuronal activity with sub-millisecond precision. Finally, if well-functioning and correctly connected to their functional network, interneurons play a pivotal role in preventing neuronal hyperexcitability – while the consequences of their dysfunction are quite severe on the brain and can be further aggravated by dysfunctions in excitatory transmissions (**Figure 11**). Indeed, the desynchronization of interneuronal activity (Shuman et al., 2020) or the loss of interneurons in the hippocampus (de Lanerolle et al., 1989; Powell et al., 2003) have been



suggested to be involved in a neurological disorder with more than 45 million active cases worldwide: epilepsy (Beghi et al., 2019).

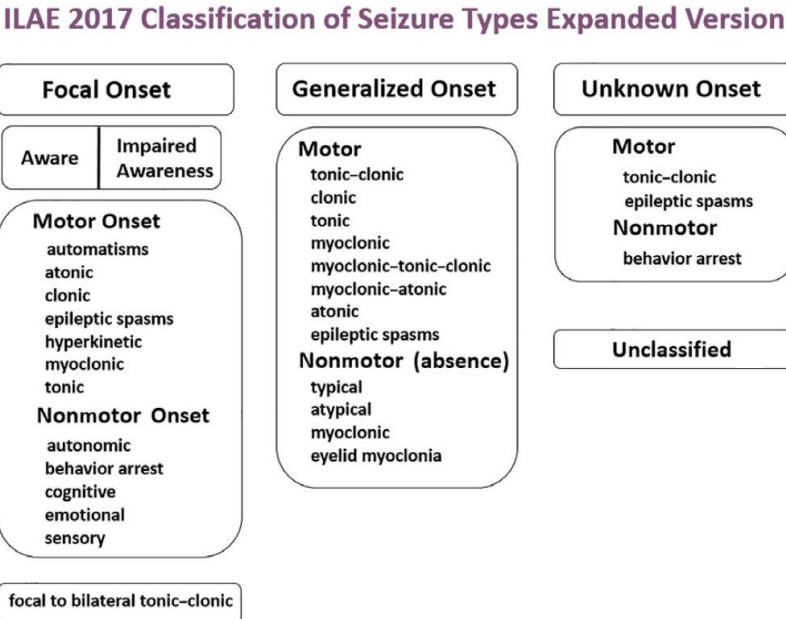


**Figure 11. Schematic illustration of factors leading to neuronal hyperexcitability.** Under physiological conditions, excitatory and inhibitory activities are well-balanced, leading to normal network function. In the case of epilepsy or subclinical neuronal hyperexcitability, the inhibitory function may be decreased (due to interneuron dysfunction or disrupted inhibitory neurotransmitter release), or excitatory function may be increased, for example due to excessive glutamate or other excitatory neurotransmitter release.

## 2.2 Clinical prevalence and classification

The International League Against Epilepsy (ILAE) and the International Bureau of Epilepsy (IBE) define epilepsy as “a disorder of the brain characterized by an enduring predisposition to generate epileptic seizures and by the neurobiological, cognitive, psychological and social consequences of this condition” (Fisher et al., 2005, p. 471). Diagnosis is posited following either two unprovoked seizures with at least 24 hours between them or with a single seizure and an estimated risk of re-occurrence in the next ten years of more than 60% (Fisher et al., 2017). Epilepsy onset can happen at any age, although it is somewhat more frequent in the early years and amongst the elderly (Chen et al., 2012). This suggests a bimodal distribution of the risk of epilepsy-onset, with age groups in-between childhood and older adulthood having a decreased risk. However, epilepsy comes in different shapes and sizes across age groups, with diverse clinical manifestations and underlying neurobiological processes, so it might be more helpful to talk about epilepsy as a broad umbrella term for the many epileptic disorders that exist (Brissart & Maillard, 2018).

In epilepsy, seizures arise from transient excessive excitatory neuronal activity or an abnormal neuronal hypersynchrony in the brain (Fisher et al., 2005). According to the current classification established by the ILAE (Fisher et al., 2017; **Figure 12**), if this aberrant excitatory activity originates from a more extensive, bilateral network, the following seizure is defined as a generalized seizure. Conversely, if it starts in an initially localized, unilateral area (even if it later propagates to other brain regions), it is considered a focal seizure (Brissart & Maillard, 2018). Focal seizures can further be subdivided into focal impaired awareness seizures (or “complex partial seizures” by the previous classification) and focal aware seizures (previously “simple partial seizures”). During the latter, the patients maintain awareness of the environment even if they are not responsive (Fisher et al., 2017). A further classification level also distinguishes between motor-onset (e.g. atonic, clonic, tonic, myoclonic or epileptic spasm) and nonmotor-onset seizures (e.g. cognitive, emotional, sensory or with behavioural arrest) based on their initial sign of onset. For generalised onset seizures, the central axis of categorisation is the motor-nonmotor one. For motor seizures, we distinguish, amongst others, clonic, tonic, atonic, tonic-clonic, myoclonic seizures or epileptic spasms. Finally, for nonmotor generalized seizures, we most often talk about absence seizures. These latter ones are short lapses in consciousness that can be characterized by a vacant stare eventually accompanied by myoclonus (often restricted to the eyelid) or other small movements (either of the hands or movements imitating chewing), or even a sudden change in movement.



**Figure 12. Suggested current classification of epileptic activities by the International League Against Epilepsy.** While focal seizures are generally subtypes as a function of the level of awareness maintained during the seizure and also on the motor-nonmotor one, generalized onset seizures only use the latter axis. From Fisher et al., (2017)

In many childhood epilepsies, the seizures do not continue into adulthood, but most adult-onset epilepsies require lifelong treatment. Today, a vast panoply of antiseizure medications (ASM) is available to patients (revisited in Sills & Rogawski, 2020, **Figure 13**). These medications act on different sides of the excitation-inhibition imbalance (**Figure 11**). Most first-generation ASMs that are still widely used (e.g. carbamazepine, phenobarbital, phenytoin, primidone) target voltage-gated ion channels. Their mechanism of action leads to the temporary blockade of sodium, calcium or potassium channels (e.g. carbamazepine, eslicarbazepine, oxcarbazepine, see **Figure 13** below), therefore preventing depolarization in a given cell, potentially mitigating the hyperexcitability and preventing seizures. Another portion of first-generation ASMs (e.g. phenobarbital, primidone) and some newer compounds (**Figure 13**) act more on inhibitory neurons or, more precisely, on their primary neurotransmitter, GABA, and their receptors on post-synaptic cells. Indeed, most of them bind to GABA<sub>A</sub>-type receptors on the post-synaptic cell, leading to prolonged opening of chloride channels, which contributes to the generation of inhibitory post-synaptic potentials, potentially preventing action potentials and increased firing rates. Other types (such as vigabatrin) inhibit the enzyme responsible for GABA breakdown or the mechanism allowing GABA reuptake, prolonging the time GABA is available at the synaptic cleft. Yet another type of medication also used as ASMs is the group of carbonic anhydrase inhibitors (e.g. Topiramate, Zonisamide, see **Figure 13**). The inhibition of their target enzyme, carbonyl anhydrases, leads to increased CO<sub>2</sub> levels both on the periphery and in the brain, making it a diuretic medication also causing urine alkalization but, most notably for epileptic patients, decreasing excess neuronal activity (Turner & Perry, 2017). Other, more novel-generation ASMs target AMPA ( $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors and selectively inhibit them, leading to decreased seizure occurrence (Franco et al., 2013). On the other hand, one of the most widely used types of ASMs directly targets the machinery behind synaptic transmission. Indeed, one subgroup (Levetiracetam and Brivaracetam) binds to SV2A, a synaptic vesicle protein, and seems to decrease vesicle release rates (Yang et al., 2007). The other subgroup (encompassing gabapentin and pregabalin) is thought to exert its antiseizure effect by binding to the voltage-gated calcium channel subunit  $\alpha 2\delta 1$  on the presynaptic end and directly inhibit excitatory neurotransmitter release (Yasaei et al., 2022). The other ASMs with known mechanisms of action are used in more specific types of epilepsy, such as Everolimus, which acts on the mTORC1 signalling pathway and is mainly used in tuberous

sclerosis complex-related epilepsy or Cerliponase alpha which is used in the treatment of another genetic disease, late-infantile neuronal ceroid lipofuscinosis type 2 where it also alleviates seizures. With the help of these medications, an estimated 80% of patients enter into remission with treatment, and 55-68% experience extended seizure-free periods in the long term (Beghi, 2020).

<b>Molecular Target</b>	<b>Antiseizure Drugs That Act on Target</b>
<i>Voltage-gated ion channels</i>	
Voltage-gated sodium channels	phenytoin, fosphenytoin <sup>1</sup> , carbamazepine, oxcarbazepine <sup>2</sup> , eslicarbazepine acetate <sup>3</sup> , lamotrigine, lacosamide, cenobamate; possibly, rufinamide, topiramate, zonisamide
Voltage-gated calcium channels	ethosuximide
Voltage-gated potassium channels	retigabine (ezogabine)
<i>GABA inhibition</i>	
GABA <sub>A</sub> receptors	phenobarbital, primidone, benzodiazepines including diazepam, lorazepam, clonazepam, midazolam, clobazam; stiripentol; possibly, topiramate, felbamate, cenobamate, retigabine (ezogabine)
GAT-1 GABA transporter	tiagabine
GABA transaminase	vigabatrin
Carbonic anhydrase inhibition	acetazolamide, topiramate, zonisamide; possibly lacosamide
<i>Synaptic release machinery</i>	
SV2A	levetiracetam, brivaracetam
α2δ	gabapentin, gabapentin enacarbil <sup>4</sup> , pregabalin
<i>Ionotropic glutamate receptors</i>	
AMPA receptor	perampanel
<i>Disease specific</i>	
mTORC1 signaling	everolimus
Lysosomal enzyme replacement	cerliponase alfa (recombinant tripeptidyl peptidase 1)
<i>Mixed/unknown</i>	valproate, felbamate, cenobamate, topiramate, zonisamide, rufinamide, adrenocorticotrophin, cannabidiol

**Figure 13. Currently used antiseizure medications and their primary mechanism of action.** While many ASMs act on voltage-gated sodium, potassium or calcium channels or GABA availability, other mechanisms of action have also been revealed. These include inhibiting AMPA receptor activation, increasing CO<sub>2</sub> levels in the brain, or directly impacting excitatory neurotransmitter release via an effect on the synaptic release machinery. From Sills and Rogawski (2020)

However, a subset of patients who present a form of pharmaco-resistant epilepsy undergoes invasive brain surgery to remove the epileptogenic zone in the hopes of decreasing or ceasing seizure activity that affects all aspects of the patient's life. This requires the precise localization of the seizure onset zone to resect only the strictly necessary areas, which is not evident to pinpoint.

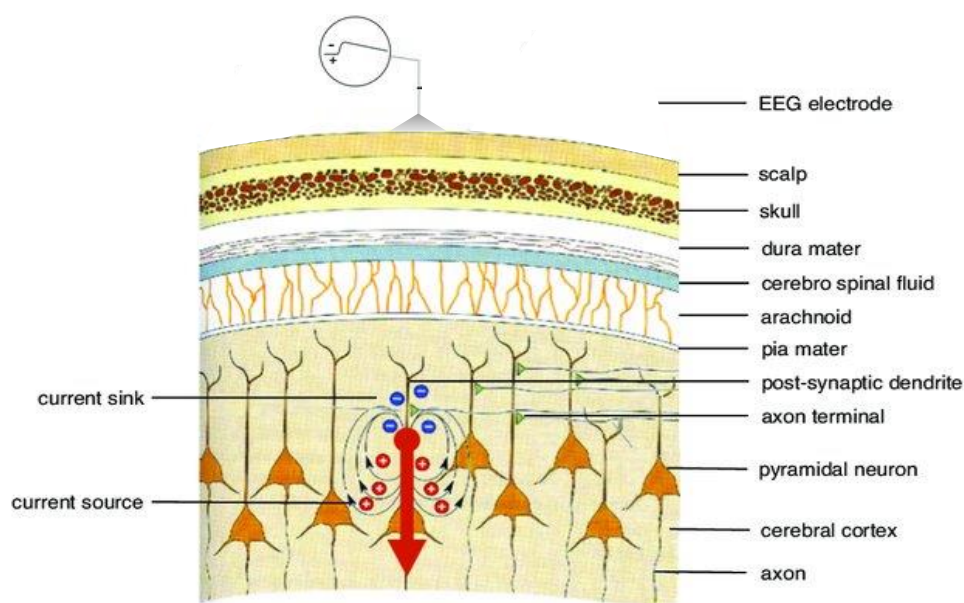
## 2.3 Detecting epileptic activities

### *2.3.1 Electroencephalography*

Indeed, localizing seizure onset zones requires a close look at brain activity which is most often achieved using electroencephalography (EEG). This method allows recording the electric activity generated by the brain. The first such recording (in humans at least) took place in 1924 and is attributed to Hans Berger, a German psychiatrist (St. Louis et al., 2016). The overwhelming majority of EEG recordings are so-called scalp EEGs, where electrodes are placed on the scalp, accompanied by either a conductive gel or a type of paste that helps reduce the interface's impedance between the electrode and the skin – the goal in both cases being improving the signal. Indeed, even if amplifiers are built into all EEG devices, signals are still relatively small and are often affected by ambient electric signals or body movements. Moreover, the signal captured by scalp electrodes is also dampened as it has to first travel through several layers, including the scalp, the skull, the dura and pia mater, as well as the arachnoid layer and a volume of cerebrospinal fluid in between (**Figure 14**).

EEG signals mainly reflect the activity of pyramidal neurons in the cortex and, more precisely, the summation of excitatory and inhibitory post-synaptic currents of the population of perpendicularly oriented neurons below a given electrode (St. Louis et al., 2016). In the case of a dendritic excitatory post-synaptic potential (EPSP), cationic channels favouring  $\text{Na}^+$  and  $\text{Ca}^{2+}$ -ion influx from the extracellular to the intracellular space open, resulting in a current sink at the extracellular level. This corresponds to a negative extracellular current balanced out by a cationic efflux (current source) from the intracellular to the extracellular space, called the return current (Buzsáki et al., 2012). A spatially mirrored sink-source localisation will be

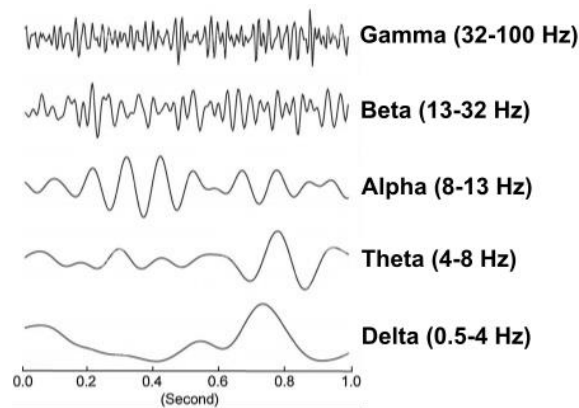
observed upon the opening of chloride channels that will potentiate the entry of anions into the intracellular space in the case of an IPSP. However, what is measured in the end is the sum of the generated currents from all ongoing cellular activities of that area that can be summed as a scalar potential  $V_e$ , in function of a reference potential (Buzsáki et al., 2012). This includes, other than this transmembrane current at the level of pyramidal cells, other sources such as non-synaptic events including calcium spikes, membrane potential fluctuations in glial cells, or intrinsic, voltage-gated currents (Buzsáki et al., 2012, p. 4). Therefore, given all the noise in the system, EEG has a relatively poor signal source-localisation capacity – this can be amended to a certain level with dense electrode layouts, albeit it would still bear some limitations.



**Figure 14. Schematic Illustration of EEG signal generation and capture by scalp electrodes.** Signals generated by the activity at the level of cortical pyramidal neurons have to pass through several layers to reach the surface that electrodes can capture, reducing the signal amplitude and quality. Adapted from the PhD thesis of Gregor Strobbe (2015)

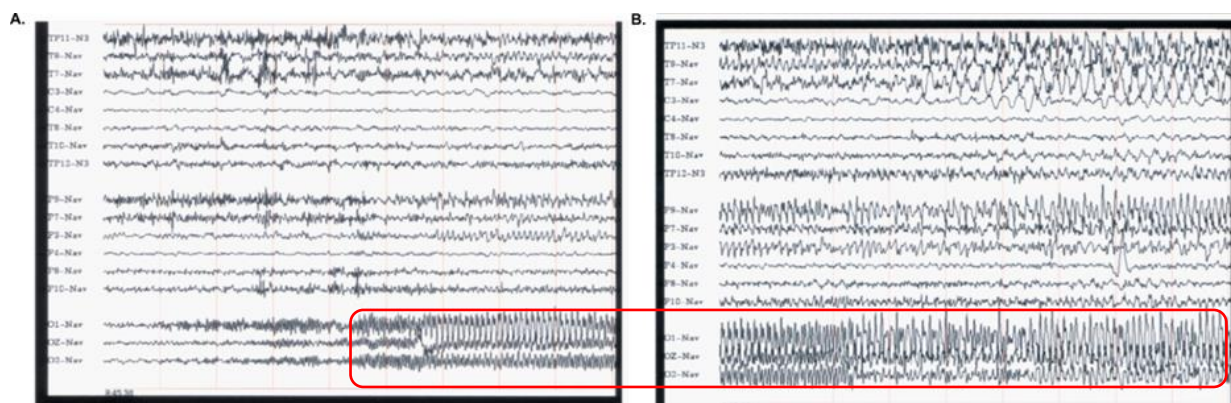
On the temporal level, however, EEG has a sub-millisecond precision and is not only a valuable tool for clinicians working with epileptic patients but was and still is instrumental in understanding brain activity and oscillations under physiological conditions. It allowed, amongst others, delimiting the main frequency bands of characteristic brain oscillations present during various behaviours and vigilance states (**Figure 15**). Higher frequencies, such as gamma waves (32-100 Hz), are generally tied to cognitive processing and learning, while beta rhythm (13-32 Hz) is the background activity accompanying alert wakefulness. Alpha waves (8-13 Hz) are more specific to wakeful rest with eyes closed, while theta rhythm (4-8 Hz) is predominant during rapid-eye-movement sleep and drowsiness and is thought to be

involved in processes including spatial navigation and memory formation. Finally, the slow delta waves ( $< 4$  Hz) are prominent during the deeper stages of sleep.



**Figure 15. Main oscillatory frequencies detected in the human brain.** Brain oscillatory rhythms have been divided into five main frequencies corresponding to different states of consciousness and behaviours. These include higher frequency activities such as gamma (32-100 Hz), as well as beta (13-32 Hz), Alpha (8-13 Hz), Theta (4-8 Hz) and delta (0.5-4 Hz). Adapted from Jafari et al. (2020)

As mentioned before, under pathological conditions, such as epilepsy, EEG is also an essential tool for localizing the seizure onset zone. After all, a seizure marks the hyperexcitability and the hypersynchrony of excitatory neurons: When an abnormally large portion of them undergo action potentials in a synchronized and sustained manner, a seizure occurs. With an EEG recording, we can follow both seizure activity and deduce the seizure onset zone in case of focal seizures (**Figure 16A, B**).

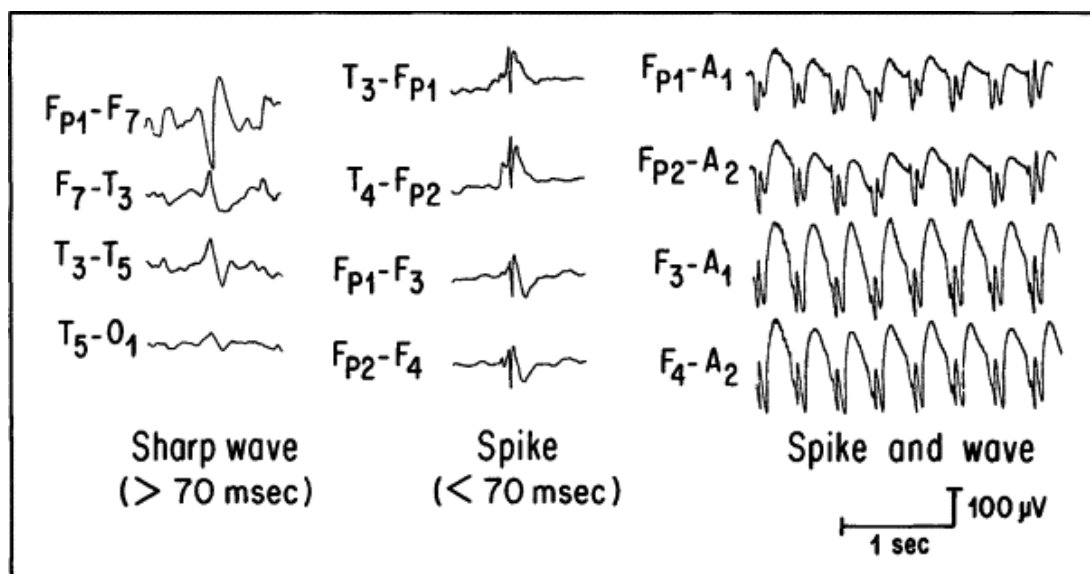


**Figure 16. EEG recording of a focal partial seizure with occipital onset.** (A) Seizure onset in the occipital zone (red circle). (B) Sequential recording, showing the propagation of the same occipital seizure to temporoparietal zones. From Cascino et al. (2002)



### 2.3.2. Interictal epileptic activities

However, not all epileptic events end in seizures. There exist so-called interictal epileptic activities (EAs, encompassing sharp waves, spikes and spike-and-wave discharges) that are also pathologic electrophysiological events. Still, they only consist of a single burst or a short series of bursts without transforming into a seizure (**Figure 17**). Furthermore, they do not usually have a detectable behavioural output – and when they happen in non-epileptic brains, they more often than not remain undetected. However, in epilepsy patients who often undergo EEGs, these EAs are distinguishable on the EEG and may give hints about seizure onset zones (**Figure 17**). It is of note that scalp EEG is limited in detecting some other epilepsy-related interictal activities, such as fast ripples that can only be visualized using intracranial electrodes implanted in pre-surgery settings for patients with pharmaco-resistant epilepsy (Despouy et al., 2020). Interictal activities such as spikes have been related to the inhibition of ictal events.



**Figure 17.** Examples of subclinical/interictal epileptic discharges. From left to right, these traces show the occurrence of sharp waves, interictal spikes and spike-and-wave discharges that are the interictal events possible to detect via scalp EEG. From Westmoreland (1996)

As was summarized by Holmes and Lenck-Santini (2006, p. 506), generally, the synchronous discharge that is the starting point of seizures is called the paroxysmal depolarization shift. During a paroxysmal depolarization shift, the membranes of the cells involved are subject to a high-voltage depolarization of 10-15 mV in the vicinity of the soma which is around ten times longer than the depolarization seen with a normal EPSP. During this prolonged depolarization,



a series of AP is generated, and this activity, in the end, is what would be reflected as an interictal spike on the EEG (see **Figure 17**) and is followed by a marked hyperpolarization that was hypothesized to reduce the duration of the epileptic event – and potentially its propagation towards an ictal event as well (Holmes & Lenck-Santini, 2006).

## 2.4 Cognitive deficits and epilepsy

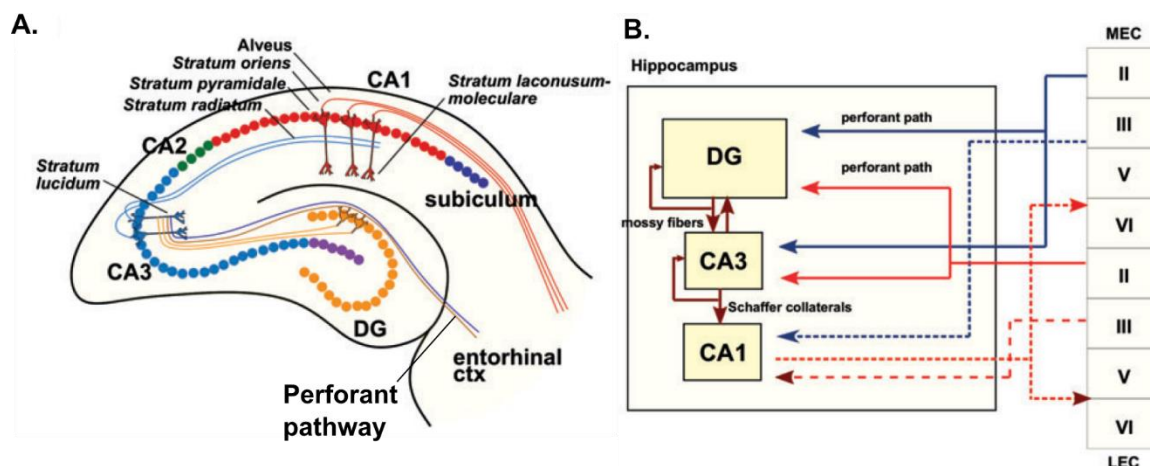
### *2.4.1. Disrupted cognition and epilepsy*

However, no matter how well-intended its generation may or may not be, these interictal events, like full-blown seizures, contribute to the cognitive complaints many epileptic patients experience. Indeed, in a study comparing objective and subjective cognitive complaints in epileptic patients, objective cognitive impairments were put forward in the majority (72%) of patients. Concordantly, almost 85% of them had high scores on the questionnaire concerning subjective cognitive impairment (Samarasekera et al., 2015). The issues with EAs and cognition are well represented in childhood epilepsies, such as benign rolandic epilepsy with centrotemporal spikes (BECTS). Children with this condition often present decreases in IQ scores and impairments in visual perception and short-term memory - and these deficits correlate better to interictal than to ictal events (Croona et al., 1999; reviewed in Holmes & Lenck-Santini, 2006). Cognitive impairment related to behaviourally asymptomatic EAs has also been shown in a study with almost fifty participants between 10 and 46 years (Aarts et al., 1984). Those participants were tested on two short-term memory tasks - one with verbal and the other with nonverbal material. Transitory cognitive impairment was present in around half of the participants, especially when EAs fell on the stimulus presentation periods. This was further elucidated by a series of experiments by Shewmon and Erwin (e.g. 1988, 1989; synthesised by Holmes & Lenck-Santini, 2006), who have shown that localized posterior EAs not only prolong reaction times during visual tasks but also lead to the non-perception or at least to an altered perception of the stimuli. In an experiment where they presented stimuli to the participants at gradually increased delays following EAs, they found that altered perception starts just before the EAs and ends with the slow wave that follows it. (Shewmon & Erwin, 1989).

### 2.4.2 Memory deficits and epilepsy

Over and above disrupted cognition by transient epileptic events, most of the cognitive symptoms in epileptic patients concern memory-related issues. This is not surprising as mesial temporal lobe epilepsy (mTLE) is the most common form of focal epilepsies. In mTLE – as the name suggests – the seizure onset zone is located at the level of the temporal lobe, which englobes the hippocampus. The hippocampi are subcortical structures embedded in the temporal lobe of both hemispheres. Both can be divided into three main parts: the dentate gyrus (DG), the *cornu ammonis* (or CA, divided into CA1, CA2 and CA3) and the subiculum (**Figure 18 A**). Information enters the hippocampus at the level of the DG from the medial and lateral entorhinal cortex (**Figure 18 B**) via the perforant pathway. There are granular cells located at the level of the DG whose axons will form de mossy fibres that will extend towards the CA3 area, where they will make extremely powerful – but rather sparse – synapses on pyramidal cells (Aimone et al., 2011). Pyramidal cells located at the CA3, on top of forming a recurrent network inside the CA3, will then continue the information transfer through their axonal projections named Schaeffer collaterals to the CA1 and the CA2 (**Figure 18 B**). Finally, the CA1 will project to the subiculum and back to the entorhinal cortex. The CA possesses a laminar structure with six layers: *the stratum moleculare* (mainly containing interneurons and pyramidal cell dendrites), the stratum radiatum, the stratum lacunosum (composed of the axons from the Schaffer collaterals and the perforant path), the stratum pyramidale (as the name suggests, encompassing the somas of the pyramidal cells), stratum oriens (with sparse basket cells and dendrites of the pyramidal cells) and the alveus (white matter).

The importance of the hippocampus in memory formation has been put forward with probably the most famous case report of an epileptic patient, H.M. In 1953, this patient underwent the bilateral ablation of the hippocampi and some of the neighbouring regions in the temporal lobe with the hopes of ridding himself of his epilepsy (Corkin, 1984). The operation was a success from the point of view of seizure occurrence. However, the patient developed anterograde amnesia as well as partial retrograde amnesia, the latter extending approximately to the decade preceding the surgery.



**Figure 18. Localization, anatomy and circuitry of the hippocampus.** (A) Schematic organization of the hippocampus, with information entering via the entorhinal cortex, passing through the dentate gyrus (DG) before traversing the different subparts of the cornu ammoni (CA) and arriving at the level of the subiculum. (B) Schematic chart of information flow from the lateral and medial entorhinal cortex towards the hippocampus. On top of the already mentioned straightforward flow of information towards the subiculum, several recurrent networks are present, most notably at the DG and the CA3. Not that information flow is also present from the hippocampus towards the entorhinal cortex. Adapted from Temido-Ferreira et al. (2019)

Another direct indication of the role of this brain structure in memory is that it is the first to show atrophy in AD patients – which is, in fact, part of the diagnostic criteria for AD (Dubois et al., 2014). These hippocampal atrophies can be translated to the progression of mnemonic deficits as different parts of the hippocampus bearing different importance for memory formation become affected. As an example, CA1 and CA3 are involved in the spatial coding and time-coding of events - explaining the crucial role of CA1 in episodic memory (Bartsch et al., 2011; see Eichenbaum, 2014; and O’Keefe, 1976), while the CA2 area is more involved in social memory formation (e.g. Oliva et al., 2020; Rey et al., 2022).

Interestingly, similar hippocampal sclerosis to that of AD accompanies many mTLE cases, associated with concomitant memory impairments, although memory complaints are also common in mTLE patients even in the absence of evident temporal lobe atrophy (Baker & Zeman, 2017). More precisely, there is an accelerated long-term forgetting (ALF) that has been described in a subset of mTLE patients, which corresponds to the pathologically rapid forgetting (less than 30 minutes) of newly learned information (Baker & Zeman, 2017). Like in the other cognitive domains above, EAs and recurrent seizures were proposed as potential culprits for ALF. Ricci and collaborators (2015) found a link between hippocampal lesions and short-term memory formation concerning the autobiographic domain that could indeed

support this claim. Furthermore, other than disrupting short-term memory, Lah and collaborators (2006) have found, following a similar pattern to what was described above for posterior EAs, that patients with right-sided temporal lobe seizures experienced more severe deficits in remote autobiographic memory, while Kapur et al. (1997) described the same phenomenon in a patient with left-sided TLE. Given that these are deficits in remote memory recall, the authors suggested the existence of a very long-term memory consolidation process with temporal lobe origins – and as we will explore in just a few paragraphs, recent results support this claim.

We have seen that AD often manifests in the beginning as autobiographic deficits. Does it mean that the initial mnemonic deficits of mTLE patients indicate the onset of dementia? It has been shown that epilepsy patients present a 2 to 3.6-fold risk of developing dementia compared to the non-epileptic population (Costa et al., 2019; Stefanidou et al., 2020). Unfortunately, in current clinical practice, the more and more severe cognitive complaints of epilepsy patients might be mislabeled as Epileptic Amnesic Syndrome (EAS), a less severe condition with good responsiveness to ASMs (Cretin et al., 2017; Gallassi, 2006). EAS patients are generally older adults whose seizures are barely symptomatic (mainly oral automatisms and transient loss of contact) and, therefore, are sometimes uncovered only when the patients consult a neurologist for memory complaints. Their amnesia extends to the period of these short focal seizures and is generally not recovered but can occasionally also expand towards an anterograde or to more retrograde amnesia – although these amnesias may not be permanent. Furthermore, ASM treatment generally alleviates cognitive symptoms (Gallassi, 2006). However, mislabeling mTLE patients as having “only” EAS while they are at the doorstep of dementia could lead to suboptimal treatment plans and worse prognostic. It is, of course, not to say that a diagnosis of dementia or AD is justified in all of these cases, yet an altered path of cognitive decline in epileptic patients compared to healthy older adults has been observed throughout the literature. These findings were summarized by Breuer and colleagues (2016) in favour of a potential accelerated cognitive ageing for people living with epilepsy. As they underscored, the brain of older adults tackles several problems at a time, such as elevated levels of inflammatory brain response, comorbidities that affect cognition (such as AD), the use of various medications with often interacting effects, and, notably, a potentially decreased brain reserve due to the temporal lobe atrophies. Brain reserve (the

solidity of the structures, e.g. brain size and surviving neurons) underlying given cognitive functions would have a decisive role in how the brain responds to further assaults, such as epileptic events, and how early cognitive symptoms would appear (Menardi et al., 2018). The decrease of this reserve in older adults with epilepsy may mean they cannot compensate for epilepsy-induced cognitive problems like younger epileptic patients, further aggravating cognitive decline (Breuer et al., 2016).

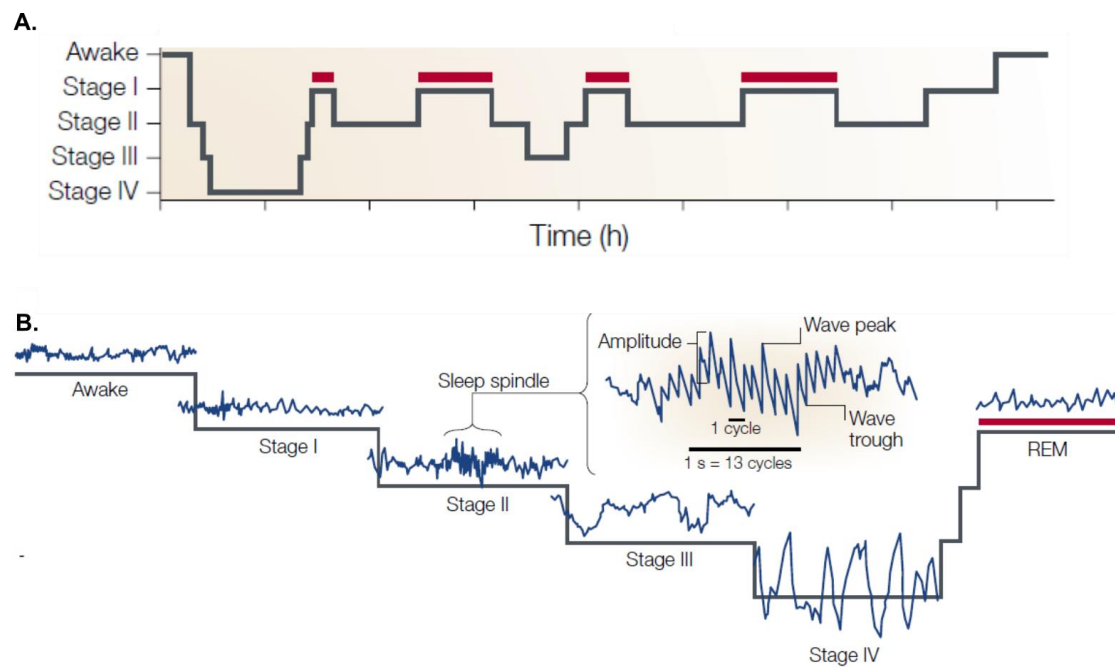
However, other than the accelerated cognitive ageing of epileptic patients, links between the pathophysiologic mechanisms between AD and epilepsy have recently been uncovered. Clinical studies suggested that age-related amyloid plaque deposition is more significant among TLE patients than in healthy older adults and that there might be an enhancing effect of the epileptic syndrome on plaque formation and amyloidosis. This is based on observations from resected cerebral tissue from epileptic patients and CSF AD-biomarker measurements of epileptic patients, as well as on observations from genetic and neurotoxic AD mouse models (Costa et al., 2016; Mackenzie & Miller, 1994; Nardi Cesarini et al., 2020). A similar increase to that of amyloid burden was found for pTau in post-surgery brain resections collected from TLE patients with focal drug-resistant epilepsy (Tai et al., 2016). Summarizing these points, a very recent review by Tombini et al. (2021) showed that TLE and AD patients share many pathophysiological associations starting with the presence of hallmark depositions of AD (A $\beta$  plaques and NFTs, or their soluble forms), a similarity that is more robust for late-onset TLE cases (Kaestner et al., 2021). Interestingly, many pathways promoting cognitive decline in epilepsy patients can be linked to one of the characteristics of epileptic events: their timing during the sleep-wake cycle. Indeed, it has been demonstrated in an elegant review by Ng et Pavlova (2013) that only about a quarter of all seizures (28%) occur during wakefulness, while the rest fall on periods of sleep, disrupting its structure and many of its functions – such as sleep-related memory consolidation or the clearance mechanisms of toxic metabolites in the brain.

## **CHAPTER III - SLEEP**

“If sleep does not serve an absolutely vital function, then it is the biggest mistake the evolutionary process has ever made” (Allan Rechtschaffen, in Mignot, 2008, p. 661). Indeed, with (ideally) around eight hours of sleep per night, humans technically spend a third of their lives asleep – a time essential for virtually all organ systems in the body to function correctly (reviewed by Mignot, 2008; and Walker, 2021). Therefore, even though, by definition, sleep is “a natural and reversible state of reduced responsiveness to external stimuli and relative inactivity, accompanied by a loss of consciousness” (Rasch & Born, 2013, p. 681), it is far from being a passive state. And just as the hormonal and immune systems, metabolism, vascular system or emotional regulation require sleep, the brain is no exception to this rule. And, as we will see, when the functions allotted to sleep time – such as the elimination of toxic metabolites or sleep-related memory consolidation – are disrupted, the probability of triggering neurologic pathologies, including AD, increases. So how are sleep and its primary roles related to cognitive functions and neurodegeneration regulated in the brain?

### **3.1. The macroarchitecture of sleep**

Sleep is not a homogenous stage, as evidenced by the use of EEG. Indeed, a night’s sleep is composed of several successive sleep cycles in which various stages repeat themselves. In humans, these include four stages of non-rapid eye movement sleep (NREM sleep) of increasing sleep depth, which can be distinguished on the EEG as high-voltage and low-frequency activity becomes more and more predominant from lighter to deeper stages (Pace-Schott & Hobson, 2002). These stages are followed by what we call rapid eye movement sleep (REM sleep), which was first described in detail by Michel Jouvet in the 1950s (1959). In the night's first half, cycles are dominated by NREM sleep, whereas REM sleep takes up disproportionately long periods during the night's second half (**Figure 19 A**). The two main stages (NREM and REM sleep) are present in almost all mammals and birds (e.g. Ungurean et al., 2021). However, the distinction between the sub-stages of NREM is rarely made in other animals.



**Figure 19. Sleep macrostructure and corresponding EEG signals.** (A) An example of a hypnogram throughout one night of sleep involving all NREM stages is REM sleep (in red). (B) Representative segments of EEG activity during the different sleep stages in one sleep cycle. Adapted from Pace-Schott and Hobson (2002).

NREM stages 1 and 2 are generally considered light sleep, while stages 3 and 4 as deeper stages, also called slow-wave sleep (SWS) – which can be confusing in preclinical literature where SWS is often used interchangeably with NREM sleep. When differentiating sleep stages from EEG recordings, by convention (Silber et al., 2007), stage 1 is identified by relatively low voltage activity but at a lower frequency than during wakefulness (2-7 Hz vs 8-13 Hz). The apparition of sleep spindles (Figure 19 B) and other morphological markers such as K-complexes and at least 20% of any given epoch at high voltage ( $> 75 \mu\text{V}$ ) and low frequency ( $< 2\text{Hz}$ ) characterizes stage 2. During stage 3, 20-50% of the epoch is defined by this type of high-voltage, low-frequency activity, while in stage 4, its fraction ramps up to at least 50%, although this latter stage is not used in the international sleep scoring system. REM sleep is marked by fast cortical activity reflecting the desynchronization and the presence of hippocampal theta rhythm ranging from 4-12 Hz (Silber et al., 2007), coupled with faster gamma oscillations (30-300 Hz). Behaviourally, REM sleep can be divided into tonic and phasic stages, with the former characterized by a total muscular atonia and the latter with phasic periods of rapid eye movements (Mignot, 2008).

The regulation of this complex cycling and switching between sleep stages and the function of their existence has long fascinated researchers, which led to a succession of discoveries in the past century concerning the neurobiology of sleep.

### 3.2. The neurobiology of sleep

For a long time, sleep was regarded more as a passive state, wherein one system would maintain wakefulness, and the dampening of its activity would automatically induce sleep. Initially, this wake-promoting system was attributed solely to the ascending reticular activating system's activity (Moruzzi & Magoun, 1949; reviewed by Jouvet, 1969). Later, several theories of sleep regulation have seen the light where a system that would actively inhibit the wake-promoting one was suggested to exist, looking at sleep as more of an actively regulated process.

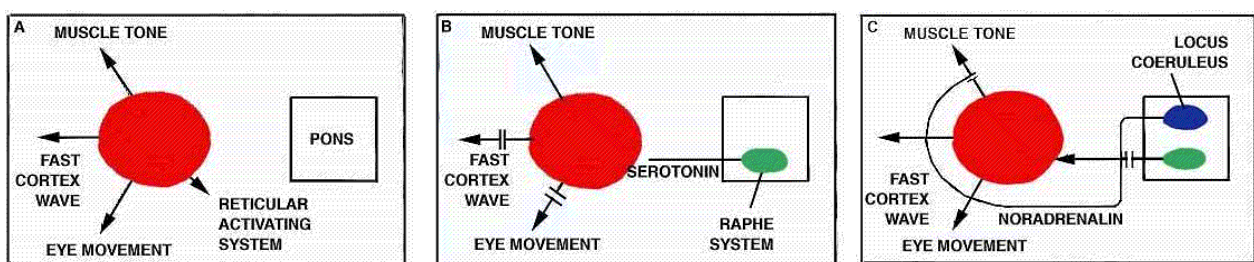
#### *3.2.1. The monoaminergic theory of sleep*

The first widely known model, the *monoaminergic theory of sleep* (Jouvet, 1972), saw the key to the switch between different vigilance states in monoaminergic transmission. Jouvet's work – which towers over sleep research with its major contributions to its understanding – initially focused on the underpinnings of the sleep stage he identified, REM sleep (1959). This work was the golden thread that led him to the proposition of his theory on sleep-wake regulation later. On the road toward the monoaminergic theory, he delimited the area responsible for cortical arousal during REM sleep to the anterior part of the oral pontine reticular nucleus and the posterior segment of the tegmentum (Jouvet, 1967b) and identified a zone essential for muscular atonia during tonic REM in the pontine tegmentum. He also pinpointed the pons as the origin of the activity responsible for rapid eye movements during phasic REM sleep (reviewed by Jones, 2018) and described the propagation of this activity from the pons to the lateral geniculate nucleus and the occipital cortex. This propagation can be observed via EEG recordings shortly before REM onset across these areas and – coherently – is named ponto-geniculo-occipital spike or PGO spike. Over and above these major contributions to the



understanding of REM sleep, he consecrated much of his career to disentangling how REM sleep – and sleep in general – is triggered.

Based on his and others' findings, he hypothesized that the system that could counter the arousal system and orchestrate sleep onset was located in the medulla or the posterior pons, where nuclei containing most neurons responsible for the monoaminergic supply of the brain are located. His work seemed to point principally toward the serotonergic neurons in the dorsal raphe nuclei (**Figure 20 A**) as the destruction of this area was shown to lead to long-lasting insomnia in cats (Jouvet, 1968).



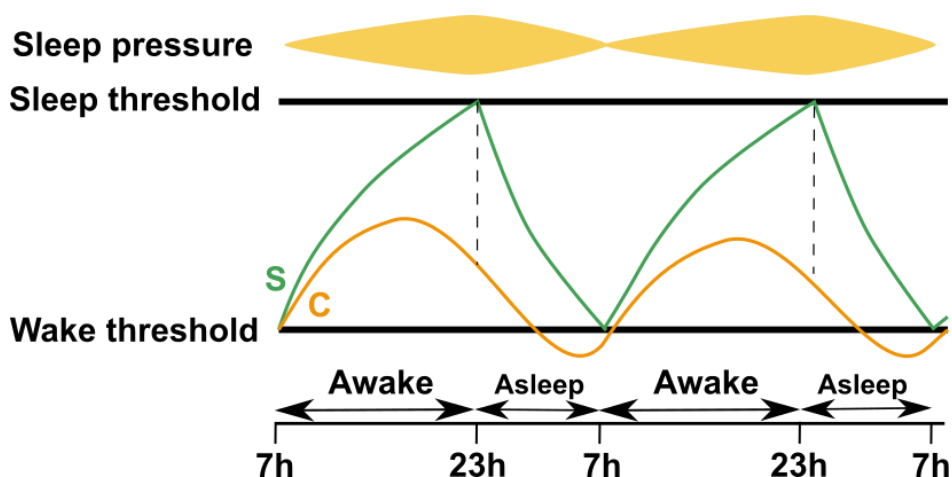
**Figure 20. The initial monoaminergic theory of sleep.** In the initial theory, the regular brain-and behavioural activity (**A**) would turn into slow-wave sleep via the activity of serotonergic neurons from pontine nuclei, more precisely from the dorsal raphe, interrupting eye movements and fast cortical activity and inhibiting the action of the reticular activating system (**B**). A switch towards REM sleep (**C**) would then be initiated with the onset of noradrenergic activity in the locus coeruleus that would re-initiate fast cortical activity and eye movements and lead to muscular atonia. From Jouvet (1967a), Scientific American

While he considered serotonin the master switch controlling NREM sleep (**Figure 20 B**), his findings identified the locus coeruleus (LC) and the noradrenergic cell bodies in it as essential for REM sleep onset (**Figure 20 C**). This was based on the result that the LC's destruction led to an absence of atonia during REM sleep in Jouvet's experiments (1969). Later on, however, single-unit recordings of the LC seemed to contradict this part of the theory, as noradrenergic cell activity was found maximal during wakefulness, decreased during NREM sleep to become quasi-null during REM sleep (Aston-Jones & Bloom, 1981). Therefore, noradrenaline turned out to be more of a wake-promoting substrate – and was later joined by dopamine in this group when it was found by Jouvet's team that ablation of either dopaminergic hub (the substantia nigra or the ventral tegmental area) led to deficits in behavioural arousals (Jones et al., 1973). In parallel, acetylcholine emerged as a trigger for REM sleep (George et al., 1964) and was later integrated into Jouvet's theory. Even refined with these precisions, the initial monoaminergic theory of sleep can be summed up as the interactions between a wake-promoting system of catecholamines and a sleep-promoting system based on serotonin

(**Figure 20**). This seems to be a relatively straightforward model, and, as was assumed later by Jouvét himself, it was a *totalitarian one* (Jouvét, 1999), and, *as for every totalitarianism, this dogma did not live very long* (Jouvét, 1999, p. 2). This “monoaminergic regime” fell when it turned out that the activity of serotonergic neurons in the raphe follows a similar dynamic as noradrenergic neurons, that is, maximal during wakefulness, decreased during NREM sleep, and virtually zero during REM sleep (McGinty & Harper, 1976; reviewed by Jones, 2018). After this discovery, Jouvét hypothesized that it might be the accumulation of serotonin throughout wakeful periods that could contribute to the homeostatic regulation of sleep (Jouvét, 1999); however, this role was later turned out to be reserved for adenosine.

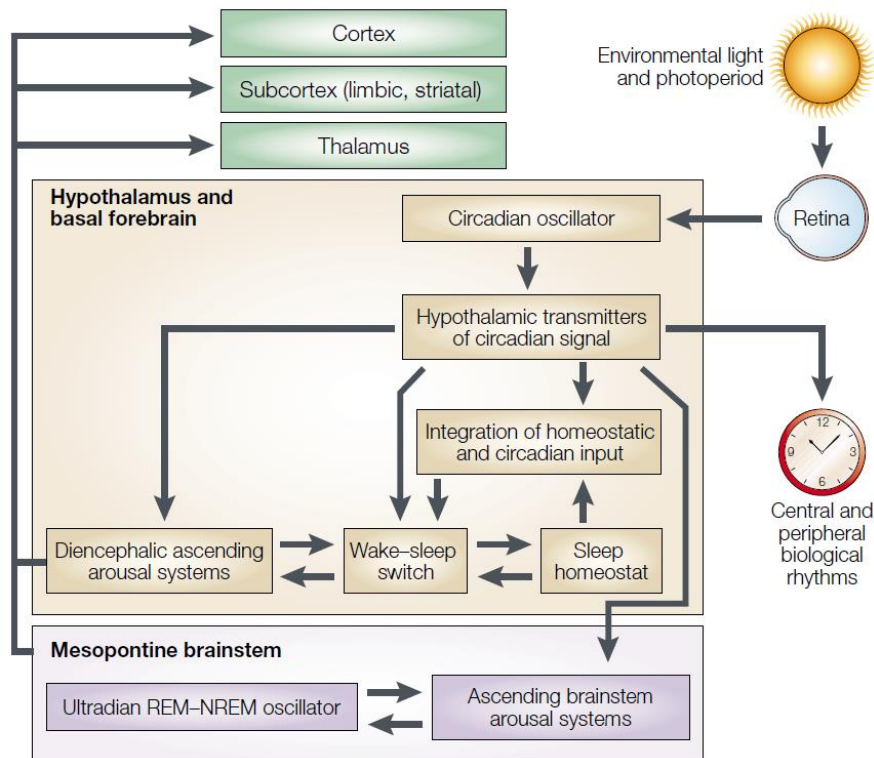
### 3.2.2. *The two-process model of sleep*

The following theory, proposed by Borbély (1982), the *Two Process Model of sleep*, suggested that sleep regulation is based on two factors: a so-called Process S and a Process C (**Figure 21**). The former is sleep-dependent, modulated by the time spent awake, and could be reformulated as an increasing sleep pressure with time spent awake and dissipating sleep pressure with longer and longer sleep episodes. Based on EEG recordings, Borbély noted that while EEG power in the lower frequency range (i.e. slow-wave range) tended to decrease during sleep time, its initial power was determined by the time spent awake preceding the sleep episode. Therefore, this power density change could be interpreted as an indicator of the force of Process S. Process C, in this theory, would be a sleep-independent component related to a circadian oscillator that would be responsible for the circadian rhythm. Interestingly, REM sleep duration seems less affected after sleep deprivation than NREM sleep; therefore, it was concluded that REM sleep would primarily depend on Process C while NREM sleep onset and duration would be regulated by the combined action of Processes S and C.



**Figure 21. The two-process model of sleep.** According to this theory, the sleep-wake cycle is regulated by the interactions between two main processes, Process S, representing mounting sleep pressure with time spent awake and decreasing sleep pressure across sleep episodes, and Process C, interpreted as a circadian oscillator that accords sleep-wake behaviour to the approximately 24-h astronomical periodicity. Modified after Borbély (1982) and Schmidt et al. (2007).

The suggested Process C, or the circadian oscillator, has been minutely scrutinized and linked to a hypothalamic structure, the suprachiasmatic nucleus (SCN), in which each cell expresses a “molecular circadian clock” regulated by genetic factors (reviewed in detail in Pace-Schott & Hobson, 2002). This “master clock” of the SCN receives photic input from glutamatergic retinal ganglion cells and exerts its effects on the body by controlling peripheral circadian oscillators responsible for regulating circadian rhythms of the different organ systems (**Figure 22**). As for Process S, the above-mentioned adenosine was suggested as the sleep-promoting agent, whose accumulation during wake time could increase sleep propensity in the organism. This is well-supported by the observation (repeated and verified many times during the writing of this document) that caffeine, which acts as a potent antagonist of all types of adenosine receptors, has a powerful wake-promoting effect (Pace-Schott & Hobson, 2002). Accumulating adenosine was suggested to exert its sleep-promoting influence indirectly by inhibiting groups of GABAergic neurons that themselves directly inhibit sleep-active neurons during wakefulness. However, as can be deduced from the previous paragraph and has been revealed by decades of research, sleep-active neurons do not correspond to serotonergic cells in the dorsal raphe; furthermore, wake-active cells are not only catecholamines.



**Figure 22. Illustration of the circadian rhythm's involvement in regulating the sleep-wake cycle.** Circadian rhythms are highly modulated by the 24h periodicity of the astronomical cycle dictating the alternation of dark and light phases. Photic information about these phases reaches the retina and is transmitted to the circadian oscillator, identified as the suprachiasmatic nucleus/SCN). This “master oscillator” relays information to peripheral oscillators and will impact the ascending arousal system and the sleep-wake onset timing. The resulting pieces of information resulting from the interactions of this highly interconnected system will then be relayed towards the thalamus and higher cortical areas. From Pace-Schott & Hobson (2002).

### 3.2.3. The interaction of neurotransmitters in wake-promotion

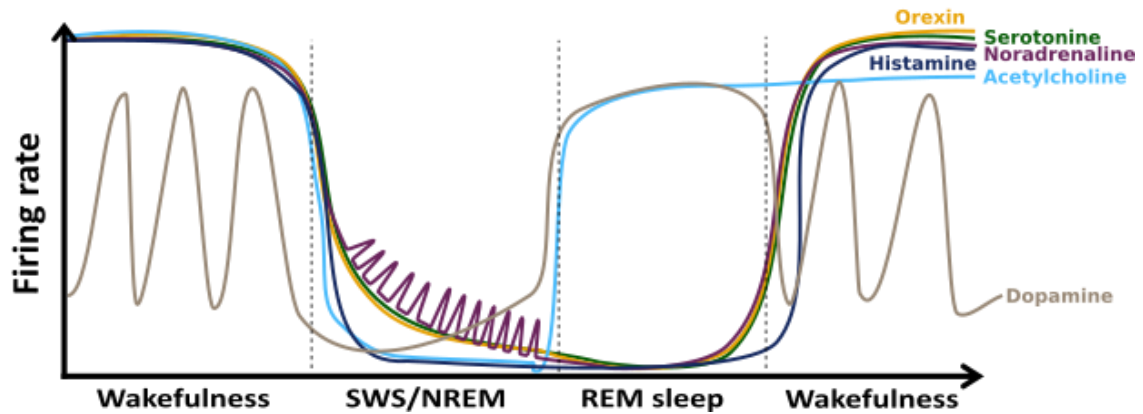
As for wakefulness, the role of monoamines and catecholamines, however, cannot be denied. For example, noradrenaline is a potent molecule in that aspect, as it can both depolarize wake-promoting neurons through  $\alpha_1$ -adrenoreceptors and hyperpolarize sleep-active ones through  $\alpha_2$ -adrenoreceptors (see Jones, 2005). Of interest, however, it has recently been shown that noradrenaline might not follow a clear-cut sleep-related decrease as it was conventionally believed before (Léna et al., 2005). Indeed, Osorio-Forero and collaborators (2021) found that instead of decreased firing rates, subpopulations of the noradrenergic neurons of the LC show burst firing approximately every 50 seconds, inducing high levels of NA concentration fluctuation at the level of thalamocortical circuits. This was hypothesized to be a wake-promoting mechanism maintaining a certain level of arousability during sleep, allowing the organism to respond to potentially hazardous stimuli (**Figure 23**). Dopamine has also

maintained its role as an arousal-inducing agent, although the dopaminergic neurons that fire in burst most in relation to positive rewards have also been shown to reach high levels of activity during REM sleep (**Figure 23**) (Dahan et al., 2007). As mentioned before, serotonin has switched sides since the monoaminergic theory and was revealed to be a wake-active neurotransmitter.

As for acetylcholine (ACh), two distinct populations participate in the regulation of the sleep-wake cycle. The cholinergic neurons of the brainstem are involved in cortical activation (Marrosu et al., 1995), especially in the generation of theta and gamma rhythms, both during wakefulness and during REM sleep (Vazquez & Baghdoyan, 2001). The other group, situated in the laterodorsal (LTD) and the pedunculopontine (PPT) nuclei, have been found to contribute to cortical activation via their ascending thalamic projections and to REM-sleep associated muscle atonia via their projections towards the brainstem reticular formation and reticulospinal neurons (reviewed by Jones, 2005). Furthermore, using selective activation of separate cholinergic populations in the PPT, the LTD and the basal forebrain, it was revealed that the PPT and LTD were more critical for REM sleep initiation, while the basal forebrain neurons played a more important role in REM sleep maintenance (Van Dort et al., 2015). The apparent paradox of the wake-and REM-active nature of cholinergic neurons can also be alleviated when considering their interaction with noradrenergic neurons: when both cell types display high activity levels, wakefulness is maintained, and the muscular atonia-related effect of ACh seems to be diminished. However, when noradrenergic neurons are inactive (during REM sleep), this seems to change, and ACh exerts its REM-on effect.

Over and above the already known molecules at the time of the monoaminergic theory, several others have been identified in the past decades with significant impacts on the sleep-wake cycle (**Figure 23**). The neurons secreting the first type, histamine, are located in the tuberomammillary nuclei, have a similar firing pattern as noradrenergic and serotonergic neurons and are known to exert an excitatory effect on the arousal system. The second agent, orexin (also known as hypocretin), has a similar secretion pattern via neurons in the lateral hypothalamus. It exerts a powerful excitatory effect on all the above-mentioned wake-promoting systems, from noradrenaline to histamine (reviewed in Jones, 2005, 2018). To further support the claim of orexin's central role, it has been shown that its depletion or an

absence of orexin receptors induces narcolepsy, a pathology marked by abrupt and involuntary switches from wakefulness to sleep (Chemelli et al., 1999).



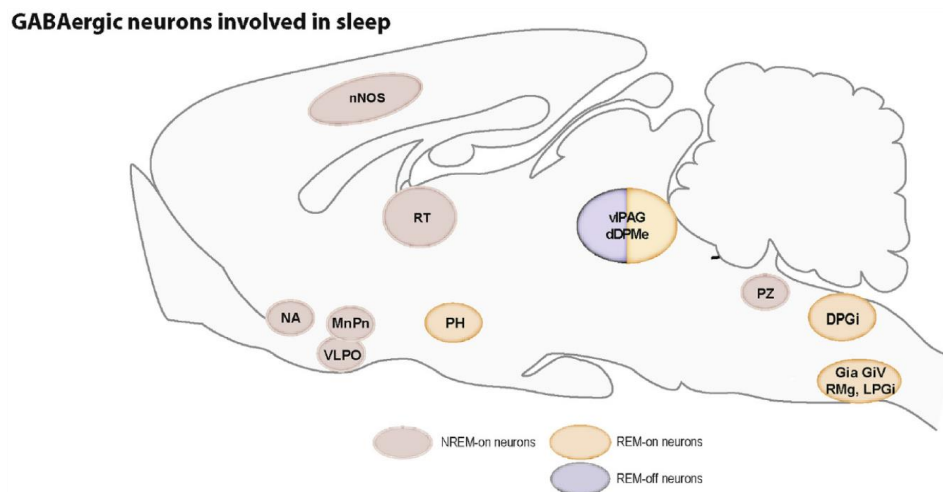
**Figure 23. Schematic illustration of the concentrations of different wake-promoting molecules across the sleep-wake cycle.** While serotonergic, histaminergic and orexinergic neurons are tonically active during wakefulness, they decrease firing rates with deepening stages of sleep to come to a quasi-complete halt in activity during REM sleep. Noradrenaline seems to follow a similar pattern except for the temporary bursts of noradrenergic transmission during NREM sleep, corresponding to periods of arousability approximately every minute (Osorio-Forero et al., 2021). For dopamine, activity is sparse during wakefulness and sleep but reaches high activity levels with REM onset.

### 3.2.4. Neurotransmitters contributing to sleep-onset

As for sleep-active neurons, the role of GABA is the most often accentuated (**Figure 24**). Its impact on sleep onset can be further underscored by the fact that most sedatives target GABAergic transmission. As was synthesized by Luppi et al. (2017), while barbiturates induce sleep by increasing GABA binding to its receptors, benzodiazepines and new generation “Z-drugs” act as positive allosteric modulators at GABA<sub>A</sub> receptors and increase the chloride current triggered by GABA binding. Finally, agonists of GABA<sub>B</sub> receptors also increase NREM sleep, especially deeper sleep stages, as they increase delta- and slow-wave activity.

One of the critical populations of the sleep-inducing GABAergic neurons seems to be located in the ventrolateral preoptic area (VLPO) and median preoptic area, where GABAergic interneurons are highly active during NREM sleep (in Luppi et al., 2017). These areas are excited by glucose and adenosine (Varin et al., 2015; Scammell et al., 2001, respectively; reviewed by Luppi et al., 2017) and provide inhibition during sleep to all brain areas that promote wakefulness described above. Reciprocally, these GABAergic NREM-on neurons are inhibited by acetylcholine and noradrenaline during wakeful periods (Gallopini et al., 2000).

Other GABAergic influences acting over sleep onset and maintenance include Parvalbumin-positive GABAergic interneurons of the reticular thalamic nuclei (Thankachan et al., 2019), a subpopulation of cortical interneurons expressing the neuronal nitric oxide synthase and GABA/glycinergic interneurons in the medullary parafacial zone, GABAergic cells in the nucleus accumbens and the median preoptic nuclei, as well as a group of interneurons in the basal forebrain (in Luppi et al., 2017) (**Figure 24**). It is of note, however, that not all sleep-active neurons are GABAergic. In fact, one of the most potent sleep-promoting molecules seems to be the hypothalamic melatonin-concentrating hormone that exerts an inhibitory influence over REM-on areas (see below), the monoaminergic systems, the wake-promoting orexinergic (Sakurai, 2007) and histaminergic systems as well.

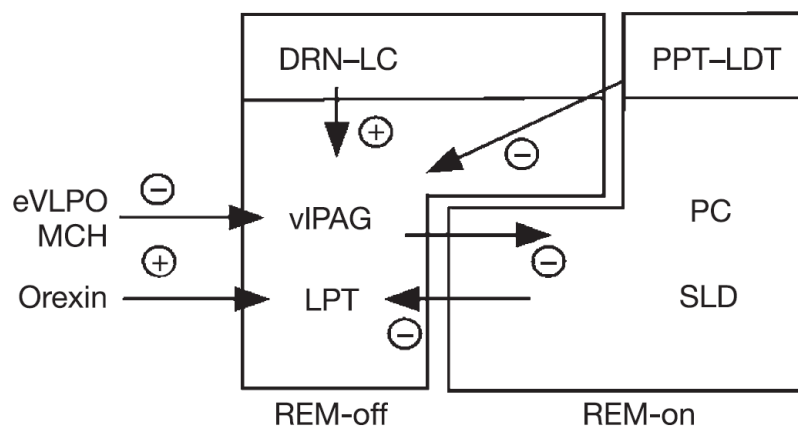


**Figure 24. Schematic representation of the main groups of interneurons involved in NREM/REM sleep initiation and maintenance.** These include the ventrolateral preoptic area (VLPO), the reticular thalamic nuclei (RT), cortical neurons secreting nitric oxide (nNOS), interneurons in the medullary parafacial zone and GABAergic neurons in the nucleus accumbens (NA). From Luppi et al., (2011)

### 3.2.5. The Flip-Flop model – or switching from NREM to REM sleep

Even if the mechanism of sleep onset starts to be more transparent, the nature of the switch between NREM and REM sleeps remains to be answered. Indeed, it is known that REM sleep onset is highly dependent on the glutamatergic activity of the pontine sublaterodorsal nucleus (SLD), where the descending REM-generating neurons are found (Luppi et al., 2011). Upon activation of the SLD, muscle atonia and the REM-sleep characteristic brain activity rapidly settle in (Boissard et al., 2002). Atonia is potentially induced via the activation of GABA and glycinergic neurons in the ventromedial medulla activated by the SLD. In return, the SLD is

reciprocally gated by the activity of the ventrolateral periaqueductal gray (vIPAG) (Weber et al., 2018). Indeed, GABAergic neurons in the vIPAG seem to inhibit REM-onset by synapses towards the SLD. This reciprocal inhibition is the core of the so-called *Flip-Flop model of REM switch*, brought forward by Lu and collaborators (2006). In this system, the GABAergic neurons of the vIPAG and the adjacent lateral pontine tegmentum (LPT) represent the REM-off system, which is reciprocally inhibited by the REM-on site, identified as the SLD and the glutamatergic neurons of the adjacent pre-coeruleus (PC on **Figure 25**). This reciprocal inhibition is unilaterally modified by numerous inputs from the already presented actors of sleep-wake regulation, including inhibitory cholinergic inputs from the PPT/LDT and excitatory inputs from monoaminergic centres, most notable the LC and the dorsal raphe (Lu et al., 2006) (**Figure 25**).



**Figure 25. The Flip-Flop model of REM-switch.** According to this theory, the reciprocal inhibition of GABAergic neurons in the REM-off (ventrolateral periaqueductal gray (vIPAG), lateral pontine tegmentum (LPT) and REM-on (sublaterodorsal nucleus (SLD), pre-coeruleus (PC)) areas determines the switch-point towards REM onset and REM offset. Non-reciprocal excitatory and inhibitory influences also arrive from diverse regions and modulate the switch, including cholinergic input from the laterodorsal and pedunculopontine tegmental areas (PPT and LDT), monoaminergic input from the dorsal raphe (DRN) and the locus coeruleus (LC), orexin and melatonin-concentrating hormone from the hypothalamus, and galanin/GABA from the extended ventrolateral preoptic area (eVLPO). From Lu et al. (2006).

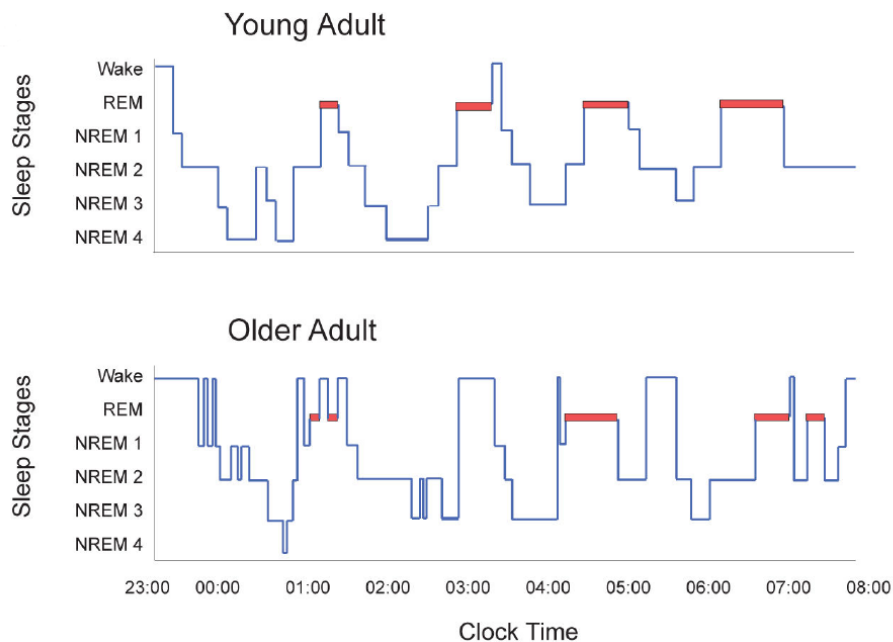
### 3.3 The decline of sleep in Alzheimer's disease

#### *3.3.1 Declines in global sleep quality*

The models above describe a system under physiological conditions, allowing for the maintenance of regular sleep cycles. However, this is not the case in many pathologies or even during healthy ageing. Ageing brings about a more fragmented sleep with extended awake periods during the night, increasing sleep latency and shortened total sleep time.



Deeper NREM stages (N3-N4) decrease while lighter stages and wakeful periods increase (**Figure 26**). N2 stage is less affected in its quantity, but its markers, sleep spindles and K-complexes, become less well-formed and decrease in quantity (Wauquier, 1993).



**Figure 26. Representative hypnograms of young and old adults.** In younger adults (upper panel), sleep latency is decreased, and the first half of the night is dominated by NREM2-NREM4 stages, while in the second half of the night, REM sleep predominates. Awakenings are rare and brief. In older adults (lower panel), sleep latency increases, and the night is interspersed with more or less prolonged awakenings altogether. Stages below NREM2 are rare and brief, while the more superficial stages take over. REM sleep is also decreasing in overall quantity. From Mander et al. (2017).

This is further aggravated in the case of AD, with a highly fragmented sleep pattern, often burdened by nighttime confusion. The duration of REM sleep further decreases, probably due to early atrophy of the brain areas in charge of cholinergic and noradrenergic transmissions that are essential for REM sleep integrity (Cummings & Back, 1998; Szot, 2012). This REM-sleep dysfunction is also considered early because the general and prominent slowing of EEG activity during REM sleep was reported to be present even at stages without apparent cognitive decline, including participants with MMSE scores as high as 29 (Petit et al., 1992). In fact, a 5% decrease in REM sleep compared to healthy older adults has already been reported in MCI patients and seemed to be worsened for APO-E4 allele carriers (Hita-Yañez et al., 2012). The gravity of sleep decline continues to climb with the later stages of AD, where patients may spend around 40% of the night awake, accompanied by numerous daytime naps (reviewed by McCurry et al., 2000). However, these naps cannot compensate for lost N3, N4, and REM sleep across the night as they mainly contain N1 and N2 sleep.

To make matters worse, an attempt is often made to treat this insomnia-like state with sedatives. However, they might add insult to injury as they were reported to worsen both nighttime confusions and sleep apnoeas in AD patients (McCurry et al., 2000). Furthermore, the decrease in sleep quantity seems more likely to be a continuum, mainly mediated by A $\beta$ -deposition. Indeed, in a group of cognitively normal middle-aged participants, they found that those with low CSF-A $\beta$  (indicative of higher A $\beta$  levels in the brain) had poorer sleep quality, independent of age, sex or even APO-E4 status (Ju et al., 2013).

Treatment with drugs counterbalancing the fallout of different molecules due to early neurodegeneration may be a better option for treating insomnia. For example, changes in REM sleep quantity were hypothesized to be due to the early neurodegeneration of the cholinergic system, specifically the Nucleus Basalis of Meynert (Montplaisir et al., 1995). Treatment across several weeks with the acetylcholinesterase inhibitor Donepezil has been reported to increase REM sleep percentage in AD patients (Mizuno et al., 2004), while similar results from healthy older adults were obtained just after six days of treatment and were accompanied by improved cognitive functions (Schredl et al., 2001).

### *3.3.2. Sleep Apnoeas*

Another major sleep-related issue in AD is Sleep Apnoea Syndrome. Sleep apnoeas are events when respiratory airflow stops. If this occurs more than five times per hour or if the airflow stops at times for over ten seconds, the diagnosis of Sleep Apnoea Syndrome (SAS) may be posited (Zhao et al., 2021). Apnoeas are more frequent in older adults, people with higher body-mass index, and men (Lyons et al., 2020). Patients addressed to the sleep clinic with a suspicion of sleep apnoeas usually report excessive daytime sleepiness and tiredness in the morning even after a whole night of sleep, headaches after awakening and decreased concentration capacity across the day. Occasionally, they might complain of sudden awakenings while gasping for air or of dry mouth or sore throat in the morning. Partners may also complete the list of symptoms by adding observed disrupted breathing patterns during sleep or loud snoring. Sleep apnoeas are diagnosed with polysomnography (PSG) at home or in hospital settings. This examination, generally extending over at least one full night of sleep, uses a series of sensors measuring a vast panoply of parameters during sleep, including brain

activity, breathing patterns, blood-oxygen levels and muscle activity. A rather important number of sensors is consecrated to breathing activity and detecting SAS: respiratory effort belts (thoracic and abdominal) measure breathing effort, pulse-oxymetry allows following of blood-oxygen saturation, while an airflow sensor generally measures nasal airflow. A microphone can further be used to detect snoring. All these indicators help pinpointing the presence of apnoeas, as well as the severity of SAS, using the Apnoea-Hypopnea Index (AHI, representative of pathologic respiratory events/hour) as well as the presence of the above-mentioned symptoms. In the current clinical practice, a mild SAS is defined as an AHI between 5 and 15, while an AHI between 15 and 30 is classified as moderate SAS. Severe SAS is diagnosed if AHI surpasses 30. As per the types of apnoeas, three categories are distinguished generally: central apnoeas, obstructive apnoeas and mixed apnoeas. Obstructive Sleep Apnoea (OSA) is the most common type, as its prevalence alone is estimated at 1 billion people between 30 and 69 years (Lyons et al., 2020) around the globe, although the vast majority (as much as 98%) of them may remain undiagnosed and, therefore, untreated. OSA is defined as “recurrent episodes of upper airway collapse and obstruction during sleep, associated with arousals with or without oxygen desaturation” (Rundo, 2019), however, respiratory rhythm is still maintained (Zhao et al., 2021). While in the case of OSA, the reason for apnoeas may be anatomical, central sleep apnoeas (CSA) are related to insufficient signalling from the brain to the muscles involved in respiratory movements. Therefore, contrary to OSA, the abdominal and thoracic belts would not detect any increased breathing effort for CSA, which helps distinguish between the two types of apnoeas during PSGs. The third type, mixed sleep apnoea (MSA), is detected when a central apnoea is followed by an obstructive one in a short interval (Zhao et al., 2021). The most common treatment is continuous positive airway pressure (CPAP) therapy, which includes a device providing constant air pressure throughout the night for the patients via a mask or a nosepiece. Given that sleep apnoea is also associated with temporary increases and drops in artery pressure as the body tries to compensate for the obstruction of the upper airways (Bitwise, 1996), if left untreated for longer periods, SAS increases the risk of high blood pressure, coronary heart disease or even of stroke (in Zhao et al., 2021).

However, SAS may also contribute to dementia and was suggested as a risk factor for MCI and, subsequently, AD (Mander et al., 2016), with a younger age of onset (Osorio et al., 2015). The

causality in this relationship may be inferred by adding the detail from the study of Osorio and colleagues (2015) that in the group of patients equipped with CPAP treatment, dementia onset was delayed by approximately two years. To highlight the weight of this delay, it is of interest to note that in 2007, it was estimated that delaying AD onset by as little as a year could lead to 9.2 million fewer cases in 2050 (Brookmeyer et al., 2007). Even a one-year delay could substantially impact people living with AD and have major socioeconomic impacts, given the global cost of dementia. Furthermore, as short as three weeks of CPAP treatment for AD patients significantly improved performances on a verbal learning task (Hopking Verbal Learning Test, Revised) and a psychomotor speed and attention-centred task (Trail making test B). Furthermore, it was also found that untreated patients with severe sleep apnoeas do not only perform worse in general on cognitive tests and depression scales but a decrease in grey matter volume at the level of the posterior parietal cortex, the superior frontal gyrus and the parahippocampal gyrus was also noted (Canessa et al., 2011). Interestingly, three months of CPAP treatment increased grey matter volume, especially at the parahippocampal gyrus. This increase was correlated with performances on several tests measuring short-term memory and executive functions (Canessa et al., 2011).

This impact of sleep apnoeas on cognition and the link with AD was first mentioned in 1981. However, a milder “intellectual deterioration” was already noted in 11 out of 15 middle-aged SAS patients in 1978 (Guilleminault et al., 1978; Smirne et al., 1981; cited in Bliwise, 2002). According to current approximations, 40-70% of AD patients are suspected of having SAS (in Peter-Derex et al., 2015). The link between dementia and apnoeas may be attributed to the continuous disruption of clearance mechanisms responsible for eliminating A $\beta$  and pTau (such as the glymphatic system detailed in the **General discussion**) and to the repetitive hypoxia to which the brain is subjected during apnoeas. Furthermore, in animal models with hypoxia, a down-regulation of cholinergic transmission and a particular decrease in cholinergic concentration in the hippocampal formation was observed (Shimada, 1981). This down-regulation in the case of sleep-apnoea-related hypoxia during sleep could potentially aggravate AD symptoms due to the primary loss of a large proportion of cholinergic neurons early in the disease course. And indeed, apnoeas may be present quite early as a positive association between higher A $\beta$  loads and SAS-related indices (e.g. AHI, oxygen desaturation indices) has already been reported in a small cohort of MCI patients (Spira et al., 2014). This

was later confirmed in even pre-MCI participants with only subjective cognitive impairment but cognitive performances still in the normal range (Liguori et al., 2017). In this latter study, a group of 25 untreated participants with *a minima* moderate OSA without CPAP (AHI > 15), 10 patients with the same level of OSA but equipped with CPAP, and 15 controls without OSA underwent a PSG, as well as a battery of neuropsychological tests, an MRI and a lumbar puncture for AD biomarker and lactate level assessment after the PSG. Interestingly, CSF-A $\beta$ <sub>42</sub> levels decreased to below-cutoff levels in the group of OSA patients without CPAP treatment only, while values were in the normal range for the other groups. This result was replicated in another study using PET scans and CSF biomarkers in healthy older adults, MCI patients and AD patients to address the link between sleep apnoea and AD. Increased annual uptake of florbetapir (indicating more important cerebral A $\beta$  deposition) and decreased CSF-A $\beta$ <sub>42</sub> levels as well as increases in CSF T-and P-tau levels were observed only in the OSA patients from the healthy older adult group and the MCI group but were no longer significant for the AD group. This gives further ground to the suggestion of Branger and collaborators (2016) that the main drive for A $\beta$  accumulation during sleep could be the pathologic respiratory events and not the disruption of sleep architecture *per se*. Furthermore, given that the correlation only exists in the early stages, this accumulation could be underway years before AD onset and potentially drive A $\beta$  deposition, at least in SAS/OSA patients.

All these factors contributing to fragmented sleep patterns, however, potentially contribute to or even accelerate cognitive decline by disrupting one of the most crucial functions of sleep for the brain: sleep-related memory consolidation.

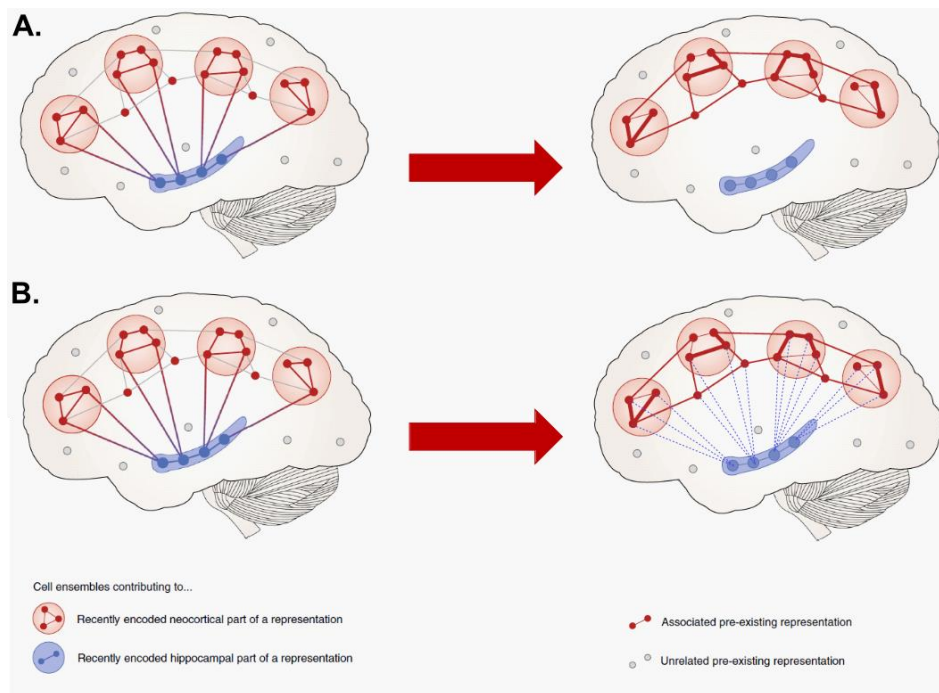
### 3.4. Sleep-related memory consolidation

#### *3.4.1. Systemic consolidation*

Over the past fifty years, an impressive panoply of theories have been proposed and refined concerning the role of sleep in memory consolidation, which was extensively reviewed recently (Klinzing et al., 2019; Rasch & Born, 2013; Watson & Buzsáki, 2015). A theory of a memory consolidation mechanism was first postulated by Marr in the early seventies (Marr, 1970, 1971). He suggested that the hippocampus might only serve as a temporal store for

memories, while remote memory formation requires reorganising such stored information at the cortical level. Reactivation (or replay) of patterns of previous waking activities during “offline” periods was later suggested as a mechanism for such reorganization (McClelland & O’Reilly, 1995; Wilson & McNaughton, 1994). Sleep provides ideal slots for such offline consolidation as no incoming input is present, allowing the system to shift from “encoding” mode to “consolidation” mode. This reorganization would also enable the mnemonic trace to become gradually independent of the hippocampus, even though the initial encoding is dependent on both distributed cortical modules and the integrative function of the hippocampus that links the distributed aspects of the trace together (**Figure 27 A**). The idea of such a temporal gradient in the hippocampus dependence of memories has been backed by many studies on anterograde amnesia that describe a temporal gradient for hippocampal dependence of more or less remote mnemonic traces. However, according to the description by Frankland and Bontempi (2005), the episodic component of mnemonic traces seems to escape such dissociation from the hippocampus, independent of the time interval. This led to the multiple trace theory, in which contextual and spatial information of a given mnemonic trace remains dependent on the hippocampus even though the semantic component transfers to “cortical only” storage (Nadel & Moscovitch, 1997) (**Figure 27 B**). This is also coherent with AD symptomatology, where episodic memory decline and a loss of hippocampal integrity are among the first signs of the disease.

These observations converged, giving rise to the currently accepted active system consolidation theory (Diekelmann & Born, 2010; Marshall & Born, 2007). This framework is still based on the theory of systemic consolidation allowing remote semantic memories to claim their independence from the hippocampus. However, it stipulates that even though encoding occurs initially at both cortical and hippocampal levels, the cortical hubs are a slow-learning store compared to the fast-learning hippocampal memory hub. Therefore, the hippocampus can act as a “trainer” for the slow-learning store as it gradually integrates the new memory in the pre-existing network (Diekelmann & Born, 2010), without creating interference with previous, eventually similar memories. According to this model, this slow learning process occurs during sleep in the form of repeated reactivations or *replays* of time-compressed activation patterns of the mnemonic trace of previous waking experiences (Buzsáki, 1998).



**Figure 27. Classic and multiple trace models of memory consolidation.** According to the standard theories of memory consolidation (**A**), the memory traces initially encoded both in the hippocampus and distributed cortical hubs would become independent of the hippocampal storage over repeated sleep episodes. Therefore, the hippocampus would not be needed for future recall. Somewhat contrary to that, according to the Multiple Trace Theory (**B**), while most aspects of a given mnemonic trace would indeed become independent of the hippocampal hub, the episodic components (marked with dashed blue lines) would always depend on the hippocampus. Figure adapted from Klinzing et al. (2019).

### 3.4.2. Sleep microarchitecture – the scaffold of memory consolidation

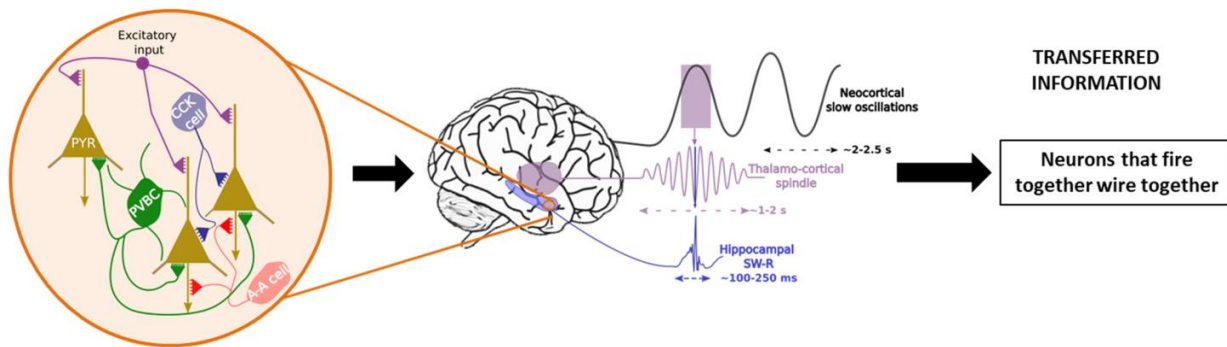
Replay occurs during NREM sleep and, to a lesser extent, during different wakeful behaviours (Buzsáki, 1989). During these states, the synchronized activity of three oscillators (discussed below but also see Diekelmann & Born, 2010; Dudai et al., 2015) enables the transmission of these traces from the hippocampus to the cortical networks (**Figure 28**).

The first group of these three oscillations are cortical slow oscillations (SO) which are approximately 0.75 Hz oscillations whose function in memory consolidation is suggested by the observed positive correlation between learning and the SO amplitude during the ensuing rest period. Moreover, inducing SOs via transcranial magnetic stimulation increases the consolidation of hippocampus-dependent memories (Marshall et al., 2006; Mölle et al., 2009). Furthermore, in AD, the quantity of SOs decreases in an  $A\beta_{1-42}$  load-dependent manner (Helfrich et al., 2018), further pointing toward the importance of these oscillations for successful memory consolidation. The other role of these large-amplitude waves is the

synchronization of neuronal activity by the creation of fluctuation between hyperpolarizing “down-states”, characterized by attenuated neuronal firing, and depolarizing “up-states” during which firing activity reaches levels observed during wakefulness (Steriade, 2006; J. Born & Wilhelm, 2012). Most notably, SOs also coordinate the occurrence of the two other oscillations vital for memory consolidation, namely thalamo-cortical spindles and hippocampal sharp-wave ripples (Sirota et al., 2003).

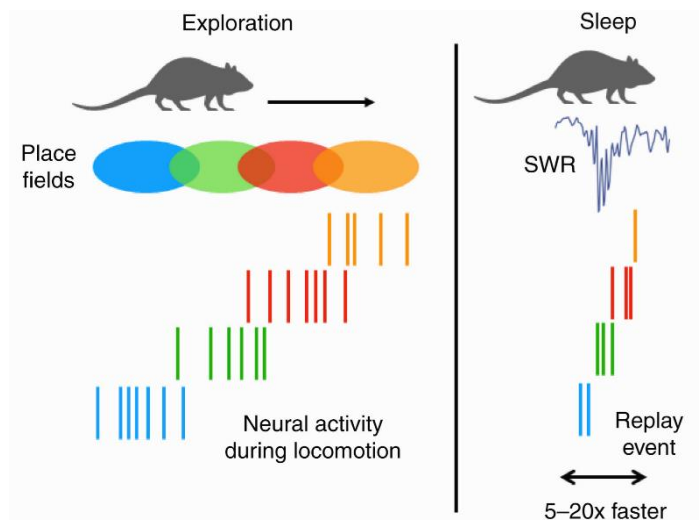
The above-mentioned thalamo-cortical spindles are waxing-and-waning oscillations of 0.5-3 seconds between approximately 10-16 Hz (see Buzsáki, 2015 for a detailed description). Spindles occur on the depolarized up-state of SOs (Clemens et al., 2007), although the literature usually differentiates further between centroparietal fast (13-15 Hz) spindles, indeed phase-locked to the ascending phase of SOs and frontal slow (10-12 Hz) spindles that occur on later (~200 ms) phases of SOs (Andrillon et al., 2011; Mölle et al., 2011). The timing of SO-spindle coupling has been reported to be related to hippocampus-dependent memory processes (Latchoumane et al., 2017), while the density and duration of spindles during post-learning sleep have been continuously shown to be positively correlated with better performances on declarative or episodic memory-related tasks (Clemens et al., 2005; Cox et al., 2012; Griessenberger et al., 2012; van der Helm et al., 2011). However, during ageing, spindles’ density and duration decrease, their frequency slightly increases (Crowley et al., 2002; Mander et al., 2014; Nicolas et al., 2001), and their precise phase-locking on SOs is disturbed (Helfrich et al., 2018). These changes are even more marked in AD and MCI (Liu et al., 2020), which seems to be especially true for fast spindles (Gorgoni et al., 2016), further impacting memory consolidation efficiency (Kam et al., 2019; Rauchs et al., 2008). Spindles are hypothesized to be essential to consolidation as they provide the network with a time window without interference from external stimuli (Dang-Vu et al., 2011; Schabus et al., 2012), during which information can travel from the hippocampus to the cortex. Interestingly, a recent work by Kjaerby et al., (2022) has shown that the descending phase of the already mentioned ultra-slow 50-s oscillations of noradrenergic signalling during NREM sleep (Osorio-Forero et al., 2021) is essential for spindle generation, and longer descending phases also promote the spindle-rich intermediate stage that often precedes REM sleep. Using optogenetics in the same study, this enhanced intermediate stage also improved memory performance, once again emphasizing the role of noradrenergic signalling in sleep, neuronal hyperexcitability and memory consolidation.





**Figure 28. Temporal control of memory consolidation-related oscillations.** Under physiological conditions (A), Parvalbumin-positive basket cells (PVBC) are the primary regulators of the firing patterns of pyramidal cells (PYR) in the hippocampus, leading to the generation and control of sharp-wave ripples (SW-R) nested in the trough of sleep spindles, themselves time-locked to the ascending phase of neocortical slow oscillations. This enables the transfer of correct information packets to the “long-term memory stores” from the temporary hippocampal reserve. CCK cells = Cholecystikinin interneurons, A-A cells = Axono-axonic cells. Adapted from B. Szabo et al. (2022)

The transfer of information relies on sharp-wave ripples (SW-Rs) that are physiological transients of 50-150 ms with the ripple component in the 140-200 Hz frequency range (Gulyás & Freund, 2015). They arise from the hippocampus and are nested on the troughs of spindles (e.g. Clemens et al., 2011; Mölle et al., 2009; Siapas & Wilson, 1998; Staresina et al., 2015). The sharp-wave component originates from the CA3, while the ripple is generated at the level of the pyramidal cells of the CA1 (Buzsáki, 2015; in Halász et al., 2019). Recent results also point towards the involvement of the CA2 sub-region as an initiator of the SW-R complex and as a potential origin of SW-Rs arising following learning tasks involving social memory (Oliva et al., 2016, 2020). Place-cell recordings in rodents (Wilson & McNaughton, 1994) also showed that the time-compressed replay of past waking behaviours mentioned above occurs during SW-R bursts that are nested in the troughs of spindles (**Figure 29**). SW-Rs are therefore regarded as the ideal “packages” of information transfer between the hippocampus and the neocortex (Chrobak & Buzsaki, 1994; Siapas & Wilson, 1998; Sirota et al., 2003; Wilson & McNaughton, 1994). Their role in memory consolidation has been suggested by experiments whereby ripple activity is selectively suppressed during post-training rest periods in rodents (Ego-Stengel & Wilson, 2009; Girardeau et al., 2009), which led to poor post-rest recall performances. Moreover, as is the case with spindles, a marked increase in ripple activity was observed following learning in both rodents and humans.

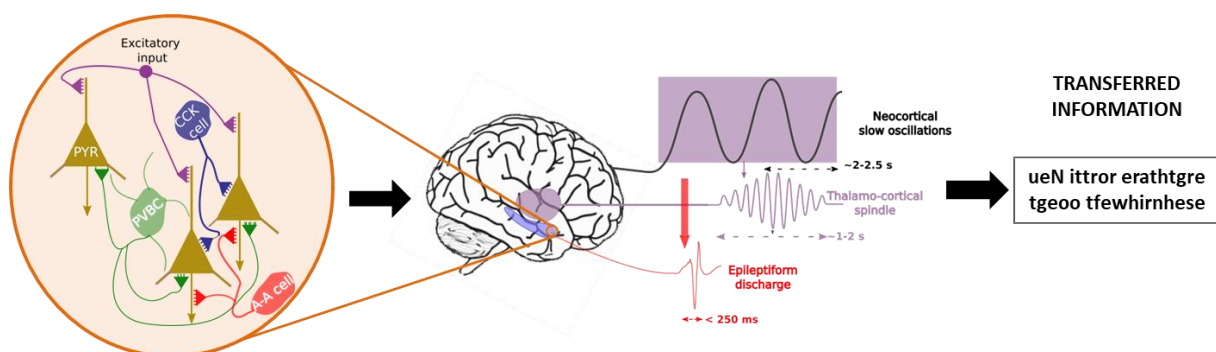


**Figure 29. Schematic representation of replay of past waking behaviour during ensuing sleep periods.** While exploring a new area, hippocampal place fields fire at specific locations as the rodent crosses the field. During the subsequent sleep periods, this firing pattern is repeated in a time-compressed manner (at a 5-20 times faster speed than the original firing pattern). This replay takes place during sharp-wave ripples. Figure modified from Lewis & Bendor (2019).

### 3.4.3. Derailed oscillations during sleep-related epileptic events

However, it is not only fragmented sleep or sleep apnoeas that can disrupt the process of mnemonic consolidation. Indeed, epileptic activities, be they ictal or subclinical, early- or late onset (Bower et al., 2015; Galer et al., 2015; Verrotti et al., 2014), may disrupt sleep-related memory consolidation itself. Indeed, the finely tuned oscillatory activity between SOs, spindles and SW-Rs can be seen as the meticulously synchronized playing of an orchestra. However, as with complex symphonies, at such high levels of precision, there is always an increased probability of errors that can quickly derail into cacophony. This same risk is highly inherent in consolidation-related oscillations where a minor discrepancy – such as an aberrant spike – suffices to push the system into a pathological state. In fact, even before the theories of memory consolidation, it was suggested that the mechanisms of remote memory formation and those of epileptic activities share many similarities (Goddard & Douglas, 1975; in Halász et al., 2019). Based on the well-known Hebbian principle (neurons that “fire together, wire together”), this early suggestion evoked a “hijacking” of learning mechanisms by epileptic discharges. Recent results even incited Halász and colleagues (2019) to suggest that epilepsy (even simple EAs) is a derailment of plasticity-related mechanisms rather than an external force that sometimes profit from a pre-existing mechanism to wreak havoc in the brain. This risk of derailment is all the more increased for SW-Rs, which are the most synchronous

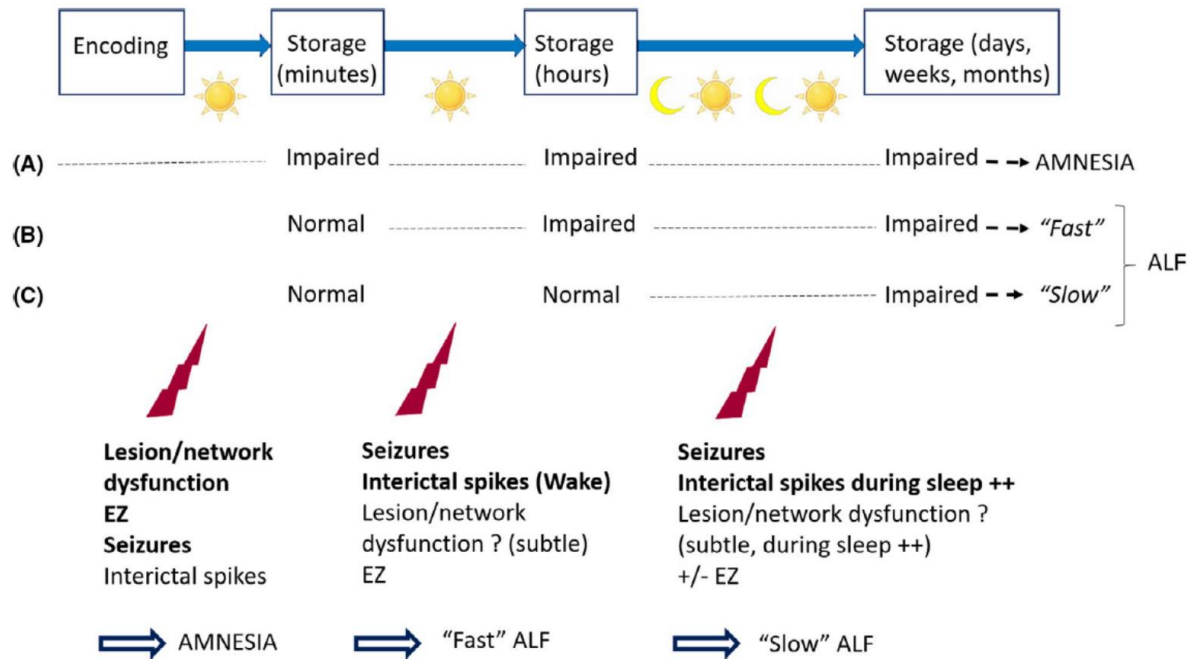
physiological oscillations that occur in all mammalian species (Buzsáki, 1989, 2015), as ~10-18% of hippocampal neurons, as well as neurons of the surrounding regions fire during SW-Rs (Watson & Buzsáki, 2015). SW-R initiation is thought to be a result of the coincidental firing of pyramidal neurons, which generates a massive increase in the activity of the recurrent CA3 network, leading to the SW-R (Gulyás & Freund, 2015). However, instead of unsupervised excitation, this activity is regulated by synchronized perisomatic inhibition by parvalbumin-positive basket cells (PVBCs), which would be essential for maintaining the behaviour of physiological SW-Rs (Hájos et al., 2013; Schlingloff et al., 2014). However, when EAs are present, for example, due to a dysfunctional transmission from PVBCs to pyramidal cells, there is a marked decrease in SW-Rs (Gelinás et al., 2016; Karlócai et al., 2014). Remarkably, during such EAs, all other interneuron types step up their firing rates in response to the fallout of the perisomatic inhibition by PVBCs. Nevertheless, their influence on pyramidal neurons via dendritic synapses is insufficient to suppress the induction of EAs (**Figure 30**) (Gulyás & Freund, 2015). What these results suggest altogether is that when PVBCs can respond to the synchronized excitatory activity of pyramidal cells, physiological transients (SW-Rs) are present, but in the absence of sufficient perisomatic inhibition, the result is a pathological SW-R, most often in the form of EA. This under-inhibition might be achieved either by dysfunctional PVBCs or by an abnormally rapid recruiting of pyramidal cells due to neuronal hyperexcitability with which PVBCs cannot keep up.



**Figure 30. Disruption of memory consolidation-related oscillations in case of interneuronal dysfunction.** As mentioned in Figure 28, in physiological conditions, PVBCs are the primary regulators of the firing patterns of pyramidal cells, leading to the generation and control of SW-Rs nested in the trough of sleep spindles, themselves time-locked to the ascending phase of neocortical slow oscillations. This enables the transfer of correct information packets to the “long-term memory stores” from the temporary hippocampal reserve. In the case of PVBC dysfunction, other interneurons (Cholecystinin interneurons, Axono-axonic cells) try to compensate for the diminished inhibitory tone. Nonetheless, they are insufficient due to their more distal synapses on pyramidal cells. This leads to uncontrolled excitation and epileptic events that may induce spindles (Gelinás et al., 2016) which leads to the consolidation of nonsense information. Adapted from B. Szabo et al. (2022)

Accumulating evidence suggests that this disruption of consolidation-related oscillatory activity inflicts serious harm on memory formation in epilepsy. Notably, Frauscher and collaborators (2015) found decreased spindle activity in relation to hippocampal epileptic EAs in epileptic patients, and recently reported specific decreases in spindle density near the epileptic focus, which were associated to attentional deficits (Schiller et al., 2022). Furthermore, in a rat model of TLE, Gelinás et al. (2016) showed that EAs are not only associated with memory consolidation deficits but that they can lead to abnormal induction of spindles, especially during wakefulness and REM sleep, even though spindles never occur during REM sleep under physiological conditions. Since spindles are the safest highway of communication between the hippocampus and the neocortex, information could travel even through such abnormally induced spindles. However, as suggested by Buzsáki (2015), these spindles might not carry learning-related traces, leading to the consolidation of potentially useless or even scribbled cacophony arising from the EAs that elicited them (**Figure 30**). This conclusion is supported by Bower and colleagues (2015), who found, via intracranial EEG experiment on mTLE patients, that neuronal assemblies activated during a seizure benefit from the most remarkable learning-associated plastic changes during the post-ictal sleep period. This suggests a potential detouring of sleep-related memory consolidation mechanisms by EA. Picking up on this thread, another team using iEEG recordings also described dysfunctions in memory consolidation stemming from sleep-related hippocampal ictal or interictal epileptic activities in epileptic patients (Lambert et al., 2020). Interestingly, they found that interictal spike frequency during the NREM sleep following learning was negatively correlated to memory recall at a one-week delay. In contrast, EA frequency during wakefulness was anti-correlated with short-term (30 min) recall. This suggests that hippocampal EAs lead to disrupted consolidation in the fast-learning memory hub (hippocampus), whereas sleep-related EAs have the same effect on the slow-learning cortical hub by disrupting the information transfer that is supposed to occur during NREM sleep-related memory consolidation. They recently completed this work by suggesting a model that explains the accelerated long-term forgetting often experienced by patients with epileptic activities during sleep (Lambert et al., 2021). According to this model (**Figure 31**), the speed of epilepsy-related accelerated forgetting would be related to the timing of the epileptic activities. In case of epileptic events (ictal or interictal) or lesions affecting the encoding process (**Figure 31 A**), organic hippocampal amnesia would ensue. In case of epileptic events

during the wakeful period following learning, the fast-learning system would bear the consequences, leading to a “fast accelerated long-term forgetting” that is measurable even on short-term recall tests (**Figure 31 B**). Finally, if epileptic events occur during sleep, the slow-learning consolidation system would be affected, leading to a “slow accelerated long term forgetting” affecting recall on a longer timescale (from days to months, **Figure 31 C**).

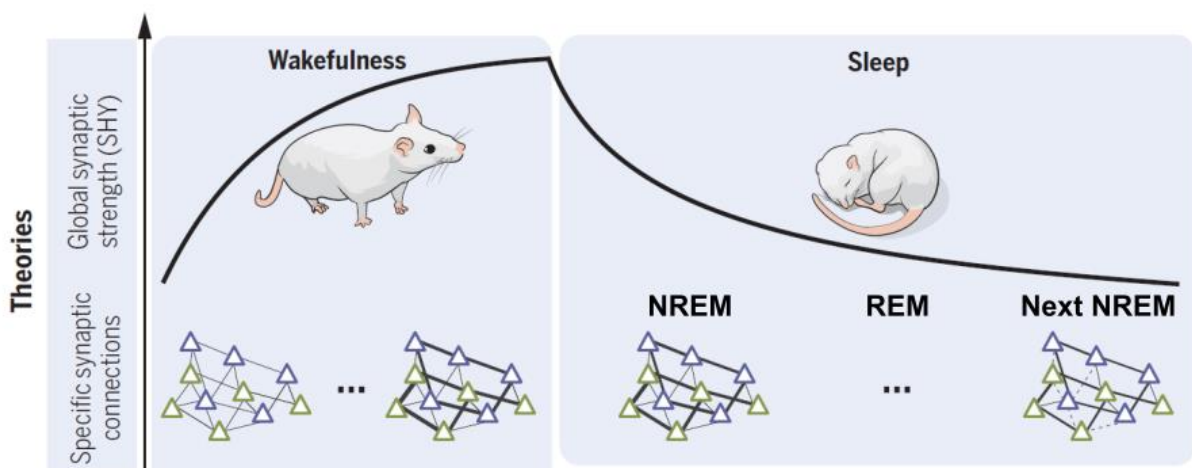


**Figure 31. Model of accelerated long-term forgetting in epilepsy due to interictal events.** According to this framework, the timing of EA or seizures would impact the timescale at which consolidation deficits become detectable. EA or lesions affecting the encoding process would result in hippocampal amnesia (A). When the fast-learning system is disturbed with EA during the wakeful period following encoding (B), a “fast” ALF would ensue, which is detectable on short-term recalls. EAs and seizures occurring during the sleep episodes in the period following learning (C), “slow” ALF would occur with recall deficit only showing after a period extending from a few days to months. From Lambert et al., (2021)

### 3.4.4. Synaptic consolidation deficits – the ancillary damage of epileptic events?

While NREM sleep is involved in the so-called systemic consolidation discussed above, REM sleep might be implicated in more local stabilizing processes (synaptic plasticity) (Ribeiro, 2004; Born & Wilhelm, 2012; Diekelmann & Born, 2010). This role of REM sleep is also consistent with previous hypotheses, which suggest a “local synaptic organizer” or “integrator” function for this specific sleep stage (Giuditta et al., 1995; Grosmark et al., 2012; Tononi & Cirelli, 2014). In fact, although the role of REM sleep in memory consolidation is still disputed, findings point to a role in local, synaptic plasticity-related processes, including an

impact on the expression of immediate early genes related to consolidation, and a general downscaling of synapses leading to the elimination of weak synapses (Synaptic Homeostasis Hypothesis). Downscaling of synapses is essential since the sum of synaptic weights in the brain should maintain a quasi-constant value to avoid hyperexcitability (Watson & Buzsáki, 2015), while global synaptic strength increases during wakeful periods involving the encoding of new pieces of information requiring the formation of new synapses. According to recent models, the SO-spindle-ripple-dependent memory consolidation could, therefore, mechanistically take place during NREM sleep to strengthen consolidation-tagged engrams, while a global downscaling would take place during the following REM sleep (Figure 32). Therefore, EAs or their induced spindles interrupting REM sleep (Gelinas et al., 2016) could increase network hyperexcitability and push the system during further EAs by disrupting downscaling functions generally attributed to REM sleep. The probability that REM sleep is implicated in such processes is all the more increased since evidence shows that firing rates decrease brain-wide after the short periods of REM sleep (Grosmark et al., 2012). Therefore, the interruption of theta oscillations during REM sleep by EAs or induced spindles might lead to an incomplete reduction of synaptic weights and elevated baseline neuronal activity, further aggravating pre-existing activity.



**Figure 32. Proposed model synergistically combining memory consolidation with synaptic downscaling.** According to this model, mechanisms responsible for synaptic homeostasis, mainly synaptic downscaling, can occur during REM episodes, while systemic consolidation, dependent on SO-spindle-SWR coupling, would be limited to NREM sleep. The increased synaptic weight during learning would be therefore downscaled during successive sleep, and while synapses tagged for consolidation would be strengthened during NREM sleep through the synchronous occurring of SO-spindle-ripple complexes, the global weight of synapses could be downscaled accordingly during the subsequent REM stages. Adapted from Girardeau et Lopes-dos-Santos (2021)

However, we have seen that sleep is also fragmented, and REM sleep is significantly decreased even in the earliest stages of AD. Would that mean that downscaling might be dysfunctional in AD, with potential neuronal hyperexcitability? Interestingly, a study by Ju and collaborators (2013) found a link between declining sleep quality and A $\beta$  load. The authors suggested that chronic sleep deficit would entail increased neuronal activity, leading to more important levels of A $\beta$  generation. This suggestion stems from the finding that at non-pathological A $\beta$  levels, A $\beta$  production and neuronal activity are part of a self-regulating feedback loop (Kamenetz et al., 2003). This has been backed by studies showing that increasing the A $\beta$  load by blocking its degradation leads to over-excitation in the hippocampus (Abramov et al., 2009), while elevated or decreased neuronal activity leads to increased or decreased A $\beta$  aggregates, respectively (Bero et al., 2011; Leal et al., 2017; Minkeviciene et al., 2009; Yuan & Grutzendler, 2016). Therefore, the link between AD and epilepsy seems to be an exciting path of investigation – and could easily meet at a crossroads with sleep.

## **CHAPTER IV – SLEEP-RELATED EPILEPTIC ACTIVITIES IN ALZHEIMER’S DISEASE**

### 4.1. The curious case of Johann F.

In fact, more and more studies took this unexplored road in the past two decades and uncovered that there exists, indeed, a bidirectional relationship between epilepsy and AD. Uncovered may indeed be more adept a word than discovered as information on this potential link dates back to the first descriptions of AD – and potentially even before. Indeed, in 1911, five years after presenting the case of Auguste D, Alois Alzheimer described a second case, a patient known in medical history as Johann F (Alzheimer, 1911; translated by Möller & Graeber, 1998). Besides the progressive loss of cognitive functions and autonomy, the 56-year-old man experienced several epileptic seizures toward the later stages of the pathology. This case, together with the detection of amyloid plaque-like structures named sclerotic plaques of neuroglia during the autopsy of an elderly epileptic patient in 1892 (Blocq & Marinesco, 1892; in Cipriani et al., 2011), provided the first clues for a link between AD and epilepsy. However, the investigation of the AD-epilepsy axis failed to become what we would call today a “hot topic” in research. Sparse publications appeared every now and then, mainly advocating a link between FAD and epilepsy, as patients with these rare forms were shown to have a high seizure incidence (Cabrejo et al., 2006; Furuya et al., 2003; Larner, 2011; Shrimpton et al., 2007; Snider et al., 2005; Velez-Pardo et al., 2004; Zarea et al., 2016). As for sporadic AD, for quite a while, this comorbidity was defined as a mere marker of the severe stages of the disease (Mendez et al., 1994; Romanelli et al., 1990). A turn in these trends started with the seminal paper by Amatniek and colleagues (2006), which described an increase in seizure incidence among sporadic AD patients as of the earliest stages of the disease and which considered it a *potential part of the natural history of AD* (Amatniek et al., 2006, p. 871). A smaller longitudinal study published the same year by Lozsadi and Larner echoed these results and suggested the existence of potentially shared pathogenetic processes between AD and epilepsy (Lozsadi & Larner, 2006). Concomitantly, a series of studies in mouse models of AD (harbouring mutations on the genes implicated in FAD) demonstrated the role of A $\beta$  plaques and NFTs in the observed neuronal hyperexcitability, manifesting as subclinical epileptic events (spikes) and rare seizures (Busche et al., 2008; Palop



et al., 2007; Roberson et al., 2007b; Busche et al., 2012). These results fit well into the newly emerging network dysfunction perspective in AD (Palop et al., 2006) and lead to an explosion of publications on the link between AD and epilepsy in preclinical and clinical research. These ended in somewhat concordant but also rather contradictory results over the past decades in terms of clinical results.

## 4.2. Filtering coherence from discordant clinical findings

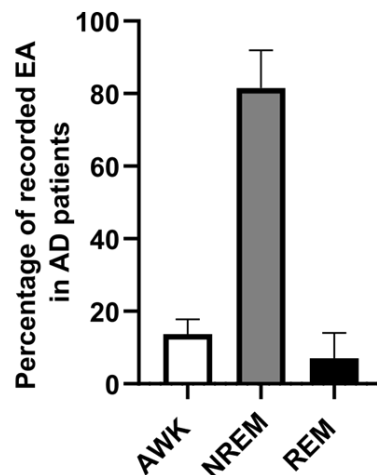
Although incoherence might as well be the keyword in clinical results on this topic, several coherent findings emerge from the apparent discordance, and these can guide our understanding of the peculiar link between AD and epilepsy.

### *4.2.1. Silent temporal epileptic events in sleep – and the challenge of their detection*

The first of these coherent findings is that AD-related EA is primarily subclinical and, therefore, complicated to detect – which might explain why this comorbidity has been long overlooked. On the one hand, these EA include subclinical epileptic spikes without a behavioural output, which are difficult to detect due to the limitations mentioned above of scalp EEG. This limitation is well underscored in a paper by Lam and collaborators (2017). They showed, from concomitant intracranial (Foramen Ovale or FO) and scalp EEG in one AD patient that 95% of epileptic events detected on the FO electrodes are not visible on the scalp EEG. These issues already present a bias toward the high underestimation of EA in AD. Another difficulty of epileptic activity detection in AD is that focal seizures with temporary loss of contact (i.e. absence seizures) might be interpreted as a symptom of AD instead of silent epileptic seizures (Horváth et al., 2018; Vossel et al., 2013). Therefore, there is a high chance that aberrant network activities go unnoticed and untreated for extended periods.

The probability of detection is further decreased by the fact that most of these silent events occur during sleep – therefore, even if they would be detectable with a scalp EEG, they will not appear on it as these examinations are generally carried out only in a 20-30 minute interval of wakefulness. However, this sleep-related increase in EA is one of the most consistent

findings in the clinical literature (Horváth et al., 2018; Lam et al., 2020; Vossel et al., 2016) (Figure 33). Such an increase is not surprising in the light of the review of Ng et Pavlova (2013) on non-AD epilepsy, in which they concluded only about a quarter of all seizures (28%) occur during wakefulness, while the rest fall during periods of sleep.



**Figure 33. Mean distribution of epileptic events in AD patients across previous clinical studies.** A NREM-sleep predominance of epileptic events in AD patients is visible, while REM sleep seems to be almost devoid of epileptic events. Data pooled from: (Horváth et al., 2018; Lam et al., 2020; K. A. Vossel et al., 2016). From the study of Lam et al., (2020), data is pooled for AD patients with and without known epilepsy. Note that REM-occurring EA frequency was not reported in Vossel et al., (2016). Figure from B. Szabo et al., (2022)

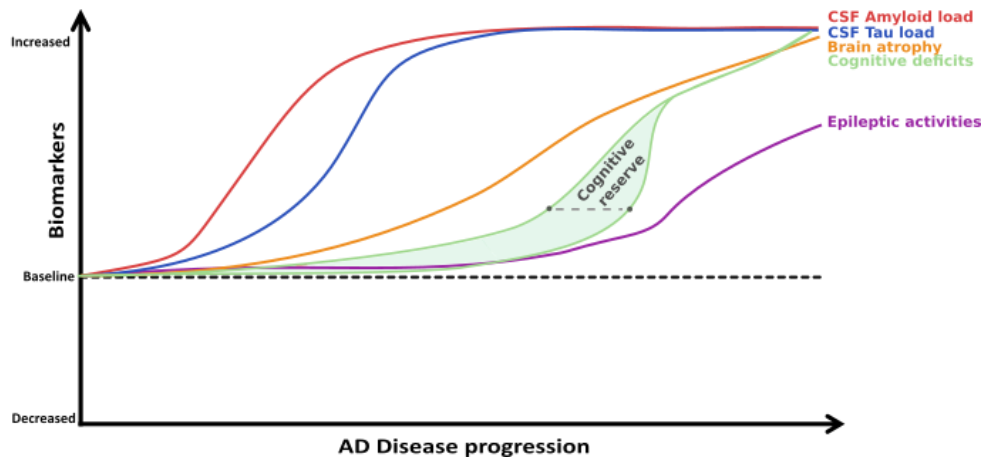
However, when detected, the localization of EA seems to be a relatively coherent cross-study finding. Similarly to AD-independent mesial-temporal lobe epilepsies (mTLE), these aberrant network activities in AD are predominant in the temporal lobes and more often detected on the left hemisphere by EEG (Lam et al., 2020; Vossel et al., 2016). However, concerning the study of Lam and colleagues (Lam et al., 2020), it is of note that while left temporal subclinical EAs were indeed highly predominant in AD patients with no history of epilepsy, patients with a history of seizures were more prone to have bilateral temporal interictal discharges. However, this brings about yet another difficulty concerning the detection of epileptic activities: temporo-basal electrodes, where EA are most readily detected, are not included in the electrode set used in most routine EEG setups. Alternative methods that rank between iEEG and surface electrodes in EA detection capacity and that could replace EEG, such as Magnetoencephalography (M/EEG), have only been applied in two studies on this topic so far with discordant results (Brunetti et al., 2020; Vossel et al., 2016), and given the costs of M/EEG, this incoherence is not likely to be remediated in the near future.

#### *4.2.2. Epileptic events - A disease-accelerating mechanism of cognitive decline in AD?*

The third point of cross-study consensus is that the progression of AD in patients with a co-occurring epileptic syndrome or EAs is faster (Vossel et al., 2016; Volicer et al., 1995; Vöglein et al., 2020; Horvath et al., 2021). AD patients with EA seem to have worse results on cognitive tests than patients without such aberrant network activities (Bernardi et al., 2010; Haoudy et al., 2020; Horváth et al., 2018; Irizarry et al., 2012). This correlation does not seem to be merely related to a longer duration of AD or more severe stages of the disease. First, Vossel et al. (2016) demonstrated that even if patients with and without EAs did not differ at baseline in their mini-mental state examination (MMSE) scores, a follow-up over several years showed a steeper cognitive decline for patients with EAs. Second, Vöglein and colleagues (2020) recently showed that even after adjusting for age and disease duration, the MMSE scores were still unexpectedly low for AD patients with underlying epileptic activity. Moreover, they found that a history of seizures significantly impacted disease severity (as measured by the Clinical Dementia Rating Scale–Sum of Boxes/CDR-SB). Finally, a recent study by Horvath and colleagues described a 1.5 times faster decline in global cognitive performances of AD patients with EA than patients without epileptic abnormalities on their EEGs (Horvath et al., 2021).

This, however, could be explained by a biasing factor that could also explain why in some studies, EAs are found to be an early phenomenon in AD (Amatniek et al., 2006; Vossel et al., 2013), while others state it is a marker of the severe stages of the disease (Mendez et al., 1994; Romanelli et al., 1990): cognitive reserve. Cognitive reserve is a concept where accumulating cognitively stimulating activities over the lifespan helps the brain to more efficiently compensate for eventual structural damages, potentially aiding patients in delaying the onset of symptoms even in the face of rather important structural atrophies (Menardi et al., 2018). However, once the macro- and microstructural damages reach a level where compensation is no longer possible, the disease seems to progress at an excessive speed (**Figure 34**). Interestingly, yet another common cross-study finding is that AD patients with epileptic activities have significantly higher educational levels (Horváth et al., 2018; Vossel et al., 2016) – indicating potentially higher cognitive reserves as well. It could therefore be the case that while these patients are, cognitively speaking, in the milder stages of AD, structural atrophy and biomarker levels are already leaning into more advanced stages, which would

explain the still existing uncertainty of the timing of occurrence of EAs in the AD progression continuum and the faster decline of patients with EA following symptom onset (Vossel et al., 2016; Vöglein et al., 2020; Haoudy et al., 2020).



**Figure 34. Proposed model of biomarker-and cognitive decline progression throughout AD progression.** High cognitive reserves compensate on a functional level for marked Amyloid and Tau aggregation and atrophy may lead to delayed diagnosis. This might explain why EA is most often detected in highly educated participants who are (seemingly) in the mild stages of the disease. However, once compensation is no longer possible, and EA becomes more frequent or probable, the cognitive decline might be steeper, which is compatible with the fast progression noted in AD patients with EA. Figure adapted from (Toniolo et al., 2020) with permission in B. Szabo et al. (2022). The grey dashed line represents the approximate time of diagnosis.

Another coherent finding is that AD patients with comorbid epileptic activities experience an earlier age of onset of cognitive symptoms despite not carrying the mutations known to be involved in FAD (Amatniek et al., 2006; Irizarry et al., 2012; Lam et al., 2020; Mendez & Lim, 2003; Scarmeas et al., 2009; Tábuas-Pereira et al., 2019; Vöglein et al., 2020; Vossel et al., 2013). This indicates a synergistic accelerating of AD-and epilepsy-related factors on cognitive decline, which could lead to a faster progression and an earlier age of onset – even in the presence of potentially higher cognitive reserves – instead of the decade-long process of insidious plaque and NFT deposition.

#### 4.2.4. Cross-study discordance on the link between AD and epilepsy

As per the cross-study incoherences on the topic of AD and epilepsy in the clinical literature, it is essential to note that the prevalence of subclinical EAs (**Table 1**) and epileptic seizures (**Table 2**) in AD patients is highly variable across studies, reaching 64% in some cases (Risse et al., 1990) while barely hitting 2% in others (Giorgi et al., 2016). This is probably due to the extreme variability in research methods (Born, 2015). In fact, several points of divergence

create a snowball effect of inconsistencies in the results. One such point is the diagnostic criteria of Alzheimer's disease fixed in a given study as inclusion criteria. While most papers follow NINCDS-ADRDA or IWG-2 criteria, some authors base the diagnosis mainly on MRI (Brunetti et al., 2020; Horváth et al., 2018) or do not differentiate between AD and other types of dementia (Johnson et al., 2020; Keret et al., 2020; McAreavey et al., 1992; Rao et al., 2009; Stefanidou et al., 2020). Finally, retrospective population-based studies or projects using datasets from large memory clinics or databases cannot verify the diagnostic method for all patients in the cohort (Baker et al., 2019; Blank & Willis, 2019; Cook et al., 2015; Imfeld et al., 2013; Lyou et al., 2019; Sherzai et al., 2014; Zelano et al., 2020). Using only imagery and neuropsychological data for diagnosis might inflate the percentage of "AD" patients with epilepsy, given that patterns of cortical atrophy and cognitive symptoms can be highly overlapping in epilepsy and AD (Noebels, 2011) or aMCI (Kaestner et al., 2021). On the other hand, while around two-thirds of all dementias are due to AD, mixed cohorts of dementia patients might also affect the observed prevalence of epilepsy and dementia due to AD.

Another vital consideration is study type. Many previous publications consisted of retrospective cohort studies, which, besides having an inherent risk of including a relatively high percentage of misdiagnosed dementia patients, are often carried out by a meticulous sweep of patients' medical history (e.g. DiFrancesco et al., 2017). Even when the problem of misdiagnosis is averted by the availability of post-mortem pathology reports confirming AD (as in Rauramaa et al., 2018), using medical reports to infer the presence of epilepsy brings in several biasing factors. First, although caregivers might observe convulsive seizures, they might not be reported by the patients themselves who might not recall such events. Secondly, while in some cases, EEG results are available in the medical history, they are primarily standard 20-30-minute EEGs without periods of sleep. However, as was mentioned before, subclinical network anomalies or silent seizures in AD occur mainly during sleep (Horváth et al., 2018; Lam et al., 2020; Vossel et al., 2016). Accordingly, Horvath et al. (2017) have shown that a 1-hour EEG recorded from AD patients between 0:00 and 8:00 (potentially including periods of sleep) is 16.5 times more sensitive in detecting epileptiform events than a recording of the same length during the day (from 8:00 to 16:00, mainly containing wakeful sections). Moreover, recording length strongly correlates with EEG sensitivity. Therefore, there is no comparability between results obtained with 30-minute recordings and those obtained after eight or even 24-hour recordings.

Publication	Mean age in years ± SD (n/group)	AD diagnostic criteria	AD severity (MMSE/ACE/CDR mean + SD*)	History of epilepsy	Excluded medications or substances	EEG type and duration	EA detection	% with EA or incidence	Risk factors for EA
Liedorp et al., 2010 (211)	1. AD-no EA: 63 ±10 (500) 2. AD+EA: 71 ± 9 (10)	NINCDS-ADRDA	MMSE = 25±5	Included	NS, but accounted for in the analyses	Standard 30-min EEG	1 neurophysiologist	2% of AD cases	Younger age, Disease severity (trend)
Vossel et al., 2013 (111)	At diagnosis: 1. AD+EPI: 69.1 ± 9 (35) 2. AD-no EA: 74.5 ± 10.3 (969) 3. MCI+EPI: 68 ± 7.8 (12) 4. MCI-no EA: 74.6 ± 9.5 (216)	1. AD: NINCDS-ADRDA 2. MCI: IWG	MMSE range = 4-30	Included	Alcohol/substance abuse	Standard 20-min EEG for a sub-group of 152 participants	Multidisciplinary team of experts	Groups 1, 3: 24/39 Groups 2, 4: 7/113	Younger age at onset
Vossel et al., 2016 (100)	1. AD 61.7 ± 7.4 for (33) 2. CTRLs: 65.3 ± 5.6 (19)	NIA-AA	MMSE range: 1. AD = 18-24 2. CTRLs = 29-30	Not included	BZD Antipsychotics Narcotics Antihistamines. Substance abuse	Overnight vEEG and 1h M-EEG	1 epileptologist + 1 neurophysiologist per exam type, blinded	1. AD: 42,4% 2. CTRLs: 10.5%	No risk factor, but faster decline
Horvath et al., 2018 (110)	1. AD-no epilepsy: 79.4±7.4 (20) 2. AD + seizures: 75.9±4.7 (10); 3. AD+IEDs: 73.6±9.3 (12)	NINCDS-ADRDA	ACE 1. AD-no EA = 67.4±9.4; 2. AD + seizures = 38.4±15.3; 3. AD + IEDs = 44.5±7.4	Included (if onset < 10y before AD)	Antipsychotics Antidepressants Antihistamine BZD	24h ambulatory EEG	Two independent neurophysiologists (1 blinded). ILAE definition of epilepsy diagnosis	28% IED without seizures + 24% with seizures	AD severity; High VLOM ratio; Higher educational level
Brunetti et al. 2020 (191)	1. AD: 73 ± 7 (50) 2. MCI: 72 ± 6.7 (50) 3. CTRL: 69 ± 6.7 (50)	NIA-AA and DSM-IV	MMSE = 18.38 ± 4.7	Not included	Psychoactive/hypnotic drugs History of alcohol/substance abuse	Full-night vEEG (134/150 participants)	Automatic followed by two neurophysiologists' verification (blind)	AD: 6.38% (3/47) MCI: 11.63 (5/43) CTL: 4.54% (2/44)	-
Lam et al., 2020 (118)	1. AD-no EA 76.3±7.2 (41) 2. AD + EA 69.6±7.6 (15) 3. CTRLs: 72.6±9.1 (43)	NIA-AA	MMSE range: 13-30	Included (separate group)	BZD/"sleep aids" AMS (for groups 1, 3)	24h ambulatory EEG	Two steps: 1. 2 epileptologists 2. A panel of 9 epileptologists	AD-no EPI: 22% AD+EPI: 53.3% CTRL: 4.7%	SSS-like waveforms; Earlier age of onset
Vossel et al., 2021 (227)	1. UCSF branch: 60.2 ± 7.1 (18) 2. UMN branch: 64.8 ± 7.8 (16)	NIA-AA	MMSE > 17 CDR < 2	Included (if onset < 5y before screening)	BZD ASM Narcotics	Overnight vEEG and 1h M-EEG	1 epileptologist and 1 clinical neurophysiologist (both blinded); + SpikeDensityV101	38,20%	Tendencies (p = 0.054) for females and APOE4 carriers towards AD + EA

\*Unless otherwise stated. Abbreviations: ACE =Addenbrooke's Cognitive Examination; AD = Alzheimer's Disease; ASM = Antiseizure Medication; BZD = Benzodiazepines; CDR = Clinical Dementia Rating; CTRL = Controls; EA = Epileptic/Epileptiform activity; IED = Interictal Epileptiform Discharge; IWG criteria = AD diagnostic criteria based on the propositions of the International Working Group ; MCI = Mild Cognitive Impairment; MMSE = Mini Mental State Examination (Folstein version); NIA-AA criteria = AD diagnostic criteria based on the propositions of the National Institute on Aging and the Alzheimer's Association; NINCDS-ADRDA criteria = AD diagnostic criteria based on the proposals of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association; NA = Not applicable; NS = Not Specified; vEEG = Video EEG; VLOM Ratio = Ratio of Verbal Fluency and Language ACE subscores over the Orientation and delayed recall subscores

Table 1. Main inclusion criteria and results of clinical studies exploring the prevalence of epileptic activities in AD patients

**Table 2. Main inclusion criteria and results of clinical studies that explore the prevalence of seizures in AD patients**

Publication	Age mean in years ± SD * (n/group)	AD diagnostic criteria	Disease severity (MMSE/ACE/CDR mean + SD*)	History of epilepsy	Excluded medications or substances	EEG type or seizure information source	Seizure detection	% with seizures per group or incidence	Risk factors for seizures/epilepsy diagnosis
Romanelli et al., 1990	1. AD: 71.5 ± 4.9 (44) 2. CTRL: 71.7 ± 4.9 (58)	Criteria from Morris et al., 1988	NA	Not included	- Alcohol abuse - "Other medical illnesses" with a higher risk of seizures	- Standard EEG - Questions at follow up - Medical/Nursing home records	Questions at follow up; medical records; nursing home records	15.9% of patients, 0% of controls	- Severe stage
McAreavey et al., 1992	Age > 55 (208 dementia cases)	ICD-9	MMSE range: 0-24 MMSE means: 1. Seizure: 2.5 2. No seizure: 4.8	Included	NS	- Questions to nursing and medical staff	- AMS prescriptions - Medical records - Nursing home staff	9.1% (84% of those with AD)	- Younger age - Earlier age at admission
Mendez et al., 1994	Age NS (446 AD patients)	Medical records and autopsy	NS	Included	NS, but alcohol abuse and "other medical illnesses" accounted for	- Questions to family members, nursing home staff and physician	- Medical records - Nursing home staff - Physician - Questionnaire for family members	17.30%	- Younger age of onset, - Advanced AD stage
Volicer et al., 1995	1. AD+EA: 70.6±4.4; 2. AD-no EA: 72.4 ±3.5 (75 DAT altogether)	NINCDS/ADRDA or DSM IIIR	NS for all patients, but severe cases	Included	NS	- Interview with caregiver if history of seizures was found	- Observation - Medical records - Interview with caregiver	21% after being institutionalized (36% when pre-existing epilepsy counted)	- Faster decline, especially in language ability
Lozsadi and Larnar, 2006	Range at diagnosis: 49-84 (177)	NINCDS-ADRDA	NS	Included	No	- Medical records	From medical records, classification based on ILEA criteria	6.8%	- NS
Amatniek et al., 2006	NS, but age > 50 (233 probable AD)	NINCDS-ADRDA	MMSE ≥ 16	Not included	Antipsychotics Drug/alcohol abuse	- Standard EEG for 58.37% of participants - Question on seizure occurrence since last visit	Two neurologists' evaluation based on questionnaires and medical records	0.87%	- Younger age - African-American ethnicity. - AD severity at initial visit - Longer duration of symptoms - Lower educational level - Focal epileptiform findings - Depression (for younger participants)
Rao et al., 2009	Mean: 76.9 in the seizure group (1,738 dementia cases altogether)	Medical records	NS	Included	NS	- EEG for 74% of patients with seizures - Medical records	Medical records	3.6% (2.24% confirmed, 48.7% of those had AD/MCI)	-

**Table 2. Continued**

<b>Scarmeas et al., 2009</b>	74.4 ± 8.9 at entry (453 AD)	NINCDS-ADRDA or DSM IIR	<b>Baseline MMSE:</b> 21 ± 3.3 All patients > 15	Included	None, but many taken into account in the analyses	- EEG for 21 out of 52 with suspected epilepsy - Follow-up interview every 6m - Medical records	2 epileptologists reviewed medical records and interviews	1.5% of patients	- Younger age
<b>Bernardi et al., 2010</b>	78 ± 7.2 (145 probable AD)	NIA-AA	<b>MMSE =</b> 19.9 ± 6.3 (Range: 3-27)	NS	None but: antidepressants + antipsychotics accounted for	- EEG for 21/145 patients at baseline (and for all with identified seizures) - Medical records	Based on ILAE criteria [57], by one epileptology expert	9.7% of AD	- Gender (male) - AD severity - Hyperlipemia
<b>Irizarry et al., 2012</b>	74.5±9.5 (3,087 AD from 10 previous studies)	NINCDS-ADRDA	<b>MMSE range:</b> 10-28	Not included	No, but taken into account in the analyses	- Only data available from the previous studies	Verbatims from clinical trials	4.84/1,000 py	- Younger age - AD severity at baseline - Antipsychotic use
<b>Imfeld et al., 2013</b>	<b>1. AD:</b> 80.7±6.7 (7,086) <b>2. CTRLs ~ age matched (7086)</b>	Algorithm, from diagnostic coding from GPs	<b>NS</b>	Not included	No, but verified for antipsychotics + antidepressants	- UK General Practice Research Database	From GP coding	<b>AD:</b> 5.6/1,000 py; <b>CTRL:</b> 0.8/1,000 py	- Longer disease duration
<b>Vossel et al., 2013</b>	<b>At diagnosis:</b> <b>1. AD+EA:</b> 69.1 ± 9 (35) <b>2. AD-no EA:</b> 74.5 ± 10.3 (969) <b>3. MCI+EA:</b> 68 ± 7.8 (12) <b>4. MCI-no EA:</b> 74.6 ± 9.5 (216)	<b>1. AD:</b> NINCDS/ADRDA <b>2. MCI:</b> IWG	<b>MMSE range:</b> 4-30	Included	Alcohol/substance abuse	- Standard 20-min EEG for a sub-group of 152 participants; - Medical History	Multidisciplinary team of experts	2.86% of AD, 5.26% of MCI	- Younger age at onset
<b>Cook et al., 2015</b>	<b>Mean</b> ≈ 80 (n = <b>11,042</b> AD and <b>11,042</b> non-AD dementia)	- Diagnosis codes CPRD	NS	NS	Antipsychotics + antidepressants accounted for	- Diagnosis codes from GPs	GP diagnosis codes and follow-up questionnaire	<b>AD:</b> 8.8/1,000 py <b>Non-AD dementia:</b> 1.7/1,000 py	- Stroke - Antipsychotics prescribed within 6m
<b>Giorgi et al., 2016</b>	<b>At diagnosis:</b> 69.6 ± 8.5 (1223 AD)	NINCDS-ADRDA	<b>MMSE Range =</b> 3-29 <b>Median =</b> 18.67	Included	NS	- EEG for a subset of patients only - Medical records	From medical records	2.45% (1.63% without patients with concomitant lesions)	- NS
<b>DiFrancesco et al., 2017</b>	<b>At onset:</b> 75 ± 7 (1,371)	NIA-AA criteria	NS, probably advanced	Included	Antipsychotics Antidepressants Alcohol/drug abuse	- EEG for a subset of patients only - Medical records	From medical records (with EEG when available)	1.68% before AD + 1.16% after AD onset	- Earlier onset of cognitive decline
<b>Horvath et al., 2018</b>	<b>1. AD-no EA:</b> 79.4±7.4 (20) <b>2. AD+seizure:</b> 75.9±4.7 (10) <b>3. AD+IEDs:</b> 73.6±9.3 (12)	NINCDS-ADRDA	<b>ACE</b> <b>1: AD-no EA =</b> 67.4±9.4; <b>2. AD + seizures =</b> 38.4±15.3; <b>3. AD + IEDs =</b> 44.5±7.4	Included (if onset < 10y pre- AD)	Antipsychotics Antidepressants Antihistamine BZD	- 24h ambulatory EEG - Epilepsy-related information collection	2 neurophysiologists (1 blind to condition), ILAE definition of epilepsy diagnosis	24%	- AD severity - High VLOM ratio - Higher educational level
<b>Rauramaa et al., 2018</b>	85 ± 8.6 (64 AD - 7 mixed dementia)	NINCDS-ADRDA and autopsy	NA	Included	NS (but no alcohol abuse)	- For 10/11 patients with epilepsy - Medical records	EEG, medical records	17.20%	- Younger age of onset - Longer disease duration



**Table 2. Continued**

<b>Baker et al., 2019</b>	75.1 ± 7.07 (n = 72 at 1-year follow-up)	NIA-AA	<b>CDR-score at BL</b> = 4.2 ± 2.69)	Included	NS	- Structured interview (with informant)	From interview	<b>At follow-up:</b> <b>Probable:</b> 18.06%; <b>Possible:</b> 19.44%	- Worse score on CBI-R at baseline - Worse cognitive functions at follow-up (problem solving, personal care, attentional capacities ↓, daytime sleeping, confusion, fluency difficulties ↑)
<b>Lyou et al., 2019</b>	<b>Age &gt; 70 (4,516 AD; 19,713 CTRL)</b>	ICD-10	NS	NS	No	- Medical database	Diagnostic codes from medical database	<b>At last follow-up</b> <b>1. AD:</b> 13.97 % <b>2. CTRL:</b> 6.05%	- Gender (male) - Hypertension or hyperlipidemia - Chronic kidney disease
<b>Tabuas-Pereira et al., 2019</b>	<b>1. AD+EA:</b> 68.4 ± 8.4 <b>2. AD-no EA:</b> 68.1 ± 9.9 (292 AD altogether)	NIA-AA	<b>MMSE</b> <b>1. AD + EA =</b> 16.2± 64 <b>2. AD – no EA:</b> 20.8 ± 7.4	Not included	NS on medication No alcohol abuse	- EEG for patients with suspected epilepsy - Medical charts	Retrospectively from medical files, backed by EEG	17.8%	- Earlier onset of dementia - Lower MMSE even at baseline - Higher tau load
<b>Stefanidou et al., 2020</b>	<b>1. Dementia:</b> 83 ± 7 (660) <b>2. CTRLs:</b> 83 ± 7 (1,980)	DSM IV criteria	NA	Not included	NS	- EEG at least for a subset, but NS - Medical charts - ICD-9 codes - Follow-up interviews	Scoring of epilepsy probability by 2 epileptologists, based on ILAE criteria	<b>1. Dementia group:</b> 2.9% <b>2. CTRLs:</b> 2%	-
<b>Vöglein et al., 2020</b>	<b>1. AD pre-symptomatic:</b> 79.8 ± 7.7 (498) <b>2. Impaired-no-MCI:</b> 74.9 ± 9.0 (43) <b>3. AD MCI:</b> 75.1 ± 8.3 (859) <b>4. AD:</b> 75.1 ± 9.9 (9,127) <b>5. CTRLs:</b> 69.8 ± 10.9 (10,218)	NINCDS/ADRDA or NIA-AA	<b>MMSE</b> <b>1. Pre-sympt.:</b> 28.2±1.8 <b>2. Impaired-no MCI:</b> 27.2±2.6 <b>3. MCI:</b> 26.1±2.7 <b>4. AD:</b> 19.5 ± 6.7 <b>5. CTRL:</b> 28.9 ± 1.4	Included	NS	- Interview with participant and “co-participant” - Medical records - Observation	- Interview with participant and “co-participant” - Medical records - Observation	- 2.93% all confounded - 3.14% of AD	- Earlier age of AD onset - Worse cognitive and functional state - Longer AD duration
<b>Zelano et al., 2020</b>	<b>Age range =</b> 32-107 ((25626 non-mixed dementia AD case); 223933 CTRLs)	ICD-10	NS, probably broad range	Included	NS	SveDem database	ICD-10 and ICD-9 codes, meeting ILAE criteria	<b>1. All cases:</b> 4%, <b>2. LOAD:</b> 2.1% <b>3. EOAD:</b> 5%	- Young age, - Gender (male) - History of stroke/head trauma/brain tumor, - Lower MMSE
<b>Blank et al., 2021</b>	<b>Median =</b> 84 (178593 probable AD)	Medical records from various sources	NS	NS	NS	- Medical records	Mention of epilepsy diagnosis in records	4.45% of AD	- Stroke - Depression - African-American ethnicity

\*Unless otherwise stated. Abbreviations: ACE =Addenbrooke’s Cognitive Examination; BZD = Benzodiazepines; CDR = Clinical Dementia Rating; CPRD = Clinical Practice Research Datalink; DSM-III-R = Diagnostic and Statistical Manual of Mental Disorders – 3<sup>rd</sup> revised edition; ICD-9/10 = International Classification of Diseases; IED = Interictal Epileptiform Discharge; IWG criteria = AD diagnostic criteria based on the work of the International Working Group ; NIA-AA criteria = AD diagnostic criteria based on the propositions of the National Institute on Aging and Alzheimer’s Association; NINCDS-ADRDA criteria = AD diagnostic criteria based on the work of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer’s Disease and Related Disorders Association; NA = Not applicable; NS = Not Specified; py = person-years; VLOM Ratio = Ratio of Verbal Fluency and Language ACE subscores over the Orientation and delayed recall subscores

**Table 2. Main inclusion criteria and results of clinical studies that explore the prevalence of seizures in AD patients**

Another critical point is the question of inclusion and exclusion criteria. While it is now well established that EA can occur at any stage of the disease, even before symptom onset (Vossel et al., 2013, 2016), its prevalence still strongly correlates with disease severity. Therefore, the form of AD or the inclusion threshold for disease severity (measured by MMSE or other commonly used neuropsychological evaluation) could also influence results. This also applies when deciding whether to include patients with a known history of epilepsy (or with ASM treatment). In such cases, separating the results according to pre-existing epilepsy, as done in Lam et al. (2020), could be a solution toward coherency. However, other neurological antecedents (e.g. stroke, tumours, previous head trauma, etc.) and pharmaceutical treatments that suggest underlying psychiatric or neurologic comorbidities (such as antipsychotics, antihistamines, benzodiazepines, and of course, ASMs) should be considered in exclusion criteria (as in Horváth et al., 2018). Moreover, a large proportion of AD patients are prescribed antidepressant medication, even in the early stages, and including these patients presents yet another issue: While such medication might not have a substantial effect on seizure susceptibility (Kanner, 2016), it strongly impacts the sleep cycle, especially REM sleep and sleep fragmentation (Wichniak et al., 2017). As mentioned earlier, EA in AD is strongly linked to sleep. Therefore, including patients on medications that significantly modify the sleep-wake cycle has a high probability of interfering with EA detection. On the negative side, such strict criteria bring about a relatively small fraction of AD patients that can potentially participate in such studies.

However, over and above the already mentioned difficulties in detecting EA with scalp EEGs, another pressing problem is the detection method and sensitivity. In fact, the majority of authors used manual detection by experts, with some papers relying on one expert (Liedorp et al., 2010), others on two neurophysiologists (Horváth et al., 2018) or epileptologists (Scarmeas et al., 2009) or a combination of these specializations (K. A. Vossel et al., 2016). Most, but not all, experts are blinded to the condition of the participants (AD or control) while analysing the data. Another problem that probably prevents EA detection, even for automatic detection, is the modified characteristics of brain activity in AD patients: their EEGs show more significant slowing than is seen during healthy ageing (Lam et al., 2020; Petit et al., 2004), which is most prominent during REM sleep (Petit et al., 1992). Therefore, these EEGs show a

significant amount of slowing-related activities, which makes the classification of some pathological or unusual but physiological activities rather complicated.

Finally, other issues regarding the scoring of EEG activity are epileptic transients of sleep or sporadic sleep spikes (SSS) and temporal intermittent rhythmic delta activity (TIRDA) which have already been linked to mTLE but which have not yet been established as pathological events. However, as Lam et al. (2020) pointed out, a surprising increase in SSS is seen in AD patients with underlying EA. Defining when these events should be re-evaluated as pathologic would be of interest to improve the characterization of EA as opposed to benign EEG patterns. It is all the more important since the boundary where EEG patterns are considered subclinical or interictal activities is already blurred. The most common definition that authors give for spikes is "sharp waveforms 20 to 200ms, clearly distinct from ongoing background activity, with an associated subsequent slow-wave" (Horváth et al., 2018; Vossel et al., 2016), but this is not unified across studies. In fact, the prevalence dropped from 42% and 48%, respectively, in the Vossel and Horváth studies to as little as ~6% in the study by Brunetti et al. (2020), who defined EA based on stricter morphologic characteristics and required at least 10 EA during the night for it to be considered a patient with EA. However, contrary to preclinical studies (Gureviciene et al., 2019; Jin et al., 2021), there are no recommendations or guidelines for EA detection in AD patients. Such recommendations would greatly facilitate the diagnosis of epilepsy or subclinical EA in AD patients. In their recent article, Sen and colleagues (2018) have already made a step toward formulating such guidelines for neuropsychological data collection. They suggest standardized, culture-independent, short batteries of tests, with baseline evaluation and longitudinal tracking. Methodology-unifying considerations concerning the measurement and detection of epileptic events in AD could help amplify data on mechanisms that may underlie this comorbidity – some of which will be discussed below. In a recent review (B. Szabo et al., 2022), we aimed to create a set of such recommendations for clinicians to facilitate EA detection in AD patients (**Textbox 1**) and a second set for researchers to try harmonizing cross-study methodologies and avoid a maximum of potential biases (**Textbox 2**). This was completed with a questionnaire created in the framework of an end of studies MSc internship by Fleur Gerard (FG). This questionnaire (**Figure 35**) is based on previous papers (Cretin et al., 2016, 2017; Horváth et al., 2018; Vossel et al., 2017) both for research and clinical practitioners to detect potential signs of underlying epileptic abnormalities.

But, in the end, what do these coherences and discrepancies of clinical research tell us about the common underlying factors of epilepsy and AD when put together with the abundant preclinical literature of the past two decades trying to uncover these underpinnings?

1. A vEEG including at least an hour of sleep should be systematically undertaken for patients with sporadic AD if at least one of the following conditions applies: early onset (< 65), presence of APOE4 allele(s), high discrepancy between brain atrophy (MRI) and cognitive functions suggestive of high cognitive reserves, or other indicators of a large cognitive reserve (educational level, occupation, etc.)
2. A vEEG including at least an hour of sleep should be systematically undertaken for patients with familial AD.
3. ASM treatment of patients with epileptic activities should be considered, mainly LEV, until further data is available on other drugs.
4. A search for AD biomarkers should be undertaken for patients with epilepsy if at least one of the following conditions applies: cognitive complaints, late onset epilepsy of unknown origin (especially with indications of a large cognitive reserve), family history of dementia.
5. The importance of improving sleep quality for AD patients should be recognized and a part of the treatment process whenever possible. Sleep apnea or other sleep-related pathologies (e.g. REM-sleep behavior disorder or restless leg syndrome, confusional arousals) should be identified in case of sleep complaints reported by the patient or by the primary caregiver.

**Textbox 1. Clinical recommendations for EA detection in AD patients** (From B. Szabo et al., 2022)

1. When possible, the diagnosis of AD should be based on CSF or amyloid-PET
2. Atypical AD patients should be clearly distinguished in the cohort and verified in the analysis (e.g. (25)).
3. EEG analyses should be based on at least two sources, either the combination of automatic detection with a manual verification *a posteriori*, or by two experts, preferably blind to the study group.
4. IED should be clearly defined, using standardized criteria: 20-200 ms spike or sharp-waves followed by disrupted background activity (25, 111), with a multiphasic character (suggested by (112)).
5. All types of detected epileptic/epileptiform events should be quantified and reported separately (IEDs, SWDs, longer rhythmic synchronizations, seizures, and preferably SSS).
6. A graded quantification according to the certainty of detection should be applied, differentiating between certain and doubtful events (e.g. (24))
7. The presence of patients with known epilepsies should be stated – and handled as a separate group if necessary.
8. Given the potential impacts of different medications on seizure activity, pharmacologic treatments should be stated and verified.
9. Given the subclinical nature of epileptic activities in AD, EEGs should be accompanied by interviews with caregivers on the occurrence of symptoms related to seizures (suggested by (122)). One such questionnaire is provided in **Suppl. material 1**.
10. VEEGs should systematically include a full EEG setup with at least 27 scalp electrodes including lower temporal electrodes.
11. Detailed sleep scoring should be conducted on VEEGs.

**Textbox 2. Methodological considerations for clinical research.** (Adapted from B. Szabo et al., 2022)

**Instructions:** This questionnaire is intended to explore the occurrence of symptoms indicative of prior epileptic seizures. Given the frequent losses of consciousness or amnesic post-ictal episodes, it is preferred for the interview to be conducted in the presence of a caregiver or a person living with the patient.

**Presence of caregiver:**

Yes

No

	ACCORDING TO THE PATIENT		ACCORDING TO CAREGIVER (IF APPLICABLE)	
	YES	NO	YES	NO
<u>1-</u> SENSORY DISTURBANCE (UNPROVOKED PAIN, TINGLING, PRICKING, NUMB SKIN)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<u>2-</u> UNEXPLAINED SENSATION OF FEAR	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<u>3-</u> TRANSITORY MEMORY IMPAIRMENT	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<u>4-</u> OLFACTORY HALLUCINATIONS	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<u>5-</u> DEJA-VU	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<u>6-</u> TRANSIENT MOTOR DISTURBANCE (LOSS OF MUSCLE TONE, PARESIS)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<u>7-</u> TRANSITORY LANGUAGE IMPAIRMENT	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<u>8-</u> FEELING OF ASCENDING EPIGASTRIC WARMTH	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<u>9-</u> OROFACIAL AUTOMATISMS	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<u>10-</u> MOTOR AUTOMATISMS (E.G. RHYTHMIC OR STEREOTYPED MOVEMENTS)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<u>11-</u> STRONG VERTIGO	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<u>12-</u> AUDITORY HALLUCINATIONS	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<u>13-</u> SUDDEN ONSET OF CONFUSION	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<u>14-</u> ABNORMAL MOVEMENTS (MUSCLE TWITCHES/MYOCLONUS)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<u>15-</u> LOSS OF CONSCIOUSNESS	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<u>16-</u> CONVULSIVE SEIZURES	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<u>17-</u> AUTONOMIC SENSATIONS (TACHYCARDY, TRANSPIRATION,	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<u>18-</u> URINARY LEAKING AT AWAKENING	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<u>19-</u> TONGUE BITING AT AWAKENING	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<u>20-</u> TRANSITORY ALTERED STATE OF MIND, LAPSSES IN AWARENESS	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

*Figure 35. Epilepsy questionnaire for quantifying symptoms of a potential epileptic syndrome.* Translated from the original French version of Fleur Gerard, in B. Szabo et al., 2022

### 4.3. Preclinical results disentangling the potential underpinnings of AD-related neuronal hyperexcitability

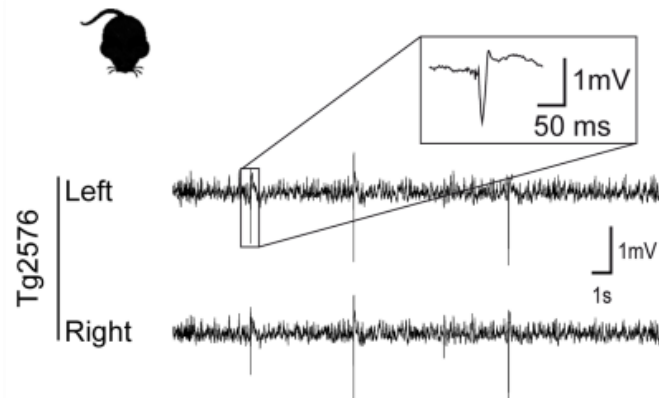
#### 4.3.1. Rodent models used in the investigation of the AD-epilepsy link

Research over the past decade has made it abundantly clear that the epileptic events found in a subgroup of AD patients are present in almost all mouse models used in AD research (**Figure 36**) (for a review see Kazim et al., 2021). Most of the models used in disentangling the intricate links between AD and epilepsy are based on APP or presenilin mutations implicated in FAD. The most widely used models in this topic (and often cited in this section) are listed in **Table 3**. Even though most of these models carry only the amyloidosis of AD without Taupathy, they generally recapitulate memory deficits and behavioural pathologies, such as disrupted circadian rhythms.

Model	Mutation
3xTg-AD	Carry three AD-linked mutations of human APP and PSEN1 transgenes: 1. Swedish (K670N/M671L) on APP 2. P301L on MAPT (coding for Tau) 3. M146V on PSEN1
5xFAD	Carry five AD-linked mutations of human APP and PSEN1 transgenes: 1. Swedish (K670N/M671L) 2. Florida (I716V) 3. London (V717I) } on APP 4. M146L and 5. L286V on PSEN1
APP23-PS45	Double transgenic mice, carrying: 1. The Swedish mutation 2. M146V mutation-burdened human PS1
hAPP-J20	Double mutant carrying human APP transgene with Swedish (K670N/M671L) and Indiana (V717F) mutations.
Tg2576	Overexpressing a double mutant form of human APP695 (Lys670-Asn, Met671-Leu [K670N, M671L]), driven by a hamster prion protein promoter.

*Table 3. Some of the commonly used mouse models in AD research, with a focus on models often referenced in the document.*

With the help of these models, significant advances have been made in elucidating the mechanistic pathways related to the aberrant network hyperactivity that has also been extensively reviewed recently (Ambrad Giovannetti & Fuhrmann, 2019; Rey et al., 2019; Vico Varela et al., 2019; Busche & Hyman, 2020; Giorgi et al., 2020; Toniolo et al., 2020; Tombini et al., 2021). The results of the main pathways of investigation are reported below or in the discussion.



**Figure 36. Examples of epileptic activities (EA) in a mouse model of amyloidosis.** Representative examples of EA in Tg2576 mice (Power 1401 mk-II, CED, Cambridge, UK). Reproduced from Bezzina et al., (2015) with permission in B. Szabo et al., (2022)

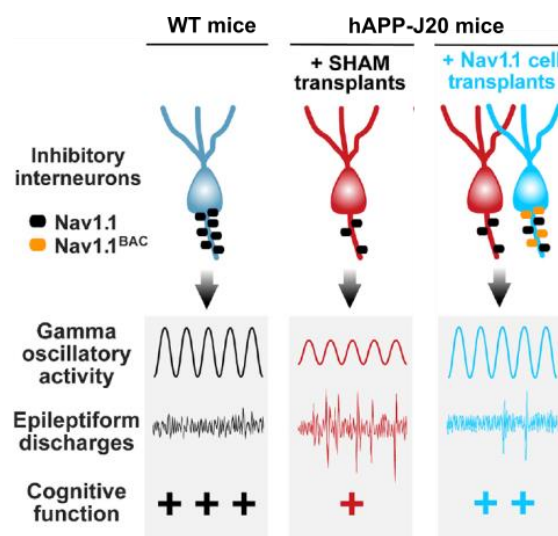
#### 4.3.2. Amyloid- $\beta$ accumulation and neuronal hyperexcitability

Throughout the literature, the excitatory impact of A $\beta$  as one of the main stimuli of neural hyperexcitability in AD is a surprisingly coherent finding. First, as already mentioned, at non-pathological A $\beta$  levels, A $\beta$  production and neuronal activity are part of a self-regulating feedback loop (Kamenetz et al., 2003). However, in AD, this loop becomes a vicious cycle. These findings are further supported by Busche and colleagues (2008, 2012). They showed in AD mouse models that (I) hyperactive neurons cluster around amyloid plaques and that (II) in the absence of plaque during the earliest stages of AD, even soluble forms of A $\beta$  can drive neuronal hyperactivity in the hippocampus of APP/PS1 mice. The results of Reyes-Marin and Nuñez (2017) point in the same direction revealing a solid correlation between plaque load and epileptic events in the same model. Finally, A $\beta$  clearance seems to decrease neuronal hyperexcitability (Kazim et al., 2017), although some results are contradictory on this subject (Busche et al., 2015). Various other candidate pathways through which A $\beta$  drives neuronal

hyperexcitability have been suggested, including differential impacts of A $\beta$  peptides on neural activity regulation depending on their state of oligomerization, with less oligomerized forms driving hyperexcitability (Orbán et al., 2010). The vicious cycle between neuronal activity and A $\beta$  generation could result in a steep A $\beta$  peptide accumulation curve and, later on, amyloid plaques. Accumulating A $\beta$  may then drive neuronal hyperexcitability through the dysregulation of calcium homeostasis due to a high plaque load (Lerdkrai et al., 2018; Sun et al., 2019), which would be left unchecked due to the early deterioration of several types of interneurons.

#### 4.3.3. A dysfunction of fast-spiking interneurons

The above-mentioned dysfunctional interneurons are mainly parvalbumin-positive basket cells (PVBC) implicated in the synchronization of the activity of large neuronal populations. Moreover, their dysfunction is strongly linked to cognitive impairment (for a summary of PVBC interneurons in AD, see Cattaud et al., (2018)). These interneurons seem to be dysfunctional in patients and hAPPJ20 mice due to decreased levels of the Nav1.1 subunit of voltage-gated sodium channels (Verret et al., 2012). Interestingly, restoring the Nav1.1 subunit rescues both aberrant network activities and the memory-impaired phenotype of the hAPPJ20 model (Martinez-Losa et al., 2018; Verret et al., 2012) (**Figure 37**).



**Figure 37. Restoration of Nav 1.1 channels of interneurons in the hAPP-J20 mouse model improve network and cognitive functions.** Transplanting Nav1.1-enhanced interneurons to compensate for their reduced numbers in hAPP-J20 mice leads to decreased network hyperexcitability, normal oscillatory rhythms and improved cognitive functions. Adapted from Martinez-Losa et al. (2018)



Moreover, in the Tg2576 mouse model (overexpressing a double mutant form of human APP695), Cattaud and colleagues recently demonstrated an early disruption of the perineuronal nets (PNN) surrounding these interneurons (Cattaud et al., 2018). PNNs protect cells from oxidative stress and play an essential role in stabilizing existing synapses and, through that function, memories (Kwok et al., 2011; Shi et al., 2019). Interestingly, restoring PV interneurons in the APP/PS1 model abolishes neuronal hyperexcitability and even improves cognitive function (Hijazi et al., 2019), which indicates that the observed PPN/PV interneuron-related deficits contribute to neuronal hyperexcitability in AD. It is of interest that the matrix metalloproteinases (MMPs) that control the integrity of PNNs surrounding the PV interneurons (Rossier et al., 2015) are increased after induced seizures in rats (Dubey et al., 2017; Rankin-Gee et al., 2015; Wilczynski et al., 2008). This means MMPs could be continuously degrading PNNs, which may further increase seizure susceptibility in the long run (Rankin-Gee et al., 2015). Therefore, seizures and epileptic events could constantly contribute to the degradation of PNNs and the dysfunction of the parvalbumin-positive interneurons in case of epileptic activities occurring in AD models or patients, creating yet another vicious cycle. More importantly, the same metalloproteases (most notably MMP-9), either secreted by astrocytes (Yin et al., 2006) or hippocampal neurons (Backstrom et al., 1996), have been reported to be increased around amyloid plaques in AD. Therefore, there is a distinct possibility that the increased MMP-9 release is a desperate attempt of the nervous system to fight off the high A $\beta$ -load and its hyperexcitability. This might be an adaptive short-term response but could lead to the exact opposite effect by degrading, in parallel with A $\beta$ -aggregates, the PNNs around the hippocampal PV+ interneurons, decreasing the inhibitory tone and still leading to hippocampal hyperexcitability all the same. Finally, PNNs need to be pruned by metalloproteases to some extent to stay at an optimal PNN/PV interneuron ratio, as too rigid PNNs can impede the formation of hippocampus-dependent memories (Cattaud et al., 2018). However, continuous slight degradation of PNNs by the potential spike-induced release of MMPs might make this system “too flexible”, leading to cognitive dysfunction, especially when it comes to memory consolidation. Indeed, intracranial EEG recordings obtained from patients with TLE have shown that neuronal assemblies that are activated during an ictal period undergo the most outstanding plastic changes during the following period of sleep (Bower et al., 2015). In a system where PNNs are too loose due to continuous deterioration, the result is a highly malleable hippocampal network where the changes

responsible for synaptic plasticity during sleep (as reviewed by Diekelmann & Born, 2010) could be altered. This could lead to altogether impaired memory consolidation, at least partially explaining memory issues reported in AD, and would explain the faster cognitive decline of AD patients with co-occurring epileptic activities that has been repeatedly reported (Horvath et al., 2021; Vöglein et al., 2020; Vossel et al., 2016b).

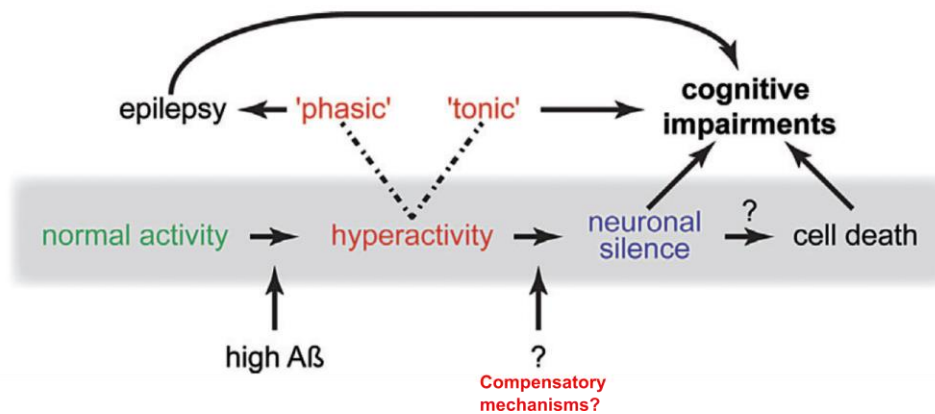
Furthermore, other populations of interneurons have also been described as suffering damage relatively early in APP/PS1 mice. This seems to be attenuated by the transplantation of embryonic interneuron progenitors that suppress the hyperexcitable phenotype (Lu et al., 2020). Nonetheless, the interneuron dysfunction observed in AD models seems to add insult to injury as it removes the brakes that could stop the runaway train of A $\beta$ -induced hyperexcitability.

Finally, recent results seem not only to link interneuronal deficits in AD to epilepsy but may also explain the accelerated memory decline of AD patients by the disruption of memory consolidation-related oscillations with epileptic activities. Indeed, Caccavano et al. (2020) demonstrated in the 5xFAD transgenic mouse model of FAD that PVBCs receive unusually low levels of excitatory input during SW-Rs, which results in a reduction of almost 50% in their firing rate during SW-Rs. This hypoactivity of the PVBCs leaves the pyramidal cells with an enhanced excitation/inhibition ratio that is highly prone to EA. Even in *in vitro* mouse brain slices that only partially reproduce the network interactions, this gives rise to abnormal SW-Rs with decreased length, increased frequency and wider amplitudes. Interestingly, Jones et al. (2019) described a predictor function of early SW-R activity decrease on subsequent spatial memory deficits in a knock-in rodent model (apoE4-KI) of AD. At the same time, two other teams observed decreased SW-R frequency and SO-SWR and SO-spindle coupling in two transgenic models of AD (Bentham et al., 2020 for 3xTg-AD and; Stoiljkovic et al., 2018 for TgF344-AD).

#### 4.3.4. *Tau in its various forms*

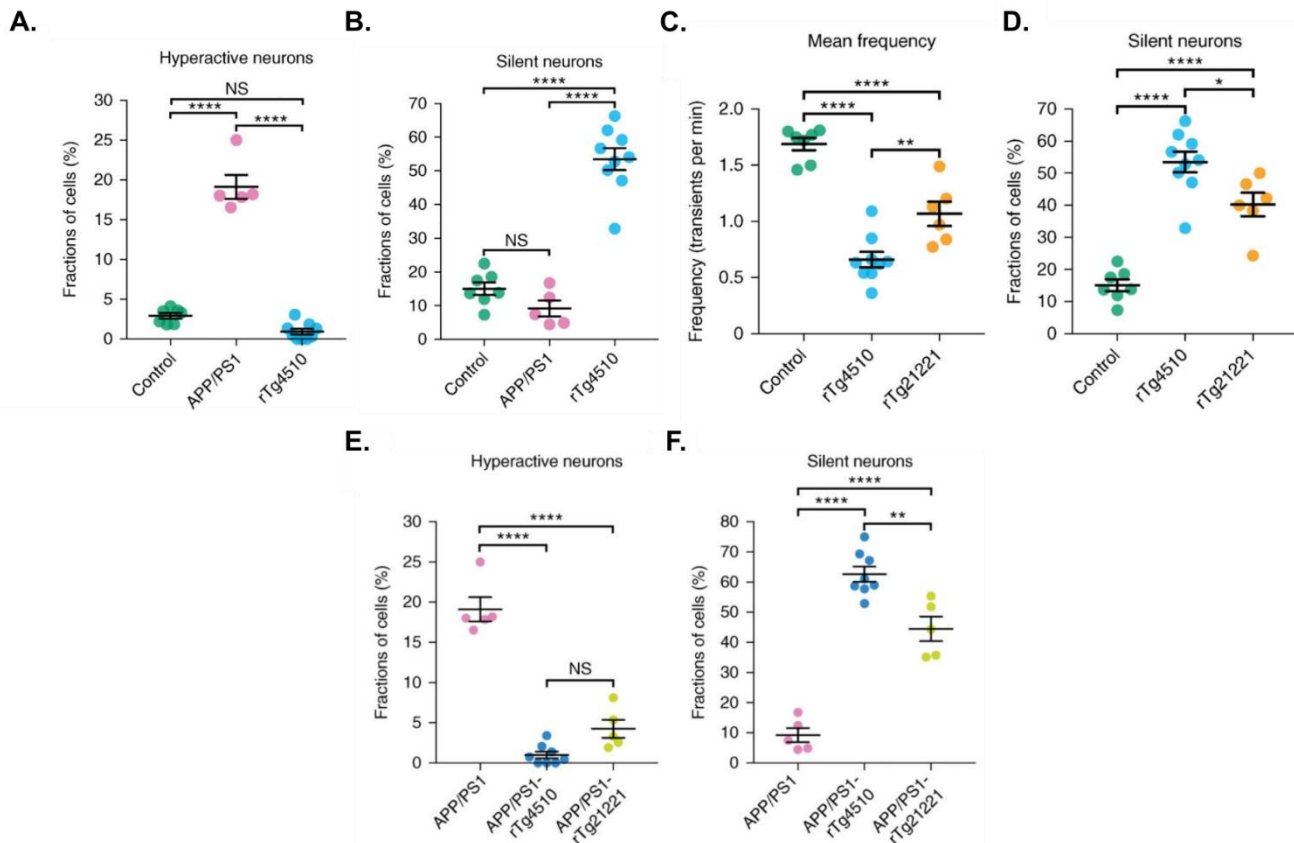
In addition, the second major pathophysiological hallmark of AD after A $\beta$  deposits, NFTs, also assist and even contribute to the hypersynchronous phenotype. While many aspects of the pathways through which pTau or its endogenous form exerts a neurotoxic effect are still under investigation, disentangling their role in the epileptic phenotype associated with AD has started. For example, reducing total tau levels relieves neuronal hyperexcitability in mouse models of AD (DeVos et al., 2013; Roberson et al., 2007). Furthermore, a recent longitudinal study in AD patients found that the risk of seizure was associated with higher total CSF tau levels (Tábuas-Pereira et al., 2019). In AD mouse models, the effect of tau on epileptogenesis seems to be driven by (I) the tau-dependent depletion of Kv4.2 potassium channels on dendrites (Hall et al., 2015) and (II) the interaction between endogenous tau, Fyn and PSD95. This latter interaction seems to be involved in the excitotoxic effect of A $\beta$  as it increases the number of post-synaptic glutamate receptors and renders neurons more receptive to excitatory inputs (Ittner et al., 2010). However, phosphorylating tau near the microtubule domain renders this interaction impossible, leading to NMDA receptor endocytosis, which induces short-term neuroprotective suppression of hyperexcitable networks but comes with a long-term potential to generate network hypoactivity (Mondragón-Rodríguez et al., 2020).

Furthermore, Tau-hyperphosphorylation seems to be accelerated in a hippocampus subfield-specific manner after status epilepticus and during epilepsy (Alves et al., 2019). At the same time, neural activity-related acceleration of tau pathology has recently been demonstrated (Tai et al., 2016; Wu et al., 2016). These results are coherent with the observations of Mondragón-Rodríguez and colleagues (2020), who suggest that tau phosphorylation in the early stages of AD might be a neuroprotective mechanism against A $\beta$ -related hyperexcitability. They are also compatible with the model proposed by Busche and collaborators that describes the hyperactivity-promoting impacts of early amyloid accumulation. This accumulation would lead to aberrant excitatory activity manifesting as more or less detectable phasic or tonic epileptic activities, with both potentially contributing to cognitive decline. The later neuronal hypoactivity, potentially driven by NFTs, could, according to results obtained since this initial model, be a compensatory activity to counteract hyperactivity. **(Figure 38).**



**Figure 38. Proposed model adapted from Busche et al., (2015) for AD progression related to aberrant network activities.** In this model, the hyperactivity-promoting impacts of early amyloid accumulation would lead to aberrant excitatory activity manifesting as more or less detectable epileptic activities, potentially contributing to cognitive decline. The later neuronal hypoactivity, potentially driven by NFTs could, according to results obtained since this initial model, could be a compensatory activity to counteract hyperactivity.

Indeed, the more recent results provided by Busche and colleagues (2019) show that the hypoactivity-inducing effect of Tau dominates over the hyperexcitability-promoting impact of amyloid. Using two-photon  $Ca^{2+}$  imaging in mice overexpressing either human A $\beta$  or either mutant or wild-type (WT) Tau, they managed to quantify the fraction of hyperactive and hypoactive neurons in layer 2/3 cortical layers of the animals. They could therefore confirm the net excitatory effect of A $\beta$  (**Figure 39 A, B**) and observed a robust hypoactivity-promoting effect in mice bearing a human form of Tau with a P301L mutation known to be related to familial frontotemporal dementia. Furthermore, in mice expressing a human WT form of Tau, they observed a somewhat less marked effect in the same direction (**Figure 39 C, D**). Finally, when they crossed A $\beta$  overexpressing mice with either Tau-overexpressing genotype, they found an increased fraction of hypoactive neurons, indicating that the hypoactivity-promoting effect of Tau prevails over the hyperexcitability inducing amyloid (**Figure 39 E, F**). A recent finding from Viney et al. (2021, bioRxiv preprint) also hinted in the THY-Tau22 mouse model of taupathy that pTau not only accumulates in deep-layer pyramidal neurons but that it later invades oligodendrocytes as well. This spread during ageing is related to less and less high-firing pyramidal cell activity (in this specific case, during navigation), which is highly coherent with the above-mentioned results and the well-known progressive hippocampal hypoactivity with more advanced stages of AD.



**Figure 39. Differential impact of Amyloid and Tau on neuronal activity levels.** (A, B) The net effect of amyloid is an increased fraction of hyperactive neurons, whereas introducing a human mutant form of Tau augments the number of hypoactive neurons. (C, D) The same tendency is observed when introducing WT human Tau. However, the introduction of mutant APP with either form of Tau still (E, F) produces a net hypoactive effect, indicating that the hypoactivity-promoting effect of Tau prevails over the hyperexcitability-inducing amyloid. Adapted From Busche et al. (2019)

#### 4.3.5. Early deficits of monoaminergic transmission

The importance of monoaminergic transmission in the induction and maintenance of sleep and their early deficits, especially that of noradrenaline, in AD have been discussed in the previous section. However, these monoamines also contribute to the delicate balance of excitation and inhibition in the brain. Therefore, the early siege of their main centres by AD-related deposits could also amplify neuronal hyperexcitability. This is an all the more intriguing possibility as the dynamics of noradrenergic transmission inversely correlate with the increase of EA frequency described by Kam and collaborators (2016) in Tg2576 mice and by Brown et al. (2018) in the hAPPJ20 model of AD. While the concentration of several monoamines are maximal during wakefulness, they decrease during SWS and become quasi-null during REM

sleep episodes (e.g. Aston-Jones & Bloom, 1981; Léna et al., 2005); the inverse has been shown for epileptic events in Tg2576 mice. That is, maximal EA frequency during REM sleep, decreased EA in SWS and minimal abnormal events during wakefulness (Brown et al., 2018; Kam et al., 2016). Of all the monoamines concerned, noradrenaline is a promising target as it 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 adrenoreceptor subtypes or via combined activation of the  $\alpha$ 2 and  $\beta$ 2 receptor subtypes (Clinckers et al., 2010). Therefore, it is plausible that a decreased noradrenergic tone can lead to decreased inhibitory activity, resulting in the unmasking of neuronal hyperexcitability and epileptic activity (Ross et al., 2015). This is all the more alluring as NFT deposition starts at the level of the center of noradrenergic neurons, the *locus coeruleus* (LC), potentially inducing an early hypoactivity at this site (Braak et al., 2011).

However, interactions with other systems cannot be excluded, and indeed, Dopamine has also been under investigation recently, even though its concentrations do not parallel EA frequency as perfectly as noradrenaline's does (Dahan et al., 2007). Most notably, Ren and collaborators revealed an interneuronal deficit behind the dysfunction of fast-spiking interneurons (such as PVBCs) in an in vitro model of acute A $\beta$  exposure (Ren et al., 2018). More precisely, they suggested a causal role of D1 receptors behind the A $\beta$ -induced hyperexcitability as their results indicated that even low concentrations of A $\beta$  (50 nM) lead to excessive dopamine release from the anterior cingulate cortex, which entails a massive activation of D1-type dopamine receptors on fast-spiking interneurons, resulting in a marked inhibition of these interneurons and, therefore, the tipping of the excitation/inhibition balance towards excitation. These results were also supported by another in vitro study finding elevated numbers of surface D1 receptors at the dentate gyrus in hippocampal slices of Tg2576 mice (Costa et al., 2016), creating one of the first bridges between sleep, epilepsy and AD.

Furthermore, a link between cholinergic transmission and neuronal hyperexcitability has been made in a study by Kam et al., (2016), who found that the REM-sleep prominent epileptic events in the Tg2576 mouse model of AD were decreased upon treatment with the muscarinic acetylcholine receptor atropine. However, it is important to note that atropine interferes with the sleep-wake cycle. Most notably, it completely suppresses REM sleep. For that reason, the

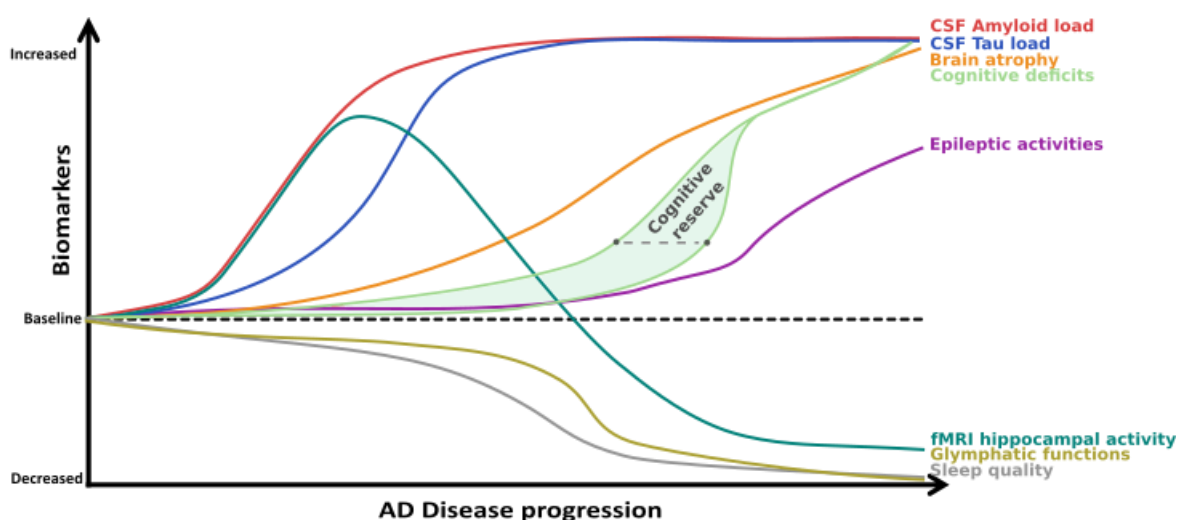
authors were focused on interictal activity during SWS. On the other hand, atropine is also known to induce a “sleep-like” slow-wave activity in the cortex with a concomitant waking behaviour in deeper, subcortical regions, a state followed by sleep rebound (Qiu et al., 2015). These might render the correct classification of sleep stages rather complicated. The eventual presence of this altered state might have interfered with their observations, although it cannot be excluded that muscarinic transmission is implicated in the early neuronal hyperactivity in Tg2576 mice.

#### *4.3.6. The role of APO-E in the AD-epilepsy comorbidity*

As mentioned earlier, many other mechanisms have been suggested, such as an early reduction in the level of either the mitochondrial protein deacetylase sirtuin 3 (Aroor & Brewster, 2020) or reelin (Chin et al., 2007; Machado et al., 2019; reviewed by Haberman et al., 2017). Another factor mentioned in the literature is the APOE-4 allele, which was mentioned earlier as the major genetic risk factor of sporadic AD. The role of this allele in neuronal hyperexcitability is supported by the findings of elevated hippocampal activation levels even in non-AD APOE-4 carriers (Bookheimer et al., 2000; Filippini et al., 2009) and by another elegant study by Andrews-Zwilling et al. (2010). Using a knock-in APOE-4 mouse model, they found an APO-E4-related decrease in hilar GABAergic interneurons. Interestingly, this decrease correlated with learning deficits during ageing in these mice. Furthermore, they found indications that APOE-4 acts upstream of Tau in AD pathogenesis. Therefore, APOE-4 alleles could contribute to network hyperexcitability by decreasing the strength of inhibition (Najm et al., 2019).

All these converging and synergistic pathways between different actors of sleep-pattern disrupting factors introduced in this section could contribute to forming a dysfunctional network in which the excitation/inhibition balance has been considerably slanted towards hyperexcitability, which makes the occurrence of epileptic events or even seizures highly probable. Neuronal hyperactivity during such events might, in turn, further aggravate the situation by reorganizing functional networks (Krezymon et al., 2013; Palop et al., 2007; Shapiro et al., 2008) and generating substantial amounts of A $\beta$ . Since the major pathological pathways that lead to AD can be triggered decades before the onset of cognitive symptoms,

these processes can slowly but steadily reorganize and destroy existing functional circuits. This also applies to sporadic forms of AD, where epilepsy might not be as clear-cut a symptom as in the case of FAD, yet silent reorganization might occur during the asymptomatic period of plaque and NFT accumulation. This could lead to aberrant network activities, even before cognitive symptoms appear (Vossel et al., 2016), leading to the proposition of the current model above (**Figure 40**), whose elements interact in complex manners, creating several vicious cycles in which AD, sleep and epileptic events aggravate one another (B. Szabo et al., 2022).



**Figure 40. Proposed model of biomarker- and cognitive decline progression over the course of AD in relationship to epileptic activities.** High cognitive reserves compensate on a functional level for marked Amyloid and Tau aggregation and atrophy may lead to delayed diagnosis. This might explain why EA is most often detected in highly educated participants who are (seemingly) in the mild stages of the disease. However, once compensation is no longer possible, and EA becomes more frequent or probable, the cognitive decline might be steeper, which is compatible with the fast progression noted in AD patients with E/EA. Figure adapted from with permission. The grey dashed line represents the approximate time of diagnosis. From B. Szabo et al. (2022)



## **CIRCUMSTANCES AND GENERAL OBJECTIVES**

It can be seen that the current literature on the topic of Alzheimer's disease and epilepsy is not only discordant but is also burdened by newer and newer questions adding to the pile of the ones already waiting in line to be answered. Therefore, the aim of the work reported in this document was to disentangle some of the knots of the intricate web of this comorbidity by addressing several of the questions reviewed above. However, as stated before, getting to the bottom of the pile of questions will probably require larger, more robust, collaborative studies across teams both on the clinical and the preclinical end.

The presented translational work also results from a collaboration between clinical and preclinical researchers interested in this topic in different research institutes in Toulouse (France). More precisely, the study stems from a large preclinical project launched in 2012 at the Research Center on Animal Cognition (CRCA) in Toulouse, aiming to further disentangle the complex links between sleep, epilepsy and Alzheimer's disease using a mouse model of AD, the Tg2576 mouse line. Using mostly intracranial electrophysiologic recordings, behavioural tests and immunohistochemistry, the project had already shown early alterations of hippocampal neurogenesis, potentially leading to network dysfunction (Krezymon et al., 2013). This was followed by the detection of early (1.5 months of age) epileptic events (Bezzina et al., 2015b), accompanied by early (3 months of age) dysfunctions of Parvalbumin positive (PV+) interneurons and their surrounding perineuronal nets (Cattaud et al., 2018) that was shown to be causally related to social memory deficits at the level of the CA2 (Rey et al., 2022). Interestingly, although it was shown that the improved cognitive functions following transitory stays in enriched environments shown previously (Verret et al., 2013) might also be related to the concomitant improvement of the same interneuronal deficit (Cattaud et al., 2018), the enriched environment did not seem to reduce network hyperexcitability *per se* in this model (Bezzina et al., 2015a). It is therefore clear that while interneuronal dysfunction contributes toward memory impairment and potentially toward network hypersynchrony as well (as is the case in other AD mouse models, Verret et al., 2012), other factors must also interfere in the epileptic phenotype of AD mouse models.

Furthermore, a parallel axis was focused on the description of **the sleep-wake cycle throughout the ageing process in the Tg2576 mouse model** to establish whether it shows

similar decline patterns as in AD patients – a recently closed large cohort study (~100 animals) that was not only part of this thesis but spanned through several thesis projects throughout the past ten years (Bezzina, 2015; Cattaud, 2018). These evaluations, when crossed with the study of epileptic events, revealed – as we will see – that the EAs are distributed in a quite peculiar manner throughout the sleep-wake cycle in Tg2576 mice, with a quasi-sleep-only presence and an especially increased EA frequency during REM sleep. This also implies that EA may affect memory functions by disrupting sleep-related memory consolidation – a hypothesis that is still awaiting confirmation both in mice and in AD patients. Furthermore, the distribution of EAs during sleep seen in our mice was seemingly contradictory to the clinical findings cited previously, however, REM sleep is often less explored due to its presumed EA-free nature – therefore, it was deemed pertinent better to explore this sleep stage in AD patients in relation to epileptic activities. This led to the conception of the clinical study EREMAD (Epileptic Activity during REM sleep in Alzheimer’s Disease), launched in 2019. During the course of the work presented here, the two studies progressed in parallel.

The main objectives of the *preclinical study* were, first, to **complete the already launched exploration of the sleep-wake cycle and EA activity during said cycle throughout the lifespan in Tg2576 mice**. This included adding new individuals to the cohort recorded with EEG to reach sufficient statistical power and a reanalysis of the quasi-totality of the cohort recorded since 2012 to unify scoring criteria and minimize bias related to inter-rater variability.

A secondary objective was to **explore the underlying factors of the epileptic phenotype of Tg2576 mice**. This was mainly achieved using signal analysis-based methods to **identify potential cell types involved in the generation of EAs**. Furthermore, by combining EEG with a pharmacological approach, we sought to **disentangle the involvement of dopaminergic and noradrenergic transmission**. This included the expansion of an already launched preliminary study and the re-analysis of the dataset to explore the role of noradrenergic transmission, as well as the addition of a preliminary exploration of the involvement of dopaminergic transmission.

A tertiary preclinical study was added following the conception of the EREMAD study that, as we will see in the next paragraph, largely focused on the potential disruption of memory consolidation by EAs. Therefore, in a translational mindset, we devised a pilot study relying on

behavioural tests (most notably on a social memory protocol) combined with EEG recordings to **explore the possibility of EA-related disruption of memory consolidation in Tg2576 mice.**

As we will see, the clinical project EREMAD, involving several laboratories and hospital units across Toulouse, was based on video-EEG combined with polysomnography, neuropsychological and genetic tests, MRI data, and the exploration of lifestyle-related factors. Its main objectives were, first and foremost, to **characterize the prevalence and the distribution of EAs in early-to-moderate stage AD patients (MMSE > 18), with a strong focus on the analysis of REM episodes.**

The secondary objectives were to **explore how EAs may disrupt sleep-related memory consolidation**, using pre-and post-sleep memory tests and a signal analysis-based methodology to analyse sleep microarchitecture (i.e. sleep spindles).

Our tertiary goal was to **investigate the potential mechanistic underpinnings of EAs in AD, in this case, brain atrophy-related and genetic factors.** Furthermore, **cognitive reserves were also evaluated to pinpoint whether this factor may indeed represent a bias in previous studies and could explain the early-onset and rapid decline of AD patients with EAs.**

Finally, the **analysis of the role of other sleep-related pathology, notably sleep fragmentation and sleep apnoeas, in memory consolidation deficits** was placed amongst our exploratory objectives.

In the sections below, the results of the preclinical study are presented and discussed first, followed by the preliminary results of the clinical study.

# **PRECLINICAL STUDY**

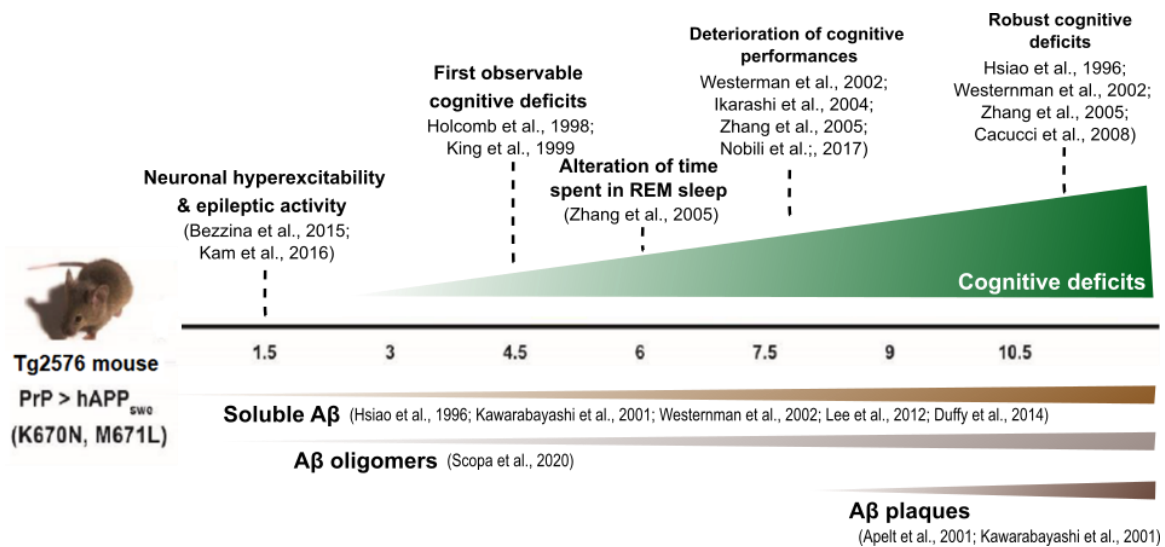
# **1. OBJECTIVES**

As mentioned above, the presence of EA in technically all AD models have been shown throughout the past decades (e.g. Kam et al., 2016; Palop et al., 2007; Roberson et al., 2007a) and have recently been synthesized in the exhaustive literature review of Kazim et al., (2021), with a comprehensive table of the presence of EA and the method of detection in the different mouse lines. Furthermore, these models give access to molecular and cellular mechanisms that are not possible to study in humans. These pieces of information, however, are highly precious when trying to understand the puzzle around network hyperexcitability and AD.

The Tg2576 mouse line is a widely used rodent model in AD research and has been used in our laboratory as well for a decade (Bezzina et al., 2015a; Bezzina et al., 2015b; Cattaud et al., 2018; Krezymon et al., 2013; Lassalle et al., 2008; Rey et al., 2022; Verret et al., 2013). Tg2576 mice show the presence of soluble A $\beta$  technically throughout their lifespan (e.g. Westerman et al., 2002) in gradually increasing concentrations, and the same pattern has been reported for oligomers appearing at 1.5 months (Scopa et al., 2020) and for plaques that become visible at around 7-8 months of age (e.g. Kawarabayashi et al., 2001) (**Figure 41**). Cognitive deficits, however, only appear at 4.5 months and show increasing severity to reach extremely low performance levels at around 12 months of age (e.g. Hsiao et al., 1996; Westerman et al., 2002) (**Figure 41**). Early sleep alterations were reported in the vicinity of six months, especially for REM sleep duration (Zhang et al., 2005). Finally, epileptic activities have been reported as early as 1.5 months of age (Bezzina et al., 2015b; Kam et al., 2016).

Therefore, in the current chapter of this thesis, the goal was to understand better the intricate links between epileptic events, sleep quantities and molecular factors in the Tg2576 mouse model of amyloidosis using electrophysiologic and pharmacologic approaches.

A part of this preclinical work has been submitted for publication in *Neurobiology of Aging*, and the manuscript, entitled “Neuronal hyperexcitability in the Tg2576 mouse model of Alzheimer's disease – The influence of sleep and noradrenergic transmission” (authored by Anna B. Szabo\*; Vanessa Cattaud\*; Charlotte Bezzina; Robin F. Dard; Fares Sayegh; Sebastien Gauzin; Camille Lejards; Luc Valton; Claire Rampon; Laure Verret and Lionel Dahan) is provided in the annexes of this thesis.



**Figure 41. Disease progression patterns of the Tg2576 mouse model.** The Tg2576 mouse model shows an early presence of soluble Aβ and a continuous buildup of oligomers and plaques throughout the first year of life. Cognitive deficits also show a gradual increase from 4.5 months. Neuronal hyperexcitability and sleep perturbations seem to be both early phenomena (starting at 1.5 months and six months, respectively). Figure modified from: Charlotte Bezzina, doctoral dissertation (2015, Université Paul Sabatier, Toulouse, France).

As mentioned above, this part of the project was aimed at elucidating the following points:

- (I) the disruption of the sleep-wake cycle in Tg2576 mice,
- (II) the frequency and distribution of epileptic activities across the lifespan and in different vigilance states,
- (III) the cell types potentially involved in such epileptic activities,
- (IV) the role of noradrenergic and dopaminergic transmission as potential triggers of epileptic activities,
- (V) the impact of epileptic events on sleep-related memory consolidation and the oscillatory patterns crucial for its success.

## **2. MATERIALS AND METHODS**

### 2.1. General Study Information

#### *2.1.1 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 authorized by the French Direction of Veterinary Services (#31-1155521, 2002; #20210, 2019). All efforts were made to improve the animals' welfare and minimize their suffering.

#### *2.1.2. Mouse Line*

All 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 13 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. Since female mice produced in the CRCA were used in other studies involving the use of enriched environment protocols, this project was carried on the remaining Tg2576 male mice, which are too aggressive and thus cannot undergo environmental enrichment protocols.

## 2.2. Determining epileptic activities and sleep-wake cycle changes across the lifespan in Tg2576 mice

### *2.2.1. Electrode preparation*

In most cases, two EEG electrodes were used for the implantation procedure (positioned at frontal and parietal sites, see below for details), along with a reference electrode (positioned over the occipital cortex) and an EMG inserted at the level of the neck muscles. EEG electrodes are prepared with Teflon-coated silver wire of 0.125 mm in diameter and ~1.2 cm in length. The coating is removed at both extremities at approximately 1 mm to allow for currents to pass. Reference electrodes are also made of silver wires (0.5 mm in diameter) of 1.2 cm in length, with the Teflon coating removed from the extremities. The EMG electrode is a multi-stranded PFA-coated stainless steel of 0.15 mm in diameter (AM-system, ref. 793200), with the insulation removed at both extremities (one of which is shaped into a loop to avoid muscle irritation). All electrodes are soldered with separate gold-plated miniature rods of 0.7 cm in length and 1 mm in diameter. The four rods, along with the electrodes, are then positioned on a six-channel plastic connector (PlasticsOne, Roanoke, NC, USA). Dental cement is then used to fix the rods in the corresponding holes of the plastic connector and can be implanted later.

### *2.2.2. Implantation of EEG electrodes for the 24-hour recordings*

For the first part of the study, aiming at evaluating the sleep-wake cycle of Tg2576 across the lifespan and assessing the distribution of EAs over the sleep-wake cycle, male Tg2576 mice and non-transgenic littermates of different age groups were implanted for EEG recordings (1.5 months: Non-transgenic (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 six 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). 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 six months.

Surgical procedures were carried out by previous PhD students, Principal Investigators and Study Engineers at the CRCA (Charlotte Bezzina/CB, Vanessa Cattaud/VC, Lionel Dahan and



Sebastien Gauzin). During the surgery, mice were anaesthetized 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 two 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. Stereotaxic coordinates were measured from Bregma (Parietal: Antero-posterior at -2.2 and lateral at 2, Frontal: Antero-posterior at 1.5, lateral at 1.5.) A silver reference 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 plugged into a miniature connector (PlasticsOne, Roanoke, NC, USA) and fixed to the skull with dental cement (Superbond, Sun Medical + Duralay). Lidocaine was applied to the flesh before suturing the skin. The animals received an injection of Metacam (0.1 ml, i.p.) and were recovering for at least a week before any experimental procedure.

### *2.2.3. 24-hour-long EEG recordings*

For recordings, mice were connected with a six-channel cable (PlasticsOne, Roanoke, NC, USA) 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 (A-M 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, corresponding to the very first set of experiments, the EEG was sampled at 200 Hz instead of 1kHz ( $Tg_{2576} = 4$ ,  $NTg = 8$ ). Experimental recordings lasted 24 hours and were preceded by at least 24 hours of habituation to the experimental setup.

### *2.2.4. Sleep scoring*

Recordings were analyzed offline using the 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 a MATLAB® script (MATLAB and Statistics Toolbox Release 2012b, The MathWorks, Inc., Natick, Massachusetts, United States) or 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. All statistical analyses were performed using the JASP software (Version 0.13.1) (Love et al., 2019).

#### *2.2.5. Detection of epileptiform abnormalities on EEG traces*

EAs were conceptualized 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 EAs and the EA frequency (spikes/minute) per vigilance state. Each REM episode was separately analyzed for spikes to establish whether EA frequency was stable or fluctuating throughout REM sleep. Then the link between episode length and EA frequency was analyzed by fitting a nonlinear model over the REM sleep episode length and spike frequencies. Statistical analyses were performed using the JASP software (Version 0.13.1)(Love et al., 2019). The figures and the nonlinear fit were generated via the Prism 5 software (GraphPad Software Inc., La Jolla, CA, USA) and Inkscape (Inkscape Project, 2020, available at [www.inkscape.org](http://www.inkscape.org))

#### *2.2.6. Spike-phase coherence analysis*

The regularity of theta oscillations during REM sleep is maintained by the perfect synchronization of several types of interneurons and pyramidal cells. Over the years, the cell types that preferentially fire on different phases of the theta oscillations have been identified

(Klausberger et al., 2003; Somogyi & Klausberger, 2005). Therefore, we hypothesized that if epileptic events are phase-locked to a given phase of the theta oscillations, it could give us a peek into the cell types potentially involved in their generation. To this end, spike-containing segments were analyzed 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 in the **Results** section, each 18° large for better readability, the statistical analysis was applied to unbinned data for more precision.

### 2.3. Examining the involvement of monoaminergic transmission in sleep-related epileptic events

#### *2.3.1. Protocols*

To explore the potential role of dopaminergic and noradrenergic transmission systems in REM-specific network hypersynchrony (Brown et al., 2018; Costa et al., 2016; Ren et al., 2018), we aimed to pharmacologically hinder dopaminergic or noradrenergic transmission by using specific antagonists.

For the dopamine experiment, SCH 23390 (D1/D5 receptor antagonist) and Haloperidol (D2/3 receptor antagonist) were injected to a group of 20 animals from 3 to 6 months of age (NTg: n = 6; Tg2576: n = 14). For the noradrenaline experiments, we administered the  $\beta_{1/2}$  adrenoreceptors (ARs) specific antagonist propranolol or the  $\alpha_1$ -AR antagonist prazosin to 18 Tg2576 and 15 NTg mice between 2 and 6.5 months of age. Mice were implanted for EEG as described above and EEG recordings were performed on four consecutive days as follows (**Figure 42 A and B**).

### **Dopamine experiment**

On the habituation day (Day 0), mice were connected to the EEG cables for approximately 8 hours (mean and SD: 7 hours 22 minutes  $\pm$  42 minutes) for habituation. Throughout the following days of the experiment (D1, D2, D3), mice were connected each morning to the EEG setup approximately at 9 a.m. and were recorded for at least 7,5 hours (7 hours 56 minutes  $\pm$  33 minutes, **Figure 42 A**). Each day, they were injected with a different pharmacological agent at two different dosages (low dose for the first injection and high dose for the second). The first injection took place around 12:15 a.m. (between 12:14 a.m. and 1 p.m.), but at least 90 minutes after the beginning of the recording, whereas the second injection took place 2 hours following the first one. The following sections were analyzed each day: the 90 minutes preceding the first injection and 90 minutes following each injection starting from t+20 minutes (t = injection) to allow for the drugs to take effect.

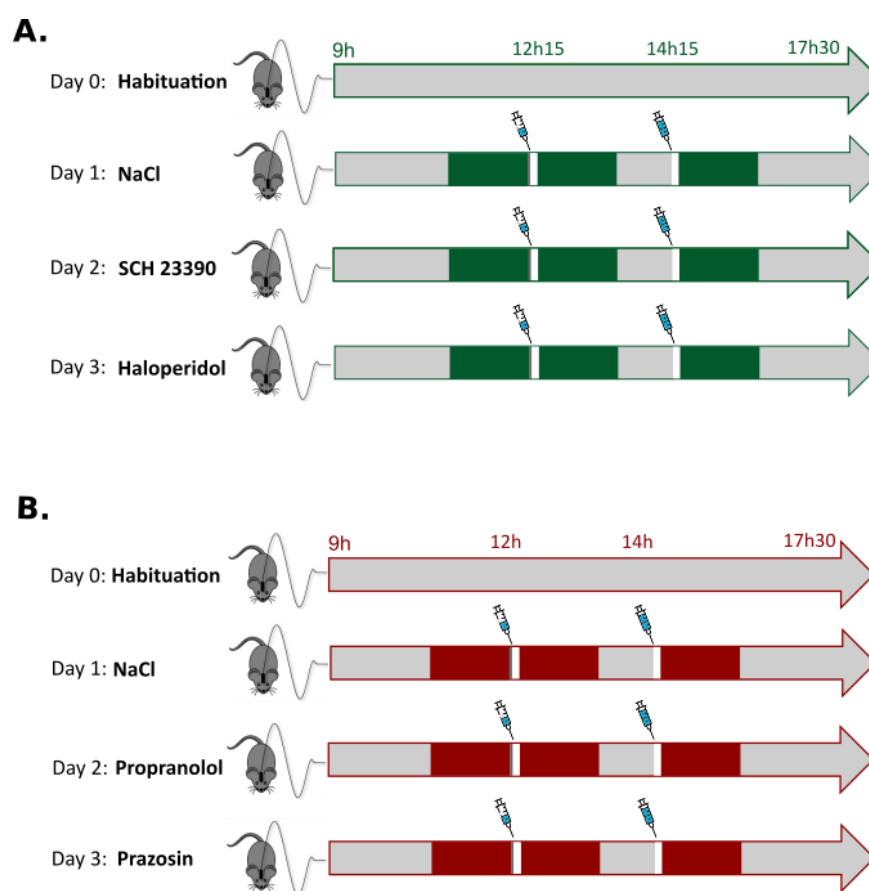
On the first day (D1), a control solution (sodium chloride 0.9 %) was injected (0.05 ml/10 g and 0.1 ml/10 g). On the second day (D2), the D1-like receptor antagonist SCH-23390 hydrochloride (Sigma-Aldrich) dissolved in sodium chloride 0.9% was injected (0.05  $\mu$ g/10g and 0.1  $\mu$ g/10g). On the last day (D3), Haloperidol (Sigma-Aldrich), dissolved in sodium chloride 0.9% was injected at 3  $\mu$ g/10g (first injection) and 6  $\mu$ g/10 g (second injection). Because of supply issues, for four animals, a solution of Haldol for human use was administered, diluted in sodium chloride 0.9% at the same concentration of Haloperidol as for the other Tg2576 mice.

### **Noradrenaline experiment**

The experimental load of this part of the pharmacology experiments was shared with a previous PhD project (VC), whereas the entire dataset was reanalyzed in the current document.

Similarly to the design of the dopamine experiment, on the habituation day, mice were connected to the EEG for 11 hours. Throughout the following three days, mice were connected to the EEG each morning at 9 a.m. and were returned to their home cage at 5 p.m. They were injected with a different pharmacological agent during these three days in a similar fashion as detailed above (**Figure 42 B**). Sodium chloride (0.9 %) was injected on the first experimental day. On the second day, propranolol hydrochloride was used. Finally, on day three, Prazosin hydrochloride was administered (for details on drug preparation, see the paragraph below).

For technical reasons, one transgenic mouse and three nontransgenic littermates had to be excluded. Following the recordings, EEGs were analyzed using Spike 2 V7.11 software (Cambridge Electronic Design, Milton, UK) for sleep scoring and EA detection as described above. For each recording day, three sections of EEG recordings were analyzed: (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 (**Figure 42 B**). For technical reasons, one batch of mice only had 76.5 minutes of recording before the first NaCl injection recording instead of 90 minutes, while four Tg2576 mice did not undergo the experiment's last day (prazosin).



**Figure 42. The four-day-long experimental protocol of pharmacological experiments coupled with EEG.** On both occasions, two doses of different pharmacologic agents were used each day following a first day of habituation. For the dopamine experiment (**A**) it included SCH-23390 (D1/D5 DA receptor antagonist) and Haloperidol (D2/D3 DA receptor antagonist). For the noradrenalin experiment (**B**) Propranolol ( $\beta$ -AR antagonist) and Prazosin ( $\alpha$ 1-AR antagonist) were used. Dark green/red squares represent the 90-minute sections that were analyzed from each recording. White areas represent the 20-minute windows that were not analyzed following the injections. Syringes placed right before the first injection of each day represent the 5-minute intervals during which the injections were prepared in the experimental room. Hence, these periods were not analyzed either.

For better solvability, Prazosin was dissolved in a solution of 2-hydroxypropyl  $\beta$ -cyclodextrin and not in sodium chloride 0.9 % solution (see below). To control for this potential biasing factor, four animals (two who underwent the initial experiments and two that were naïve to the experiment, mean age = 8.9 months) underwent a two-day long supplementary control experiment. In this protocol, NaCl was administered identically to the original experiment on the first day, and 2-hydroxypropyl  $\beta$ -cyclodextrin on the second, in the same volumes as used in the main experiment.

### *2.3.2. Drug preparation*

**Dopamine experiment:** The D1-like receptor antagonist SCH-23390 hydrochloride (Sigma-Aldrich) was dissolved in sodium chloride 0.9% and was injected (0.05  $\mu$ g/10g and 0.1  $\mu$ g/10g). Haloperidol was dissolved in sodium chloride 0.9% and was injected at 3  $\mu$ g/10g (first injection) and 6  $\mu$ g/10 g (second injection). For four animals, Haldol dissolved in sodium chloride 0.9% was used instead of Haloperidol at the same concentration as for the other Tg2576 mice.

**Noradrenaline experiment:** 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 at 0.05 ml/ 10 g for the first injection and 0.1 ml/ 10 g for the second one.

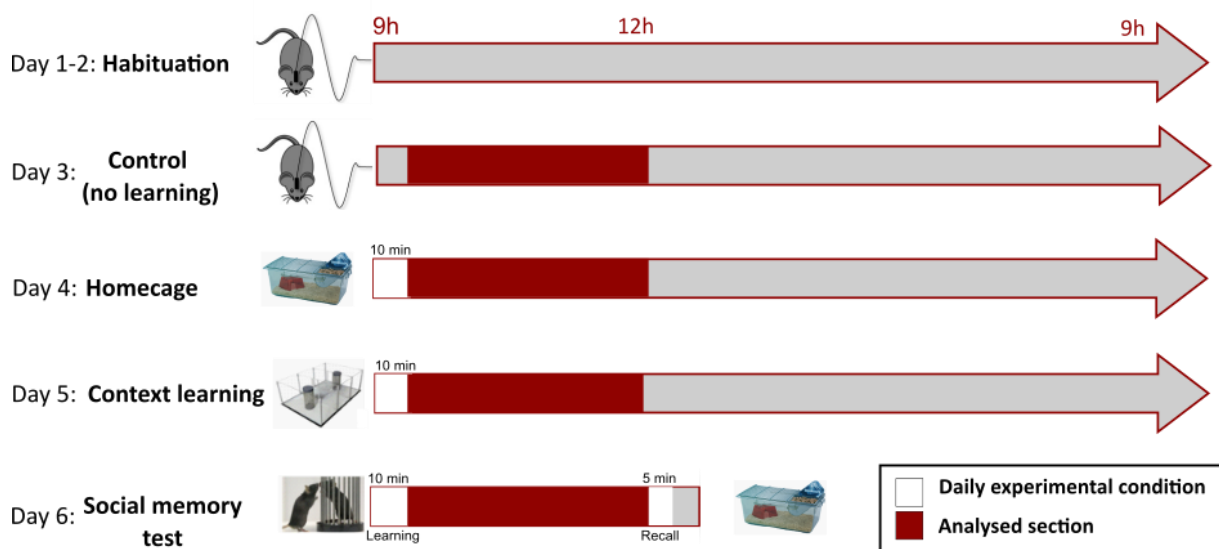
### **Statistical analysis**

All statistical analyses were performed using the JASP software (Version 0.13.1)(Love et al., 2019). Figures were created via the Prism 5 software (GraphPad Software Inc., La Jolla, CA, USA) and Inkscape (Inkscape Project, 2020, available at [www.inkscape.org](http://www.inkscape.org)).

## 2.4. The impact of epileptic activities on sleep microarchitecture and memory consolidation

### 2.4.1. Study Protocol

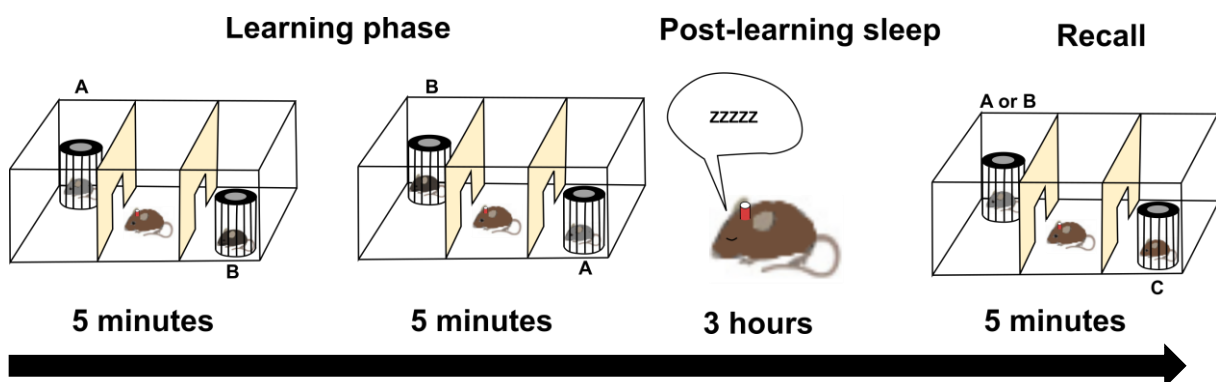
To evaluate how post-learning sleep microarchitecture would be impacted by epileptic events, a pilot study was implemented during the end-of-studies internship of Amel Bouloufa. In this protocol, 9 Tg2576 mice and 6 NTg littermates between 5 and 7 months were implanted for EEG recordings and underwent a six-day-long social memory protocol (**Figure 43**). Mice were implanted as described above and left to recover for at least a week before the start of the protocol.



**Figure 43. Experimental protocol of the social learning project.** On the first two days, mice were habituated to the recording conditions, followed by a day similar in its course, without any stimulation that served as a control condition (Day 3). Day 4 was consecrated to another control, this time for the impact of handling: mice were replaced in their homecage for ten minutes, then back to the recording chamber. The next day (Day 5) consisted of ten minutes spent alone in the three-chamber apparatus to explore it, serving as a “spatial learning” condition. Finally, the social learning test took place on the sixth day, with a recall phase at the end of the 3 hours of recording. Mice were then returned to their homecages.

During the first two days, mice were connected to the EEG system for habituation. Their health status was checked daily. The third day was used as a control “no-learning” condition (*Control*) to evaluate the baseline levels of the variables of interest without any intervention or stimulation. Then, on the fourth day (*Homecage*), we aimed to control for the potential impact of handling. Therefore, the mice were put from the recording cage back to their individual

homecages for 10 minutes, then moved back to their recording cage and re-recorded for 24 hours. The fifth day (*Context*) served as a spatial learning procedure wherein animals were placed one by one in the three-chamber cage devised for the social learning experiment for ten minutes of exploration, but with empty cup cages. Afterwards, they were again recorded until the following morning. On the last day (*Social*), mice were placed again in the same three-chamber cage, but this time, the cup cages were occupied by two animals never seen before (genotype: DAT:CRE, mice A and B in **Figure 44**) from two different litters. The test mice (Tg2576 or NTg) spent 10 minutes altogether in the three-chamber apparatus, with the cup cages' position being switched after 5 minutes to control for a potential place preference. After this procedure, mice were placed back in the recording cages and were recorded for approximately 3 hours (given that 3-4 mice were tested in each batch and the social learning protocol took around 20 minutes per animal, post-learning recordings could not start simultaneously.) A recall test was undertaken when each animal had spent at least 3 hours in the post-learning recording condition. During this part, they were placed back in the three-chamber cage and were confronted yet again with two mice in the cup cages: one already seen during the social learning condition earlier, and another DAT:CRE mice from a third litter that they never saw before (mouse C). Given the instinct of mice to explore new objects/individuals compared to already known ones, it can be expected that the animal will spend more time exploring the new animal in case of a correct social learning and consolidation process from the morning session. After the recall phase, mice were returned to their homecages.



**Figure 44. Social Memory protocol.** The animal tested was placed in the middle of the three-chamber dispositive with two new mice (A and B) placed in the right and left chambers. The mouse was free to explore them for five minutes, then the cages were switched, and the Tg256/NTg animal was again free to explore the apparatus and the other mice for 5 minutes. This was followed by a 3-hour post-sleep interval recorded via EEG. Finally, a recall phase took place with one of the previously presented animals and a new mouse (C) in the two cages. The animal explored them for five minutes. Exploration times were quantified with the Ethoc software. Note that all three animals presented to the tested mouse were black, here the colour difference serves only illustration purposes. Figure adapted from Rey et al., (2022)



### 2.4.2. Data analysis

#### Behavioural data

The time spent exploring the two compartments containing cup cages was measured via the Ethoc software (version 3.5, CNRS, CRCA/CBI, Maud COMBE (2019)) either online during the experiment or after the experiments from the video recording made during the session. For the recall part, we compared the percentage of time spent with the new animal via a t-test against 50% (marking an absence of preference in exploration time) for both genotypes.

#### EEG data – Sleep macro-and microarchitecture analysis and EA detection

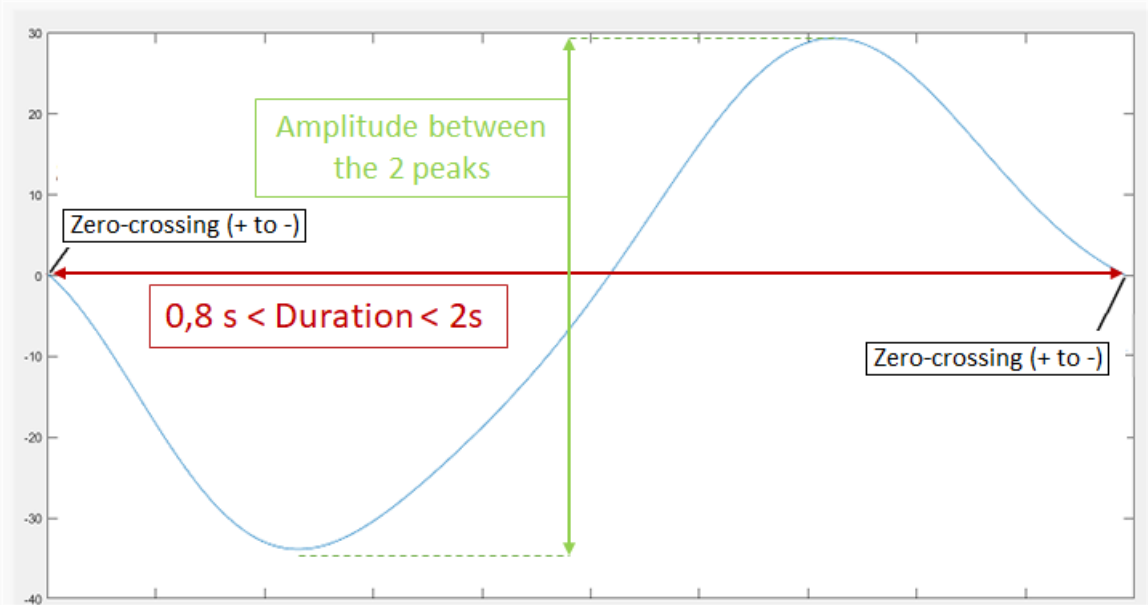
On each experimental day, the first three hours of recording were used, starting each day 5 minutes after the last animal of the batch had been connected to the EEG setup and the experimenter had left the room (see dark red intervals in **Figure 43**). Sleep stages were determined as detailed above. EA detection was also undertaken using the same methodology as introduced in the previous paragraph, with the exception of the detection threshold: here, all deflections exceeding 150% of the unidirectional baseline amplitude (determined on a 20-second-long section of quiet wakefulness) were verified manually to determine whether they are EAs or artefacts. The time spent in different sleep stages was compared between genotypes and experimental conditions using 2-factor RM-ANOVAs (genotype as between-subject factor, experimental condition as within-subject condition).

To detect how sleep microarchitecture changes throughout the lifespan in standard conditions in Tg2576 mice, we also analysed spindle characteristics (number, frequency, duration, amplitude). **Spindle detection** was carried out using the Detect Spindles plugin of EEGLAB (Ray et al., 2015) in Matlab (MATLAB and Statistics Toolbox Release 2022a, The MathWorks, Inc., Natick, Massachusetts, United States). This detector is based on complex signal demodulation that allows extracting the power in a chosen frequency band for each time point. As detailed in the article of Ray and collaborators (2015), this method straightforwardly transforms the signal, meaning that the temporal resolution is not distorted compared to the original signal, but all data points after transformation become positive as this method uses  $\mu V^2$  instead of

the original  $\mu\text{V}$  scale. To account for individual differences and the modulation of spindle parameters across the night without introducing individual-specific thresholds or other biasing methods, this detector uses a sliding window of one minute of NREM-sleep/SWS and transforms every timepoint of the complex demodulated signal into z-scores that are obtained from the mean and SD of each centred time window. This method, therefore, allows for normalizing each value compared to the mean signal, creating a unique fixed amplitude threshold and accounting for eventual changes in the sigma activity during NREM sleep (such as the one related to noradrenergic modulation, Osorio-Forero et al., 2021). The plugin filters the signal in the expected frequency of sleep spindles (11-16 Hz), then accepts only those events as spindles where the signal falls in the 1% highest amplitudes detected throughout the potential spindle candidates. It is of note that this detector is best calibrated for human data. Therefore, the results of spindle characteristics remain preliminary until a manual verification of the analysis is carried out or a more adapted analysis method is implemented in future projects. Not all animals had a frontal electrode implanted; therefore, only one parietal electrode was analyzed for all animals for consistency. The analysis was run via a home-built Matlab code.

To implement the detector of **slow oscillations (SO)**, the methodology section of a previously published article detailing their SO analysis was followed in a step-by-step fashion (Staresina et al., 2015). Data was once again analyzed in 1-hour segments, using only SWS segments. We started by filtering the signal between 0.5-1.25 Hz using a band-pass filter and cleaned artefacts using the Clean Rawdata plugin of EEGLAB (Kothe, 2013). The next step was to find each zero-crossing (i.e. time points where the signal crosses 0  $\mu\text{V}$ ), only in the positive-to-negative direction. In the following step, distances between zero-crossings, between 0.8 and 2 seconds, were maintained as potential slow oscillations (**Figure 45**). The amplitude of each remaining SO candidate was then determined using the amplitude between the positive and negative peaks in the segment. Finally, the 75<sup>th</sup> percentile was calculated, and only segments passing this threshold (i.e. corresponding to 25% of the amplest segments) were retained as SOs. The manual verification of the reliability and specificity of the detector is currently pending. Two-way RM-ANOVAs were again solicited to find inter-group and condition-related differences in SO characteristics.

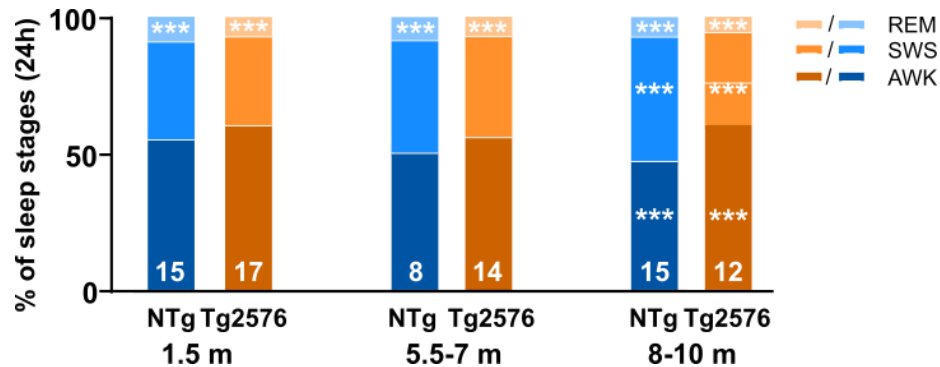
It is of note that the manual verification of the efficacy of the automated analysis is currently pending. Therefore, all results pertaining to sleep microarchitecture remain preliminary and were only applied, for now, in this behavioural pilot study.



**Figure 45. Illustration of the first steps implemented in the SO detector.** Positive-to-negative zero crossings were identified in the already filtered NREM 3 data. Segments between 0.8-2 seconds were selected for the next second. Amplitudes of remaining segments were calculated, and the amplest 25% of them were retained as SOs. Figure adapted from the MSc Thesis of Amel Bouloufa (2022, Université Paul Sabatier, Toulouse, France).

### 3. RESULTS

#### 3.1. Alteration of REM sleep and SWS of Tg2576 across the lifespan



**Figure 46.** Changes in the sleep-wake cycle for Tg2576 mice and nontransgenic littermates over the lifespan. While REM sleep percentages are lower for Tg2576 mice as early as 1.5 months of age, the difference in SWS becomes visible only later in life (8-10 months). Only significant Age-Genotype interactions are shown when applicable (SWS and WK), and genotype differences when no interaction was observed (REM sleep). For Age-related effects, see Tables 4 and 5 below. \*\*\*:  $p < 0.001$ . Data represented as group means for each vigilance state.

Concerning the modification of the sleep-wake cycle across the lifespan, a significant impact of both genotype and age in the time spent in each vigilance state (wakefulness, Slow-wave sleep/SWS, REM sleep) over 24 hours was observed, accompanied by an interaction between these factors for SWS ( $p = 0.027$ ) as well as a marginally significant interaction for wakefulness ( $p = 0.058$ , **Figure 46**, **Table 4 and 5**). This was due, on the side of nontransgenic animals, to a gradual decrease of time spent awake throughout the lifespan (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), while REM sleep duration decreased with age (from  $8.9 \pm 1.8$  % at 1.5 months to  $7.1 \pm 1.6$  % by 8-10 months). For Tg2576 mice, no clear tendencies toward age-related changes 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, the decrease in REM sleep was also captured in Tg2576 mice, but with significantly lower initial percentages compared to NTg animals (NTg vs Tg2576:  $p < 0.001$ ), independent of age and as early as 1.5 months of age ( $7 \pm 1.7$  % at 1.5 months,  $7 \pm 3.4$  % at 5.5-7 months and  $5.3 \pm 1.8$  % by 8-10 months). In sum, even though the sleep profile of Tg2576 mice does not vary much with age, the tendency to an increased time spent awake and to a reduced time spent in SWS as compared to NTg mice only gets significant at 8-10 months, when the time spent awake is reduced in NTg mice (Tg2576 vs NTg at 8-10 months:  $p < 0.001$  for both wakefulness and SWS, no significant differences at other ages).

Vigilance stage	Descriptives	NTg 1.5 m	NTg 5.5-7m	NTg 8-10 m	Tg2576 1.5 m	Tg2576 5.5-7 m	Tg2576 8-10 m
AWK	Mean ± SD	55.23 ± 5.1 %	50.32 ± 5.26 %	47.33 ± 8.82 %	60.4 ± 4.88 %	56.06 ± 6.55 %	60.22 ± 7.24 %
	N	15	8	15	17	14	12
SWS	Mean ± SD	35.84 ± 4.85 %	41.26 ± 4.53 %	45.55 ± 7.56 %	32.58 ± 4.47 %	36.96 ± 5.47 %	34.53 ± 6.09 %
	Shapiro-Wilk p-value	0.77	0.32	0.4	0.13	0.7	0.2
	N	15	8	15	17	14	12
REM	Mean ± SD	8.92 ± 1.82 %	8.41 ± 1.92 %	7.12 ± 1.57 %	7 ± 1.65 %	7 ± 2.35 %	5.25 ± 1.78 %
	Shapiro-Wilk p-value	0.72	0.99	0.35	0.45	0.1	0.29
	N	15	8	15	17	14	12

**Table 4. Sleep-wake cycle analysis over the lifespan - Descriptive statistics.** Mean duration and standard deviation of time spent in different sleep stages at different ages for both genotypes, with Shapiro-Wilk normality test p-values and group sizes (N).

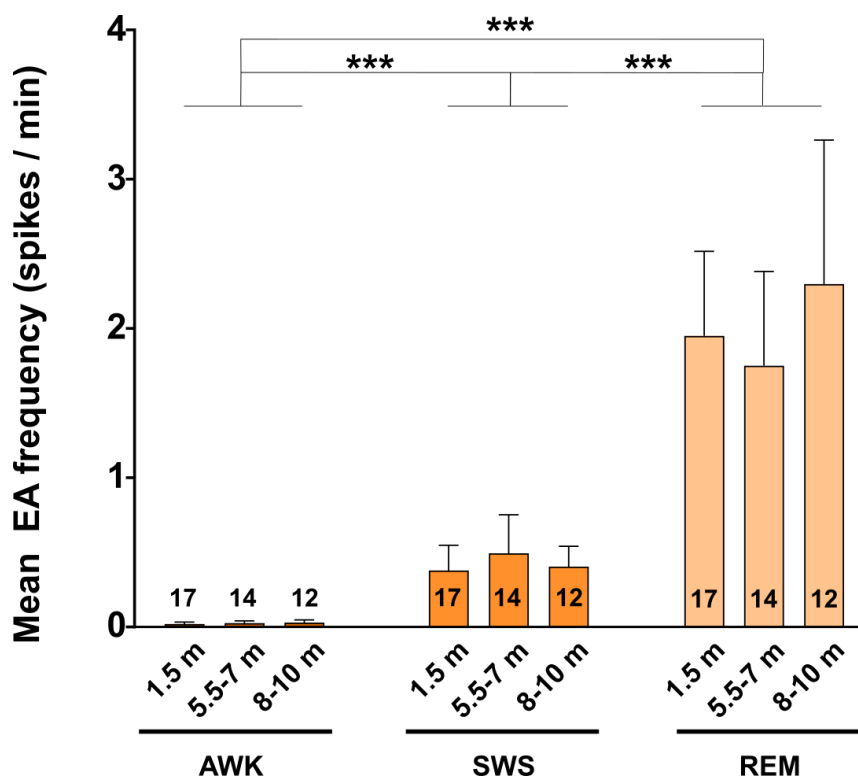
Vigilance stage	Two-way ANOVAs				Tukey Post Hoc Tests				
	Factors	df	F	p	Comparisons	Mean diff.	SE	t	Adjusted P <sub>Tukey</sub>
AWK	Age	2	4.202	<b>0.019</b>	1.5 m vs 5.5-7 m	4.625	1.842	2.511	<b>0.037</b>
					1.5 m vs 8-10 m	4.042	1.704	2.372	0.052
					5.5-7 vs 8-10 m	-0.583	1.911	-0.305	0.95
	Age * Genotype	2	2.955	0.058	1.5 m: NTg vs. Tg2576	-5.171	2.3	-2.248	0.229
					5.5-7 m: NTg vs. Tg2576	-5.734	2.878	-1.992	0.356
					<b>8-10 m: NTg vs. Tg2576</b>	-12.89	2.515	-5.126	<b>&lt; 0.001</b>
Genotype	1	28.458	<b>&lt; 0.001</b>	NA					
SWS	Age	2	8.91	<b>&lt; 0.001</b>	1.5 m vs 5.5-7 m	-4.893	1.601	-3.057	<b>0.009</b>
					1.5 m vs 8-10 m	-5.818	1.481	-3.929	<b>&lt; 0.001</b>
					5.5-7 vs 8-10 m	-0.924	1.661	-0.557	0.843
	Age * Genotype*	2	3.793	<b>0.027</b>	NTg 1.5 m vs. 8-10 m	-9.692	2.06	-4.704	<b>&lt; 0.001</b>
					<b>NTg 8-10 m: vs. Tg2576 8-10 m</b>	11.022	2.185	5.043	<b>&lt; 0.001</b>
	Genotype	1	23.018	<b>&lt; 0.001</b>	NA				
REM	Age	2	7.371	<b>0.001</b>	1.5 m vs 5.5-7 m	0.255	0.525	0.486	0.878
					1.5 m vs 8-10 m	1.777	0.486	3.659	<b>0.001</b>
					5.5-7 vs 8-10 m	1.522	0.545	2.794	<b>0.018</b>
	Genotype	1	16.707	<b>&lt; 0.001</b>	NA				
	Age * Genotype	2	0.127	0.881	NA				

**Table 5. Sleep-wake cycle analysis over the lifespan – Main analysis and post hoc tests.** Significant differences are shown in bold. \*: only significant differences between genotypes at the same age or the same genotype at different ages are shown.

Altogether, these results show an age-related decrease in REM sleep and wakefulness in NTg mice and a deficit in the time spent sleeping in Tg2576, which is already significant at 1.5 months for REM sleep and only gets significant much later, at 8-10 months, when considering the time spent in SWS.

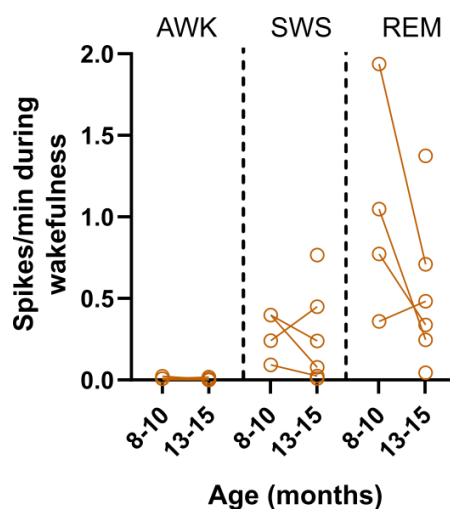
### 3.2. Epileptic events preferentially occur during REM sleep in Tg2576 mice and increase with REM sleep episode length

Given that only six spikes were detected across the entire non-transgenic cohort (n = 38 mice), we restricted the analysis of epileptic events to Tg2576 animals. First of all, spikes were detected in all but one Tg2576 tested. However, what shines through **Figure 47** is the extreme inter-individual variability. Indeed, when looking at inter-individual variability pooled across all ages in the different sleep stages, while spikes were rare in wakefulness in general, they were still widely distributed across the mean (Mean EA/minute +/- SD = 0.03 +/- 0.53). The inter-individual variability was also present during SWS (0.41 +/- 0.73) and REM sleep (1.96 +/- 2.61). From these values, it is also clear that EA frequency (spikes/minute) was highly dependent on the sleep-wake cycle (Friedman test:  $p < 0.001$ , **Figure 47**). Specifically, post hoc tests revealed a gradual increase in EA from wakefulness to SWS ( $p < 0.001$ ) and from SWS to REM ( $p < 0.001$ ).



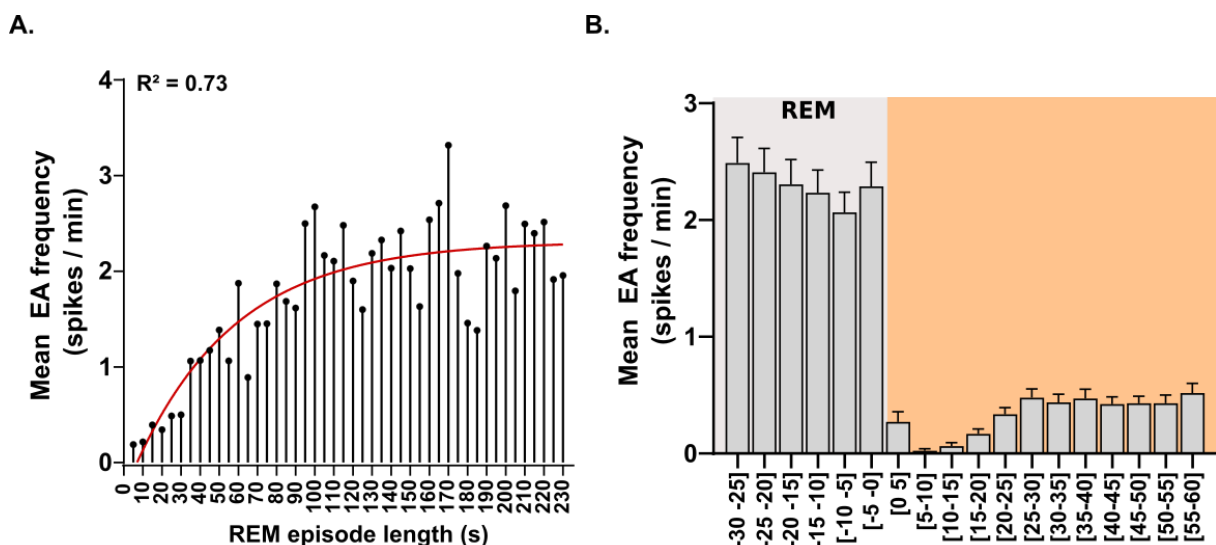
**Figure 47. Epileptiform activities increase during REM sleep and with REM sleep episode length, independent of disease progression.** Spike frequency over the sleep-wake cycle in Tg2576 mice throughout the lifespan. AWK = Awake, SWS = Slow-wave sleep, REM = Rapid-eye movement sleep. Data represented as Mean + SEM. \*\*\*:  $p < 0.001$

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 from 1.5 months to 8-10 months of age (Kruskal-Wallis tests: AWK:  $p = 0.901$ ; SWS:  $p = 0.891$ ; REM:  $p = 0.919$ ). Furthermore, the EEG of a subset of seven Tg2576 mice (four of which were initially recorded at 8-10 months for the main analysis) was also recorded at 13-15 months. In these aged mice, there is a tendency toward 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), further implying that EA does not increase with age. However, this latter result from this restricted cohort of 13-15 month-old mice might have resulted from higher mortality rates of individuals with more frequent epileptic events – and potentially, seizures in the first year of life. This would lead to the mice with higher levels of epileptic activity not surviving up to this later age while less epilepsy-prone mice would, biasing EA frequencies. Therefore, given this potential bias and the small sample size and epilepsy-related deaths in our model, data from the 13-15 month-old animals were excluded from the main analyses. However, results are shown for visualization in **Figure 48 A-C** for the full cohort of 13-15 month-old Tg2576 mice, and also for the 4 animals for whom comparative data at 8-10 months was available. As can be seen, three out of four mice show remarkable decreases, which suggests that it may indeed be a decrease in spike frequency that is behind the observed decrease and not higher mortality rates of epilepsy-prone mice – although the two hypotheses are not mutually exclusive. Future, longitudinal explorations may shine light on this point.



**Figure 48. Spike frequencies do not increase with disease severity even in the later stages.** Spike frequencies for the subset of 6 mice recorded at later ages, with data recorded at 8-10 months in the same individuals when available.  $n = 4$  at 8-10 months and  $n=6$  at 13-15 months of age.

The REM-sleep-related dramatic increase of EA is a robust result, and we tried to further understand its mechanical underpinnings. It has been previously suggested that hippocampal excitability levels are progressively decreasing throughout successive REM sleep episodes during the night (Grosmark et al., 2012). Therefore, we analyzed spike frequencies throughout REM-sleep episodes in all Tg2576 mice (all ages confounded) to determine whether EA frequency evolves toward increase or decrease during the length of single REM episodes. Based on 3891 REM sleep episodes from the cohort of Tg2576 mice, our data seem to contradict the proposition about decreasing hippocampal excitability during REM sleep – at least in this model (**Figure 49 A**). Indeed, the data were well-fitted by a nonlinear fit using an exponential plateau model (adjusted R<sup>2</sup> = 0.73). It is also clear that the offset of REM sleep led to an instant drop rather than a gradual decrease in EA (**Figure 49 B**). Altogether, these data suggest that hippocampal excitability does not decrease in Tg2576 mice across REM sleep episodes.



**Figure 49. Epileptiform activities increase during REM sleep and with REM sleep episode length, independent of disease progression.** (A) 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. (B) 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).

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

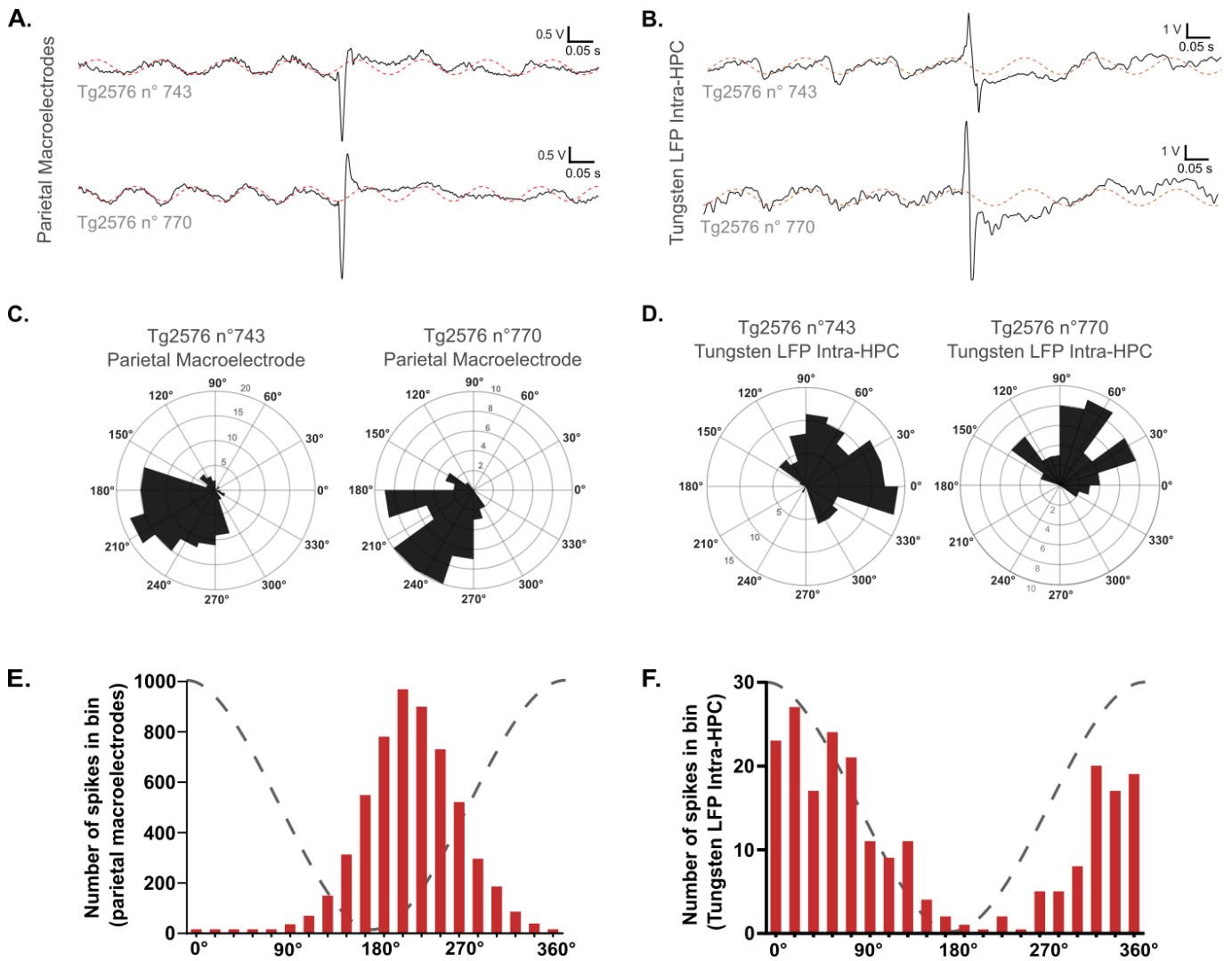
To better understand how this peculiar EA is generated in Tg2576 mice, we dug deeper into analyzing the spikes occurring during REM sleep. We looked at the timing of these spikes during theta oscillations, the dominant brain oscillation of REM sleep (Jouvet, 1967b). To do



so, we analyzed the REM sleep episodes of all Tg2576 mice (all ages confounded) 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 train 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 (See **Figure 50 A** for an illustration). Second, on a more quantitative level, individual analyses revealed that every animal had spikes whose phase distribution was significantly locked to 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 seven spikes and another with only nine, respectively). Actually, 73.41% of all spikes (pooled for all mice) appeared between  $162^\circ$  and  $270^\circ$ . This angle range covers mainly the trough and the first half of the ascendant phase of the theta cycle recorded from the parietal EEG (See **Figure 50 C** for individual illustrations and **Figure 50 E** for the grouped analysis), which corresponds to the phase when hippocampal pyramidal cells are most likely to fire action potentials (Klausberger et al., 2003).

As mentioned earlier, this theta rhythm is recorded from parietal macroelectrodes in the subdural zone, which begs the question of whether our recordings reflect activity at the cortical level or volume conduction from deeper, potentially hippocampal areas. Therefore, we decided to back our analyses with the exploration of data from the Tungsten LFP intrahippocampal electrodes with which 6 animals (one NTg and five Tg2576) were also implanted, allowing us to see the signal at the hippocampal level. The same analysis was repeated as described before for Tg2576 mice, revealing similar levels of phase-locking in four of the animals analysed ( $p < 0.001$ ) and  $p = 0.12$  in the fifth animal with only 12 recorded spikes on the intrahippocampal electrodes. However, as expected, it turned out that the phase of theta oscillation at the hippocampal level is the exact opposite of the one observed on the macroelectrodes (**Figure 50 B, D, F**). The phase reversal of the theta oscillations was also observed for the NTg mouse with intrahippocampal recordings, even though spikes were still not present. Thus, we showed that during REM sleep, epileptic spikes appear preferentially during the peak and the flanking phases of the hippocampal theta cycle ( $0 - 90^\circ$  and  $300 - 360^\circ$ ), which can further inform us on the cell types potentially involved in their generation (Somogyi & Klausberger, 2005).



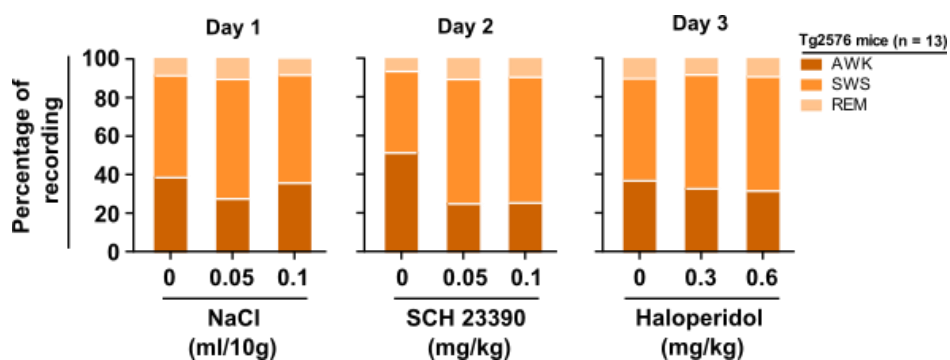
**Figure 50. Epileptiform activities are phase-locked on theta activity.** (A) and (B) Examples of interictal spikes (continuous line) on parietal macroelectrodes (A) and intrahippocampal Tungsten-LFP electrodes (B) compared to a regular theta oscillation (dashed red line) revealing a preferential spike-timing around the trough and the ascending phase of the cortical theta cycle. (C) and (D) Examples of spiking activity from two Tg2576 mice on the theta cycle on parietal macroelectrodes (C) and intrahippocampal Tungsten-LFP electrodes (D), with 180° representing the trough. Spike numbers are represented by inner circles. Each bin corresponds to 18°. (E) Spike-timing around the theta cycle recorded with parietal macroelectrodes during REM sleep for all recorded spikes from n=38 Tg2576 mice. Total number of spikes = 5675. (F) Spike-timing around the theta cycle recorded with intrahippocampal Tungsten LFP electrodes during REM sleep for all recorded spikes from n=5 Tg2576 mice. Total number of spikes = 227.

### 3.4. The impact of dopaminergic transmission on sleep and epileptic activity

We tested the impact of dopaminergic transmission on EA frequency in our mouse model as previous *in vitro* reports have suggested that dopaminergic transmission may play a role in EA generation in AD (Costa et al., 2016; Ren et al., 2018). Given that dopamine secretion fluctuates during wakefulness, becomes rather low during SWS and reaches high levels during REM sleep (Dahan et al., 2007), it could be the case that dopamine contributes to EA, especially during REM sleep. Therefore, blocking dopaminergic transmission during REM sleep should lead to a decrease of EA frequency.

#### *3.4.1. D1/D5 and D2 receptor blockade impacts REM sleep latency but not sleep duration*

One animal had to be removed from the Dopamine-study due to concerns over its well-being during the experiment as a subcutaneous oedema developed at the level of the neck muscles, potentially due to the EMG electrode. Due to technical issues, another mouse's baseline (Day1, pre-injection) was not exploitable. Still, data from this animal for the other experimental days were taken into account for the analyses whenever possible by the analysis design.

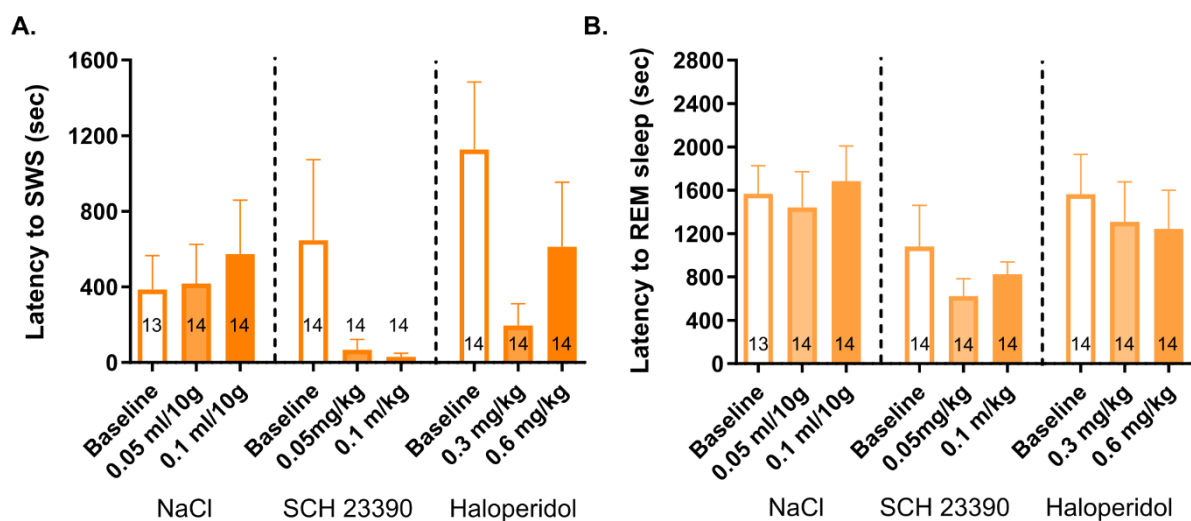


**Figure 51. The sleep-wake cycle is not significantly affected by dopaminergic antagonists.** Sleep-wake cycle modulation of SCH 23390 (D1 receptor antagonist) and Haloperidol (D1 and D2 receptor antagonist) in varying doses over 1.5-hour-long periods compared to control injections (NaCl). The treatments affected mostly sleep latencies without thoroughly altering sleep percentages per se. The increased sleep quantity following SCH 23390 treatments may be a result of a joint effect of the drug and a sleep-rebound for the two animals having spent the whole pre-injection period awake.

As per the effect of the different dopamine-receptor targeting treatments on sleep parameters **Figure 51** already suggests relatively stable values on Day 1 (NaCl), while the baseline of the second day (SCH 23390) comes with high levels of wakefulness – which was due to two of the mice not sleeping technically at all (or only minimal quantities) during this

period – while sleep quantities do not differ vastly from post-injection periods seen on Day 1. As per the third day, Haloperidol does not seem to impact sleep durations.

Indeed, statistics do confirm the quasi-absence of an impact of the drugs used: the two-way ANOVA for repeated measures (factors: treatment, dose) on slow-wave sleep duration (**Figure 51, Tables 6 and 7**) revealed no main effect of treatment ( $p = 0.95$ ), a main effect of dose ( $p = .003$ ) albeit with a feeble effect size (Cohen’s  $d = 0.156$ ), and a significant interaction ( $p = 0.04$ ) with a negligible effect size (Cohen’s  $d = 0.08$ ). Posthoc tests revealed that the baseline intervals of the three experimental days did not differ significantly (Day 1 vs Day 2:  $p = 0.55$ ; Day 2 vs Day 3:  $p = 0.77$ ; Day 1 vs Day 3:  $p = 1$ ), and the significant differences behind the interaction effect were all related to the baseline interval of Day 2 where SWS duration was reduced, which was probably due, as mentioned above, to the biasing factor of two mice not sleeping at all during this period. As per the latencies to slow-wave sleep, one-way ANOVAs with repeated measures were run between the same dosages of different treatments. No significant results were found even though **Figure 52 A** suggests a strong sedative effect of SCH-23390 (small and large dose) and of haloperidol (small dose) with visibly lower latencies to sleep, potentially hindered by the high inter-individual variabilities.



**Figure 52. Sleep latencies are not significantly affected by the dopaminergic treatments. (A)** Latency to first SWS episode (in seconds). **(B)** Latency to REM sleep (in seconds).

	Descriptives	NaCl Baseline	NaCl Small Dose	NaCl Large Dose	SCH 23390 BL	SCH 23390 Small Dose	SCH 23390 Large dose	Haloperidol Baseline	Haloperidol Small dose	Haloperidol Large dose
REM	Mean ± SD	8,65 ± 4,56	10,42 ± 3,64	8,29 ± 4,61	6,89 ± 4,86	10,94 ± 4,36	10,07 ± 3,89	10,3 ± 4,61	8,51 ± 4,48	9,6 ± 4,44
	Shapiro-Wilk p-value	0,236	0,203	0,26	0,641	0,13	0,044	0,626	0,597	0,076
	N	13	14	14	14	14	14	14	14	14
SWS	Mean ± SD	52,94 ± 9,3	62,18 ± 9,02	56,19 ± 13,35	42,16 ± 19,72	64,41 ± 8,82	64,7 ± 9,26	53,04 ± 14,08	59,05 ± 12,34	59,01 ± 19,61
	Shapiro-Wilk p-value	0,123	0,062	0,032	0,01	0,602	0,165	0,664	0,406	0,086
	N	13	14	14	14	14	14	14	14	14
WK	Mean ± SD	38,42 ± 11,83	27,39 ± 10,5	35,52 ± 16,31	50,95 ± 23,03	24,65 ± 8,71	25,23 ± 9,86	36,66 ± 17,22	32,45 ± 14,59	31,39 ± 21,1
	Shapiro-Wilk p-value	0,088	0,236	0,279	0,016	0,167	0,261	0,549	0,411	0,015
	N	13	14	14	14	14	14	14	14	14

Table 6. Sleep characteristics in response to NaCl, SCH 23390 or Haloperidol injections - Descriptive statistics

As for REM sleep durations (**Figure 51**), only a meager interaction was discovered ( $p = 0.02$ ; Cohen's  $d = 0.1$ ) with neither the main effect of treatment ( $p = 0.95$ ) nor that of dose ( $p = .379$ ) being significant. Furthermore, post hoc comparisons for the Interaction factor hindered all significance levels, leading to the conclusion that our treatments did not affect REM sleep durations. As for the latencies, using the same analyses as for the SWS latencies, we only uncovered a mild tendency for the larger dose of SCH-23390 to induce REMS faster than it occurs after NaCl injections (main effect of treatment:  $p = 0.1$ ; posthoc comparison:  $p = 0.1$ , **Figure 52 B**). Altogether, the doses used in this study did not significantly alter REM sleep, while SCH-23390 led to slightly increased SWS durations and slightly lower latencies to SWS. However, the low quantities of SWS observed in the baseline recording of the second day (SCH 23390) poses a potential bias and require a cautious interpretation of the data.

	Two-Way RM ANOVA			Post hoc tests				
	Factor	F	P value	Comparisons	SE	t	P holm	
REM	Treatment	0,054	0,948	NA				
	Dose	1,009	0,379	NA				
	<b>Dose * Treatment</b>	3,433	<b>0,015</b>	No significant effect in the post hoc analyses				
SWS	Treatment	0,052	0,949	NA				
	<b>Dose</b>	7,231	<b>0,003</b>	<b>Baseline vs Small</b>	-12,066	3,443	-3,505	<b>0,005</b>
				<b>Baseline vs Large</b>	-10,435	3,443	-3,031	<b>0,012</b>
				Small vs Large	1,632	3,443	0,474	0,64
	<b>Treatment * Dose*</b>	2,775	<b>0,037</b>	<b>SCH 23990 vs NaCl Baselines</b>	-19,775	5,146	-3,843	<b>0,008</b>
				<b>SCH 23990 Baseline vs small dose</b>	-22,359	5,349	-4,18	<b>0,003</b>
				<b>SCH 23990 Baseline vs large dose</b>	-22,856	5,349	-4,273	<b>0,002</b>
WK	Treatment	0,015	0,985	NA				
	<b>Dose</b>	6,204	<b>0,007</b>	<b>BL vs SD</b>	13,379	4,067	3,29	<b>0,009</b>
				<b>BL vs LD</b>	11,123	4,067	2,735	<b>0,023</b>
				<b>SD Vs LD</b>	-2,256	4,067	-0,555	0,584
	<b>Treatment * Dose</b>	3,257	<b>0,019</b>	<b>SCH 23990 vs NaCl Baselines</b>	23,328	6,044	3,86	<b>0,008</b>
				<b>SCH 23990 Baseline vs Small dose</b>	26,642	6,222	4,282	<b>0,002</b>
				<b>SCH 23390 Baseline vs large dose</b>	25,874	6,222	4,159	<b>0,003</b>

Table 7. Sleep characteristics in response to NaCl, SCH 23390 or Haloperidol injections - Main analysis and post hoc tests.

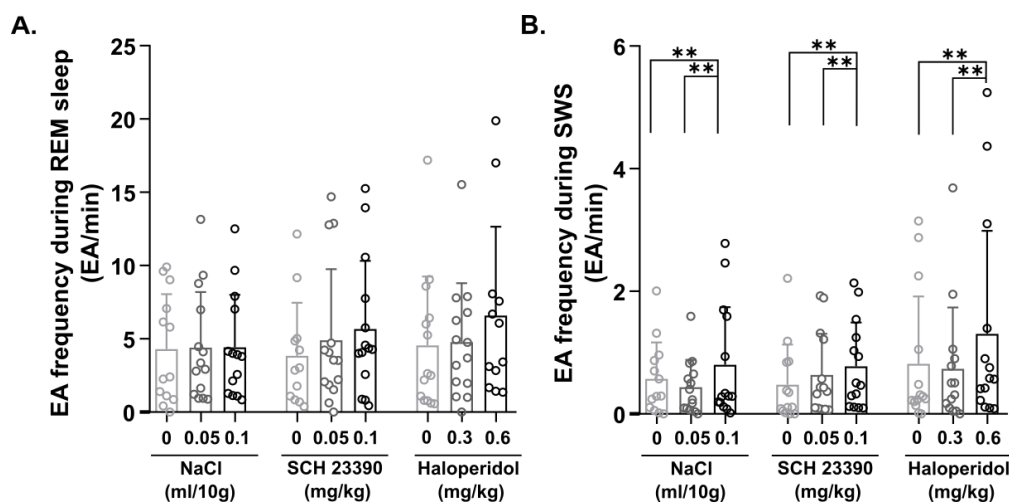
### 3.4.2. Dopaminergic antagonists do not impact EA frequency

Our hypothesis based on previous results stated that the blockade of D1-like receptors should decrease spiking activity during REM sleep. Given that the pharmacological treatments did not have major effects on REM sleep duration, the analyses could be carried out without important sleep-wake cycle-related biases.

On an exploratory basis, we also verified whether the treatments affected spiking activity in other vigilance states, starting with wakefulness. This analysis turned out to be a futile one as neither the main effect of treatment ( $p = 0.56$ ), nor that of dose ( $p = 0.39$ ) or that of the interaction ( $p = 0.3$ ) was significant. However, even though the RM-ANOVA run on the slow-wave sleep data did not find significant effects of the treatment ( $p = 0.29$ ) or of the interaction

( $p = 0.56$ ), it revealed a significant effect of dose ( $p < 0.001$ ). Post-hoc analysis clarified that the effect lay in the increase in spike frequency on each day after the large dose ( $p = 0.003$  both for Baseline-Large dose and Small dose – Large dose comparisons, Baseline – Small dose not significant). This hints at a potential circadian effect. To explore that, we ran a supplementary One-way ANOVA for repeated measures comparing the spike frequency during SWS following the large doses from Day 1 to Day 3. This analysis revealed no significant main effect ( $p = 0.78$ ), confirming that spike frequency did not differ following large doses of either treatment and was probably due to a circadian effect.

Finally, to make sure that the two types of haloperidol treatments used (soluble haloperidol chloride or ready-to-use solution) did not affect the results, one-way ANOVAs of repeated measures were run for each vigilance state during Day 3, taking into account the type of haloperidol treatment as between-subject factor. No significant effect was found for this potentially confounding factor during REM sleep ( $p = 0.16$ ), SWS ( $p = 0.77$ ) or wakefulness ( $p = 0.52$ ).



**Figure 53. Epileptiform activities are not modulated by dopaminergic transmission in Tg2576 mice.** Modulation of SCH 23390 (D1 receptor antagonist) and Haloperidol (D1 and D2 receptor antagonist) in varying doses over 1.5-hour-long periods on epileptiform activity (A) during REM sleep ( $n = 13$  on NaCl baseline and  $n = 12$  on SCH 23390 baseline and Haloperidol post large-dose, otherwise  $n = 14$ ) and (B) during SWS for Tg2576 mice, with  $n = 14$  except Day 1 and Day 2 baselines ( $n = 13$ ). Data represented as Mean + SEM. \*\*:  $p < 0.01$ .

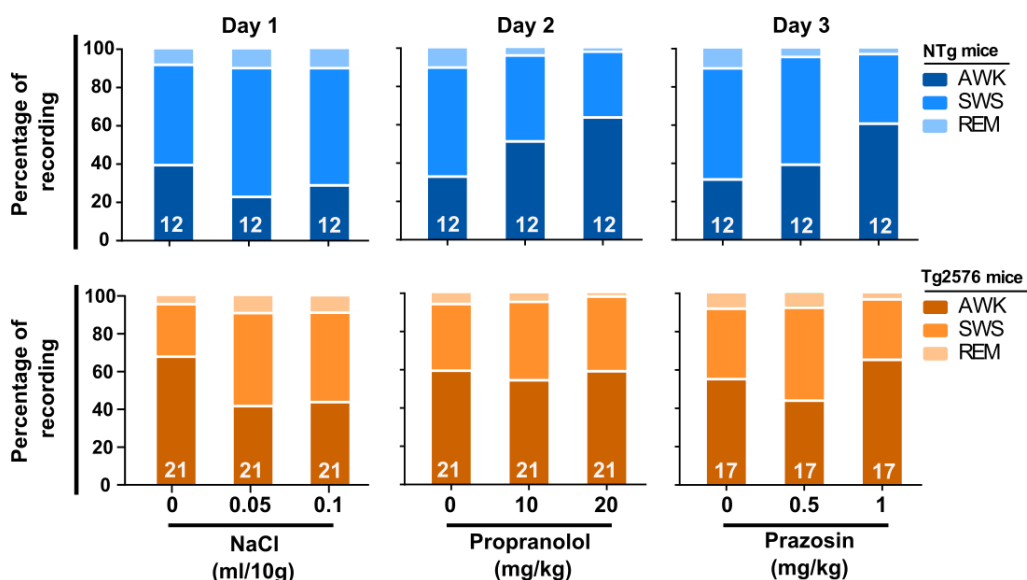
### 3.5. The impact of noradrenergic transmission on sleep and epileptic activity

Noradrenaline release follows an inverse dynamic to the EA frequency observed in Tg2576 mice (that is, maximal activity of noradrenergic neurons during wakefulness, decreasing activity during SWS and quasi-silence during REM sleep). Furthermore, this neurotransmitter has already been suggested as possessing antiepileptic properties. In light of these

informations, we hypothesised that noradrenaline might exert an anti-epileptic effect, especially during wakefulness and, to a lesser extent, during SWS, while its protective effect would disappear during REM sleep, unmasking network hyperexcitability. Therefore, we tested the effect of propranolol ( $\beta$  adrenoreceptor antagonist) and prazosin ( $\alpha$ 1 adrenoreceptor antagonist) on EA during wakefulness and SWS.

### 3.5.1. Modulation of sleep-activity by $\alpha$ 1- and $\beta$ 2-AR blockade

We first verified that pharmacological treatments did not drastically reduce wakefulness and SWS in Tg2576 mice (**Figure 54, Table 8**). In NTg animals, they both dose-dependently increased wakefulness (61-64% following high dose vs 32-40% at baseline) and concomitantly decreased SWS (34-36% following high dose vs 57-58% at baseline) as well as REM sleep (1-2% following high dose vs 9-10% at baseline). In Tg2576 mice, which exhibit high levels of wakefulness and low levels of SWS at baseline, these treatments had no significant effect on these two vigilance states (**Table 9-10**), while they decreased REM sleep (1-2.5% following high doses vs 5-7% at baseline **Table 11**). Therefore, 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.



**Figure 54.** Sleep-wake cycle modulation of propranolol ( $\beta$ -AR antagonist) and prazosin ( $\alpha$ 1 antagonist) in varying doses over 1.5-hour-long periods in Tg2576 (orange) mice and NTg littermates (blue). In NTg animals, both drugs led to dose-dependent decreases of both SWS and REM sleep. Given that SWS percentages were already lower in Tg2576 mice, the treatments did not seem to further decrease their amount, however, a drop in REM percentage is perceptible.



		NaCl Baseline	NaCl Small Dose	NaCl Large Dose	Propranolol Baseline	Propranolol Small Dose	Propranolol Large Dose	Prazosin Baseline	Prazosin Small Dose	Prazosin Large Dose
WK	N (Tg2576; NTg)	21; 12	21; 12	21; 12	21; 12	21; 12	21; 12	17; 12	17; 12	17; 12
	Shapiro-Wilk P value Tg2576	0.02	0.053	0.624	0.147	0.435	0.255	0.01	0.081	0.383
	Shapiro-Wilk P value NTg	0.15	0.133	0.049	0.022	0.088	0.37	0.001	0.314	0.946
	Mean ± SD Tg2576	68.59 ± 27.703	42.37 ± 24.160	44.5 ± 19.1	60.31 ± 24.3	55.36 ± 19.28	59.93 ± 16.9	56.05 ± 30.92	44.77 ± 23.26	66.06 ± 24.83
	Mean ± SD NTg	40.20 ± 22.5	23.65 ± 13.59	29.6 ± 12.9	33.72 ± 20.48	52.06 ± 13.94	64.52 ± 23.02	32.15 ± 21.8	40.06 ± 21.86	61.47 ± 22.52
SWS	N (Tg2576; NTg)	21; 12	21; 12	21; 12	21; 12	21; 12	21; 12	17; 12	17; 12	17; 12
	Shapiro-Wilk P value Tg2576	0.023	0.049	0.76	0.26	0.64	0.38	0.014	0.11	0.3
	Shapiro-Wilk P value NTg	0.059	0.32	0.024	0.047	0.04	0.343	0.001	0.314	0.946
	Mean ± SD Tg2576	27.8 ± 24.11	49.26 ± 20.61	47.3 ± 15.97	34.78 ± 21.28	40.95 ± 18.66	39.09 ± 16.64	36.69 ± 25.99	48.53 ± 19.85	31.49 ± 22.96
	Mean ± SD NTg	52.46 ± 19.74	67.3 ± 11.05	61.26 ± 10.14	57.03 ± 17.07	44.91 ± 13.79	34.53 ± 21.83	58.18 ± 18.05	56.43 ± 19.41	36.49 ± 21.49
REM	N (Tg2576; NTg)	21; 12	21; 12	21; 12	21; 12	21; 12	21; 12	17; 12	17; 12	17; 12
	Shapiro-Wilk P value Tg2576	0.001	0.73	0.56	0.11	0.14	< 0.001	0.04	0.14	< 0.001
	Shapiro-Wilk P value NTg	0.8	0.69	0.11	0.08	0.09	< 0.001	0.99	0.05	0.003
	Mean ± SD Tg2576	3.6 ± 4.3	8.37 ± 4.67	7.81 ± 4.4	4.91 ± 3.72	3.73 ± 2.87	0.98 ± 1.68	7.27 ± 5.61	6.71 ± 5.05	2.45 ± 3.51
	Mean ± SD NTg	7.34 ± 3.84	9.05 ± 4.24	9.14 ± 4.31	9.25 ± 4.17	3.03 ± 1.97	0.96 ± 1.46	9.67 ± 4.77	3.51 ± 3.86	2.04 ± 2.73

*Table 8. Descriptive statistics for the analysis of noradrenergic receptor blockade on the sleep-wake cycle for Tg2576 and NTg animals.*

Sleep stage	Treatment	2-WAY RM ANOVA (Dose, Genotype)				Holm Post hoc tests				
		Cases	df	F	P value	Comparison	Mean diff.	SE	t	P holm
WK	NaCl	Dose	2	9.83	< 0.001	Baseline vs Small Dose	21.384	5.126	4.172	<0.001
						Baseline vs Large Dose	17.342	5.126	3.383	0.002
		Genotype	1	16.08	< 0.001					
		Dose * Genotype	2	0.92	0.4					
	Propra-nolol	Dose*	1.58**	5.32**	0.013**	Baseline vs Large Dose	-15.21	ppt	-3.26	0.006
		Dose * Genotype*	1.58**	6.03**	0.008**	NTg Baseline vs Tg2576 Baseline	-26.59	7.27	-3.66	0.006
						NTg Baseline vs Tg2576 Small Dose	21.61	7.27	-2.97	0.046
						NTg Baseline vs NTg Large Dose	-30.8	7.45	-4.13	0.002
						NTg Baseline vs Tg2576 Large Dose	-26.21	7.2	-3.61	0.007
		Genotype	1	2.99	0.09	NA				
	Prazosin	Dose	2	8.15	<0.001	Baseline vs Small Dose	1.68	5.88	0.29	0.776
						Baseline vs Large Dose	-19.69	5.88	-3.34	0.003
						Small Dose vs Large Dose	-21.35	5.88	-3.63	0.002
		Genotype	1	2.96	0.097	NA				
		Dose * Genotype	2	1.79	0.177	NA				

Table 9. Proportion of wakefulness following NaCl, Propranolol or Prazosin injections - TWO-WAY RM ANOVAs and post hoc tests. \*: Only Significant Interactions shown

Sleep stage	Treatment	2-WAY RM ANOVA (Dose, Genotype)				Holm Post Hoc tests				
		Cases	df	F	P value	Comparison	Mean diff.	SE	t	P holm
SWS	NaCl	Dose	2	10.01	< 0.001	Baseline vs Small Dose	-18.05	4.263	-4.26	< 0.001
						Baseline vs Large Dose	-14.15	4.263	-3.32	0.003
						Small Dose vs Large Dose	4	4.263	0.94	0.352
		Genotype	1	17.14	< 0.001	NA				
		Dose * Genotype	2	0.8	0.453	NA				
	Propranolol	Dose * Genotype *	1.63**	5.13**	0.014**	NTg vs Tg2576 Baseline	22.25	6.72	3.31	0.02
						NTg Baseline vs NTg Large Dose	22.502	6.824	3.298	0.023
		Genotype	1	2.51	0.124	NA				
		Dose	1.63**	2.35**	0.115**	NA				
	Prazosin	Dose	1.66**	6.82**	0.004	Baseline vs Small Dose	-5.05	5.15	-0.98	0.332
						Baseline vs Large Dose	13.44	5.15	2.61	0.023
						Small Dose vs Large Dose	18.49	5.15	3.59	0.002
		Genotype	1	4.12	0.052	NA				
		Dose * Genotype	1.66**	1.46**	0.243	NA				

Table 10. Proportion of SWS following NaCl, Propranolol or Prazosin injections – TWO-WAY RM ANOVAs and post hoc tests \*: Only Significant Interactions shown

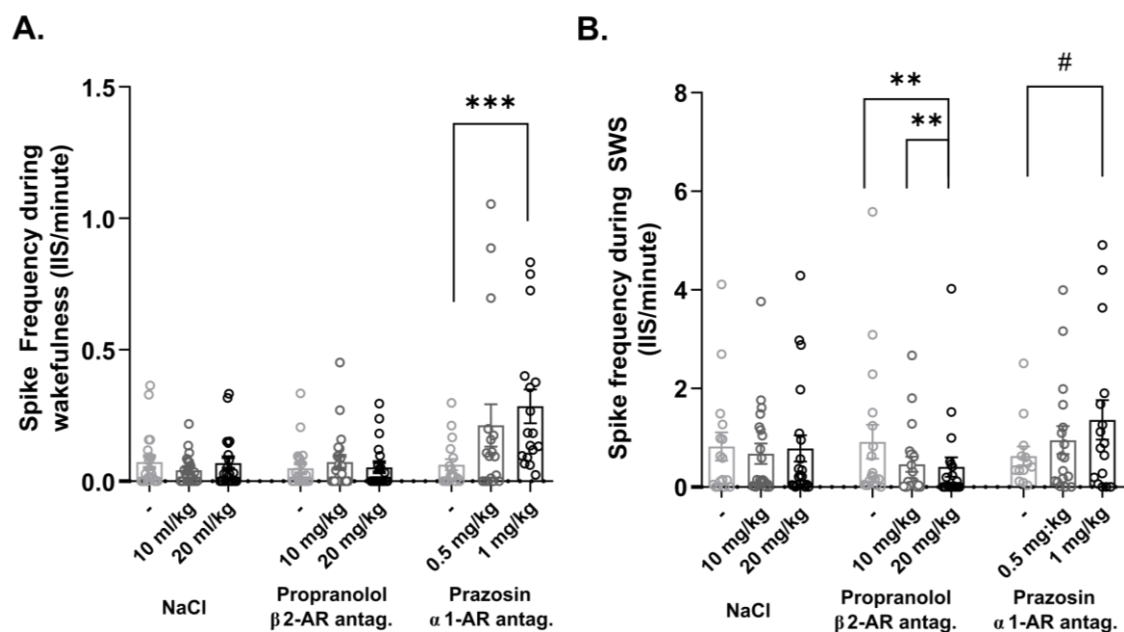
Sleep stage	Treatment	2-WAY RM ANOVA (Dose, Genotype)				Holm Post hoc tests				
		Cases	df	F	P value	Comparison	Mean diff.	SE	t	P holm
REM	NaCl	Dose	2	6.17	<b>0.004</b>	Baseline vs Small Dose	-3.24	1.03	-3.15	<b>0.008</b>
						Baseline vs Large Dose	-3	1.03	-2.92	<b>0.01</b>
						Small Dose vs Large Dose	0.23	1.03	0.23	0.82
		Genotype	1	3.45	0.073	NA				
		Dose * Genotype	2	1.23	0.3	NA				
	Propra-nolol	Dose	1.43**	43.21**	<b>&lt;0.001**</b>	Baseline vs Small Dose	3.703	0.662	5.591	<b>&lt; 0.001</b>
						Baseline vs Large Dose	6.112	0.662	9.227	<b>&lt; 0.001</b>
						Small Dose vs Large Dose	2.409	0.662	3.637	<b>&lt; 0.001</b>
		Genotype x Dose*	1.43**	8.51**	<b>0.002**</b>	NTg Baseline vs Tg2576 Baseline	4.338	1.031	4.206	<b>&lt; 0.001</b>
						NTg Baseline vs NTg Small Dose	6.218	1.057	5.884	<b>&lt; 0.001</b>
						NTg Baseline vs Tg2576 Small Dose	5.526	1.031	5.358	<b>&lt; 0.001</b>
						NTg Baseline vs NTg Large Dose	8.295	1.057	7.849	<b>&lt; 0.001</b>
						NTg Baseline vs Tg2576 Large Dose	8.267	1.031	8.016	<b>&lt; 0.001</b>
						Tg2576 Baseline vs NTg Large Dose	3.957	1.031	3.837	<b>0.002</b>
						Tg2576 Baseline vs Large Dose	3.929	0.799	4.918	<b>&lt; .0001</b>
						Tg2576 Small vs Large Dose	2.741	0.799	3.431	<b>0.009</b>
		Genotype	1	3.04	0.091	NA				
	Prazosin	Dose	2	16.84	<b>&lt; 0.001</b>	Baseline vs Small Dose	3.36	1.07	3.13	<b>0.006</b>
						Baseline vs Large Dose	6.22	1.07	5.8	<b>&lt;0.001</b>
						Small Dose vs Large Dose	2.86	1.07	2.66	<b>0.01</b>
		Genotype x Dose*	2	3.41	<b>0.04</b>	NTg Baseline vs NTg Small Dose	6.17	1.64	3.75	<b>0.006</b>
						NTg Baseline vs NTg Large Dose	7.63	1.64	4.64	<b>&lt;0.001</b>
						NTg Baseline vs Tg2576 Large Dose	7.23	1.68	4.31	<b>&lt;0.001</b>
						Tg2576 Baseline vs NTg Large Dose	5.22	1.68	3.11	<b>0.029</b>
						Tg2576 Baseline vs Tg2576 Large Dose	4.816	1.381	3.487	<b>0.012</b>
						Tg2576 Small Dose vs NTg Large Dose	4.26	1.38	3.08	<b>0.032</b>
			Genotype	1	0.12	0.73	NA			

Table 11. Proportion of REM sleep following NaCl, Propranolol or Prazosin injections - TWO-WAY RM ANOVAs and post hoc tests. \*: Only Significant Interactions shown

### 3.5.2. Opposing effects of the $\alpha$ 1- and $\beta$ 2-AR blockade on spike frequency

The next step was to quantify the impact of these interventions on EA frequency. This analysis was run only for Tg2576 mice since NTg littermates still did not exhibit any EAs whatever the experimental condition.

The frequency of EAs during wakefulness was similar across experimental days in the pre-injection period, establishing a stable baseline (Friedman test for the three pre-injection periods,  $p = 0.479$ , **Figure 55 A, Table 12**). NaCl or propranolol injections had no effect on EA frequency during wakefulness (Friedman tests:  $p = 0.34$  and  $p = 0.35$ , respectively) while prazosin dose-dependently increased it (Friedman test:  $p = 0.002$ , **Figure 55 A, Table 15**). Post-hoc tests revealed a significant difference between the baseline and prazosin 1mg/kg and but, given the high inter-individual variability, failed to show a significant difference between Prazosin 0.5mg/kg and the two other conditions.



**Figure 55. Epileptiform activities are modulated by noradrenergic transmission via  $\alpha$ 1 adrenergic receptors in Tg2576 mice.** (A) Spike-frequency modulation of propranolol ( $\beta$ -AR antagonist) and prazosin ( $\alpha$ 1 antagonist) in varying doses over 1.5-hour-long periods in Tg2576 mice during wakefulness ( $n = 21$  on Day 1 and 2,  $n = 17$  on Day 3). (B) Spike-frequency modulation of propranolol ( $\beta$ -AR antagonist) and prazosin ( $\alpha$ 1 antagonist) in varying doses over 1.5-hour-long periods in Tg2576 mice during wakefulness ( $n = 16$  on Day 1  $n = 18$  on Day 2,  $n = 17$  on Day 3). Data represented as Mean + SEM. \*:  $p < 0.05$ ; \*\*:  $p < 0.01$ ; \*\*\*:  $p < 0.001$

For the analysis of the frequency of EA during SWS (**Figure 55 B, Table 12**), only animals that slept during all three conditions of a given day were taken into account (that is, 16, 18 and 13 animals for NaCl, Propranolol, Prazosin, respectively). We observed no difference between the baseline EA frequency across experimental days and confirmed the proepileptic impact of prazosin on EA frequency (Mean EA frequency per minute:  $0.63 \pm 0.68$  at baseline,  $1.36 \pm 1.6$  after the large dose;  $p = 0.05$ ). 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 ( $p = 0.081$ ). Altogether, these results are consistent with those obtained for awake periods and point towards an antiepileptic effect of noradrenaline (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, **Figure 55 B**; baseline vs small dose:  $p = 0.93$ ; baseline vs large dose:  $p_{\text{bonf}} = 0.007$ , small vs large dose:  $p_{\text{bonf}} = 0.03$ ). These results indicate a SWS-specific pro-epileptic effect of NA via  $\beta$ -ARs. Note that no general circadian effect for SWS similar to that seen in the dopamine experiment was not found here.

Finally, the control experiment verifying whether the use of  $\beta$ -cyclodextrin for the Prazosin solution modified EA frequency reassuringly affirmed that  $\beta$ -cyclodextrin did not modify the sleep structure compared to NaCl. This was true for Wakefulness ( $p_{\text{Wilc}}$  pre-injection = 0.25;  $p_{\text{Wilc}}$  post-injection 1 = 0.63;  $p_{\text{Wilc}}$  post-injection 2 = 0.13), SWS ( $p_{\text{Wilc}}$  pre-injection = 0.25;  $p_{\text{Wilc}}$  post-injection 1 = 0.63;  $p_{\text{Wilc}}$  post-injection 2 = 0.13) and REM sleep ( $p_{\text{Wilc}}$  pre-injection = 0.38;  $p_{\text{Wilc}}$  post-injection 1 = 0.38;  $p_{\text{Wilc}}$  post-injection 2 = 0.88).

Altogether, these data support, on the one hand, the hypothesis that the drop in NA release at the beginning of sleep would unmask network hyperexcitability in Tg2576 mice and that noradrenaline would play a protective role against it via  $\alpha 1$  adrenoreceptors during wakefulness.

Descriptive Statistics						Main analyses				Post Hoc tests								
	Groups Compared	Shapiro-Wilk P value Tg2576	N	Mean ± SD	Median	Test used	Friedman tests				Conover Post Hoc tests							
							Treatment	df	p	Kendall's W	Factors	T-Stat	df	W <sub>i</sub>	W <sub>j</sub>	P value	P <sub>Bonf</sub>	
Spike Frequency AWK	NaCl Baseline	< 0.001	21	0.073 ± 0.11	0.029	Friedman Tests (one per treatment).	NaCl	2	0.308	0.056	Baseline vs Small Dose	NA						
	NaCl Small Dose	< 0.001	21	0.04 ± 0.06	0.023						Baseline vs Large dose							
	NaCl Large dose	< 0.001	21	0.07 ± 0.1	0.027						Small Dose vs Large dose							
	Propranolol Baseline	< 0.001	21	0.05 ± 0.08	0.033		Friedman Tests (one per treatment).	Propranolol	2	0.33	0.053	Baseline vs Small Dose	NA					
	Propranolol Small Dose	< 0.001	21	0.07 ± 0.11	0.028							Baseline vs Large dose						
	Propranolol Large dose	< 0.001	21	0.05 ± 0.09	0							Small Dose vs Large dose						
	Prazosin Baseline	< 0.001	17	0.06 ± 0.09	0.02		Friedman Tests (one per treatment).	Prazosin	2	<b>0.002</b>	<b>0.357</b>	Baseline vs Small Dose	1.742	32	24	34	0.091	0.273
	Prazosin Small Dose	< 0.001	17	0.21 ± 0.33	0.096							<b>Baseline vs Large dose</b>	3.485	32	24	44	<b>0.001</b>	<b>0.004</b>
	Prazosin Large dose	0.003	17	0.29 ± 0.26	0.183							Small Dose vs Large dose	1.742	32	34	44	0.091	0.273
Spike Frequency SWS	NaCl Baseline	0.009	16	0.83 ± 1.15	0.368	Friedman Tests (one per treatment).	NaCl	2	0.032	0.071	Baseline vs Small Dose	NA						
	NaCl Small Dose	< 0.001	16	0.68 ± 0.93	0.146						Baseline vs Large dose							
	NaCl Large dose	< 0.001	16	0.78 ± 1.22	0.214						Small Dose vs Large dose							
	Propranolol Baseline	< 0.001	18	0.92 ± 1.45	0.22		Friedman Tests (one per treatment).	Propranolol	2	<b>&lt; 0.001</b>	0.51	Baseline vs Small Dose	1.031	34	46	40	0.31	0.93
	Propranolol Small Dose	< 0.001	18	0.46 ± 0.7	0.109							<b>Baseline vs Large dose</b>	4.123	34	46	22	<b>&lt; 0.001</b>	<b>&lt; 0.001</b>
	Propranolol Large dose	< 0.001	18	0.41 ± 0.92	0.026							<b>Small Dose vs Large dose</b>	3.092	34	40	22	<b>0.004</b>	<b>0.012</b>
	Prazosin Baseline	0.002	13	0.63 ± 0.68	0.408		Friedman Tests (one per treatment).	Prazosin	2	<b>0.05</b>	0.231	Baseline vs Small Dose	1.765	24	19	28	0.09	0.271
	Prazosin Small Dose	0.002	13	0.95 ± 1.17	0.598							<b>Baseline vs Large dose</b>	2.353	24	19	31	0.027	0.081
	Prazosin Large dose	0.002	13	1.36 ± 1.6	0.841							Small Dose vs Large dose	0.588	24	28	31	0.562	1

Table 12. Changes in Spike frequency during wakefulness and SWS following NaCl, Propranolol and Prazosin injections during SWS and WK. Significant results in bold.

### 3.6. The impact of epileptic activities on post-learning sleep and memory consolidation

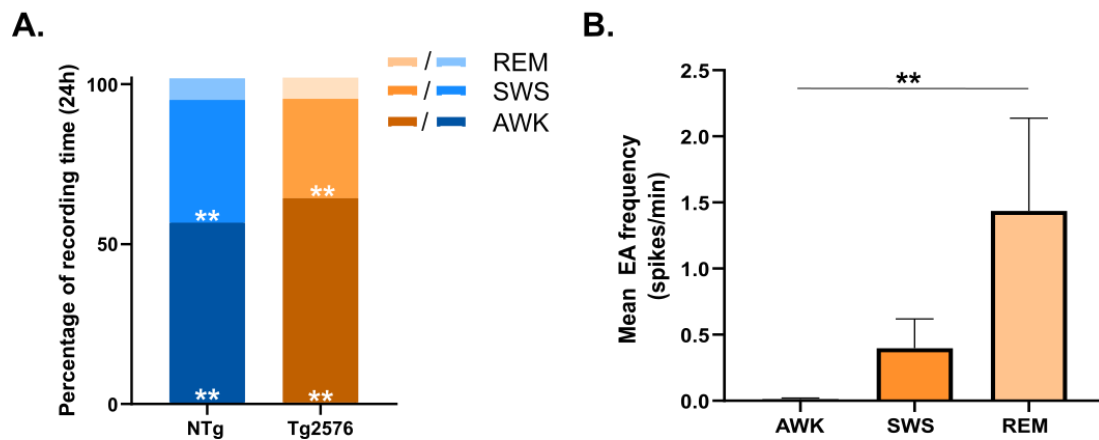
We devised a preliminary experiment to evaluate how sleep microarchitecture would be impacted by epileptic events, especially following hippocampo-dependent learning protocols where post-learning increases in spindle density have been shown before (Oliva et al., 2020). This pilot study was also implemented during the end-of-studies internship of Amel Bouloufa. Sleep macro-and microarchitecture and spike frequency were evaluated in four different conditions spun around a six-day-long social memory protocol (see **Figure 43**) modified after the study of Oliva and collaborators (2020). The social memory test seemed to be an ideal behavioural measure since deficits of this ability are well-established in our model (Rey et al., 2022), while the area associated with social memory, the CA2 region of the hippocampus, has been shown to participate in the generation of memory-consolidation-related oscillatory activities (Oliva et al., 2016, 2020). In our protocol, 9 Tg2576 mice and 6 NTg littermates were tested at 5 months of age in a 6-day-long protocol, including the exploration of a new environment and a social memory test, with post-learning sleep periods and corresponding control intervals from previous days of the experiment recorded with EEG. One NTg animal was discarded from corresponding analyses due to a technical error on the control day of the recording.

As a first step, we verified the sleep architecture over the full 24 hours of the *Control* day to ensure that the changes in sleep architecture (**Figure 56 A**) and epileptic activity (**Figure 56 B**) are still present compared to the main experiment. In fact, between the recording presented before this point and this pilot study, the mouse line was re-derived, and the facility moved to a Specific Pathogen Free (SPF) facility from the non-SPF that was used before.

This control analysis on the sleep-wake cycle (**Figure 56 A**) still finds decreased SWS and increased wakefulness for Tg2576 mice compared to NTg animals, but the difference this time is already significant at 5 months of age ( $p = 0.004$  and  $p = 0.007$ , respectively) compared to 8-10 months on the main experiment, with corresponding effect sizes of 0.91 and -0.87 even at this low sample size. Although the difference in percentage is approximately the same at five months of age between the two genotypes in both experiments, the difference in statistics



could be due to the better-controlled conditions of the new facility allowing for less disruption and ambient noise and leading to less variability across individuals.



**Figure 56. Sleep-wake cycle and spike frequency analysis over 24h from the social memory protocol. (A)** Percentages of time spent in different vigilance states over 24 hours for NTg and Tg2576 mice, with asterisks marking significant differences between the two genotypes. Data recorded on the Control day, without any experimental manipulation. **(B)** Mean EA (+ SEM) frequency in Tg2576 mice during the same day, over 24 hours.  $n_{Tg2576} = 9$ ,  $n_{NTg} = 5$ .

As for spiking activity (**Figure 56 B**), a Friedman test (chosen due to the low sample size high inter-individual variability, and notably to one animal with extremely high EA frequencies compared to the rest of the cohort) still finds a strong difference in EA frequency in different sleep stages ( $p < 0.001$ ). Conover post hoc tests indicate, however, that while the difference is highly significant between REM and wakefulness even with Bonferroni-corrected p-values, the difference is no longer significant between WK and SWS and SWS and REM ( $p = 0.15$  in both cases). However, the cohort here is only a third of what has been tested in the main experiments, and the tendency towards a statistically significant difference is visible. Given the rather coherent results with our previous observations, we proceeded with the analyses of the first three hours of recording from each experimental day as planned.

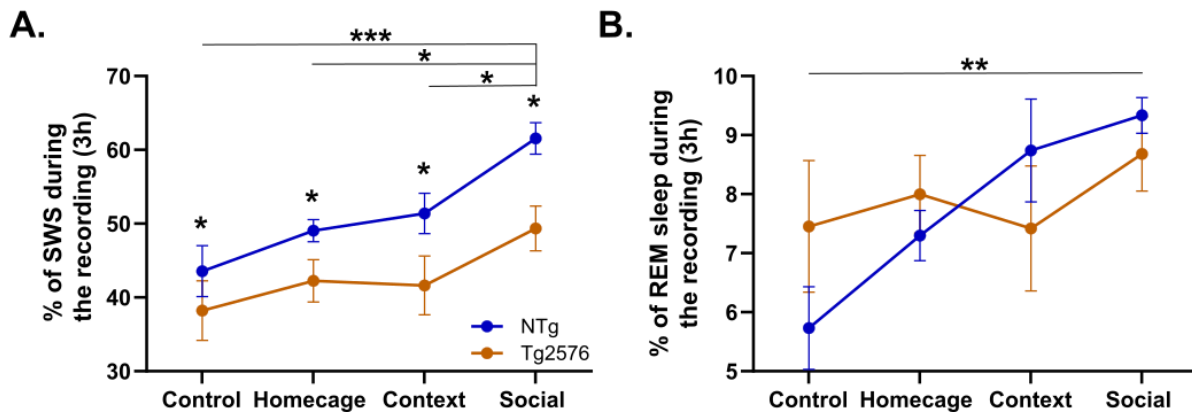
### 3.6.1. Sleep macroarchitecture changes following spatial-and social learning tasks

The most striking change across experimental days is the increase in SWS percentage after the social learning task, which seems to be more accentuated for NTg animals (Mean  $\pm$  SD<sub>control</sub> = 43.57  $\pm$  7.72; Mean  $\pm$  SD<sub>homecage</sub> = 49.05  $\pm$  3.34%; Mean  $\pm$  SD<sub>context</sub> = 51.34  $\pm$  6.11%; Mean  $\pm$  SD<sub>social</sub> = 61.55  $\pm$  4.74 %) compared to Tg2576 mice (starting at 38.22  $\pm$  12.09%,

stagnating at 42.25 +/- 8.56 on the *Homecage day* and at 41.62 +/- 11.94 on the *Context day* to reach only 49.34 +/- 9.12% following the learning phase of the social memory task; **Figure 57 A**). Statistically speaking, this translates to a main effect of the experimental day and of genotype on the RM-ANOVA ( $p < 0.001$  and  $p = 0.04$ , respectively, **Table 13**) without an interaction ( $p = 0.66$ ). Post-hoc tests confirm the difference to be due to all experimental days being significantly different from the social learning task (**Table 13**). Therefore, the experiment confirms a general decrease of SWS time for Tg2576 mice, and a sleep-enhancing impact of the social learning task independent of genotype, but not that of the exploration task.

RM-ANOVA - Social memory protocol - SWS changes					
Within Subjects Effects					
Cases	Sum of Squares	df	Mean Square	F	p
<i>Day</i>	1422.190	3	474.063	8.387	< .001
<i>Day * Genotype</i>	90.457	3	30.152	0.533	0.662
<i>Residuals</i>	2034.864	36	56.524		
Note. Type III Sum of Squares					
Between Subjects Effects					
Cases	Sum of Squares	df	Mean Square	F	p
Genotype	935.327	1	935.327	5.482	0.037
<i>Residuals</i>	2047.248	12	170.604		
Note. Type III Sum of Squares					
Post Hoc Comparisons - Day					
		Mean Difference	SE	t	p <sub>holm</sub>
<i>Control</i>	<i>Homecage</i>	-4.756	2.965	-1.604	0.235
	<i>Context</i>	-5.614	2.965	-1.893	0.199
	<i>Social</i>	-14.552	2.965	-4.907	< .001
<i>Homecage</i>	<i>Context</i>	-0.858	2.965	-0.289	0.774
	<i>Social</i>	-9.796	2.965	-3.304	0.011
<i>Context</i>	<i>Social</i>	-8.937	2.965	-3.014	0.019
Note. P-value adjusted for comparing a family of 6					

**Table 13.** Changes in SWS percentages across experimental days for NTg and Tg2576 mice - Repeated Measures ANOVA



**Figure 57.** Percentage of time spent in SWS and REM sleep in different experimental conditions for Tg2576 and NTg littermates. **(A)** Percentage of time spent in SWS in the 3 hours following the different experimental manipulations for Tg2576 (orange) and NTg mice (blue). **(B)** Percentage of time spent in REM sleep in the 3 hours following the different experimental manipulations. \*\*\*:  $p < 0.001$ ; \*\*:  $p < 0.01$ ; \*:  $p < 0.05$ . Inter-group differences are shown above the symbols representing a given day's mean and SEM. Inter-day differences are shown above the graphs. Data represented with mean  $\pm$  SEM.  $N = 9$  for Tg2576 and 5 for NTg animals.

As for REM sleep, only a main effect of *Day* is revealed ( $p = 0.01$ ) but no *genotype* effect ( $p = 0.91$ ), or interaction ( $p = 0.13$ , **Figure 57 B**). Post-hoc tests indicate that the main effect is due to the progressively increasing proportion of REM sleep for all mice, culminating in a significant difference between the *Control* and the *Social learning* day ( $p = 0.006$ ), without any other significant difference. Therefore, REM sleep percentages increase following social learning, independent of genotype in the mouse models tested here.

### 3.6.2. Sleep microarchitecture changes following spatial and social learning tasks

Regarding sleep microarchitecture, spindle characteristics were evaluated, including their density (number/minute), mean amplitude and duration, using the same method as described above. As mentioned before, however, the manual verification of the success rate of the automated method calibrated mostly for human EEGs is still pending, therefore, these preliminary results should be taken with precaution. Spindle densities are slightly deficient for Tg2576 mice compared to NTg littermates (**Table 14, Figure 58 A**). Indeed, the RM-ANOVA revealed only a poor tendency ( $p = 0.095$ ) – which was highly impacted by the increased variability in the NTg group ( $n = 5$ ), contrary to the more stable values of the Tg2576 mice

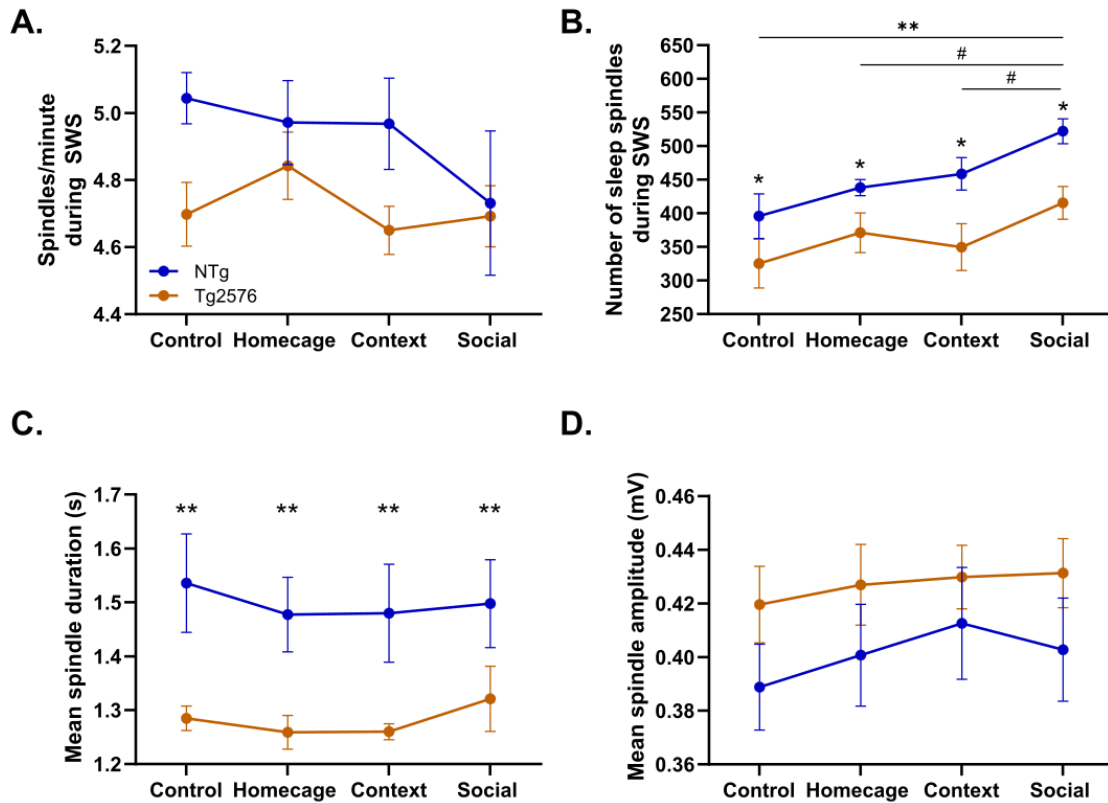
(n=9). However, spindle densities were not affected significantly across experimental conditions (*Day* factor:  $p = 0.19$ ).

However, the number of spindles increased significantly from one day to another following the gradual increase in the time spent in SWS, and that, independent of genotype (Main effect of *Day*:  $p = 0.02$ ) and even in spite of the apparent decrease of spindle density for NTg mice on day four (**Figure 58 B**). Indeed, this apparent drop in spindle density was led by one NTg whose spindle density decreased the most on day four (from 5.09 spindles/min on the *Context* day to 3.98 on the *Social learning* day for that individual).

Density of sleep spindles in the social memory protocol							
		NTg			Tg2576		
		<i>Mean</i>	<i>SD</i>	<i>N</i>	<i>Mean</i>	<i>SD</i>	<i>N</i>
Spindles	Control	5.04	0.17	5	4.7	0.26	9
	Homecage	4.97	0.28	5	4.84	0.3	9
	Context	4.97	0.3	5	4.65	0.22	9
	Spatial	4.73	0.48	5	4.7	0.27	9

*Table 14. Main descriptives of sleep density-related variables across experimental days in the social learning protocol.*

As for other spindle-related characteristics, a main effect of genotype ( $p = 0.01$ ) was found for spindle duration (**Figure 58 C**), but once again, the experimental conditions did not seem to impact this variable (main effect of *Day*:  $p = 0.45$ ). Finally, concerning spindle amplitudes, descriptive statistics hint at higher amplitudes for Tg2576 compared to NTg (**Figure 58 D**); however, the high variability in the NTg group had probably masked this effect, as no significant main effect or interaction was found.

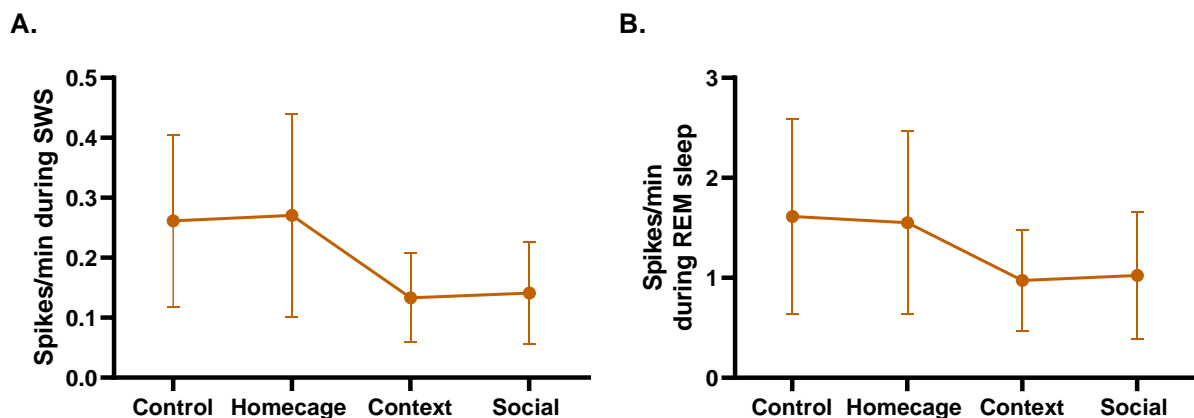


**Figure 58. Changes in spindle characteristics following different experimental conditions for Tg2576 (orange) and NTg mice (blue).** Spindle characteristics: Difference in spindle frequency ((A) spindles/minute) and spindle number (B), mean spindle amplitude (C) and mean spindle duration (D) during slow-wave sleep across experimental conditions. \*\*:  $p \leq 0.01$ . Inter-group differences are shown above the symbols representing a given day's mean and SEM. Inter-day differences are shown above the graphs. Data represented with mean  $\pm$  SEM.  $n_{Tg2576} = 9$ ,  $n_{NTg} = 5$

Somewhat similar conclusions can be drawn for slow oscillations, where no significant effect of genotype or day (or their interaction) was revealed for the SO density ( $p > 0.05$  for all comparisons, *not shown*). As for SO amplitude, only a slight tendency towards increased amplitudes for NTg mice was found ( $p = 0.09$ ), independent of the experimental condition. However, the experimental condition seemed to impact the SO duration (or, in other terms, their frequency), as the mean SO duration increased from one experimental day to another (Main effect of *Day*:  $p = 0.003$ ). More precisely, the *Social learning* day differed from the *Control* day ( $p = 0.002$ ), the *Context* day ( $p = 0.04$ ), and showed a tendency also to be increased compared to the *Context learning* day ( $p = 0.1$ ). Furthermore, SO duration also showed slightly increased means for Tg2576 altogether (*Genotype*:  $p = 0.02$ ), but no interaction ( $p = 0.89$ ). Altogether, these results indicate that sleep microarchitecture is altered for Tg2576 mice even at this relatively young age. Still, this protocol did not seem suitable for inducing post-learning sleep spindles or increasing the density of slow oscillations.

### 3.6.3. Spatial and learning tasks do not impact EA frequency during post-learning sleep periods

A crucial aim of this preliminary study was to infer some information about the possibility that epileptic events during sleep may indeed be consolidation-related oscillations that are turned pathological (Halász et al., 2019). If so, EA should increase after learning a new context or meeting a conspecific. However, EA frequency exhibited a non-significant trend ( $p > 0.05$ ) toward a reduction during both SWS (**Figure 59 A**) and REM sleep (**Figure 59 B**) following both learning sessions. Given the high variability of EA frequencies and the low sample size ( $n = 9$  for Tg2576,  $n = 5$  for NTg), our starting hypothesis cannot be directly rejected. Therefore, further tests (including electrodes allowing to detect SW-Rs directly) are needed after this pilot study to investigate this question better.

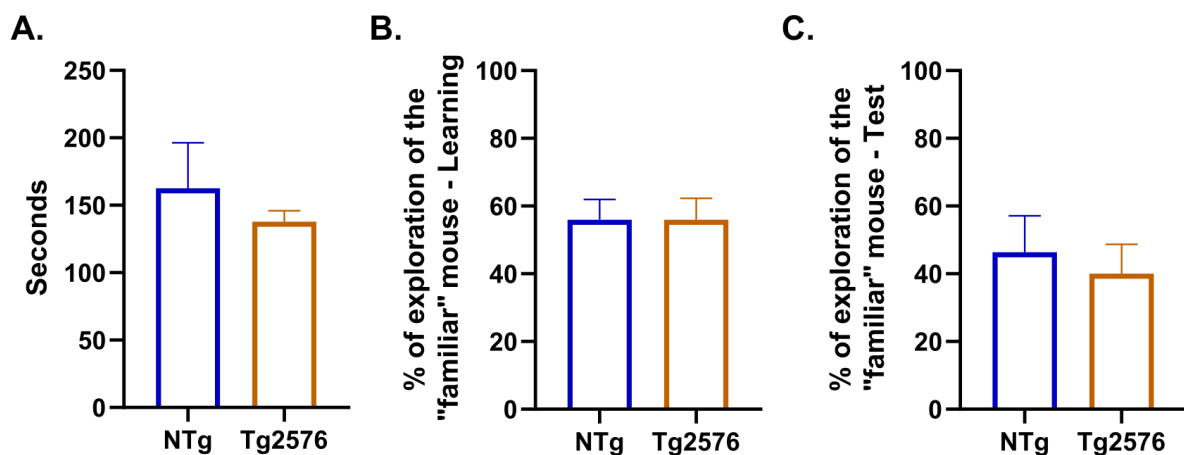


**Figure 59. No modification of spike frequency following spatial learning and social memory tasks.** Spike frequency during SWS (A) and REM sleep (B) for Tg2576 mice ( $n=9$ ) following the last three days of the social memory protocol, normalized to the control day.

### 3.6.4. Behavioural results of the social memory task

In the social learning task, we first quantified the exploration times of both conspecifics by tested animal during the learning phase, especially the duration of exploration to ensure that both animals have been explored during the task, and the percentage of the total exploration time. In the text below, the term “familiar mouse” will always be applied to the conspecific that was presented both during the learning task and the recall part. As for the recall task, the percentage of exploration time was quantified.

Our experiment failed to show a behavioural evidence of social learning with the protocol used. Indeed, while total exploration times or the percentage of exploration of the “familiar mouse” during the learning phase did not differ between genotypes (**Figure 60 A, B**), no efficient learning was shown during the recall test for either genotype ( $p > 0.05$  for both genotypes on the One-Sample T-test against 50%, **Figure 60 C**). Unfortunately, given the high levels of intra-group variability, further conclusions are difficult to draw from these data without improving the protocol and expanding the groups, all the more so as no link between task performance and sleep macro-or microarchitecture-related variables could be put to evidence (all correlations:  $p > 0.1$ ). Note that only data of 5 NTg and 8 Tg2576 are shown here for the behavioural part, as one Tg2576 with a strong place preference (only entering the left chamber immediately and never leaving it during any of the sessions on the *Social learning* day) had to be excluded from this part.



**Figure 60. Absence of efficient recall on the social memory task.** (A) Total exploration times of the two individuals for NTg (blue) and Tg2576 mice (orange) during the learning task. (B) Percentage of total exploration time spent exploring the cage of the “familiar” mice during the learning task. (C) Percentage of total exploration time spent exploring the cage of the “familiar” mice during the test phase. All comparisons were non-significant. Data represented as Mean +/- SEM.  $n_{NTg} = 5$ ,  $n_{Tg2576} = 8$

## **4. DISCUSSION – Preclinical Study**

In summary, this work – together with previous theses' in our laboratory based on this project (Bezzina, 2015; Cattaud, 2018) – shows that Tg2576 mice present an altered sleep-wake cycle as early as 1.5 months, especially relating to REM sleep quantity. Wakefulness also increases at the detriment of REM sleep and SWS, an effect that turns significant by 8-10 months. Slight disruptions of sleep microarchitecture, especially in spindle duration, are also present from an early age. Spontaneous epileptic activities are present as young as 1.5 months of age in this mouse model; however, high inter-individual variabilities are observed in their frequencies. Furthermore, contrary to previous findings, the frequency of these events does not aggravate with disease progression. Interestingly, these aberrant events are absent during quiet wakefulness and appear almost exclusively during sleep, with a drastic increase during REM sleep, when they occur phase-locked to the trough and the first half of the ascending phase of theta oscillations measured at the cortical level, suggesting abnormal pyramidal cell activity behind epileptic events. What is more, 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. As per the molecular underpinnings of this spontaneous epileptic activity,  $\alpha$ 1 adrenoreceptor blockade during wakefulness and SWS increases the frequency of EA in adult Tg2576 mice, suggesting an antiepileptic role of noradrenaline via these channels during wakefulness and SWS, while this protective effect would be lost during REM sleep when noradrenergic neurons cease firing. However, contrary to *in vitro* studies, dopaminergic antagonists had no effect on epileptic events *in vivo*, even if the sleep-inducing effect of SCH-23390 was visible and the same dose had already been tested in our laboratory and found to induce changes both in locomotor activity and in the encoding efficiency of contextual information (Sayegh et al., 2022).

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

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. On the one hand,



this is discordant with a previous study by Wisor et al. (2005), who found that the percentage of SWS and REM sleep were similar between Tg2576 mice and NTg littermates even at 8-15 months. On the other hand, it corroborates the data obtained by Zhang et al. (2005), who reported that Tg2576 (bred on a different genetic background than our cohort) exhibited a 30% decrease in the time spent in REM sleep as early as six 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, qualitative observations of our data 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 analyze 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, slow-wave sleep or even REM sleep, as the studies mentioned above only used activity count to determine locomotor activity levels and did not use any EEG/EMG recordings.

It would seem 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 characterized by EEG desynchronization 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 vital information concerning amyloid 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 (Cattaui 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 EAs observed during REM sleep in Tg2576 mice, which interrupt theta oscillations, impair mnemonic processes.

#### *4.4. The impact of epileptic event on sleep microarchitecture, learning and memory consolidation – a question for future studies*

However, our current pilot study using a social memory protocol to evaluate the impact of EAs on memory consolidation failed to show such an effect. On the one hand, the absence of a learning effect altogether both in Tg2576 and NTg animals points toward a potentially faulty experimental design, that unfortunately does not allow to draw further conclusions from the present results. On the other hand, an almost identical social learning protocol had been successfully applied, albeit with a 1 h post-learning sleep instead of a three-hour long one (Oliva et al., 2020). Given the hyperactivity of Tg2576 mice after manipulations, however, such short intervals would almost certainly contain only wakeful periods and could, therefore, not be analysed. It is also possible that for this genotype and genetic background (in the case of NTgs), longer periods are necessary for successful consolidation processes. Yet another potential issue is the use of males both for the Tg2576 animals and for the mice presented during the social memory task. Indeed, social learning protocols have already been successfully implemented in the laboratory with female mice from the same genotype (Rey et al., 2022). The high aggressivity levels of Tg2576 mice towards other males (requiring individual housing for the same reason) indeed make it probable that social behaviours are altered in the animals used. Repeating the experiment with female mice recorded with intrahippocampal electrodes and detecting sleep spindles and sharp-wave ripples at the same time would be necessary to further elaborate on this part.

The interest in CA2-related oscillatory activity and memory function is all the more intriguing as the role of social memory and of CA2 is getting more and more attention for Alzheimer's disease, epilepsy and memory consolidation as well. Indeed, as for AD, Cattaud and collaborators (2018) found that the deterioration of the perineuronal nets surrounding PV+ interneurons was the most deficient at the level of the CA2 in the hippocampus, while Rey et

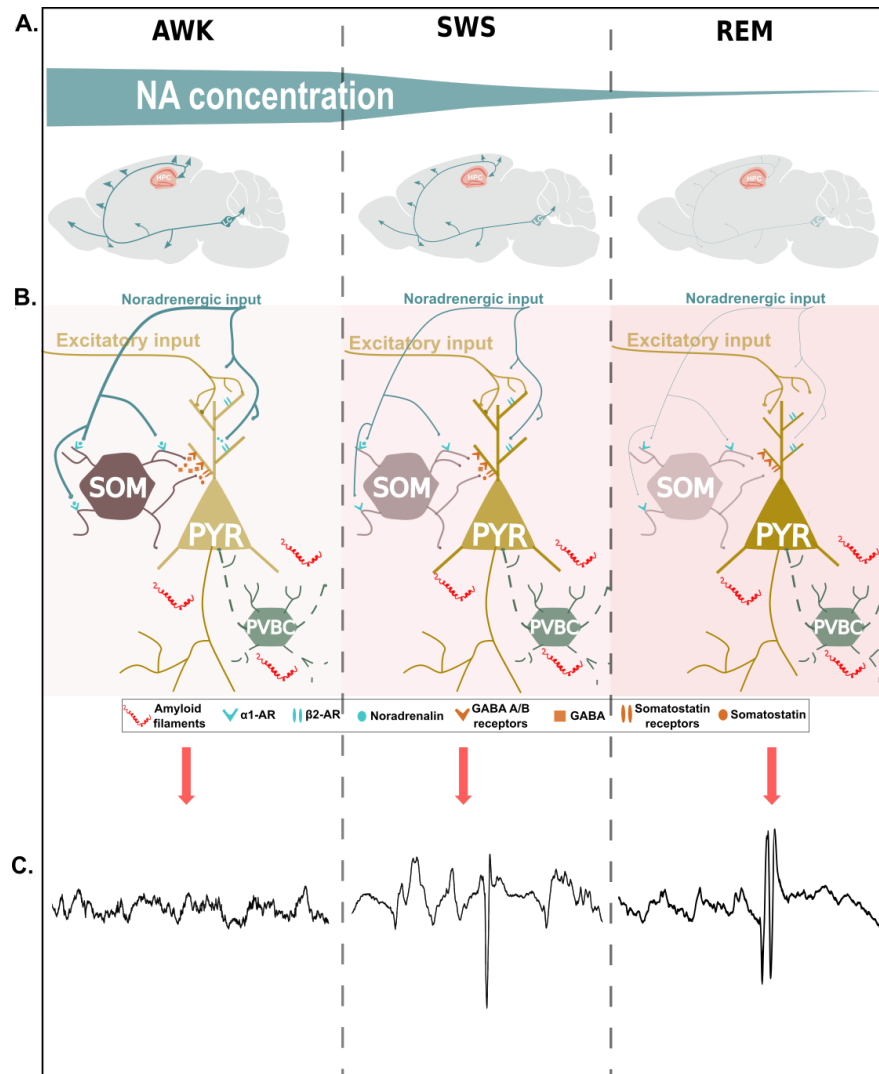
al. (2022) further found that this deterioration could be casually related to social memory deficits in Tg2576 mice. Concerning findings from epilepsy research, Whitebirch and collaborators (2022) found, in a mouse model of TLE, that the increased intrinsic excitability of pyramidal cells in this region may be particularly related to spontaneous seizure generation. Finally, in the experiments of Oliva et al. (2016, 2020) it was found that SW-Rs may not arise exclusively from the CA3 but also from the CA2, at that SW-Rs generated at the CA2 play a role in social memory formation.

Finally, it is of note that the current study and especially the microarchitecture analysis was preliminary and solely based on an automated method adapted principally for human data. Therefore, the manual verification of the success rate of the analysis, as well as more adapted methods for sleep microarchitecture analysis, will be needed in the future to acquire a more precise picture of the current dataset. It is of note, however, that the number of detected spindles does not seem to be outstandingly different from spindle rates presented in other mouse models (Aguilar et al., 2020; Kim et al., 2012).

#### *4.5. 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, the results reported above indicate that noradrenergic transmission, primarily through  $\alpha_1$ -ARs, could eventually counterbalance the neuronal hyperexcitability in Tg2576 mice (**Figure 61**). 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 antiepileptic effect of NA has been suggested by previous publications describing higher seizure rates in NA-deficient 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 Weinschenker & 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 hypothesized to contribute to the onset of seizures (Brière et al., 1986), yet again hinting at an antiepileptic effect of noradrenaline.



**Figure 61. 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 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.

Interestingly, Hillman and collaborators (2005, 2007, 2009) 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, **Figure 61 B**). 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 (**Figure 61 C**).

Finally, it is of note that several antiseizure medications have been shown to induce increased levels of NA, as 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 (**Figure 61 A, B**).

In the future, it would be of interest to measure EA frequency in Tg2576 mice injected with prazosin during the exploration of a novel environment. Indeed, during exploratory activity, theta rhythms are prominent, and EAs appear during REM sleep phase-locked to theta oscillations in the absence of noradrenaline. Reproducing this similar oscillatory activity during wakefulness without noradrenergic inputs through  $\alpha$ 1-ARs could further inform us of the validity of our results.

Altogether, these preclinical results have the potential to unveil molecular and cellular underpinnings and their association with behavioural outputs that are impossible to investigate *in vivo* in human participants. However, the one-dimensionality (i.e. the reproducibility of only one side of the pathology) of the genetic mouse models of models used in much AD-related research undeniably adds biases to the results and their interpretations. Therefore, keeping a translational mindset and aiming to explore as much as possible about this phenomenon in clinical settings is crucial, and this is what the aims of the clinical study ERERMAD detailed below were.

# **CLINICAL STUDY**

# **1. OBJECTIVES**

The last decades of research trying to pinpoint the prevalence of EAs in AD patients were highly variable (Brunetti et al., 2020; Horváth et al., 2018; Lam et al., 2020; Vossel et al., 2021; Vossel et al., 2016). This may as well be due to the various methodological issues listed above, including AD patients taking medications potentially interfering with the sleep-wake cycle and, therefore, with EAs. Furthermore, even though an increase in EAs during sleep has been reported and it has been known that EAs are predominant during REM sleep in several mouse models (Brown et al., 2018; Kam et al., 2016), very few studies reported on REM-sleep related EAs. Moreover, it is still a matter of debate whether EAs appear in the early or late stages of AD (Romanelli et al., 1990; Vossel et al., 2013), which might be due to the time of diagnosis compared to the start of neurodegeneration, which itself is dependent on cognitive reserve (Horváth et al., 2018). However, what seems to be clear is that EA leads to a faster cognitive decline (Horvath et al., 2021; Vöglein et al., 2020; K. A. Vossel et al., 2016). Yet, the mechanisms behind this fast decline and the potential link between sleep and especially sleep-related memory consolidation and EAs have not yet been explored, especially when correcting for other sleep-related pathologies, such as apnoeas and sleep fragmentation. Based on the above-mentioned pieces of information, the objectives of the clinical part of this thesis were, for lack of a better word, manifold:

- (I) Estimate the prevalence and distribution of epileptic activities across the sleep-wake cycle in mild-stage AD patients and age-matched healthy controls.
- (II) Examine potential underlying factors behind the onset of epileptic events (genetic or structural factors).
- (III) Evaluate differences in cognitive functions, with a specific accent on memory and the efficiency of sleep-related memory consolidation.
- (IV) Estimate the extent of sleep fragmentation and the frequency and severity of other sleep-related pathologies (e.g. Sleep Apnoea Syndrome) in the same cohort.
- (V) Understand how epileptic activities may interfere with memory consolidation and examine how other biasing factors (such as apnoeas) may contribute to dysfunctional consolidation processes.



## **2. METHODOLOGY**

### 2.1. Study design and evolution

The EREMAD (Epileptic activity during **REM** sleep in **Alzheimer's Disease**) study was designed as an observational, monocentric prospective case-control study, with the initial aim of including a group of 40 early-to-moderate stage AD patients and an age-matched control group of the same size. The study was a collaboration between research facilities across Toulouse (Research Center on Animal Cognition/CRCA; Brain and Cognition Research Center/CerCo and the Toulouse NeuroImaging Center/ToNIC), the Clinical Investigation Center/CIC of the University Hospital of Toulouse and the departments of the Neurology Unit at the same hospital: the Electrophysiology, Epilepsy & Sleep Unit, as well as the Movement Disorders & Cognition Unit. The main funding covering the cost of the study over the first three years of the project's duration was obtained from the French organisation *Fondation Alzheimer*. Supplementary funding from the organisations *France Alzheimer* and the *Agence Régionale de Santé d'Occitanie (France)* was received to cover the salaries of the two students involved in the study for one year, and from the *Toulouse Mind and Brain Institute (TMBl)* to cover the salary-related costs of an end of studies MSc internship (5 months). The designated French ethical committee (CPP) gave its permission to initiate the study on 8 March 2019 (**Reference:** RC31/18/0265 – Etude EREMAD; **N° ID RCB:** 2018-A02229-46).

Initial eligibility criteria for participants differed only in a few points between the two groups: patients had to receive an AD diagnosis based on expert neurological, neuropsychological and biological evaluation, supported by neuroimagerY (MRI or PET scan) and by biomarker rates in the CSF (attested via a lumbar puncture) prior to participation and had to have an MMSE score (Folstein version, Folstein et al., 1975) of at least 20 upon inclusion. Initial neuropsychological evaluation for the diagnosis took place at least six months prior to V2 to minimize test/retest effects. None of the patients had FAD.

As for controls, the MMSE score had to be over 25. Furthermore, controls also had to undergo the Dubois 5-word test (Dubois et al., 2002) and score at least 9 over 10. The rest of the eligibility criteria were identical across the two groups (**Table 15**): The age limit was set at 50-75 years. Given that the protocol included Magnetic Resonance Imagery (MRI), pregnancy and

other contraindications for this procedure (metallic prostheses, claustrophobia) were part of the exclusion criteria, along with other neurologic antecedents or any other serious disease (cancer, addiction, etc., **Table 16**). The primary goal of the study was to examine epileptic activities during REM sleep in a cohort of AD patients without other concomitant neurological disorders or sleep-architecture modifying treatments that could bias the results. Therefore, known sleep apnoeas, depression or anxiety for more than three months (attested by a score over ten on the Beck Depression Inventory), concomitant psychiatric diseases, the use of antidepressants, antiseizure medication (and/or a documented history of epilepsy), more than one dose of neuroleptic or benzodiazepine intake a day or a restless leg syndrome treated by dopaminergic agonists also figured amongst the exclusion criteria (**Table 16**).

<b>Inclusion criteria of the EREMAD study for patients and controls</b>	
<b>Controls</b>	<b>Patients</b>
-	Previously diagnosed AD
MMSE > 25	MMSE >18
5 Words of Dubois > 8	-
Age between 50-75 years (50-90 after SA3)	
Affiliated to the French healthcare system	
Having signed the informed consent form to participate before the start of the experiment	
Capable of understanding instructions and the information provided	

*Table 15. Inclusion criteria for patients and controls, with most criteria shared between the two groups.*

<b>Non-inclusion criteria of the EREMAD study (shared between patients and controls)</b>
Contraindications to MRI examinations (pregnancy, metallic implants, etc.)
Neurologic antecedents or concomitant psychiatric disease (other than AD for the patients' group)
Severe systemic disease (cancer, addiction)
Previously diagnosed but untreated Sleep Apnoea Syndrome
Depression/anxiety in the past three months
Antidepressant treatment
Antiseizure medications or documented history of epilepsy
More than one dose of neuroleptics or benzodiazepines a day
Restless leg syndrome treated by dopaminergic agonists

*Table 16. Non-inclusion criteria for patients and controls, with most criteria shared between the two groups.*

For organizational reasons, all inclusions took place on Friday afternoons, ending Saturday morning. After eligibility criteria were verified and the consent form was signed, patients (but not controls) underwent a blood draw for genotyping of the APO-E alleles. Afterwards, the protocol was identical for both groups, including an MRI examination of approximately 60 minutes, followed by a neuropsychological evaluation (~1h15-2h) and then a full-night video-EEG with polysomnography (an illustration of the final version of the experimental protocol can be found in **Figure 62** below). On the morning following the sleep examination, the second part of the neuropsychological evaluation took place, in which the recall phase of all memory tests was included. All tests are detailed below in the *“Detailed presentation of the experimental protocol”* section. Over the years, four substantial amendments (SA) were added to the protocol (**Table 17**).

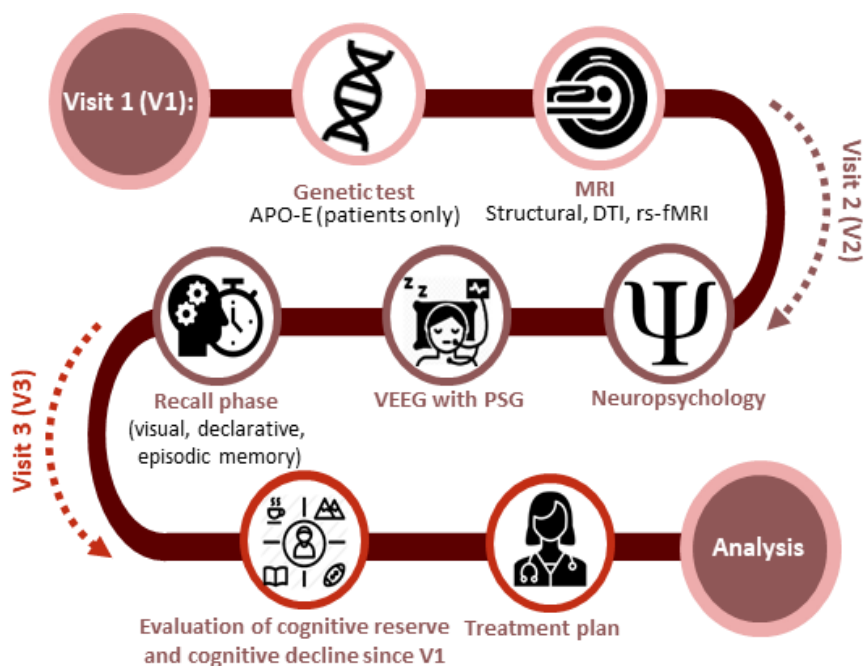
The first modification (SA1) mainly contained rectifications on the consent form for participants that were noted during the first inclusions (e.g. the MRI sequences lasted 15 minutes longer than initially estimated). Over and above that, given the already high number of participants detected with apnoeas, the eligibility criterion over that point was modified to *“known but untreated sleep apnoeas”*. A questionnaire on the subjective quality of sleep was also added (PSQI) to have an estimation of participants' sleep quality outside of experimental conditions. During the first few inclusions, however, it was also observed that the protocol

was quite tiring for most participants, so an SA2 was prepared and validated that allowed for the protocol to be divided into two visits. The first visit encapsulated the genetic test and the MRI, while the second (in the two months following the first visit) the remaining tests. Furthermore, we noticed a substantial decrease in the MMSE scores of patients upon the first visit compared to the score noted by the referring neurologist, leading to recurrent screenfails. We concluded upon observations with the medical staff that these decreases were potentially transient and related to the initial stress of the new situation for the patients. To account for this effect, the MMSE score required for inclusion was lowered to at least 18 for patients.

The strict inclusion criteria on medications, especially on antidepressant treatment, introduced an unexpected obstacle to patient recruitment. However, this criterion could not have been modified because of these medications' effect on our primary outcome measure, notably on REM sleep quantities. Instead, we decided to extend the age limit in SA3 to 50-90 years. At the same time, we also aspired to increase the patient recruitment rate by expanding recruitment sites to a pre-existing network of neurologists in the region of Occitanie working outside of the University Hospital of Toulouse.

In the final modification (SA4), in the aftermath of the COVID-19 pandemic that halted the project for six months, the target number of participants included was lowered to 62 (31 per group), which still gives a fair power estimate of 0.85. Furthermore, a third visit was added to the protocol, taking place after all data of a given participant was analyzed by clinicians (MRI, neuropsychological assessment and a long-time Video EEG with Polysomnography/VEEG-PSG) (the final version of the protocol is presented in **Figure 62**). This supplementary visit served as a possibility to improve the medical care of the participants by adjusting their treatment plans if needed (for example, by equipping them with CPAP machines if severe apnoeas were detected). Given the debate mentioned in the introduction over the disease-accelerating nature of EAs and the role of cognitive reserve, the third visit also introduced questionnaires on this point. More precisely, the Cognitive Reserve Inventory questionnaire/CRIq (Nucci et al., 2012) and the French National Adult Reading Test/fNART (Mackinnon & Mulligan, 2005) were used for this purpose. An MMSE test was added to evaluate the slope of cognitive decline since the first visit, along with the above-mentioned Epilepsy Questionnaire (EQ, **Figure 35**). This latter contains four overt questions on potential seizures (i.e. sudden temporary loss of consciousness) and twenty close-ended (yes/no) questions about potential symptoms (the

latter ones are found in **Figure 35**). Importantly, the primary outcome measure was modified to include this EQ in the detection rates of EA in participants. Given that most questionnaires of the third visit required insight from family members for more optimal estimations, participants were all encouraged to arrive accompanied to this visit. It is of note that this modification was added after more than half of the participants had already finished both visits indicated in the original protocol. However, the third visit was proposed to them as well *a posteriori*, conditioned to the signature of a supplementary consent form. One control declined this supplementary visit. Furthermore, the delay between the first two visits and the third one was significantly increased for those having signed the supplementary consent form compared to those included after the implementation of the third visit. To address this issue, when calculating the slope of cognitive decline, an estimation of yearly MMSE decline was used (taking into account the time between visits) instead of the raw MMSE scores.



**Figure 62. The final form of the experimental design.** The first visit included a genetic test of the APO-E alleles (for patients only) and an MRI. The second visit encompassed a neuropsychological examination followed by polysomnography and the second part of the neuropsychological tests right after the VEEG-PSG. The third visit englobed the evaluation of cognitive reserve and the cognitive decline since V1 as well as the restitution of results and an adjustment of the treatment plan if needed.

<b>Substantial modifications to the EREMAD protocol during the course of the study</b>		
<b>SA n°</b>	<b>Submitted to the committee</b>	<b>Major changes</b>
<b>SA 1</b>	20/05/2019	Only known <b>but untreated</b> apnoeas were considered as exclusion criteria.  A questionnaire on the subjective quality of sleep was added
<b>SA 2</b>	18/12/2019	Division of the protocol into two visits  Lowering the required MMSE score to 18 for patients
<b>SA 3</b>	01/09/2020	Age criteria extended to 50-90 years of age  Patient recruitment extended to a network of neurologists in the region of Occitanie working outside of the University Hospital of Toulouse.
<b>SA 4</b>	27/05/2021	Supplementary secondary objectives were added concerning the microarchitecture of sleep.  The target participant number was lowered to 62 from 80. Addition of a third visit including: <ul style="list-style-type: none"> <li>- Questionnaires related to the cognitive reserve</li> <li>- MMSE for estimating cognitive decline since V1</li> <li>- A questionnaire on the presence of symptoms suggesting an epileptic syndrome was added.</li> <li>- Restitution of the clinical results and modification of the treatment plan, if applicable</li> </ul>

*Table 17. List of substantial amendments to the original protocol.*

## 2.2. Detailed presentation of the experimental protocol

### *2.2.1. First Visit*

#### **APO-E genotyping**

After verification of the eligibility criteria and signature of the consent form, a blood sample was obtained from each patient (7 ml EDTA tube) by a trained professional of the Clinical Investigation Center of Toulouse and genotyping of the APO-E alleles took place at the Federal Institute of Biology of Toulouse.

#### **MRI Examination**

Each participant underwent an approximately 60-minute-long MRI examination at the MRI platform of the Toulouse Neuroimaging Center on the site of the University Hospital in a 3T Philips ACHIEVA dStream machine (Intera Achieva, Philips, Best, The Netherlands).

The primary sequences run during the examination included an anatomic sequence (T1), a diffusion tensor imaging sequence (DTI) to estimate white matter organization and microstructural integrity, as well as a resting-state functional MRI (fMRI). Participants had the opportunity to sleep, watch a documentary or listen to music during the examination, except for the fMRI sequence. Only T1-related data is reported in the current manuscript, for which the following parameters were applied during image acquisition: 180 sagittal slices in 3D scan mode; multishot, voxel resolution (mm<sup>3</sup>): 1 x 1 x 1. Data analysis was carried out by an expert associated with the project, using the same pipeline and methodology in FreeSurfer (Fischl et al., 2002, 2004; Fischl & Dale, 2000; Reuter et al., 2010, 2012; Reuter & Fischl, 2011) as described in Yroni et al., (2019).

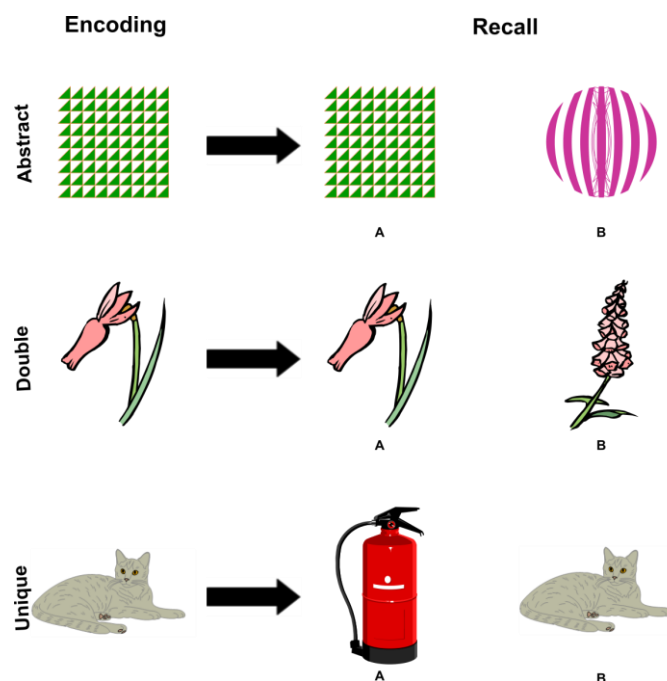
### *2.2.2. Second Visit*

#### **Neuropsychological evaluation – Pre-sleep**

This evaluation took place in the late afternoon preceding the vEEG-PSG examination. 94% of all evaluations (n = 47) were done by the same experimenter (BSA).

**1. The cognitive difficulties scale** (McNair & Kahn, 1983) is an auto-questionnaire containing 39 items of difficulties of everyday life (e.g. “I forget the ingredients of recipes I know well”). Participants are asked to declare the frequency with which they encountered these difficulties in the past three weeks (never, rarely, sometimes, often, very often).

**2. DMS-48:** A visual memory test consisting of several steps (Barbeau et al., 2004). First, during encoding, participants are asked to flip through a set of 48 images and state whether each of them contains more than three colours, less than three colours or precisely three colours. They are not asked to memorize the images to keep encoding as incident as possible. Then, test n°3 (see below) is carried out to introduce a short delay before participants are presented with 48 pairs of images (recall phase). Amongst each pair, they are asked to identify the one seen before while counting colours. Figures (and pairs during the second part) are classified into three categories: unique, double and abstract images. An example of each is found in **Figure 63**. Another, delayed, recall at approximately one hour post-learning takes place right before the end of this first evaluation, using a different set of image pairs. The last recall occurs the next morning, using once again the first set of 48 pairs (**Figure 63**).



**Figure 63. Examples of the DMS-48 test.** During encoding, a set of 48 images are presented to the participants, classified into abstract, double and unique images. Participants are solely asked to count the colours on the images, not to memorize them. During the recall phases of which they are not aware in advance, a set of 48 pairs of images are shown to them, and amongst each pair, they have to identify the one (A or B) seen during encoding.



**3. The brief screening scale of Mahieux-Laurent evaluating praxic abilities** (Mahieux-Laurent et al., 2009): This quick test of praxic abilities contains three parts, each lasting approximately 1 minute. During the first part (symbolic gestures), participants are asked to perform five well-known gestures (e.g. showing that it smells terrible). The second part (pantomimes) consists of imagining handling a particular object (e.g. a hammer or a box of matches) and carrying out movements with them (hammering a nail into the table or lighting a match). The last part (imitation) consists of mimicking the same abstract gestures the experimenter carries out, using the same hand the experimenter uses. As stated above, the first recall phase of the DMS-48 takes place after this test.

**4. RL/RI-16** (Van der Linden et al., 2004): This declarative memory task starts with the gradual learning of 16 words of separate categories by associating a category to each word (e.g. flower - daffodil). Patients are then asked to try to recall the words three times, each time starting with a free recall, then continuing (if necessary) by the cued recall for the remaining words, using the categories as cues. After the third recall, a list of 48 words is presented to the participants, containing the 16 belonging to the list the participant learnt, 16 semantic distractors (words belonging to the same categories but not on the initial list) and 16 neutral distractors (words from categories not on the initial list). During this recognition task, participants have to identify the 16 words that have been on their list. Between the learning and the first recall, as well as between each subsequent recall phase, participants are asked to count backwards one by one from a given number for 20 seconds to prevent rote rehearsal. Note that if participants do not give a good answer during the cued recall of the first two recalls, the correct answer is provided, but it is not the case during the third recall that precedes the recognition task. A delayed recall is carried out approximately 30 minutes after the first (right before the PEGV test, see below), then another one the next morning.

**5. Rey-Osterrieth Complex Figure (Rey, 1941):** in this classic neuropsychological test, participants are first asked to copy a complex geometric figure. This test does not only evaluate visuospatial functions but gives indications about planning and attentional capacities as well. In the second part, following a brief delay (in our experiment, the time to execute test

n°6 presented below), participants are asked to reproduce the figure from memory, therefore assessing short-term memory functions as well.

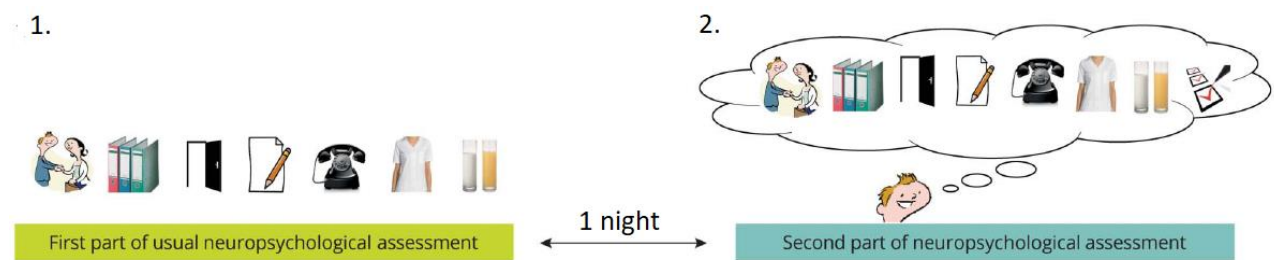
**6. Forward and Reverse Digit Span test from the WAIS-IV** (Drozdick et al., 2012): In this subtest of the Weschler Adult Intelligence Scale's 4th edition, participants are asked to repeat several series of digits of increasing length. For the first subtask, they have to repeat the numbers in the same order the experimenter read them (forward) – and then in reverse order for the second subtask. Both subtests are interrupted when the participant misses two consecutive series of the same length. The recall phase of the Rey-Österrich Figure takes place after this test.

**7. Visual Gnosis Evaluation Protocol (PEGV)** (Agniel, 1982): In this rapid task of ten items devised to detect visual agnosia, participants have to find, in under 30 seconds per item, a geometric figure that is identical to a given target amongst a group of 5 distractors.

**8. Verbal Fluency task (GREFEX)** (Godefroy et al., 2008): Here, participants are first asked to list words of any type – except for proper nouns and names – starting with the letter “P” for two minutes. Then, during the second part, they once again have two minutes to list words, but this time belonging to the animal category.

**9. Epi-Real encoding phase** (Lemesle et al., 2022): The tests chosen for the pre-sleep evaluation were in fact adjusted to the Epi-Real episodic memory test recently developed at the Brain and Cognition Research Center (CerCo) of Toulouse and the Neuropsychology Unit of the University Hospital of Toulouse, Epi-Real (Lemesle et al., 2022). This test includes a series of built-in ecological mini-events in the pre-sleep test battery that takes place in a finely orchestrated order and timing for every participant. These events include, amongst others, a phone ringing always during the same test, the experimenter leaving the room for five minutes always under the same pretext and then returning in a different outfit, or the examiner offering a choice of drink (water or orange juice) to the participant during the evaluation

(**Figure 64**). The type and order of the neuropsychological tests during the evaluation are also counted as an event. Encoding of the events is considered incident as participants are unaware of the events being part of the tests, even though the next day they are asked to recall these events and their main episodic components (i.e. **what** happened, **when** did it occur/in which order during the evaluation, **where** was it placed) by free recall (FR), then cued recall (CR), and as a forced-choice recognition (REC) for the remaining items not recalled during the FR or the CR.



**Figure 64. The main events of the Epi-Real protocol.** Eight ecological mini-events are systematically interleaved with the standard neuropsychological tests that also take place in a specific order, with the same events happening during the same time for each participant. Participants undergo a recall phase of these events the next day, following the VEEG-PSG examination. Figure adapted from Lemesle et al., (2022)

## Neuropsychological evaluation – Post-sleep

As mentioned above, the neuropsychological evaluation of the second day, following the VEEG-PSG (detailed below), started with the recall phase of the Epi-Real protocol, first as a free recall, followed by a cued recall for remaining items, and ending with a forced-choice recognition task for the events and details still missing. Furthermore, during the morning evaluation, a recall of the RL/RI-16 and the DMS-48 was also carried out following the Epi-Real recall, along with the complementary tests evaluating emotional state, executive functions and instrumental functions listed below.

**9. The Frontal Assessment Battery at Bedside (FAB, Dubois et al., 2000):** This task assesses frontal lobe functions using the succession of six rapid subtests evaluating conceptualization and mental flexibility capacities, motor programming, sensitivity to interference, inhibitory control and environmental autonomy. A score lower than 16 (or 15 if the participant does not fall into the highest sociocultural category) out of the 18 is considered deficient.

**10. Digit-Symbol subtest of the Wais IV** (Drozdick et al., 2012): In this test, the numbers from 1 to 9 are presented as being associated with easy-to-draw symbols that remain visible throughout the task. On the task part itself, 135 cases (plus nine practice cases) containing the numbers between 1 and 9 in random order are shown, with empty cases below each number. Participants have two minutes to draw the correct symbol associated with each number without skipping cases or lines. This task measures working memory and visuospatial processing capacity, attention, and manual dexterity.

**11. Confrontation naming subtest of the GREMOTs** (Bézy et al., 2016): In this computerized test, a series of 36 images (along with two practice images to ensure correct understanding of the task) are shown to the participants. Images are of everyday objects or living things (e.g. of a hammer or a duck), and a subclassification is also possible for scoring based on the frequency of that word in the commonly spoken language and/or on the length of the word (one, two or three syllables). Participants have to give the name of what they see on the image in under 5 seconds on the strict scoring level or under 10 s (or with one autocorrection) to get the point on the more relaxed “large scoring”.

**12. Beck Depression Inventory – Short version** (Beck et al., 1987): This questionnaire filled out by the participants contains thirteen series, each containing four propositions pertaining to the current emotional state of the participant. The propositions in each series are centred on one topic, and participants have to choose the proposition(s) that best describes their current state for each series. A score from 0 to 3 is associated with each proposition, adding up, therefore, to a maximum of 39 points on the 13-item test. Scores under four do not indicate any underlying depressive syndrome, while scores between 4 and 7 are interpreted as mild-, between 8 and 15 as moderate- and over 15 as severe depression.

**13. Starkstein Apathy Scale** (Pedersen et al., 2011; Starkstein et al., 1992): This questionnaire was devised to measure the presence and/or severity of apathy with 14 items (e.g. “Are you

interested in learning new things?”) to which the participants have to respond on a four-point scale (Not at all, Slightly, Some, A Lot). Responses are once again graded from 0 to 3, leading to a maximum of 42 points, wherein a score equal or superior to 14 is considered pathological.

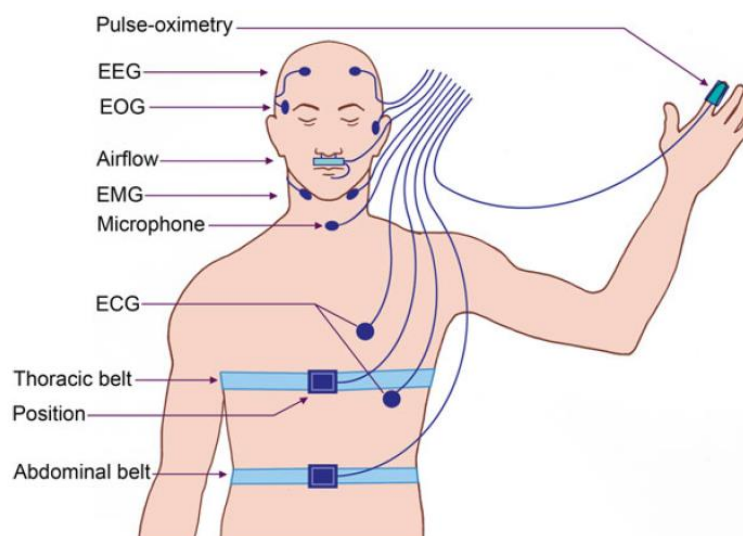
**14. Pittsburgh Sleep Quality Index** (Buysse et al., 1989): This questionnaire evaluates subjective sleep quality over the 30 days preceding its administration. Items include questions around sleep time, latency to sleep, frequency of nighttime awakenings for diverse reasons and daytime sleepiness, amongst others. For scoring purposes, items are clustered into seven components, each with a total score ranging from 0 to 3, depending on the participant’s answers. Final scores are therefore ranging from 0 to 21, and scores over five were suggested to mark the threshold from which significant sleep disturbances are present.

## Video-EEG with Polysomnography

In the current study, in order to quantify sleep-related parameters and detect epileptic events, a full-night VEEG completed with PSG sensors was carried out using custom-made portable equipment and sensors from Natus (Pleasanton, CA, USA). Note that, in our protocol, to avoid increased stress and agitation for patients, one person could accompany patients and stay with them during the night. This was also extended to controls to avoid lifting the blinded analyses by the experts.

Our exam allows registering the PSG and the VEEG at the same time with a large sample of scalp electrodes. Electrodes were placed with respect to the International 10/20 (and 10/10) system, where neighbouring electrodes are placed at a distance of 10 or 20% of the full front-to-back or right-left distance of the head. Electrodes are labelled based on the lobe above which they are supposed to be placed (FP – Prefrontal, F – Frontal, T – Temporal, P – Parietal, O – Occipital). Central (C) electrodes are used as well but only for facilitating localization. Midline electrodes further receive a “z” annotation, while electrodes at increasing distances from the midline over a given lobe are numbered (odd on the left, even on the right.) In the current protocol, an electrode setup used in standard sleep-pathology targeted PSGs was

completed with additional lower temporal electrodes (F9, P9, T9, F10, P10, T10) usually used in setups focusing on the detection of epileptic activities were applied. Before placing electrodes, each site was gently scraped with a gel devised for this purpose (Gionta Erasmo, Milan, Italy) to increase conductance and remove dead skin tissue. This was followed by the placement of Grass® Reusable Cup electrodes filled with Ten20® Conductive Paste (both Natus, Pleasanton, CA, USA) on pre-measured electrode locations. Individual electrodes were held in place by a Conductive Adhesive Gel (Natus). A homemade hood made of medical-quality tubular elastic net dressing was used to keep the complete setup in place during the night.



**Figure 65. Example of a Polysomnography setup.** Polysomnographies include a varying number of EEG electrodes, ECG for cardiac activity, EMG for muscle activity, EOG for eye-movement detection, pulse-oxymetry as well as breathing-related sensors (microphone, airflow sensor and thoracic and abdominal belts). From Pandi-Perumal et al., (2014).

As for polysomnography sensors (**Figure 65**), electrooculography (EOG) served as an additional marker for identifying REM sleep (associated with increased eye movements), but was also useful for identifying eye-movement-related activities on the EEG.

Electromyography (EMG) electrodes placed on the chin served similar purposes: first, the REM-related atonia can be easily perceived, and muscle activity-related signals on the EEG can be identified. Extra EMG electrodes were also placed over the legs, which allowed for the detection of excessive movements (e.g. REM-sleep behaviour disorder or periodic limb movements).

Electrocardiography (ECG) additionally followed cardiac activity, serving first to identify potential anomalies but also for identifying cardiac activity-related artefacts (which often resemble regular micro-spikes and can be confounding for epileptic activity detection) on the EEG (Pandi-Perumal et al., 2014). In the EREMAD protocol, two disposable adhesive EOG and two chin EMG were used (in some cases, both were replaced with 2 Grass® cup electrodes), along with 4 Grass® cup leg EMG electrodes and two cup ECG electrodes.

A rather substantial number of sensors was consecrated to breathing activity, with the following specifications: Two Piezo respiratory effort belts (thoracic and abdominal) measured breathing effort. Pulse-oxymetry (Nonin® xPod and Nonin® PureLight® disposable adhesive sensor, Natus) allowed following blood-oxygen saturation, while a Thermistor Airflow Sensor, connected through an Ultima™ Dual Pressure Flow/Snore Sensor box (Natus) measured nasal airflow. A microphone (Ultima™ reusable snore microphone, Natus) was further used to detect snoring. These sensors are highly sensitive in detecting sleep apnoea syndrome (SAS). Finally, to facilitate sleep scoring and disentangle doubtful segments, a video recording took place concomitantly over the night and, in the absence of epileptic seizures during the night, was destroyed following the analyses. The VEEG-PSG setup was prepared by a nurse who specialized in electroencephalography and polysomnography, assisted by the experimenter (BSA in 94% of the cases). The same nurse then monitored the recording from an adjacent room throughout the night and changed electrodes when necessary.

### *2.2.3. Third Visit*

This supplementary visit was added in the last substantial modification of the protocol (n°4), with three main objectives: evaluating the level of cognitive decline since the last visit, determining the participants' premorbid/pre-protocol cognitive reserves and adjusting or introducing treatment plans based on the results of the medical examinations included in the protocol, whenever necessary. This visit was carried out by a medical student in residency at the University Hospital of Toulouse, Fleur Gerard, under the supervision of trained sleep experts and epileptologists (Dr Jonathan Curot and Pr Jérémie Pariente) of the hospital associated with the study. Given the nature of the questionnaires detailed below, participants were encouraged to arrive accompanied by a caregiver/family member, as their input on

several questionnaires was valuable. During this last visit of approximately one hour, the following short tests were carried out:

1. **Mini Mental State Evaluation** (Folstein version, Folstein et al., 1975), detailed above.
2. **The Epilepsy Questionnaire** (B. Szabo et al., 2022): This questionnaire, presented above (**Figure 35**), was devised to detect eventual signs of an underlying epileptic syndrome that might not have come across during the vEEG-PSG. The questionnaire is ideally filled out in the presence of a family member who also gives input concerning his/her observations of the participant on each item of the questionnaire. The questionnaire contains two open-ended questions about a potential history of transitory loss of consciousness or a temporarily altered state of mind/consciousness. This is followed by twenty close-ended (yes/no) questions about symptoms often associated with epilepsy (**Figure 35**).
3. **Cognitive Reserve Index questionnaire/CRIq** (Nucci et al., 2012): This standardized questionnaire aims at estimating the cognitive reserve of participants accumulated throughout the years. Therefore, it contains questions relative to educational level and workplace-related information, as well as several items related to hobbies and lifestyle (e.g. driving, use of new technology, social life, reading, pet care). Final scoring gives three separate scores on educational level, workplace and hobbies, which are moulded into a final Cognitive Reserve Index (CRI). Scores under 70 are considered low CRI, 70-84 as low-to-medium, 85-114 as medium, while scores of 115-130 are deemed medium-high CRI and anything over 130 as high CRI.
4. **National Adult Reading Test – French version/fNART** (Mackinnon & Mulligan, 2005; based on Nelson & O’Connell, 1978): This test of 40 words with irregular pronunciation (e.g. charisme, caecal) is used as a proxy of premorbid intelligence for patients diagnosed with dementia. Indeed, the faculty to read words with irregular pronunciation has been shown to be relatively preserved over the course of cognitive decline and is well correlated with verbal and total IQ scores in non-demented participants. Total scores can be compared against norms established over a French-speaking Swiss cohort of non-demented participants along three axes: sex, educational level and dementia status (no dementia, possible dementia, confirmed dementia) (Mackinnon & Mulligan, 2005).



Following these tests, participants were informed about their clinical results on each examination included in the study (genetics, MRI, VEEG-PSG, neuropsychological examination). If the introduction of a treatment plan was needed, for example, CPAP treatment or a follow-up of vascular risk factors detected on the MRI, the necessary procedures were undertaken to ensure rapid treatment.

## 2.3. Imaging Data Analysis

### *2.3.1 MRI examination*

Data analysis was carried out by an expert associated with the project (Pr Patrice Péran), using the same pipeline and methodology in FreeSurfer v. 5.3.0 (Fischl et al., 2002, 2004; Fischl & Dale, 2000; Reuter et al., 2010, 2012; Reuter & Fischl, 2011) as described in Yroni et al., (2019). This pipeline includes automatic processing of the images with the longitudinal pipeline of the software to obtain more reliable estimates of volume and thickness. The pipeline generates a template space via robust coregistration (Reuter et al., 2010, 2012; Reuter & Fischl, 2011). To increase reliability and statistical power, this method uses common information from the internal model for surface maps and parcellations which follow other processing steps (skull extraction, Talairach transformations, atlas recording). This allows for a later step of automated volumetric segmentation of grey matter in the structures of interest (Yroni et al., 2019).

### *2.3.2 VEEG-Polysomnography*

Data were analysed in groups of four to six recordings, each anonymized and undated. To ensure maximal anonymity and independence between the experts examining the recordings, for each of the experts that analysed a given recording, the participants' IDs were shuffled randomly to not correspond between experts. Experts were blinded to the group of the participant as well (control or patient).

## Sleep analysis

Sleep scoring and detection of sleep apnoeas, cardiac anomalies and eventual periodic leg movements were carried out by one of three somnology experts of the University Hospital of Toulouse associated with the project (RD, CG and JC). Note that NREM 3 and 4 were not differentiated in the current study and are referred to as NREM3. The following pieces of information were obtained for each recording. Note that many of them were only used for clinical interpretation of the data, and the ones used for research purposes in the current study are marked in **bold**:

### a) Quantitative and qualitative organization of sleep:

- **Total sleep time (TST) and Time in bed (TIB)**
- **Sleep efficiency indexes** (TTS/TIB, TTS/(time from first N2/REM to awakening))
- **Latencies** until the first occurrence of each sleep stage
- **Percentages of each stage** of sleep compared to the total sleep time
- **Duration of each sleep stage** over the night
- Number of stage changes and its corresponding index (number of changes/hour)
- **Number and duration of intra-sleep awakenings**

### b) Sleep microstructure

- **Number of arousals and index of arousals (number/hour)**
- Number of arousals due to movements
- **Number of arousals due to pathological respiratory events and their corresponding index (number/hour)**

### c) Respiratory events

- **Number, maximum duration, average duration, total time and index of central, obstructive and mixed apnoeas, as well as the number and index of all apnoeas combined.**
- **Number, duration (maximum and average) and index of hypopneas.**
- Number and index of desaturation superior to 3% and 4%
- **Apnoea-Hypopnea index (AHI)** and the total number of respiratory events.

- Index (number/hour) of undifferentiated, obstructive, central and mixed apnoeas in REM, NREM 1, NREM2 and NREM3.
  - Number of desaturation superior to 3% or 4% by sleep stage (REM, NREM 1, NREM2 and NREM3)
  - Respiratory events occurring while the participant was lying on the back
  - Total snoring index (number/hour)
  - Snoring index for the time the participant spent lying on his/her back or his left or right side.
- d) Oxygen Saturation levels: Maximum, minimum and average saturation levels during wakefulness and in the different sleep stages (REM, NREM 1-3), as well as projected over the total time spent asleep.
- e) Cardiac evaluation: Minimum, maximum and average heartrate over the full recording, during time spent asleep and during time spent awake.
- f) Periodic leg movements:
- Number and index (number/hour) of total leg movements
  - Number of periodic leg movements and the corresponding index (number/hour)
  - Percentage of periodic leg movements of all leg movements
  - Index (number/hour) of periodic leg movements leading to arousals/awakenings.

### **Detection of epileptic activities**

For the detection of epileptic activities, each VEEG examination was analysed independently by two of three epileptology experts of the University Hospital of Toulouse associated with the study (Drs Luc Valton, Jonathan Curot and Marie Denuelle). The objective was to detect subclinical epileptic activities (spikes, spike-waves, etc.) or clinical epileptic activities (seizures) and to describe their time of onset, morphological characteristics and spatial distribution, together with the level of certainty of the given event (confirmed or doubtful). Confirmed events refer to EAs that at least one expert marked as such with high confidence levels. The

two analyses were then compared, leading to three potential outcomes and consequences for further analysis:

1. None of the experts identified **confirmed** epileptic events in a given recording: In this case, the recording was considered normal without epileptic events, and no further steps were taken.

2. Both experts found **confirmed** epileptic events in the same recording: In that scenario, the recording was considered as a recording **containing confirmed epileptic events**. For the quantification of events, the sum of **confirmed** events noted by Expert 1 and the sum of events noted by Expert 2 was used – when both noted a given event, it was counted as only one. Doubtful events (that is, no certainty expressed over it by either expert) were discarded.

3. Only one of the experts detected epileptic events in the recording in question: If such a discrepancy was detected, the third expert – not involved in the original scoring of the given recording – re-read the recording, with the annotations left by the expert having previously found epileptic events already in it. The third expert re-read the whole recording, and only events he/she deemed confirmed were kept from the initial scoring. If the third expert found confirmed epileptic events not identified before in the recording, they were also counted towards the final number of retained epileptic events.

In scenarios number 2 and 3, the retained events were then associated with sleep stages resulting from the sleep scoring.

## 2.4. Statistics

All statistical analyses were carried out using the JASP software (Love et al., 2019) or via the Prism 5 software (GraphPad Software Inc., La Jolla, CA, USA). Figures were also created using the Prism 5 software as well as InkScape (Inkscape Project. (2020). *Inkscape*. Retrieved from <https://inkscape.org>). Spindle and Slow-oscillation analysis related variables were obtained using MATLAB (MATLAB and Statistics Toolbox Release 2022a, The MathWorks, Inc., Natick, Massachusetts, United States).

## **3. RESULTS**

### **3.1. Demographics**

Given that inclusions are still ongoing for the EREMAD study and are set to end in October 2022, only a part of the planned cohort is available for analysis. More precisely, for the current document, patients with the following criteria were included in the analyses:

- At least two visits completed
- EEGs analysed for sleep scoring and epileptic activities
- Having a matched control fitting the same criteria for data availability

As of August 2022, 31 AD patients and 29 controls were screened at the Clinical Investigation Center of Toulouse. Of those, 28 patients and 28 controls could be included. However, one patient (impossibility to execute the polysomnography and the post-sleep evaluation) and a control (pathological neuropsychology scores and MRI) had to be excluded *a posteriori*. Moreover, one of the controls has not yet undergone the second visit. Finally, given the constraints of analyzing PSGs by groups of 3-7 participants, the VEEG-PSG of another control has not yet been interpreted. This leaves 27 patients and 25 controls whose data is analyzable, which drops to 25 per group when considering the last criteria (matching control) stated above. Forty-one of those participants have also undergone the third visit as well. Patients were sex- and age-matched, leading to no significant differences in age (Mean  $\pm$  SD = 68.2  $\pm$  5.46 for controls and 69.48  $\pm$  6.39 for patients ( $p = 0.45$ ) or sex, although we should note that a slight predominance of women is present in the cohort (**56%, Table 18**). This latter is coherent with the higher percentage of women amongst AD patients already reported on many occasions in the literature (~62% as estimated by the Alzheimer's Association's report 2018). One participant per group (a patient and the control assigned to him) had a known and treated Sleep Apnoea Syndrome (SAS), for which the equipment was worn throughout the PSG. As expected, MMSE scores differed across the two groups (28.68  $\pm$  1.07 for controls, 24.6  $\pm$  2.94 for patients,  $p < 0.001$ ). 20% of AD patients had atypical forms of AD (3 with aphasic/logopenic variants and 2 with agnostic variants). As mentioned earlier, none of the participants had severe neurological antecedents.

Demographic variables of the included EREMAD participants			
Variable	Controls (n = 25)	AD patients (n = 25)	p-value
Age (mean ± SD)	68.2 ± 5.46	69.48 ± 6.39	0.45
Women (n; %)	14; 56%	14; 56%	NA
MMSE (mean ± SD)	28.68 ± 1.07	24.6 ± 2.94	< 0.001
Known SAS	1	1	NA
Atypical AD	NA	20% (5/25)	NA
APO-E4 carrier	NA	76%	NA

Table 18. Demographic variables of the included EREMAD participants in the current manuscript.

### 3.2. Epileptic activities – prevalence and link with the sleep-wake cycle

Inter-rater coherence of the two experts on each recording in detecting the presence of confirmed EAs was 0.9. No seizures were observed in either group. One of the 25 controls had subclinical epileptic activities detected on her EEG during the night (a total of 21 events, **Figure 66 A**), corresponding to 4% of controls. A different picture is seen in the patients' group, where subclinical epileptic events were detected in 20% of participants (5 patients, with a total of 455 events, **Figure 66 B**). EAs were distributed unevenly across patients, ranging between 4 and 404 per night (Mean ± SD = 91 ± 175.15). The risk for sleep-related epileptic activities was therefore 5 times higher across mild-to-moderate stage AD patients than among age-matched controls. However, a two-sided z-test for equality of two proportions revealed that this difference was only marginally significant ( $z = 1.74$ ,  $p = 0.08$ ).

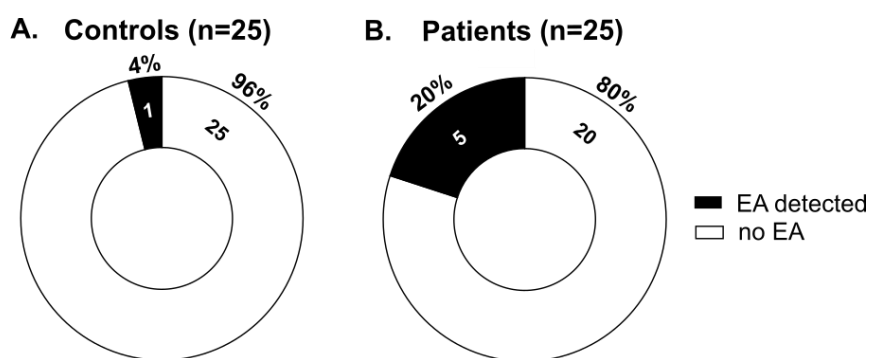


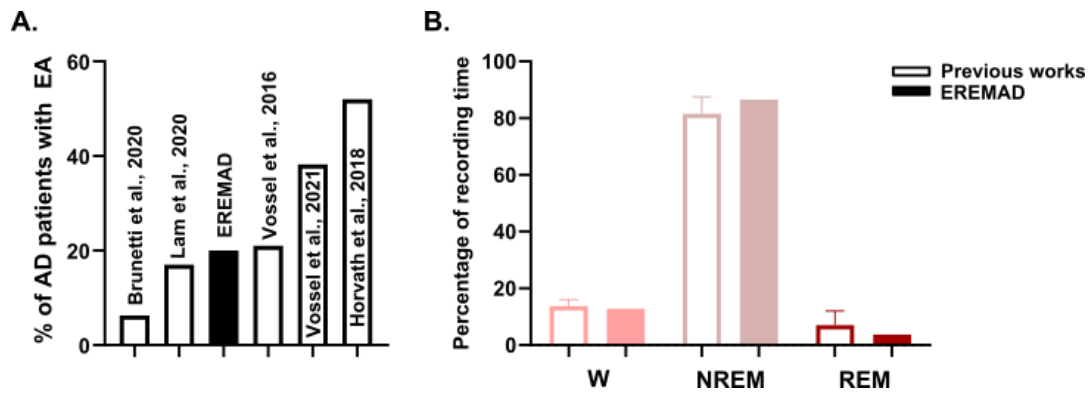
Figure 66. Detection of subclinical epileptic activities amongst controls and AD patients. Percentage and number of controls (A) and patients (B) with and without epileptic events detected during the video-EEG. The number of participants is marked inside the circles, while the percentage this number represents is represented on the outer rim of the circle.

Patients with and without EA were similar in age (**Table 19**), and the ratio between the sexes was not tilted in either group from the original ratio. Atypical AD rates were also identical across the two groups. Initial MMSE scores and the proportion of APO-E4 carriers amongst patients with and without EA did not differ, and this was also true for the proportion of participants with 2 APO-E4 alleles (**Table 21**).

<b>Demographic variables of AD patients with and without EAs</b>			
<b>Variable</b>	<b>AD – no EA (n = 20)</b>	<b>AD + EA (n = 5)</b>	<b>p-value</b>
<b>Age</b> (mean ± SD)	69.1 ± 6.22	71 ± 7.58	0.56
<b>Women</b> (n; %)	11; 55 %	3; 60%	NA
<b>MMSE</b> (mean ± SD)	24.26 ± 2.79	25.8 ± 3.83	0.32
<b>Known SAS</b>	1	0	NA
<b>Atypical AD</b> (% , n)	20% (4)	20% (1)	NA
<b>At least 1 APO-E4 allele</b>	80%	60%	0.34
<b>2 APO-E4 alleles</b>	31.58% (6)	0% (0)	0.16

*Table 19. Demographic variables of AD patients with and without epileptic activities detected on their EEG.*

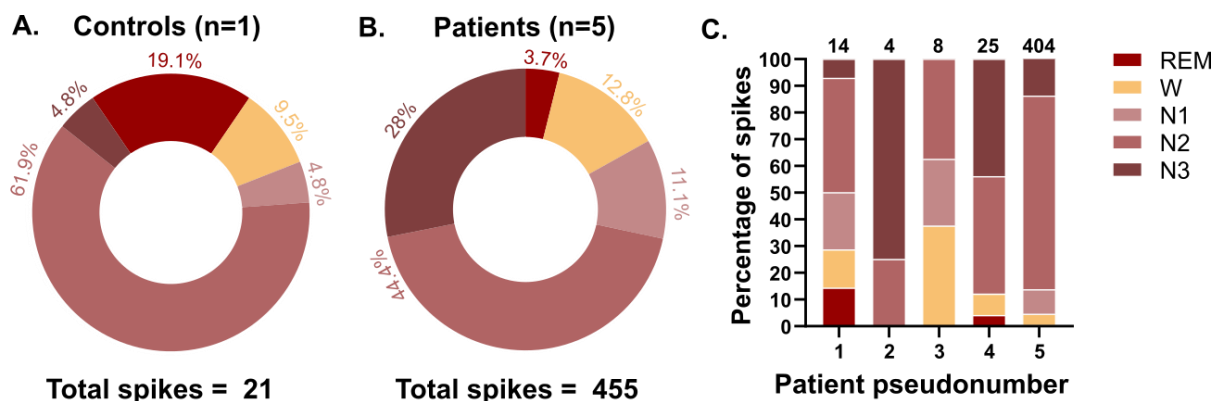
Regarding EA frequency in AD, compared to studies with similar methodologies, EREMAD reports AD patients with EA in the mid-range of previous studies and, most notably, falls in similar ranges as the studies with the strictest patient inclusion criteria (Lam et al., 2020 and Vossel et al., 2016, **Figure 67 A**). As per the distribution of EA over the different sleep stages, we also observe percentages comparable with the three previous studies detecting a relatively high number of AD patients with epileptic events and reporting sleep-related results (**Figure 67 B**, filled bars marking the current results).



**Figure 67. Comparison of EA frequency and sleep-cycle distribution with previous studies.** (A) Frequency of EAs in previously published studies with similar methodologies, wherein the current study reports approximately the mean of other studies (20%) and falls in the mid-range compared to those studies. Differences with some of the reported studies can be explained by methodological factors detailed in the Introduction section. Note that for Vossel et al., 2016, only the percentage reported with EEG (and not with only M-EEG) are shown. (B) Distribution of EA over a full-night PSG in the studies of Brunetti et al., Vossel et al., (2016) and Lam and collaborators from panel (A), having reported sleep-related data (empty bars) and in the current study (filled bars). Data presented as Mean +/- SEM for previous studies.

Before Looking more in detail at the distribution of subclinical epileptic events across the different sleep stages we have to add that these data are drawn from a small patient pool and therefore are challenging to interpret in terms of robustness. More precisely, results for this analysis are reported as mean percentages of EAs in different sleep stages across the 5 AD patients – however, as mentioned above, the number of EAs varied vastly across patients, and data for only one person is represented for the control group. The latter point also made all potential statistical inter-group comparisons futile with a sample size of 1 in the control group. However, the overall distributions can still be appreciated, even if their interpretations must remain qualitative. First, the predominance of N2-related activities is evident, with 61.9% and 44.4% ± 17.4 of all events detected during this stage for controls and patients, respectively (**Figure 68 A and B**, only means shown, and **Figure 68 C** for individual data in the patients' group). Overall percentages of N1-related epileptic events were all modest (4.8% and 11.1% ± 11.7, respectively). In comparison, EA occurred quite frequently during N3 sleep in patients (28% ± 31.2) and visibly less frequently in the case of the sole control with EAs (4.8%). Epileptic events during wakeful periods before sleep and during the night amounted to 9.5 and 12.8 ± 14.8% of all EAs among controls and patients, respectively. Finally, contrary to what is observed in murine models introduced above, only 3.7 ± 6.2% of all EA fell during periods of REM sleep in patients - even with the extended electrode layout over the lower temporal regions. Interestingly, it was 19.1% in the case of the control with EA, which is entirely unexpected in epilepsy patients (Ng & Pavlova, 2013) – but the feeble number of points this 19.1% represents (4 spikes) may explain this discrepancy.





**Figure 68. Distribution of epileptic activities during the polysomnography recording.** Percentage of EA over different sleep stages in the control with EA (A) and mean percentage of EA during the different sleep stages in patients (B). W = Wakefulness. Percentages mark the percentage of total detected epileptic events across all participants of a given group. (C) Distribution of EAs per patient over the sleep-wake cycle, with the total number of events marked on the top of each column.

An interesting final point about epileptic events detected in the current study is the morphology of these events. In many cases, experts remarked that EAs, albeit present, were challenging to detect due to their small amplitudes, in many cases termed “microspikes” (*micropointes*) by the experts. These activities required reading the EEGs at 5  $\mu$ V instead of the usual 10  $\mu$ V. First, this implies that EAs may often be missed due to the low amplitude of activities. Secondly, they may signal different underlying mechanisms compared to non-AD-related epileptic activities.

### 3.3. Neuropsychological evaluation

Given the low number of participants per group, for the following analyses, a more modest and prudent stepwise method was chosen when aiming to compare patients and controls with and without EAs. As a first step, this included establishing the differences between the two main groups (controls and patients) in cognitive functions and sleep quality – with showing, in each case, the points corresponding to participants with epileptic events on all figures below for data visualization. Once differences between the two groups were established, patients with and without EAs were also compared for these variables, without including the control group. Finally, an attempt toward a more complex model, taking into account neuropsychology, MRI-volumetry, EA and sleep-quality related variables was built to understand better the impact of EAs, sleep apnoeas and sleep fragmentation on sleep-related memory consolidation.

Note that the sample sizes may differ for the following neuropsychology results, especially for the *patient* group. Indeed, to avoid psychological distress, some tests had to be skipped for a few patients. If the test was omitted entirely, the participant was not considered for the given test. In the case of the RL/RI-16, where the third recall had to be overlooked for several patients due to fatigue, the score from the second recall was taken into account instead (4 patients). Similarly, several participants have not yet undergone the third visit ( $n = 9$ ), therefore, V3-related variables are also reported in reduced sample sizes per group. Finally, on all tests detailed below, better scores are always associated with controls' performance.

### *3.3.1. Memory functions*

As mentioned above, the three memory tests included in the study were the episodic memory test Epi-Real, the declarative verbal memory test RL/RI-16 and the visual memory test DMS-48. Of these three, Epi-Real contains a single recall after the VEEG-PSG in three steps: a free recall, a cued recall and a recognition task. Elements found in a given step are not revisited during the following ones. The RL/RI-16 contains a learning phase with three post-learning recalls immediately after (Post-learning), a single recall approximately 30 minutes after learning (30m-delay) and another recall after the polysomnography (Post-sleep). Given that age and education-level-related norms are only available for the post-learning and 30m delayed phases but not for the post-sleep recall, only brute scores were used for most of the following analyses, which potentially limits the extent of the conclusions we can draw from our results. For the DMS-48, three recalls are undertaken, one almost immediately after learning (post-learning), another approximately 1 hour post-learning, and the last one post-sleep. Due to the same issue with existing norms as for the RL/RI-16, only brute scores were used. Finally, given that normality of the distribution of many variables or assumption of equal variances was not maintained, mainly nonparametric tests were applied.

As seen in **Table 20**, all recall phases of the Epi-Real (**Figure 69 A**) are significantly different between the two groups ( $p < 0.001$  in all cases). Furthermore, based on results from 41 participants who have been seen at the third visit and for whom the rate of cognitive decline can be estimated based on their MMSE scores at V1 and at V3, Epi-Real performance (especially the score summing free and cued recalls/FR+CR) seems to be the best predictor of future cognitive decline of all the results ( $R^2 = 0.28$ , **Figure 69 B**. Note, however that one control was excluded from this analysis due to an extremely short follow-up time (45 days) leading to high outlier values in spite of minimal MMSE change.) Notably, while both the

RL/RI-16 and the DMS-48 differentiated well between patients (**Table 20, Figure 69 C-E**), they did not possess the same predictive values concerning future cognitive decline ( $R^2$  ranging from 0.09 to 0.18 for the different recalls of the RL/RI-16 and from 0.03 to 0.08 for the three DMS-48 recalls). This further underlines the importance of catching episodic memory decline as early as possible with highly sensitive and specific measures.

	Median		Mean $\pm$ SD		W	p
	Controls (25)	Patients (25)	Controls (25)	Patients (25)		
<i>EPI-REAL Free Recall</i>	9	5	8.8 $\pm$ 3.36	5.22 $\pm$ 3.69	487	< .001
<i>EPI-REAL Free+Cued Recall</i>	29.5	15	28.34 $\pm$ 3.17	16.76 $\pm$ 7.11	581	< .001
<i>EPI-REAL Total Recall</i>	36	27	35.78 $\pm$ 1.97	27.36 $\pm$ 5.87	560	< .001
<i>RL/RI6-16 Post-learning Free Recall 3</i>	12	3	11.44 $\pm$ 1.92	4.08 $\pm$ 3.87	574	< .001
<i>RL/RI6-16 30m-delay Free Rec.</i>	12	2	11.92 $\pm$ 1.87	3.84 $\pm$ 4.58	549	< .001
<i>RL/RI6-16 Post-Sleep Free Rec.</i>	10	3	10.2 $\pm$ 3.03	3.16 $\pm$ 3.78	571	< .001
<i>RL/RI6-16 Post-learning Total Recall 3</i>	16	9	15.8 $\pm$ 0.5	9.52 $\pm$ 4.46	564	< .001
<i>RL/RI6-16 30m-delay Total Recall</i>	16	9	15.88 $\pm$ 0.44	9.4 $\pm$ 4.37	582.5	< .001
<i>RL/RI6-16 Post-Sleep Total Rec.</i>	16	6	15.28 $\pm$ 1.43	7.28 $\pm$ 5.45	564.5	< .001
<i>DMS-48 Post-learning score</i>	45	41	45.2 $\pm$ 2.57	41.16 $\pm$ 5.19	461	0.004
<i>DMS-48 1h-delay score</i>	45	43	44.76 $\pm$ 2.65	41.48 $\pm$ 6.23	406	0.070
<i>DMS-48 Post-sleep score</i>	46	41	45.2 $\pm$ 2.72	40.24 $\pm$ 6.71	455	0.006

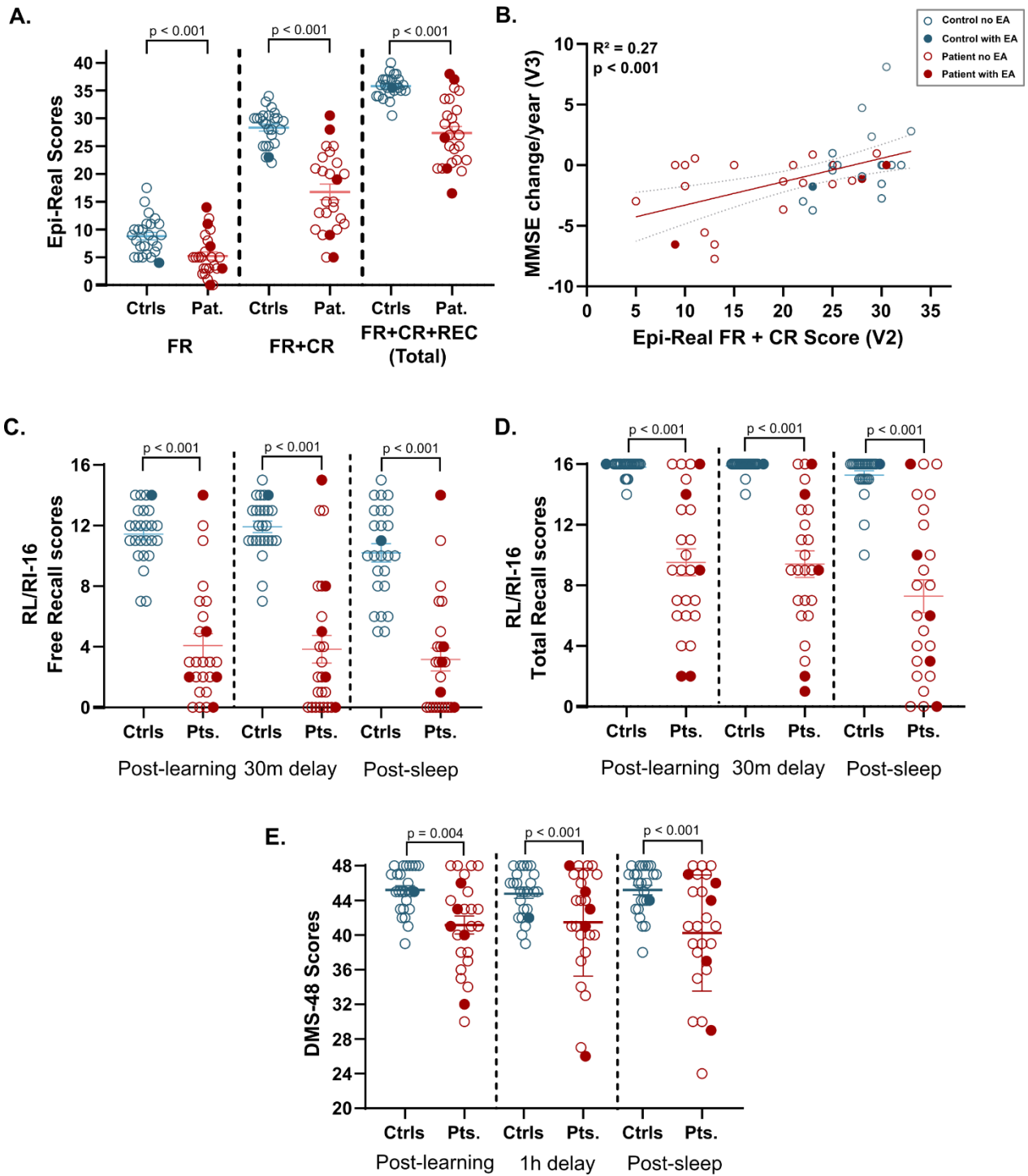
*Table 20. Descriptives and statistical differences (Mann-Whitney U Test) on tests measuring memory functions across the group of controls and AD patients.*

Furthermore, as secondary analyses, RM-ANOVAs were also run (Within-subject factor: length of *delay* post-learning; Between subject factor: *Group*, Covariate: *Age*) on the total recall scores on the RL/RI-16 recall scores as well as on the three DMS-48 recalls. For the free recall scores on the RL/RI-16, only a significant *Group* effect was found ( $p < 0.001$ ) without any effect of the *Delay* ( $p = 0.48$ ), *Age* ( $p = 0.385$ ) or of interaction between *Delay* and *Group* ( $p = 0.23$ ) or between *Delay* and *Age* ( $p = 0.3$ ). For the total recall scores of the RL/RI-16, the *group* effect was still significant ( $p < 0.001$ , Greenhouse-Geisser corrected values reported for all comparisons of this RM-ANOVA), while neither the *Delay* nor *Age* seemed to intervene on their own ( $p = 0.5$  and  $p = 0.17$ , respectively). However, a significant interaction between the *Delay* and the *Group* factors was found ( $p = 0.01$ ). Post hoc tests revealed that, over and above the better performances of controls compared to AD patients at all levels, this was due to the fact that control participants' performance did not decline between the recalls (**Table 21**), but that of patients did. More precisely, while patients' performance remained stable from the

immediate to the 30-min recall ( $p = 1$ ), it dropped significantly for the post-sleep recall ( $p < 0.001$  for comparing post-sleep recall to both immediate and 30-min recalls). This indicates that while encoding is already compromised (as seen in the between-group differences), successfully encoded information remains available in the short-term (i.e. stable performance on a 30-min span), but the consolidation of these pieces of information is further deficient.

RM-ANOVA - RL/RI-16, Total Recall Values					
Within Subjects Effects					
Cases	Sum of Squares	df	Mean Square	F	P <sup>a</sup>
<i>Delay</i>	2.532	1.396	1.815	0.707	0.449
<i>Delay * Group</i>	20.712	1.396	14.842	5.780	0.011
<i>Delay * Age</i>	3.545	1.396	2.540	0.989	0.351
<i>Residuals</i>	168.428	65.589	2.568		
<i>Note.</i> Type III Sum of Squares					
<sup>a</sup> All values reported are corrected values (Greenhouse-Geissner correction)					
Between Subjects Effects					
Cases	Sum of Squares	df	Mean Square	F	p
<i>Group</i>	1846.766	1	1846.766	58.868	< .001
<i>Age</i>	62.529	1	62.529	1.993	0.165
<i>Residuals</i>	1474.457	47	31.371		
<i>Note.</i> Type III Sum of Squares					
Post Hoc Comparisons - Group * Delay					
Comparison 1	Comparison 2	Mean Difference	SE	t	p <sub>holm</sub>
<i>CTRL, Post-Learning</i>	<i>Patients, Post-Learning</i>	6.463	0.971	6.657	< .001
	<i>CTRL, 30m-delay</i>	-0.047	0.380	-0.124	1.000
	<i>Patients, 30m-delay</i>	6.550	0.970	6.749	< .001
	<i>CTRL, Post-sleep</i>	0.556	0.380	1.464	0.463
	<i>Patients, Post-sleep</i>	8.667	0.970	8.931	< .001
<i>Patients, Post-Learning</i>	<i>CTRL, 30m-delay</i>	-6.510	0.970	-6.708	< .001
	<i>Patients, 30m-delay</i>	0.087	0.380	0.229	1.000
	<i>CTRL, Post-sleep</i>	-5.907	0.970	-6.087	< .001
	<i>Patients, Post-sleep</i>	2.204	0.380	5.805	< .001
<i>CTRL, 1h-delay</i>	<i>Patients, 30m-delay</i>	6.597	0.971	6.795	< .001
	<i>CTRL, Post-sleep</i>	0.603	0.380	1.588	0.463
	<i>Patients, Post-sleep</i>	8.714	0.970	8.979	< .001
<i>Patients, 1h-delay</i>	<i>CTRL, Post-sleep</i>	-5.994	0.970	-6.176	< .001
	<i>Patients, Post-sleep</i>	2.117	0.380	5.576	< .001
<i>CTRL, Post-sleep</i>	<i>Patients, Post-sleep</i>	8.111	0.971	8.354	< .001

Table 21. Results of the RM-ANOVA run on the RL/RI-16 results between patients and controls, taking into account the variables "group", "delay since learning" and "age".



**Figure 69. Performances of controls and AD patients on neuropsychological tests evaluating memory functions.** (A) Performances of controls (blue) and patients (red) on the free recall (FR) the free and cued recall (FR+CR) and the total scores (FR+CR+REC) of the Epi-Real test. (B) Predictive value of EPI-REAL FR+CR performances on future cognitive decline, as measured with the approximation of yearly MMSE score decline (estimated from the differences between the first and the third visit). Note that one participant with an extremely short follow-up time (45 days) was excluded from this analysis due to the biasing effect of the year-long projection. (C) RL/RI-16 performances on the free recall task post-learning, ~30 minutes after learning and post-sleep. (D) RL/RI-16 performances, as summed after the free and the cued recalls, post-learning, ~30 minutes after learning and post-sleep. (E) Performances of controls and AD patients on the DMS-48 at three intervals: Post-learning, 1 hour after learning and post-sleep. Participants with epileptic activities are marked with filled circles in all panels. Filled circles represent participants with epileptic activities. Data represented as mean +/- SEM.

As for the RM-ANOVA on the DMS-48 scores following the same pattern, a *Group* effect was once more evident ( $p = 0.003$ ), with no other main effects ( $p = 0.44$  and  $p = 0.15$  for *Delay* and *Age*, respectively) and only a tendency towards an interaction on the *Delay \* Group* condition ( $p = 0.07$ ). However, these were due to expected inter-group differences and not intra-group differences indicative of performance changes over time. This may entice us to conclude that the consolidation of visual information is more successful than that of declarative-memory-related information in the case of mild-to-moderate stage AD patients.

### 3.3.2. Executive functions

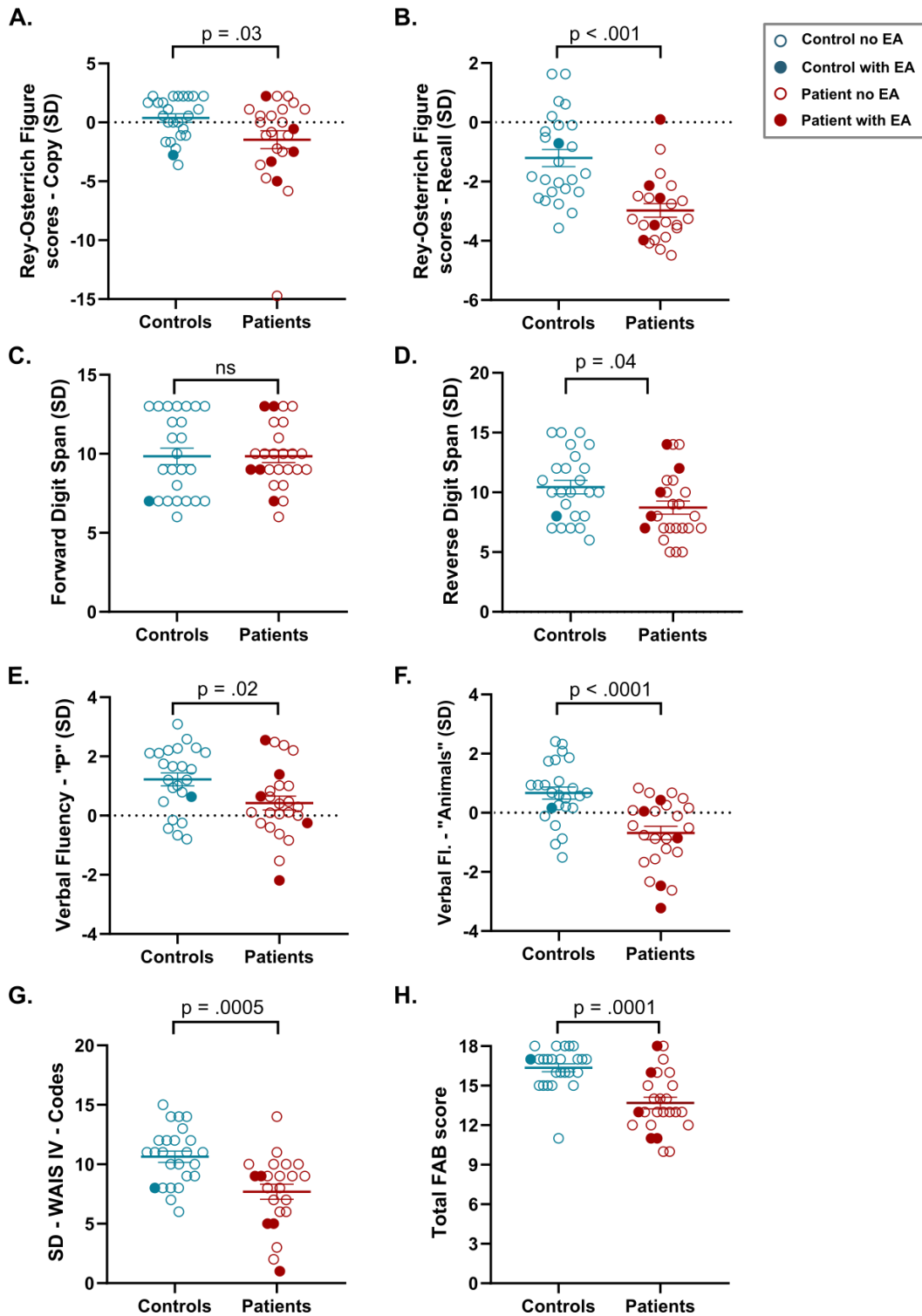
As seen in **Table 22** and in **Figure 70**, and as expected based on previous results, most measures of executive functions included in the study revealed significant differences between the two groups in favour of controls. Indeed, both subtests of the Rey-Österrich figure revealed significant differences, with a visibly more important deficit for patients on the *recall* part than on the *copy* subtask ( $p = 0.035$  for the *copy*,  $p < 0.001$  for the *recall*, **Figure 70 A and B**). As per the Digit Span tests of the WAIS-IV, only the Reverse Digit Span revealed a significant between-group difference ( $p = 0.034$ , **Figure 70 C and D**). The verbal fluency test of the GREFEX, however, found significant differences on both subparts ( $p = 0.02$  for the “*p*” subtest and  $p < 0.001$  for the “*animal-citing*” part, **Figure 70 E and F**). The other task from the WAIS-IV, the *Digit-Symbol* subtest, found important differences as well ( $p < 0.001$ , **Figure 70 G**) just as the Frontal Assessment Battery (FAB,  $p < 0.001$ , **Figure 70 H**). These results confirm the substantial deficits in verbal fluency, processing speed, short-term and working memory, inhibitory control and sensibility to interference in the mild-to-moderate stages of AD.

#### Independent Samples T-Test - Executive Functions

	Mean ± SD (n)		t	df	p
	Controls	Patients			
<i>SD - Rey-Osterrich Figure - Copy</i>	0.37 ± 1.79 (25)	-1.47 ± 3.69 (24)	2.203	32.915	0.035
<i>SD - Rey-Osterrich Figure - Recall</i>	-1.21 ± 1.45 (25)	-2.97 ± 1.11 (23)	4.770	44.495	< .001
<i>SD - Forward Digit Span Test</i>	9.84 ± 2.58 (25)	9.84 ± 1.97 (25)	0.000	44.935	1.000
<i>SD - Reverse Digit Span Test</i>	10.44 ± 2.82 (25)	8.72 ± 2.75 (25)	2.187	47.971	0.034
<i>SD - Verbal Fluency "P"</i>	1.22 ± 1.09 (24)	0.42 ± 1.18 (25)	2.469	46.947	0.017
<i>SD - Verbal Fluency "Animals"</i>	0.67 ± 1.02 (25)	-0.69 ± 1.14 (25)	4.440	47.427	< .001
<i>SD - WAIS IV Coding Subtest</i>	10.64 ± 2.38 (25)	7.7 ± 3.04 (23)	3.718	41.672	< .001
<i>Total FAB score</i>	16.36 ± 1.52 (25)	13.68 ± 2.25 (25)	4.931	42.202	< .001

Note. Welch's t-test.

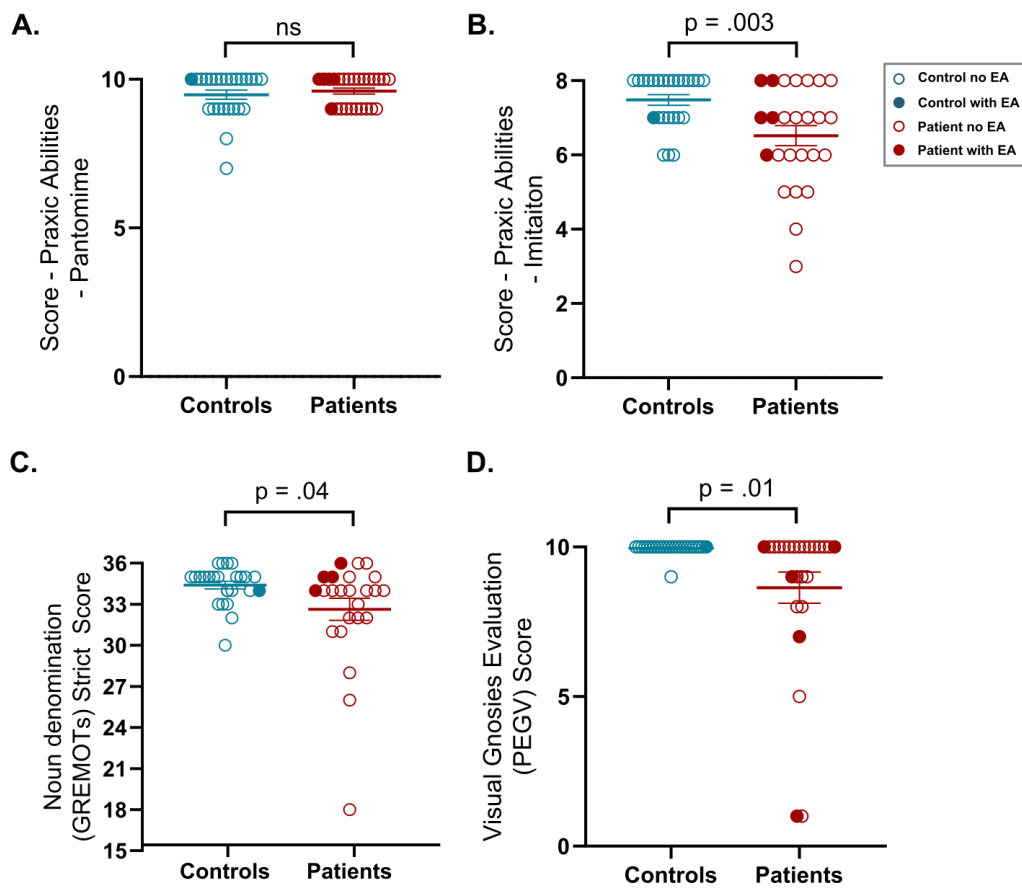
*Table 22. Descriptives and statistical differences on tests measuring executive functions between patients and controls. Test used: Welch's t-test.*



**Figure 70. Performances of controls and AD patients on neuropsychological tests evaluating executive functions.** Performances of controls (blue) and patients (red) on the copy (A) and the recall (B) of the Rey-Osterrich Figure, reported as standard scores according to norms. (C) and (D) show the standard scores of controls and patients on the Forward and Reverse Digit Span subtest of the WAIS-IV. (E) and (F) represent the norm-adjusted results of participants on the verbal fluency subtest of the GREFEX (Godefroy et al., 2008). (G) Standardized scores on the Codes subtest if the WAIS-IV for controls and patients. (H) Total scores on the Frontal Assessment Battery for the two groups (Dubois et al., 2000). Participants with epileptic activities are marked with filled circles in all panels. Data represented as Mean +/- SEM.

### 3.3.3. Instrumental Functions

Instrumental functions, however, seemed less affected (**Table 23**), with only the most complex part of the measure evaluating praxic abilities (Imitation) being significantly different between the two groups ( $p = 0.005$ , see **Figure 71 A and B**). Furthermore, the noun denomination subtest was only marginally significant ( $p = 0.04$ ), and this difference was mainly due to the outliers belonging to the performances of the atypical AD patients (aphasic – logopenic AD, **Figure 71 C**). The PEGV scores evaluating the presence of visual agnosia were significant ( $p = 0.002$ ), however, it was also attributable to the presence of atypical (agnosic) AD patients' in the cohort (**Figure 71 D**). In sum, while milder deficits in instrumental functions are present in our cohort of mild-to-moderate AD patients, they concern mainly atypical AD patients at this stage of disease progression.



**Figure 71. Performances of controls and AD patients on neuropsychological tests evaluating executive functions.** (A) and (B) represent scores on the second and third subtasks of the test of Mahieux-Laurent (Pantomime and Imitation, respectively) between controls (blue) and patients (red). (C) Shows scores on the noun denomination subtask of the GREMOTs, while (D) represents PEGV performance, evaluating the presence of agnosia. Participants with epileptic activities are marked with filled circles in all panels. Data represented as Mean +/- SEM.



	Median (n)		Mean ± SD		W	p
	Controls	Patients	Controls	Patients		
<i>Praxic - Symbolic</i>	5 (25)	5 (25)	4.8 ± 0.5	4.8 ± 0.52	324.5	0.738
<i>Praxic - Pantomime</i>	10 (25)	10 (25)	9.6 ± 0.5	9.5 ± 0.77	322.5	0.830
<i>Praxic - Imitation</i>	8 (25)	7 (25)	7.48 ± 0.71	6.52 ± 1.36	448	0.005
<i>Noun denomination (GREMOTS)</i>	35 (25)	34 (24)	34.4 ± 1.38	32.64 ± 3.94	399	0.043
<i>Visual Gnosies (PEGV)</i>	10 (25)	10 (25)	9.96 ± 0.2	8.64 ± 2.6	428	0.002

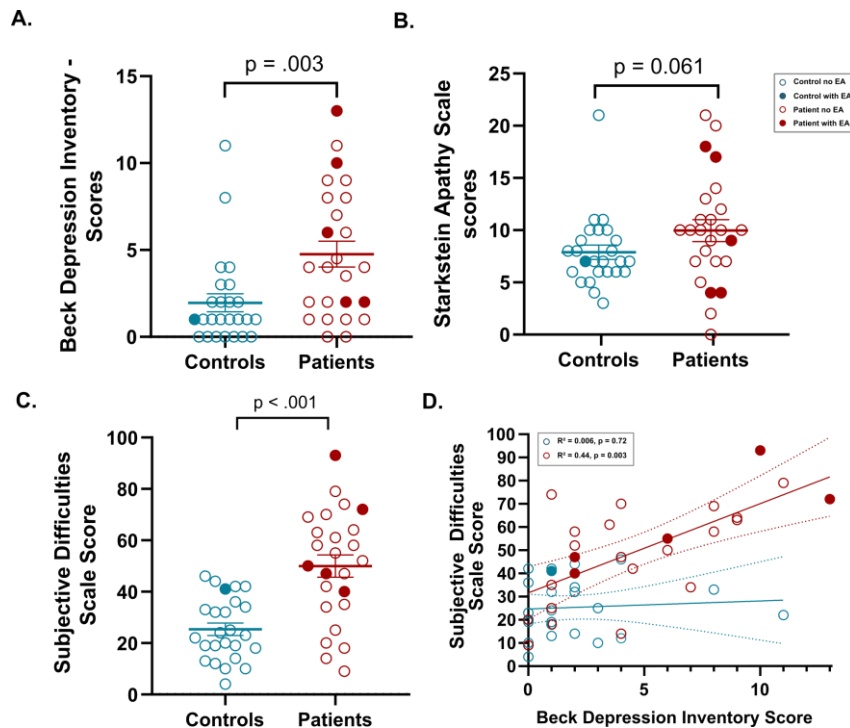
Table 23. Results of the Mann-Whitney U test of tests measuring instrumental functions between patients and controls.

### 3.3.4. Subjective difficulties and emotional state

Even though only participants without antidepressants or severe identified depression were included in the study, patients still showed significantly higher scores on the Beck Depression Inventory ( $p = 0.003$ , **Figure 72 A**), with the mean reaching scores associated with mild depression (4-7 points). The Starkstein scale only revealed a tendency towards higher levels of apathy for patients ( $p = 0.06$ , **Figure 72 B**). Concerning the subjective daily living difficulties perceived by participants, patients reported significantly more difficulties ( $p < 0.001$ , **Figure 72 C**, see **Table 24** for a sum of the results reported above). Interestingly, when bringing the level of subjective difficulties together with the Beck Depression Inventory, we observed a satisfying fit for patients (Linear Regression,  $R^2 = 0.44$ ,  $p = 0.003$ ), but not for controls ( $R^2 = 0.005$ ,  $p = 0.72$ , **Figure 72 D**). Although causality remains questionable, these results may indicate that the more or less important subjective difficulties of daily living may be perceived as a part of the natural ageing process without reducing the emotional state of healthy older adults. However, when these difficulties are aggravated and complemented with a diagnosis foreshadowing further rapid decline, it might trigger the rapid degradation of perceived quality of life that can, together with the diagnosis itself, lead to depression.

	Controls	Patients	Controls	Patients	W	p
<i>Subjective difficulties scale Score</i>	23 (25)	52 (25)	25.36 ± 12.11	49.96 ± 21.74	107.5	< .001
<i>Beck Depression Inventory Score</i>	1 (25)	4 (25)	1.96 ± 2.61	4.76 ± 3.74	158.5	0.003
<i>Starkstein Apathy Scale Score</i>	7 (25)	10 (25)	7.88 ± 3.44	9.96 ± 5.24	216	0.061

Table 24. Results of the Mann-Whitney U tests comparing subjective difficulties and emotional/motivational states between controls and patients.



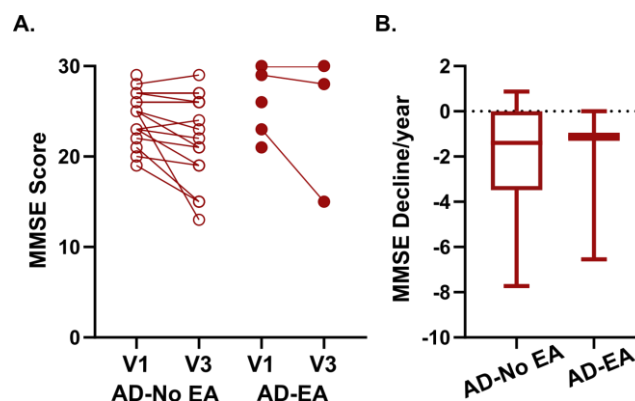
**Figure 72. Scores on questionnaires evaluating emotional state and subjective difficulties of daily living.** (A) Scores on the Beck Depression Inventory for controls (blue) and AD patients (red). (B) Scores on the Starkstein Apathy Scale for controls (blue) and AD patients (red). (C) Scores on the Subjective Difficulties Questionnaire of McNair and Kahn for controls (blue) and AD patients (red). (D) Linear regression for both groups on the Subjective Difficulties Scale scores and the Beck Depression Inventory scores. Participants with epileptic activities are marked with filled circles in all panels. Data represented as Mean +/- SEM.

Still pertaining to the subjective difficulties of daily living, when running correlations of this questionnaire's scores compared to the other neuropsychological tests for controls, apart from the scores on the *Copy* subtest of the Rey-Österrich Figure that showed a significant correlation (Spearman's rho = -0.43, p = 0.03), only the Epi-Real scores, and more precisely the sum of free and cued recall (FR+CR), turned out to be significantly negatively correlated to subjective difficulties (Spearman's rho = -0.56, p = 0.004). These results once again underline how early deficits of episodic memory decline may signal even slight levels of cognitive decline, be it related to healthy cognitive ageing or to AD. Interestingly, this relationship between Epi-Real scores and subjective difficulties was not present in the patients' group (Spearman's rho = -0.054, p = 0.8), yet correlations with other tests became more prominent in this group, including both the *Copy* and the *Recall* parts of the Rey-Österrich Figure (Spearman's rho = -0.51, p = 0.01 and Spearman's rho = -0.53, p = 0.009, respectively), the *Digit-Symbol* subtest of the WAIS IV (Spearman's rho = -0.51, p = 0.01), the PEGV test scores (Spearman's rho = -0.46, p = 0.02) and the MMSE scores (Spearman's rho = -0.53, p = 0.008). This indicates that the significant increase in subjective difficulties may settle in when executive functions start their more pronounced decline in AD patients.

### 3.3.5. The effect of EAs on cognitive functions and the steepness of cognitive decline

After establishing a solid difference in most neuropsychological measures between the two main groups of analysis (controls and patients), we were interested in how epileptic events may have impacted cognitive decline and memory consolidation. Given the statistical challenge of vastly different group sizes in this case (one control and five patients with EAs), only patients with and without EAs are compared instead of the inclusion of the control group. Even if the biasing impact of multiple comparisons burdens these analyses, hopefully, adding the remaining ten participants in the near future will ameliorate statistical power and will allow for more sophisticated analyses.

Concerning the results of the neuropsychological test battery, Mann-Whitney U tests did not reveal any differences between patients with and without EAs, except for a slight difference on the noun denomination test ( $p = 0.05$ ) in favour of patients with EAs; however, it may be explained by the fact that one patient with EAs having an aphasic form of AD could not perform this test due to psychological distress, further decreasing the sample size in the AD-EA group and tilting the scores toward better performances. Concerning the steepness of cognitive decline, unfortunately, data is not yet available for all patients and is crucially lacking for 2 out of 5 patients with the most epileptic events. However, a preliminary look at the data indicates similar scores and estimations of yearly MMSE decline in the two groups (**Figure 73 A and B**), which is contradictory to previous publications on this matter (Haoudy et al., 2020; Vöglein et al., 2020; Vossel et al., 2016).



**Figure 73. Cognitive decline measured by the MMSE for patients with and without epileptic activities between the first and the last visit. (A)** Change in total MMSE scores between the two visits between patients without EAs (AD-No EA, empty circles) and patients with EAs (AD-EA, full circles). **(B)** Estimated yearly change of the MMSE, adjusting for the differences in the delay between two visits in the cohort. Note that data is based on  $n=18$  for patients without AE and  $n=3$  on patients with AE.

### 3.4. Sleep Macroarchitecture

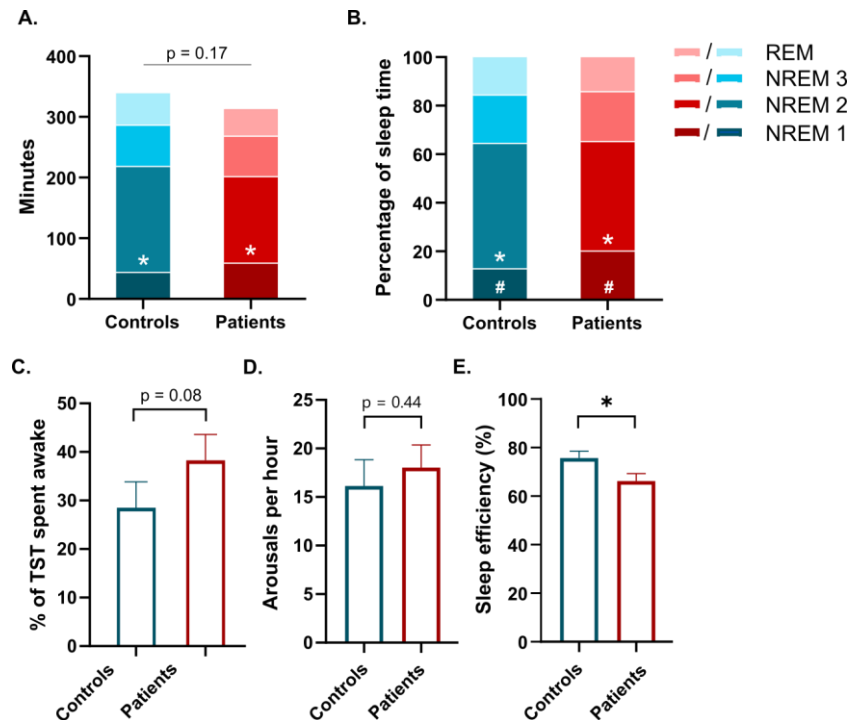
#### *3.4.1. Sleep quality differences in Alzheimer's disease*

As mentioned earlier, in the first step, general sleep quality-related variables were assessed between the two groups before taking into account the impact of epileptic events. Note that in many cases, nonparametric tests were used due to the violation of one or several prerequisites of their parametric counterparts. As for the previous results, given the exploratory nature of the study, corrections for multiple comparisons are not applied.

Sleep latencies were not different between controls (Mdn = 10 min) and AD patients (Mdn = 15 min,  $U = 268.5$ ,  $p = 0.4$ ), however, the high inter-individual variability is of note for the group of controls (Mean = 26.6, SD = 39.11, Min = 2, Max = 143) and also for patients (Mean = 28.44, SD = 33.32, Min = 0, Max = 132). This could be due to the novelty of the experimental setup, especially since there was no observed correlation between subjectively perceived general sleep latency outside of the experimental condition (measured by the second component of the PSQI test) and actual sleep latencies observed during the VEEG-PSG for controls (Kendall's Tau B = -0.02,  $p = 0.88$ ) nor for patients (Kendall's Tau B = -0.01,  $p = 0.94$ ). Interestingly, patients' subjective evaluation of sleep latency was significantly better compared to controls' (indicating that subjectively, patients experience fewer difficulties in falling asleep) as measured by a multinomial test ( $\chi^2(3) = 9.73$ ;  $p = 0.02$ ). Unfortunately, the confounding bias of the new environment and the vEEG+PSG setup on sleep latencies impedes confirming these subjective descriptions by the PSG data.

Concerning sleep macroarchitecture, when looking at Total Sleep Time (TST), descriptive statistics suggest lesser sleep time for patients, however, this difference does not reach significance ( $p = 0.15$ , **Figure 74 A**). On the other hand, the distribution of this difference is disproportionate across sleep stages between the two groups, as it lies in more time spent in NREM 2 stage by controls ( $t(48) = 2.49$ ;  $p = 0.02$ ), while no difference in the other sleep stages was observed. When looking at normalized values (expressed as percentages of TST), the difference is still almost completely accountable by decreased NREM 2% for patients (Mean = 45.23) compared to controls (Mean = 51.89,  $t(48) = 2.4$ ;  $p = 0.02$ ). However, this decrease was accompanied by a concomitant increase of more superficial (N1) sleep for patients (Mean<sub>Pat</sub> =

20.1,  $\text{Mean}_{\text{ctrl}} = 12.65$ ,  $t(48) = -2.44$ ;  $p = 0.02$ , **Figure 74 B**), but N3 and REM percentages did not differ ( $p = 0.85$  and  $p = 0.39$ , respectively). Altogether, these results suggest a slight increase in superficial sleep at the detriment of the deeper, spindle-rich N2 sleep in AD patients.



**Figure 74. Sleep macroarchitecture for patients and controls.** (A) Time spent in different sleep stages across the night, expressed in minutes, revealing a significant group difference for N2 that is also maintained when data are expressed in terms of percentages of total sleep time (B). (C) Differences in the time spent awake during the night, expressed as the percentage of total sleep time, showing a tendency toward increased nighttime wakefulness for AD patients. (D) Number of arousals per hour. (E) Sleep efficiency differences indicating slightly better sleep quality for healthy controls. \*:  $p < 0.05$ ; #  $p < 0.1$ .

When looking at sleep quality, a slight difference toward more time spent awake during the night is revealed for patients ( $U(48) = 213$ ;  $p = 0.05$ , **Figure 74 C**), without any differences in micro-arousals during the night ( $U(48) = 268$ ;  $p = 0.4$ , **Figure 74 D**). However, these slight alterations are still sufficient to be visible in sleep efficiency calculated either by dividing total sleep time by the time spent in bed (TST/TIB;  $p = 0.03$ , **Figure 74 E**) or by dividing total sleep time by sleep period time (SPT) from the first N2/REM event until awakening in the morning (TTS/SPT N2-REM,  $p = 0.03$ , *not shown*). Altogether, these results point toward an increase of superficial sleep to the detriment of deeper sleep stages (especially N2 sleep) and decreased sleep efficiency for mild-to-moderate stage AD patients.

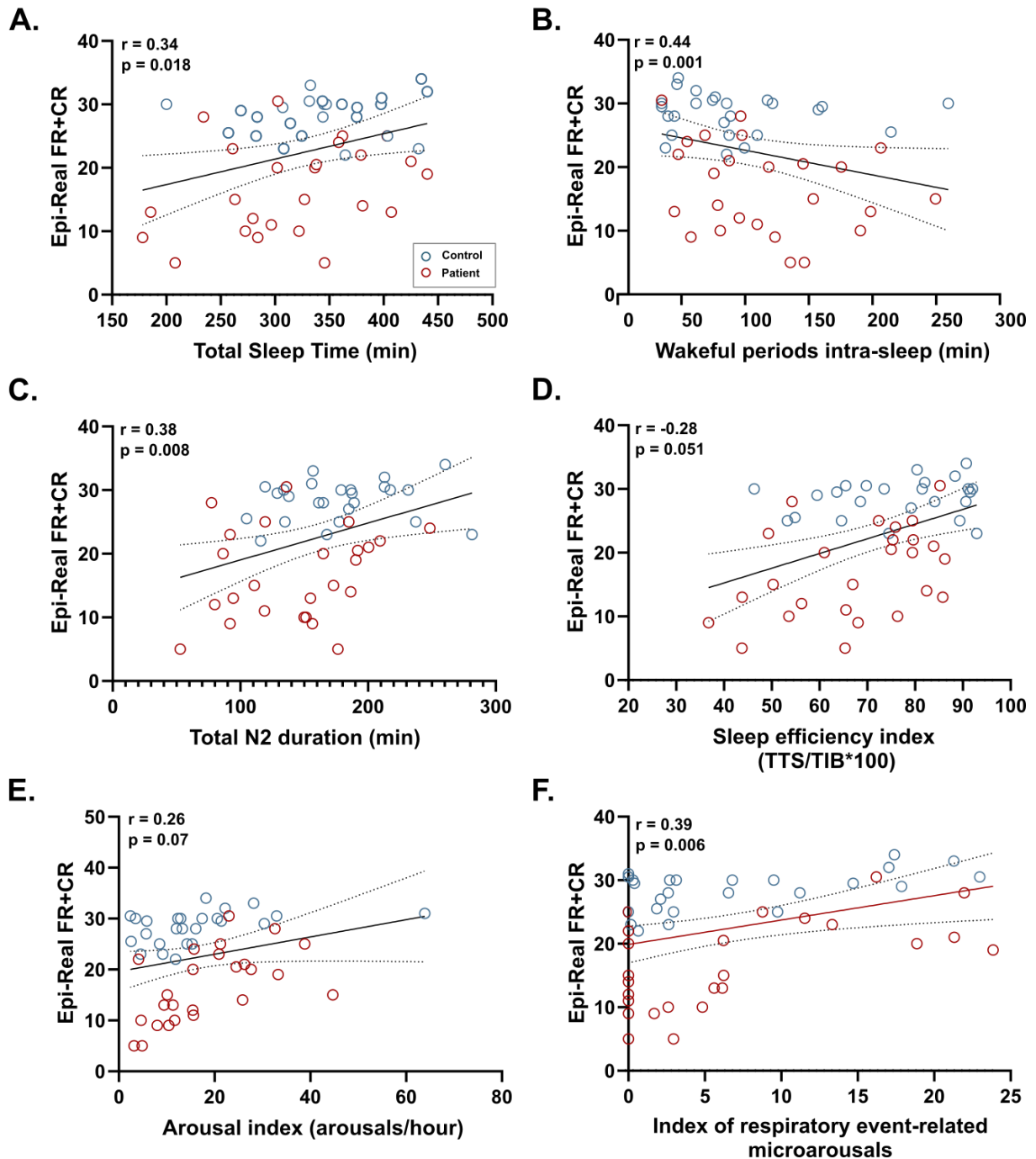
### 3.4.2. The impact of sleep quality decline on memory consolidation

To evaluate the evaluation of memory consolidation efficiency, we used three scores: the Free + Cued recall score on the Epi-Real, as well as the difference between the pre-sleep and post-sleep recall scores on the RL/RI-16 and the DMS-48. Interestingly, independent of group and even when controlling for age, many test results measuring memory consolidation efficiency were correlated to sleep quality-related variables (**Table 25**). This concerned total sleep time, both sleep efficiency indices and the duration of N2 sleep, for which an expected positive correlation was found. Expected negative correlations were discovered for the latency to N2 sleep, percentage and time spent awake during the night after the first sleep episode. Modest correlations between arousal-related indices and the Epi-Real scores were also discovered but were surprisingly positive (that is, more arousals, related to pathological respiratory events or not, resulted in better scores on the episodic memory test, **Table 25**). However, when looking at the data amongst the concerned variables (**Figure 75 A-F**), it is visible that differences are mostly due to differences in initial cognitive status (healthy or with dementia) – therefore, potentially, in initial encoding capacity.

<b>Pearson's Partial Correlations - Epi-Real scores and sleep quality</b>			
<b>Variable 1</b>	<b>Variable 2</b>	<b>Pearson's r</b>	<b>p</b>
<b>Epi-Real FR+CR</b>	TST	0.337	0.018
	Sleep efficiency index (TTS/TIB)	0.442	0.001
	Sleep eff. index (TTS/(Time from first N2/REM to awakening))	0.333	0.019
	Latency to N2 sleep	-0.329	0.021
	Total duration of N2 sleep	0.377	0.008
	% of TST spend awake	-0.278	0.053
	Time spent awake after first sleep period	-0.281	0.051
	Number of microarousals	0.293	0.041
	Number of microarousals due to respiratory events	0.314	0.028
	Index of microarousals due to respiratory events	0.385	0.006
	<i>Note.</i> Conditioned on variables: Age.		
<i>Note2.</i> Only significant correlations shown.			

*Table 25. Partial correlations between episodic memory test results and sleep-quality related variables.*

This is further underscored by looking at the regressions only for the group of patients, in which case the only correlations with the Epi-Real scores still standing are for the TTS/TIB sleep efficiency index ( $r = 0.43$ ,  $p = 0.03$ ), and, surprisingly, the microarousal-related variables that become even stronger (e.g. Microarousal index:  $r = 0.58$ ,  $p = 0.003$ ; Microarousal index related to respiratory events:  $r = 0.54$ ,  $p = 0.006$ ).



**Figure 75. Mild correlations between sleep quality-related measures and episodic memory.** Links are shown between episodic memory (measured by the Epi-Real Free+Cued recall test) and Total sleep time (A), Sleep efficiency index (B), duration of N2 sleep (C), intra-sleep wakefulness (D), Total microarousal index (E) and the microarousal index related to respiratory events (F). Linear regression was run on the full dataset, patients (red) and controls (blue) are shown in different colours only for visualization purposes.  $N = 25$  per group.

Similar correlations were observed for the pre vs post-sleep RL/RI-16 scores (calculated as post minus pre-sleep scores), although solely for Total Sleep Time, the Sleep efficiency index (measured as TTS/TIB), N2 duration and REM duration (**Table 26**). However, none of the correlations were present solely for the group of patients, while control's data is highly challenging to interpret alone due to the ceiling effect observed on the RL/RI-16 (**Figure 76**).

Pearson's Partial Correlations – RL/RI-16 pre and post sleep scores and sleep quality			
Variable 1	Variable 2	Pearson's r	p
<b>RL/RI-16 pre vs. Post sleep score</b>	TST	0.305	0.033
	Sleep efficiency index (TTS/TIB)	0.372	0.008
	Sleep eff. index (TTS/(Time from first N2/REM to awakening))	0.271	0.060
	Total duration of N2 sleep	0.289	0.044
	Total duration of REM sleep	0.320	0.025
<i>Note.</i> Conditioned on variables: Age.			
<i>Note2.</i> Only significant correlations shown.			

*Table 26. Partial correlations between post-sleep declarative memory test results and sleep-quality related variables.*

Finally, for DMS-48 pre-and post sleep performances, only sleep efficiency index (TTS/TBI,  $r = 0.298$ ,  $p = 0.038$ ) and total sleep time ( $r = 0.331$ ,  $p = 0.02$ ) were significantly correlated to the performances of all the above-mentioned variables but were joined by sleep latency related variables instead, which all showed negative correlations with the pre vs. post-sleep performances on the DMS-48, independent of the sleep stage concerned (**Table 27**). Interestingly, this is the test the data is more dispersed both for controls and patients and suggests an effect of sleep *per se* on memory consolidation instead of a confounding effect with initial cognitive status – although the strength of the correlations remains modest (**Figure 76 B**).



Pearson's Partial Correlations - DMS-48 pre vs post-sleep performances and sleep quality			
Variable 1	Variable 2	Pearson's r	p
<b>DMS-48 pre vs. Post-sleep performance</b>	TST	0.331	0.020
	Latency N1	-0.272	0.059
	Latency N2	-0.355	0.012
	Latency N3	-0.284	0.048
	Latency REM	-0.244	0.091
	Sleep efficiency index (TTS/TIB)	0.298	0.038

Note. Conditioned on variables: Age.  
Note2. Only significant correlations shown.

Table 27. Partial correlations between post-sleep visual memory test results and sleep-quality related variables.

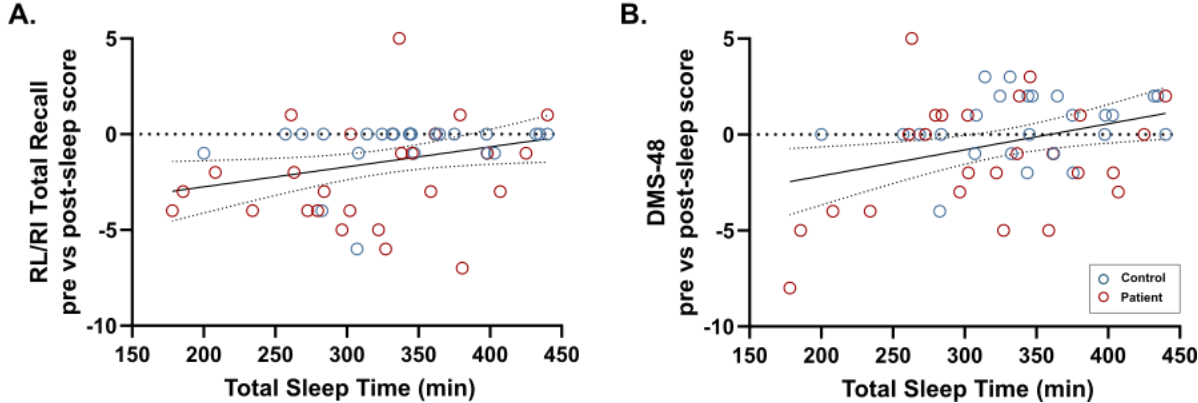
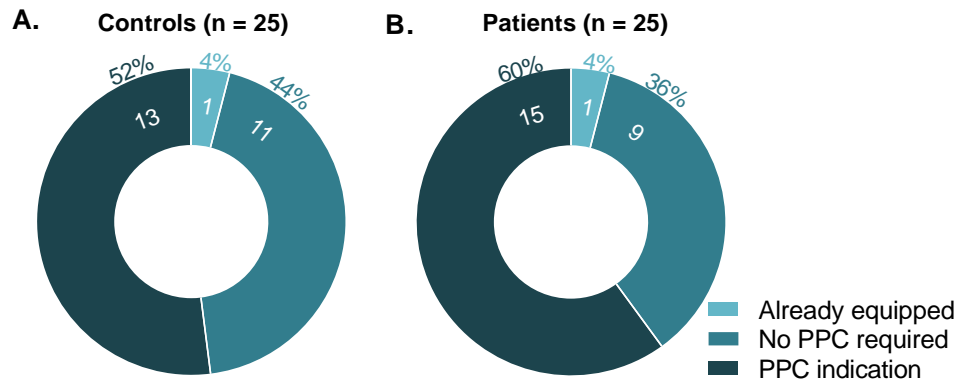


Figure 76. Correlation between total sleep time and memory consolidation efficiency on the verbal declarative and the visual memory test. Pre vs. post-sleep performances of patients (red) and controls (blue) on the RL/RI 16 total recall (A) and on the DMS-28. N = 25 per group.

### 3.4.3. The prevalence and impact of Sleep Apnoea Syndrome on AD-related cognitive decline

As mentioned earlier, apnoeas may be important biasing factors when looking at sleep-related memory consolidation and age-related cognitive decline (or, as in our case, when seeking to understand both better). And indeed, even in this cohort where a known and untreated sleep apnoea syndrome was considered a non-inclusion criterion, an unexpectedly high number of

participants were diagnosed with moderate-to-severe sleep apnoeas following the vEEG-PSG, with an indication for positive airway pressure equipment (**Figure 77 A, B**). Indeed, 60% of patients and 52% of controls received an indication for positive airway pressure treatment. The two-sided z-test for equality of two proportions for participants with or without SAS revealed that the between-group difference was not significant ( $z = 0.57, p > 0.05$ ).



**Figure 77. Newly diagnosed sleep apnoeas in the EREMAD cohort.** Percentage of participants with newly diagnosed sleep apnoea syndrome, participants with already known and treated apnoeas and participants without moderate or severe sleep apnoeas across the group of controls (**A**) and patients (**B**). Proportion of participants with PPC indication per group: ns. The number of participants in a different segment is marked inside the circle, while the percentage this number represents is noted on the outer rim of the circle.

Note that for the analyses on apnoea-related variables below, the two participants (one per group) with already equipped SAS were not included. For these further analyses, indexes (number/hour) of different types of apnoeas (central, obstructive or mixed), plus the total number of apnoeas per hour were considered, together with the hypopnea index and the combined apnoea-hypopnea index (AHI). The total apnoea index showed no inter-group difference ( $p = 0.13$ ), nor did the separate indexes for central ( $p = 0.49$ ), obstructive ( $p = 0.16$ ) or mixed apnoeas ( $p = 0.92$ ), however, slight increases for the patients' group, especially for obstructive sleep apnoeas and the total apnoea index can be seen from the descriptive statistics (**Table 28, Figure 78**). Note that mixed apnoeas were relatively rare (with a Median of 0 for both groups). Similarly to the apnoea results, the hypopnea index was not distinguishable between the two groups ( $p = 0.62$ ), excluding the possibility of significant differences in the composite AHI index as well ( $p = 0.3$ , **Figure 78**). Altogether, sleep apnoeas are extremely frequent even in healthy older adults, making it important to consider their potential bias on cognitive decline.

	Med. Control (24)	Med Patients (24)	W	P
Total apnoea index	2.07	5.62	214.5	0.13
Central apnoeas	0.18	0.29	255	0.49
Obstructive apn.	1.27	4.24	219	0.16
Mixed Apnoeas	0	0	293	0.92
Hypopneas	13.91	14.87	263	0.62
AHI	16.51	22.57	237	0.3

Table 28. Results of the Mann-Whitney U tests on apnoea-related variables between controls and patients.

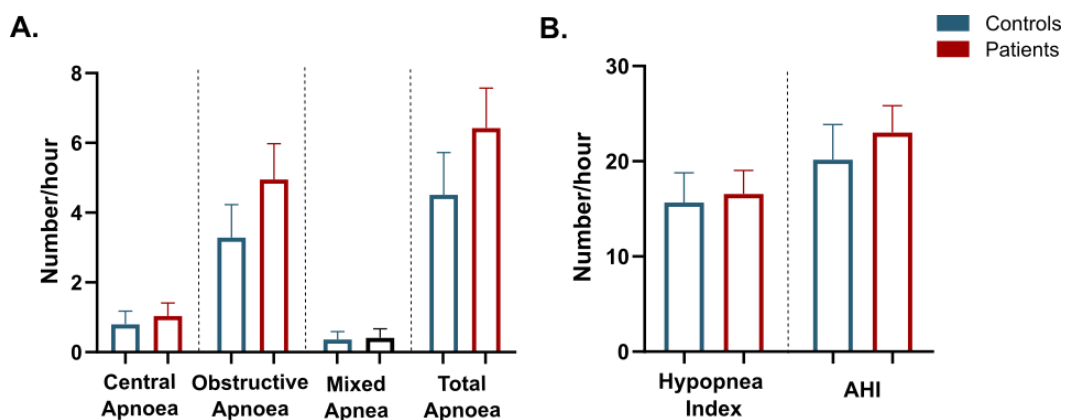
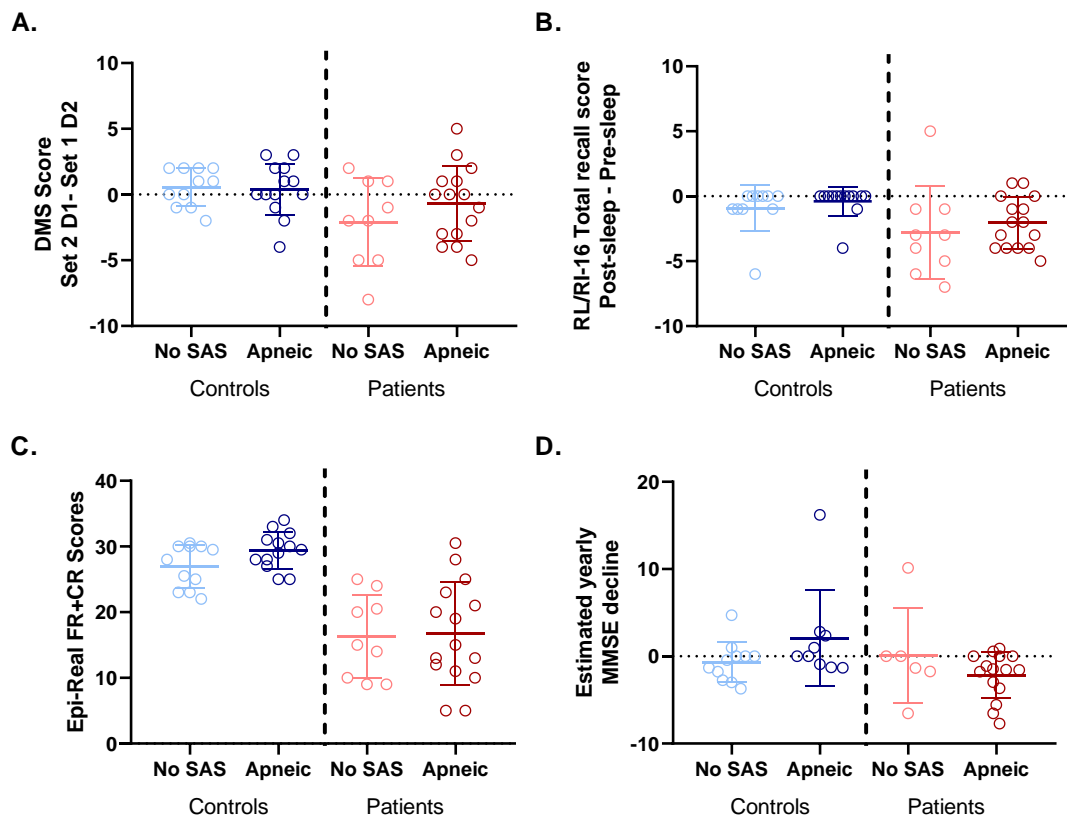


Figure 78. Apnoea and hypopnea-related indices for patients and AD patients. (A) Hourly quantities of central, obstructive and mixed apnoeas, and their total numbers for controls (blue) and patients (red). (B) Hypopnea index and total apnoea-hypopnea index for controls and patients. All comparisons  $> 0.05$ .  $N = 25$  in both groups.

Furthermore, the presence of moderate-to-severe sleep apnoeas requiring PPC treatment did not seem to impact memory consolidation, as the RM-ANOVA run with group and PPC indication status on the post-sleep recall phases as between-subject factors and the time of test (Post-learning, Delayed pre-sleep, Post-sleep) as within-subject factors was negative for each memory test. Indeed, when looking at the difference between post-learning as well as pre- and post-sleep scores on the DMS-48, no main effect or interaction could be found, with all measures at  $p > 0.05$ ; **Figure 79 A**). Concerning the pre- and post-sleep scores of the RL/RI-16 total recall scores, the same effects of *group*, *delay* and *interaction* were found as described in *Section 3.3.1*, without any added impact of PPC indication (main effect and interactions including PPC indication all at  $p > 0.05$ , **Figure 79 B**). On the Epi-Real scores, on all types of recall (here, only FR+CR shown on **Figure 79 C**), only the effect of the group was once again significant ( $p < 0.001$  in all three cases), without an impact of SAS or a significant interaction. For participants having already passed the third visit, the impact of SAS on the slope of

cognitive decline was also explored ( $n = 41$ ), without any impact of the PPC indication, once again with a significant group effect ( $p = 0.02$ , **Figure 79 D**). Note that two participants were excluded from the last analysis on cognitive decline as the unusually short delay between their V1 and V3 led to potentially biasing values in their yearly estimated MMSE change (both  $> 10$  points, for 1-2 point overall differences).

Interestingly, no correlations were found between tests requiring memory consolidation and apnoea-hypopnea-related variables, except for a mild contribution of the hypopnea index to a linear regression when looking at the link between Epi-Real FR+CR scores and Group, MMSE and Apnoea-related variables. Indeed, the best fit ( $R^2 = 0.72$ ,  $F = 37.41$ ,  $p < 0.001$ ) was achieved when taking into account Group, initial MMSE scores ( $p < 0.001$  for both) and the hypopnea index ( $p = 0.02$ ). Altogether, these results indicate a potential triangle between sleep apnoea, AD and episodic memory consolidation. However, these analyses cannot infer causality with the current sample sizes and experimental protocol. Repeating the tests months after the initiation of the PPC treatment in larger samples could respond to these points in the future, taking into account the potential impact of test-retest effect in the case of this specific task.

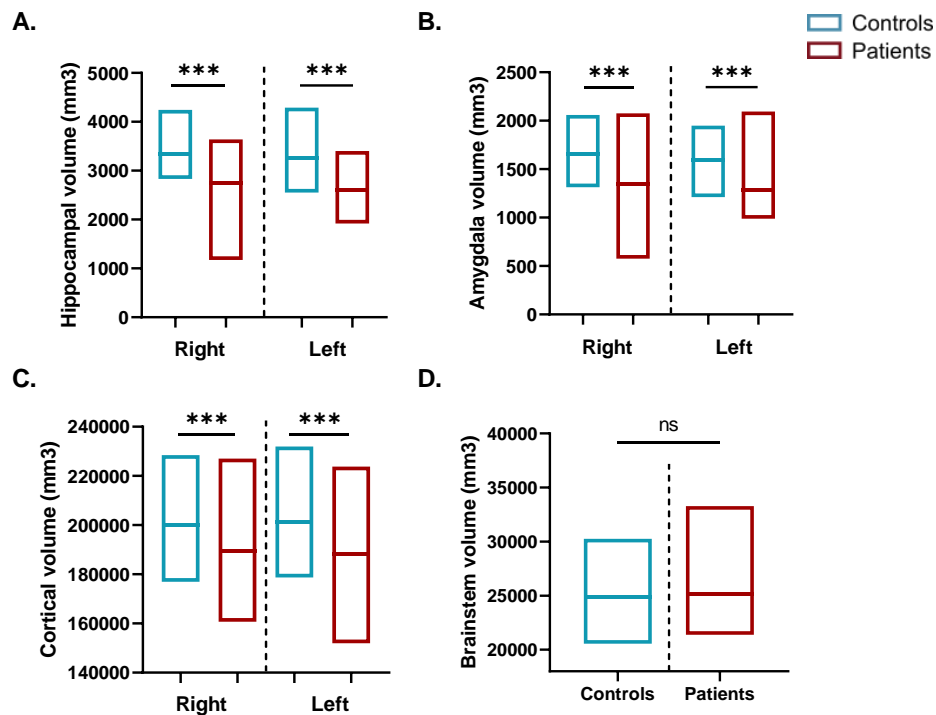


**Figure 79. The impact of moderate-to-severe SAS on memory consolidation.** (A) Post-sleep minus pre-sleep scores on the DMS-48 test, separated by group and PPC indication (signaling the presence of moderate-to-severe SAS). (B) Post-sleep minus pre-sleep scores on the RL/RI-16 test, once again divided by group and SAS indication. (C) Epi-Real cued+free recall scores, divided by the presence or absence of SAS. (D) Estimated yearly change of the MMSE scores for participants having already passed the third visit, in function of PPC indication ( $n = 41$ ). Data shown as mean  $\pm$  SD.

### 3.5 Structural MRI analysis

As mentioned earlier, the volumetric analysis itself was run by an expert (Pr Patrice Péran) using already existing pipelines. Note that one patient's data is not yet included in the current sample, leading to 25 controls and 24 patients for this analysis.

Concerning the statistical analyses, expected differences between patients and controls were found in hippocampal and amygdala volumes ( $p < 0.0001$  for both structures in both hemispheres, **Figure 80 A** and **B**), total cortical volumes (once again,  $p < 0.0001$  for both hemispheres, **Figure 80 C**) but full gray-and white matter estimations showed only tendencies towards decreases in patients ( $p = 0.09$  and  $p = 0.053$ , respectively).

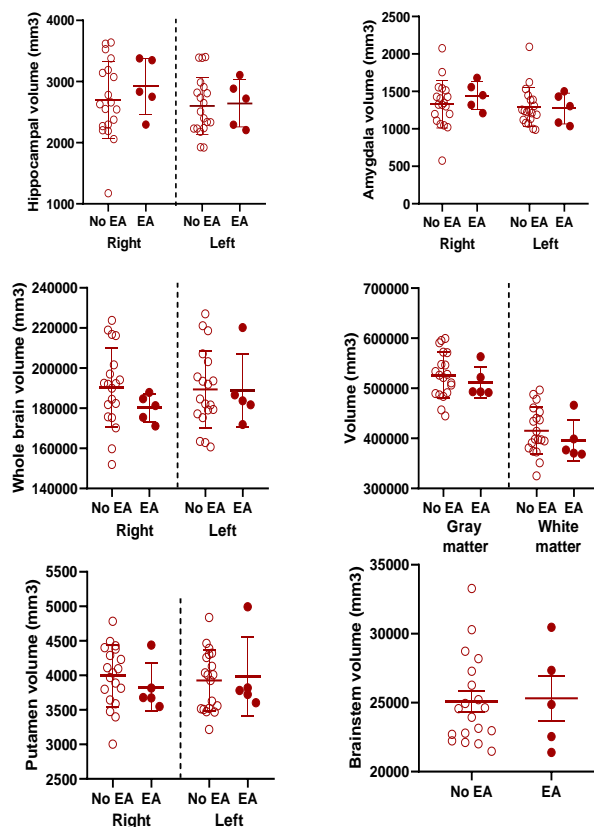


**Figure 80. Between-group differences on structural MRI results.** Differences of right and left hippocampal (A), amygdalar (B) and cortical (C) volumes between controls (blue) and patients (red). (D) Differences in brainstem volumes between controls (blue) and patients (red). N = 25 for controls, N = 24 for patients. Lines in the boxes represent the means. \*\*\*:  $p < 0.001$

Differences were also observed at the level of the basal ganglia, notably at the level of the left ( $p = 0.03$ ) and right ( $p = 0.05$ ) putamens, the only part of the basal ganglia considered for this explorative analysis. However, the between-group differences did not propagate towards the brainstem's total volume ( $p = 0.71$ , **Figure 80 D**) nor towards its subparts (medulla:  $p = 0.69$ ; pons:  $p = 0.71$ ; midbrain:  $p = 0.89$ , *not shown*). This is an interesting point as the brainstem nucleus *locus coeruleus* has not only been reported as being one of the first structures

burdened by NFT deposits (Braak et al., 2011), but it has been shown that the integrity of the LC is correlated to tau burden (Dahl et al., 2022). Therefore, early signs of total volume decrease could be expected around the brainstem, and especially at the pontine level. However, it is to be kept in mind that these results are not a correct estimate of the LC volumes, therefore, more precise measures in future studies (see Poe et al., 2020) would be necessary to get a more complete picture of the state of the *locus coeruleus*.

Concerning the link between given early deterioration of certain brain regions of interest and the presence of epileptic activities, no differences were found between patients with and without epileptic activities (all comparisons of the above-mentioned regions, Mann-Whitney tests:  $p > 0.1$ , **Figure 81**).

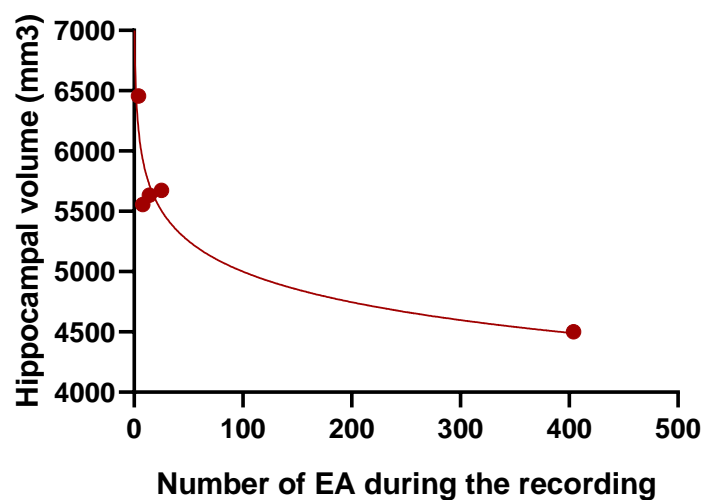


**Figure 81.** Differences on structural MRI results for patients with and without epileptic activities (EA) on their EEG. Differences of right and left hippocampal (A), amygdalar (B), estimated whole brain (C), cortical (D) and putamen volumes (E) between patients without (no EA) and with (EA) epileptic events on their EEGs. (F) Differences in brainstem volumes between controls (blue) and patients (red). No significant comparisons.  $n = 24$  for the No EA group and  $n = 25$  for the EA group.

It is of interest, though, that patients with epileptic events seem to be mainly under the mean values of patients without epileptic activities, especially for putamen volumes, whole brain volumes, and gray-and white matter volumes, except for one participant of the five. Interestingly, this is the participant having EAs diagnosed with an atypical (agnosic) form of

AD, with not only different atrophy and/or biomarker deposition patterns (Vogel et al., 2021), but also potentially distinct mechanisms of EA onset. Once again, the small sample size impedes robust conclusions but may imply the necessity for further inquiries on this matter in future studies.

Finally, correlations between volumetric values and the severity of epileptic events (defined by their number over the night) revealed a tendency towards a negative correlation between the number of EA and total hippocampal volume (even with  $n=5$ , Spearman's  $\rho = -0.9$ ,  $p = 0.08$ ). Indeed, the linear regression reached an  $R^2$  of 0.75 ( $p = 0.058$ ). However, the link turned out to be rather due to a nonlinear connection between the variables, best fitting a semilog line (**Figure 82**), with intermediate EA numbers (14-25) clustered around the same volumes, while the patient with only 4 EAs having less atrophy, and the patients with over 400 EAs detected presenting the lowest hippocampal volumes. This could indicate either more advanced stages of the disease for patients with EAs, or a potential accelerating impact of epileptic events on hippocampal atrophy. Although causality cannot be inferred, given that almost all recorded spikes had been detected at the level of the temporal lobe and only hippocampal values seem to be affected, the latter option seems to be a more viable possibility.



*Figure 82. Nonlinear fit of the number of EAs and total hippocampal volume for AD patients with EA.*

### 3.6. Multifactorial preliminary models

Even though our sample sizes are too feeble to draw strong conclusions from multifactorial analyses as the analysis rapidly loses statistical power, an attempt was made to create models

including sleep quality data, spindle characteristics, volumetric information, SAS-and epilepsy-related variables and memory-consolidation tests. Unfortunately, given the absence of genetic results for controls – which would have too strongly restricted the number of participants included even in these preliminary models, genetic factors were not considered. The first test verified was the Epi-Real, with the Free+Cued recall scores.

When selecting the factors for the model, the variables with the most salient between-group differences were chosen in the first step, that is: Dementia status (*Group*), *Age*, the *number of EAs during the night*, the *sleep efficiency index*, *whole hippocampal volumes* and the *total apnea-hypopnoea index (AHI)*. In fact, while the model was already a satisfying fit ( $R^2 = 0.54$ ,  $p < 0.001$ ) when adding the *Group* factor, all the above-mentioned variables were also significant within the model (See **Table 29**). Adding *hippocampal volume* increased the model fit to  $R^2 = 0.6$ , the *sleep efficiency index* to further 0.62, the *number of EAs* to 0.66, *Age* to 0.7 and finally, the *AHI index* to 0.74. That is, even if the presence of AD explains most of the variability in episodic memory across our cohort, a complex interaction with pathologic breathing during sleep, as well as with sleep quality, brain reserve and epileptic events is potentially present.



**Preliminary Linear Regression of EREMAD results – Epi-Real FR+CR**

**Model Summary – Epi-Real FR+CR TOTAL**

Model	R	R <sup>2</sup>	Adjusted R <sup>2</sup>	RMSE
H <sub>0</sub>	0	0	0	8.002
H <sub>1</sub>	0.861	0.741	0.704	4.352

**ANOVA**

Model		Sum of Squares	df	Mean Square	F	p
H <sub>1</sub>	Regression	2278.073	6	379.679	20.049	< .001
	Residual	795.386	42	18.938		
	Total	3073.459	48			

*Note.* The intercept model is omitted, as no meaningful information can be shown.

**Coefficients**

Model	Variables	Unstandardized	Standard Error	Standardized <sup>a</sup>	t	p
H <sub>0</sub>	(Intercept)	22.704	1.143		19.861	< .001
H <sub>1</sub>	(Intercept)	-14.864	11.837		-1.256	0.216
	Group	-7.488	1.591	0.329	-4.707	< .001
	Whole hippocampal volume	0.002	8.046e-4	-0.197	3.033	0.004
	Number of EAs	-0.194	0.080	0.213	-2.436	0.019
	Sleep efficiency index (TTS/TIB)	0.113	0.046	0.180	2.482	0.017
	Age	0.242	0.121	0.207	1.994	0.053
	Apnoea-Hypopnea Index	0.101	0.040		2.522	0.016

<sup>a</sup> Standardized coefficients can only be computed for continuous predictors.

*Table 29. Preliminary Linear Regression of the current EREMAD results, focusing on Epi-Real Free + Cued recall scores.*

As of pre-and post-sleep scores on the verbal declarative memory test (RL/RI-16, measured by the difference in total recall scores pre-and post-sleep), the same model was only marginally significant, and most of the variables included in the precedent model were not contributing towards it. As an example, the dementia status only explained 0.13 of the total variance. Whole hippocampal volumes still remained highly significant ( $p = 0.005$ , although  $R^2$  only reached 0.16). No other variables from the model used on the Epi-Real scores were significant. However, of all the sleep duration variables tested, REM sleep durations seemed to marginally ameliorate the model ( $R^2 = 0.22$ ). At this point, we were interested in whether premorbid intelligence levels obtained from a test measuring, in a sense, ease with unusual words may explain results on this particular word-learning test, and indeed, the fNART scores

adjusted for dementia diagnoses helped the  $R^2$  reach 0.35 (**Table 30**). Preliminary analyses ran on sleep spindles (not included in the current document) also suggested a contribution of spindle duration during N2 and spindle number during N3 sleep to the model. Given that the manual verification of spindle-related analyses is currently pending, these variables were taken out of the model.

These preliminary results indicate an essential role both of brain reserve and cognitive reserve for verbal declarative memory consolidation, as well as the importance of REM sleep for consolidation processes – be it at the systemic or synaptic level. Note, however, that including the fNART scores meant decreasing the participant number to 41, as not all participants had undergone the last visit when the fNART scores are established.

<b>Preliminary Linear Regression of current EREMAD results – RL/RI-16</b>						
<b>Model Summary -RL/RI-16 Total Recall: Post-sleep - Pre-sleep scores</b>						
<b>Model</b>		<b>R</b>	<b>R<sup>2</sup></b>	<b>Adjusted R<sup>2</sup></b>	<b>RMSE</b>	
H <sub>0</sub>		0	0	0	2.15	
H <sub>1</sub>		0.59	0.35	0.3	1.8	

<b>ANOVA</b>						
<b>Model</b>		<b>Sum of Squares</b>	<b>df</b>	<b>Mean Square</b>	<b>F</b>	<b>p</b>
H <sub>1</sub>	Regression	63.61	3	21.21	6.52	0.001
	Residual	117.161	36	3.25		
	Total	180.78	39			

*Note.* The intercept model is omitted, as no meaningful information can be shown.

<b>Coefficients</b>						
<b>Model</b>	<b>Variables</b>	<b>Unstandardized</b>	<b>Standard Error</b>	<b>Standardized<sup>a</sup></b>	<b>t</b>	<b>p</b>
H <sub>0</sub>	(Intercept)	-1.325	0.340		-3.892	< .001
H <sub>1</sub>	(Intercept)	-7.954	1.687		-4.714	< .001
	Whole hippocampal volume	6.853e-4	2.690e-4	0.349	2.547	0.015
	REM duration	0.040	0.015	0.361	2.649	0.012
	fNART adjusted for dementia	0.827	0.454	0.249	1.823	0.077

<sup>a</sup> Standardized coefficients can only be computed for continuous predictors.

*Table 30. Preliminary Linear Regression of the current EREMAD results, focusing on RL/RI-16 Total Recall performance, and notably on the difference between post-sleep and pre-sleep scores*

Finally, concerning the visual memory test (DMS-48, once again looking at the difference in score between post-and pre-sleep performance), the best fit ( $R^2 = 0.46$ ,  $F = 9.47$ ,  $p < .001$ ,

**Table 31)** was found when including the variables *Hippocampal volume* and *EA number* from the initial model and *Total sleep time* from sleep-related variables. *Sleep efficiency index* also contributed to the model, but interestingly, not the same as in the Epi-Real model (Total sleep time/Time in bed), but the one calculated as “Total Sleep Time/The time between the first N2 or REM stage and the awakening”. Interestingly, separating participants by dementia status did not impact the model’s fit. Even though these results are to be taken with high levels of precaution given the small sample size, they still hint at a complex link between visual memory consolidation, sleep quantity and quality, epileptic activities and brain reserves – both in AD and healthy cognitive ageing.

<b>Preliminary Linear Regression of current EREMAD results - DMS-48</b>						
<b>Model Summary - DMS-48 Post-sleep – Pre-sleep scores</b>						
<b>Model</b>		<b>R</b>	<b>R<sup>2</sup></b>	<b>Adjusted R<sup>2</sup></b>	<b>RMSE</b>	
H <sub>0</sub>		0	0	0	2.49	
H <sub>1</sub>		0.68	0.46	0.414	1.9	
<b>ANOVA</b>						
<b>Model</b>		<b>Sum of Squares</b>	<b>df</b>	<b>Mean Square</b>	<b>F</b>	<b>p</b>
H <sub>1</sub>	Regression	137.122	4	34.281	9.469	< .001
	Residual	159.286	44	3.620		
	Total	296.408	48			
<i>Note.</i> The intercept model is omitted, as no meaningful information can be shown.						
<b>Coefficients</b>						
<b>Model</b>	<b>Variables</b>	<b>Unstandardized</b>	<b>Standard Error</b>	<b>Standardized<sup>a</sup></b>	<b>t</b>	<b>p</b>
H <sub>0</sub>	(Intercept)	-0.306	0.355		-0.862	0.393
H <sub>1</sub>	(Intercept)	-4.164	2.045		-2.036	0.048
	Whole hippocampal volume	9.087e-4	2.775e-4	0.395	3.274	0.002
	Number of EA	0.077	0.035	0.252	2.209	0.032
	Sleep efficiency index 2	-0.136	0.033	-0.691	-4.091	< .001
	Total Sleep Time	0.028	0.006	0.744	4.402	< .001
<sup>a</sup> Standardized coefficients can only be computed for continuous predictors.						

*Table 31. Preliminary Linear Regression of the current EREMAD results, focusing on DMS-48 Post sleep – pre sleep scores.*

## **4. DISCUSSION – Clinical study**

### 4.1 Epileptic activities are slightly more frequent in AD than during healthy ageing and are not predominant during REM sleep

The current study revealed a risk for sleep-related epileptic events that was five times higher for AD patients (20%) than for age-matched controls (4%) – a difference that was only marginally significant given the low sample size. Reaching the full sample size in the upcoming months may tip the balance in favour of more robust results. However, similar prevalences have been found in previous studies (see **Figure 67 A**), especially in those with the strictest inclusion criteria (Lam et al., 2020; Vossel et al., 2016), starting to reach a cross-study convergence – at least in terms of detection with scalp EEGs. Indeed, in the current study, EAs in AD patients were reported as unusual in morphology, presenting low-amplitude aspects and reading at scales rarely used in clinical settings – and not applied to all recordings of the current dataset. Furthermore, concomitant scalp and *Foramen Ovale* (FO) recordings in AD patients have revealed that only about 5% of all EAs detected at the FO level are captured with non-invasive scalp EEG (Lam et al., 2017).

The difficulty of EA detection may also explain part of the heterogeneity in terms of EA frequency: indeed, of the 455 events detected, around 400 were reported in the same patient. Notably, a reading at 5  $\mu$ V (instead of the usual 10) was undertaken for this patient, while this was not forcefully the case for the other patients. Therefore, a second reading at this lower scale of the full dataset may lead to the detection of further EAs – although this will probably not change the heterogeneity of EAs thoroughly. Most plausibly, the heterogeneity points rather towards interactions of underlying factors that may play a role in EA occurrence.

However, the distribution of EAs over the sleep-wake cycle was more coherent even in spite of vastly differing EA numbers across patients and is also comparable to previous studies (**Figure 67 B**): Almost 85% of all EAs were detected during NREM sleep with a predominance during stages N2 and N3, while only ~4% of the EAs occurred during REM sleep, already refuting our initial hypothesis. Indeed, the main objective of the EREMAD study was to evaluate whether EAs are distributed similarly during the sleep-wake cycle as can be seen in

AD mouse models, where EAs are predominant during REM sleep (Brown et al., 2018; Kam et al., 2016; B. Szabo, Cattaud et al., *under revision*).

## 4.2. The impact of epileptic events on AD

### *4.2.1. The impact of epileptic activities on disease progression*

However, where our results diverge from the results of both the above-mentioned study of Vossel and collaborators, as well as others (Haoudy et al., 2022; Horvath et al., 2021; Vöglein et al., 2020), is the steepness of cognitive decline for patients with EA. In fact, previous reports found an accelerated cognitive decline for AD patients with EA burden compared to AD patients without EAs. However, a confounding factor may impose on the interpretation of the results: cognitive reserves diverged in several of the above-cited papers between patients with and without EAs, in favour of the former group. The issue with this difference is that scores of patients with high cognitive reserves are generally higher on tests required for inclusion or for disease-stage determination in many research protocols (e.g. MMSE) even in spite of high levels of amyloid-and pTau burden and more advanced levels of brain atrophy until compensation is possible due to the cognitive reserve. However, passed this compensation threshold, these patients' cognitive decline slope becomes quite steep (**Figure 40**). In our study, we aimed to understand whether EAs really accelerate disease progression or whether it is a result stemming from the bias of high cognitive reserves that better explain the faster progression. Unfortunately, the COVID-19 outbreak caught up with the current project as well. It introduced significant delays in patient inclusions, leading to a decrease in the target sample size and to the fact that currently, many participants have not yet been seen at the follow-up visit (V3), where initial cognitive reserves and the steepness of cognitive decline are estimated, both of which could provide important insight into the real impact of epileptic events on cognition. Therefore, currently, this objective cannot be certainly answered. However, three of the 5 patients with EAs have passed the third visit, and some inferences can be drawn from these data. Indeed, two out of the three patients with EA having passed the follow-up visit did not show any decrease in their MMSE score even in the face of a rather long hiatus between the two evaluations (437 and 325 days for the two patients),

which seems incompatible with the above-mentioned previous results. On the other hand, these participants had the fewest EAs of all AD patients with epileptic events in the study, which once again complexifies the interpretation of the results.

However, the general tendency for lower volumes on the MRI analysis in most areas tested for all but one patient with EAs (and atypical form of AD) is an interesting finding – even if it requires further verifications. In fact, brain atrophy patterns similar to that seen in MCI are known in mTLE patients (Kaestner et al., 2021). Our results could, therefore (and especially if confirmed on the full dataset), indicate either more advanced stages of the disease for patients with EAs (that is not yet reflected in MMSE score), or an aggravating impact of EAs on neuronal integrity and, therefore, on hippocampal volume. However, the tendency toward a negative correlation between total hippocampal volume and the number of EAs during the night suggests a potential aggravating impact on EAs instead of more advanced stages – even though one does not exclude the other, and further investigations are needed to gain a better insight into this point. Altogether, our study cannot confirm nor infirm a direct impact of EAs on brain atrophy levels but points towards the necessity of further investigations.

Indeed, even in spite of the current results, it is still a possibility that EAs contribute to cognitive decline and treating them could improve cognitive functions in patients. At least, this is what the recent study of Vossel et al. (2021) suggests: when treating AD patients with low doses of the ASM Levetiracetam (2 daily doses of 125 mg for 4 weeks), they found improved performances on a test measuring executive functions and on a navigation learning task, but only for patients with EAs, while the treatment did not affect the performances of patients without EAs. These results are also echoed by a previous study where lowering hippocampal excitability levels in aMCI patients using Levetiracetam led to better performances on a visual memory/pattern separation task. (Bakker et al., 2012). Altogether, the various unknown factors determining one's slope of cognitive decline in AD make it complicated to evaluate the accelerating effect of EAs on disease progression, however, recent studies – and our preliminary models discussed below – suggest that they do have such an impact, and their treatment may help delay disease progression or even temporarily improve cognitive functions.

#### *4.2.2. The impact of epileptic events on memory consolidation*

Initially, we hypothesised that if EAs do impact memory, it would be via the disruption or at least the derailment of memory consolidation. Indeed, epileptic events have been hypothesized to replace physiological memory consolidation-related oscillations (Halász et al., 2019), and previous studies have found anticorrelations between sleep spindle densities and EAs both in children with childhood epilepsy with centrotemporal spikes (Kramer et al., 2021) and in a rodent model of epilepsy (Gelinas et al., 2016). Furthermore, EAs coinciding with sleep spindles also show morphological alterations, including higher amplitudes and frequencies as well as longer durations (Sákovics et al., 2022). These results suggest that EAs may indeed replace or alter physiological sleep spindles, which can have detrimental impacts on memory consolidation and cognition – as shown by the findings of Kramer et al. (2021), who found that spindle rate (but not spike rate) is correlated to performances on several cognitive tests. However, when looking at the neuropsychological results of the EREMAD participants, the scores of patients with epileptic events are widely distributed amongst the spectrum seen in the full AD group on all tests – including those requiring memory consolidation and those simply measuring executive or instrumental functions – and do not show any statistical differences. However, the already deficient encoding of information in AD patients (as measured by immediate recalls of declarative and visual memory tests) and the ceiling effect seen in most controls on the same tests make the interpretation of the data on memory consolidation rather challenging. This is further aggravated by the absence of normalized scores for post-sleep tests, which forced us to use uncorrected raw scores or post-sleep versus pre-sleep scores on each test. Obtaining age and education level-adjusted scores for recalls at approximately one day (or simply post-sleep) would be extremely useful for future research on sleep-related memory consolidation. Furthermore, we have already launched the analysis of sleep microarchitecture (including sleep spindles and slow oscillations, as well as their coupling) on our dataset, although the results of the implemented algorithms are currently awaiting manual verification. Therefore, the preliminary results from these analyses were not included in the current document – but could help to interpret the data in the upcoming months better.

### 4.3. The impact of sleep pathologies on cognition and memory consolidation

However, what shone through all our results and observations, was the decreased sleep quality and high SAS prevalence – and that, not only for AD patients, but also for the majority of healthy older adults, which incited us to look at their potential effects on the cognitive measures we were interested in.

#### *4.3.1. The prevail of superficial sleep and sleep apnoeas – at the detriment of memory consolidation*

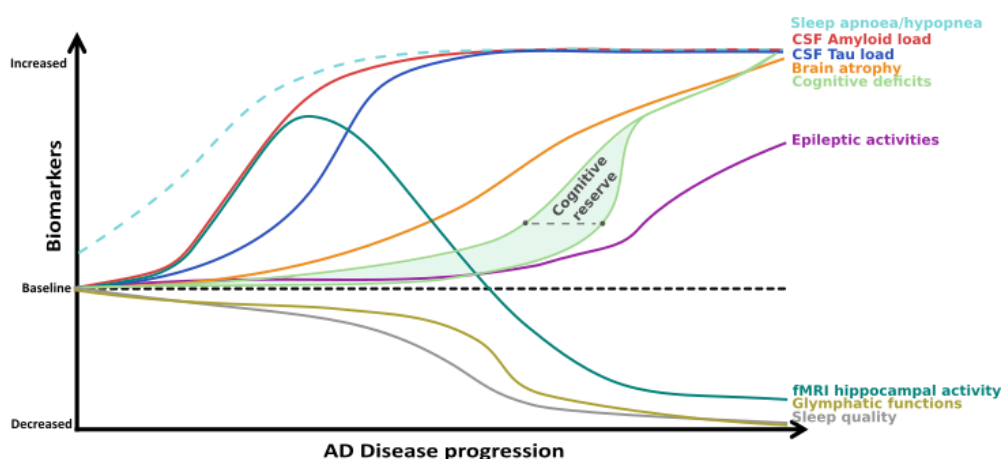
Our investigation revealed that, although most participants had decreased sleep quality compared to what is generally reported in younger adults –, the sleep of AD patients seemed the most disorganized. An increased percentage of superficial (N1) sleep at the detriment of N2 sleep was observed - a modification that was, in fact, reflected in the worse performance of patients on memory tests requiring memory consolidation. Indeed, N2 sleep is a state rich in sleep spindles – potentially accompanied by slow oscillations and sharp-wave ripples essential for systemic consolidation (see Rasch & Born, 2013). Our results suggest that this N2 decrease could negatively impact memory consolidation as we observed strong links between episodic memory and several sleep-related variables – including sleep efficiency, total sleep time and N2 sleep, among others.

Since we observed high percentages of SAS, especially with obstructive sleep apnoeas, both in patients and controls, we suspected that it might be explicative of the observed effect of declining sleep quality on memory consolidation. Finding high levels of SAS is not such a surprising result in itself, given that it is the most common type of apnoeas, with a prevalence estimated at 1 billion people between 30 and 69 years (Lyons et al., 2020) around the globe, with only 2% estimated to get a proper diagnosis and treatment. Furthermore, sleep apnoea severity increases linearly with age (Gabbay & Lavie, 2012), which implies that moderate-to-severe levels of SAS would be overrepresented in our cohort with participants ranging from 54 to 84 years of age. While the impact of main sleep apnoea-related indices by itself was not a successful predictor of memory consolidation efficiency in our study, SAS and EAs have been



shown to contribute to arousals from sleep (Malhotra et al., 2018; Peter-Derex et al., 2020) and decreased sleep quantity, therefore, indirectly playing a role in the results. Indeed, we did find a correlation between both the number and the index of micro-arousals linked to respiratory events and the episodic memory test scores, however, they were associated with better scores on the Epi-Real test. However, a closer look at the data helped show that this correlation is largely mediated by the initial differences in cognitive status (presence of AD or not) and the sleep-quality differences between the group of patients and controls – including slightly elevated hypopnea indices, potentially leading to more arousals for patients than for controls. However, it is possible that the declining sleep quality – due to apnoeas, for example – contributes to faster cognitive decline in AD patients and could be even an early event driving the pathophysiological hallmark depositions of AD.

Indeed, sleep apnoeas were shown to inflict damage on cholinergic signalling, known to be an early element of AD pathophysiology as well (Shimada, 1981). Moreover, as was mentioned in the **Introduction**, positive associations between A $\beta$  accumulation and SAS have been shown even in pre-MCI participants with only subjective cognitive impairment but cognitive performances still in the normal range (Liguori et al., 2017). Furthermore, the correlation between A $\beta$  load and SAS was primarily observed in the early stages, which would imply that SAS may play a crucial, potentially causal role in A $\beta$  accumulation – but would not show marked impacts on cognition once the diagnosis have been posed – which often occurs when A $\beta$  levels have already reached a plateau (**Figure 83**).



**Figure 83. Proposed model of biomarker- and cognitive decline progression over the course of AD in relationship to epileptic activities and sleep pathologies.** Amyloid and Tau aggregation may be driven by pathological breathing patterns disrupting glymphatic function during sleep early on. On the other end, high cognitive reserves compensate on a functional level for atrophy which may lead to delayed diagnosis, explaining why EA is most often detected in highly educated participants, seemingly in the mild stages of the disease. However, once compensation is no longer possible, and EA becomes more frequent, the cognitive decline becomes rapid, compatible with the fast progression noted in AD patients with E/EA. Grey dashed line represents the approximate time of diagnosis. Adapted from B. Szabo et al. (2022)

Unfortunately, pre-test lumbar punctures were not added to the protocol for either group to account for this possibility. Even if it remains hypothetical for our sample if such a relationship does exist between apneas and A $\beta$  accumulation, one of the mechanisms through which this may be achieved could be the disruption of clearance mechanisms active during sleep, such as the glymphatic system.

#### *4.3.2. Dysfunctional glymphatic system – the link between AD, epilepsy and declining sleep quality?*

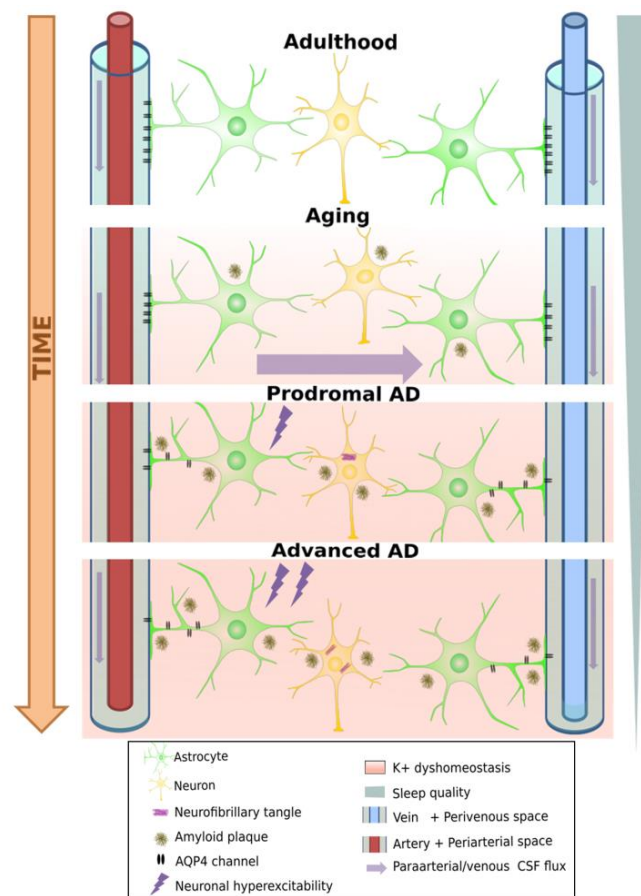
Indeed, the pathophysiological processes behind AD may further be complicated and risk entering a vicious cycle between sleep, AD and, as we will see, epilepsy, by the brain's incapacity to clear excess A $\beta$  in case of a disrupted sleep-wake cycle – which could happen in the case of sleep apnoeas. In fact, A $\beta$  load, as measured in CSF samples, fluctuates diurnally and increases during wakefulness (Kang et al., 2009) and even after a single night of sleep deprivation (Shokri-Kojori et al., 2018). In response, plaque accumulation was shown to aggravate sleep fragmentation in a drosophila AD model, while enhancing sleep duration led to decreased deposits (Tabuchi et al., 2015). Interestingly, the same authors described a net excitatory effect of sleep loss and A $\beta$  deposition leading to neuronal hyperexcitability that was responsive to ASM treatment (Levetiracetam, LEV) and that coincidentally prolonged the animals' lifespan as well. The disruptive effect of A $\beta$  on sleep integrity and related memory consolidation has already been described (Mander et al., 2015), and in light of previous evidence on the subject, a bidirectional link (or rather a vicious cycle) between A $\beta$  deposition and sleep fragmentation was proposed by Ju et al. (2014). This link is such that low sleep efficiency turned out to be a good predictor of A $\beta$  deposition rates over several years even for rather long durations (mean follow-up of 3.7 years +/- 2.4) in healthy elderly adults (Winer et al., 2020). Another sleep-related marker, the decrease in Non-REM (NREM) slow-wave activity, was linked to A $\beta$  deposition (Mander et al., 2015), and a similar or even stronger relationship was observed for tau deposits (Lucey et al., 2019). More importantly, tau also seems to follow a sleep-wake cycle-dependent accumulation similar to A $\beta$  (Holth et al., 2019).

This daily fluctuation of tau and A $\beta$  is strongly linked to one of the crucial mechanisms by which the brain can eliminate excess or potentially toxic metabolites, including soluble A $\beta$  and Tau: the glymphatic system. This relatively newly discovered clearance pathway (Iliff et al.,

2012; Xie et al., 2013) is a highly organized fluid transport mechanism that accommodates an influx of subarachnoid CSF into the brain interstitium via intracerebral arterial perivascular spaces. Due to anterograde flow towards the venous equivalent of perivascular and perineuronal spaces that ends in the meningeal lymphatic drainage system, this mechanism can eliminate unwanted metabolites (**Figure 84**, see Rasmussen et al. (2018)). As our understanding of this system grows, it is becoming clear that dysfunctions in this pathway can cause rapid deposition of both A $\beta$  and tau (Boespflug & Iliff, 2018). Correct functioning of this pathway is highly dependent on water transport through aquaporin-4 (AQP4) water channels at astrocyte endfeet that line both the arterial and venous end of the perivascular spaces. These channels ensure low-resistance flow between perivascular spaces and brain interstitium (Iliff et al., 2012), and several pathways by which these channels become dysfunctional in AD were recently uncovered (Silva et al., 2021).

First, these channels show an age-dependent decline even in wild-type mice (Kress et al., 2014), leading to a decrease by 40% of intraparenchymally injected A $\beta$ -clearance efficiency. This is consistent with the findings that approximately 40-80% of all proteins and soluble metabolites are cleared via this mechanism (Iliff et al., 2012). Importantly, it would appear that the glymphatic system is 90% more active during sleep than during waking hours (Xie et al., 2013). Given the considerable disruption of sleep and decreased total sleep time from the earliest stages of AD, it is plausible that the decrease in glymphatic clearance may further aggravate A $\beta$  and tau accumulation (Christensen et al., 2021). Moreover, fragmented sleep might do more harm than just decrease the “ON-time” of glymphatic clearance: Using two-photon in vivo imaging in non-transgenic mice, Liu et al. (2017) showed that sleep deprivation leads to a mislocalization of AQP-4 channels that might render glymphatic clearance impaired. It is fascinating to note that in a transgenic model of AD (Tg-ArcSwe), Yang et al. (2011) demonstrated an A $\beta$  deposition-dependent mislocalization of AQP-4 channels on perivascular astrocytes even before the discovery of the glymphatic system. Importantly, Yang and collaborators also drew attention to an earlier paper by Eid and colleagues (2005), who found a dysfunction in perivascular AQP-4 channels in resected hippocampal sections from epileptic patients undergoing surgery. At that time, having no information about the existence of the glymphatic system, they hypothesized that such impairments may not only disturb water homeostasis but could also reduce K<sup>+</sup> clearance, a function in which these channels were

already known to be implicated. This K<sup>+</sup> clearance deficit is yet another pathway that further spirals the hyperexcitable phenotype of AD mouse models (**Figure 84**).



**Figure 84. Degeneration of the glymphatic clearance system during healthy ageing and Alzheimer's disease.** White band represents time passing throughout ageing, turning into neural pathology, including loss of AQP4 channels and their eventual loss of polarity on astrocyte endfeet. This leads to a loss of K<sup>+</sup> homeostasis, which can already induce neuronal hyperexcitability, increasing A- $\beta$  production, further aggravating the process. On the other hand, progressive sleep loss also decreases glymphatic function, amplifying all the other pathological processes mentioned. From B. Szabo et al. (2022)

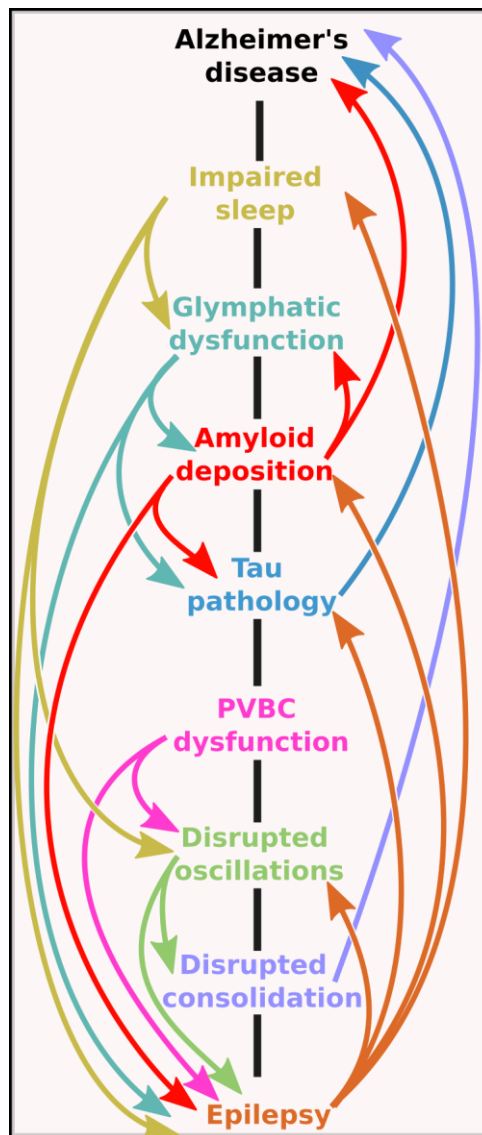
Finally, a reactive state can also induce loss of polarity in astrocytes, as has been shown to occur near amyloid- $\beta$  plaques in a transgenic FAD model (APP/PS1) (Peng et al., 2016). Deleting AQP-4 channels from the same model led to aggravated A $\beta$  deposition in both the cortex and the hippocampus and increased spatial memory deficits (Xu et al., 2015). In another “cross-transgenic” FAD model devoid of AQP-4 channels (5xFAD/AQP4 KO mice), an increase of approximately 6-fold in EA frequency was observed compared to 5xFAD mice at ten months of age (Abe et al., 2020). Many questions remain about the glymphatic system and its relationship with AD (Peng et al., 2016; Christensen et al., 2021). Nevertheless, the possibility that yet another vicious cycle that amplifies amyloid deposition and epileptic susceptibility might exist seems plausible. In fact, age-related functional impairment of the glymphatic

system may lead to increased plaque deposition. Plaque or A $\beta$ <sub>1-42</sub> oligomers could, in turn, create deposits in the vicinity of perivascular astrocytes, leading to a reactive glial state or the mislocalization of AQP-4 channels on astrocytes, further diminishing the process of glymphatic clearance (Yang et al., 2011; Peng et al., 2016). This could lead not only to further acceleration of plaque deposition but also to a loss of K<sup>+</sup> homeostasis, increasing the risk of epileptic activities, which, once again, leads to an increase in A $\beta$  (and tau) load (**Figure 84**).

Moreover, the respiratory cycle also modulates glymphatic clearance. Inspiration is notably a strong driving force for the process in humans by its modulation of hydrostatic pressure, which facilitates CSF flow towards the interstitial spaces of the brain (Dreha-Kulaczewski et al., 2015). Importantly, breath-holding suppresses this flow and potentially decreases clearance, a scenario that may happen quite often in patients with sleep apnoea.

Therefore, even if sleep apnoeas may not be an assault serious enough to have a direct effect on memory consolidation or AD onset – as was suggested by Branger and collaborators (2016) –, small, additional impacts, such as the addition of sleep apnoeas and inflammation leading to a reactive state in astrocytes, may lead to more severe disruption of the glymphatic system that could be sufficient for an A $\beta$  accumulation important enough to drive AD onset over several years, adding additional layers to the pre-existing vicious cycles discussed throughout this manuscript (**Figure 84, 85**).

Drawing conclusions from this possibility for our study, future protocols should include biomarker measures pre-and post-sleep both in controls and patients. With less invasive methods such as plasma biomarker measures becoming more and more available and sophisticated (Hansson et al., 2022), these measures will probably be more and more frequently done and will provide more detailed answers to these questions. Furthermore, even if not during a full night's sleep, measures of glymphatic clearance in humans are now available (Lee et al., 2021; Myllylä et al., 2018; R. Watts et al., 2019), therefore, future studies could also add measures pertaining to clearance efficiency, even if the validation of these methods will probably require several years.



*Figure 85. The bidirectional road between Alzheimer's disease and epilepsy.* Note that the mechanistic pathways contain several vicious cycles, meaning that no matter at which level the initial imbalance is on the road, the self-amplifying potential of the system could lead to the onset of one (or possibly both) pathologies. From B. Szabo et al. (2022).

#### 4.4. Strong points and Limitations

Advantages of the study include the precise following of sleep-related variables due to the quasi-elimination of sleep-modifying agents, such as high levels of benzodiazepines and antidepressants, whose impact on sleep quality and quantity (both on macro- and microarchitecture) is well-known (A. Watts et al., 2012; Wichniak et al., 2017).

Yet another advantage is the strict inclusion criteria regarding AD diagnosis: it had to depend not only on expert neuropsychological evaluation and brain imaging results supporting the diagnosis but also on biomarkers from lumbar puncture, avoiding misdiagnosis – for example

with epileptic patients who can show similar neuropsychological profiles and hippocampal atrophy as aMCI patients (Kaestner et al., 2021).

Another strong aspect is the strict scoring of epileptic activities, determined by two independent experts – and occasionally taking into account the opinion of a third expert on doubtful cases. In the current study, only events marked as certain by at least one of the experts were included in the analyses, decreasing the possibility of over-estimation of the occurrence of epileptic events. The detection of EAs was further supported by the use of lower temporal electrodes (P9, P10, T9, T10, F9, F10) that are not always added in PSG explorations. We suggest the use of these electrodes in sleep-and epilepsy explorations both in clinical settings and in research protocols, as the current study also indicates a positive impact on the precision of EA detection.

However, by aiming at preserving sleep structure as natural as possible by excluding the above-mentioned medications, we may have introduced other biases, especially when it comes to the representativeness of the participants. Indeed, by excluding participants on antidepressants and higher doses of benzodiazepines, which are used in more than half of AD patients, the sample may not be deemed representative of the AD population. To that end, larger, potentially multi-centric studies with the possibility of including another group of AD patients on antidepressants could clarify the actual impact of these drugs on the frequency of epileptic activities.

A further point to consider is the representativeness of sleep quality recorded: it is not only the problem of an artificial environment of the hospital setting, but that only one night of polysomnography was recorded, which could bias the results especially for the patients' group who may be more agitated or anxious and have more difficulty falling asleep in the new environment, therefore modifying sleep-related variables in the outcome. Further studies with portable devices that can be used over several nights in the home environment of the patients may improve on this aspect and could give more robust results. Such measures may also be helpful in a more widespread apnoea-and sleep-quality screening in the general population, especially among middle-aged and older adults where it could have important impacts on prevention. Indeed, our results suggest that EAs, sleep apnoeas and decreasing sleep qualities may all play a role in decreased memory functions – especially in the case of longer-term, consolidation-dependent memory.

Another limitation is the capacity of scalp EEGs to detect EAs: indeed, as has been shown by Lam et al. (2017) as much as 95% of all EA may remain undetected with scalp EEGs, compared to intracranial EEGs. The challenge of detecting epileptic events in AD patients and the high risk of omitting their presence is further underlined by the subjective observations of medical experts on unusually low-amplitude epileptic events, in spite of the generally good-quality signal. On occasion, epileptic events could only be detected at 5  $\mu$ V scales, which is below the usually used scales in most clinical settings. Furthermore, a large number of events were marked doubtful by the experts and were discarded (both for participants with eventually retained EAs and for participants for whom no definitively characteristic EAs could be discerned). This peculiar epileptic activity and the difficulties of detection could be due to the general slowing of the EEG for patients (Montplaisir et al., 1998), making EEG reading even more challenging – and indicating a potential underestimation of EA prevalence, especially in the patients' group. A potential option for increasing confidence levels in EA detection would be to analyze EEG events that are generally characterized as benign, such as wicket spikes or temporal intermittent rhythmic delta activity (TIRDA) and small sharp spikes (SSS), the latter two of which have been proposed as a marker for AD-related mTL hyperexcitability (Lam et al., 2020).

A final limitation is the preliminary nature of the data presented, which comes without the correct corrections for multiple comparisons and gives the data a rather descriptive value that will be amended once the full cohort will be included in the study.

#### 4.5. Data analysis perspectives

Several perspectives for future study designs have been evoked in the past section. However, it is not to forget that the currently presented EREMAD study is not finished, and the results reported here remain preliminary. Indeed, the upcoming months will see the addition of many critical analyses to the final work.

- First of all, the inclusion phase is set to finish by the end of October 2022, allowing to expand the results and obtain correct statistical power for the analyses that have been presented merely as preliminary and often nonparametric inter-group comparisons. Hopefully, the full



dataset will allow for more precise and better fits on complex analyses, including our linear regression models presented at the very end of the **Results** section.

- Secondly, the manual verification of the automated sleep microarchitecture analysis both in rodents and in the human data will be carried out, which could give us a better insight into sleep microarchitecture changes related to AD – and AD-related epileptic activities.

- A third major step will be the completion of the third visit by all participants so that our hypothesis concerning the biasing effect of cognitive reserves (and the discrepancy between behavioural and brain-atrophy-defined AD stage it induces) on the findings about the disease-accelerating role of epileptic events in AD can be tested better.

- Lastly, wherein only structural MRI data are presented in the current report, adding the results of the DTI and rs-fMRI sequences will represent an important addition to our models and results. More precisely, the functional MRI results may give an interesting insight into hippocampal hyperexcitability in AD patients, as increased hyperexcitability has already been reported in MCI patients before (Bakker et al., 2012). Looking at the correspondence between the resting-state hippocampal activity levels and the presence of epileptic activities on the EEG, amongst others, is planned. Concerning the DTI data, together with the fMRI data, it could also be a valuable addition as it may allow looking into potential aberrant network reorganisation amongst the AD-EA group as has been already done in other cases of epilepsy (Balter et al., 2019). Finally, a last addition to the structural MRI analysis is planned, notably the analysis of the precuneus, which has been reported to bear decreased volumes in AD patients with epileptic activities (Horvath et al., 2019).

# **GENERAL DISCUSSION**

## 1. The role of noradrenergic transmission in AD-related epileptic activities

When trying to braid the clinical and preclinical results presented above, one of the crucial coherences that shines through the dissimilarities is the high inter-individual variability in the frequency of epileptic activities during sleep. Indeed, in the Tg2576 mouse model, the frequency during the most EA-prone state (REM-sleep) varied from 0 to 12.65 spikes per minute (with a mean of 1.98 and a standard deviation of 2.6), while in patients, the number of epileptic events during the night varied from 4 to 404 across the 5 patients with epileptic events, in spite of the absence of high levels of variance in sleep quantity *per se* in those participants. It is also of note that most patients (80%) did not show epileptic activities on their EEG, and none of the patients seen in the last visit and having filled out the Epilepsy Questionnaire (**Figure 35**) gave answers that could have suggested the presence of an underlying epileptic syndrome that might not have surfaced during a single night of sleep recording. This variability is intriguing and once again confirms the multifactorial origin of epileptic activities in AD, with several potential aggressions to the fine balance of the central nervous system that tips the balance toward hyperexcitability. These factors could include soluble A $\beta$  load or plaque load and could be further complicated by the hypoexcitability-prone impact of Tau hyperphosphorylation in specific brain areas. To this end, our results on noradrenergic transmission seem of interest, given that it is the first place where NFTs appear in the brain (Braak et al., 2011).

Indeed, one of the major finds of our preclinical study was the impact of noradrenergic signalling on epileptic activities. Our results indicate that noradrenaline can impede the onset of epileptic events in the Tg2576 mouse models during wakefulness and partially during slow-wave sleep, when noradrenaline concentrations start to decrease. Finally, with the offset of noradrenergic signalling at the beginning of REM-sleep episodes, the shield provided by noradrenaline against the excitatory-inhibitory imbalance is shattered, and epileptic events can appear with astounding frequency in some individuals (sometimes 8-12 spikes per minute). Unfortunately, given the parallel design of the two studies, sequences allowing for the precise measuring of locus coeruleus volume by new imagery methods (see Poe et al., 2020) were not included in the MRI sequences of the EREMAD project. Therefore, the current

results are limited in the extent to which we can explore the role of noradrenergic transmission in our clinical study. However, an estimate of the whole brainstem volume could be computed, which indicated no difference between patients and controls in general ( $p = 0.78$ ), nor between patients with and without epileptic events ( $p = 1$ ). Unfortunately, these preliminary results are to be taken with a grain of salt as whole brainstem volumes do not depend only on the LC volume itself. Future studies using MRI sequences with more precise LC estimates, together with vEEG-PSGs could give better insights into the role of noradrenergic transmission in AD-related epileptic activities. This would be all the more of interest since, as mentioned earlier, the antiepileptic effect of NA has been suggested by previous publications in rodents (Raedt et al., 2011; Szot et al., 1999; see Weinschenker & Szot, 2002 for a review). More importantly, our results indicate that this effect is specific to  $\alpha 1$ -ARs, which were shown to be decreased in epileptic rats (Nicoletti et al., 1986) and in the epileptic foci of epilepsy patients (Brière et al., 1986). Therefore, differential levels of atrophy to the LC could explain part of the phenotypic variability as per to epileptic activities in patients – and, potentially in mouse models as well, a question that future histological analysis on rodent brain slices could answer (following EEG recordings to get an estimate of each animal's baseline EA levels).

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 6.5-8 months of age (Guérin et al., 2009), indicating that in the Tg2576 mice included in the pharmacology experiment (between 2.5-6 months of age), this structure was mostly 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 (**Figure 47**). 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. 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 that might show inter-individual differences. 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 at later ages (as of 8-10 months).

Interestingly, Fitzgerald (2021) posited that early harmful events at the level of the *locus coeruleus* (LC) (e.g. inflammation or oxidative stress) could lead to abnormal hyperactivity of LC neurons and, therefore, to increased noradrenalin levels in the earliest (prodromal) stages of 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). These results are further echoed by a clinical study revealing higher  $A\beta$  and pTau/Tau ratios in AD patients with higher levels of plasma noradrenaline (Pillet et al., 2020) or CSF noradrenaline (Riphagen et al., 2021), especially in its soluble form (Busche et al., 2019).

The negative impact of noradrenaline on AD biomarker generation, however, could be counteracted by (I) the  $A\beta$ -induced internalization and degradation of  $\beta$ -ARs (Wang et al., 2011; cited in Fitzgerald, 2021) and due to the well-known relatively early degradation of the *locus coeruleus* in AD (Braak et al., 2011). However, in the same review Of Fitzgerald (2021), it is also postulated that the early increase of noradrenaline concentrations would activate  $\alpha 2$ -ARs as well, which has also been shown to promote  $A\beta$  generation (Chen et al., 2014; in Fitzgerald, 2021). Therefore, it might be the case that early hyperactivity of the LC could cause increased noradrenaline levels, leading to an increase in the activation of the pathogenic cleavage process of APP, favouring amyloid-beta deposition. This  $A\beta$ , in return, would increase network hyperexcitability by mechanisms due to the vicious cycle between neuronal activity and  $A\beta$  generation in AD (see B. Szabo et al., 2022 for a review), which seems to be a pathologically distorted version of the self-regulating feedback loop that is present during physiological conditions (Kamenetz et al., 2003). Moreover, the increasing  $A\beta$  would, by hijacking  $\alpha 2$ -ARs to activate the GSK3 $\beta$ -dependent pathway that leads to tau hyperphosphorylation, also contribute to the deposition of neurofibrillary tangles (Zhang et al., 2020).

This effect on tau hyperphosphorylation is all the more interesting as tau phosphorylation near the microtubule domain was suggested to be a counter-measure to relieve neuronal hyperexcitability, as it renders the tau-Fyn-PSD95 interaction impossible (Ittner et al., 2010), which leads to the endocytosis of NMDA receptors, therefore decreasing the excitotoxic effect of A $\beta$  as a short-term emergency measure, but inducing network hypoactivity on the long term, which also had been tied to AD progression (Mondragón-Rodríguez et al., 2020). Interestingly, this induced Tau-hyperphosphorylation is present in epilepsy as well, potentially also as a counter-balancing measure, given that neuronal activity-related acceleration of tau pathology has been shown (Tai et al., 2016; Wu et al., 2016). However, in AD, if this compensatory hyperphosphorylation takes place in the *locus coeruleus* in response to the above-hypothesized hyperactivity, it would lead to the hypoactivity of noradrenergic neurons – which, in this case, could unmask other sources of neuronal hyperexcitability that may be repressed by noradrenaline – such as dysfunctional interneurons.

It is also plausible, given the antiepileptic impact of NA in Tg2576 mice where LC degeneration is relatively late and Tau pathology is not present, that the early decrease of noradrenergic tone due to LC degeneration/hypoactivity in AD patients (Braak et al., 2011) may explain why they exhibit epileptic events also during wakefulness and slow-wave sleep (Horváth et al., 2018; Lam et al., 2020; Vossel et al., 2016). In conclusion, the suggested antiepileptic effect of noradrenaline 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.

Furthermore, it is of note that many currently used antidepressants act on noradrenaline concentrations, including monoamine oxidase inhibitors, serotonin-noradrenaline reuptake inhibitors (SNRIs) or noradrenaline-dopamine reuptake inhibitors. Of these, notably, SNRIs have been reported to possess anticonvulsant effects (see Kanner, 2016). All of these medications have, as an effect, an increase in noradrenaline concentration as they impede its recapture in most cases. As noted earlier, one of the almost unsurmountable obstacles of the current study (except the COVID-19 pandemic) was an unexpectedly high percentage of screened AD patients taking antidepressant treatment, which was a non-inclusion criterion for EREMAD. We cannot exclude the possibility that in studies where a lower percentage of AD patients with epileptic activities were found, antidepressants may have biased the results.

However, Vessel and collaborators (2016) did not find a significant difference in the proportion of patients with and without EAs on antidepressant treatment (57.9% for patients without EA and 50% for patients with EA). On the other hand, the type of antidepressants taken by those patients remains unknown, and given that not all antidepressants act on noradrenergic transmission (e.g. selective serotonin reuptake inhibitors/SSRIs), these medications may have still impacted the results.

It is all the more interesting since chronic treatment with the SSRI fluoxetine leads to seizure activity in the APP<sub>swe</sub>/PS1dE9 mouse model of AD (Sierksma et al., 2016). This suggests that serotonin may also be implicated in neuronal hyperactivity in AD and could potentially have a pro-epileptic effect as its presence seems to aggravate the already present epileptic phenotype (Gurevicius et al., 2013; Gureviciene et al., 2019). Future preclinical studies in AD mouse models modulating both serotonergic and noradrenergic transmission could help disentangle the role of monoamines in AD-related epilepsy.

However, over and above the coherences across the two studies, starting with the inter-individual variability, many discrepancies were uncovered between the clinical and the preclinical study. Starting with the prevalence of EAs in the two cases (virtually 100% for Tg2576 mice but only 20% for patients), to their distribution over the sleep-wake cycle (REM sleep-predominant in Tg2576, NREM 2 and 3 predominant in patients). This implies, once again, that although the basic pathophysiological processes underlying general network hyperexcitability may be shared in the two cases, factors not reproduced in the mouse models are at play in patients that may make this network hyperexcitability less prominent – such as the above-cited hypoexcitability-enforcing effect of tau.

## *2. Preclinical perspectives - Bringing animal models closer to the disease*

Indeed, one of the major issues with currently used mouse models is the inherent problem that most of them reproduce but the amyloid-end of the pathology, without the co-occurring tau pathology. We have seen, however, that while amyloid renders networks highly hyperexcitable, tauopathy lays at the opposite end of the continuum and leads to general

network hypoexcitability – which is even dominant over the impact of amyloid (Busche et al., 2019). Therefore, it is not surprising to see such high levels of epilepsy are observed in mouse models of AD, and that they reflect the high seizure occurrence also seen in familial forms of AD where amyloid seeding happens at accelerated rates (Cabrejo et al., 2006; Furuya et al., 2003; Larner, 2011; Shrimpton et al., 2007; Snider et al., 2005; Velez-Pardo et al., 2004; Zarea et al., 2016). To avoid this option, using genetic models such as the one presented in the work of Busche and collaborators (2019) is a viable option, however, still carries the inherent problem of genetic mutations and potential uncontrolled lifelong compensation mechanisms. One more alluring pathway is the development of non-genetic animal models that have the potential to reproduce both sides of the pathology and be closer in phenotype to the sporadic AD cases. Such could be the case of species where pathologies closely mimicking AD occur naturally in a subset of animals, like the mouse lemur, or the *Microcebus murinus*, a small Lemurian primate, with as much as 20% of animals in a given sample described as developing an AD-like pathology with amyloid plaques, tauopathy and loss of cholinergic neurons (Bons et al., 2006). Furthermore, to avoid the use of more-than-necessary numbers of animals in research and using five times more animals than what will eventually develop the disease, a method in the same species has recently been described, based on inoculation with AD-burdened brain homogenates, leading to gradual cognitive decline and altered neuronal activity, as well as brain atrophy and, in many cases, depositions of the hallmark biomarkers of AD in the same model (Gary et al., 2019). Testing the presence of neuronal hyperactivity in these models could be of interest in the future, as well as the development of similar, more ecologically valid models for AD research.

### 3. Clinical perspectives – and the importance of early screening

However, methodological issues not only burden the preclinical end: on the clinical side, our results also indicate an underestimation of EA-prevalence in Alzheimer's disease, as well as a substantial underestimation of sleep quality decline and SAS amongst the general elderly population – and call for the development of home-based, self-applied methods allowing a more widespread screening of these pathologies. Such screening and concomitant treatments



could have an important impact on AD prevention and future patient care but could also be stimulating for research.

### *3.1 The importance of early screening of sleep apnoeas*

As was mentioned earlier, sleep-related pathologies are widespread across the whole population and can foreshadow the onset of neurodegenerative disorders such as AD. Even during the healthy ageing process, general sleep quality decreases as sleep becomes more fragmented and superficial, with less and less memory consolidation-related oscillations (Wauquier, 1993). These declines, however, are frequently burdened by other pathologies, such as sleep apnoeas, which are the most frequent in the elderly population (Lyons et al., 2020). Even if OSA alone is estimated at a global prevalence of one billion in the age range of 30 to 69 years alone, 98% may remain undiagnosed for the condition. This unbeknownst aggression on the sleeping brain could, however, increase their risk of developing MCI and, subsequently, AD and could also bring down the age of dementia onset (Mander et al., 2016; Osorio et al., 2015). On the other hand, diagnosing SAS and treating it with CPAP could delay dementia onset by as much as two years (Osorio et al., 2015), potentially by avoiding hypoxia-related neuronal death, as CPAP was shown to positively act on grey matter volume after as little as three months of use (Canessa et al., 2011). However, sleep examinations are complicated to carry out among the general population with a screening purpose, as they require scarcely available equipment and special training for the nurses handling it either in hospital settings or during mobile, home-based screenings. Hence, waiting lists are long for those who visit their doctors complaining about apnoea symptoms; adding population-level screenings (even if only amongst the elderly) would not be a sustainable solution for the healthcare system. The issue of long waiting lists may be resolved by the improvement of current home-based, self-applied PSG systems (Ayappa et al., 2008; Bruyneel & Ninane, 2014), however, the high cost of these systems and difficulties with handling new technologies especially amongst the elderly may represent further obstacles. However, over and above the waiting lists, the situation is also burdened by the time-consuming analysis of vEEG-polysomnography examinations, which are more often than not carried out manually and can take up to 3-4 hours per recording. This issue of tedious analyses may be avoided in the future with the help of machine learning. Deep learning algorithms, often trained on tens of

thousands of recordings, are approaching scoring quality at the level of somnology experts (Fiorillo et al., 2019; Guillot et al., 2020; Vallat & Walker, 2021; L. Zhang et al., 2019). If such systems are wrapped in user-friendly graphical user interfaces, they may become better accepted and more widely used by clinicians. Working towards solutions to allow for more widespread apnoea screening would be of absolute importance, given the early links between SAS and A $\beta$  deposition even in pre-MCI participants (Liguori et al., 2017). Even though our results do not show direct, large-scale impacts of sleep apnoeas on memory consolidation, sleep apnoeas are significant contributors to post-sleep performances in our preliminary models, implying that the combined impact of age-related sleep-pathology burden (SAS included) does impede consolidation processes and, therefore, could aggravate age-related cognitive decline.

### *3.2. The importance of early screening of epileptic events*

A similar issue is present in the detection of epileptic activities – both for older adults with and without AD. Even if, in our cohort, the number of participants with epileptic activities was low, and the number of epileptic events across participants highly variable, many previous studies with larger sample sizes have found detrimental impacts of even subclinical epileptic activities (e.g. Aarts et al., 1984; Croona et al., 1999; Samarasekera et al., 2015). Even if clear-cut epilepsy cases with easily detectable seizures do not suffer from diagnostic issues, detecting more subtle forms of epilepsy does. Such an example is the case of epileptic amnesic syndrome (EAS), with quasi-silent seizures (absence seizures) that are often detected not because of the seizures but because of the memory complaints they cause to patients who seek medical help (Cretin et al., 2017; Gallassi, 2006). EAS, on the other hand, responds well to ASM treatment, therefore, the main issue remains its detection. A similar example is seen with AD patients, who may also undergo focal seizures with temporary loss of contact (i.e. absence seizures) that even the caregivers can interpret as a consequence of AD rather than a seizure (Horváth et al., 2018; Vossel et al., 2013). Furthermore, even if a few EAs may not break havoc on the brain in normal conditions, the brain of older adults faces many other challenges, including inflammation, neurological comorbidities, and medications acting upon the central nervous system and decreasing brain volumes. Therefore, the supplementary

assault by epileptic events may not be as well tolerated as it may be without these complications (Breuer et al., 2016). This is what seems to shine through from the preliminary models of the EREMAD data, where the frequency of epileptic events did seem to impact post-sleep performances on various memory tests. Unfortunately, given the unsuccessful implementation of the social memory test in our mouse model, our preclinical data cannot confirm this claim for the moment.

However, AD and epilepsy have been recently shown to share pathophysiologic mechanisms, including significant amyloid deposition (Costa et al., 2016; Mackenzie & Miller, 1994; Nardi Cesarini et al., 2020) and high levels of pTau burden (Tai et al., 2016). Therefore, a more general screening of epileptic events amongst the elderly, especially in the case of cognitive complaints, should be undertaken. Yet again, standard EEGs of 20-30 minutes with regular electrode layouts may not be the ideal setup. Indeed, EAs are far more frequently captured in EEGs involving at least an hour of sleep in AD patients (Horváth et al., 2017), and potentially in all types of epilepsy as such events are more frequent during sleep (Ng & Pavlova, 2013). This is also confirmed in our study, where almost 90% of all EAs detected occurred during sleep in patients, a percentage that was even higher for our mouse model. Furthermore, while in mice, EAs are easily detected with the help of intracranial electrodes, such invasive methods are barely used in clinical settings. Therefore, adding lower temporal electrodes to the setup whenever possible is of utmost importance, as they bear the highest probability of capturing epileptic events from deeper temporal structures, such as the hippocampus. However, even if home-based, self-applied methods are becoming more and more available and could once again solve the difficulty of bed-or personal shortage to a certain point at least, these contain mostly frontal electrodes or electrode setups avoiding lower temporal areas – an issue that should be addressed in the future. On the analysis-end, most spike-sorting algorithms are optimized for intracranial EEG, however, some for scalp EEG have recently been developed as well (Bagheri et al., 2019). Once again, using these algorithms with user-friendly interfaces has the potential to become a widely used clinical tool and save several hours a day for clinicians. Furthermore, these methods allow for less subjectivity and, potentially, more robust results. In fact, even in the case of the EREMAD study, we noted that while inter-rater coherence was high when evaluating the absence/presence of EAs per recording, but it was low when looking at the intra-recording EA detection rates (i.e. experts declared relatively unanimously if EAs

were present in a given recording, but often they did not annotate the same events in that recording). While alternatives exist, such as in the article of Lam and collaborators (2020), where a panel of several epileptologists verified the initial scoring of the two independent experts, this is a rather time-consuming practice that increases, rather than decreases, the time necessary for the analysis. Therefore, it is highly possible that automated methods will be the solution, at least to the data-analysis issues in the future. However, the logistic and cost-related problems of recordings (especially longer, sleep-containing periods) will depend not only on technological advances but also on healthcare reform that considers that in our ageing society new health issues have to be tackled with new strategies, taking into account recommendations stemming from geroscience (De Souto Barreto et al., 2020; Sierra, 2016).

Last, but not at least, the use of home-based, self-applied methods relying on automated analyses could also have a stimulating effect on research. Indeed, as an example, in the current study, recordings were only possible once a week (Friday evenings) when a hospital bed was reserved for research purposes at the Sleep and Epilepsy unit of the hospital. However, each participant required the presence of an extra nurse (specialized in EEG and PSG) at the unit during the night, which was not always possible logistics-wise. Finally, sleep scoring and EA detection often took several months per recording, as the medical experts had to undertake this task on top of the sleep scorings they are tasked with for clinical practice. Even if manual verification of automated analyses will be necessary (but potentially only on shorter durations of a given recording), home-based methods with AI-assisted analysis would facilitate the work both for researchers and clinicians involved in research.

### *3.3 The importance of early detection of episodic memory decline*

Episodic memory has long been known to be one of the earliest signs of AD. As an example, most MCI patients who later progress towards AD complain of episodic memory deficits as a first symptom (Albert et al., 2011). Unfortunately, the number of ecologic episodic memory tests is rather limited, and such tests are generally substituted by tasks measuring only one component of the “what-when-where” triad of episodic memory (e.g. the RL/RI-16, a test of verbal declarative memory that has been used as a proxy for episodic memory in previous studies). In the current study, we opted for a recently validated ecologic memory test (Lemesle

et al., 2022), that did not only distinguish between patients and controls with extremely high efficiency but seemed to be a convenient predictor of the slope of the subsequent cognitive decline, independent of dementia status. Furthermore, difficulties in episodic memory were found to be virtually the only faculty that was correlated to the subjective difficulties of everyday life tasks in controls, while in patients, the episodic memory deficits were replaced by executive dysfunctions in the correlation. Therefore, the use of ecologic episodic memory tests for general cognitive screening could be an efficient tool for the early detection of cognitive decline and could allow for preventive actions, potentially delaying further declines.

## CONCLUSION

Altogether, our clinical and preclinical data further confirm the peculiar association between Alzheimer's disease and epilepsy, although indicates that this phenomenon may be less pronounced (~20%) than previously estimated and comes with high levels of inter-individual variability as per to the frequency of epileptic activities. Our findings also indicate that the impact of these activities on memory decline, by themselves, may not be highly prominent. However, our results point towards a broadening of screening necessity for sleep-related pathologies in general – including epileptic events, sleep apnoeas and decreasing sleep efficiency – for AD patients, but also for healthy older adults. Indeed, our results draw attention to a composite impact of sleep quality and quantity, epileptic events and sleep apnoeas on post-sleep memory performances in several domains (episodic, visual and verbal declarative memory) over and above the expected impact of age, dementia status and brain atrophy levels. Furthermore, the clinical results presented here highlight the importance of developing or using the already developed more ecological tests of episodic memory instead of tests often used in current clinical practice as it seems to be the most sensitive predictor of subsequent cognitive decline.

Our preclinical results also point towards a potential beneficial impact of noradrenergic transmission via  $\alpha_1$  adrenoreceptors in preventing network hyperexcitability-related epileptic events, which could be an interesting point of clinical exploration in the future – especially in view of the high percentages of AD patients under antidepressant treatments modifying noradrenergic transmission. However, the incoherence between preclinical and clinical data, notably concerning the prevalence and distribution of epileptic activities during the sleep-wake cycle, urges the use of models with more ecological validity concerning disease mechanisms and phenotypes in the future.

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# **ANNEXES**



# Sleep: The Tip of the Iceberg in the Bidirectional Link Between Alzheimer's Disease and Epilepsy

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The observation that a pathophysiological link might exist between Alzheimer's disease (AD) and epilepsy dates back to the identification of the first cases of the pathology itself and is now strongly supported by an ever-increasing mountain of literature. An overwhelming majority of data suggests not only a higher prevalence of epilepsy in Alzheimer's disease compared to healthy aging, but also that AD patients with a comorbid epileptic syndrome, even subclinical, have a steeper cognitive decline. Moreover, clinical and preclinical investigations have revealed a marked sleep-related increase in the frequency of epileptic activities. This characteristic might provide clues to the pathophysiological pathways underlying this comorbidity. Furthermore, the preferential sleep-related occurrence of epileptic events opens up the possibility that they might hasten cognitive decline by interfering with the delicately orchestrated synchrony of oscillatory activities implicated in sleep-related memory consolidation. Therefore, we scrutinized the literature for mechanisms that might promote sleep-related epileptic activity in AD and, possibly dementia onset in epilepsy, and we also aimed to determine to what degree and through which processes such events might alter the progression of AD. Finally, we discuss the implications for patient care and try to identify a common basis for methodological considerations for future research and clinical practice.

**Keywords:** Alzheimer's disease, epilepsy, memory consolidation, sleep, neuronal hyperexcitability, glymphatic clearance, interictal spike, EEG

## A BRIEF HISTORY OF ALZHEIMER'S DISEASE AND EPILEPSY

In 1911, five years after presenting the first patient with the pathology that would later be named after him, Alois Alzheimer described a second case, a patient known in medical history as Johann F. Besides the progressive loss of cognitive functions and autonomy, the 56-year-old man experienced several epileptic seizures toward the later stages of the pathology (1, 2). This case, together with the detection of amyloid plaque-like structures called sclerotic plaques of neuroglia during the autopsy of an elderly epileptic patient in 1892, provided the first clues for a link between AD and epilepsy (3) [cited by Cipriani et al. (4)].

During the following century, the potential pathophysiological pathways of AD were meticulously investigated in a race to discover a treatment as the number of cases exploded, reaching ~33–38 million patients worldwide. However, the investigation of the AD-epilepsy axis failed to become what we would call today a “hot topic” in research. Sparse publications appeared every now and then, mainly advocating a link between familial forms of AD (FAD) and epilepsy. Patients with these rare forms, which account for ~1% of all AD cases and are due to mutations on the APP, PSEN1 or PSEN2 genes, were shown to have a high seizure incidence (5–11). As for sporadic AD, for quite a while, this comorbidity was defined as a mere marker of the severe stages of the disease (12, 13). A turn in these trends started with the seminal paper by Amati and colleagues in 2006 (14) which described an increase in seizure incidence among sporadic AD patients as of the earliest stages of the disease and which considered it a potential *part of the natural history of AD* (14). A smaller longitudinal study published the same year by Lozsadi and Larner echoed these results and suggested the existence of potentially shared pathogenetic processes between AD and epilepsy (15). Concomitantly, a series of studies in mouse models of AD (harboring mutations on the genes implicated in FAD) demonstrated the role of the AD-related protein-aggregates, namely A $\beta$  plaques and neurofibrillary tangles [NFTs, (16)], in the observed neuronal hyperexcitability and spontaneous epileptic or epileptiform activity (17–20). (Note that for the remainder of this review, we will use Epileptic Activity/EA to refer to epileptic/epileptiform events). This brought about the introduction of the network dysfunction perspective in AD (21) and an explosion of publications on the link between AD and epilepsy in preclinical and clinical research.

The main objective of this review is to organize the available information fragments from these publications on the potential underlying causes and clinical consequences of the AD-epilepsy comorbidity and its apparent link to the sleep-wake cycle. Our secondary aim is to find ways to identify the pieces of the puzzle of the AD-epilepsy connection that are still missing by adapting current clinical practice and research methods. Therefore, we will first scrutinize the literature on pathophysiological processes to see how significant alterations in AD might trigger epileptic events. We will then attempt to distill coherence from the seemingly discordant clinical results on the characteristics of epileptic activity in AD patients. In the third and fourth parts,

**Abbreviations:** A $\beta$ , Amyloid- $\beta$  peptide; ACE, Addenbrooke's Cognitive Examination; AD, Alzheimer's disease; APP, Amyloid Precursor Protein; AQP-4, Aquaporin-4; ASM, Antiseizure medication; (a)MCI, (amnestic) Mild Cognitive Impairment; BZD, Benzodiazepines; CDR-SB, Clinical Dementia Rating Scale-Sum of Boxes; CSF, Cerebrospinal Fluid; EOAD, Early-onset Alzheimer's disease; EA, Epileptic/Epileptiform activity; FAD, Familial Alzheimer's disease; IED, Interictal epileptic discharge; iEEG, intracranial EEG; LTG, Lamotrigine; LEV, Levetiracetam; LOAD, Late-onset Alzheimer's disease; LOEU, Late-onset epilepsy of unknown origin; MMSE, Mini-mental State Examination (Folstein Version); (m)TLE, (Mesial) Temporal Lobe Epilepsy; NFT, Neurofibrillary tangle; PH, Phenytoin; (N)REMS, (Non)-Rapid-eye movement sleep; PSEN, Presenilin; PSG, Polysomnography; PVBC, Parvalbumin positive basket cells; SSS, sporadic sleep spikes; SWD, Sharp-wave discharge; SW-R, Sharp-wave ripple; SO, Slow oscillation; v-EEG, video-electroencephalography.

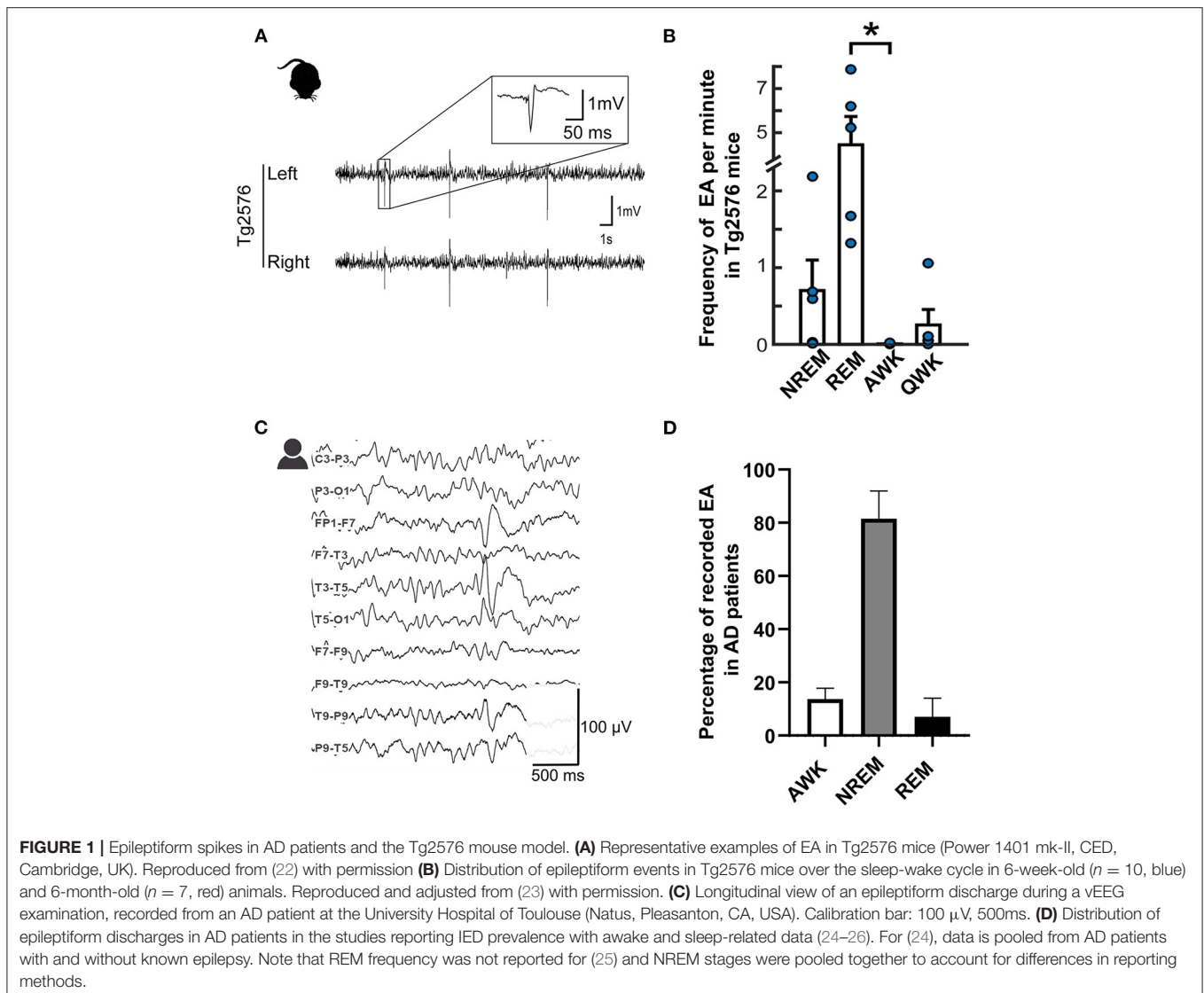
we will attempt to understand the possible consequences of these aberrant brain activities that occur during sleep on the progression of cognitive deficits in AD. Finally, we will examine how these pieces of information could be integrated into current clinical practice and research methodology that could increase coherence and comparability across studies.

## POTENTIAL MECHANISTIC UNDERPINNINGS OF AD-RELATED NEURONAL HYPEREXCITABILITY

Research over the past decade has made it abundantly clear that almost all mouse models used in AD research present aberrant network hypersynchrony and hyperexcitability quite early during the disease. These anomalous activities manifest as EA mostly during sleep or periods of inactivity in several models (Figures 1A,B), an aspect that, as we will see, seems to be similar in patients (Figures 1C,D). [For a more exhaustive list, see Kazim et al. (27)]. With the help of these models, significant advances have been made in elucidating the mechanistic pathways related to the aberrant network hyperactivity which have also been extensively reviewed recently (27–34). This review will only focus on the major pathways and the pathophysiological mechanisms related to clinical findings on the subject.

### Amyloid- $\beta$ Accumulation and Neuronal Hyperexcitability

AD is well known for the diffuse presence of senile plaques in the brain. These deposits are composed of A $\beta$  peptide aggregates resulting from the activation of the amyloidogenic proteolytic cleavage process of the APP protein by the  $\beta$ 1 (BACE-1) and  $\gamma$ -secretases which leads to a particularly aggregation-prone form, A $\beta$ <sub>1–42</sub> [see (35) for an illustration]. Under physiological conditions, this pathway is only secondary to the non-amyloidogenic pathway which depends on cleavage by the  $\alpha$ - and  $\gamma$ -secretases and leads to less aggregation-prone forms of A $\beta$ . However, there seems to be slanting toward the BACE-1-dependent pathways in AD, resulting in the well-known extracellular aggregates first described by Alois Alzheimer (36), and translated by Stelzmann and colleagues in 1995 (37). Throughout the literature, the excitatory impact of A $\beta$  as one of the main stimuli of neural hyperexcitability in AD is a surprisingly coherent finding. At non-pathological A $\beta$  levels, A $\beta$  production and neuronal hyperactivity seem to be part of a self-regulating feedback loop (38). However, in AD, this loop becomes a vicious cycle. In fact, increasing the A $\beta$  load by blocking its degradation leads to over-excitation in the hippocampus (39), while elevated or decreased neuronal activity leads to increased or decreased A $\beta$  aggregates, respectively (40–43). These findings are further supported by Busche and colleagues (17, 20). They showed that (I) hyperactive neurons cluster around Amyloid plaque and that (II) in the absence of plaque during the earliest stages of AD, even soluble forms of A $\beta$  can drive neuronal hyperactivity in the hippocampus of APP/PS1 mice. The results obtained by Reyes-Marín and Nuñez (44) point in the same direction revealing a strong correlation between plaque load and



epileptic events in the same model. Finally, A $\beta$  clearance seems to decrease neuronal hyperexcitability (45), although some results are contradictory on this subject (46).

Various candidate pathways through which A $\beta$  drives neuronal hyperexcitability have been suggested, including differential impacts of A $\beta$  peptides on neural activity regulation depending on their state of oligomerization [(47), see (48) for a review on further hypotheses]). This could result in a steep A $\beta$  peptide accumulation curve and, later on, amyloid plaques. Accumulating A $\beta$  may then drive neuronal hyperexcitability through the dysregulation of calcium homeostasis due to a high plaque load (49, 50), which would be left unchecked due to the early deterioration of several types of interneurons.

## A Dysfunction of Fast-Spiking Interneurons

The above-mentioned dysfunctional interneurons are mainly parvalbumin-positive basket cells (PVBC) implicated in the synchronization of the activity of neuronal populations. Moreover, their dysfunction is strongly linked to cognitive

impairment [for a summary of PVBC in AD, see Cattaud et al. (51)]. These interneurons seem to be dysfunctional in patients and hAPPJ20 mice due to decreased levels of the Nav1.1 subunit of voltage-gated sodium channels (52, 53). Interestingly, restoring the Nav1.1 subunit rescues both aberrant network activities and the memory-impaired phenotype of the hAPPJ20 model (52). Moreover, in the Tg2576 model (overexpressing a double mutant form of human APP695), Cattaud and colleagues recently demonstrated an early disruption of the perineuronal nets (PNN) surrounding the PVBC (51). PNNs not only protect cells from oxidative stress but they also play an essential role in stabilizing existing synapses, and through that function, memories (54, 55). Interestingly, restoring PVBC in the APP/PS1 model abolishes neuronal hyperexcitability and even improves cognitive function (56), which indicates that the observed PPN/PVBC-related deficits contribute to neuronal hyperexcitability in AD. Other populations of interneurons have also been described as suffering massive damage relatively early in APP/PS1 mice. This seems to be attenuated by the



transplantation of embryonic interneuron progenitors that suppress the hyperexcitable phenotype (57). Nonetheless, the interneuron dysfunction observed in AD models seems to add insult to injury as it removes the brakes that could stop the runaway train of A $\beta$ -induced hyperexcitability.

## Tau in Its Various Forms

In addition, the second major pathophysiological hallmark of AD after A $\beta$  deposits, the intracellular aggregations called neurofibrillary tangles (NFT), also assist and even contribute to the hypersynchronous phenotype (21). NFT aggregations, which are thought to appear downstream to A $\beta$  aggregation, are made of deposits of the hyperphosphorylated version of the microtubule-associated protein Tau (pTau). This hyperphosphorylated form leads to a loss of protein function and an increased probability of aggregation described by Mokhtar et al. (58). While many aspects of the pathways through which pTau or its endogenous form exerts a neurotoxic effect is still under investigation, the disentangling of their role in the epileptic phenotype associated with AD has started. For example, the reduction of total tau levels relieves neuronal hyperexcitability in mouse models of AD (19, 59). Furthermore, a recent longitudinal study in AD patients found that a risk of seizure was associated with higher total CSF tau levels (60), but not with higher pTau levels alone. In AD mouse models, the effect of tau on epileptogenesis seems to be driven by (I) the tau-dependent depletion of Kv4.2 potassium channels on dendrites (61) and (II) the interaction between endogenous tau, Fyn and PSD95. This latter interaction seems to be involved in the excitotoxic effect of A $\beta$  as it increases the number of post-synaptic glutamate receptors and renders neurons more receptive to excitatory inputs (62). However, phosphorylating tau near to the microtubule domain renders this interaction impossible, leading to NMDA receptor endocytosis, which induces short-term neuroprotective suppression of hyperexcitable networks but comes with a long-term potential to induce network hypoactivity (63). Furthermore, Tau-hyperphosphorylation seems to be accelerated in a hippocampus subfield-specific manner after *status epilepticus* and during epilepsy (64). At the same time, neural activity and epilepsy-related acceleration of tau pathology has recently been demonstrated (65, 66). These results are coherent with the observations of Mondragón-Rodríguez and colleagues (63), who suggest that tau phosphorylation in the early stages of AD might be a neuroprotective mechanism against A $\beta$ -related hyperexcitability to suppress neuronal hyperexcitability. However, in light of the recent results provided by Busche et al. (67), the respective roles of soluble and aggregated forms of pTau and the different ways through which endogenous Tau can increase or decrease neuronal activity in the presence of high A $\beta$  loads needs further investigation.

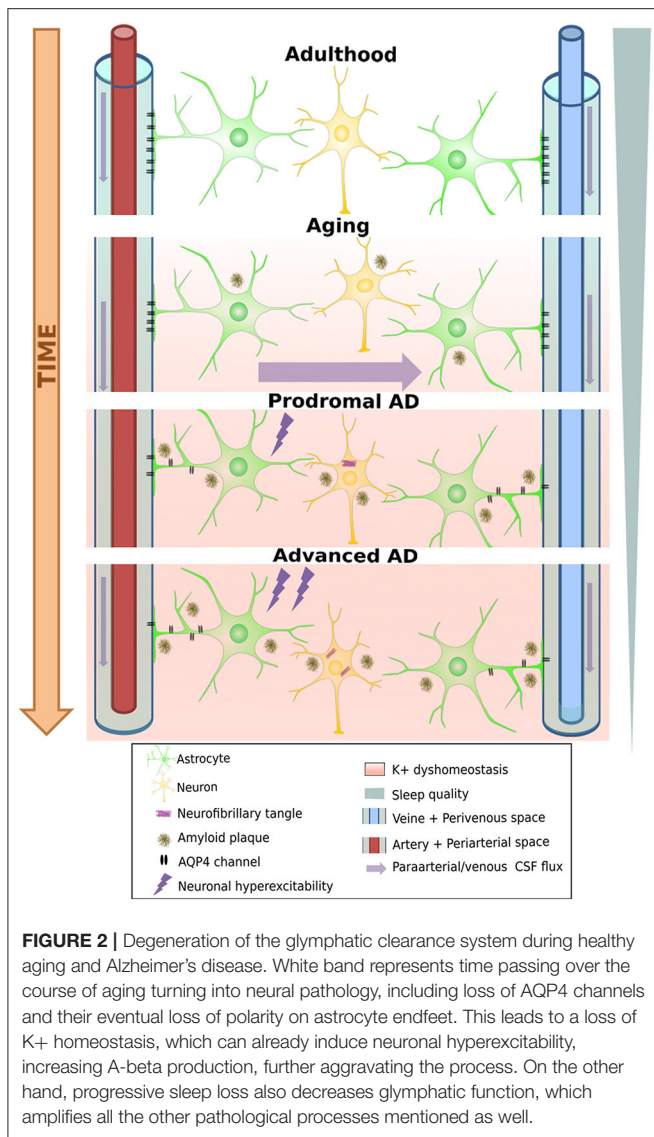
## Dysfunctional Sleep-Related Systems in AD

Furthermore, the accumulation of all the aggregates and the subsequent aggravation of the epileptic phenotype are linked to one of the earliest clinical signs of AD: declining sleep quality.

Firstly, the progression of AD parallels a more and more fragmented sleep with extended awake periods during the night, increasing sleep latency and shortened total sleep time. The duration of REM sleep decreases, probably due to early atrophy of the brain areas in charge of cholinergic and noradrenergic transmissions that are essential for REM sleep integrity (68, 69). These disturbed sleep patterns may also contribute to the deposition of both A $\beta$  and pTau and aggravate network hyperexcitability. In fact, A $\beta$  load, as measured in CSF samples, fluctuates diurnally and increases during wakefulness (70) and even after a single night of sleep deprivation (71). In response, plaque accumulation was shown to aggravate sleep fragmentation in a drosophila AD model, while enhancing sleep duration led to decreased deposits (72). Interestingly, the same authors described a net excitatory effect of sleep loss and A $\beta$  deposition leading to neuronal hyperexcitability that was responsive to ASM treatment (Levetiracetam, LEV) and that coincidentally prolonged the animals' lifespan as well. The disruptive effect of A $\beta$  on sleep integrity and related memory consolidation has already been described (73), and in light of previous evidence on the subject, a bidirectional link between A $\beta$  deposition and sleep fragmentation was proposed by Ju et al. (74). This link is such that low sleep efficiency turned out to be a good predictor of A $\beta$  deposition rates over several years even for rather long durations (mean follow-up of 3.7 years  $\pm$  2.4) in healthy elderly adults (75). Another sleep-related marker, the decrease in Non-REM (NREM) slow-wave activity, was linked to A $\beta$  deposition (73), and a similar or even stronger relationship was observed for tau deposits (76). More importantly, tau also seems to follow a sleep-wake cycle-dependent accumulation similar to A $\beta$  (77).

This daily fluctuation is strongly linked to one of the crucial mechanisms by which the brain can eliminate excess or potentially toxic metabolites, including soluble A $\beta$  and Tau: the glymphatic system. This relatively newly discovered clearance pathway (78, 79) is a highly organized fluid transport mechanism that accommodates an influx of subarachnoid CSF into the brain interstitium via intracerebral arterial perivascular spaces. Due to anterograde flow toward the venous equivalent of perivascular and perineuronal spaces that ends in the meningeal lymphatic drainage system, this mechanism can eliminate unwanted metabolites [Figure 2, see Rasmussen et al. (80)]. As our understanding of this system grows, it is becoming clear that dysfunctions in this pathway can cause rapid deposition of both A $\beta$  and tau (81). Correct functioning of this pathway is highly dependent on water transport through aquaporin-4 (AQP4) water channels at astrocyte endfeet that line both the arterial and venous end of the perivascular spaces. These channels ensure low-resistance flow between perivascular spaces and brain interstitium (78), and several pathways by which these channels become dysfunctional in AD were recently uncovered (82).

First, these channels show an age-dependent decline even in wild-type mice (83), leading to a decrease by 40% of intraparenchymally injected A $\beta$ -clearance efficiency. This is consistent with the findings that ~40–80% of all proteins and soluble metabolites are cleared via this mechanism (78). Importantly, it would appear that the glymphatic system is 90% more active during sleep than during waking hours (79). Given



the considerable disruption of sleep and decreased total sleep time from the earliest stages of AD, it is plausible that the decrease induced in glymphatic clearance may further aggravate A $\beta$  and tau accumulation (84). Moreover, fragmented sleep might do more harm than just decrease the “ON-time” of glymphatic clearance: Using two-photon *in vivo* imaging in non-transgenic mice, Liu et al. (85) showed that sleep deprivation leads to a mislocalization of AQP-4 channels that might render glymphatic clearance impaired. It is fascinating to note that in a transgenic model of AD (Tg-ArcSwe), Yang et al. (86) demonstrated an A $\beta$  deposition-dependent mislocalization of AQP-4 channels on perivascular astrocytes even before the discovery of the glymphatic system. Importantly, they also drew attention to an earlier paper by Eid and colleagues (87), who found a dysfunction in perivascular AQP-4 channels in resected hippocampal sections from epileptic patients undergoing surgery. At that time, having no information about the existence of the glymphatic system, they hypothesized that such impairments may not only disturb

water homeostasis but could also reduce K<sup>+</sup> clearance, a function in which these channels were already known to be implicated. This K<sup>+</sup> clearance deficit is yet another pathway that further spirals the hyperexcitable phenotype of AD mouse models (Figure 2). Moreover, a reactive state can also induce loss of polarity in astrocytes, as has been shown to occur near amyloid- $\beta$  plaque in a transgenic FAD model (APP/PS1) (88). Deleting AQP-4 channels from the same model led to aggravated A $\beta$  deposition both in the cortex and the hippocampus and resulted in an increased spatial memory deficits (89). In another “cross-transgenic” FAD model devoid of AQP-4 channels (5xFAD/AQP4 KO mice), an increase of ~6-fold in EA frequency was observed compared to 5xFAD mice at 10 months of age (90). Many questions remain about the glymphatic system and its relationship with AD (84, 88). Nevertheless, the possibility that yet another vicious cycle that amplifies amyloid deposition and epileptic susceptibility might exist seems plausible. In fact, age-related functional impairment of the glymphatic system may lead to increased plaque deposition. Plaque or A $\beta$ <sub>1–42</sub> oligomers could, in turn, create deposits in the vicinity of perivascular astrocytes, leading to a reactive glial state or the mislocalization of AQP-4 channels on astrocytes, further diminishing the process of glymphatic clearance (86, 88). This could lead not only to further acceleration of plaque deposition but also to a loss of K<sup>+</sup> homeostasis, increasing the risk of epileptic activities, which, once again, leads to an increase in A $\beta$  (and tau) load (Figure 2). Finally, the respiratory cycle also modulates glymphatic clearance. Inspiration is notably a strong driving force for the process in humans by its modulation on hydrostatic pressure which facilitates CSF flow toward the interstitial spaces of the brain (91). Importantly, breath-holding suppresses this flow and potentially decreases clearance, a scenario that may happen quiet often in patients with sleep apnea, which is a frequent comorbidity in AD patients (92). This is supported by the finding that the severity of obstructive sleep apnea seems to contribute to the build-up of amyloid deposition even in healthy older adults (93, 94).

Finally, it is of note that the glymphatic system, just as many physiological processes including the sleep-wake cycle, has been suggested to be under circadian control (95). Circadian rhythms are maintained via a complex transcriptional machinery that are present in most cells in the human body and are modulated via the suprachiasmatic nucleus of the hypothalamus that is thought to serve as a “master body clock” (96). Interestingly, both clinical and preclinical research have suggested a dysfunction in the circadian rhythms in AD that might happen even before the onset of the clinical symptoms of the pathology. Albeit the mechanistic pathways of the circadian clocks are outside of the scope of this review, this angle needs further investigation as mending this dysfunction that is upstream of several pathways by which AD might progress and by which EA might appear could have therapeutic implications (96).

## A Potential Bidirectional Road to Accelerated Disease Progression

As mentioned earlier, many other mechanisms have been suggested, such as the APO- $\epsilon$ 4 allele, (97–99), and an early reduction in the level of either the mitochondrial protein



deacetylase sirtuin 3 (100) or reelin [(101, 102), reviewed by (103)].

All these converging and synergistic pathways introduced in this paragraph could contribute to forming a dysfunctional network in which the excitation/inhibition balance has been considerably slanted toward hyperexcitability, which makes the occurrence of epileptic events or even seizures highly probable. Neuronal hyperactivity during such events might in turn further aggravate the situation by reorganizing functional networks (18, 104, 105) and generating substantial amounts of A $\beta$ . Since the important pathological pathways that lead to AD can be triggered decades before the onset of cognitive symptoms, these processes can slowly but steadily reorganize and destroy existing functional circuits. This also applies to sporadic forms of AD, where epilepsy might not be as clear-cut a symptom as in the case of FAD, yet silent reorganization might occur during the asymptomatic period of plaque and NFT accumulation. This could lead to aberrant network activities, even before cognitive symptoms appear (25).

The suggestion of a disease-accelerating synergistic phenomenon between AD and epilepsy also begs the question of the possible bidirectionality of the link between the two pathologies (48, 106). Clinical research has suggested that age-related senile plaque deposition is more significant among TLE patients than healthy older adults. Moreover, an enhancing effect of the epileptic syndrome on plaque formation and amyloidosis has been suggested. This is based on observations from resected cerebral tissue and CSF AD-biomarker measurements on epileptic patients, as well as observations from genetic and neurotoxic AD mouse models (107–109). A similar increase to that of amyloid burden was found for pTau in post-surgery brain resections collected from TLE patients with focal drug-resistant epilepsy (65). Moreover, a very recent review by Tombini et al. (34) showed that TLE and AD patients share many pathophysiological associations starting with the presence of hallmark depositions of AD (A $\beta$  plaques and NFTs, or their soluble forms), a similarity that is more robust for late-onset TLE cases (110).

However, does the current clinical research confirm (I) the vicious cycle of A $\beta$ , NFTs and EA promoting a fast accumulation of aggregates and a consequent fast progression of AD, and/or (II) the bidirectional relationship between AD and epilepsy?

## FILTERING COHERENCE FROM DISCORDANT FINDINGS

First of all, it is essential to note that the prevalence of subclinical epileptic activities (**Table 1**) and epileptic seizures (**Table 2**) in AD patients is highly variable across studies, reaching 64% (133) in some cases while barely hitting 2% in others (124). This is probably due to the extreme variability in data collection methods (134), an issue we will discuss in detail in the last paragraph. However, several coherent findings emerge from the apparent discordance that can guide our understanding of this peculiar link between AD and epilepsy, and we will first focus on these points.

The first of these coherent findings is that AD-related EA is primarily subclinical and, therefore, hard to detect. This might explain why this comorbidity has been long overlooked. On one hand, these EA include subclinical epileptic spikes without a behavioral output and which are, as we shall see, difficult to detect by conventional non-invasive methods (135). On the other hand, focal seizures with temporary loss of contact in AD patients might be interpreted as a symptom of AD instead of silent epileptic seizures (26, 112). Therefore, there is a high chance that aberrant network activities go unnoticed and untreated for extended periods.

The second point of cross-study consensus is that the progression of AD in patients with a co-occurring epileptic syndrome or epileptiform activities is faster (25, 117, 130, 136). Moreover, AD patients with EA seem to have worse results on cognitive tests than patients without such aberrant network activities (26, 120, 121, 137). This correlation does not seem to be merely related to a longer duration of AD or more severe stages of the disease. First, Vossel et al. (25) demonstrated that even if patients with and without EA did not differ at baseline in their mini-mental state examination (MMSE) scores, a follow-up over several years showed a steeper cognitive decline for patients with EA. Second, Vöglein and colleagues (130) recently showed that even after adjusting for age and disease duration, the MMSE scores were still unexpectedly low for AD patients with underlying epileptic activity. Moreover, they found that a history of seizures had a significant negative impact on disease severity (as measured by the Clinical Dementia Rating Scale–Sum of Boxes/CDR-SB). Finally, a recent study of Horvath and colleagues described a 1.5 times faster decline of global cognitive performances of AD patients with EA compared to patients without epileptic abnormalities on their EEGs (136).

Another coherent finding is that AD patients with comorbid epileptic activities experience an earlier age of onset of cognitive symptoms (14, 24, 60, 112, 119, 121, 130, 138). This, as we will detail further below, is indicative of a synergistic accelerating of AD- and epilepsy-related factors on cognitive decline which could lead to a faster progression and an earlier age of onset instead of the decade-long process of insidious plaque and NFT deposition.

The localization of EA also seems to be a relatively coherent cross-study finding. Similarly to AD-independent mesial-temporal lobe epilepsies (mTLE), these aberrant network activities in AD are predominant in the temporal lobes and more often detected on the left hemisphere by EEG (24, 25). However, concerning the study of Lam and colleagues (24), it is of note that while left temporal subclinical EA were indeed highly predominant in AD patients with no history of epilepsy, patients with a history of seizures were more prone to have right and left temporal interictal discharges as well.

Last but not least, one of the cornerstones of this review is the strong relationship between the sleep-wake cycle and EA, since sleep-related increase in EA is one of the most consistent findings across both clinical and preclinical literature (24–26) (**Figures 1C,D**). This sleep-related increase in EA implies that they could plausibly interfere with sleep-related memory

**TABLE 1** | Main inclusion criteria and results of clinical studies exploring the prevalence of epileptic activities in AD patients.

References	Mean age in years $\pm$ SD (n/group)	AD diagnostic criteria	AD severity (MMSE/ACE/CDR mean + SD*)	History of epilepsy	Excluded medications or substances	EEG type and duration	EA detection	% with EA or incidence	Risk factors for EA
Liedorp et al., (111)	<b>1. AD-no EA:</b> 63 $\pm$ 10 ( <b>500</b> ) <b>2. AD+EA:</b> 71 $\pm$ 9 ( <b>10</b> )	NINCDS-ADRDA	<b>MMSE</b> = 25 $\pm$ 5	Included	NS, but accounted for in the analyses	Standard 30-min EEG	1 neurophysiologist	<b>2%</b> of AD cases	Younger age, Disease severity (trend)
Vossel et al., (112)	<b>At diagnosis:</b> <b>1. AD+EPI:</b> 69.1 $\pm$ 9 ( <b>35</b> ) <b>2. AD-no EA:</b> 74.5 $\pm$ 10.3 ( <b>969</b> ) <b>3. MCI+EPI:</b> 68 $\pm$ 7.8 ( <b>12</b> ) <b>4. MCI-no EA:</b> 74.6 $\pm$ 9.5 ( <b>216</b> )	<b>1. AD:</b> NINCDS-ADRDA <b>2. MCI:</b> IWG	<b>MMSE range</b> = 4–30	Included	Alcohol/substance abuse	Standard 20-min EEG for a sub-group of 152 participants	Multidisciplinary team of experts	<b>Groups 1, 3:</b> 24/39 <b>Groups 2, 4:</b> 7/113	Younger age at onset
Vossel et al., (25)	<b>1. AD</b> 61.7 $\pm$ 7.4 for ( <b>33</b> ) <b>2. CTRLs:</b> 65.3 $\pm$ 5.6 ( <b>19</b> )	NIA-AA	<b>MMSE range:</b> <b>1. AD</b> = 18–24 <b>2. CTRLs</b> = 29–30	Not included	BZD Antipsychotics Narcotics Antihistamines. Substance abuse	Overnight vEEG and 1h M-EEG	1 epileptologist + 1 neurophysiologist per exam type, blinded	<b>1. AD:</b> 42,4% <b>2. CTRLs:</b> 10.5%	No risk factor, but faster decline
Horvath et al., (26)	<b>1. AD-no epilepsy:</b> 79.4 $\pm$ 7.4 ( <b>20</b> ) <b>2. AD + seizures:</b> 75.9 $\pm$ 4.7 ( <b>10</b> ); <b>3. AD+IEDs:</b> 73.6 $\pm$ 9.3 ( <b>12</b> )	NINCDS-ADRDA	<b>ACE</b> <b>1: AD-no EA</b> = 67.4 $\pm$ 9.4; <b>2. AD + seizures</b> = 38.4 $\pm$ 15.3; <b>3. AD + IEDs</b> = 44.5 $\pm$ 7.4	Included (if onset < 10y before AD)	Antipsychotics Antidepressants Antihistamine BZD	24 h ambulatory EEG	Two independent neurophysiologists (1 blinded). ILAE definition of epilepsy diagnosis	28% IED without seizures + 24% with seizures	AD severity; High VL0M ratio; Higher educational level
Brunetti et al. (113)	<b>1. AD:</b> 73 $\pm$ 7 ( <b>50</b> ) <b>2. MCI:</b> 72 $\pm$ 6.7 (50) <b>3. CTRL:</b> 69 $\pm$ 6.7 ( <b>50</b> )	NIA-AA and DSM-IV	<b>MMSE</b> = 18.38 $\pm$ 4.7	Not included	Psychoactive/hypnotic drugs History of alcohol/substance abuse	Full-night vEEG (134/150 participants)	Automatic followed by two neurophysiologists' verification (blind)	<b>AD:</b> 6.38% (3/47) <b>MCI:</b> 11.63 (5/43) <b>CTL:</b> 4.54% (2/44)	-
Lam et al., (24)	<b>1. AD-no EA</b> 76.3 $\pm$ 7.2 ( <b>41</b> ) <b>2. AD + EA</b> 69.6 $\pm$ 7.6 ( <b>15</b> ) <b>3. CTRLs:</b> 72.6 $\pm$ 9.1 ( <b>43</b> )	NIA-AA	<b>MMSE range:</b> 13–30	Included (separate group)	BZD/"sleep aids" AMS (for groups 1, 3)	24 h ambulatory EEG	<b>Two steps:</b> <b>1.</b> 2 epileptologists <b>2.</b> A panel of 9 epileptologists	<b>AD-no EPI:</b> 22% <b>AD+EPI:</b> 53.3% <b>CTRL:</b> 4.7%	SSS-like waveforms; Earlier age of onset

(Continued)

TABLE 1 | Continued

References	Mean age in years ± SD (n/group)	AD diagnostic criteria	AD severity (MMSE/ACE/CDR mean + SD*)	History of epilepsy	Excluded medications or substances	EEG type and duration	EA detection	% with EA or incidence	Risk factors for EA
Vessel et al., (114)	<b>1. UCSF branch:</b> 60.2 ± 7.1 (18) <b>2. UMN branch:</b> 64.8 ± 7.8 (16)	NIA-AA	MMSE > 17 CDR < 2	Included (if onset < 5y before screening)	BZD ASM Narcotics	Overnight vEEG and 1 h M-EEG	1 epileptologist and 1 clinical neurophysiologist (both blinded); + Persist EEG software	38,20%	Tendencies (p = 0.054) for females and APOE4 carriers toward AD + EA

\*Unless otherwise stated. ACE, Adenbrooke's Cognitive Examination; AD, Alzheimer's Disease; ASM, Antiepileptic Medication; BZD, Benzodiazepines; CDR, Clinical Dementia Rating; CTRL, Controls; EA, Epileptic/Epileptiform activity; IED, Interictal Epileptiform Discharge; IWG criteria, AD diagnostic criteria based on the propositions of the International Working Group; MCI, Mild Cognitive Impairment; MMSE, Mini Mental State Examination (Folstein version); NIA-AA criteria, AD diagnostic criteria based on the propositions of the National Institute on Aging and the Alzheimer's Association; NINCDS-ADRDA criteria, AD diagnostic criteria based on the proposals of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association; NA, Not applicable; NS, Not Specified; vEEG, Video EEG; VLOM Ratio, Ratio of Verbal Fluency and Language ACE subscores over the Orientation and delayed recall subscores.

consolidation, one of the major potential consequences of AD-related epilepsy which we will examine in the next paragraph.

## THE IMPACT OF SUBCLINICAL EPILEPTIC ACTIVITIES ON MEMORY DEFICITS IN AD

An increase in EA during sleep in AD patients is not surprising as such a sleep-related increase in the occurrence of epileptic events has already been shown in non-AD epileptic patients (139). However, in epileptic patients, EA seems to fall during periods of NREM sleep and is extremely rare in REM sleep (139). In FAD mice (hAPPJ20 or Tg2576), this is not entirely the case. While EA is still predominant during inactive periods (52) such as sleep, their frequency shows a drastic increase during REM sleep compared to NREMS (23, 140), pinning down a visible discrepancy between animal and human results (140). This pattern is undoubtedly puzzling, as REM sleep is thought to be highly protective against EA due to the desynchronized EEG patterns that make the occurrence of hypersynchrony-related events rather unlikely (139).

It is of note that the predominance of EA during NREM sleep in clinical results might be partially due to difficulties in detecting REM sleep in AD patients, as notable EEG slowing specifically during REM sleep has been observed in AD, which might impede correct scoring of REM sleep (141, 142). However, currently available clinical data (albeit scarce for the moment, **Figure 1C**) are still more compatible with what is observed in non-AD epileptic patients, with EA predominantly occurring during NREM sleep (**Table 2**). Consequently, while we look at the potentially detrimental impact of EA on memory consolidation in the next paragraph, we mainly focus on NREM sleep and less on REM sleep.

## The Orchestra of Oscillations Behind Memory Consolidation

Throughout the past fifty years, an impressive panoply of theories have been proposed and refined concerning the role of sleep in memory consolidation and this was extensively reviewed (143–145). A theory of a memory consolidation mechanism was first postulated by Marr in the early seventies (146, 147). He suggested that the hippocampus might only serve as a temporal store for memories, while remote memory formation requires the reorganization of such stored information at the cortical level. Reactivation (or replay) of patterns of previous waking activities during “offline” periods was later suggested as a mechanism for such reorganization (148, 149). Sleep provides ideal slots for such offline consolidation as no incoming input is present, which allows the system to shift from “encoding” mode to “consolidation” mode. Such reorganization would also enable the mnemonic trace to become gradually independent of the hippocampus, despite the fact that the initial encoding is dependent on both distributed cortical modules and the integrative function of the hippocampus which links the distributed aspects of the trace together. The idea of such a temporal gradient in the hippocampus-dependence of memories has been backed by many studies on anterograde

**TABLE 2 |** Main inclusion criteria and results of clinical studies that explore the prevalence of seizures in AD patients.

References	Age mean in years $\pm$ SD* (n/group)	AD diagnostic criteria	Disease severity (MMSE/ACE/CDR mean + SD*)	History of epilepsy	Excluded medications or substances	EEG type or seizure information source	Seizure detection	% with seizures per group or incidence	Risk factors for seizures/epilepsy diagnosis
Romanelli et al., (12)	<b>1. AD:</b> 71.5 $\pm$ 4.9 ( <b>44</b> ) <b>2. CTRL:</b> 71.7 $\pm$ 4.9 ( <b>58</b> )	Criteria from Morris et al., (115)	NA	Not included	- Alcohol abuse - "Other medical illnesses" with a higher risk of seizures	- Standard EEG - Questions at follow up - Medical/ Nursing home records	Questions at follow up; medical records; nursing home records	15.9% of patients, 0% of controls	- Severe stage
McAreavey et al., (116)	<b>Age &gt; 55</b> (208 dementia cases)	ICD-9	<b>MMSE range:</b> 0-24 <b>MMSE means:</b> <b>1. Seizure:</b> 2.5 <b>2. No seizure:</b> 4.8	Included	NS	- Questions to nursing and medical staff	- ASM prescriptions - Medical records - Nursing home staff	9.1% (84% of those with AD)	- Younger age - Earlier age at admission
Mendez et al., (13)	<b>Age NS</b> (446 AD patients)	Medical records and autopsy	NS	Included	NS, but alcohol abuse and "other medical illnesses" accounted for	- Questions to family members, nursing home staff and physician	- Medical records - Nursing home staff - Physician - Questionnaire for family members	17.30%	- Younger age of onset, - Advanced AD stage
Volicer et al., (117)	<b>1. AD+EA:</b> 70.6 $\pm$ 4.4; <b>2. AD-no EA:</b> 72.4 $\pm$ 3.5 ( <b>75</b> DAT altogether)	NINCDS/ADRDA or DSM III-R	NS for all patients, but severe cases	Included	NS	- Interview with caregiver if history of seizures was found	- Observation - Medical records - Interview with caregiver	21% after being institutionalized (36% when pre-existing epilepsy counted)	- Faster decline, especially in language ability
Lozsadi and Larnar, (15)	<b>Range at diagnosis:</b> 49–84 ( <b>177</b> )	NINCDS-ADRDA	NS	Included	No	- Medical records	From medical records, classification based on ILEA criteria	6.8%	- NS
Amatniek et al., (14)	NS, but age > <b>50</b> ( <b>233</b> probable AD)	NINCDS-ADRDA	<b>MMSE <math>\geq</math> 16</b>	Not included	Antipsychotics Drug/alcohol abuse	- Standard EEG for 58.37% of participants - Question on seizure occurrence since last visit	Two neurologists' evaluation based on questionnaires and medical records	0.87%	- Younger age - African-American ethnicity. - AD severity at initial visit - Longer duration of symptoms - Lower educational level - Focal epileptiform findings - Depression (for younger participants)

(Continued)

TABLE 2 | Continued

References	Age mean in years $\pm$ SD* (n/group)	AD diagnostic criteria	Disease severity (MMSE/ACE/CDR mean + SD*)	History of epilepsy	Excluded medications or substances	EEG type or seizure information source	Seizure detection	% with seizures per group or incidence	Risk factors for seizures/epilepsy diagnosis
Rao et al., (118)	<b>Mean: 76.9</b> in the seizure group ( <b>1,738</b> dementia cases altogether)	Medical records	NS	Included	NS	- EEG for 74% of patients with seizures - Medical records	Medical records	3.6% (2.24% confirmed, 48.7% of those had AD/MCI)	-
Scarmeas et al., (119)	74.4 $\pm$ 8.9 at entry ( <b>453</b> AD)	NINCDS-ADRDA or DSM IIIR	<b>Baseline MMSE:</b> 21 $\pm$ 3.3 All patients > 15	Included	None, but many taken into account in the analyses	- EEG for 21 out of 52 with suspected epilepsy - Follow-up interview every 6m - Medical records	2 epileptologists reviewed medical records and interviews	1.5% of patients	- Younger age
Bernardi et al., (120)	78 $\pm$ 7.2 (145 probable AD)	NIA-AA	<b>MMSE</b> = 19.9 $\pm$ 6.3 (Range: 3-27)	NS	None but: antidepressants + antipsychotics accounted for	- EEG for 21/145 patients at baseline (and for all with identified seizures) - Medical records	Based on ILAE criteria (63), by one epileptology expert	9.7% of AD	- Gender (male) - AD severity - Hyperlipemia
Irizarry et al., (121)	74.5 $\pm$ 9.5 ( <b>3,087</b> AD from 10 previous studies)	NINCDS-ADRDA	<b>MMSE range:</b> 10–28	Not included	No, but taken into account in the analyses	- Only data available from the previous studies	Verbatims from clinical trials	4.84/1,000 py	- Younger age - AD severity at baseline - Antipsychotic use
Imfeld et al., (122)	<b>1. AD:</b> 80.7 $\pm$ 6.7 ( <b>7,086</b> ) <b>2. CTRLs ~ age matched (7,086)</b>	Algorithm, from diagnostic coding from GPs	<b>NS</b>	Not included	No, but verified for antipsychotics + antidepressants	- UK General Practice Research Database	From GP coding	<b>AD:</b> 5.6/1,000 py; <b>CTRL:</b> 0.8/1,000 py	- Longer disease duration

(Continued)

TABLE 2 | Continued

References	Age mean in years $\pm$ SD* (n/group)	AD diagnostic criteria	Disease severity (MMSE/ACE/CDR mean + SD*)	History of epilepsy	Excluded medications or substances	EEG type or seizure information source	Seizure detection	% with seizures per group or incidence	Risk factors for seizures/epilepsy diagnosis
Vossel et al., (112)	<b>At diagnosis:</b> <b>1. AD+EA:</b> 69.1 $\pm$ 9 ( <b>35</b> ) <b>2. AD-no EA:</b> 74.5 $\pm$ 10.3 ( <b>969</b> ) <b>3. MCI+EA:</b> 68 $\pm$ 7.8 ( <b>12</b> ) <b>4. MCI-no EA:</b> 74.6 $\pm$ 9.5 ( <b>216</b> )	<b>1. AD:</b> NINCDS/ADRDA <b>2. MCI:</b> IWG	<b>MMSE range:</b> 4–30	Included	Alcohol/substance abuse	- Standard 20-min EEG for a sub-group of 152 participants; - Medical History	Multidisciplinary team of experts	2.86% of AD, 5.26% of MCI	- Younger age at onset
Cook et al., (123)	<b>Mean</b> $\approx$ 80 (n = <b>11,042</b> AD and <b>11,042</b> non-AD dementia)	- Diagnosis codes CPRD	NS	NS	Antipsychotics + antidepressants accounted for	- Diagnosis codes from GPs	GP diagnosis codes and follow-up questionnaire	<b>AD:</b> 8.8/1,000 py <b>Non-AD dementia:</b> 1.7/1,000 py	- Stroke - Antipsychotics prescribed within 6 m
Giorgi et al., (124)	<b>At diagnosis:</b> 69.6 $\pm$ 8.5 (1223 AD)	NINCDS-ADRDA	<b>MMSE Range</b> = 3–29 <b>Median</b> = 18.67	Included	NS	- EEG for a subset of patients only - Medical records	From medical records	2.45% (1.63% without patients with concomitant lesions)	- NS
DiFrancesco et al., (125)	<b>At onset:</b> 75 $\pm$ 7 (1,371)	NIA-AA criteria	NS, probably advanced	Included	Antipsychotics Antidepressants Alcohol/drug abuse	- EEG for a subset of patients only - Medical records	From medical records (with EEG when available)	1.68% before AD + 1.16% after AD onset	- Earlier onset of cognitive decline
Horvath et al., (26)	<b>1. AD-no EA:</b> 79.4 $\pm$ 7.4 ( <b>20</b> ) <b>2. AD+seizure:</b> 75.9 $\pm$ 4.7 ( <b>10</b> ) <b>3. AD+IEDs:</b> 73.6 $\pm$ 9.3 ( <b>12</b> )	NINCDS-ADRDA	<b>ACE</b> <b>1. AD-no EA</b> = 67.4 $\pm$ 9.4; <b>2. AD + seizures</b> = 38.4 $\pm$ 15.3; <b>3. AD + IEDs</b> = 44.5 $\pm$ 7.4	Included (if onset < 10y pre- AD)	Antipsychotics Antidepressants Antihistamine BZD	- 24h ambulatory EEG - Epilepsy-related information collection	2 neurophysiologists (1 blind to condition), ILAE definition of epilepsy diagnosis	24%	- AD severity - High VLOM ratio - Higher educational level

(Continued)

TABLE 2 | Continued

References	Age mean in years $\pm$ SD* (n/group)	AD diagnostic criteria	Disease severity (MMSE/ACE/CDR mean + SD*)	History of epilepsy	Excluded medications or substances	EEG type or seizure information source	Seizure detection	% with seizures per group or incidence	Risk factors for seizures/epilepsy diagnosis
Rauramaa et al., (126)	85 $\pm$ 8.6 (64 AD - 7 mixed dementia)	NINCDS-ADRDA and autopsy	NA	Included	NS (but no alcohol abuse)	- For 10/11 patients with epilepsy - Medical records	EEG, medical records	17.20%	- Younger age of onset - Longer disease duration
Baker et al., (127)	75.1 $\pm$ 7.07 (n = 72 at 1-year follow-up)	NIA-AA	<b>CDR-score at BL</b> = 4.2 $\pm$ 2.69)	Included	NS	- Structured interview (with informant)	From interview	<b>At follow-up:</b> <b>Probable:</b> 18.06%; <b>Possible:</b> 19.44%	- Worse score on CBI-R at baseline - Worse cognitive functions at follow-up (problem solving, personal care, attentional capacities $\downarrow$ , daytime sleeping, confusion, fluency difficulties $\uparrow$ )
Lyou et al., (128)	<b>Age &gt; 70 (4,516 AD; 19,713 CTRL)</b>	ICD-10	NS	NS	No	- Medical database	Diagnostic codes from medical database	<b>At last follow-up</b> <b>1. AD:</b> 13.97 % <b>2. CTRL:</b> 6.05%	- Gender (male) - Hypertension or hyperlipidemia - Chronic kidney disease
Tabuas-Pereira et al., (60)	<b>1. AD+EA:</b> 68.4 $\pm$ 8.4 <b>2. AD-no EA:</b> 68.1 $\pm$ 9.9 ( <b>292</b> AD altogether)	NIA-AA	<b>MMSE</b> <b>1. AD + EA =</b> 16.2 $\pm$ 6.4 <b>2. AD - no EA:</b> 20.8 $\pm$ 7.4	Not included	NS on medication No alcohol abuse	- EEG for patients with suspected epilepsy - Medical charts	Retrospectively from medical files, backed by EEG	17.8%	- Earlier onset of dementia - Lower MMSE even at baseline - Higher tau load

(Continued)



TABLE 2 | Continued

References	Age mean in years $\pm$ SD* (n/group)	AD diagnostic criteria	Disease severity (MMSE/ACE/CDR mean + SD*)	History of epilepsy	Excluded medications or substances	EEG type or seizure information source	Seizure detection	% with seizures per group or incidence	Risk factors for seizures/epilepsy diagnosis
Stefanidou et al., (129)	<b>1. Dementia:</b> 83 $\pm$ 7 ( <b>660</b> ) <b>2. CTRLs:</b> 83 $\pm$ 7 ( <b>1,980</b> )	DSM IV criteria	NA	Not included	NS	- EEG at least for a subset, but NS - Medical charts - ICD-9 codes - Follow-up interviews	Scoring of epilepsy probability by 2 epileptologists, based on ILAE criteria	<b>1. Dementia group:</b> 2.9% <b>2. CTRLs:</b> 2%	-
Vöglein et al., (130)	<b>1. AD pre-symptomatic.:</b> 79.8 $\pm$ 7.7 (498) <b>2. Impaired-no-MCI:</b> 74.9 $\pm$ 9.0 (43) <b>3. AD MCI:</b> 75.1 $\pm$ 8.3 (859) <b>4. AD:</b> 75.1 $\pm$ 9.9 (9,127) <b>5. CTRLs:</b> 69.8 $\pm$ 10.9 (10,218)	NINCDS/ADRDA or NIA-AA	<b>MMSE</b> <b>1. Pre-sympt.:</b> 28.2 $\pm$ 1.8 <b>2. Impaired-no-MCI:</b> 27.2 $\pm$ 2.6 <b>3. MCI:</b> 26.1 $\pm$ 2.7 <b>4. AD:</b> 19.5 $\pm$ 6.7 <b>5. CTRL:</b> 28.9 $\pm$ 1.4	Included	NS	- Interview with participant and "co-participant" - Medical records - Observation	- Interview with participant and "co-participant" - Medical records - Observation	- 2.93% all confounded - 3.14% of AD	- Earlier age of AD onset - Worse cognitive and functional state - Longer AD duration
Zelano et al., (131)	<b>Age range =</b> 32–107 [(25,626 non-mixed dementia AD case); 223,933 CTRLs]	ICD-10	NS, probably broad range	Included	NS	SveDem database	ICD-10 and ICD-9 codes, meeting ILAE criteria	<b>1. All cases:</b> 4%, <b>2. LOAD:</b> 2.1% <b>3. EOAD:</b> 5%	- Young age, - Gender (male) - History of stroke/head trauma/brain tumor, - Lower MMSE
Blank et al., (132)	<b>Median =</b> 84 (178,593 probable AD)	Medical records from various sources	NS	NS	NS	- Medical records	Mention of epilepsy diagnosis in records	4.45% of AD	- Stroke - Depression - African-American ethnicity

\*Unless otherwise stated. ACE, Addenbrooke's Cognitive Examination; AD, Alzheimer's Disease; ASM, Antiseizure Medication; BZD, Benzodiazepines; CDR, Clinical Dementia Rating; CPRD, Clinical Practice Research Datalink; CTRL, Controls; DSM-III-R, Diagnostic and Statistical Manual of Mental Disorders – 3<sup>rd</sup> revised edition; EA, Epileptic/Epileptiform activity; ICD-9/10, International Classification of Diseases; IED, Interictal Epileptiform Discharge; IWG criteria, AD diagnostic criteria based on the propositions of the International Working Group; MCI, Mild Cognitive Impairment; MMSE, Mini Mental State Examination (Folstein version); NIA-AA criteria, = AD diagnostic criteria based on the propositions of the National Institute on Aging and Alzheimer's Association; NINCDS-ADRDA criteria, AD diagnostic criteria based on the propositions of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association; NA, Not applicable; NS, Not Specified; py, person-years; vEEG, Video EEG; VLOM Ratio, Ratio of Verbal Fluency and Language ACE subscores over the Orientation and delayed recall subscores.



amnesia which describe a temporal gradient for hippocampal-dependence of more or less remote mnemonic traces. However, according to the description by Frankland and Bontempi (150), the episodic component of mnemonic traces seems to escape such dissociation. This led to the multiple trace theory, according to which contextual and spatial information of a given mnemonic trace remain dependent on the hippocampus despite the fact that the semantic component transfers to “cortical only” storage (151). Once again, this is coherent with AD, where episodic memory decline and a loss of hippocampal integrity are amongst the first signs of the disease. These observations converged giving rise to the currently accepted *active system consolidation* theory (152, 153). This framework is still based on the theory of systemic consolidation allowing remote semantic memories to claim their independence from the hippocampus and stipulates that this takes place with the help of repeated reactivation or replay of time-compressed activation patterns of previous wakeful periods (154).

Replay is thought to occur during NREM sleep and, to a lesser extent, during different wakeful behaviors (155). During these states, the synchronized activity of several oscillators enables the transmission of these traces from the hippocampus to the cortical networks. While this allows for stabilization and transfer of the mnemonic traces to cortico-cortical centers during NREM sleep (systemic consolidation), REM sleep might be implicated in more local stabilizing processes (synaptic plasticity) (152, 156, 157). This role of REM sleep is also consistent with previous hypotheses which suggest a “local synaptic organizer” or “integrator” function for this specific sleep stage (158–160).

As for the role of NREM sleep-related consolidation, three hierarchically organized, nested oscillatory patterns seem to be essential (**Figure 3**) (152, 162): cortical slow oscillations, thalamo-cortical spindles and hippocampal sharp-wave ripples. But what makes these oscillations so crucial for memory consolidation?

**Cortical slow oscillations (SO)** are  $\sim 0.75$  Hz oscillations whose function in memory consolidation is suggested by the observed positive correlation between learning and the SO amplitude during the ensuing rest period (163). Moreover, inducing SOs via transcranial magnetic stimulation increases the consolidation of hippocampus-dependent memories (163, 164). The other role of these large-amplitude waves is the synchronization of neuronal activity by the creation of fluctuation between hyperpolarizing “down-states,” characterized by attenuated neuronal firing, and depolarizing “up-states” during which firing activity reaches levels observed during wakefulness (157, 165). Most notably, SOs also coordinate the occurrence of thalamo-cortical spindles and sharp-wave ripples (166) whose role in memory consolidation seems to be vital. However, in AD, the quantity of SOs decreases in an  $A\beta_{1-42}$  load-dependent manner (73).

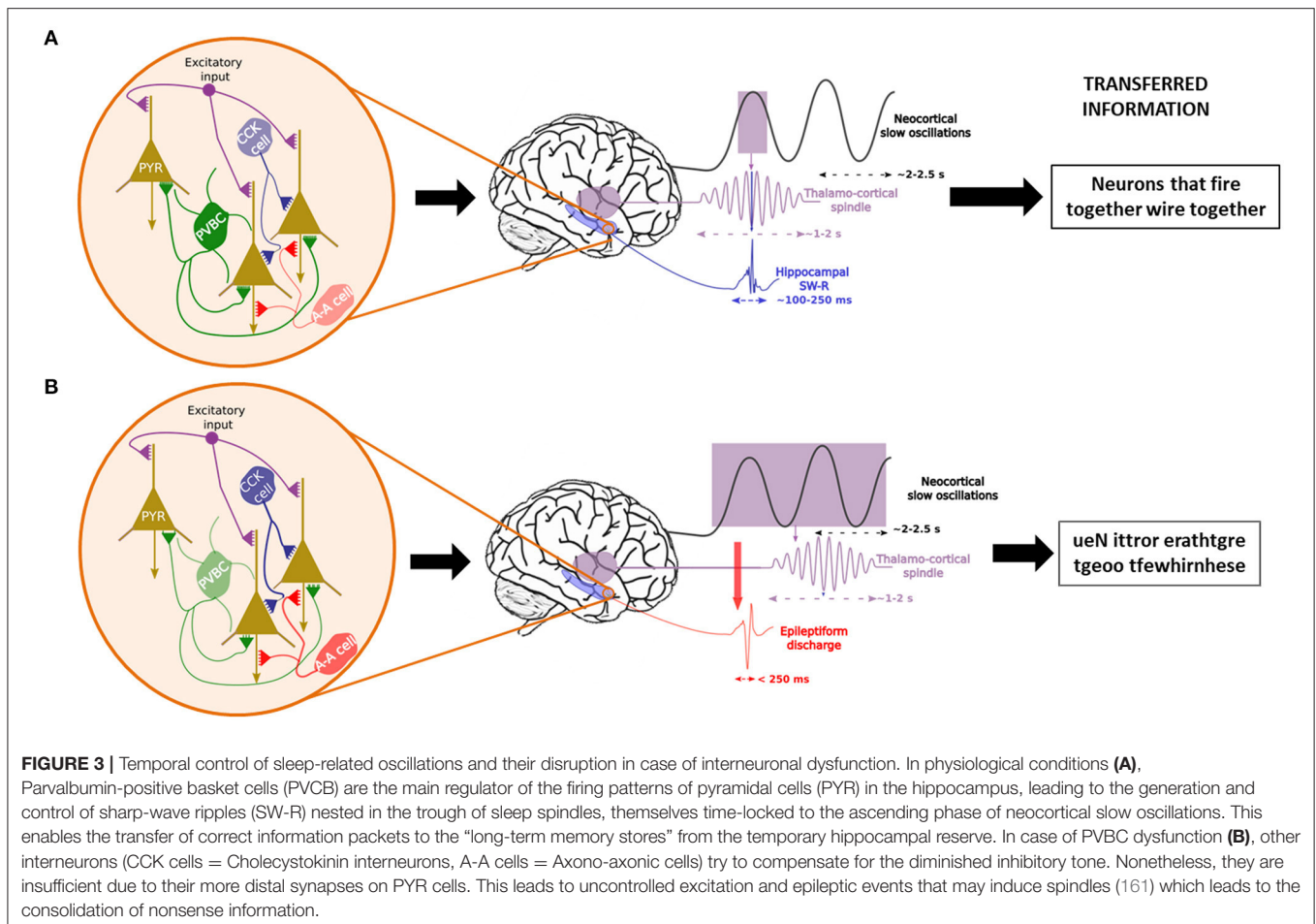
The above-mentioned **thalamo-cortical spindles** are waxing-and-waning oscillations of 0.5–3 s between  $\sim 10$ –16 Hz. Spindles occur on the depolarized up-state of SOs (167), although the literature usually differentiates further between centroparietal fast (13–15 Hz) spindles, indeed phase-locked to the ascending phase of SOs and frontal slow (10–12 Hz) spindles that occur

on later ( $\sim 200$  ms) phases of SOs reviewed by (168, 169). The timing of SO-spindle coupling have been reported to be related to hippocampus-dependent memory processes (170), while the density and duration of spindles during post-learning sleep has been continuously shown to be positively correlated with better performances on declarative or episodic memory-related tasks (171–174). However, during aging, their density and duration decrease, their frequency slightly increases (175–177), and their precise phase-locking on SOs is disturbed (178). These changes are even more marked in AD and MCI (179), which seems to be especially true for fast spindles (180), potentially further impacting memory consolidation efficiency (181, 182). Spindles are hypothesized to be essential to consolidation as they provide the network with a time window without interference from external stimuli (183, 184) during which information can travel from the hippocampus to the cortex.

The transfer of information itself relies on **sharp-wave ripples (SW-Rs)** that are physiological transients of 50–150 ms, with the ripple component in the 140–200 Hz frequency range (185). They arise from the hippocampus, with the sharp-wave component originating from the CA3 and the ripple being generated at the level of the pyramidal cells of the CA1 (185). Recent results also point toward the involvement of the CA2 sub-region as an initiator of the SW-R complex and as a potential origin of SW-Rs arising following learning tasks involving social memory (186, 187). Place-cell recordings in rodents (188) showed that time-compressed replay of past waking behaviors occurs during SW-R bursts that are nested in the troughs of spindles (189–191). SW-Rs are therefore regarded as the ideal “packages” of information transfer between the hippocampus and the neocortex (149, 166, 190, 192). Their role in memory consolidation has been suggested by experiments whereby ripple activity is selectively suppressed during post-training rest periods in rodents (193, 194) which led to poor post-rest recall performances. Moreover, as is the case with spindles, a marked increase in ripple activity was observed following learning in both rodents and humans. As we will see in the next paragraph, they are also altered throughout AD progression.

## Derailed Oscillations During Sleep and Epileptic Activity

The finely tuned oscillatory activity between SOs, spindles and SW-Rs can be seen as the meticulously synchronized playing of the many members of an orchestra. However, as with complex symphonies, at such high levels of precision there is always an increased probability for errors that can quickly derail into cacophony. This same risk is highly inherent in consolidation-related oscillations where a minor discrepancy suffices to push the system into a pathological state. In fact, even before the theories of memory consolidation, it was suggested that the mechanisms of remote memory formation and those of epileptic activities share many similarities (195, 196). Based on the well-known Hebbian principle (neurons that “fire together, wire together”), this early suggestion evoked a “hijacking” of learning mechanisms, be it learning or epileptic discharges. However,



more recent results incited Halász and colleagues (197) to suggest that epilepsy (even simple EAs) is a derailment of plasticity-related mechanisms rather than an external force that sometimes profit from a pre-existing mechanism to wreak havoc in the brain. This risk is all the more increased for SW-Rs which are the most synchronous physiological oscillations that occur in all mammalian species (155, 185), as ~10–18% of hippocampal neurons, as well as neurons of the surrounding regions fire during SW-Rs (145). SW-R initiation is thought to be a result of the coincidental firing of pyramidal neurons, which generates a massive increase in the activity of the recurrent CA3 network, which leads to the SW-R [see (198) for more detail]. However, instead of unsupervised excitation, this activity is regulated by synchronized perisomatic inhibition by PVBCs which would be essential for maintaining the behavior of physiological SW-Rs [see (196, 199)]. However, when EAs are present, for example due to a dysfunctional transmission from PVBCs to pyramidal cells, there is a marked decrease in SW-Rs (161, 200). Remarkably, during such EAs, all other interneuron types step up their firing rates in response to the fallout of the perisomatic inhibition by PVBCs. Nevertheless, their influence on pyramidal neurons via dendritic synapses is not sufficient to suppress the induction of EAs (Figure 3) (196). What these results suggest altogether is that when PVBCs can respond to the synchronized excitatory activity of pyramidal cells,

physiological transients (SW-Rs) are present, but in the absence of sufficient perisomatic inhibition, the result is a pathological SW-R, most often in the form of EA. This under-inhibition might be achieved either by dysfunctional PVBCs or by an abnormally rapid recruiting of pyramidal cells due to neuronal hyperexcitability with which PVBCs cannot keep up. As we have seen, both of these conditions are present in the AD brain and could explain the sleep-related nature of epileptic abnormalities. These results seem to converge harmoniously with the recent results provided by Caccavano et al. (201), who demonstrated in the 5xFAD transgenic mouse model of FAD that PVBCs receive unusually low levels of excitatory input during SW-Rs, which results in a reduction of almost 50% in their firing rate during SW-Rs. This hypoactivity of the PVBCs leaves the pyramidal cells with an enhanced excitation/inhibition ratio that is highly prone to EA. Even in *in vitro* mouse brain slices that only partially reproduce the network interactions, this gives rise to abnormal SW-Rs with decreased length, increased frequency and wider amplitudes. Interestingly, Jones et al. (202) described a predictor function of early SW-R activity decrease on subsequent spatial memory deficits in a knock-in rodent model (apoE4-KI) of AD. At the same time, two other teams observed decreased SW-R frequency and SO-SWR and SO-spindle coupling in two transgenic models of AD [(203) for 3xTg-AD and (204) for TgF344-AD].

Based on these findings, decreased SW-Rs would lead to less efficient memory consolidation. However, EA-transformed SW-Rs can inflict even more harm than detailed above. Notably, in a rat model of TLE, Gelinas et al. (161) provided further support for the SW-R-to-EA transformation and showed that EAs are associated with memory consolidation deficits. Moreover, they also found that EAs can lead to abnormal induction of spindles, especially during wakefulness but also during REM sleep, even though spindles never occur during this state in physiological conditions. Given that spindles are the safest highway of communication between the hippocampus and the neocortex, information could travel even for the abnormally induced spindles. However, as suggested by Buzsáki (185), these spindles might not carry learning-related traces which would lead to the consolidation of potentially useless or even scribbled cacophony arising from the EAs that elicited them (Figure 3). This conclusion is supported by Bower and colleagues (205), who, in an intracranial EEG (iEEG) experiment on mTLE patients, showed that neuronal assemblies activated during a seizure benefit from the most remarkable learning-associated plastic changes during the post-ictal sleep period. This suggests a potential detouring of sleep-related memory consolidation mechanisms by EA. Picking up on this thread, another team using iEEG recordings also described dysfunctions in memory consolidation stemming from sleep-related hippocampal ictal or interictal epileptic activities in epileptic patients (206). They recently completed this work by suggesting a model that explains the accelerated long-term forgetting often experienced by patients with epileptic activities during sleep (207).

## ANCILLARY DAMAGE OF SLEEP-RELATED EPILEPTIC ACTIVITY IN AD

But sleep-related EA may not only inflict harm on the brain by derailed oscillatory patterns but also by disrupting sleep itself which fulfills many other essential functions besides memory consolidation. We have already noted that EAs are predominant during REMS in rodent models of AD. Therefore, EAs, or their induced spindles, which interrupt REMS, could increase network hyperexcitability by disrupting downscaling functions generally attributed to REMS. In fact, although the role of REMS in memory consolidation is still disputed, findings point to a role in local, synaptic plasticity-related processes, including an impact on the expression of immediate early genes related to consolidation (*ASH framework*), and a general downscaling of synapses leading to the elimination of weak synapses (*Synaptic Homeostasis Hypothesis*). Downscaling of synapses is essential since the sum of synaptic weights in the brain should maintain a quasi-constant value to avoid hyperexcitability (145). The probability that REMS is implicated in such processes is all the more increased since evidence shows that firing rates decrease brain-wide after the short periods of REMS (159). Therefore, the interruption of theta oscillations during REMS by EAs or induced spindles might lead to an incomplete reduction of synaptic weights and elevated baseline neuronal

activity, further aggravating pre-existing activity. This self-aggravating cycle between sleep and epilepsy could add yet another vicious cycle to the path between epilepsy and AD (Figure 4). Finally, as we mentioned earlier (see paragraph 1.4), we should not forget that even without EA, sleep structure in itself is already impaired early in the course of AD (208), which might further aggravate both memory consolidation and the epileptic phenotype.

We have seen the various pathways through which epileptic activity might aggravate the progression of AD (summarized in Figure 4). The concerning number of vicious cycles in this system seems to justify considering an amelioration of epilepsy-detection in AD patients and the treatment options of these aberrant network activities.

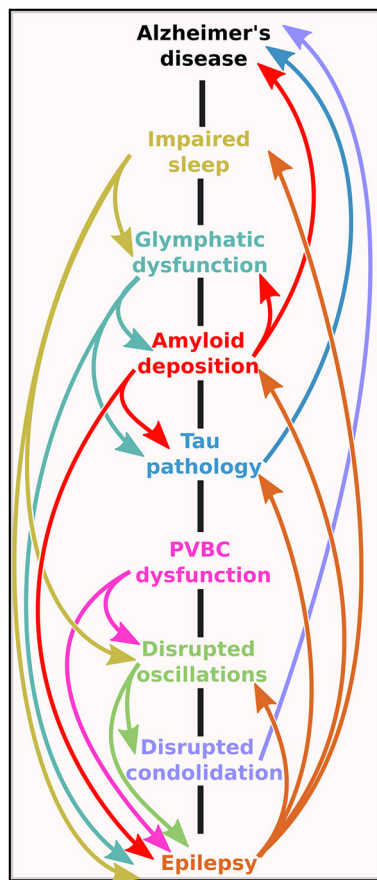
## METHODOLOGICAL CONSIDERATIONS, DIAGNOSTIC HURDLES AND TREATMENT OPTIONS

### Methodological Considerations for Research

As it has been emphasized throughout this review, the prevalence of EA in AD is highly variable across studies, which we suspect is the result of methodological choices. In fact, several points of divergence create a snowball effect of inconsistencies in the results.

One such point is the diagnostic criteria of Alzheimer's disease. While most papers follow NINCDS-ADRDA or IWG-2 criteria, some authors base the diagnosis on MRI markers (26), or do not differentiate between AD and other types of dementia (116, 118, 129, 209, 210). Finally, retrospective or prospective population-based studies or projects using datasets from large memory clinics or databases cannot verify the diagnostic method for all patients in the cohort (122, 123, 127, 128, 131, 132, 211). Using only imagery and neuropsychological data for diagnosis might inflate the percentage of "AD" patients with epilepsy, given that patterns of cortical atrophy and cognitive symptoms can be highly overlapping in epilepsy and AD (212) or aMCI from which the conversion rate to AD is exceptionally high (110). On the other hand, while approximately two-thirds of all dementias are due to AD, mixed cohorts might also impact the observed prevalence of epilepsy and dementia due to AD.

Another important consideration is study type. Many previous publications consisted of retrospective cohort studies, which, besides having an inherent risk of including a relatively high percentage of misdiagnosed dementia patients, are often carried out by a meticulous sweep of patients' medical history [e.g., (123)]. Even when the problem of misdiagnosis is averted by the availability of post-mortem pathology reports confirming AD [as in (126)], using medical reports to infer the presence of epilepsy brings in several biasing factors. First of all, although convulsive seizures might be observed by caregivers, they might not be reported by the patients themselves, who might not recall such events. Secondly, while in some cases EEG results are available in the medical



**FIGURE 4 |** The bidirectional road between Alzheimer's disease and epilepsy. Note that the mechanistic pathways contain several vicious cycles, meaning that no matter at which level the initial imbalance is on the road, the self-amplifying potential of the system could lead to the onset of one (or possibly both) pathologies.

history, they are primarily standard 20–30-min EEGs without periods of sleep (25, 26). In fact, we have discussed at large that subclinical network anomalies or silent seizures in AD occur mainly during sleep (24–26). Accordingly, Horvath et al. (213) have shown that a 1-h EEG recorded between 0:00 and 8:00 (potentially including periods of sleep) is 16.5 times more sensitive in detecting epileptiform events than a recording of the same length during the day (from 8:00 to 16:00, mainly containing wakeful sections). Moreover, recording length strongly correlates with EEG sensitivity. Therefore, there is no comparability between results obtained with 30-min recordings and those obtained after eight or even 24-h recordings.

Another critical point is the question of inclusion and exclusion criteria. While it is now well established that EA can occur at any stage of the disease, even before symptom onset (25, 112), its prevalence still shows strong correlations with disease severity. Therefore, the form of AD or the inclusion threshold for disease severity (measured by MMSE, CDR, ACE or any other commonly used neuropsychological evaluation) could

also influence results. This also applies when deciding whether or not to include patients with a known history of epilepsy (or even with ASM treatment). In such cases, separating the results according to pre-existing epilepsy, as done in the recent article by Lam et al. (24), could be a solution to avoid bias. However, other neurological antecedents (e.g., stroke, tumors, previous head trauma, etc.) and pharmaceutical treatments that suggest underlying psychiatric or neurologic comorbidities (such as antipsychotics, antihistamines, benzodiazepines, and of course, ASMs) should be considered in exclusion criteria (24). Moreover, a large proportion of AD patients are prescribed antidepressant medication, even in the early stages, and including these patients presents another issue. While such medication might not have a substantial effect on seizure susceptibility (214), it strongly impacts the sleep cycle, especially REM sleep and sleep fragmentation (215). As mentioned earlier, EA in AD is strongly linked to sleep. Therefore, including patients who are on medications that significantly modify the sleep-wake cycle has a high probability of interfering with EA detection.

Another critical question is whether, technically speaking, EA in AD can be diagnosed. First of all, for obvious medical and ethical reasons, EEG is recorded using scalp electrodes in the absence of drug-resistant epilepsy. However, as it was demonstrated in a study using implanted Foramen Ovale (FO) electrodes in two AD patients, ~95% of the spikes captured by iEEG are not detected on simultaneous scalp EEG recordings (135). Furthermore, this finding was also echoed by a case-study using FO electrodes in a fronto-temporal dementia patient, indicative of the epileptic phenotype being a shared feature of several neurodegenerative diseases (216). Moreover, even with surface electrodes, most of the EA are captured at the level of the temporal lobe (24, 25, 135). However, they are often only detected by temporo-basal electrodes, which are not included in the electrode set used in most setups. Finally, other methods such as MEG have the potential to rank between iEEG and surface electrodes, but MEG has only been applied in two studies on this topic so far with quiet discordant results (25, 113), and given the scarcity of studies having access to MEGs, this incoherence is not likely to be remediated in the near future. These issues already present a bias toward high underestimation of EA in AD. However, the more pressing problems are the detection method and sensitivity. In fact, the majority of authors used manual detection by experts, with some papers relying on one expert (111), or two neurophysiologists (26) or epileptologists (119) or a combination of these specializations (25). Most, but not all experts are blinded to the condition of the participants (AD or control) while analyzing the data, which could introduce further bias. Another problem that probably prevents EA detection, even for automatic detection, is the modified characteristics of brain activity in AD patients. In fact, their EEGs show more significant slowing than is seen during healthy aging (141, 142), which is most prominent during REM sleep (217). Therefore, these EEGs show a significant amount of slowing-related activities, which makes the classification of some pathological or unusual but physiological activities rather tricky.



Finally, other issues regarding the scoring of EEG activity are epileptic transients of sleep or sporadic sleep spikes (SSS) and temporal intermittent rhythmic delta activity (TIRDA) which have already been linked to mTLE, but which have not yet been established as pathological events. However, as Lam et al. (24) pointed out, a surprising increase in SSS is seen in AD patients with underlying EA. Defining when these events should be re-evaluated would be of interest to improve the characterization of EA as opposed to benign EEG patterns. It is all the more important since the boundary where EEG patterns are considered as subclinical or interictal activities, is already blurred. The most common definition that authors give for spikes is “sharp waveforms 20 to 200 ms, clearly distinct from ongoing background activity, with an associated subsequent slow-wave” (25, 26), but this is not unified across studies. In fact, the prevalence dropped from 42 to 48% respectively in the Vossel and Horváth studies to as little as ~6% in the study by Brunetti et al. (113), who defined EA based on stricter morphologic characteristics. However, contrary to preclinical studies (218, 219), there are no recommendations or guidelines for EA detection in AD patients, considering the many abnormalities and specificities already present on EEGs. Such recommendations would greatly facilitate the diagnosis of epilepsy or subclinical EA in AD patients. In their article, Sen and colleagues (220) have already made a step toward formulating such guidelines for neuropsychological data collection. They suggest standardized, culture-independent, short batteries of tests, with baseline evaluation and longitudinal tracking. Based on snippets from existing studies and an ongoing clinical study (ClinicalTrials identification: NCT03923569), we suggest some methodology-unifying considerations concerning the measurement and detection of epileptic events in AD in **Textbox 1** in the hope of amplifying data on mechanisms that have already been suggested but which have still not been replicated.

## Diagnostic Hurdles in Clinical Practice

As is the case of all AD-related issues, AD-related neuronal hyperactivity should be treated as soon as possible, especially since AD patients with EA seem to benefit from such treatment, as we will see below. However, the detection of subclinical epileptic activity is subject to high methodological variations, not only in research but also in clinical practice. This variability in methodology might be one of the reasons why this issue is not yet profoundly nested in current clinical practice. As we have emphasized throughout this review, EA is mainly subclinical in AD (221) and often goes unnoticed by the patient and the caregiver(s). Hence, even if periods of loss of contact or stereotypical behaviors/automatisms are present during ictal or interictal activities, they might be considered part of the disease progression rather than an epilepsy-related event. To address this issue, questionnaires or interviews targeting these symptoms could be applied preferentially in the presence of caregivers [an example of such a questionnaire, elaborated from previous observations (26, 221–223) is provided in **Supplementary Material 1**].

1. When possible, the diagnosis of AD should be based on CSF or amyloid-PET
2. Atypical AD patients should be clearly distinguished in the cohort and verified in the analysis [e.g. (25)].
3. EEG analyses should be based on at least two sources, either the combination of automatic detection with a manual verification a posteriori, or by two experts, preferably blind to the study group.
4. IED should be clearly defined, using standardized criteria: 20-200 ms spike or sharp-waves followed by disrupted background activity (25, 112), with a multiphasic character [suggested by (113)].
5. All types of detected epileptic/epileptiform events should be quantified and reported separately (IEDs, SWDs, longer rhythmic synchronizations, seizures, and preferably SSS).
6. A graded quantification according to the certainty of detection should be applied, differentiating between certain and doubtful events [e.g. (24)]
7. The presence of patients with known epilepsies should be stated - and handled as a separate group if necessary.
8. Given the potential impacts of different medications on seizure activity, pharmacologic treatments should be stated and verified.
9. Given the subclinical nature of epileptic activities in AD, EEGs should be accompanied by interviews with caregivers on the occurrence of symptoms related to seizures [suggested by (124)]. One such questionnaire is provided in **Supplementary Material 1**.
10. VEEGs should systematically include a full EEG setup with at least 27 scalp electrodes including lower temporal electrodes.

**Textbox 1.** Methodological considerations for clinical research.

In any event, unless overt signs of epilepsy are present, EEG, especially of longer duration including periods of sleep, is rarely requested for AD patients. An exception applies to patients with suspected sleep apnea who undergo a polysomnographic examination coupled with EEG. In fact, until portable, easily applicable, useable-at-home technologies that measure brain activity during the night become widely available, more systematic screening for epileptiform activities in AD is unlikely to take place. Although FO electrodes are especially well-positioned to capture EA from anterior temporal and mesial-temporal structures, they are not feasible for the entire AD population due to the invasive nature of the procedure. Video-EEG also seems to be a relatively reliable method. However, most healthcare systems across the globe are probably unprepared to screen the entire AD population. The study by Vossel (25) indicated that MEG might be a highly sensitive non-invasive detector of EA, especially if it can be backed by computational approaches that enable the detection of signatures

of EA even in the absence of large amplitude deflections (33, 224). However, MEG also requires expensive, specific and, more often than not, inaccessible equipment for EA screening.

On the plus side, although methodological differences led to highly variable incidence rates across previous studies (see **Table 1**), the different inclusion criteria helped to narrow down the patient group that is at higher risk for AD and EA comorbidity and for whom a vEEG screening would be the most important. For sporadic cases, this includes patients with an earlier age of onset (< 65 years), APOE4 allele(s), higher educational levels or a very apparent discrepancy between cognitive symptoms and the degree of brain atrophy during the early stages (indicative of a high cognitive reserve). In FAD, given the extremely high prevalence of epilepsy (11) and the relatively low number of patients affected by this condition (1% of all AD cases), systematic screening by v-EEG at cognitive decline onset should be conducted. Detecting and treating EA before seizures with high levels of network reorganization potential might help to slow down cognitive decline.

Another crucial diagnostic consideration is that the link between AD and epilepsy is increasingly considered to be a bidirectional road (106, 225). It has been shown that while AD patients have an increased risk of developing epilepsy, epilepsy patients also present a 2-3.6-fold risk of developing dementia compared to the non-epileptic population (106, 129). Unfortunately, in current clinical practice, just as AD-related silent EA often goes unnoticed, there is also a risk that instead of being diagnosed as AD, the cognitive complaints of epilepsy patients might be mislabeled as Epileptic Amnesic Syndrome, a less severe condition with good responsiveness to ASMs (221, 226). This could lead to suboptimal treatment plans for dementia. But even if a diagnosis of AD is not justified, an altered path of cognitive decline in epileptic patients compared to healthy older adults has been observed throughout the literature, as was summarized by Breuer and colleagues (2016) (227) in favor of *accelerated cognitive aging* for people living with epilepsy. According to their review, while a younger age of epilepsy onset seems to be a risk factor for such an increase in cognitive decline, it was emphasized that LOEU patients are no exception. As they underscored, the brain of older adults tackles several problems at a time, such as elevated levels of inflammatory brain response, comorbidities that affect cognition (such as AD), the use of several medications with often interacting effects, and, notably, a potentially decreased cognitive reserve. This decrease means that they cannot compensate for epilepsy-induced cognitive problems like younger epileptic patients, which leads to an even more accelerated cognitive decline (227). This is consistent with the finding that AD patients with epilepsy generally have higher educational levels (25, 26). In fact, patients with high cognitive reserves can compensate even for comorbid AD and (subclinical) epilepsy for longer periods, and therefore present only mild symptoms even in more advanced stages of AD in terms of biomarkers. However, once the macro- and microstructural damages reach a level where compensation is no longer possible, the disease seems to progress at an excessive speed, as is the case for AD patients with EA (**Figure 5**) (25, 130, 137). However, this conclusion might beg the question of what happens to AD

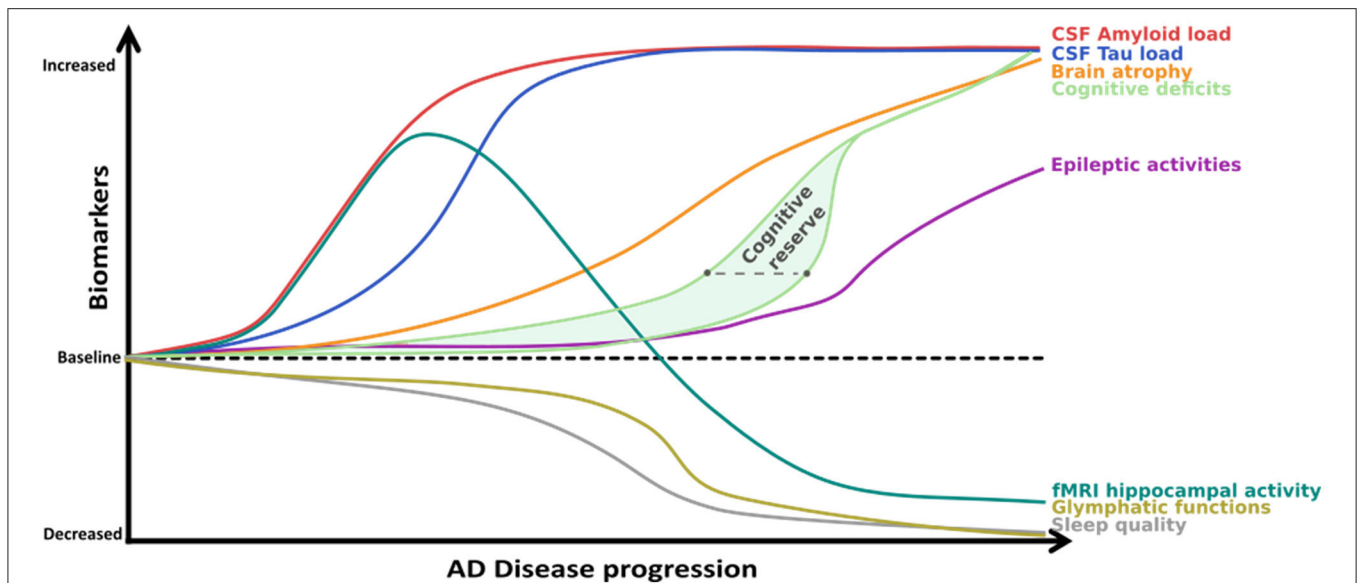
patients with EA with more modest cognitive reserve. It might be that the accelerating effect of the comorbid pathologies without the shield of cognitive reserve is such that polysomnographic examination might not be possible in some cases even shortly after diagnosis due to agitation.

To conclude on the bidirectionality of the link between AD and epilepsy, we would like to address a notion from recent work by Sen and colleagues (220). They noted that it could not be ascertained whether epilepsy simply facilitates dementia by lowering brain reserves or whether it produces it. According to our interpretation, both suggestions are valid: (sub)clinical epilepsy, by continuous insults to neuronal circuits and through all the harmful effects we reviewed, leads to an increase in “AD-inducing” substances (especially if clearance mechanisms are impaired) and, through the reorganization of neuronal networks, reduces brain reserve that compensates for dementia-related cognitive loss. In any event, screening of epilepsy patients for AD biomarkers, especially those at higher risk for dementia, should be systematic to enable earlier detection which might ensure a more efficient, partially preventive (228), partially medical treatment. This applies to patients at risk for AD, including those with LOEU, patients with cognitive complaints, or a family history of dementia. The importance of this screening came to light through a recent publication (106) which found abnormally decreased CSF A $\beta$ 1-42 levels in LOEU patients, with a hazard ratio of 3.4 for progressing to AD. That is why the medical recommendations that summarize the most important notions for clinical practice from this paragraph (**Textbox 2**) include both pathologies. This seems all the more justified since epileptic auras have also been described as preceding MCI onset by 4–7 years, leading to the suggestion of an “epileptic variant” of AD (222). Of note, cognitive functions (measured by MMSE scores) remained relatively stable at the one-year follow-up for almost all participants, suggesting a potentially positive impact of ASMs in preventing a disease course that is noted to be generally worse for the AD-epilepsy comorbidity (222).

## Treatment Options and Considerations

Despite the widespread harmful effects of EA, it is quite probable that its attenuation in AD patients is not the star that sashays on the red carpet and dazzles the world with an all-in-one cure to AD. However, silencing network hyperactivity might lead to slower disease progression, a decrease in disease burden and should be considered as part of the standard medical treatment plan for AD patients. In 2007, it was estimated that delaying AD onset by as little as a year could lead to 9.2 million fewer cases in 2050 (229). A one-year delay could have a substantial impact on people living with AD and could also have major socioeconomic impacts, given that the global cost of dementia was estimated at US \$818 billion per year in 2015 (230), most of which can be attributed to AD.

We have just explored whether neuronal hyperactivity might accelerate disease onset and progression. If this is the case, treatment in AD patients at the earliest possible moment might delay disease progression. A rather impressive quantity of literature is now available on treatment options for AD-related epilepsy and was recently reviewed at length by two separate



**FIGURE 5 |** Proposed model of biomarker and cognitive decline progression over the course of AD. High cognitive reserves compensate on a functional level for marked Amyloid and Tau aggregation and atrophy may lead to delayed diagnosis. This might explain why EA is most often detected in highly educated participants who are (seemingly) in the mild stages of the disease. However, once compensation is no longer possible and EA becomes more frequent or probable, the cognitive decline might be steeper, which is compatible with the fast progression noted in AD patients with E/EA. Figure adapted from (33) with permission. The gray dashed line represents the approximate time of diagnosis.

research groups (33, 231). These results accentuate the positive effects of Levetiracetam (LEV) both in rodent models and in AD patients. In fact, LEV led to improved memory performance in aged rats in the study by Koh et al. (232). Interestingly, the treatment only benefited older but not younger animals, suggesting that this ASM treats excess neuronal activity without inducing potentially detrimental decreases in baseline activity. This preclinical result was also replicated in a recent clinical study with AD patients in mild-moderate stages of the disease (MMSE > 18) in which a marked improvement in executive functions (measured by the Stroop test) and in navigation capacities (as shown by results on a virtual route learning test) were noted after only 4 weeks of treatment with low doses of LEV (125 mg/day) (114). Importantly, beneficial effects in the above-mentioned tests were only observed for patients with EA, further suggesting that LEV targets only excess neuronal activity and that this excess activity impairs cognitive functions. Among the other clinical studies on ASM treatment in AD-related EA, that of Cumbo and Lorigi (233) should be noted, where the impact of long-term use of several ASMs (LEV, phenobarbital (PB) and lamotrigine (LTG) in AD patients with concomitant epilepsy was examined (n = 38, 28 and 29 respectively in the three treatment arms). They found similar responder rates between the three ASMs (71% responded to LEV, 59% to LTG and 64% to PB) which were also comparable to the rate noted in epilepsy unrelated to AD (~66%). However, while LEV had relatively few side effects and slightly improved cognitive functions, PB negatively impacted cognitive functions. On the other hand, LTG was associated with a slight decline in cognitive functions but with an improvement in mood. Given the encouraging results

with LEV, it is not surprising that most clinical trials tested this medication rather than other ASMs. For example, another study of chronic treatment with LEV in cases of severe AD with late-onset seizure found that 72% of the patients were seizure-free after the one-year follow-up, only 8% were non-responsive, while 16% had undesirable side effects (234). In addition, in a study with a small sample size of 7 AD patients, Musaeus et al. (235) described normalized EEG activity after a single dose of 7.5 mg/kg (~500 mg) of LEV but not after 2.5 mg/kg. This normalization was manifested by decreased coherence in slower and increased coherence in faster bands. In two other studies with aMCI patients, Bakker et al. (236, 237) showed that a 2-week treatment with lower doses of LEV (most notably 125 mg) lead to decreased hippocampal hyperactivity (as measured by fMRI signals in the CA3 and the entorhinal cortex). Although this relatively short-term treatment did not seem to affect long-term memory performances, it did improve pattern separation, which is an important component of episodic memory. Furthermore, it appears that ASMs (including LEV) do elicit such a positive effect on cognition even with chronic treatment in LOEU patients without AD (238), especially in patients diagnosed with focal seizures. Although the amount of randomized case-control studies remains too low for further conclusions (231), preclinical results of antiepileptic treatments in AD are promising [reviewed by (27), and (239)]. They suggest decreases in excess neuronal activity (61, 240), improved cognitive functions (240–242), reduced amyloid plaque load in some cases (242), or a rescue from the depletion of neuronal stem cells due to network hyperactivity [(241), see (27) for a review]. LEV also seems to prevent tau-dependent depletion of the potassium channels of

1. A vEEG including at least an hour of sleep should be systematically undertaken for patients with sporadic AD if at least one of the following conditions applies: early onset (< 65), presence of APOE4 allele(s), high discrepancy between brain atrophy (MRI) and cognitive functions suggestive of high cognitive reserves, or other indicators of a large cognitive reserve (educational level, occupation, etc.)
2. A vEEG including at least an hour of sleep should be systematically undertaken for patients with familial AD.
3. ASM treatment of patients with epileptic activities should be considered, mainly LEV, until further data is available on other drugs.
4. A search for AD biomarkers should be undertaken for patients with epilepsy if at least one of the following conditions applies: cognitive complaints, late onset epilepsy of unknown origin (especially with indications of a large cognitive reserve), family history of dementia.
5. The importance of improving sleep quality for AD patients should be recognized and a part of the treatment process whenever possible. Sleep apnea or other sleep-related pathologies (e.g. REM-sleep behavior disorder or restless leg syndrome, confusional arousals) should be identified in case of sleep complaints reported by the patient or by the primary caregiver.

**Textbox 2.** Recommendations for clinical practice.

the Kv4.2 type which were mentioned earlier as being implicated in neuronal hyperexcitability and further aggravate the above-mentioned vicious cycles (61). Finally, LEV restores impaired LTP function in the perforant path toward dentate granule cell synapses and normalizes levels of NPY, Fos and calbindin proteins, all of which are related to excess neuronal activity (240). Moreover, many potential pathways for treatment are under investigation, and have been reviewed recently (33).

It is important to note that the data that is currently available mainly describes the effect of LEV although other, less explored molecules should also be tested, especially given the circadian variations of EA activity in AD patients. In fact, since EA are predominant during sleep, a posology including a bedtime dose of ASM might be the most effective way to tackle these anomalies. Moreover, limiting the strongest effect of the ASMs to sleep intervals could also prevent most of their side effects during wakefulness. On the other hand, the half-life of LEV is rather short, and even residual impacts are improbable after an entire night. Consequently, taking it before sleep could lead to an overspill of EA during wakefulness. This might be a rationale for testing other available treatments with a longer half-life. However, until more data are available on the subject, LEV seems to be the most effective treatment of EA for AD patients of all the rarely tested ASMs in this field.

Finally, sleep-related EA might only be one of the ways through which sleep (and related consolidation processes) is

altered in AD. We have seen that fragmented sleep could affect amyloid and Tau clearance from the brain by impeding glymphatic functions, and it has been shown that altered sleep quality is linked to Tau and Amyloid deposition alike (76, 243). Attempts at remediating fragmented sleep (due to apnea, confusional arousals, restless leg syndrome, REM sleep behavior disorder or other causes) should be made as early as complaints concerning sleep quality are reported by the patient or by their caregiver(s), especially since the literature on the close relationship between sleep quality and cognitive decline is growing daily. A morning dose of donepezil has been suggested to counteract REM sleep decline and REM-sleep related EEG slowing. However, other pharmacological treatments are still under investigation for AD (208). Non-pharmaceutical treatments such as bright-light therapy show encouraging results and could be considered, along with psycho-educational measures and behavioral changes aimed at improving sleep (daily physical activity, less time spent in bed during the day, more time spent outside in natural light) (208).

## CONCLUSIONS

In this paper, we reviewed how the often behaviorally masked epileptic phenotypes in AD (or possibly, vice versa) might be unmasked and aggravated during sleep. Sleep is a crossroads where AD-related proteins can propagate unsupervised and when epileptic activities are more likely to happen through the occurrence of highly synchronized consolidation-related neuronal activities in under-inhibited networks. We also aimed to draw attention to the risk that such activities might cause cognitive decline, in particular, difficulties with memory consolidation and further insult to the already fragile hippocampal formation by chronic reorganization of neuronal circuits. We emphasized the importance, or rather, the absolute necessity of early screening during medical care, for both epileptic events in AD and AD biomarkers in epilepsy, to enable early medical intervention and to prevent the escalation of neuronal and generalized brain damage. Given that such screening is impossible in the entire AD and epileptic population, we filtered homogenous results from previous findings to characterize patients at risk. Finally, we briefly summarized the available knowledge on the relationship between AD and epilepsy, which has grown incredibly since the first observations of Blocq and Marinesco (3) and is still growing daily. However, it will be challenging to arrive at clinically relevant conclusions from coherent results without unified methodologies across research teams. As others have noted before us, such coherence will only be possible by comparing cross-study samples, or through joint data acquisition in large-scale collaborations.

## AUTHOR CONTRIBUTIONS

ABS contributed to the conceptualization of the review, collected references, and drafted the manuscript. BC, LD, EB, JC, JP, and LV contributed to the conceptualization of the review and provided



feedback. JC and BC organized references. FG and JC devised the supplementary questionnaire and provided feedback. LV and LD supervised the project. All authors contributed to the article and approved the submitted version.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fneur.2022.836292/full#supplementary-material>

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**Title:**

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 (I) to better characterise epileptic events in the Tg2576 mouse model throughout the sleep-wake cycle and disease progression *via* electrophysiological recordings and (II) 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.

## KEYWORDS<sup>1</sup>

Epilepsy, Tg2576, Alzheimer's disease, Sleep, Noradrenaline, theta oscillations

## 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 subclinical epileptiform events on their electroencephalogram (EEG) compared to age-matched healthy individuals

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<sup>1</sup> **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.

(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.

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 three 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 six 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 to 7 months and 8 to 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 (Cattaui 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  $\alpha_{1D}$  adrenoreceptor subtypes or *via* combined activation of the  $\alpha_2$  and  $\beta_2$  receptor subtypes (Clinckers 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  $\alpha_1$  adrenoreceptors.

## **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 six 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 two 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 six-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 (A-M 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



two 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 four consecutive days as follows (**Suppl. Figure 1.**):

On the habituation day, mice were connected to the EEG for 11 hours. Throughout the following three days, mice were connected to the EEG each morning at 9 a.m. and were returned to their home cage at 5 p.m. They were injected with a different pharmacological agent during each of these three days at two 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 three, Prazosin hydrochloride was administered (for details on drug preparation, see the paragraph below). One transgenic mouse and two 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, three 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. Figure 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 four 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, Massachusetts, United States). Finally, the figures and the nonlinear fit were generated via the Prism 5 software (GraphPad Software Inc., La Jolla, CA, USA).

## 3. RESULTS

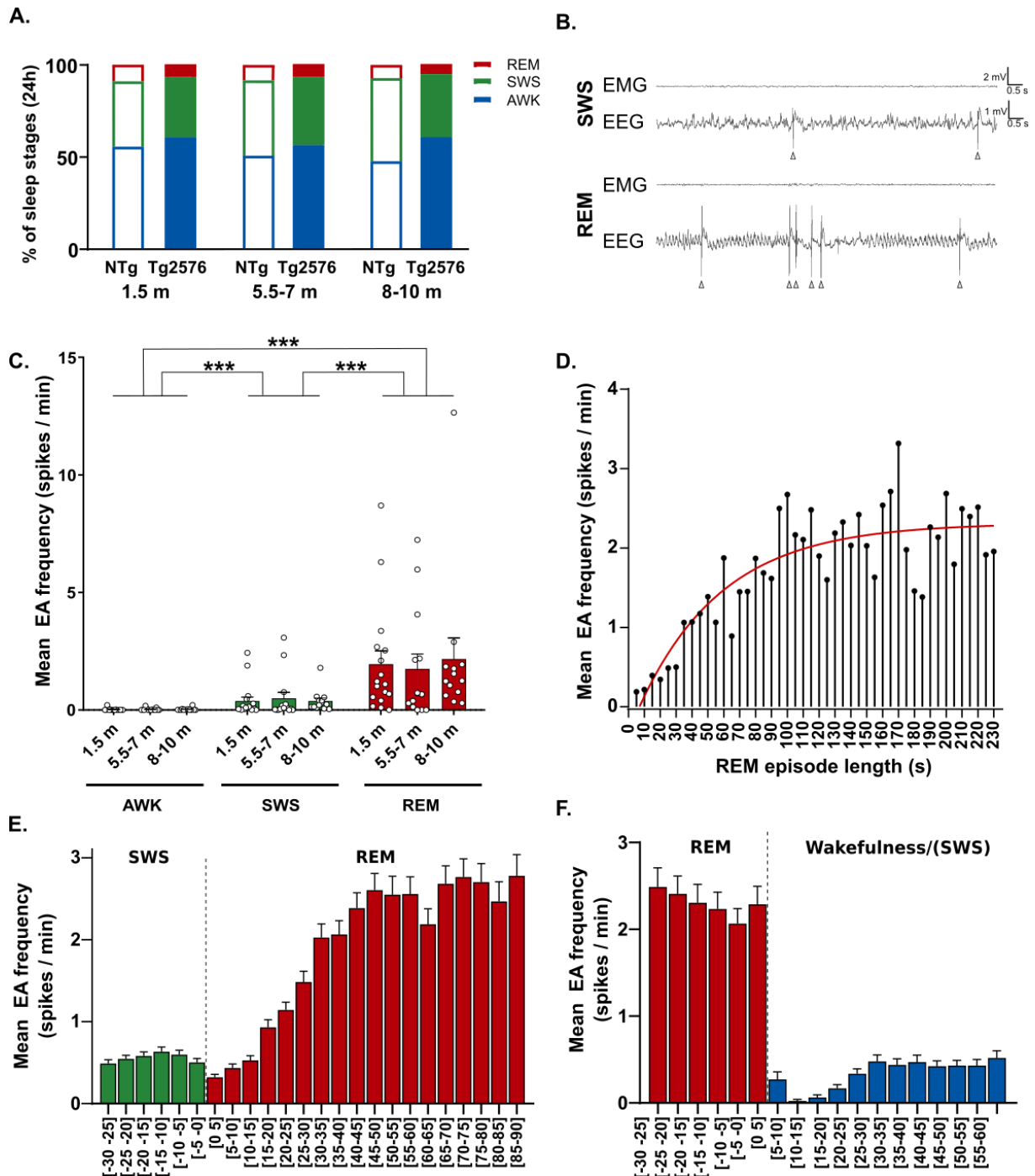
### 3.1. Alterations of REM sleep and SWS in Tg2576 mice across the lifespan

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 ( $F(2, 75) = 2.96$ ,  $p = 0.06$ ; **Figure 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 six spikes were observed across all NTg animals recorded).



**Figure 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 three 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. AWK = Awake, SWS = Slow-wave sleep, REM = Rapid-eye movement sleep. \*\*\*:  $p < 0.001$ .

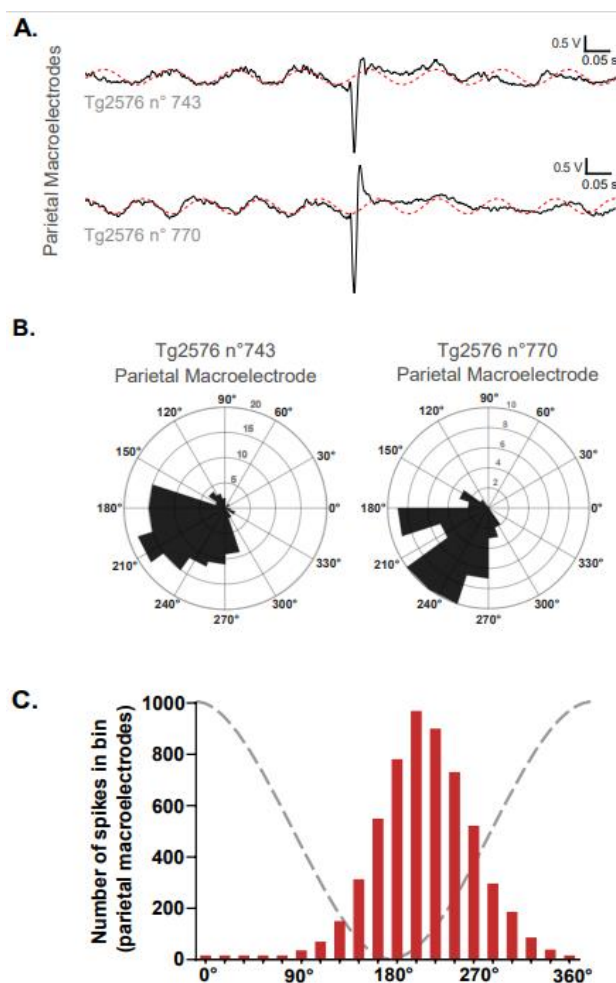
First of all, we observed that EA frequency (spikes/min) was highly dependent on the sleep-wake cycle (**Figures 1B, C**. Friedman test:  $\chi^2(2) = 75.26$ ;  $p < 0.001$ , **Suppl. Table 3**). Specifically, post hoc tests revealed a gradual increase in EA from wakefulness to SWS ( $t(84) = 4.67$ ,  $p < 0.001$ ) 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 six Tg2576 mice (four 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 (**Figures 1D, E**). Indeed, the data were well fitted by a nonlinear fit using an exponential

plateau model (adjusted  $R^2 = 0.72$ , **Supp. Table 4**, 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 (**Figure 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.



**Figure 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.

First of all, on a qualitative level, spikes brutally disrupted ongoing theta oscillations, sometimes for seconds and most of the time disrupting its rhythmicity (**Figure 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 seven spikes and another with only nine, **Figure 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 (**Figure 2C**), which corresponds to the phase when pyramidal cells are most likely to fire action potentials (Klausberger et al., 2003).

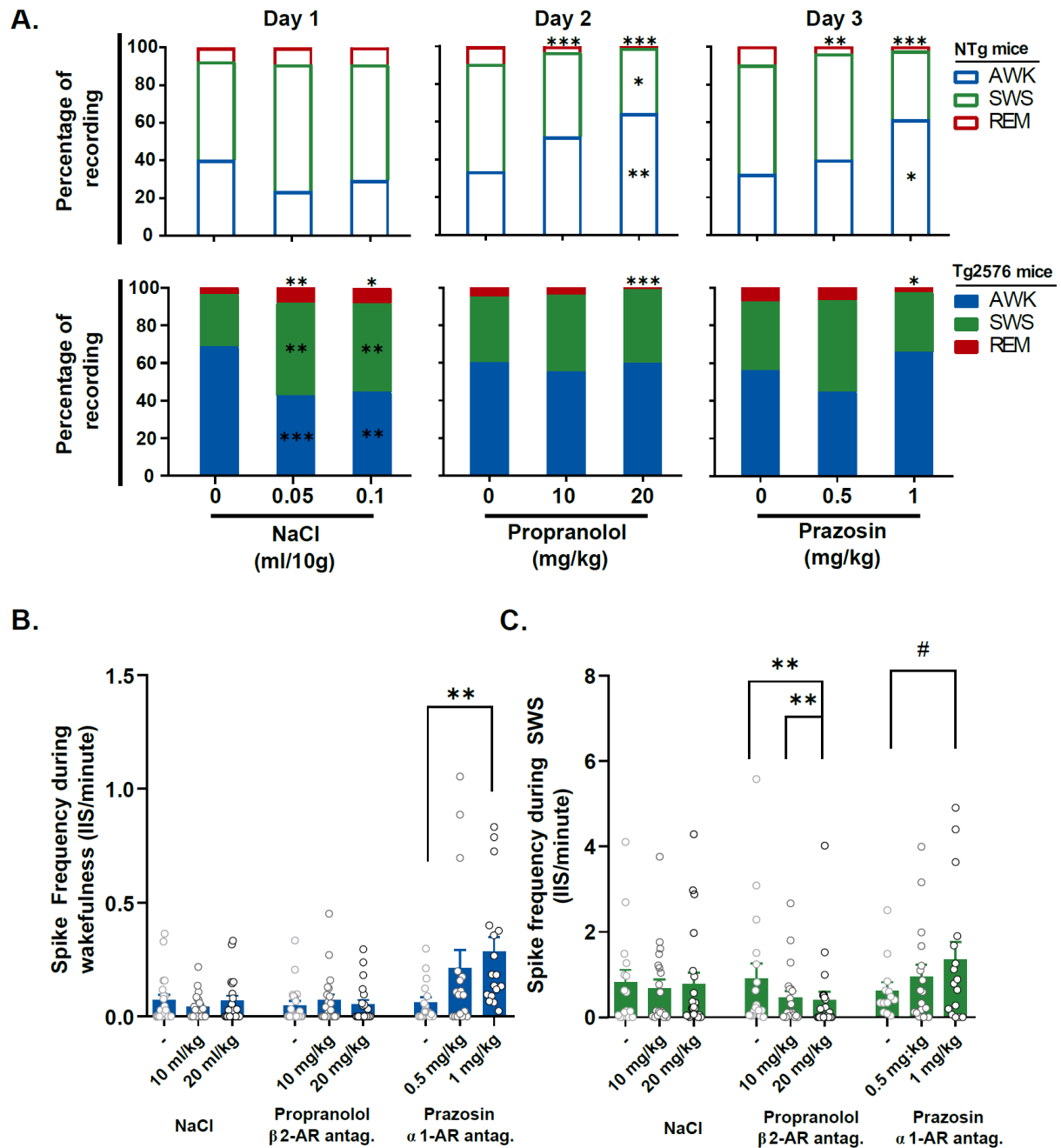
#### **3.4. Opposing effects of the $\alpha 1$ - and $\beta 2$ -AR blockade on spike frequency**

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 two 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, Figure 3A, Suppl. Table 5). As mentioned above, one 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 ( $M_{wake} = 64.5 \pm 23.0$  % 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 ± 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 two vigilance states (Suppl. Table 5). However, the treatments still decreased REM sleep in Tg2576 mice (REM = 1.0 ± 1.67 % of the total recording time after the large dose of propranolol and 2.5 ± 3.5 % following the same dose of prazosin vs 7.8 ± 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 (**Figure 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 three pre-injection periods,  $\chi^2(2) = 1.79$ ,  $p = 0.41$ ). The Friedman tests on the three 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 ± 0.09 at baseline, 0.29 ± 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.





**Figure 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.

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 (**Figure 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:  $\chi^2(2) = 18.35$ ,  $p < 0.001$ ) (**Figure 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 three analysed intervals of a given day) were taken into account in the Friedman test leading to 16, 18 and 13 animals analysed on the three experimental days (NaCl, Propranolol, Prazosin), respectively. These results indicate an SWS-specific pro-epileptic 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 noradrenalin 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 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 ascending 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 even 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 six 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 (**Figures 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.

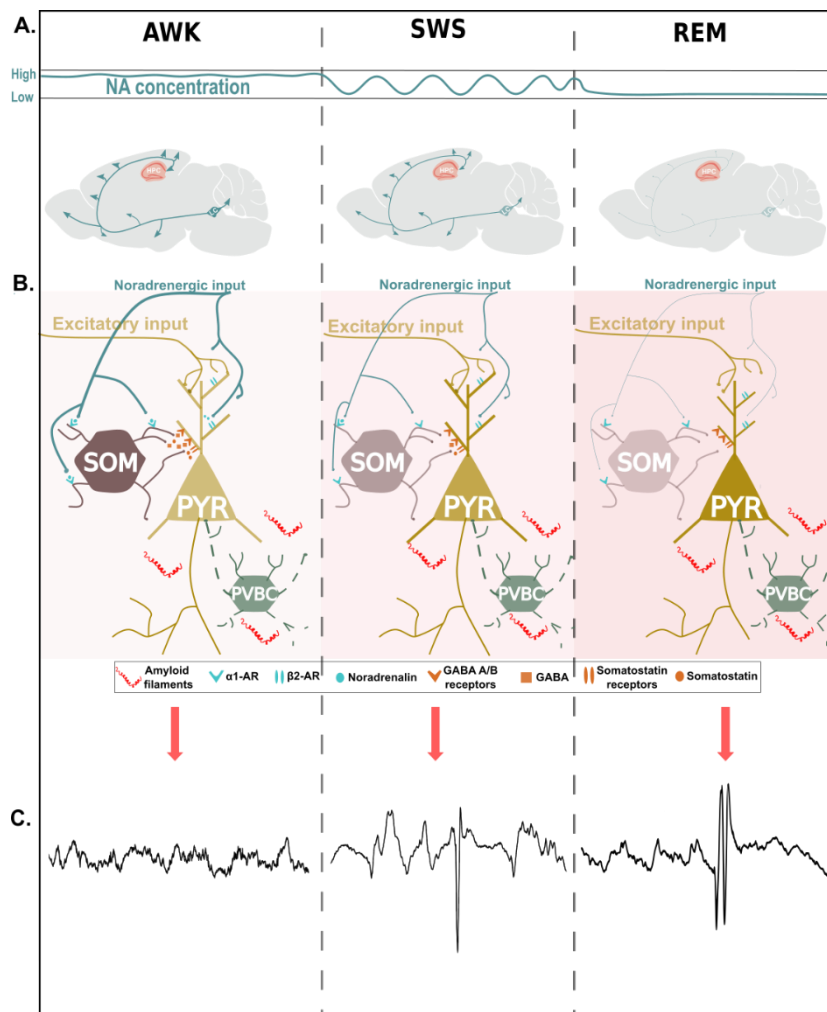
First, the antiepileptic effect of NA has been suggested by previous publications describing higher seizure rates in NA-deficient rodents (Szot et al., 1999) or that the decrease in seizure susceptibility observed after vagus nerve stimulation might be mediated by the increased NA concentration that 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 antiseizure 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 (**Figures 4A, 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 (Gelinas 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, **Figure 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 (**Figure 4C**).



**Figure 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.

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 6.5-8 months of age (Guérin et al., 2009), indicating that in the Tg2576 mice included in the pharmacology experiment (between 2.5-6.5 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 (**Figure 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 (**Figure 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 µg/µl of the neurotoxin 6-hydroxydopamine three weeks post-treatment (Szot et al., 2016). Indeed, extracellular NA concentrations 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 ten 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  $\alpha_1$  adrenoreceptors. 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.

### **Conflict of interest**

The authors declare no current or potential conflict of interest.

### **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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## Abstract

With more than 30 million people diagnosed worldwide, Alzheimer's disease (AD) is the most frequent neurodegenerative disorder. Previous research on murine models of AD highlighted the potential role of cerebral excitatory-inhibitory imbalance in the pathophysiology of AD. Clinical research has since reported that it may give rise to epileptic activities in as much as 50% of AD patients. However, these epileptic events may remain undetected due to their silent, mostly sleep-occurring nature.

Therefore, the first part of this work corresponded to a preclinical study in which we aimed at (I) characterizing epileptic activities over the sleep-wake cycle in the Tg2576 mouse model and (II) gaining better insight into the mechanistic underpinnings of these peculiar epileptic events. Tg2576 mice displayed frequent epileptic activities that were predominant during REM sleep and highly locked to the phase of the theta cycle corresponding to the maximal firing probability of pyramidal cells, hinting at a potential deficit of inhibitory activity leading to hyperexcitability – which is coherent with previous results in our laboratory. Secondly, this epileptic activity seems to be prevented during wakefulness - and partially during slow-wave sleep - by noradrenergic signalling via  $\alpha 1$  adrenoreceptors. In contrast, EA would be unmasked during REM sleep when noradrenergic cells cease firing. Given the early degeneration of the locus coeruleus – the primary source of noradrenaline in the brain – in AD patients, this could also have important clinical implications.

In the second part of this thesis, the objective was to explore the prevalence and the distribution of epileptic activities during sleep in a cohort of 30 AD patients compared to a group of age-matched, healthy controls and to understand the impact of these activities on cognitive decline and sleep-related memory consolidation. To this end, a full-night video-EEG combined with polysomnography was undertaken, together with a two-part neuropsychological test battery with pre-and post-sleep memory tests included. These measures were combined with brain imaging (MRI), genetic analyses (only for AD patients), and the evaluation of lifestyle-related factors to evaluate, with the strict minimum of biasing factors, the impact of epileptic activities on AD progression. Preliminary results with 25 participants per group are reported here. Our results hint at a risk ratio for EA occurrence five times higher in patients than controls. In contrast to our preclinical results, epileptic events are predominant during non-REM sleep and especially during deeper (N2 and N3) stages. We also report increased percentages of superficial sleep in patients at the detriment of deeper ones. Coherently, slight alterations of the sleep-microarchitecture, and namely, of sleep spindle quantities that are otherwise the most pronounced during N2 sleep, are observed in the patient group. Notably, the prevalence of sleep apnoea syndrome is unexpectedly high: whilst still higher in AD patients (63%) than in controls (50%), concerns at least half of the participants in both groups. While our sample size is currently insufficient to draw steady conclusions from our results, our preliminary models suggest a combined impact of sleep quantity, sleep apnoea, epileptic events and AD itself on memory consolidation, especially on episodic memory. We suggest that treating EAs, sleep apnoea and improving sleep quality could potentially slow down or lessen memory complaints in AD patients.