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# Epilepsy and Alzheimer's disease: shared pathology, clinical presentations, and targets for treatment

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# Epilepsy and Alzheimer's disease: shared pathology, clinical presentations, and targets for treatment

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# Editorial: Epilepsy and Alzheimer's disease: shared pathology, clinical presentations, and targets for treatment

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Editorial on the Research Topic
Epilepsy and Alzheimer's disease: shared pathology, clinical presentations, and targets for treatment

While epilepsy incidence peaks in older adults (1, 2), the association between epilepsy and Alzheimer's disease (AD) extends beyond the increased risk of AD with age. Epilepsy and AD share clinical manifestations, with approximately 50% of epilepsy patients demonstrating cognitive dysfunction (3, 4) and prevalence estimates of seizures in AD ranging widely from 1.5 to 75% (5, 6). Epilepsy and AD can also have similar pathological findings, with beta-amyloid and tau accumulation, and selective vulnerability of the hippocampus, in both disorders (7, 8). Many questions remain unanswered, however, regarding similarities and differences in cognitive profiles, identification of biomarkers, underlying mechanisms, and treatment implications. Articles in this collection address these fundamental questions.

## Clinical presentations

Risks of developing epilepsy and dementia are bidirectional, with an estimated two-fold risk of one disorder in the setting of the other (9). Hence, we must know when to suspect a dual diagnosis. Reyes et al. described cognitive phenotypes of late onset epilepsy (LOE), finding that 62.5% declined in cognitive performance over a median of 4 years. The authors concluded that developing seizures in older age can accelerate cognitive decline. Performance decrements, however, may be challenging to distinguish from AD. Liu and Barr highlighted differing patterns of memory deficits corresponding to cell loss in different hippocampal subfields in LOE and AD. With early neuronal loss in the dentate gyrus and CA1/CA3 regions in temporal lobe epilepsy (TLE), there is corresponding difficulty with separation of details, and association and consolidation between present and past events, with relatively spared encoding and retrieval. In contrast, AD involves early cell

loss in the entorhinal cortex, impairing all stages of memory formation and retrieval. The authors proposed that in early stages, TLE and AD could be distinguished based on these differing patterns of memory dysfunction.

## Biomarkers

Liu and Barr and Lu et al. reviewed similarities between AD and epilepsy, including amyloid and tau pathology. Adults with epilepsy can exhibit early AD pathology, including lower Aβ42 in cerebrospinal fluid (CSF) and hyperphosphorylated tau in the temporal lobes (10, 11). AD patients with comorbid epilepsy have greater abnormalities in CSF Aβ42, total tau, and phosphorylated tau than AD patients without epilepsy (12). Hickman et al. recommended that all patients with late onset epilepsy of unknown cause (LOEU) have an evaluation for preclinical or prodromal AD and categorized LOEU based on presence or absence of amyloid and tau biomarkers. These categories will likely become more refined as we develop more comprehensive biomarkers of seizure-associated proteinopathies, including alpha-synuclein, TDP-43, and immune factors.

Martin and Leeman-Markowski proposed a mechanism by which hyper-phosphorylated tau and neurofibrillary tangles accumulate in epilepsy, resulting from an imbalanced endoplasmic reticulum stress response, inflammatory signaling, and a failed "last ditch effort" of amyloid-beta to revert the cell to programmed cell death. They presented a hypothesis of tau phosphorylation as an acute neuroprotective response to seizures that may transition to an injurious process when these pathways are chronically activated by repeated seizures.

Leitner et al. examined proteins within the choroid plexus (13, 14) of human post-mortem tissue. They identified protein differences in the choroid plexus of AD compared to controls, associated with a shift from glucose-mediated energy production to fatty acid beta-oxidation activation and glycolysis inhibition, coupled with activated branched-chain amino acid degradation. Greater variability and fewer protein differences were evident in the epilepsy group compared to controls, but similar trends in protein changes were present in epilepsy and AD. Proteomics of the choroid plexus and other brain regions (15) may inform future mechanistic and therapeutic studies.

## Genetics

Epigenetic regulation of gene expression can translate intermittent seizures to long-lasting cognitive changes. The neuronal activity-induced transcription factor ΔFosB is robustly increased in the dentate gyrus in AD and correlates with cognitive impairment (16). Although seizure-induced ΔFosB accumulation occurs in TLE (16, 17), whether it is associated with cognitive deficits in epilepsy is unknown. Fu et al. found increased ΔFosB expression in pediatric epilepsies that was inversely related to IQ in patients with intellectual disabilities. Thus, ΔFosB expression may contribute to cognition in a range of epilepsy syndromes.

Multiple ΔFosB target genes in the hippocampus play critical roles in calcium handling and synaptic plasticity, which may explain why their suppression by ΔFosB leads to cognitive deficits (16–18). However, prolonged ΔFosB expression may also enable neuroprotective and homeostatic pathways. In Clasadonte et al., prolonged ΔFosB reduction exacerbated neuroinflammatory pathways in mouse models of epilepsy. Their newly developed shRNA tool for reducing ΔFosB expression was effective and long-lasting, revealing that ΔFosB maintains neuroprotection, in part by limiting astrocyte and microglial engagement in neuroinflammation. These results are consistent with prior studies demonstrating that prolonged blockade of ΔFosB exacerbates seizures and memory deficits in an AD mouse model (19). Together, these data reveal how engagement of ΔFosB by recurrent seizures contributes to long-lasting impacts on hippocampal gene expression and function.

## Treatment

Lu et al. provided an overview of AD medication effects on seizure threshold, which can guide clinicians when selecting individualized treatments. We must also better understand anti-seizure medications (ASMs) in the context of AD with epileptiform activity. Lehmann and Barker-Haliski evaluated acute ASM potency and tolerability in a presenilin-2 (PSEN2) knockout (KO) early onset-AD mouse model in comparison to wild type controls, using a 6-Hz limbic seizure test. Acute potency and tolerability across multiple ASMs were altered with PSEN2 loss, providing support for targeted ASM therapy analyses in familial early-onset AD patients.

Overlapping clinical presentations and neuropathological changes of AD and epilepsy could lead to shared treatments (20–24). Further, interictal epileptiform discharges (IEDs), may serve as a target for treatment in AD. Lu et al. highlighted that seizures and IEDs in AD are associated with accelerated cognitive decline and that ASMs may improve cognitive function in AD patients with epileptiform activity, which is most commonly seen in sleep (25–28). Lemus and Sarkis advised a measured approach to considering ASMs in AD patients with IEDs, taking into account the patient's age and the frequency, morphology, and other characteristics of the epileptiform activity.

## Related dementias

The bidirectional risk of epilepsy and dementia is not limited to AD (29). Vicente et al. noted the increased risk of epilepsy in dementia with Lewy bodies (DLB). Many of the same pathological changes and pathways are implicated in AD and DLB, including glutamate transporter imbalance, cholinergic neuron degeneration, mechanistic target of rapamycin (mTOR) overactivation, and disruption of glial immunoinflammatory function, such that mechanistic insights into epileptic activity in one disease could be informative for the other.

## Conclusion

The studies highlighted in this collection contribute to a greater understanding of the relationships between

epilepsy and AD, with the hope of improving diagnosis and identifying effective treatments, so patients can have improved cognition.

## Author contributions

BL-M: Conceptualization, Funding acquisition, Project administration, Writing – original draft, Writing – review & editing. JC: Writing – original draft, Writing – review & editing. DL: Writing – original draft, Writing – review & editing, Funding acquisition. KV: Funding acquisition, Writing – original draft, Writing – review & editing.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## References

1. Hauser WA, Annegers JF, Kurland LT. Incidence of epilepsy and unprovoked seizures in Rochester, Minnesota: 1935–1984. *Epilepsia.* (1993) 34:453–8.

2. Stephen LJ, Brodie MJ. Epilepsy in elderly people. *Lancet (London, England).* (2000) 355:1441–6. doi: 10.1016/S0140-6736(00)02149-8

3. Taylor J, Kolamunnage-Dona R, Marson AG, Smith PEM, Aldenkamp AP, Baker GA, et al. Patients with epilepsy: cognitively compromised before the start of antiepileptic drug treatment? *Epilepsia.* (2010) 51:48–56. doi: 10.1111/j.1528-1167.2009.02195.x

4. Witt JA, Helmstaedter C. Should cognition be screened in new-onset epilepsies? A study in 247 untreated patients. *J Neurol.* (2012) 259:1727–31. doi: 10.1007/S00415-012-6526-2

5. Friedman D, Honig LS, Scarmeas N. Seizures and epilepsy in Alzheimer's disease. *CNS Neurosci Ther.* (2012) 18:285–94. doi: 10.1111/J.1755-5949.2011.00251.x

6. Sourander P, Sjögren H. The concept of Alzheimer's disease and its clinical implications. In: Wolstenholme GEW, O'Connor M, editors. *Ciba Foundation Symposium-Alzheimer's Disease and Related Conditions*. Chichester, UK: John Wiley & Sons, Ltd. (1970). p. 11-36.

7. Ball MJ. Topographic distribution of neurofibrillary tangles and granulovacuolar degeneration in hippocampal cortex of aging and demented patients. A quantitative study. *Acta Neuropathol.* (1978) 42:73–80. doi: 10.1007/bf00690970

8. Gourmaud S, Shou H, Irwin DJ, Sansalone K, Jacobs LM, Lucas TH, et al. Alzheimer-like amyloid and tau alterations associated with cognitive deficit in temporal lobe epilepsy. *Brain.* (2020) 143:191–209. doi: 10.1093/brain/awz381

9. Stefanidou M, Beiser AS, Himali JJ, Peng TJ, Devinsky O, Seshadri S, et al. Bi-directional association between epilepsy and dementia: the Framingham Heart Study. *Neurology.* (2020) 95:e3241–7. doi: 10.1212/wnl.0000000000011077

10. Costa C, Romoli M, Liguori C, Farotti L, Eusebi P, Bedetti C, et al. Alzheimer's disease and late-onset epilepsy of unknown origin: two faces of beta amyloid pathology. *Neurobiol Aging.* (2019) 73:61–7. doi: 10.1016/j.neurobiolaging.2018.09.006

11. Tai XY, Koepp M, Duncan JS, Fox N, Thompson P, Baxendale S, et al. Hyperphosphorylated tau in patients with refractory epilepsy correlates with cognitive decline: a study of temporal lobe resections. *Brain.* (2016) 139:2441–55. doi: 10.1093/brain/aww187

12. Banote RK, Håkansson S, Zetterberg H, Zelano J. CSF biomarkers in patients with epilepsy in Alzheimer's disease: a nation-wide study. *Brain Commun.* (2022) 4:fcac210. doi: 10.1093/braincomms/fcac210

13. Benarroch EE. Choroid plexus–CSF system: recent developments and clinical correlations. *Neurology.* (2016) 86:286–96. doi: 10.1212/wnl.0000000000002298

14. Krzyzanowska A, García-Consuegra I, Pascual C, Antequera D, Ferrer I, Carro E. Expression of regulatory proteins in choroid plexus changes in early stages of Alzheimer disease. *J Neuropathol Exp Neurol.* (2015) 74:359–69. doi: 10.1097/nen.0000000000000181

15. Leitner D, Pires G, Kavanagh T, Kanshin E, Askenazi M, Ueberheide B, et al. Similar brain proteomic signatures in Alzheimer's disease and epilepsy. *Acta Neuropathol.* (2024) 147:27. doi: 10.1007/s00401-024-02683-4

16. You JC, Muralidharan K, Park JW, Petrof I, Pyfer MS, Corbett BF, et al. Epigenetic suppression of hippocampal calbindin-D28k by ΔFosB drives seizure-related cognitive deficits. *Nat Med.* (2017) 23:1377–83. doi: 10.1038/nm.4413

17. Stephens GS, Fu CH, St Romain CP, Zheng Y, Botterill JJ, Scharfman HE, et al. Genes bound by ΔFosB in different conditions with recurrent seizures regulate similar neuronal functions. *Front Neurosci.* (2020) 14:472. doi: 10.3389/fnins.2020.00472

18. Corbett BF, You JC, Zhang X, Pyfer MS, Tosi U, Iascone DM, et al. ΔFosB regulates gene expression and cognitive dysfunction in a mouse model of Alzheimer's disease. *Cell Rep.* (2017) 20:344–55. doi: 10.1016/j.celrep.2017.06.040

19. Stephens GS, Park J, Eagle A, You J, Silva-Pérez M, Fu C-H, et al. Persistent ΔFosB expression limits recurrent seizure activity and provides neuroprotection in the dentate gyrus of APP mice. *Prog Neurobiol.* (2024) 237:102612. doi: 10.1016/j.pneurobio.2024.102612

20. Fisher RS, Bortz JJ, Blum DE, Duncan B, Burke H. A pilot study of donepezil for memory problems in epilepsy. *Epilepsy Behav.* (2001) 2:330–4. doi: 10.1006/ebeh.2001.0221

21. Hamberger MJ, Palmese CA, Scarmeas N, Weintraub D, Choi H, Hirsch LJ, et al. A randomized, double-blind, placebo-controlled trial of donepezil to improve memory in epilepsy. *Epilepsia.* (2007) 48:1283–91. doi: 10.1111/j.1528-1167.2007.01114

22. Marimuthu P, Varadarajan S, Krishnan M, Shanmugam S, Kunjuraman G, Ravinder JR, et al. Evaluating the efficacy of memantine on improving cognitive functions in epileptic patients receiving anti-epileptic drugs: a double-blind placebo-controlled clinical trial (Phase IIIb pilot study). *Ann Indian Acad Neurol.* (2016) 19:344–50. doi: 10.4103/0972-2327.179971

23. Leeman-Markowski BA, Meador KJ, Moo LR, Cole AJ, Hoch DB, Garcia E, et al. Does memantine improve memory in subjects with focal-onset epilepsy and memory dysfunction? A randomized, double-blind, placebo-controlled trial. *Epilepsy Behav.* (2018) 88:315–24. doi: 10.1016/j.yebeh.2018.06.047

24. Oustad M, Najafi M, Mehvari J, Rastgoo A, Mortazavi Z, Rahiminejad M. Effect of donepezil and memantine on improvement of cognitive function in patients with temporal lobe epilepsy. *J Res Med Sci.* (2020) 25:29. doi: 10.4103/jrms.jrms_209_19

25. Vossel KA, Ranasinghe KG, Beagle AJ, Mizuiri D, Honma SM, Dowling AF, et al. Incidence and impact of subclinical epileptiform activity in Alzheimer's disease. *Ann Neurol.* (2016) 80:858–70. doi: 10.1002/ana.24794

26. Vossel K, Ranasinghe KG, Beagle AJ, La A, Ah Pook K, Castro M, et al. Effect of levetiracetam on cognition in patients with Alzheimer disease with and without epileptiform activity: a randomized clinical trial. *JAMA Neurol.* (2021) 78:1345–54. doi: 10.1001/jamaneurol.2021.3310

27. Szabo AB, Cretin B, Gérard F, Curot J, Barbeau EJ, Pariente J, et al. Sleep: the tip of the iceberg in the bidirectional link between Alzheimer's disease and epilepsy. *Front Neurol.* (2022) 13:836292. doi: 10.3389/fneur.2022.836292

28. Csernus EA, Werber T, Kamondi A, Horvath AA. The significance of subclinical epileptiform activity in Alzheimer's disease: a review. *Front Neurol.* (2022) 13:856500. doi: 10.3389/fneur.2022.856500

29. Chen L, Yang W, Yang F, Yu Y, Xu T, Wang D, et al. The crosstalk between epilepsy and dementia: a systematic review and meta-analysis. *Epilepsy Behav.* (2024) 152:109640. doi: 10.1016/j.yebeh.2024.109640

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# Localized proteomic differences in the choroid plexus of Alzheimer's disease and epilepsy patients

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**Introduction:** Alzheimer's disease (AD) and epilepsy are reciprocally related. Among sporadic AD patients, clinical seizures occur in 10–22% and subclinical epileptiform abnormalities occur in 22–54%. Cognitive deficits, especially short-term memory impairments, occur in most epilepsy patients. Common neurophysiological and molecular mechanisms occur in AD and epilepsy. The choroid plexus undergoes pathological changes in aging, AD, and epilepsy, including decreased CSF turnover, amyloid beta (Aβ), and tau accumulation due to impaired clearance and disrupted CSF amino acid homeostasis. This pathology may contribute to synaptic dysfunction in AD and epilepsy.

**Methods:** We evaluated control ($n$ = 8), severe AD ($n$ = 8; A3, B3, C3 neuropathology), and epilepsy autopsy cases ($n$ = 12) using laser capture microdissection (LCM) followed by label-free quantitative mass spectrometry on the choroid plexus adjacent to the hippocampus at the lateral geniculate nucleus level.

**Results:** Proteomics identified 2,459 proteins in the choroid plexus. At a 5% false discovery rate (FDR), 616 proteins were differentially expressed in AD vs. control, 1 protein in epilepsy vs. control, and 438 proteins in AD vs. epilepsy. There was more variability in the epilepsy group across syndromes. The top 20 signaling pathways associated with differentially expressed proteins in AD vs. control included cell metabolism pathways; activated fatty acid beta-oxidation ($p = 2.00 \times 10^{-7}$, z = 3.00), and inhibited glycolysis ($p = 1.00 \times 10^{-12}$, z = −3.46). For AD vs. epilepsy, the altered pathways included cell metabolism pathways, activated complement system ($p = 5.62 \times 10^{-5}$, z = 2.00), and pathogen-induced cytokine storm ($p = 2.19 \times 10^{-2}$, z = 3.61). Of the 617 altered proteins in AD and epilepsy vs. controls, 497 (81%) were positively correlated ($p < 0.0001$, $R^2 = 0.27$).

**Discussion:** We found altered signaling pathways in the choroid plexus of severe AD cases and many correlated changes in the protein expression of cell metabolism pathways in AD and epilepsy cases. The shared molecular mechanisms should be investigated further to distinguish primary pathogenic changes from the secondary ones. These mechanisms could inform novel

therapeutic strategies to prevent disease progression or restore normal function. A focus on dual-diagnosed AD/epilepsy cases, specific epilepsy syndromes, such as temporal lobe epilepsy, and changes across different severity levels in AD and epilepsy would add to our understanding.

KEYWORDS

**Alzheimer's disease, epilepsy, choroid plexus, proteomics, laser capture microdissection**

# Introduction

Alzheimer's disease (AD) and epilepsy are reciprocally related: AD increases the risk of late-onset seizures, and epilepsy increases the risk of cognitive impairment (1–10), suggesting common molecular mechanisms. Seizures occur in 10–22% of sporadic AD (sAD) patients, subclinical epileptiform abnormalities in 22–54% of AD patients, (11–17) and cognitive deficits occur in up to 80% of epilepsy patients (1–3, 18). Non-convulsive seizures and subclinical electroencephalography (EEG) abnormalities are common and underrecognized in AD patients and may accelerate structural and cognitive disorders (4, 14, 15, 17, 19). In AD patients with epileptiform activity, the Mini-Mental State Examination (MMSE) score decreased faster compared to AD patients without epileptiform activity (15). Furthermore, anti-seizure medications [ASMs; e.g., levetiracetam (LEV)] decreased neuronal hyperexcitability and improved cognition in animal models and in patients with mild cognitive impairment (MCI) and are being investigated in ongoing studies for AD (20–24). Cognitive deficits are common in patients with chronic epilepsy, particularly in temporal lobe epilepsy (TLE), and late-onset epilepsy (8, 9, 18, 25, 26). Epilepsy patients had a faster MMSE decline than non-epilepsy patients (27), a 2-fold increased dementia risk when compared to controls (28), and a 3-fold increased dementia incidence in late-onset epilepsy when compared to non-epilepsy patients (9). Cognitive deficits and epileptiform activity are linked with amyloid beta (Aβ) and tau pathology in AD and epilepsy (3, 19, 25, 29, 30). Cognitive performance was impaired with altered cerebrospinal fluid (CSF) Aβ42 and EEG abnormalities in patients with late-onset epilepsy of unknown etiology and MCI when compared to MCI patients without epilepsy (26). Furthermore, some patients with late-onset epilepsy of unknown etiology develop pathogenic levels of AD biomarkers Aβ42 and tau that indicate an ongoing neurodegeneration process and a risk factor for AD (31). Compared to AD patients without seizures, those with seizures had increased Aβ and tau pathology via mTOR activation in the temporal cortex (32). An mTOR inhibitor improved cognition and ameliorated AD pathology in a 5xTg AD model (32), highlighting the therapeutic potential of exploring the pathways involved in the bidirectional relationship between AD and seizures.

The choroid plexus is impacted in both AD and epilepsy. It is the primary source for CSF production and is essential in the maintenance and function of the brain (33). This region undergoes age-related pathological changes (e.g., altered volume, epithelial atrophy, thickened basement membrane, and stroma fibrosis) that decrease CSF turnover (33–36). Aβ accumulation in the choroid plexus results from mitochondrial deficits, oxidative stress, and cytoskeletal dysregulation (34, 37–39). These pathogenic changes alter nutrient and ion secretion, impairing brain homeostasis (33, 35, 40). In epilepsy, choroid plexus and hippocampal inflammation occur ipsilateral to the seizure focus (41). CSF amino acid homeostasis is disrupted in epilepsy patients and animal epilepsy models (42–45).

We and others have identified AD protein changes in multiple brain regions over the disease course (46). These include glial proteins (47), Aβ, and tau levels that correlate with spliceosome activity (48–50), synaptic dysfunction (51, 52), and tau interacting proteins involved in ubiquitination and phagosome maturation (29, 53). In epilepsy, we identified protein changes associated with increased translation and decreased oxidative phosphorylation and synaptogenesis (54). The molecular mechanisms in the choroid plexus of AD and epilepsy are not well-understood. Limited proteomic studies in AD choroid plexus (55) and CSF revealed protein changes in CSF, indicating altered astrocyte/microglial and sugar metabolism (56), neuroinflammation, cerebrovascular dysfunction, and apoptosis (57, 58). There are no proteomics studies in human epilepsy choroid plexus. With most AD clinical trials failing (59–66) and drug-resistant epilepsy rates stable for decades (67, 68), proteomics approaches may reveal unbiased comprehensive datasets to identify shared druggable protein targets. Identifying these mechanisms can inform therapeutic strategies to improve network function, limit disease progression, and potentially reverse functional and pathological changes.

# Materials and methods

## Brain tissue

Specimens were acquired under protocols with Institutional Review Board (IRB) approval at NYU Grossman School of Medicine, including autopsy tissues from the North American SUDEP Registry (NASR) at NYU CEC, NYU ADRC, and NYU Center for Biospecimen Research and Development (CBRD)/Department of Pathology. For epilepsy cases ($n = 12$), the inclusion criteria were those cases with temporal lobe epilepsy or likely temporal lobe involved epilepsy as determined from the review of available medical records, as well as additional epilepsy cases that were age-matched to the other groups and enrolled in NASR. For AD cases ($n = 8$), the inclusion criteria were those cases with severe AD pathology as indicated by the neuropathology score A3B3C3 (69) and part of the NYU ADRC, which allowed for age matching to the other groups. Control cases ($n = 8$) were selected to include those cases with no known significant neurology or

TABLE 1 Case history summary.

| Study group | n | Sex (M/F) | Age (years) | PMI (hours) | Brain weight (grams) |
|---|---|---|---|---|---|
| Control | 8 | 5/3 | 57.8 ± 6.1 | 59.1 ± 14.3 | 1249.0 ± 130.7 |
| AD | 8 | 2/6 | 72.6 ± 9.5 | 23.6 ± 22.5 | 1063.4 ± 102.3 |
| Epilepsy | 12 | 11/1 | 45.4 ± 14.3 | 35.8 ± 19.7 | 1392.9 ± 169.3 |

From the available case information. See Supplementary Table 1 for detailed case history. Mean is indicated for age, PMI, and brain weight ± standard deviation (mean ± SD).

neuropathology. Cases were further selected to include those that were age-matched and with hippocampal sections available at the level of the lateral geniculate nucleus (LGN) with adjacent choroid plexus present. The sample size was informed by ours and other prior studies (47, 49, 52, 54, 56, 70, 71). Case history is summarized in Table 1 and detailed in Supplementary Table 1.

## Laser capture microdissection

Formalin-fixed, paraffin-embedded (FFPE) tissue was cut into 8 μg sections from autopsy hippocampal tissue at the level of the LGN with adjacent choroid plexus onto LCM PET membrane slides (54, 70, 72, 73) and stained with cresyl violet (74) for the localization of choroid plexus. Microdissected samples were collected at a consistent area per case of 3 $mm^2$ into LC-MS grade water (Thermo Fisher Scientific) with the Leica LMD6500 LCM system. Samples were stored at −80°C until further processing. The schematic overview in Figure 1 was partially generated with Biorender.com.

## Label-free quantitative mass spectrometry LFQ-MS

### Protein extraction and digestion

LCM-excised tissue samples were solubilized and digested using the SPEED sample prep workflow (75). In brief, tissue sections were incubated in 10 μl of LC-MS grade trifluoroacetic acid (TFA) for 5 min at 73°C. TFA was neutralized by 10x dilution (v:v) with 2M TRIS containing 10 mM Tris (2-carboxyethyl) phosphine TCEP and 20 mM chloroacetic acid (CAA) and incubated at 95°C for 10 min. For enzymatic digestion, samples were diluted 6x (v:v) with water containing 1 μg of sequencing-grade trypsin. Digestion was carried out at 37°C overnight and halted by acidification to 2% of TFA.

### LC-MS/MS

LC separation was performed online on an Evosep One (Evosep) LC utilizing Dr. Maisch ReproSil-Pur 120 C18 AQ, 1.9-μm bead (150 μm ID, 15 cm long, cat# EV-1106) analytical column. Peptides were gradient eluted from the column directly into an Orbitrap HFX mass spectrometer using the 88-min extended Evosep method (SPD15) at a flow rate of 220 nl/min. The mass spectrometer was operated in data-independent acquisition (DIA) mode (76) acquiring MS/MS fragmentation across 22 m/z windows after every MS full-scan event.

High-resolution full MS spectra were acquired with a resolution of 120,000, an AGC target of 3e6, with a maximum ion injection time of 60 ms, and a scan range of 350 to 1650 m/z. Following each full MS scan, 22 data-independent HCD MS/MS scans were acquired at a resolution of 30,000, an AGC target of 3e6, and a stepped normalized collision energy (NCE) of 22.5, 25, and 27.5.

## Data analysis

MS data were analyzed using the Spectronaut® software (https://biognosys.com/shop/spectronaut) and searched in direct DIA mode against the *homo sapiens* UniProt database (http://www.uniprot.org/). The database search used the integrated search engine Pulsar. For searching, enzyme specificity was set to trypsin with two or fewer missed cleavages. Oxidation of methionine was searched as a variable modification, and carbamidomethylation of cysteines was searched as a fixed modification. The false discovery rate (FDR) for peptide, protein, and site identification was set to 1%. Protein quantification was done on the MS/MS level using the three most intense fragment ions per precursor. Subsequent data analysis used Perseus (77) (http://www.perseus-framework.org/), R environment (http://www.r-project.org/), or Prism GraphPad for statistical computing and graphics. Raw data are available on the MassIVE server (https://massive.ucsd.edu/) under accession MSV000091370.

The protein expression matrix ($n$ = 2,498) was filtered to remove the proteins that were non-human, common lab contaminants, and those proteins observed in less than half of all the three groups ($n$ = 2,459). For principal component analysis (PCA), missing values were imputed from the normal distribution with a width of 0.3 and a downshift of 1.8 (relative to measured protein intensity distribution) in Perseus (77). Unpaired $t$-tests were performed in Perseus v. 1.6.2.3 (77) to detect significant changes in protein expression. A comparison of the significant proteins common to each pairwise comparison was evaluated by a Venn diagram generated from InteractiVenn (78). Cell-type annotations for each protein were evaluated in comparison to a reference choroid plexus dataset (79), as we have similarly done previously in other brain regions with enrichment evaluated by a Fisher's exact test (54, 70, 71, 73, 80, 81). The signaling pathways associated with the differentially expressed proteins were assessed by Ingenuity Pathway Analysis (IPA, Qiagen). All detected proteins were included in the dataset for each pairwise comparison, including the UniProtID, fold change, and $p$-value. Core analysis was performed in each brain region for proteins at an FDR of

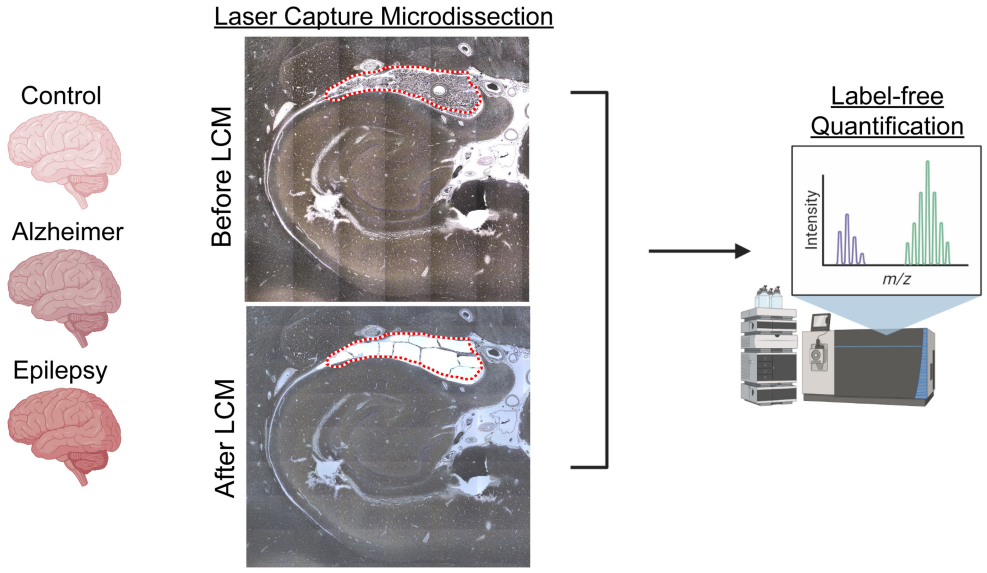


FIGURE 1
LCM and schematic approach overview. Choroid plexus (3 mm$^2$), adjacent to the hippocampus at the level of LGN, was microdissected by LCM from FFPE autopsy brain tissue from control ($n = 8$), AD ($n = 8$), and epilepsy ($n = 12$) cases. Proteins were quantified by label-free quantitative mass spectrometry to identify protein differences.

<5%. Pathways were considered enriched at a $p$-value of overlap of <0.05 and to be activated/inhibited as a result of combined protein fold changes in a pathway as reflected by a |z-score| of ≥2. Correlation analyses were performed by Pearson's correlation in GraphPad Prism. Data were also compared to previous AD studies and recently compiled in our NeuroPro database v1.12 (https://neuropro.biomedical.hosting/) (82). To identify basement membrane proteins (by cell component GO term), 616 proteins in AD vs. control were evaluated by STRING v11.5 (https://string-db.org/).

### Immunohistochemistry

Immunohistochemistry was performed to validate the protein of interest, transmembrane protein 106B (TMEM106B) (52, 73, 83, 84). The FFPE sections (8 μm) were deparaffinized and rehydrated in a series of xylenes and ethanol dilutions. A heat-induced antigen retrieval was performed with 10 mM sodium citrate, 0.05% Triton-X 100; pH 6. Blocking with 10% normal donkey serum was followed by a TMEM106B primary antibody (1:100, Sigma HPA058342) and AQP1 (1:100, Santa Cruz sc-25287) overnight at 4°C. Sections were incubated with donkey anti-rabbit Alexa-Fluor 647 and Alexa-Fluor 488 secondary antibodies (1:500, Thermo Fisher Scientific, Invitrogen), counterstained with DAPI (Sigma D9542), and coverslipped.

Whole-slide scanning was performed at ×20 magnification with a Leica Aperio Versa 8 microscope using the same settings for each slide. There were three to four images at ×10 magnification collected for each case ($n = 5$ control, $n = 5$ AD, $n = 5$ epilepsy). Images were analyzed using Fiji ImageJ to compare the average amount of TMEM106B positive area among the groups. The same binary threshold was used for all images to determine the number of TMEM106B positive pixels in each image, which was reported as a percentage of the total image area. A Mann–Whitney U-test was performed for statistical analysis; a $p$-value of $< 0.05$ was considered significant.

## Results

### Protein differential expression

Protein differential expression analysis was evaluated in control ($n = 8$), AD ($n = 8$), and epilepsy cases ($n = 12$) from the autopsy brain tissue with LFQ-MS in the microdissected choroid plexus (Table 1, Figure 1, Supplementary Table 1). LFQ-MS identified 2,459 proteins in the choroid plexus of the cases analyzed, detected in at least 50% of the cases in any of the groups. PCA showed significant segregation of AD cases from control ($p < 0.0001$) and epilepsy ($p < 0.0001$) cases in PCA1 (Figures 2A–C). There was more variability in the epilepsy group that included various syndromes. In addition to the disease group, sex contributed to some differences observed on the PCA ($p = 0.023$), while age did not ($p = 0.89$) as observed by a multiple variable linear regression analysis (Supplementary Table 2).

With an unpaired $t$-test followed by permutation-based FDR at 5%, there were significant differences between AD and control cases in 616 proteins, between epilepsy and control cases in 1 protein, and between AD and epilepsy cases in 438 proteins (Figures 2D–G, Supplementary Table 3). There were 303 proteins different in AD when compared to both control and epilepsy cases (Figure 2D). The top 20 most significant proteins altered in the AD vs. control and AD vs. epilepsy pairwise comparisons are summarized in Tables 2, 3. For epilepsy vs. control, the differentially expressed protein FUCA2 (alpha-L-fucosidase 2) was increased

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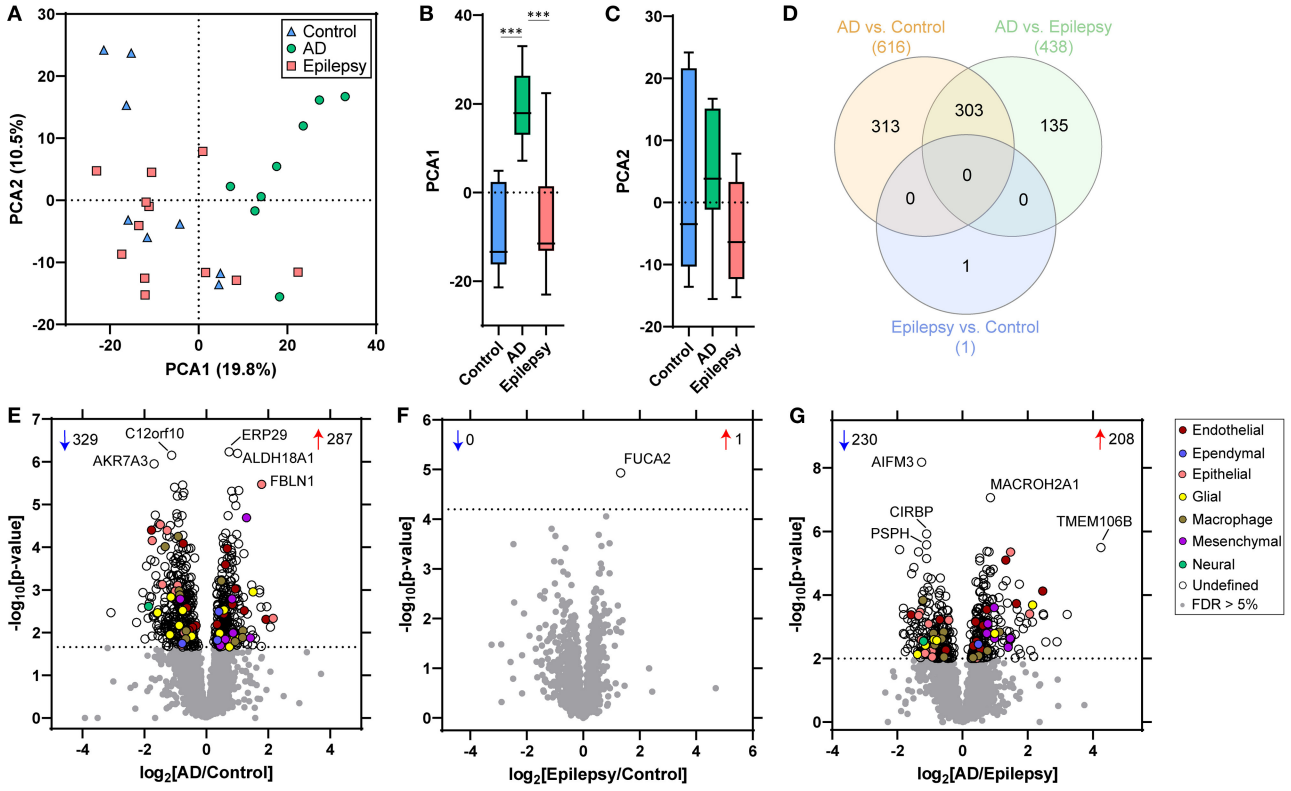


FIGURE 2
PCA and proteomic differences in the choroid plexus of control, epilepsy, and AD patients. **(A)** Principal component analysis (PCA) shows the distribution of control ($n = 8$), AD with A3, B3, C3 neuropathology ($n = 8$), and epilepsy ($n = 12$) for the 2,459 proteins detected in choroid plexus. **(B, C)** There is a segregation of AD from control ($p < 0.0001$) and epilepsy ($p < 0.0001$) in PCA1, but no segregation in PCA2 (one-way ANOVA with *post-hoc* Tukey's test). **(D)** Differential expression analysis for each pairwise comparison is indicated, as well as an overlap in the number of significant proteins, at a 5% false discovery rate (FDR; dotted line) when comparing **(E)** AD vs. control (616 proteins), **(F)** epilepsy vs. control (1 protein), and **(G)** AD vs. epilepsy (438 proteins). Annotations include the number of significantly increased (red arrows) and decreased (blue arrows) proteins. The top five altered proteins are annotated by gene name, and choroid plexus cell-type annotations for each significant protein are indicated.

by 2.5-fold ($p = 1.17 \times 10^{-5}$). There were trending differences ($p < 0.05$, FDR >5%) in epilepsy vs. control for 216 proteins (Supplementary Table 3).

After cell-type annotation of proteins, most proteins were "undefined" and likely expressed by multiple cell types, or their association is unknown (Figures 2, 3, Supplementary Table 3). After "undefined," the most abundant annotation for significant proteins was for endothelial proteins (2.4%, 15 proteins) in AD vs. control and both endothelial and epithelial proteins (3.2%, 14 proteins each) in AD vs. epilepsy. Cell-type enrichment analysis (Fisher's exact test) indicated that glial proteins (1.9%, 12 proteins) were trending in enrichment ($p = 0.051$) in AD vs. control, and endothelial proteins were enriched ($p = 0.031$) in AD vs. epilepsy (Figure 3).

## Pathway analysis

In AD vs. control (Figures 3B, C), pathway analysis of the significantly altered proteins identified 142 signaling pathways associated with the 616 proteins ($p$-value of overlap $< 0.05$); 20 of these pathways were significantly impacted by fold change as reflected by the z-score ($|z| \geq 2$; Supplementary Table 4). Top signaling pathways were associated with cell metabolism, including activated fatty acid beta-oxidation ($p = 2.00 \times 10^{-7}$, $z = 3.00$) and inhibited glycolysis ($p = 1.00 \times 10^{-12}$, $z = -3.46$; Figure 4). Three branched-chain amino acid degradation pathways were activated: valine degradation I ($p = 1.17 \times 10^{-5}$, $z = 2.45$), leucine degradation I ($p = 5.13 \times 10^{-5}$, $z = 2.00$), and isoleucine degradation I ($p = 7.59 \times 10^{-5}$, $z = 2.24$). There was BAG2 signaling activation ($p = 1.12 \times 10^{-5}$, $z = 2.00$) with several decreased proteasome proteins, as well as 14-3-3-mediated signaling inhibition ($p = 1.82 \times 10^{-2}$, $z = -2.12$).

In AD vs. epilepsy (Figures 3E, F), pathway analysis of the significantly altered proteins identified 137 signaling pathways associated with the 438 proteins ($p$-value of overlap <0.05) and 17 pathways were significantly impacted by fold change as reflected by the z-score ($|z| \geq 2$; Supplementary Table 5). The top 20 signaling pathways similar to AD vs. control included five pathways associated with cell metabolism (gluconeogenesis I, glycolysis I, oxidative phosphorylation, and glutaryl-CoA degradation) and the GP6 signaling pathway that is related to platelet activation and thrombus formation. Unique to AD vs. epilepsy, there were two activated inflammation signaling pathways: complement system ($p = 5.62 \times 10^{-5}$, $z = 2.00$) and pathogen-induced cytokine storm ($p = 2.19 \times 10^{-2}$, $z = 3.61$).

TABLE 2 Top 20 significant proteins in AD vs. control.

| Gene ID | Protein name | UniProt ID | *p*-value | Fold change |
|---|---|---|---|---|
| Increased | | | | |
| ERP29 | Endoplasmic reticulum resident protein 29 | P30040 | 5.83E-07 | 1.7 |
| ALDH18A1 | Delta-1-pyrroline-5-carboxylate synthase | P54886 | 6.33E-07 | 2.0 |
| FBLN1 | Fibulin-1 | P23142 | 3.38E-06 | 3.4 |
| FAHD1 | Acylpyruvase FAHD1, mitochondrial | Q6P587 | 4.73E-06 | 2.1 |
| HIBADH | 3-hydroxyisobutyrate dehydrogenase, mitochondrial | P31937 | 4.91E-06 | 1.8 |
| NUCB2 | Nucleobindin-2 | P80303 | 7.16E-06 | 1.8 |
| HADH | Hydroxyacyl-coenzyme A dehydrogenase, mitochondrial | Q16836 | 1.07E-05 | 1.9 |
| LETM1 | Mitochondrial proton/calcium exchanger protein | O95202 | 1.83E-05 | 1.6 |
| FBN1 | Fibrillin-1 [Cleaved into: Asprosin] | P35555 | 2.04E-05 | 2.5 |
| Decreased | | | | |
| C12orf10 | UPF0160 protein MYG1, mitochondrial | Q9HB07 | 7.02E-07 | 2.2 |
| AKR7A3 | Aflatoxin B1 aldehyde reductase member 3 | O95154 | 1.12E-06 | 3.2 |
| DCTN2 | Dynactin subunit 2 | Q13561 | 3.50E-06 | 1.7 |
| YWHAB | 14-3-3 protein beta/alpha | P31946 | 3.94E-06 | 2.0 |
| EIF3A | Eukaryotic translation initiation factor 3 subunit A | Q14152 | 5.28E-06 | 1.7 |
| NAP1L4 | Nucleosome assembly protein 1-like 4 | Q99733 | 6.21E-06 | 1.7 |
| AKR7A2 | Aflatoxin B1 aldehyde reductase member 2 | O43488 | 6.36E-06 | 1.9 |
| EZR | Ezrin | P15311 | 1.65E-05 | 1.9 |
| ALDOA | Fructose-bisphosphate aldolase A | P04075 | 1.78E-05 | 3.1 |
| PPM1B | Protein phosphatase 1B | O75688 | 1.82E-05 | 1.7 |
| RDX | Radixin | P35241 | 1.97E-05 | 1.9 |

In epilepsy vs. control, there were no pathways associated with the one altered protein FUCA2. Pathways associated with the 216 trending proteins at a $p$-value of $< 0.05$ with an FDR of >5% are detailed in Supplementary Table 6.

## TMEM106B validation and localization

TMEM106B (Q9NUM4) was among the top 20 most significantly altered proteins when comparing AD vs. epilepsy (Table 3) with the highest fold change at an 18.9-fold increase ($p = 3.22 \times 10^{-6}$) and was a top protein candidate for validation with cell and regional localization. For AD vs. control by LFQ-MS, there was a 3.5-fold increase ($p = 0.04$, not significant at 5% FDR). By immunohistochemistry, TMEM106B was predominantly localized in epithelial cells at the basal membrane (Figure 5). The epithelial cell marker in the choroid plexus, aquaporin 1 (AQP1), was evaluated for colocalization and was present in the apical membrane of epithelial cells. Validation of the LFQ-MS findings in five cases per group with the semiquantification of immunohistochemistry similarly showed the same trends for TMEM106B, with a 3.9-fold increase in AD vs. epilepsy ($p = 0.095$) and a 5.0-fold increase in AD vs. control ($p = 0.095$).

## AD and epilepsy correlation analysis

Although few proteomic differences in epilepsy vs. control reached the 5% FDR, 617 proteins altered in AD and epilepsy vs. controls had a positive correlation in expression levels ($p < 0.0001$, $R^2 = 0.27$, Figure 6A). There were 81% (497/617) of proteins changing in the same direction and 19% (120/617) of proteins changing in the opposite direction, indicating that many protein changes in AD also trend in epilepsy cases but do not reach significance in these cohorts. The top 10 pathways associated with these proteins were specified by those up in both disease groups, down in both, or changing in the opposite direction (Figures 6B–E, Supplementary Tables 7–10).

## Comparison to other AD studies

We compared the choroid plexus protein differences in AD vs. control to AD-related proteomics studies in our NeuroPro database (82) that compiles results from 38 other proteomics studies, with multiple brain regions, subtypes of disease progression, and types of pathology. There was an overlap of the identified proteins from the choroid plexus with 525 confirmed from previous studies and 91 unique proteins via proteomics to the choroid plexus

TABLE 3 Top 20 significant proteins in AD vs. epilepsy.

| Gene ID | Protein name | UniProt ID | *p*-value | Fold change |
|---|---|---|---|---|
| Increased | | | | |
| MACROH2A1 | Core histone macro-H2A.1 | O75367 | 8.64E-08 | 1.8 |
| TMEM106B | Transmembrane protein 106B | Q9NUM4 | 3.22E-06 | 18.9 |
| ERLIN2 | Erlin-2 | O94905 | 4.23E-06 | 1.7 |
| HIBADH | 3-hydroxyisobutyrate dehydrogenase, mitochondrial | P31937 | 4.35E-06 | 1.8 |
| FBLN1 | Fibulin-1 | P23142 | 4.43E-06 | 2.8 |
| VAPA | Vesicle-associated membrane protein-associated protein A | Q9P0L0 | 6.84E-06 | 1.4 |
| TGM2 | Protein-glutamine gamma-glutamyltransferase 2 | P21980 | 8.01E-06 | 2.5 |
| XRCC5 | X-ray repair cross-complementing protein 5 | P13010 | 8.42E-06 | 1.4 |
| ATP5PD | ATP synthase subunit d, mitochondrial | O75947 | 1.54E-05 | 1.9 |
| FAHD1 | Acylpyruvase FAHD1, mitochondrial | Q6P587 | 1.56E-05 | 1.7 |
| PNPLA6 | Patatin-like phospholipase domain-containing protein 6 | Q8IY17 | 1.74E-05 | 1.8 |
| NUCB2 | Nucleobindin-2 | P80303 | 1.98E-05 | 1.8 |
| Decreased | | | | |
| AIFM3 | Apoptosis-inducing factor 3 | Q96NN9 | 6.58E-09 | 2.4 |
| CIRBP | Cold-inducible RNA-binding protein | Q14011 | 1.19E-06 | 2.2 |
| PSPH | Phosphoserine phosphatase | P78330 | 2.63E-06 | 2.1 |
| MPI | Mannose-6-phosphate isomerase | P34949 | 3.70E-06 | 3.8 |
| KCNJ13 | Inward rectifier potassium channel 13 | O60928 | 4.40E-06 | 2.6 |
| SLC39A12 | Zinc transporter ZIP12 | Q504Y0 | 7.08E-06 | 2.1 |
| AKR7A3 | Aflatoxin B1 aldehyde reductase member 3 | O95154 | 1.36E-05 | 2.8 |
| GLUL | Glutamine synthetase | P15104 | 2.12E-05 | 3.0 |

(Supplementary Tables 11, 12). Of the 525 confirmed proteins, 114 proteins were altered in AD when compared to controls from 9 other brain regions in previous studies. Among the 91 unique proteins by proteomics to choroid plexus, there were several increased collagen and aldehyde dehydrogenase proteins.

# Discussion

We identified protein differences in the choroid plexus of AD cases with severe neuropathology when compared to control and epilepsy cases, with top significant pathways related to activated fatty acid beta-oxidation and inhibited glycolysis. The protein differences in the AD group correlated with the same trends in epilepsy when compared to control cases, with more variability in the epilepsy group.

## AD vs. control

We identified pathways associated with altered cell energy metabolism indicating a shift from glucose-mediated energy production to fatty acid beta-oxidation activation and glycolysis inhibition, coupled with activated branched-chain amino acid degradation. This shift was further reflected by trends in ketogenic pathways, with mild activation of ketolysis ($p = 8.41 \times 10^{-5}$, z = 1.00) and ketogenesis ($p = 1.29 \times 10^{-4}$, z = 1.00). There was oxidative phosphorylation activation, with many increased proteins in complex I (NDUF proteins), as well as complexes II and V. The elevated abundance of these mitochondrial proteins may indicate increased expression or mitochondrial biogenesis that occurs with ketosis (85). Brain imaging studies found hypometabolism in AD patients consistent with low glucose in some brain regions (86). We detected the glucose transporter GLUT1 (SLC2A1) (87) altered in some cells in an AD mouse model (88), but this was not different from controls in the choroid plexus. Future studies should evaluate this further in specific choroid plexus cell types and correlate with neighboring brain tissues and CSF protein levels, as well as clinical variables such as disease progression. Evaluating how these altered pathways may impact ketosis induction may provide insights into the mechanisms of cognitive dysfunction and resilience (89–92).

Other altered pathways associated with AD include BAG2 and 14-3-3 signaling. In the current study, BAG2 signaling activation included nine decreased proteasome proteins and two increased heat shock proteins. This pathway is associated with multiple functions such as cytoskeleton maintenance, including proteasome-independent phosphorylated tau degradation (93). We detected total tau (MAPT) in most cases ($n = 7$ control, $n = 2$ AD, $n = 9$ epilepsy), but this was not different among the groups. Regarding proteasome proteins, we detected a number of these

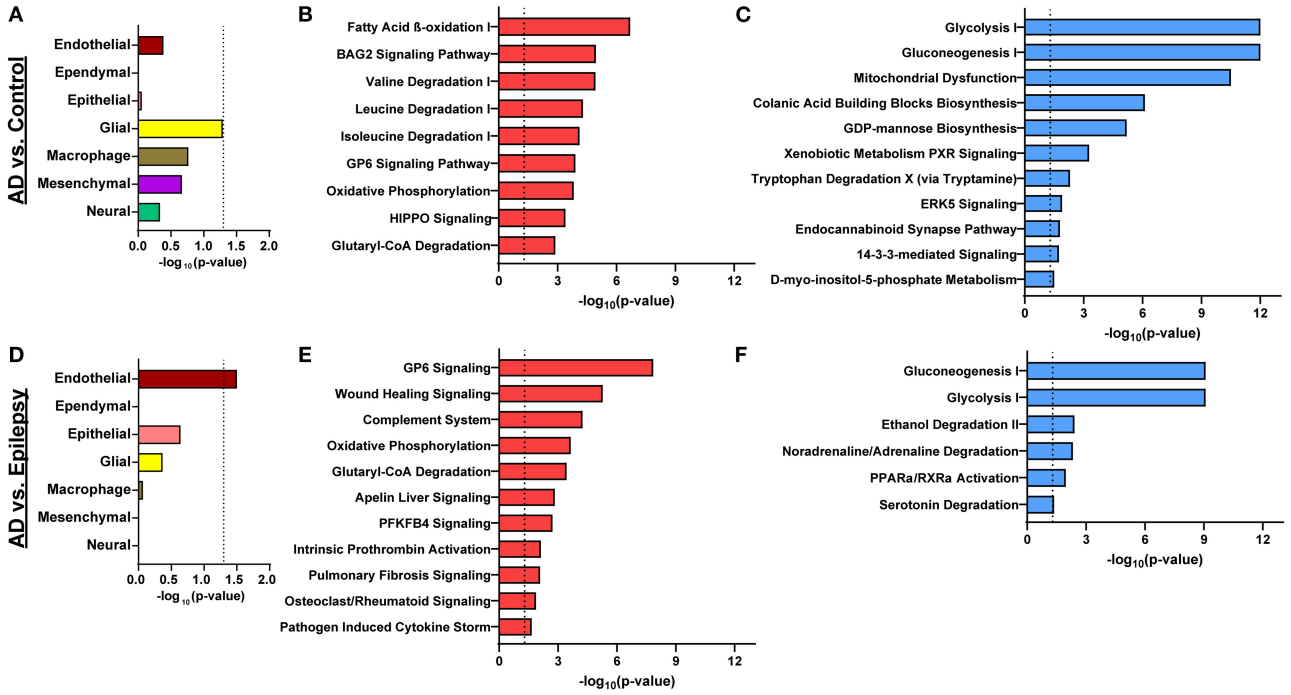


FIGURE 3
Cell-type enrichment and signaling pathways associated with proteomics differences. **(A)** Cell-type annotation analysis of differentially expressed proteins in AD vs. control by Fisher's exact test indicates a trend in enrichment ($p = 0.051$) for glial proteins. **(B, C)** For AD vs. control, the 616 differentially expressed proteins are significantly associated with 9 activated pathways (red) and 11 inhibited pathways (blue; $p$-value of overlap $p < 0.05$, z-score $\geq |2|$). **(D)** Cell-type enrichment analysis for differentially expressed proteins in AD vs. epilepsy indicates enrichment for endothelial cell proteins ($p = 0.031$). **(E, F)** For AD vs. epilepsy, the 438 differentially expressed proteins are significantly associated with 11 activated pathways and 6 inhibited pathways. The dotted lines indicate $p = 0.05$.

proteins, but those that were significant were all decreased and associated with this pathway. Previous studies have shown that proteasome proteins tend to be increased in AD when compared to controls in other brain regions when searched in our NeuroPro database (82). Follow-up studies should evaluate this finding in choroid plexus to determine whether these decreased proteins are associated with the dysfunction of protein clearance, altered in specific cell types, or present in another insoluble fraction for example. Additionally, 14-3-3-mediated signaling was inhibited with decreased 14-3-3 proteins (YWHAB, YWHAE, YWHAG, YWHAQ, and YWHAZ). The proteins in this pathway are also associated with multiple cellular functions, and in AD, they colocalize with neurofibrillary tau tangles and are increased in CSF, with correlations to clinical variables (94, 95). Evidence suggests that 14-3-3 proteins are decreased in the frontal cortex tissue, as well as in some studies from our NeuroPro database in most brain regions and in a limited choroid plexus proteomics study (55, 82, 96).

Proteomics analyses in human AD choroid plexus have been limited to less sensitive approaches (55), and transcriptomic studies have been limited to two RNA microarray analyses (97, 98). In the first RNA microarray study, choroid plexus epithelial cells were microdissected from AD and controls with differences related to increased oxidative stress and protein ubiquitin pathways and decreased glutathione-mediated detoxification and urea cycle pathways (99). In the second RNA microarray study, bulk AD choroid plexus were compared to controls with differences related to upregulated metabolic and immune-related pathways and downregulated methionine degradation and protein translation (98). We identified trends in these signaling pathways ($p$-value of overlap $< 0.05$, z-score n.s.), including mTOR signaling (98), methionine degradation pathways (98), unfolded protein response (99), protein ubiquitination pathway, (99) urea cycle, (99) and glutathione-mediated detoxification (99). In contrast to previous studies, NRF2 oxidative stress (99) and aldosterone signaling in epithelial cells (99) trended down.

Other altered proteins in aging or AD choroid plexus were identified by non-proteomic studies (33, 35, 100), including basement membrane thickening, decreased clusterin, TTR, LRP2, IGF1, and gelsolin, and increased LRP1 and PGP. We identified 17 proteins associated with the basement membrane (GO cellular component GO:0005604) that were all increased and may be consistent with basement membrane thickening. Clusterin (CLU, also known as APOJ; P10909), an extracellular chaperone that traffics multiple proteins including Aβ in addition to other functions (100), was increased by 2.3-fold ($p = 1.42 \times 10^{-4}$). LRP1 was detected but not different. LRP2, TTR, PGP, gelsolin, and IGF1 were not detected.

We expected some similarities of proteins when comparing the choroid plexus to other studies evaluating CSF and blood vessel protein expression levels, as the choroid plexus produces

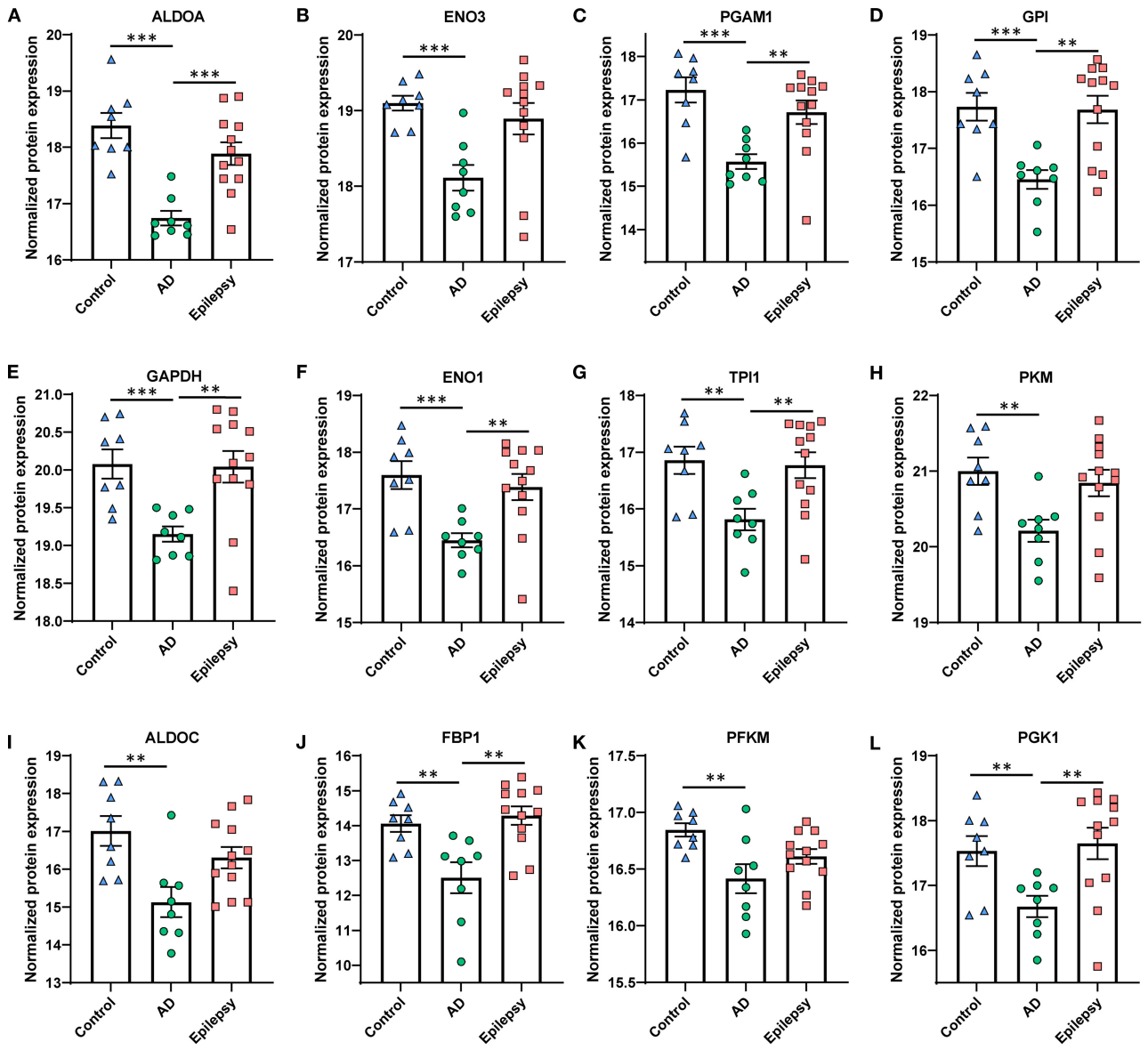


FIGURE 4
Proteins in AD vs. control associated with the top altered pathway, glycolysis inhibition. The glycolysis signaling pathway was the most significantly altered and was the most affected by the fold change of proteins in AD vs. control ($p = 1.00 \times 10^{-12}$, $z = -3.46$). **(A–L)** The proteins are depicted by order of decreasing significance. Those proteins that are significant at 5% FDR are indicated for all pairwise comparisons, with the *p*-values as indicated. ***$p < 0.001$, **$p < 0.01$. Error bars indicate SEM.

CSF and also contains blood vessels. CSF proteomics analyses had identified altered metabolism proteins in AD vs. controls, some differing from the brain tissue (56, 101). Increased glycolysis proteins were identified in CSF, including a top candidate aldolase fructose-bisphosphate A (ALDOA) (101). Whereas, we identified a significant 3.1-fold decrease ($p = 1.78 \times 10^{-5}$) in ALDOA in the choroid plexus of AD. In a proteomics analysis of Aβ accumulation in blood vessels of cases with cerebral amyloid angiopathy (CAA) in the occipital/parietal lobes, one of the top altered proteins was high-temperature requirement serine peptidase 1 (HTRA1) which is suggested to remove misfolded or mislocalized peptides in an ATP-independent manner (102). From our NeuroPro database, this protein is also increased in a number of other studies in AD from various brain regions (82). Similarly, we identified a 2.8-fold increase in HTRA1 ($p = 1.11 \times 10^{-3}$).

## Epilepsy vs. control

In the epilepsy vs. control comparison, only one protein (FUCA2) was elevated with many trending proteins in this heterogeneous disease group. In the 216 proteins trending in epilepsy, FUCA1 was also increased and has a similar function to FUCA2 adding fucose to glycoproteins and can be associated with cell migration as suggested from elevation in various tumor types (103). From the pathways associated with trending proteins,

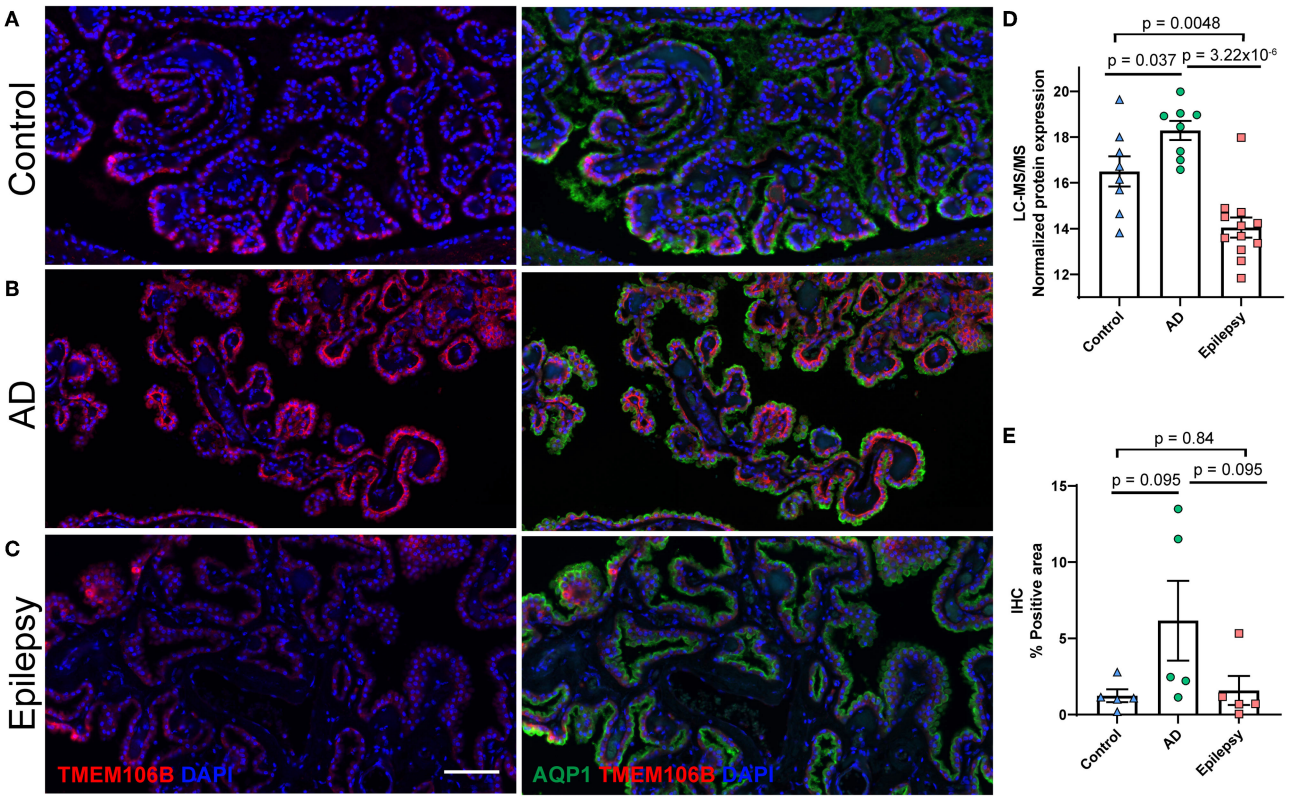


FIGURE 5
Protein candidate TMEM106B histological localization and quantification. Representative images from the **(A)** control, **(B)** AD, and **(C)** epilepsy groups of TMEM106B (red) localized in the basal membrane and epithelial marker AQP1 (green) in the apical membrane of epithelial cells of the choroid plexus adjacent to the hippocampus at the level of LGN. **(D)** TMEM106B quantification by LFQ-MS in control ($n = 8$), AD ($n = 8$), and epilepsy cases ($n = 12$). As determined by Student's two-tailed $t$-test with permutation correction at a 5% FDR, for AD vs. epilepsy, there was an 18.9-fold increase ($p = 3.22 \times 10^{-6}$, FDR <5%), for AD vs. control, there was a 3.5-fold increase ($p = 0.037$, FDR >5%), and for epilepsy vs. control, there was a 5.5-fold decrease ($p = 0.0048$, FDR >5%). **(E)** Immunohistochemistry from five cases/group shows using semiquantitative analysis that TMEM106B expression follows a similar trend observed in LFQ-MS, AD vs. epilepsy (3.9-fold increase, $p = 0.095$), AD vs. control (5.0-fold increase, $p = 0.095$), and epilepsy vs. control (1.3-fold increase, $p = 0.84$) by the Mann–Whitney U-test. Scale bar 100 um. Error bars indicate SEM.

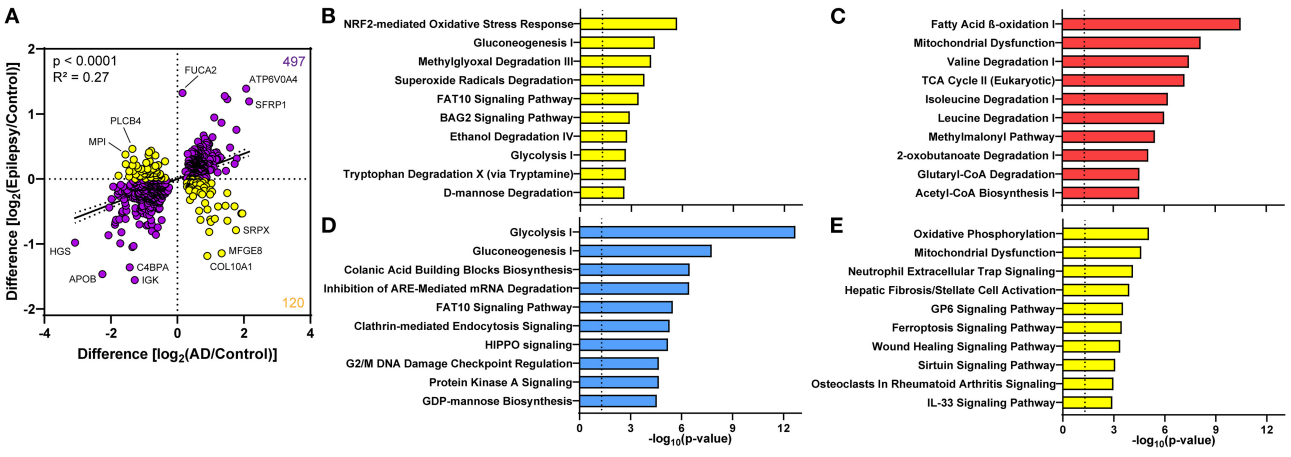


FIGURE 6
Proteomic differences in the choroid plexus of AD and epilepsy cases positively correlate. **(A)** Of the 617 altered proteins in AD and epilepsy cases when compared to controls, 497/617 (81%) changed in the same direction (purple) and 120/617 (19%) in the opposite direction (yellow) with an overall positive correlation ($p < 0.0001$, $R^2 = 0.27$). Several of the proteins with the highest fold change are annotated by the gene name. The top 10 signaling pathways associated with the proteins in each quadrant from the correlation show those pathways **(B)** down in AD and up in epilepsy, **(C)** up in both AD and epilepsy, **(D)** down in both AD and epilepsy, and **(E)** up in AD and down in epilepsy.

there were similarities to those observed in AD when compared to controls that included fatty beta-oxidation and 14-3-3 signaling. There have been no related proteomics or transcriptomics studies in human epilepsy choroid plexus for comparison. It will be of interest in future studies to evaluate larger homogeneous cohorts to identify whether there are additional protein differences, as well as comparison to other AD groups with more mild pathology and AD cases with an epilepsy diagnosis.

## AD vs. epilepsy

In the AD vs. epilepsy comparison, most of the protein differences were also found when comparing AD to controls, and so many of the same signaling pathways were identified. Additionally, there was the activation of inflammatory-related pathways such as complement system and pathogen-induced cytokine storm that were associated with a number of complement and collagen proteins. Although there were many differences, the changes in AD also correlated with trends in epilepsy when compared to controls.

TMEM106B was a top protein candidate that was elevated in AD when compared to the epilepsy group. TMEM106B is a type II transmembrane protein that localizes to late endosomes and lysosomes in many cell types, including in both neurons and oligodendrocytes (104). Previous studies have shown that TMEM106B can fibrilize in a similar way as Aβ in AD and that TMEM106B filaments may form in an age-dependent manner (105–107). There was a similar trend for expression levels on LFQ-MS and histology, with differences related to the detection method (i.e., sensitivity and normalization).

The correlation of AD and epilepsy to controls from those proteins significant in at least one pairwise comparison identified a positive correlation, with the majority of proteins changing in the same direction. With these similar trends, as expected, many of the same signaling pathways were identified and were associated with a shift in cellular energy production. Among the top correlated proteins with the highest fold changes, there was increased ATP6V0A4 and decreased APOB. ATP6V0A4 is a vacuolar ATPase (108) and can be involved in several signaling pathways, including those associated with endocytosis. The top pathway associated with ATP6V0A4 (increased by 4.2-fold in AD and by 2.6-fold in epilepsy compared to controls) from the increased proteins in the correlation was the iron homeostasis signaling pathway ($p = 3.80 \times 10^{-4}$). APOB is an apolipoprotein that transports lipids in plasma and CSF (109) and is also involved in several signaling pathways including endocytosis. APOB is increased in AD CSF and plasma. (109) It is unclear whether these cases have lower APOB levels relative to the many controls with atherosclerosis (110) that were observed on neuropathology and whether these levels reflect those in the adjacent brain tissue or CSF. Some of the top protein differences between AD and epilepsy with the highest fold change from the correlation included increased MFGE8 (milk fat globule EGF and Factor V/VIII domain containing) by 2.5-fold in AD and decreased by 2.2-fold in epilepsy. An increase in AD may be expected as MFGE8 vascular deposition increases with age and it can interact with Aβ (111). As noted above, it will be of interest to evaluate these protein differences further in larger homogeneous epilepsy cohorts, as well as across the AD and epilepsy spectrums of disease. Furthermore, future mechanistic studies will be essential to elucidate the implications of these protein differences, i.e., how the altered signaling pathways directly or indirectly impact CSF production, turnover, and content.

## Limitations

Our study had limitations, including a small sample size. Our technique is less sensitive in detecting large membrane proteins, insoluble proteins, and low-abundance proteins (i.e., TTR, AQP1, and APP were not detected). Among the AD and epilepsy disease groups, heterogeneous clinical variables warrant further evaluation in future studies with larger samples, as do genetic risk factors (e.g., APOE, MTOR, APP, and PSEN1). Differences we identified in bulk choroid plexus should be explored with regard to specific cell types.

# Conclusion

We identified a shift in cell energy metabolism in the choroid plexus of AD patients with severe neuropathology and similar trends in epilepsy patients. Follow-up studies should evaluate the spectrum of AD and epilepsy, including those cases with dual diagnoses to identify potential molecular drivers of epilepsy and AD. This could empower novel and targeted therapies.

# Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary material.

# Ethics statement

The studies involving human participants were reviewed and approved by NYU Grossman School of Medicine Institutional Review Board (IRB). The patients/participants provided their written informed consent to participate in this study.

# Author contributions

TW, OD, and DL contributed to the conception and design of the manuscript. DL, EK, AF, MT, DF, SD, and BU contributed to data collection. DL and EK performed data analyses. DL wrote the first draft of the manuscript. All authors contributed to the manuscript revision, read, and approved the submitted version.

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## Conflict of interest

DF receives salary support for consulting and clinical trial-related activities performed on behalf of The Epilepsy Study Consortium, a non-profit organization, and receives no personal income for these activities. NYU receives a fixed amount from the Epilepsy Study Consortium toward Dr. DF's salary. Within the past 2 years, The Epilepsy Study Consortium received payments for research services performed by DF from: Biohaven, BioXcell, Cerevel, Cerebral, Epilex, Equilibre, Jannsen, Lundbeck, Praxis, Puretech, Neurocrine, SK Life Science, Supernus, UCB, and Xenon. He has also served as a paid consultant for Neurelis Pharmaceuticals. He has received travel support from the Epilepsy Foundation. He has received research support from NINDS, NSF, and CDC for work unrelated to this study. He holds equity interests in Neuroview Technology. He received royalty income from Oxford University Press.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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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.2023.1221775/full#supplementary-material

## References


1. Mendez M, Lim G. Seizures in elderly patients with dementia: epidemiology and management. *Drugs Aging.* (2003) 20:791–803. doi: 10.2165/00002512-200320110-00001

2. Born HA. Seizures in Alzheimer's disease. *Neuroscience.* (2015) 286:251–63. doi: 10.1016/j.neuroscience.2014.11.051

3. Sánchez MP, García-Cabrero AM, Sánchez-Elexpuru G, Burgos DF, Serratosa JM. Tau-induced pathology in epilepsy and dementia: notions from patients and animal models. *Int J Mol Sci.* (2018) 19:4. doi: 10.3390/ijms19041092

4. Palop JJ, Mucke L. Epilepsy and cognitive impairments in Alzheimer disease. *Arch Neurol.* (2009) 66:435–40. doi: 10.1001/archneurol.2009.15

5. Rabinowicz AL, Starkstein SE, Leiguarda RC, Coleman AE. Transient epileptic amnesia in dementia: a treatable unrecognized cause of episodic amnestic wandering. *Alzheimer Dis Assoc Disord.* (2000) 14:231–3. doi: 10.1097/00002093-200010000-00008

6. Sen A, Capelli V, Husain M. Cognition and dementia in older patients with epilepsy. *Brain.* (2018) 141:1592–608. doi: 10.1093/brain/awy022

7. Miller LA, Galioto R, Tremont G, Davis J, Bryant K, Roth J, et al. Cognitive impairment in older adults with epilepsy: characterization and risk factor analysis. *Epilepsy Behav.* (2016) 56:113–7. doi: 10.1016/j.yebeh.2016.01.011

8. Kaestner E, Reyes A, Chen A, Rao J, Macari AC, Choi JY, et al. Atrophy and cognitive profiles in older adults with temporal lobe epilepsy are similar to mild cognitive impairment. *Brain.* (2020) 3:397. doi: 10.1093/brain/awaa397

9. Johnson EL, Krauss GL, Kucharska-Newton A, Albert MS, Brandt J, Walker KA, et al. Dementia in late-onset epilepsy: the atherosclerosis risk in communities study. *Neurology.* (2020) 95:e3248–e56. doi: 10.1212/WNL.0000000000011080

10. Xu Y, Lavrencic L, Radford K, Booth A, Yoshimura S, Anstey KJ, et al. Systematic review of coexistent epileptic seizures and Alzheimer's disease: Incidence and prevalence. *J Am Geriatr Soc.* (2021) 69:2011–20. doi: 10.1111/jgs.17101

11. Cortini F, Cantoni C, Villa C. Epileptic seizures in autosomal dominant forms of Alzheimer's disease. *Seizure.* (2018) 61:4–7. doi: 10.1016/j.seizure.2018.07.015

12. Ezquerra M, Carnero C, Blesa R, Gelpí JL, Ballesta F, Oliva R. A presenilin 1 mutation (Ser169Pro) associated with early-onset AD and myoclonic seizures. *Neurology.* (1999) 52:566–70. doi: 10.1212/WNL.52.3.566

13. Vöglein J, Willem M, Trambauer J, Schönecker S, Dieterich M, Biskup S, et al. Identification of a rare presenilin 1 single amino acid deletion mutation (F175del) with unusual amyloid-β processing effects. *Neurobiol Aging.* (2019) 84:241e5–11. doi: 10.1016/j.neurobiolaging.2019.08.034

14. Vossel KA, Beagle AJ, Rabinovici GD, Shu H, Lee SE, Naasan G, et al. Seizures and epileptiform activity in the early stages of Alzheimer disease. *JAMA Neurol.* (2013) 70:1158–66. doi: 10.1001/jamaneurol.2013.136

15. Vossel KA, Ranasinghe KG, Beagle AJ, Mizuiri D, Honma SM, Dowling AF, et al. Incidence and impact of subclinical epileptiform activity in Alzheimer's disease. *Ann Neurol.* (2016) 80:858–70. doi: 10.1002/ana.24794

16. Lam AD, Sarkis RA, Pellerin KR, Jing J, Dworetzky BA, Hoch DB, et al. Association of epileptiform abnormalities and seizures in Alzheimer disease. *Neurology.* (2020) 95:e2259–e70. doi: 10.1212/WNL.0000000000010612

17. Horvath AA, Papp A, Zsuffa J, Szucs A, Luckl J, Radai F, et al. Subclinical epileptiform activity accelerates the progression of Alzheimer's disease: a long-term EEG study. *Clin Neurophysiol.* (2021) 132:1982–9. doi: 10.1016/j.clinph.2021.03.050

18. Helmstaedter C, Witt JA. Epilepsy and cognition—A bidirectional relationship? *Seizure.* (2017) 49:83–9. doi: 10.1016/j.seizure.2017.02.017

19. Lam AD, Cole AJ, Cash SS. New approaches to studying silent mesial temporal lobe seizures in Alzheimer's disease. *Front Neurol.* (2019) 10:959. doi: 10.3389/fneur.2019.00959

20. Sanchez PE, Zhu L, Verret L, Vossel KA, Orr AG, Cirrito JR, et al. Levetiracetam suppresses neuronal network dysfunction and reverses synaptic and cognitive deficits in an Alzheimer's disease model. *Proc Natl Acad Sci U S A.* (2012) 109:E2895–903. doi: 10.1073/pnas.1121081109

21. Musaeus CS, Shafi MM, Santarnecchi E, Herman ST, Press DZ. Levetiracetam alters oscillatory connectivity in Alzheimer's disease. *J Alzheimers Dis.* (2017) 58:1065–76. doi: 10.3233/JAD-160742

22. Bakker A, Krauss GL, Albert MS, Speck CL, Jones LR, Stark CE, et al. Reduction of hippocampal hyperactivity improves cognition in amnestic mild cognitive impairment. *Neuron.* (2012) 74:467–74. doi: 10.1016/j.neuron.2012.03.023

23. Vossel K, Ranasinghe KG, Beagle AJ, La A, Ah Pook K, Castro M, et al. Effect of levetiracetam on cognition in patients with Alzheimer disease with and without epileptiform activity: a randomized clinical trial. *JAMA Neurol (.* (2021). doi: 10.1001/jamaneurol.2021.3310

24. Sen A, Akinola M, Tai XY, Symmonds M, Davis Jones G, Mura S, et al. An Investigation of Levetiracetam in Alzheimer's Disease (ILiAD): a double-blind, placebo-controlled, randomised crossover proof of concept study. *Trials.* (2021) 22:508. doi: 10.1186/s13063-021-05404-4

25. Gourmaud S, Shou H, Irwin DJ, Sansalone K, Jacobs LM, Lucas TH, et al. Alzheimer-like amyloid and tau alterations associated with cognitive deficit in temporal lobe epilepsy. *Brain.* (2020) 143:191–209. doi: 10.1093/brain/awz381

26. Nardi Cesarini E, Babiloni C, Salvadori N, Farotti L, Del Percio C, Pascarelli MT, et al. Late-onset epilepsy with unknown etiology: a pilot study on neuropsychological profile, cerebrospinal fluid biomarkers, and quantitative EEG characteristics. *Front Neurol.* (2020) 11:199. doi: 10.3389/fneur.2020.00199

27. Choi H, Thacker EL, Longstreth WT, Elkind MSV, Boehme AK. Cognitive decline in older adults with epilepsy: the Cardiovascular Health Study. *Epilepsia.* (2021) 62:85–97. doi: 10.1111/epi.16748

28. Stefanidou M, Beiser AS, Himali JJ, Peng TJ, Devinsky O, Seshadri S, et al. Bi-directional association between epilepsy and dementia: the Framingham heart study. *Neurology.* (2020) 95:e3241–e7. doi: 10.1212/WNL.0000000000011077

29. Drummond E, Pires G, MacMurray C, Askenazi M, Nayak S, Bourdon M et al. Phosphorylated tau interactome in the human Alzheimer's disease brain. *Brain.* (2020) 3:5492. doi: 10.1002/alz.045492

30. Paudel YN, Angelopoulou E, Jones NC, O'Brien TJ, Kwan P, Piperi C, et al. Tau related pathways as a connecting link between epilepsy and Alzheimer's disease. *ACS Chem Neurosci.* (2019) 10:4199–212. doi: 10.1021/acschemneuro.9b00460

31. Fernandes M, Manfredi N, Aluisantonio L, Franchini F, Chiaravalloti A, Izzi F, et al. Cognitive functioning, cerebrospinal fluid Alzheimer's disease biomarkers and cerebral glucose metabolism in late-onset epilepsy of unknown aetiology: a prospective study. *Eur J Neurosci.* (2022) 56:5384–96. doi: 10.1111/ejn.15734

32. Gourmaud S, Stewart DA, Irwin DJ, Roberts N, Barbour AJ, Eberwine G, et al. The role of mTORC1 activation in seizure-induced exacerbation of Alzheimer's disease. *Brain.* (2022) 145:324–39. doi: 10.1093/brain/awab268

33. Benarroch EE. Choroid plexus–CSF system: Recent developments and clinical correlations. *Neurology.* (2016) 86:286–96. doi: 10.1212/WNL.0000000000002298

34. Krzyzanowska A, Carro E. Pathological alteration in the choroid plexus of Alzheimer's disease: implication for new therapy approaches. *Front Pharmacol.* (2012) 3:75. doi: 10.3389/fphar.2012.00075

35. Marques F, Sousa JC, Sousa N, Palha JA. Blood-brain-barriers in aging and in Alzheimer's disease. *Mol Neurodegener.* (2013) 8:38. doi: 10.1186/1750-1326-8-38

36. Choi JD, Moon Y, Kim HJ, Yim Y, Lee S, Moon WJ. Choroid plexus volume and permeability at brain MRI within the alzheimer disease clinical spectrum. *Radiology.* (2022) 4:212400. doi: 10.1148/radiol.212400

37. Dietrich MO, Spuch C, Antequera D, Rodal I, de Yébenes JG, Molina JA, et al. Megalin mediates the transport of leptin across the blood-CSF barrier. *Neurobiol Aging.* (2008) 29:902–12. doi: 10.1016/j.neurobiolaging.2007.01.008

38. Perez-Gracia E, Blanco R, Carmona M, Carro E, Ferrer I. Oxidative stress damage and oxidative stress responses in the choroid plexus in Alzheimer's disease. *Acta Neuropathol.* (2009) 118:497–504. doi: 10.1007/s00401-009-0574-4

39. González-Marrero I, Giménez-Llort L, Johanson CE, Carmona-Calero EM, Castañeyra-Ruiz L, Brito-Armas JM, et al. Choroid plexus dysfunction impairs beta-amyloid clearance in a triple transgenic mouse model of Alzheimer's disease. *Front Cell Neurosci.* (2015) 9:17. doi: 10.3389/fncel.2015.00017

40. Serot JM, Zmudka J, Jouanny P, A. possible role for CSF turnover and choroid plexus in the pathogenesis of late onset Alzheimer's disease. *J Alzheimers Dis.* (2012) 30:17–26. doi: 10.3233/JAD-2012-111964

41. Hirvonen J, Kreisl WC, Fujita M, Dustin I, Khan O, Appel S, et al. Increased in vivo expression of an inflammatory marker in temporal lobe epilepsy. *J Nucl Med.* (2012) 53:234–40. doi: 10.2967/jnumed.111.091694

42. Dolgodilina E, Camargo SM, Roth E, Herzog B, Nunes V, Palacín M, et al. Choroid plexus LAT2 and SNAT3 as partners in CSF amino acid homeostasis maintenance. *Fluids Barriers CNS.* (2020) 17:17. doi: 10.1186/s12987-020-0178-x

43. Akanuma S, Sakurai T, Tachikawa M, Kubo Y, Hosoya K. Transporter-mediated L-glutamate elimination from cerebrospinal fluid: possible involvement of excitatory amino acid transporters expressed in ependymal cells and choroid plexus epithelial cells. *Fluids Barriers CNS.* (2015) 12:11. doi: 10.1186/s12987-015-0006-x

44. Rainesalo S, Keränen T, Palmio J, Peltola J, Oja SS, Saransaari P. Plasma and cerebrospinal fluid amino acids in epileptic patients. *Neurochem Res.* (2004) 29:319–24. doi: 10.1023/B:NERE.0000010461.34920.0c

45. Scheyer RD. Involvement of glutamate in human epileptic activities. *Prog Brain Res.* (1998) 116:359–69. doi: 10.1016/S0079-6123(08)60448-3

46. Xu J, Patassini S, Rustogi N, Riba-Garcia I, Hale BD, Phillips AM, et al. Regional protein expression in human Alzheimer's brain correlates with disease severity. *Commun Biol.* (2019) 2:43. doi: 10.1038/s42003-018-0254-9

47. Seyfried NT, Dammer EB, Swarup V, Nandakumar D, Duong DM, Yin L, et al. A multi-network approach identifies protein-specific co-expression in asymptomatic and symptomatic Alzheimer's disease. *Cell Syst.* (2017) 4:60–72.e4. doi: 10.1016/j.cels.2016.11.006

48. Hales CM, Dammer EB, Deng Q, Duong DM, Gearing M, Troncoso JC, et al. Changes in the detergent-insoluble brain proteome linked to amyloid and tau in Alzheimer's disease progression. *Proteomics.* (2016) 16:3042–53. doi: 10.1002/pmic.201600057

49. Johnson ECB, Dammer EB, Duong DM, Yin L, Thambisetty M, Troncoso JC, et al. Deep proteomic network analysis of Alzheimer's disease brain reveals alterations in RNA binding proteins and RNA splicing associated with disease. *Mol Neurodegener.* (2018) 13:52. doi: 10.1186/s13024-018-0282-4

50. Hsieh YC, Guo C, Yalamanchili HK, Abreha M, Al-Ouran R, Li Y, et al. Tau-mediated disruption of the spliceosome triggers cryptic RNA splicing and neurodegeneration in Alzheimer's disease. *Cell Rep.* (2019) 29:301–16e10. doi: 10.1016/j.celrep.2019.08.104

51. Mendonça CF, Kuras M, Nogueira FCS, Plá I, Hortobágyi T, Csiba L, et al. Proteomic signatures of brain regions affected by tau pathology in early and late stages of Alzheimer's disease. *Neurobiol Dis.* (2019) 130:104509. doi: 10.1016/j.nbd.2019.104509

52. Drummond E, Nayak S, Faustin A, Pires G, Hickman RA, Askenazi M, et al. Proteomic differences in amyloid plaques in rapidly progressive and sporadic Alzheimer's disease. *Acta Neuropathol.* (2017) 133:933–54. doi: 10.1007/s00401-017-1691-0

53. Pires G, Ueberheide B, Wisniewski T, Drummond E. Use of affinity purification-mass spectrometry to identify phosphorylated tau interactors in Alzheimer's disease. *Methods Mol Biol.* (2023) 2561:263–77. doi: 10.1007/978-1-0716-2655-9_14

54. Pires G, Leitner D, Drummond E, Kanshin E, Nayak S, Askenazi M, et al. Proteomic differences in the hippocampus and cortex of epilepsy brain tissue. *Brain Commun.* (2021) 3:fcab021. doi: 10.1093/braincomms/fcab021

55. Krzyzanowska A, García-Consuegra I, Pascual C, Antequera D, Ferrer I, Carro E. Expression of regulatory proteins in choroid plexus changes in early stages of Alzheimer disease. *J Neuropathol Exp Neurol.* (2015) 74:359–69. doi: 10.1097/NEN.0000000000000181

56. Johnson ECB, Dammer EB, Duong DM, Ping L, Zhou M, Yin L, et al. Large-scale proteomic analysis of Alzheimer's disease brain and cerebrospinal fluid reveals early changes in energy metabolism associated with microglia and astrocyte activation. *Nat Med.* (2020) 26:769–80. doi: 10.1038/s41591-020-0815-6

57. Whelan CD, Mattsson N, Nagle MW, Vijayaraghavan S, Hyde C, Janelidze S, et al. Multiplex proteomics identifies novel CSF and plasma biomarkers of early Alzheimer's disease. *Acta Neuropathol Commun.* (2019) 7:169. doi: 10.1186/s40478-019-0795-2

58. Dammer EB, Ping L, Duong DM, Modeste ES, Seyfried NT, Lah JJ, et al. Multi-platform proteomic analysis of Alzheimer's disease cerebrospinal fluid and plasma reveals network biomarkers associated with proteostasis and the matrisome. *Alzheimers Res Ther.* (2022) 14:174. doi: 10.1186/s13195-022-01113-5

59. Cummings JL, Morstorf T, Zhong K. Alzheimer's disease drug-development pipeline: few candidates, frequent failures. *Alzheimers Res Ther.* (2014) 6:37. doi: 10.1186/alzrt269

60. Banik A, Brown RE, Bamburg J, Lahiri DK, Khurana D, Friedland RP, et al. Translation of pre-clinical studies into successful clinical trials for Alzheimer's disease: what are the roadblocks and how can they be overcome? *J Alzheimers Dis.* (2015) 47:815–43. doi: 10.3233/JAD-150136

61. Schneider LS, Mangialasche F, Andreasen N, Feldman H, Giacobini E, Jones R, et al. Clinical trials and late-stage drug development for Alzheimer's disease: an appraisal from 1984 to 2014. *J Intern Med.* (2014) 275:251–83. doi: 10.1111/joim.12191

62. Kwon S, Iba M, Kim C, Masliah E. Immunotherapies for aging-related neurodegenerative diseases-emerging perspectives and new targets. *Neurotherapeutics.* (2020) 17:935–54. doi: 10.1007/s13311-020-00853-2

63. Long JM, Holtzman DM. Alzheimer disease: an update on pathobiology and treatment strategies. *Cell.* (2019) 179:312–39. doi: 10.1016/j.cell.2019.09.001

64. Reiss AB, Glass AD, Wisniewski T, Wolozin B, Gomolin IH, Pinkhasov A, et al. Alzheimer's disease: many failed trials, so where do we go from here? *J Investig Med.* (2020) 68:1135–40. doi: 10.1136/jim-2020-001297

65. Wisniewski T, Drummond E. Future horizons in Alzheimer's disease research. *Prog Mol Biol Transl Sci.* (2019) 168:223–41. doi: 10.1016/bs.pmbts.2019.08.001

66. Kim CK, Lee YR, Ong L, Gold M, Kalali A, Sarkar J. Alzheimer's disease: key insights from two decades of clinical trial failures. *J Alzheimers Dis.* (2022) 87:83–100. doi: 10.3233/JAD-215699

67. French JA. Refractory epilepsy: clinical overview. *Epilepsia.* (2007) 48(Suppl 1):3–7. doi: 10.1111/j.1528-1167.2007.00992.x

68. Dalic L, Cook MJ. Managing drug-resistant epilepsy: challenges and solutions. *Neuropsychiatr Dis Treat.* (2016) 12:2605–16. doi: 10.2147/NDT.S84852

69. Montine TJ, Phelps CH, Beach TG, Bigio EH, Cairns NJ, Dickson DW, et al. National Institute on Aging-Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease: a practical approach. *Acta Neuropathol.* (2012) 123:1–11. doi: 10.1007/s00401-011-0910-3

70. Leitner DF, William C, Faustin A, Askenazi M, Kanshin E, Snuderl M, et al. Proteomic differences in hippocampus and cortex of sudden unexplained death in childhood. *Acta Neuropathol.* (2022) 143:585–99. doi: 10.1007/s00401-022-02414-7

71. Leitner D, Kanshin E, Askenazi M, Faustin A, Friedman D, Devore S, et al. Raphe and ventrolateral medulla proteomics in epilepsy and sudden unexpected death in epilepsy. *Brain Commun.* (2022) 3:186. doi: 10.1093/braincomms/fcac186

72. Drummond E, Nayak S, Pires G, Ueberheide B, Wisniewski T. Isolation of amyloid plaques and neurofibrillary tangles from archived Alzheimer's disease tissue using laser-capture microdissection for downstream proteomics. *Methods Mol Biol.* (2018) 1723:319–34. doi: 10.1007/978-1-4939-7558-7_18

73. Leitner DF, Mills JD, Pires G, Faustin A, Drummond E, Kanshin E, et al. Proteomics and transcriptomics of the hippocampus and cortex in SUDEP and high-risk SUDEP patients. *Neurology.* (2021) 96:e2639–e52. doi: 10.1212/WNL.0000000000011999

74. Drummond E, Nayak S, Ueberheide B, Wisniewski T. Localized proteomics of individual neurons isolated from formalin-fixed, paraffin-embedded tissue sections using laser capture microdissection. *Curr Prot Appr Appl Brain Fun.* (2017) 4:289–301. doi: 10.1007/978-1-4939-7119-0_18

75. Doellinger J, Schneider A, Hoeller M, Lasch P. Sample preparation by easy extraction and digestion (SPEED)—A universal, rapid, and detergent-free protocol for proteomics based on acid extraction. *Mol Cell Proteomics.* (2020) 19:209–22. doi: 10.1074/mcp.TIR119.001616

76. Ludwig C, Gillet L, Rosenberger G, Amon S, Collins BC, Aebersold R. Data-independent acquisition-based SWATH-MS for quantitative proteomics: a tutorial. *Mol Syst Biol.* (2018) 14:e8126. doi: 10.15252/msb.20178126

77. Tyanova S, Temu T, Sinitcyn P, Carlson A, Hein MY, Geiger T, et al. The Perseus computational platform for comprehensive analysis of (prote)omics data. *Nat Methods.* (2016) 13:731–40. doi: 10.1038/nmeth.3901

78. Heberle H, Meirelles GV, da Silva FR, Telles GP, Minghim R. InteractiVenn: a web-based tool for the analysis of sets through Venn diagrams. *BMC Bioinformatics.* (2015) 16:169. doi: 10.1186/s12859-015-0611-3

79. Yang AC, Kern F, Losada PM, Agam MR, Maat CA, Schmartz GP, et al. Dysregulation of brain and choroid plexus cell types in severe COVID-19. *Nature.* (2021) 595:565–71. doi: 10.1038/s41586-021-03710-0

80. Leitner DF, Lin Z, Sawaged Z, Kanshin E, Friedman D, Devore S, et al. Brain molecular mechanisms in Rasmussen encephalitis. *Epilepsia.* (2023) 64:218–30. doi: 10.1111/epi.17457

81. Leitner DF, Siu Y, Korman A, Lin Z, Kanshin E, Friedman D, et al. Metabolomic, proteomic, and transcriptomic changes in adults with epilepsy on modified atkins diet. *Epilepsia.* (2023) 3:7540. doi: 10.1111/epi.17540

82. Askenazi M, Kavanagh T, Pires G, Ueberheide B, Wisniewski T, Drummond E. Compilation of all known protein changes in the human Alzheimer's disease brain. *bioRxiv*. (2023) 4:2023.04.13.536828. doi: 10.1101/2023.04.13.536828

83. Pires G, Leitner D, Drummond EE, Kanshin E, Nayak S, Askenazi M, et al. Proteomic differences in the hippocampus and cortex of epilepsy brain tissue. *bioRxiv*. (2020) 5:2020.07.21.209163. doi: 10.1101/2020.07.21.209163

84. Leitner DF, Kanshin E, Askenazi M, Siu Y, Friedman D, Devore S, et al. Pilot study evaluating everolimus molecular mechanisms in tuberous sclerosis complex and focal cortical dysplasia. *PLoS One.* (2022) 17:e0268597. doi: 10.1371/journal.pone.0268597

85. Bough KJ, Wetherington J, Hassel B, Pare JF, Gawryluk JW, Greene JG, et al. Mitochondrial biogenesis in the anticonvulsant mechanism of the ketogenic diet. *Ann Neurol.* (2006) 60:223–35. doi: 10.1002/ana.20899

86. Cunnane SC, Courchesne-Loyer A, Vandenberghe C, St-Pierre V, Fortier M, Hennebelle M, et al. Can ketones help rescue brain fuel supply in later life? Implications for cognitive health during aging and the treatment of alzheimer's disease. *Front Mol Neurosci.* (2016) 9:53. doi: 10.3389/fnmol.2016.00053

87. Simpson IA, Carruthers A, Vannucci SJ. Supply and demand in cerebral energy metabolism: the role of nutrient transporters. *J Cereb Blood Flow Metab.* (2007) 27:1766–91. doi: 10.1038/sj.jcbfm.9600521

88. Winkler EA, Nishida Y, Sagare AP, Rege SV, Bell RD, Perlmutter D, et al. GLUT1 reductions exacerbate Alzheimer's disease vasculo-neuronal dysfunction and degeneration. *Nat Neurosci.* (2015) 18:521–30. doi: 10.1038/nn.3966

89. Avgerinos KI, Egan JM, Mattson MP, Kapogiannis D. Medium Chain Triglycerides induce mild ketosis and may improve cognition in Alzheimer's disease. A systematic review and meta-analysis of human studies. *Ageing Res Rev.* (2020) 58:101001. doi: 10.1016/j.arr.2019.101001

90. Giannos P, Prokopidis K, Lidoriki I, Triantafyllidis KK, Kechagias KS, Celoch K, et al. Medium-chain triglycerides may improve memory in non-demented older adults: a systematic review of randomized controlled trials. *BMC Geriatr.* (2022) 22:817. doi: 10.1186/s12877-022-03521-6

91. Lilamand M, Mouton-Liger F, Di Valentin E, Sànchez Ortiz M, Paquet C. Efficacy and safety of ketone supplementation or ketogenic diets for Alzheimer's disease: a mini review. *Front Nutr.* (2021) 8:807970. doi: 10.3389/fnut.2021.807970

92. Rusek M, Pluta R, Ułamek-Kozioł M, Czuczwar SJ. Ketogenic diet in Alzheimer's disease. *Int J Mol Sci.* (2019) 20:3892. doi: 10.3390/ijms20163892

93. Qin L, Guo J, Zheng Q, Zhang H. BAG2 structure, function and involvement in disease. *Cell Mol Biol Lett.* (2016) 21:18. doi: 10.1186/s11658-016-0020-2

94. Layfield R, Fergusson J, Aitken A, Lowe J, Landon M, Mayer RJ. Neurofibrillary tangles of Alzheimer's disease brains contain 14-3-3 proteins. *Neurosci Lett.* (1996) 209:57–60. doi: 10.1016/0304-3940(96)12598-2

95. Lu Y. Early increase of cerebrospinal fluid 14-3-3ζ protein in the alzheimer's disease continuum. *Front Aging Neurosci.* (2022) 14:941927. doi: 10.3389/fnagi.2022.941927

96. Gu Q, Cuevas E, Raymick J, Kanungo J, Sarkar S. Downregulation of 14-3-3 proteins in Alzheimer's disease. *Mol Neurobiol.* (2020) 57:32–40. doi: 10.1007/s12035-019-01754-y

97. Berg AT, Langfitt J, Shinnar S, Vickrey BG, Sperling MR, Walczak T, et al. How long does it take for partial epilepsy to become intractable? *Neurology.* (2003) 60:186–90. doi: 10.1212/01.WNL.0000031792.89992.EC

98. Stopa EG, Tanis KQ, Miller MC, Nikonova EV, Podtelezhnikov AA, Finney EM, et al. Comparative transcriptomics of choroid plexus in Alzheimer's disease, frontotemporal dementia and Huntington's disease: implications for CSF homeostasis. *Fluids Barriers CNS.* (2018) 15:18. doi: 10.1186/s12987-018-0102-9

99. Bergen AA, Kaing S. ten Brink JB, Gorgels TG, Janssen SF, Bank NB. Gene expression and functional annotation of human choroid plexus epithelium failure in Alzheimer's disease. *BMC Genomics.* (2015) 16:956. doi: 10.1186/s12864-015-2159-z

100. Foster EM, Dangla-Valls A, Lovestone S, Ribe EM, Buckley NJ. Clusterin in Alzheimer's disease: mechanisms, genetics, and lessons from other pathologies. *Front Neurosci.* (2019) 13:164. doi: 10.3389/fnins.2019.00164

101. Higginbotham L, Ping L, Dammer EB, Duong DM, Zhou M, Gearing M, et al. Integrated proteomics reveals brain-based cerebrospinal fluid biomarkers in asymptomatic and symptomatic Alzheimer's disease. *Sci Adv.* (2020) 6:9360. doi: 10.1126/sciadv.aaz9360

102. Zellner A, Müller SA, Lindner B, Beaufort N, Rozemuller AJM, Arzberger T, et al. Proteomic profiling in cerebral amyloid angiopathy reveals an overlap with CADASIL highlighting accumulation of HTRA1 and its substrates. *Acta Neuropathol Commun.* (2022) 10:6. doi: 10.1186/s40478-021-01303-6

103. Zhong A, Chen T, Xing Y, Pan X, Shi M. FUCA2 is a prognostic biomarker and correlated with an immunosuppressive microenvironment in pan-cancer. *Front Immunol.* (2021) 12:758648. doi: 10.3389/fimmu.2021.758648

104. Feng T, Lacrampe A, Hu F. Physiological and pathological functions of TMEM106B: a gene associated with brain aging and multiple brain disorders. *Acta Neuropathol.* (2021) 141:327–39. doi: 10.1007/s00401-020-02246-3

105. Schweighauser M, Arseni D, Bacioglu M, Huang M, Lövestam S, Shi Y, et al. Age-dependent formation of TMEM106B amyloid filaments in human brains. *Nature.* (2022) 605:310–4. doi: 10.1038/s41586-022-04650-z

106. Perneel J, Neumann M, Heeman B, Cheung S, Van den Broeck M, Wynants S, et al. Accumulation of TMEM106B C-terminal fragments in neurodegenerative disease and aging. *Acta Neuropathol.* (2023) 145:285–302. doi: 10.1007/s00401-022-02531-3

107. Chang A, Xiang X, Wang J, Lee C, Arakhamia T, Simjanoska M, et al. Homotypic fibrillization of TMEM106B across diverse neurodegenerative diseases. *Cell.* (2022) 185:1346–55. doi: 10.1016/j.cell.2022.02.026

108. Song Q, Meng B, Xu H, Mao Z. The emerging roles of vacuolar-type ATPase-dependent Lysosomal acidification in neurodegenerative diseases. *Transl Neurodegener.* (2020) 9:17. doi: 10.1186/s40035-020-00196-0

109. Picard C, Nilsson N, Labonté A, Auld D, Rosa-Neto P, Ashton NJ, et al. Apolipoprotein B is a novel marker for early tau pathology in Alzheimer's disease. *Alzheimers Dement.* (2022) 18:875–87. doi: 10.1002/alz.12442

110. Williams KJ, Wu X. Imbalanced insulin action in chronic over nutrition: Clinical harm, molecular mechanisms, and a way forward. *Atherosclerosis.* (2016) 247:225–82. doi: 10.1016/j.atherosclerosis.2016.02.004

111. Wagner J, Degenhardt K, Veit M, Louros N, Konstantoulea K, Skodras A, et al. Medin co-aggregates with vascular amyloid-β in Alzheimer's disease. *Nature.* (2022) 612:123–31. doi: 10.1038/s41586-022-05440-3

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# Loss of normal Alzheimer's disease-associated Presenilin 2 function alters antiseizure medicine potency and tolerability in the 6-Hz focal seizure model

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**Introduction:** Patients with early-onset Alzheimer's disease (EOAD) experience seizures and subclinical epileptiform activity, which may accelerate cognitive and functional decline. Antiseizure medicines (ASMs) may be a tractable disease-modifying strategy; numerous ASMs are marketed with well-established safety. However, little information is available to guide ASM selection as few studies have rigorously quantified ASM potency and tolerability in traditional seizure models in rodents with EOAD-associated risk factors. Presenilin 2 (PSEN2) variants evoke EOAD, and these patients experience seizures. This study thus established the anticonvulsant profile of mechanistically distinct ASMs in the frontline 6-Hz limbic seizure test evoked in PSEN2-knockout (KO) mice to better inform seizure management in EOAD.

**Methods:** The median effective dose (ED50) of prototype ASMs was quantified in the 6-Hz test in male and female PSEN2-KO and wild-type (WT) C57BL/6J mice (3–4 months old). Minimal motor impairment (MMI) was assessed to estimate a protective index (PI). Immunohistological detection of cFos established the extent to which 6-Hz stimulation activates discrete brain regions in KO vs. WT mice.

**Results:** There were significant genotype-related differences in the potency and tolerability of several ASMs. Valproic acid and levetiracetam were significantly more potent in male KO than in WT mice. Additionally, high doses of valproic acid significantly worsened MMI in KO mice. Conversely, carbamazepine was significantly less potent in female KO vs. WT mice. In both male and female KO mice vs. WTs, perampanel and lamotrigine were equally potent. However, there were marked genotype-related shifts in PI of both carbamazepine and perampanel, with KO mice exhibiting less MMI at the highest doses tested. Gabapentin was ineffective against 6-Hz seizures in KO mice vs. WTs without MMI changes. Neuronal activation 90 min following 6-Hz stimulation was significantly increased in the posterior parietal association cortex overlying CA1 and in the piriform cortex of WT mice, while stimulation-induced increases in cFos immunoreactivity were absent in KO mice.

**Discussion:** Acute ASM potency and tolerability in the high-throughput 6-Hz test may be significantly altered with loss of normal PSEN2 function. Seizures in discrete EOAD populations may benefit from precisely selected medicines optimized for primary ASM pharmacological mechanisms.

KEYWORDS

**perampanel, levetiracetam, gabapentin, lamotrigine, valproic acid, mouse seizure model, piriform cortex, cFos**

## 1. Introduction

Alzheimer's disease (AD) poses a pressing global health challenge due to the rapidly aging world population and the relative lack of effective disease-modifying agents. AD may also benefit from targeted personalized medicine strategies to combat disease progression. Heterogeneity permeates all aspects of the disease, from age of onset to genetic variants, and comorbid conditions, highlighting that individualized treatment strategies may be necessary. Patients with AD experience seizures at a higher rate than the general age-matched population (1, 2). Individuals with familial early-onset AD (EOAD) experience the highest risk of undetected focal seizures (3). Genetic risk factors that lead to EOAD include variations or duplications in amyloid precursor protein (APP), presenilin 1 (PSEN1), and presenilin 2 (PSEN2) genes, all of which are also associated with seizures (3–6). Preclinical studies also reinforce this heterogeneity in seizure risk associated with EOAD-related genetic variants (7–13). Despite genotype-related variability in seizure risk, uncontrolled focal seizures likely contribute to and/or worsen overall AD burden (14, 15), similar to that which arises in uncontrolled or drug-resistant epilepsy. A longitudinal study of AD patients detected subclinical epileptiform activity in 42.4% of cases; patients with seizures also had a more rapid decline in cognition and executive functioning (14). The extent of network hyperexcitability in AD has likely been vastly underestimated (14); prolonged EEG monitoring studies are infrequently conducted in individuals with AD (1, 15, 16). Foramen ovale electrode recordings detected hippocampal hyperexcitability, mesial temporal lobe seizures, and spikes in the absence of scalp EEG abnormalities or clinical manifestations (17), suggesting that seizures in AD may be easily missed and thus untreated, further accelerating functional decline in these individuals. Epileptiform abnormalities are common in AD but inconsistent in presentation across individuals (18). Nonetheless, subclinical seizures are likely a major contributor to cognitive impairments in AD as opposed to being a late-onset sequela of AD neurodegeneration (17). Limited clinical studies have assessed the benefit of selected antiseizure medicines (ASMs) administration in people with AD, although some studies are ongoing (6, 19–22). However, it is presently unknown whether mechanistically diverse ASMs may be differentially effective or tolerated in EOAD vs. the general epilepsy population, an insight that could potentially benefit intervention selection to slow the functional decline of AD.

Presenilins are intramembrane proteases that form the catalytic component of the γ-secretase enzyme. Variants in these proteins lead to the aberrant cleavage of APP to the subsequent neurotoxic Aβ1-42 hallmark of AD and accumulation of Aβ plaques (23). However, presenilin (PSEN) variants actually reduce overall proteolytic activity, thereby indirectly increasing Aβ protein aggregation (24). PSEN2 is also the predominant γ-secretase in microglia (25, 26) and worsens inflammatory response in response to stimuli (27), making it an attractive target to study non-neuronal mechanisms of AD pathology. Clinically, PSEN1 variants are much more commonly causative for AD than either PSEN2 variants or APP duplications (28, 29). Studies frequently assess how PSEN1 variants may promote AD in the setting of APP duplication mutations (6, 30, 31). Furthermore, seizure susceptibility in mouse AD models with APP duplication and PSEN1 variants has been extensively studied (7, 9, 10, 12, 32–34). However, PSEN2 is also an attractive target to interrogate the biological heterogeneity of AD risk and AD-associated comorbidities, especially considering that hyperexcitability and seizures in people with PSEN2 variants are as common within 5 years of AD diagnosis as in people with APP duplications (3). Although PSEN2 variants are fewer in number in the EOAD population, the relevance of PSEN2 function to contribute to subsequent AD pathology and pathobiology carries the potential to uncover non-neuronal mechanisms associated with AD pathogenesis and epileptiform activity (5, 25–27).

The preclinical profiling of ASM efficacy and tolerability has been historically defined in young, neurologically intact male wild-type rodents (35–37), which does not wholly reflect the extent of epilepsy prevalence across the lifespan (38). There has been a concerted effort to improve twenty-first-century ASM discovery efforts to address these remaining unmet medical needs of people with epilepsy (39, 40), including increasing the integration of syndrome-specific models of pediatric epileptic encephalopathies and models of drug-resistant epilepsy into early ASM discovery (41–43). However, this approach does not go far enough to address the pressing global increase in seizures in older adults including individuals with seizures in AD. Thus, we sought to address this preclinical gap by quantifying ASM potency and tolerability in PSEN2-knockout (KO) mice to determine whether mice with an AD-related genotype and a breeding strategy suitable for efficient high-throughput ASM discovery could reflect a useful preclinical ASM screening platform for seizures in individuals with AD. Most pathogenic PSEN2 variants in AD lead to a biochemical loss of normal γ-secretase enzyme function (24, 28). PSEN2-KO mice are therefore a reasonable surrogate to evaluate the functional impacts of evoked or chronic seizures due to their facile breeding strategy (KO x KO), longevity (13), and adaptability to high-throughput drug assessments or subsequent cognitive comorbidity evaluations (6, 11, 13). Thus, we quantified the potency of distinct pharmacological classes of ASMs that are commonly prescribed to older adults with epilepsy (38) in this rodent model with an AD-related genotype to potentially guide the selection of ASMs in the clinical management of seizures in AD. We employed the well-characterized and high-throughput evoked mouse 6-Hz seizure model of limbic seizures to address this major gap (44–47). The 6-Hz model electrically induces acute, secondarily generalized focal seizures in the rodent forebrain with a high-throughput capacity (44–47). The 6-Hz seizures engage limbic structures at higher current intensity (44), regions that are also hyperexcitable in AD (17, 18), and suitably differentiate ASMs vs. other seizure and epilepsy models [i.e., maximal electroshock test, subcutaneous pentylenetetrazol, kindling models, and status epilepticus-induced chronic epilepsy models (37, 41, 48)]. We thus hypothesized that the loss of normal PSEN2 function would alter the anticonvulsant activity profile of mechanistically distinct ASMs in this preclinical seizure model and establish the differentiation capacity of the 6-Hz seizure test evoked in PSEN2-KO mice as a suitable strategy for ASM discovery for seizures in AD.

# 2. Materials and methods

## 2.1. Animals

Male and female PSEN2-KO mice were bred at the University of Washington (UW) from stock originally acquired from the Jackson Laboratory. PSEN2-KO mice breed normally (49) and are viable for at least 14 months in our laboratory (13); therefore, breeding was between PCR-confirmed PSEN2-KO males and females. Age-matched male and female WT mice were acquired from the Jackson Laboratory at 7 weeks of age, and housed alongside PSEN2-KO mice at the UW until behavioral testing 1–2 months later. All animal studies were approved by the UW Institutional Animal Care and Use Committee (protocol 4387-01), with housing conditions previously published (50). All tests were performed during the hours of 900 and 1,700. All mice were tested between 3 and 4 months of age. Mice were used for no more than two ASM efficacy tests separated by a minimum of three stimulation-free days (48). Prior to all experimentation, mice were given a minimum of 1 h to acclimate to the procedure room. Animals were euthanized by $CO_2$ asphyxiation or live decapitation after all seizure testing, as specified.

Two cohorts of mice were used for testing (Figure 1). Cohort #1 was used for ASM efficacy and tolerability testing in the 6-Hz assay ($n = 156$ female PSEN2-KO and 143 female WT mice; 128 male PSEN2-KO and 134 male WT mice). Cohort #2 was used solely for immunohistochemistry studies and did not receive ASMs ($n = 9$ female PSEN2-KO and 20 female WT mice; 10 male PSEN2-KO and 20 male WT mice).

## 2.2. 6-Hz seizure test

The 6-Hz test is considered a model of evoked secondarily generalized focal seizures that engages limbic structures at higher intensities (44). Seizures were induced by a low-frequency (6 Hz) and long-duration (3 s) stimulus delivered to anesthetized corneas through bilateral electrodes (41, 48). The evoked 6-Hz seizure is characterized by an initial momentary stun followed immediately by forelimb clonus, twitching of the vibrissae, and Straub tail (44). Animals not displaying this behavior were considered "protected." Prior to commencing ASM studies, the median convulsive current (CC50) for both male and female PSEN2-KO mice aged 3–4 months was confirmed to be consistent with our previously reported values in male PSEN2-KO mice [i.e., 41.9 mA (95% confidence interval 39.3–46.9)] and female PSEN2-KO mice [34.4 mA (30.4–38.5); Supplementary Figure 1 (11)]. Notably, we have previously demonstrated that the 6-Hz CC50 of PSEN2-KO mice is not different from WT male and female mice at this age range, but we also confirmed that the CC50 of WT female mice [35.7 mA (30.5–39.8)] was not different from PSEN2 KO (Supplementary Figure 1). For all *in vivo* ASM testing and cFos immunohistochemistry, a 6-Hz stimulation current equivalent to the male PSEN2-KO CC95 [49.7 mA (45.3–75.6)] was used. Notably, this value was not different from the calculated CC95 in PSEN2-KO female mice [51.2 mA (43.6–89.0)].

## 2.3. Acute ASM efficacy

ASM efficacy studies in the 6-Hz test were conducted in Cohort #1 PSEN2-KO and WT mice. Seizure scores were assessed as a binary outcome of "protected" or "not protected." ASMs were tested at their previously established time of peak anticonvulsant effect presented in Table 1 (41, 48, 51).

## 2.4. Minimal motor impairment

Immediately prior to ASM activity testing in the 6-Hz test, minimal motor impairment (MMI) was assessed in all mice, consistent with our prior reports (41, 48, 51). MMI was assessed using the fixed-speed rotarod (52). Mice were considered impaired if they fell 3 or more times off this rod over the course of 1 min. The extent of impairment ("impaired"/number of mice tested) at each dose was tabulated for all experimental groups. A median behaviorally impairing dose (TD50) was not calculated for any ASM. However, MMI data were used to estimate a protective index (PI; TD50/ED50) for each ASM in each sex and strain.

## 2.5. Antiseizure medicines

All ASMs were formulated in 0.5% methylcellulose (VEH; Sigma-Aldrich, M0430) and administered by the intraperitoneal (i.p.) route (Table 1), as previously described (41, 48, 51). ASMs represented distinct pharmacological classes commonly used in epilepsy and epilepsy in older adults (38, 53, 54): broad spectrum (valproic acid; VPA), sodium channel blockers (carbamazepine; CBZ; lamotrigine; LTG), AMPA receptor antagonist (perampanel; PER), SV2A modulator (levetiracetam; LEV), and α2δ-1 calcium channel subunit modulator (gabapentin; GBP).

## 2.6. Immunohistochemistry for cFos neuronal activation marker

Cohort #2 mice were stimulated at the same CC95 current used for the acute ASM testing. Mice were then left undisturbed for 90 min before being euthanized via live decapitation for the collection of brains directly into 4% paraformaldehyde (PFA); 24 h later, the brains were transferred into 30% sucrose solution in PBS for 48–72 h, flash frozen, and stored at −80°C until cryosectioning. Brains were sectioned between Bregma—AP: 1.58 and 2.38 using a Leica CM1860 cryostat at 20 μm onto charged superfrost slides (Fisher) for immunohistochemical processing.

The protein product of the immediate early-gene cFos was used as a marker of seizure-induced neuronal activation to identify the brain structures engaged by 6-Hz corneal stimulation (55), as previously published (44). After cryosectioning, slides were washed (3 × 5 min) in 0.1 M PBS. Slides were then permeabilized for 15 min with 0.2% Triton X-100 in 0.1 M PBS before being incubated in a 4% BSA blocking solution in 0.1 M PBS with 0.03% Triton X-100 under coverwells in a humid chamber for 2 h. The cFos antibody (1:1000; AB222699-1001, Abcam) was applied under 200

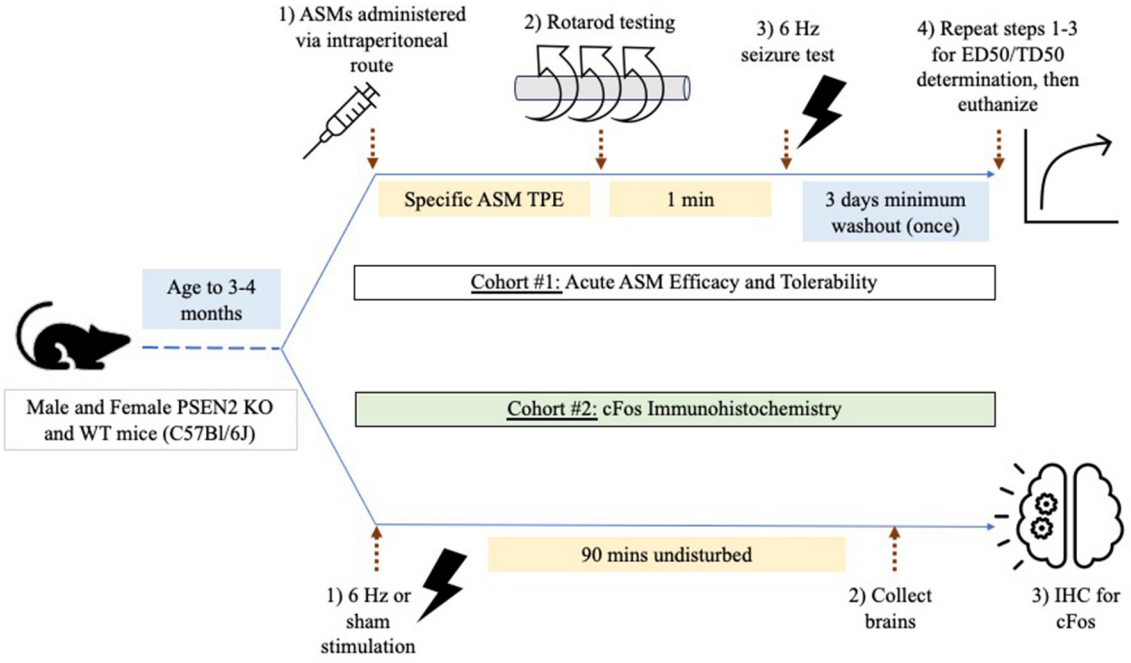


FIGURE 1
Experimental study design. Male and female PSEN2-KO and WT mice aged 3–4 months were divided into two experimental cohorts. Cohort 1 was used to assess the impact of acute administration of prototype antiseizure medicines (ASMs) administered by the intraperitoneal route and tested at the previously determined time of peak effect (TPE) for each agent. Mice were challenged on a fixed-speed (6 rpm) rotarod 1 min prior to 6-Hz seizure testing to determine a median effective (ED50) or median behaviorally impairing (TD50) dose for each ASM. Cohort 2 was used to quantify the extent of cFos immunoreactivity 90 min after a sham or 6-Hz transcorneal stimulation.

μL coverwells in a 1% BSA, 1% goat serum, and 0.03% Triton X-100 in 0.1 M PBS solution overnight at 4°C. The following day, coverwells were removed and slides washed in 0.1 M PBS (3 × 5 min) before being incubated with a goat anti-rabbit IgG H&L 555 nm secondary antibody (1:1000; AB150078 Abcam) in a 1% BSA, 1% goat serum, and 0.03% Triton X-100 in 0.1 M PBS solution light protected for 2 h at room temperature. The slides were again washed 3 × 5 min with 0.1 M PBS before being coverslipped with Prolong Gold with DAPI (ThermoFisher).

Photomicrographs were captured with a fluorescence microscope (Leica DM-4) with a 20x objective (80x final magnification) with acquisition settings held constant. cFos expression, given as average area, was automatically quantified as total field area with an immunofluorescent signal using Leica Thunder software. Additionally, the number of cells within each brain region that were positive for cFos labeling were hand-counted by two independent investigators blinded to the experimental group (Supplementary Figure 2), adapted from an ordinal scale similar to that previously reported (44). Brain regions assessed included subregions of the dorsal hippocampus, the posterior parietal association cortex overlaying CA1 of the dorsal hippocampus, and the piriform cortex.

## 2.7. Statistical analysis

The CC50s, CC95s, and ED50s were calculated by probit regression of binary data (56) using XLStat Life Sciences version 2019.1, or later with values confirmed to fall within the range of currents/doses tested. All binary response datasets for the ED50 calculations for male and female WT and PSEN2-KO mice are included in Supplementary Table 1. Statistical differences in ED50 or CC50 values were defined as values in which 95% confidence intervals did not overlap, consistent with probit methodology (56–58), which indicates with 95% probability that the true median value lies within this range. Importantly, confidence intervals provide an indication of the direction and strength of the effect studied and provide critical information about statistical differences between values that is more relevant than the *p*-value alone (59). All other statistical analyses were conducted in GraphPad Prism v8.0 or later, with $p < 0.05$ considered significant. Immunohistochemistry data for cFos labeling were checked for normality with a D'Agostino and Pearson test. A Mann–Whitney *U*-test was used to determine statistical differences in MMI following the administration of high-dose ASMs. Quantitative assessment of total area with cFos-positive signal was quantified with a two-way ANOVA with Sidak's *post-hoc* test.

TABLE 1 Acute minimal motor impairment (MMI) on the fixed-speed rotarod was assessed in male and female wild-type (WT) and PSEN2-KO mice aged 3–4 months old following intraperitoneal administration of mechanistically distinct ASMs delivered at the previously determined time of peak effect for each agent.

| ASM (highest dose tested) | Vendor (catalog #) | Time of peak effect (h) | Female WT | Female WT PI | Female PSEN2 KO | Female PSEN2-KO PI | Male WT | Male WT PI | Male PSEN2 KO | Male PSEN2-KO PI |
|---|---|---|---|---|---|---|---|---|---|---|
| VPA (300 mg/kg) | Sigma-Aldrich (P4543) | 0.25 | 3/8 | ~2.1 | 1/8 | >4.1 | 2/8 | >2.2 (†1.7) | ***6/8**** | ***~4.7*** |
| LEV (54 mg/kg) | TCI Chemicals (L0234) | 1.0 | 0/8 | >5.4 | 0/8 | >9.6 | 0/8 | >2.7 (†18.6) | 0/8 | >11.3 |
| LTG (56 mg/kg) | AK Scientific (K499) | 0.5 | 0/8 | >2.3 | ***3/8**** | ***~5.4*** | 0/8 | >4.1 (†3.6) | ***4/8**** | ***~4.2*** |
| PER (2 mg/kg) | Cayman Chemical Co (23003) | 1.0 | 3/8 | ~2.5 | ***0/8**** | ***>2.6*** | 5/8 | ~2.8 | 3/8 | ~4.9 |
| GBP (500 mg/kg) | TCI Chemicals (G0318) | 2.0 | 1/8 | >2.0 | 0/8 | ~1.0 | 3/7 | ~5.3 | 1/8 | ~1.0 |
| CBZ (40 mg/kg) | Sigma-Aldrich (C4024) | 0.25 | 3/5 | ~5.0 | 1/8 | >1.8 | 6/6 | <2.5 (†3.0) | ***0/10**** | ***>2.5*** |

Testing in this behavioral assay immediately preceded acute transcorneal 6-Hz stimulation needed to evoke a single focal seizure. Data are presented as the number of mice impaired/the number of mice tested at each dose. Impairment proportion values in italics and bold are significantly different from sex-matched WT mice with $^{*}p < 0.05$. Notably, some of the ASMs tested were associated with significant genotype- or sex-related differences in MMI at the highest dose tested. An approximate protective index (PI) was also estimated for all animals based on the highest dose tested and the median effective dose of each agent (ED50).

†Indicates previously published PI for 6 Hz equivalent in male C57BL/6N mice from Charles River (48).

# 3. Results

## 3.1. Valproic acid and levetiracetam are more potent in the 6-Hz seizure test in PSEN2-KO mice

We sought to establish the ED50 of several mechanistically distinct ASMs in WT and PSEN2-KO mice to define the extent to which an AD-related genotype alone can influence the ASM activity profile in a well-characterized focal seizure model. There was some marked divergence in anticonvulsant activity in both male and female PSEN2-KO mice (Figure 2). The ED50 of VPA in male PSEN2-KO mice was 62.7 mg/kg [95% CI 48.6 – 90.4], which was significantly lower than the ED50 of VPA in male WT mice [135 mg/kg (96.2 – 219); Figure 2]. The female PSEN2-KO and WT mice followed a similar trend to the males, in that the VPA ED50 for PSEN2-KO females [72.7 mg/kg (44.3 – 126)] was lower than the VPA ED50 for WT females [143 mg/kg (108 – 192)], though this difference did not achieve statistical significance (Figure 2). Similarly, the ED50 of LEV in male PSEN2-KO mice [2.63 mg/kg (1.22 – 4.80)] was significantly reduced vs. that of male WT mice [12.1 mg/kg (6.44 – 24.6), Figure 2]. Females followed a similar trend, although the ED50s were not significantly different [PSEN2 KO: 3.59 mg/kg (1.76 – 7.90); WT: 9.92 mg/kg (4.66 – 19.8), Figure 2]. PSEN2-KO male mice were thus more sensitive to the broad-spectrum ASM, VPA, and the SV2A modulator, LEV, vs. WT in the 6-Hz assay. These data suggest that these ASMs, which act on glutamatergic synaptic vesicle release, were more potent in PSEN2-KO mice vs. age-matched WT mice.

## 3.2. There is no difference in the potency of lamotrigine and perampanel in PSEN2-KO mice in the 6-Hz seizure test

We sought to similarly establish the ED50 for both LTG and PER in PSEN2-KO mice as both ASMs are likely to be well-tolerated and used frequently in older adults with epilepsy (38, 53, 54). There was no difference in the ED50 of LTG in PSEN2-KO males [10.8 mg/kg (6.94 – 15.9)] relative to WT males [8.09 mg/kg (4.06 – 14.5)]. Furthermore, the ED50 of PER was not significantly different in PSEN2-KO males [0.399 mg/kg (0.299 – 0.479)] vs. WT males [0.482 mg/kg (0.262 – 0.879); Figure 2]. This effect was similarly evident in female PSEN2-KO mice; there was no difference between the female PSEN2-KO and WT mice ED50′s for LTG [PSEN2 KO: 8.45 mg/kg (4.89 – 12.7); WT: 15.0 mg/kg (6.73 – 28.6)]. There was also no difference in the potency of PER [PSEN2 KO: 0.545 mg/kg (0.330 – 0.886); WT: 0.631 mg/kg (0.419 – 0.951), Figure 2]. Thus, neither the sodium channel blocker, LTG, nor the AMPA receptor antagonist, PER, exhibited differences in antiseizure potency in PSEN2-KO mice relative to age- and sex-matched WT controls in the 6-Hz assay.

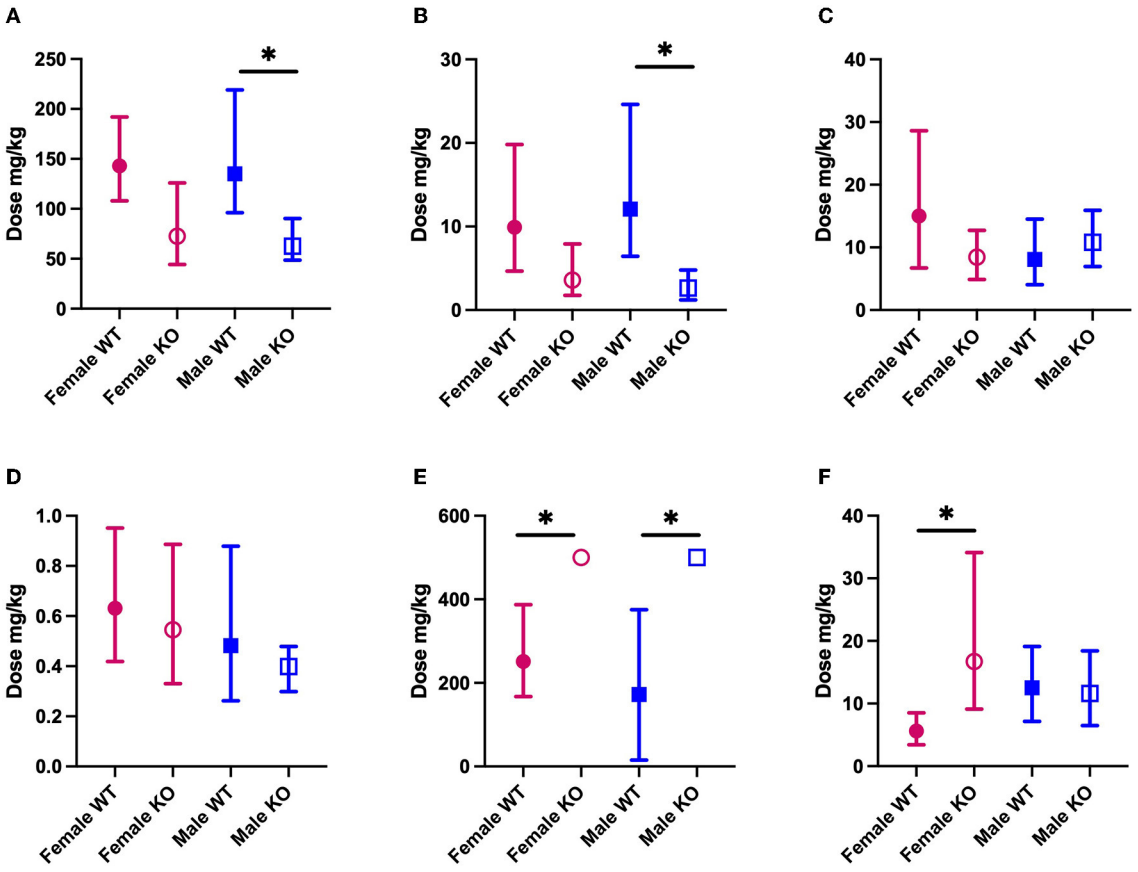


FIGURE 2
The median effective dose (ED50; the dose of an ASM that blocks seizures in 50% of animals tested) and 95% confidence intervals of each of the ASMs **(A)** VPA; **(B)** LEV; **(C)** LTG; **(D)** PER; **(E)** GBP; **(F)** CBZ in both male and female PSEN2-KO mice and their age-matched WT counterparts in the 6-Hz assay. Loss of normal PSEN2 function leads to significantly increased potency of VPA **(A)** and LEV **(B)** in male mice. There are no significant differences in the potency of LTG **(C)** of PER **(D)** between genotypes. Loss of normal PSEN2 function leads to significantly decreased potency of GBP **(E)** in male and female mice and of CBZ **(F)** in female mice. *Indicates non-overlapping 95% confidence intervals between WT and KO.

## 3.3. The potency of gabapentin in PSEN2-KO mice and of carbamazepine in female PSEN2-KO mice is reduced in the 6-Hz seizure test

There were two significant differences in ASM potency with GBP and CBZ, two agents frequently recommended for older adults with epilepsy (38, 53, 54), that largely work through fast neurotransmission via presynaptic ion channels (60). The ED50 of GBP was determined to be 172 mg/kg [15.4 – 375] for WT males and 251 mg/kg [167 – 387] for WT females (Figure 2). The ED50 for GBP could not be calculated for male or female PSEN2-KO mice (Figure 2), as only four of eight males and five of eight females were protected from a seizure at the highest dose tested (500 mg/kg, i.p.). At this same dose, six of seven WT males and seven of eight WT females were protected. Despite the inability to calculate an ED50 in PSEN2-KO mice, these results suggest that loss of normal PSEN2 function reduces the sensitivity to acute administration of the α2δ-1 calcium subunit channel modulator, GBP, in the 6-Hz assay. We also observed markedly reduced potency of CBZ in female PSEN2-KO mice. The ED50 of CBZ in female PSEN2-KO mice was 16.7 mg/kg [9.11 – 34.1] significantly higher than the CBZ ED50 in female WT mice [5.61 mg/kg (3.41 – 8.50), Figure 2]. Males were not significantly different [PSEN2 KO: 11.6 mg/kg (6.49 – 18.4); WT: 12.5 mg/kg (7.16 – 19.1), Figure 2]. Thus, female PSEN2-KO mice appear to be less sensitive to the acute administration of the sodium channel blocker CBZ compared with age-matched WT mice in the 6-Hz test; however, this trend was not conserved between the sexes. Altogether, these findings suggest that ASMs that exclusively target presynaptic ion channels necessary for fast neurotransmission may be less potent in PSEN2-KO mice in the 6-Hz limbic seizure test.

## 3.4. The protective index of selected ASMs is altered in PSEN2-KO mice

In addition to the assessment of anticonvulsant activity, mice were challenged on the rotarod immediately prior to seizure testing to determine the potential for MMI, consistent with routine ASM discovery practice (37, 41). While we did not determine a median motor-impairing dose (TD50) for any agent in this seizure model,

TABLE 2 A single 6-Hz stimulation in male WT and PSEN2-KO mice aged 3–4 months induces qualitative regional differences in cFos expression, as evaluated by two independent investigators blinded to experimental conditions.

| Region | WT sham | WT stim | PSEN2-KO sham | PSEN2-KO stim |
|---|---|---|---|---|
| CTX | 3 | 3 | 3 | 3 |
| PIR | 3 | 4 | 2 | 3 |
| CA1 | 1 | 1 | 1 | 0.5 |
| CA3 | 2 | 1 | 2 | 2 |
| DG | 2 | 2 | 1 | 1 |

TABLE 3 A single 6-Hz stimulation in female WT and PSEN2-KO mice aged 3–4 months induces qualitative regional differences in cFos expression, as evaluated by two independent investigators blinded to experimental conditions.

| Region | WT sham | WT stim | PSEN2-KO sham | PSEN2-KO stim |
|---|---|---|---|---|
| CTX | 2 | 3.5 | 1.5 | 3 |
| PIR | 3 | 4 | 2.5 | 3 |
| CA1 | 0.5 | 1 | 0.5 | 0.5 |
| CA3 | 1 | 1 | 1 | 1 |
| DG | 2 | 1 | 1 | 2 |

the number of mice with motor impairment at the highest dose tested for each agent allowed us to estimate a relative PI for all compounds across the sexes and strains (Table 1) to directly compare with previously published values in other WT mouse strains (48, 61). There were no significant differences in MMI between PSEN2 KO and WT mice of either sex with the highest doses of GBP or LEV tested (Table 1). However, there were marked differences in tolerability for CBZ, PER, VPA, and LTG (Table 1). These findings altogether demonstrate that while some ASMs were not differentially potent in PSEN2-KO mice, there were marked and impactful differences in MMI and PI with these ASMs, which carries the potential to adversely affect tolerability in humans.

### 3.5. 6-Hz stimulation increases cFos protein expression in the posterior parietal association cortex and piriform cortex of WT mice, but not of PSEN2-KO mice

We performed immunohistological detection of the cFos protein product 90 min after a single 6-Hz stimulation to assess whether the observed differences in ASM potency or tolerability could be attributed to differences in regional activation in the brains of seizure-naïve male and female 3- to 4-month-old PSEN2-KO and WT mice. cFos is an immediate early gene that is activated and expressed in response to neuronal activity (55). The extent of cFos immunoreactivity was first qualitatively rated by two blinded, independent investigators to confirm 6-Hz stimulation-induced neuronal activation (Tables 2, 3). Expression of the protein product of cFos was then quantified for all mice in discrete brain regions (Figures 3, 4). There was notable upregulation of cFos expression in the brains of male WT mice, including a genotype x stimulation interaction on cFos expression in the posterior parietal association cortex [$F_{(1,24)} = 10.08$, $p = 0.004$; Figure 3A] and the piriform cortex [$F_{(1,25)} = 6.649$, $p = 0.016$; Figure 3B]. Only WT male mice demonstrated significant increases in cFos immunoreactivity in these regions in response to 6-Hz stimulation; PSEN2-KO male mice did not show similar neuronal activation in these regions.

The extent of cFos immunoreactivity in female mice subjected to 6-Hz stimulation (Figure 4) was also assessed, which expands on a study by Barton and colleagues who only assessed 6-Hz-induced cFos expression in male WT mice (44). There was a significant main effect of 6-Hz stimulation on cFos immunoreactivity in the posterior parietal association cortex [$F_{(1,24)} = 17.35$, $p = 0.0003$; Figure 4A] and the piriform cortex [$F_{(1,25)} = 23.20$, $p < 0.0001$; Figure 4B]. *Post-hoc* assessment in the posterior parietal association cortex revealed that cFos expression was only upregulated by 6-Hz stimulation in WT females ($p = 0.0004$); this assessment also showed only upregulated expression in the piriform cortex of WT females. In DG, there was a significant interaction on cFos expression in DG [$F_{(1,21)} = 8.457$, $p = 0.0084$; Figures 4E, H]. Although overall cFos immunoreactivity in the DG of female PSEN2-KO and WT mice was generally light (Figure 4H), *post-hoc* tests also revealed marked differences in stimulation-induced cFos expression in DG in WT females ($p = 0.0031$) that were not observed in PSEN2-KO mice. Thus, cFos immunoreactivity was significantly induced in the posterior parietal association cortex and piriform cortex of WT male and female mice, but this was not similarly observed in PSEN2-KO male and female mice. There were no major stimulation-induced changes in cFos immunoreactivity in the DG of PSEN2-KO mice, unlike effects observed in WT female mice. These findings suggest disrupted 6-Hz stimulation-induced brain region activation in PSEN2-KO mice relative to similarly stimulated WT counterparts.

## 4. Discussion

Seizures in people with AD are an emerging and untapped therapeutic opportunity to potentially alter the trajectory of the disease (6). These seizures also offer the opportunity to uncover potentially novel and biologically impactful, universally conserved mechanisms associated with seizures in older individuals (6, 11–13), which may benefit epilepsy patient populations more broadly (62). We have previously demonstrated that PSEN2-KO mice are useful to assess seizure susceptibility in an AD-associated genetic background (11), ASM response (11), and the impacts of chronic seizures on cognitive function (13). We herein demonstrate marked differences in ASM potency and tolerability in male and female PSEN2-KO mice vs. WT mice subjected to the 6-Hz model of evoked limbic seizures. We also demonstrate that loss of normal PSEN2 function may alter the PI of mechanistically distinct ASMs. While differences in mouse genetic strain can alone influence ASM potency (48), patterns of anticonvulsant activity are generally similar across strains, with differences also

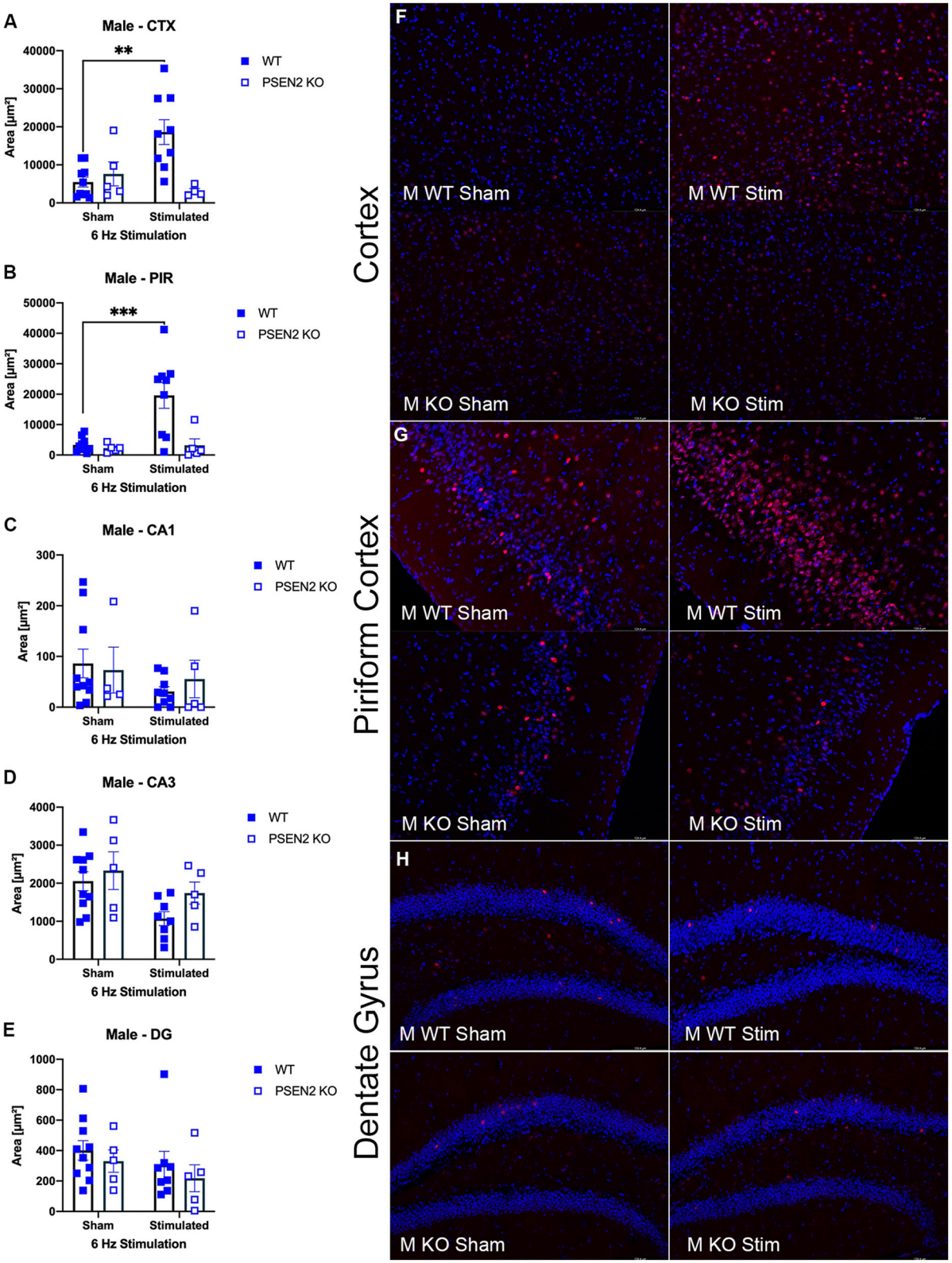


FIGURE 3
Immunohistochemical detection of the immediate early-gene cFos protein product was assessed in male wild-type (WT) and PSEN2-KO mice aged 3- to 4-month 90 min after a sham or single transcorneal 6-Hz stimulation. Brain regions analyzed for cFos expression by the automated Leica Thunder software include the following: **(A)** posterior parietal association cortex (region of cortex overlaying dorsal hippocampus at approximately Bregma −2.06); **(B)** piriform cortex; **(C)** area CA1 of the dorsal hippocampus; **(D)** area CA3 or dorsal hippocampus; **(E)** dentate gyrus (DG) of the dorsal hippocampus. The expression of cFos was analyzed by two-way ANOVA, and significance indicated within respective groups, where present (**indicates $p < 0.01$; ***indicates $p < 0.001$). Representative photomicrographs (80x final magnification) from regions where significant

*(Continued)*

FIGURE 3 (Continued)
differences in cFos expression were appreciated in either males or females are included from **(F)** posterior parietal association cortex; **(G)** piriform cortex; and **(H)** dentate gyrus of the dorsal hippocampus. Representative photomicrographs of non-significant regions (CA1 and CA3) are included in Supplementary material.

attributable to chemical source, formulation protocol, time of testing, route of administration, animal housing conditions, and animal age (44, 63–65). We herein demonstrated that age- and sex-matched PSEN2-KO mice exhibit notable sex- and strain-related differences in the patterns of ASM activity profiles relative to co-housed WT mice; findings suggest that loss of normal PSEN2 function disrupts ASM sensitivity beyond any variance attributable to genetic background strain alone. Furthermore, the WT mice in this study exhibited ED50 values and antiseizure activity patterns that are consistent relative to other WT strains (48, 63, 65). The ASMs VPA and LEV were substantially more potent in PSEN2-KO mice vs. age-matched WT animals. However, this change in potency was not universally observed with all ASMs tested. GBP was surprisingly ineffective in this seizure test in PSEN2-KO mice. CBZ demonstrated intriguing sex-related differences; it was more potent in female PSEN2-KO vs. WT mice, whereas it showed no differences in male PSEN2-KO vs. WT mice. Conversely, the potency of PER and LTG was unaltered in PSEN2-KO mice vs. WT mice. There were also substantial differences in the acute motor impairing effects of ASM administration in PSEN2-KO vs. WT mice (Table 1), suggesting that ASMs may be differentially tolerated in the setting of disrupted PSEN2 function. Finally, we demonstrate that 6-Hz stimulation in PSEN2-KO mice is associated with blunted cortical and piriform cortex activation, as assessed by cFos immunoreactivity. These findings cumulatively point to a substantial shift in ASM sensitivity and hyperexcitability in the context of loss of normal PSEN2 function.

While the PSEN2-KO mouse does not harbor a known EOAD PSEN2 gene variant (5, 25–27), clinical PSEN2 variants lead to a biochemical loss of normal function (66) such that PSEN-KO models are relevant to *a priori* assess the biological consequences of PSEN dysfunction in the setting of evoked secondarily generalized focal seizures. Despite the greater frequency of PSEN1 variants in EOAD, global PSEN1-KO mice are non-viable, whereas PSEN2-KO mice develop normally (49) and are viable up to at least 14 months old (13). Therefore, PSEN2-KO mice are useful to understand how global disruptions in PSEN signaling modify ASM activity profiles in the well-characterized evoked 6-Hz limbic seizure model, which is routinely used for frontline ASM discovery (35, 37, 41, 46, 47, 67). Notably, PSEN2-KO mice demonstrate high-frequency oscillations (68) and seizure-induced cognitive deficits (13), representing a suitably valid model of seizure-induced behavioral effects in an AD-associated genetic background. Until now, no study of ASM efficacy against evoked or spontaneous seizures has yet established a PI or defined the tolerability profile in a rodent AD-associated model, leaving a significant gap in knowledge with regard to the therapeutic window for the management of seizures in AD. Thus, our present study reveals likely mechanism-specific differences in ASM potency and acute tolerability in the AD-associated PSEN2-KO mouse that warrant more in-depth clinical study in genetically confirmed EOAD patients with seizures.

Limited prior clinical studies have investigated ASM use in older adults with mild-to-moderate AD and reported mixed therapeutic benefits. While VPA is generally acceptable for use in older adults with epilepsy (38, 53, 54), it may be contraindicated in patients with seizures in AD; a small study demonstrated that VPA administration led to increased brain volume loss and accelerated decline in MMSE scores (69, 70). Our present study revealed that MMI was worsened in PSEN2-KO male mice at the highest dose of VPA tested, but VPA was actually more potent against the 6-Hz secondarily generalized focal seizures in the PSEN2-KO mice at low doses. Our findings suggest that the therapeutic window of VPA may be shifted in this AD-associated model, which warrants further clinical study. The precise mechanism by which VPA exerts anticonvulsant effects is unclear, but it has been postulated to act through a diversity of molecular targets relevant to neuronal hyperexcitability (60) and AD (71, 72). While our current study was limited to the acute effects of VPA administration in relatively young animals, our findings of a shift in the PI of this agent suggest that perhaps the dose of VPA used in AD patients was higher than necessary to elicit neuroprotective and anticonvulsant benefits (69, 70) and thus resulted in the observed higher likelihood of treatment-related adverse side effects.

Chronic administration of the SV2A modulator, LEV, is both efficacious and well-tolerated in patients with mild-to-moderate AD (19, 73). Chronic administration of low-dose LEV may even improve performance on spatial memory and executive function tasks in patients with AD and epileptiform activity (22). In line with this clinical evidence, LEV did not elicit MMI in either genotype at the highest dose tested in our present study, and it potently blocked 6-Hz seizures at very low doses in the PSEN2-KO mice, suggesting a widened PI with this agent in PSEN2-KO mice. Our prior study with 60-Hz corneal-kindled PSEN2-KO mice also pointed to the increased potency of LEV in the absence of motor-impairing effects (11). Notably, this present study starkly contrasts with our earlier findings for reduced potency of LEV (and brivaracetam) in 6-Hz corneal-kindled mice in APP overexpressing AD models, revealing potential heterogeneity in ASM activity profiles in the setting of AD-related genotypes or intrinsic differences in the evoked seizure paradigms (12, 74). Thus, our current study suggests that the use of the acute 6-Hz limbic seizure model evoked in PSEN2-KO mice may beneficially identify both effective and well-tolerated agents for future clinical investigation to better therapeutically manage seizures in people with AD.

GBP is a calcium channel modulator that is a commonly prescribed ASM for older adults with epilepsy because of the minimal risk for drug–drug interactions and favorable cognitive side effect profile in this age group (38, 53, 54). However, studies of the safety and efficacy of GBP in older adults with seizures in AD are scant (75). GBP was entirely ineffective against 6-Hz focal seizures in PSEN2-KO mice in our study; whether PSEN2 or other AD-related variants are associated with altered GBP sensitivity requires

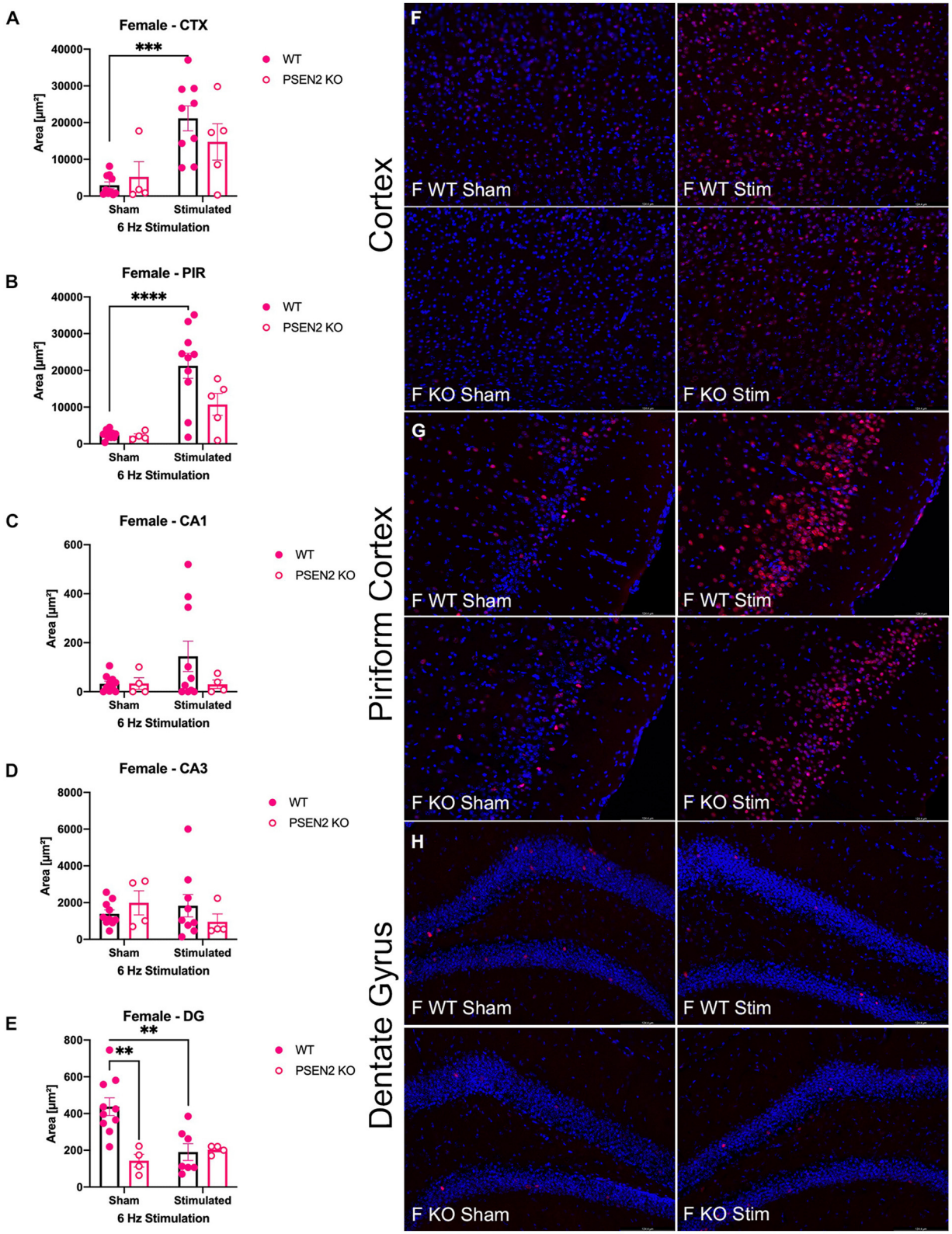


FIGURE 4
Immunohistochemical detection of the immediate early-gene cFos protein product was assessed in female wild-type (WT) and PSEN2-KO mice aged 3- to 4-month 90 min after sham or a single transcorneal 6-Hz stimulation. Brain regions analyzed for cFos expression by the automated Leica Thunder software include the following: **(A)** posterior parietal association cortex (region of cortex overlaying dorsal hippocampus at approximately Bregma −2.06); **(B)** piriform cortex; **(C)** area CA1 of the dorsal hippocampus; **(D)** area CA3 or dorsal hippocampus; **(E)** dentate gyrus (DG) of the dorsal hippocampus. The expression of cFos was analyzed by two-way ANOVA, and significance indicated within respective groups, where present (**indicates $p < 0.01$; ***indicates $p < 0.001$; ****indicates $p < 0.0001$). Representative photomicrographs (80x final magnification) from regions

*(Continued)*

FIGURE 4 (Continued)
where significant differences in cFos expression were appreciated in either males or females are included from **(F)** posterior parietal association cortex; **(G)** piriform cortex; and **(H)** dentate gyrus of the dorsal hippocampus. Representative photomicrographs of non-significant regions (CA1 and CA3) are included in Supplementary material.

further scrutiny as few preclinical studies have included this ASM for anticonvulsant testing in AD models. However, the PSEN2 protein is known to play a role in mitochondrial-dependent calcium homeostasis (76), which underlies normal neuronal signaling and seizures in epilepsy. Studies also suggest that EOAD-linked presenilin variants lower the calcium ion content of intracellular stores. By deleting the PSEN2 gene, it is likely that disrupted calcium homeostasis negatively influences the anticonvulsant potential of GBP, a calcium channel modulator, leading to our observed outcomes; a finding that warrants additional study.

While both LTG and CBZ are sodium channel blockers, the two ASMs illustrated very different anticonvulsant activity profiles likely owing to the additional effects of LTG on calcium channels (60). There were no differences in the potency of LTG between PSEN2-KO and WT mice of either sex. However, both male and female PSEN2-KO mice exhibited significant MMI at the highest dose of LTG tested relative to WT mice, revealing a narrower PI with LTG in PSEN2-KO mice. In contrast, both sexes of PSEN2-KO mice were less susceptible to the MMI-inducing effects of a high dose of CBZ compared with WT mice. PSEN2-KO females were also less sensitive to the anticonvulsant properties of CBZ than WT females in the 6-Hz test, reflective of a widened PI in this sex and strain. Thus, while the primary mechanism of action of these two ASMs is similar, differences exist in the metabolism and clearance of CBZ vs. LTG (77, 78), which may have also influenced our observed tolerability differences. Our present study did not evaluate plasma or brain concentrations of the selected ASMs; thus, future studies are needed to define the pharmacokinetic properties of candidate ASMs in this model and other AD-associated mouse models with evoked or spontaneous seizures to better establish the therapeutic potential of ASM use in people with AD.

AMPA receptor trafficking critically mediates normal synaptic plasticity and long-term potentiation (79, 80). In AD, AMPA receptor expression and trafficking are substantially dysregulated by the presence of amyloid β oligomers (81–84), thereby making modulation of AMPA receptors a relevant therapeutic target in AD. The non-competitive AMPA receptor antagonist PER has been shown to improve cognitive function and mediate psychiatric symptoms in an AD patient with myoclonic epilepsy due to its demonstrated anticonvulsant effects in this individual (85). Considering that PER is generally cognitively neutral in people with epilepsy (86), including in older adults with epilepsy (38), we sought to quantify the anticonvulsant potency and tolerability of PER against acute 6-Hz focal seizures in PSEN2-KO mice to further define therapeutic potential of this agent for older adults with seizures and AD. While the potency of PER in PSEN2-KO mice did not differ from WT animals, PER was much better tolerated in both male and female PSEN2-KO mice vs. their age-matched WT counterparts. Our results indicate a widened PI for PER in the PSEN2-KO genotype; mice were largely unimpaired by the highest dose of PER tested. These findings suggest that further detailed studies to assess the clinical benefit of PER use in older adults with seizures, including people with AD, are necessary. Given the distinct molecular anticonvulsant mechanism of PER coupled with the widened PI in our mouse model and the absence of cognitive liability in people with epilepsy, PER may be a reasonable therapeutic option to manage seizures in AD populations.

cFos is a useful marker of neuronal network activation following evoked seizures, including in response to a 6-Hz stimulation (44, 74). We observed that cFos was robustly upregulated in response to 6-Hz stimulation in the posterior parietal association cortex overlying dorsal hippocampus, as well as in the piriform cortex, in WT mice. There was blunted cFos expression in these regions in PSEN2-KO mice in response to this stimulation, despite the presentation of evoked seizures. These findings suggest that stimulation-induced neuronal network activation in PSEN2-KO mice is disrupted, in particular at the level of the cortex and piriform cortex. The piriform cortex is responsible for producing olfactory experiences (87) and memory encoding. It also frequently shows heavy cFos immunoreactivity in response to all 6-Hz stimulation currents (44). In fact, animal studies indicate that the piriform cortex is more prone to electrical stimulation-induced epileptic seizures than the hippocampus, amygdala, and entorhinal cortex (88). Moreover, evoked seizure activity tends to damage piriform cortex neurons (89, 90). While our current study was not designed to quantify longitudinal changes in piriform cortex size or volume, piriform cortex volume loss has been shown to be approximately twice as large as in the hippocampus in people with mild cognitive impairment (MCI) and AD (91), and also larger than the loss in the amygdala. Furthermore, piriform cortex atrophy is similarly apparent in patients with MCI as in those with AD, suggesting that piriform cortex atrophy may be a novel biomarker for early AD stages. Considering that we presently demonstrate reduced piriform cortex activation following a single 6-Hz electrical stimulation in mice with an AD-related genotype, further studies to assess the bidirectional relationship between seizures in AD and involvement of the piriform cortex are needed.

There is high translational value in this present study to improve ASM selection in patients with seizures in AD. The 6-Hz evoked seizure model is a well-characterized, frontline ASM discovery model that is routinely used to identify the anticonvulsant potential of novel therapeutic agents for epilepsy (37, 41, 44, 46, 47, 92). Importantly, the acute 6-Hz seizure model activates limbic structures known to be hyperexcitable in AD. However, aged rodents and rodent models with AD-related genotypes are infrequently integrated into initial ASM efficacy and tolerability studies (6, 37). Based on the bimodal age distribution of epilepsy prevalence being higher in the very young and the very old (38), it is necessary to more frequently include aged rodents or models of aging-related neurological disorders in ASM discovery practice (6, 93). Exclusively relying on efficacy studies in young, male, neurologically intact WT rodents will not adequately address the clinical needs of the world's rapidly

increasing population of older adults (93). Consistent with efforts to increasingly integrate syndrome-specific models of rare pediatric epilepsies into routine ASM discovery practice (42, 43, 47), a strategy that also includes aging models or models of aging-related diseases into the ASM discovery pipeline could substantially benefit therapeutic innovation for older adults with seizures. Our present studies provide proof-of-concept demonstration that the use of mice with AD-associated genetic risk factors can uncover biologically relevant differences in ASM potency and tolerability. This study highlights a potential untapped opportunity to apply precision medicine strategies in the management of seizures in AD. Furthermore, the characterization of the ASM response of PSEN2-KO mice in the acute 6-Hz model closely aligns with NINDS Research Benchmarks for epilepsy to prioritize discovery for the many forms in which epilepsy presents clinically (39, 40). Thus, ASM screening in the acute 6-Hz model in PSEN2-KO mice addresses an urgent need to diversify preclinical research for ASM discovery so that therapeutic options for people with seizures in AD can be more rationally discovered and, ultimately, prescribed to minimize the burden of AD.

## Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## Ethics statement

The animal study was reviewed and approved by University of Washington Institutional Animal Care and Use Committee.

## Author contributions

LL and MB-H contributed to the conception and design of the study, conducted the experiments, performed the statistical analysis, and contributed to the manuscript revision, read, and approved the submitted version. LL wrote the first draft of the manuscript. All authors contributed to the article and approved the submitted version.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note



## Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2023.1223472/full#supplementary-material

## References

1. Vossel KA, Beagle AJ, Rabinovici GD, Shu H, Lee SE, Naasan G, et al. Seizures and epileptiform activity in the early stages of Alzheimer disease. *J Am Med Assoc Neurol.* (2013) 70:1158–66. doi: 10.1001/jamaneurol.2013.136

2. Beagle AJ, Darwish SM, Ranasinghe KG, La AL, Karageorgiou E, Vossel KA. Relative incidence of seizures and myoclonus in Alzheimer's disease, dementia with lewy bodies, and frontotemporal dementia. *J Alzheimers Dis.* (2017) 60:211–23. doi: 10.3233/JAD-170031

3. Zarea A, Charbonnier C, Rovelet-Lecrux A, Nicolas G, Rousseau S, Borden A, et al. Seizures in dominantly inherited Alzheimer disease. *Neurology.* (2016) 87:912–9. doi: 10.1212/WNL.0000000000003048

4. Amatniek JC, Hauser WA, Delcastillo-Castaneda C, Jacobs DM, Marder K, Bell K, et al. Incidence and predictors of seizures in patients with Alzheimer's disease. *Epilepsia.* (2006) 47:867–72. doi: 10.1111/j.1528-1167.2006.00554.x

5. Jayadev S, Leverenz JB, Steinbart E, Stahl J, Klunk W, Yu CE, et al. Alzheimer's disease phenotypes and genotypes associated with mutations in presenilin 2. *Brain.* (2010) 133:1143–54. doi: 10.1093/brain/awq033

6. Lehmann L, Lo A, Knox KM, Barker-Haliski M. Alzheimer's disease and epilepsy: a perspective on the opportunities for overlapping therapeutic innovation. *Neurochem Res.* (2021) 21:3332. doi: 10.1007/s11064-021-03332-y

7. Ziyatdinova S, Gurevicius K, Kutchiashvili N, Bolkvadze T, Nissinen J, Tanila H, et al. Spontaneous epileptiform discharges in a mouse model of Alzheimer's disease are suppressed by antiepileptic drugs that block sodium channels. *Epilepsy Res.* (2011) 94:75–85. doi: 10.1016/j.eplepsyres.2011.01.003

8. Chan J, Jones NC, Bush AI, O'brien TJ, Kwan P. A mouse model of Alzheimer's disease displays increased susceptibility to kindling and seizure-associated death. *Epilepsia.* (2015) 56:e73–7. doi: 10.1111/epi.12993

9. Ziyatdinova S, Viswanathan J, Hiltunen M, Tanila H, Pitkanen A. Reduction of epileptiform activity by valproic acid in a mouse model of Alzheimer's disease is not long-lasting after treatment discontinuation. *Epilepsy Res.* (2015) 112:43–55. doi: 10.1016/j.eplepsyres.2015.02.005

10. Ziyatdinova S, Ronnback A, Gurevicius K, Miszczuk D, Graff C, Winblad B, et al. Increased epileptiform EEG activity and decreased seizure threshold in arctic APP transgenic mouse model of Alzheimer's disease. *Curr Alzheimer Res.* (2016) 13:817–30. doi: 10.2174/1567205013666160129095508

11. Beckman M, Knox K, Koneval Z, Smith C, Jayadev S, Barker-Haliski M. Loss of presenilin 2 age-dependently alters susceptibility to acute seizures and kindling acquisition. *Neurobiol Dis.* (2020) 136:104719. doi: 10.1016/j.nbd.2019.104719

12. Vande Vyver M, Barker-Haliski M, Aourz N, Nagels G, Bjerke M, Engelborghs S, et al. Higher susceptibility to 6 Hz corneal kindling and lower responsiveness to antiseizure drugs in mouse models of Alzheimer's disease. *Epilepsia.* (2022) 2022:17355. doi: 10.1111/epi.17355

13. Knox KM, Beckman M, Smith CL, Jayadev S, Barker-Haliski M. Chronic seizures induce sex-specific cognitive deficits with loss of presenilin 2 function. *Exp Neurol.* (2023) 361:114321. doi: 10.1016/j.expneurol.2023.114321

14. Vossel KA, Ranasinghe KG, Beagle AJ, Mizuiri D, Honma SM, Dowling AF, et al. Incidence and impact of subclinical epileptiform activity in Alzheimer's disease. *Ann Neurol.* (2016) 80:858–70. doi: 10.1002/ana.24794

15. Horvath AA, Papp A, Zsuffa J, Szucs A, Luckl J, Radai F, et al. Subclinical epileptiform activity accelerates the progression of Alzheimer's disease: a long-term EEG study. *Clin Neurophysiol.* (2021) 132:1982–9. doi: 10.1016/j.clinph.2021.03.050

16. Vossel KA, Tartaglia MC, Nygaard HB, Zeman AZ, Miller BL. Epileptic activity in Alzheimer's disease: causes and clinical relevance. *Lancet Neurol.* (2017) 16:311–22. doi: 10.1016/S1474-4422(17)30044-3

17. Lam AD, Deck G, Goldman A, Eskandar EN, Noebels J, Cole AJ. Silent hippocampal seizures and spikes identified by foramen ovale electrodes in Alzheimer's disease. *Nat Med.* (2017) 23:678–80. doi: 10.1038/nm.4330

18. Lam AD, Sarkis RA, Pellerin KR, Jing J, Dworetzky BA, Hoch DB, et al. Association of epileptiform abnormalities and seizures in Alzheimer disease. *Neurology.* (2020) 95:e2259–70. doi: 10.1212/WNL.0000000000010612

19. Cumbo E, Ligori LD. Levetiracetam, lamotrigine, and phenobarbital in patients with epileptic seizures and Alzheimer's disease. *Epilepsy Behav.* (2010) 17:461–6. doi: 10.1016/j.yebeh.2010.01.015

20. Bakker A, Krauss GL, Albert MS, Speck CL, Jones LR, Stark CE, et al. Reduction of hippocampal hyperactivity improves cognition in amnestic mild cognitive impairment. *Neuron.* (2012) 74:467–74. doi: 10.1016/j.neuron.2012.03.023

21. Sen A, Akinola M, Tai XY, Symmonds M, Davis Jones G, Mura S, et al. An investigation of levetiracetam in Alzheimer's disease (ILiAD): a double-blind, placebo-controlled, randomised crossover proof of concept study. *Trials.* (2021) 22:508. doi: 10.1186/s13063-021-05404-4

22. Vossel K, Ranasinghe KG, Beagle AJ, La A, Ah Pook K, Castro M, et al. Effect of levetiracetam on cognition in patients with Alzheimer's disease with and without epileptiform activity: a randomized clinical trial. *J Am Med Assoc Neurol.* (2021) 78:1345–54. doi: 10.1001/jamaneurol.2021.3310

23. Wiley JC, Hudson M, Kanning KC, Schecterson LC, Bothwell M. Familial Alzheimer's disease mutations inhibit gamma-secretase-mediated liberation of beta-amyloid precursor protein carboxy-terminal fragment. *J Neurochem.* (2005) 94:1189–201. doi: 10.1111/j.1471-4159.2005.03266.x

24. De Strooper B, Iwatsubo T, Wolfe MS. Presenilins and gamma-secretase: structure, function, and role in Alzheimer's disease. *Cold Spring Harb Perspect Med.* (2012) 2:a006304. doi: 10.1101/cshperspect.a006304

25. Jayadev S, Case A, Eastman AJ, Nguyen H, Pollak J, Wiley JC, et al. Presenilin 2 is the predominant gamma-secretase in microglia and modulates cytokine release. *PLoS ONE.* (2010) 5:e15743. doi: 10.1371/journal.pone.0015743

26. Jayadev S, Case A, Alajajian B, Eastman AJ, Moller T, Garden GA. Presenilin 2 influences miR146 level and activity in microglia. *J Neurochem.* (2013) 127:592–9. doi: 10.1111/jnc.12400

27. Fung S, Smith CL, Prater KE, Case A, Green K, Osnis L, et al. Early-onset familial Alzheimer's disease variant PSEN2 N141I heterozygosity is associated with altered microglia phenotype. *J Alzheimer's Dis.* (2020) 77:675–88. doi: 10.3233/JAD-200492

28. De Strooper B. Loss-of-function presenilin mutations in Alzheimer disease. Talking point on the role of presenilin mutations in Alzheimer disease. *EMBO Rep.* (2007) 8:141–6. doi: 10.1038/sj.embor.7400897

29. Dai MH, Zheng H, Zeng LD, Zhang Y. The genes associated with early-onset Alzheimer's disease. *Oncotarget.* (2018) 9:15132–43. doi: 10.18632/oncotarget.23738

30. Barthet G, Moreira-De-Sa A, Zhang P, Deforges S, Castanheira J, Gorlewicz A, et al. Presenilin and APP regulate synaptic kainate receptors. *J Neurosci.* (2022) 42:9253–62. doi: 10.1523/JNEUROSCI.0297-22.2022

31. Restrepo LJ, Depew AT, Moese ER, Tymanskyj SR, Parisi MJ, Aimino MA, et al. gamma-secretase promotes Drosophila postsynaptic development through the cleavage of a Wnt receptor. *Dev Cell.* (2022) 57:1643–1660.E7. doi: 10.1016/j.devcel.2022.05.006

32. Palop JJ, Chin J, Roberson ED, Wang J, Thwin MT, Bien-Ly N, et al. Aberrant excitatory neuronal activity and compensatory remodeling of inhibitory hippocampal circuits in mouse models of Alzheimer's disease. *Neuron.* (2007) 55:697–711. doi: 10.1016/j.neuron.2007.07.025

33. Palop JJ, Mucke L. Epilepsy and cognitive impairments in Alzheimer's disease. *Arch Neurol.* (2009) 66:435–40. doi: 10.1001/archneurol.2009.15

34. Sanchez PE, Zhu L, Verret L, Vossel KA, Orr AG, Cirrito JR, et al. Levetiracetam suppresses neuronal network dysfunction and reverses synaptic and cognitive deficits in an Alzheimer's disease model. *Proc Natl Acad Sci USA.* (2012) 109:E2895–903. doi: 10.1073/pnas.1121081109

35. Loscher W. Fit for purpose application of currently existing animal models in the discovery of novel epilepsy therapies. *Epilepsy Res.* (2016) 126:157–84. doi: 10.1016/j.eplepsyres.2016.05.016

36. Loscher W. Animal models of seizures and epilepsy: past, present, and future role for the discovery of antiseizure drugs. *Neurochem Res.* (2017) 42:1873–88. doi: 10.1007/s11064-017-2222-z

37. Barker-Haliski M, White HS. Validated animal models for antiseizure drug (ASD) discovery: advantages and potential pitfalls in ASD screening. *Neuropharmacology.* (2019) 2019:107750. doi: 10.1016/j.neuropharm.2019.107750

38. Sen A, Jette N, Husain M, Sander JW. Epilepsy in older people. *Lancet.* (2020) 395:735–48. doi: 10.1016/S0140-6736(19)33064-8

39. Caplan R, Mefford H, Berl M, Chang B, Lin J, Mazarati A, et al. 2014 epilepsy benchmarks area I: understanding the causes of the epilepsies and epilepsy-related neurologic, psychiatric, and somatic conditions. *Epilepsy Curr.* (2016) 16:182–6. doi: 10.5698/1535-7511-16.3.182

40. Dlugos D, Worrell G, Davis K, Stacey W, Szaflarski J, Kanner A, et al. 2014 epilepsy benchmarks area III: improve treatment options for controlling seizures and epilepsy-related conditions without side effects. *Epilepsy Curr.* (2016) 16:192–7. doi: 10.5698/1535-7511-16.3.192

41. Barker-Haliski ML, Johnson K, Billingsley P, Huff J, Handy LJ, Khaleel R, et al. Validation of a preclinical drug screening platform for pharmacoresistant epilepsy. *Neurochem Res.* (2017) 42:1904–18. doi: 10.1007/s11064-017-2227-7

42. Pernici CD, Mensah JA, Dahle EJ, Johnson KJ, Handy L, Buxton L, et al. Development of an antiseizure drug screening platform for Dravet syndrome at the NINDS contract site for the Epilepsy Therapy Screening Program. *Epilepsia.* (2021) 62:1665–76. doi: 10.1111/epi.16925

43. Del Pozo A, Barker-Haliski M. Cannabidiol reveals a disruptive strategy for 21st century epilepsy drug discovery. *Exp Neurol.* (2023) 360:114288. doi: 10.1016/j.expneurol.2022.114288

44. Barton ME, Klein BD, Wolf HH, White HS. Pharmacological characterization of the 6 Hz psychomotor seizure model of partial epilepsy. *Epilepsy Res.* (2001) 47:217–27. doi: 10.1016/S0920-1211(01)00302-3

45. Walrave L, Maes K, Coppens J, Bentea E, Van Eeckhaut A, Massie A, et al. Validation of the 6 Hz refractory seizure mouse model for intracerebroventricularly administered compounds. *Epilepsy Res.* (2015) 115:67–72. doi: 10.1016/j.eplepsyres.2015.06.003

46. Barker-Haliski M, Harte-Hargrove LC, Ravizza T, Smolders I, Xiao B, Brandt C, et al. A companion to the preclinical common data elements for pharmacologic studies in animal models of seizures and epilepsy. A report of the TASK3 pharmacology working group of the ILAE/AES joint translational task force. *Epilepsia Open.* (2018) 3:53–68. doi: 10.1002/epi4.12254

47. Wilcox KS, West PJ, Metcalf CS. The current approach of the Epilepsy Therapy Screening Program contract site for identifying improved therapies for the treatment of pharmacoresistant seizures in epilepsy. *Neuropharmacology.* (2020) 166:107811. doi: 10.1016/j.neuropharm.2019.107811

48. Koneval Z, Knox K, Memon A, Zierath DK, White HS, Barker-Haliski M. Antiseizure drug efficacy and tolerability in established and novel drug discovery seizure models in outbred versus inbred mice. *Epilepsia.* (2020) 2020:16624. doi: 10.1111/epi.16624

49. Herreman A, Hartmann D, Annaert W, Saftig P, Craessaerts K, Serneels L, et al. Presenilin 2 deficiency causes a mild pulmonary phenotype and no changes in amyloid precursor protein processing but enhances the embryonic lethal phenotype of presenilin 1 deficiency. *Proc Natl Acad Sci USA.* (1999) 96:11872–7. doi: 10.1073/pnas.96.21.11872

50. Meeker S, Beckman M, Knox KM, Treuting PM, Barker-Haliski M. Repeated intraperitoneal administration of low-concentration methylcellulose leads to systemic histologic lesions without loss of preclinical phenotype. *J Pharmacol Exp Ther.* (2019) 119:257261. doi: 10.1124/jpet.119.257261

51. Koneval Z, Knox KM, White HS, Barker-Haliski M. Lamotrigine-resistant corneal-kindled mice: a model of pharmacoresistant partial epilepsy for moderate-throughput drug discovery. *Epilepsia.* (2018) 59:1245–56. doi: 10.1111/epi.14190

52. Dunham MS, Miya TA. A note on a simple apparatus for detecting neurological deficit in rats and mice. *J Amer Pharm Ass Sci Ed.* (1957) 46:208–9. doi: 10.1002/jps.3030460322

53. Thomas RJ. Seizures and epilepsy in the elderly. *Arch Intern Med.* (1997) 157:605–17. doi: 10.1001/archinte.1997.00440270035003

54. Roberti R, Palleria C, Nesci V, Tallarico M, Di Bonaventura C, Cerulli Irelli E, et al. Pharmacokinetic considerations about antiseizure medications in the elderly. *Expert Opin Drug Metab Toxicol.* (2020) 16:983–95. doi: 10.1080/17425255.2020.1806236

55. Hoffman GE, Smith MS, Verbalis JG. c-Fos and related immediate early gene products as markers of activity in neuroendocrine systems. *Front Neuroendocrinol.* (1993) 14:173–213. doi: 10.1006/frne.1993.1006

56. Finney DJ. *Probit Analysis. A Statistical Treatment of the Sigmoid Response Curve*. Cambridge: University Press (1952).

57. Bliss CI. The method of probits. *Science.* (1934) 79:38–9. doi: 10.1126/science.79.2037.38

58. Du Prel JB, Hommel G, Rohrig B, Blettner M. Confidence interval or *p*-value? Part 4 of a series on evaluation of scientific publications. *Dtsch Arztebl Int.* (2009) 106:335–9. doi: 10.3238/arztebl.2009.0335

59. Shakespeare TP, Gebski VJ, Veness MJ, Simes J. Improving interpretation of clinical studies by use of confidence levels, clinical significance curves, and risk-benefit contours. *Lancet.* (2001) 357:1349–53. doi: 10.1016/S0140-6736(00)04522-0

60. Sills GJ, Rogawski MA. Mechanisms of action of currently used antiseizure drugs. *Neuropharmacology.* (2020) 168:107966. doi: 10.1016/j.neuropharm.2020.107966

61. Guignet M, Campbell A, White HS. Cenobamate (XCOPRI): can preclinical and clinical evidence provide insight into its mechanism of action? *Epilepsia.* (2020) 61:2329–39. doi: 10.1111/epi.16718

62. Goldman AM, Lafrance WCJr, Benke T, Asato M, Drane D, Pack A, et al. 2014 epilepsy benchmarks area IV: limit or prevent adverse consequence of seizures and their treatment across the lifespan. *Epilepsy Curr.* (2016) 16:198–205. doi: 10.5698/1535-7511-16.3.198

63. Esneault E, Peyon G, Castagne V. Efficacy of anticonvulsant substances in the 6Hz seizure test: comparison of two rodent species. *Epilepsy Res.* (2017) 134:9–15. doi: 10.1016/j.eplepsyres.2017.05.002

64. Loscher W, Ferland RJ, Ferraro TN. The relevance of inter- and intrastrain differences in mice and rats and their implications for models of seizures and epilepsy. *Epilepsy Behav.* (2017) 73:214–35. doi: 10.1016/j.yebeh.2017.05.040

65. Luszczki JJ, Panasiuk A, Zagaja M, Karwan S, Bojar H, Plewa Z, et al. Polygonogram and isobolographic analysis of interactions between various novel antiepileptic drugs in the 6-Hz corneal stimulation-induced seizure model in mice. *PLoS ONE.* (2020) 15:e0234070. doi: 10.1371/journal.pone.0234070

66. Sannerud R, Esselens C, Ejsmont P, Mattera R, Rochin L, Tharkeshwar AK, et al. Restricted location of PSEN2/gamma-secretase determines substrate specificity and generates an intracellular abeta pool. *Cell.* (2016) 166:193–208. doi: 10.1016/j.cell.2016.05.020

67. Kehne JH, Klein BD, Raeissi S, Sharma S. The national institute of neurological disorders and stroke (NINDS) epilepsy therapy screening program (ETSP). *Neurochem Res.* (2017) 17:2275. doi: 10.1007/s11064-017-2275-z

68. Lisgaras CP, Scharfman HE. High-frequency oscillations (250–500 Hz) in animal models of Alzheimer's disease and two animal models of epilepsy. *Epilepsia.* (2023) 64:231–46. doi: 10.1111/epi.17462

69. Fleisher AS, Truran D, Mai JT, Langbaum JB, Aisen PS, Cummings JL, et al. Chronic divalproex sodium use and brain atrophy in Alzheimer disease. *Neurology.* (2011) 77:1263–71. doi: 10.1212/WNL.0b013e318230a16c

70. Tariot PN, Schneider LS, Cummings J, Thomas RG, Raman R, Jakimovich LJ, et al. Chronic divalproex sodium to attenuate agitation and clinical progression of Alzheimer disease. *Arch Gen Psychiatry.* (2011) 68:853–61. doi: 10.1001/archgenpsychiatry.2011.72

71. Muyllaert D, Terwel D, Borghgraef P, Devijver H, Dewachter I, Van Leuven F. Transgenic mouse models for Alzheimer's disease: the role of GSK-3B in combined amyloid and tau-pathology. *Rev Neurol.* (2006) 162:903–7. doi: 10.1016/S0035-3787(06)75098-6

72. Farr SA, Ripley JL, Sultana R, Zhang Z, Niehoff ML, Platt TL, et al. Antisense oligonucleotide against GSK-3beta in brain of SAMP8 mice improves learning and memory and decreases oxidative stress: involvement of transcription factor Nrf2 and implications for Alzheimer disease. *Free Radic Biol Med.* (2014) 67:387–95. doi: 10.1016/j.freeradbiomed.2013.11.014

73. Belcastro V, Costa C, Galletti F, Pisani F, Calabresi P, Parnetti L. Levetiracetam monotherapy in Alzheimer patients with late-onset seizures: a prospective observational study. *Eur J Neurol.* (2007) 14:1176–8. doi: 10.1111/j.1468-1331.2007.01907.x

74. Albertini G, Walrave L, Demuyser T, Massie A, De Bundel D, Smolders I. 6 Hz corneal kindling in mice triggers neurobehavioral comorbidities accompanied by relevant changes in c-Fos immunoreactivity throughout the brain. *Epilepsia*. (2017) 2017:13943. doi: 10.1111/epi.13943

75. Oh GY, Moga DC, Abner EL. Gabapentin utilization among older adults with different cognitive statuses enrolled in the National Alzheimer's Coordinating Center (2006-2019). *Br J Clin Pharmacol.* (2023) 89:410–5. doi: 10.1111/bcp.15532

76. Zatti G, Burgo A, Giacomello M, Barbiero L, Ghidoni R, Sinigaglia G, et al. Presenilin mutations linked to familial Alzheimer's disease reduce endoplasmic reticulum and Golgi apparatus calcium levels. *Cell Calcium.* (2006) 39:539–50. doi: 10.1016/j.ceca.2006.03.002

77. Italiano D, Perucca E. Clinical pharmacokinetics of new-generation antiepileptic drugs at the extremes of age: an update. *Clin Pharmacokinet.* (2013) 52:627–45. doi: 10.1007/s40262-013-0067-4

78. Van Dijkman SC, Rauwe WM, Danhof M, Della Pasqua O. Pharmacokinetic interactions and dosing rationale for antiepileptic drugs in adults and children. *Br J Clin Pharmacol.* (2018) 84:97–111. doi: 10.1111/bcp.13400

79. Bramham CR, Worley PF, Moore MJ, Guzowski JF. The immediate early gene arc/arg3.1: regulation, mechanisms, and function. *J Neurosci.* (2008) 28:11760–7. doi: 10.1523/JNEUROSCI.3864-08.2008

80. Anggono V, Huganir RL. Regulation of AMPA receptor trafficking and synaptic plasticity. *Curr Opin Neurobiol.* (2012) 22:461–9. doi: 10.1016/j.conb.2011.12.006

81. Hsieh H, Boehm J, Sato C, Iwatsubo T, Tomita T, Sisodia S, et al. AMPAR removal underlies Abeta-induced synaptic depression and dendritic spine loss. *Neuron.* (2006) 52:831–43. doi: 10.1016/j.neuron.2006.10.035

82. Liu SJ, Gasperini R, Foa L, Small DH. Amyloid-beta decreases cell-surface AMPA receptors by increasing intracellular calcium and phosphorylation of GluR2. *J Alzheimer's Dis.* (2010) 21:655–66. doi: 10.3233/JAD-2010-091654

83. Whitcomb DJ, Hogg EL, Regan P, Piers T, Narayan P, Whitehead G, et al. Intracellular oligomeric amyloid-beta rapidly regulates GluA1 subunit of AMPA receptor in the hippocampus. *Sci Rep.* (2015) 5:10934. doi: 10.1038/srep10934

84. Costa C, Parnetti L, D'amelio M, Tozzi A, Tantucci M, Romigi A, et al. Epilepsy, amyloid-beta, and D1 dopamine receptors: a possible pathogenetic link? *Neurobiol Aging.* (2016) 48:161–71. doi: 10.1016/j.neurobiolaging.2016.08.025

85. Kumamoto A, Chiba Y, Suda A, Hishimoto A, Kase A. A severe dementia case in end of life care with psychiatric symptoms treated by perampanel. *J Epilepsy Res.* (2021) 11:93–5. doi: 10.14581/jer.21012

86. Witt JA, Helmstaedter C. The impact of perampanel on cognition: a systematic review of studies employing standardized tests in patients with epilepsy. *Seizure.* (2022) 94:107–11. doi: 10.1016/j.seizure.2021.12.001

87. Vaughan DN, Jackson GD. The piriform cortex and human focal epilepsy. *Front Neurol.* (2014) 5:259. doi: 10.3389/fneur.2014.00259

88. Mcintyre DC, Gilby KL. Mapping seizure pathways in the temporal lobe. *Epilepsia.* (2008) 49(Suppl.3):23–30. doi: 10.1111/j.1528-1167.2008.01507.x

89. Peredery O, Persinger MA, Parker G, Mastrosov L. Temporal changes in neuronal dropout following inductions of lithium/pilocarpine seizures in the rat. *Brain Res.* (2000) 881:9–17. doi: 10.1016/S0006-8993(00)02730-X

90. Roch C, Leroy C, Nehlig A, Namer IJ. Magnetic resonance imaging in the study of the lithium-pilocarpine model of temporal lobe epilepsy in adult rats. *Epilepsia.* (2002) 43:325–35. doi: 10.1046/j.1528-1157.2002.11301.x

91. Steinbart D, Yaakub SN, Steinbrenner M, Guldin LS, Holtkamp M, Keller SS, et al. Automatic and manual segmentation of the piriform cortex: method development and validation in patients with temporal lobe epilepsy and Alzheimer's disease. *Hum Brain Mapp*. (2023) 2023:26274. doi: 10.1002/hbm.26274

92. Metcalf CS, West PJ, Thomson KE, Edwards SF, Smith MD, White HS, et al. Development and pharmacologic characterization of the rat 6 Hz model of partial seizures. *Epilepsia.* (2017) 58:1073–84. doi: 10.1111/epi.13764

93. Del Pozo A, Lehmann L, Knox KM, Barker-Haliski M. Can old animals reveal new targets? The aging and degenerating brain as a new precision medicine opportunity for epilepsy. *Front Neurol.* (2022) 13:833624. doi: 10.3389/fneur.2022.833624

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# Cognitive phenotypes in late-onset epilepsy: results from the atherosclerosis risk in communities study

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**Introduction:** Cognitive phenotyping is a widely used approach to characterize the heterogeneity of deficits in patients with a range of neurological disorders but has only recently been applied to patients with epilepsy. In this study, we identify cognitive phenotypes in older adults with late-onset epilepsy (LOE) and examine their demographic, clinical, and vascular profiles. Further, we examine whether specific phenotypes pose an increased risk for progressive cognitive decline.

**Methods:** Participants were part of the Atherosclerosis Risk in Communities Study (ARIC), a prospective longitudinal community-based cohort study of 15,792 individuals initially enrolled in 1987–1989. LOE was identified from linked Centers for Medicare and Medicaid Services claims data. Ninety-one participants with LOE completed comprehensive testing either prior to or after seizure onset as part of a larger cohort in the ARIC Neurocognitive Study in either 2011–2013 or 2016–2017 (follow-up mean = 4.9 years). Cognitive phenotypes in individuals with LOE were derived by calculating test-level impairments for each participant (i.e., ≤1 SD below cognitively normal participants on measures of language, memory, and executive function/processing speed); and then assigning participants to phenotypes if they were impaired on at least two tests within a domain. The total number of impaired domains was used to determine the cognitive phenotypes (i.e., Minimal/No Impairment, Single Domain, or Multidomain).

**Results:** At our baseline (Visit 5), 36.3% met criteria for Minimal/No Impairment, 35% for Single Domain Impairment (with executive functioning/ processing speed impaired in 53.6%), and 28.7% for Multidomain Impairment. The Minimal/No Impairment group had higher education and occupational complexity. There were no differences in clinical or vascular risk factors across phenotypes. Of those participants with longitudinal data (Visit 6; $n$ = 24), 62.5% declined (i.e., progressed to a more impaired phenotype) and 37.5% remained stable. Those who remained stable were more highly educated compared to those that declined.

**Discussion:** Our results demonstrate the presence of identifiable cognitive phenotypes in older adults with LOE. These results also highlight the high prevalence of cognitive impairments across domains, with deficits in executive function/processing speed the most common isolated impairment. We also

demonstrate that higher education was associated with a Minimal/No Impairment phenotype and lower risk for cognitive decline over time.

KEYWORDS

epilepsy, phenotypes, cognition, aging, dementia

# 1. Introduction

Older adults represent the fastest growing population of patients with epilepsy (1–4), including those with early-onset, chronic epilepsy, and those with late-onset epilepsy (LOE) (3). The incidence of epilepsy among adults 65 years and older is approximately 1 per 1,000/year, with rates increasing as a function of age (1). As the population age continues to increase, the number of older adults with LOE is also expected to rise, thus increasing the overall global burden of epilepsy.

There is great heterogeneity in the cognitive impairments observed in individuals with LOE, which may reflect heterogeneity in etiologies. Stroke is the most common cause of LOE, followed by brain tumor, head injury, and neurodegenerative disorders (2–7). However, in approximately 13%–40% of cases the cause remains unknown. In individuals with epilepsy of unknown etiology, occult cerebrovascular disease has been proposed as an etiology given the high prevalence of vascular risk factors in this population such as hypertension and diabetes (8, 9). Another potential etiology is the shared neuropathology with neurodegenerative disease, including a bidirectional relationship between epilepsy and dementia (3, 4, 10). Specifically, several prospective and retrospective studies have reported an increased risk of dementia in individuals with epilepsy (11–16) and increased risk of epilepsy in patients with Alzheimer's disease (AD) (17–20). Further, there is evidence of AD-related pathology in patients with epilepsy including accumulation of β-amyloid (Aβ) (3, 4, 21–23) and tau (3, 4, 23), and the APOE4 genotype has been liked to an increased risk of developing epilepsy (24, 25). Together, these diverse etiologies may be expected to manifest in different cognitive profiles and differential risk for cognitive progression.

Despite increased awareness of the elevated risk of dementia in individuals with LOE and identification of risk factors for the development of LOE, the nature of cognitive deficits in this clinical population has not been fully characterized. Although several studies have examined cognitive impairments in older adults with epilepsy (25–34), only a few studies have exclusively focused on LOE (22–24, 35–39), and most of these studies have used neuropsychological screening tools with limited sensitivity that do not enable a comprehensive analysis of cognitive profiles in this growing population.

In this study, we implement an approach called *cognitive phenotyping* to better characterize the cognitive complications observed in LOE. Cognitive phenotyping has been successfully implemented across a range of disorders including chronic epilepsy (40), mild cognitive impairment (MCI) (41, 42), multiple sclerosis (43, 44), Parkinson's disease (45), autism spectrum disorders (46), and COVID-19 (47, 48) to better define the cognitive heterogeneity inherent in a disease. This approach is a patient-centered method that considers the pattern of scores within a comprehensive battery of tests rather than individual test scores. Individuals are aggregated into distinct groups or phenotypes based on this pattern and the relationship between disease related features (e.g., clinical characteristics, brain pathology, patient outcomes) can then be examined within and across phenotypes.

Our group has shown that in young-to-middle aged adults, the phenotyping approach better captures the heterogeneity inherent both within and across epilepsy syndromes compared to analyzing individual scores in isolation (40, 49–55). We have demonstrated that cognitive phenotypes are stable and robust across cohorts and are associated with distinct patterns of brain imaging abnormalities (49, 51, 56, 57). Furthermore, other studies have utilized the phenotype approach to examine cognitive progression (56) and postoperative cognitive decline (58). Thus, identifying cognitive phenotypes in LOE could help identify individuals at increased risk for cognitive progression and development of dementia, as well as delineate LOE subtypes that may be associated with distinct clinical, vascular, and lifestyle profiles.

In this study, we identify cognitive phenotypes in a group of older adults with LOE. We also examine the demographic, clinical, and vascular profiles across cognitive phenotypes and examine whether specific phenotypes confer increased risk for cognitive decline.

# 2. Materials and methods

## 2.1. Participants

The Atherosclerosis Risk in Communities (ARIC) study is a community-based, longitudinal cohort study of 15,792 men and women recruited from 1987 to 1989 via probability sampling from four US communities (Jackson, MS; Forsyth County, North Carolina; Washington County, Maryland; and suburbs of Minneapolis, MN) (59). Participants have completed 9 in-person visits (1987–2022) as of the time of manuscript preparation and are continuing to be followed via in-person visits and semi-annual telephone calls. For the purposes of this study, data from Visits 1 and 5–6 are included in the analyses (Figure 1). We included Black participants recruited in Mississippi and North Carolina and White participants recruited in Maryland, Minnesota, and North Carolina, and excluded participants of other races due to small sample sizes as is standard in ARIC.

## 2.2. Identification of LOE

Cases of LOE were identified in ARIC using an ICD code screening method that has been developed and validated in epilepsy (60) and previously used in ARIC (8, 16, 24, 61, 62). LOE was defined as two or more seizure-related ICD-9 or ICD-10 primary diagnostic codes (345.00–345.91: epilepsy; 780.39: seizure/convulsion; G40.0-G40.919: epilepsy; or R56.9: seizure/convulsion) identified from Centers for Medicare & Medicaid Services (CMS) fee-for-service (FFS) outpatient, inpatient, and Carrier claims from 1991 to 2018. To identify incident LOE, we included participants with at least 2 years of continuous CMS data prior to the first seizure-related code. Due to age at first CMS eligibility for most participants, the first seizure-related code had to occur at age 67 or older.

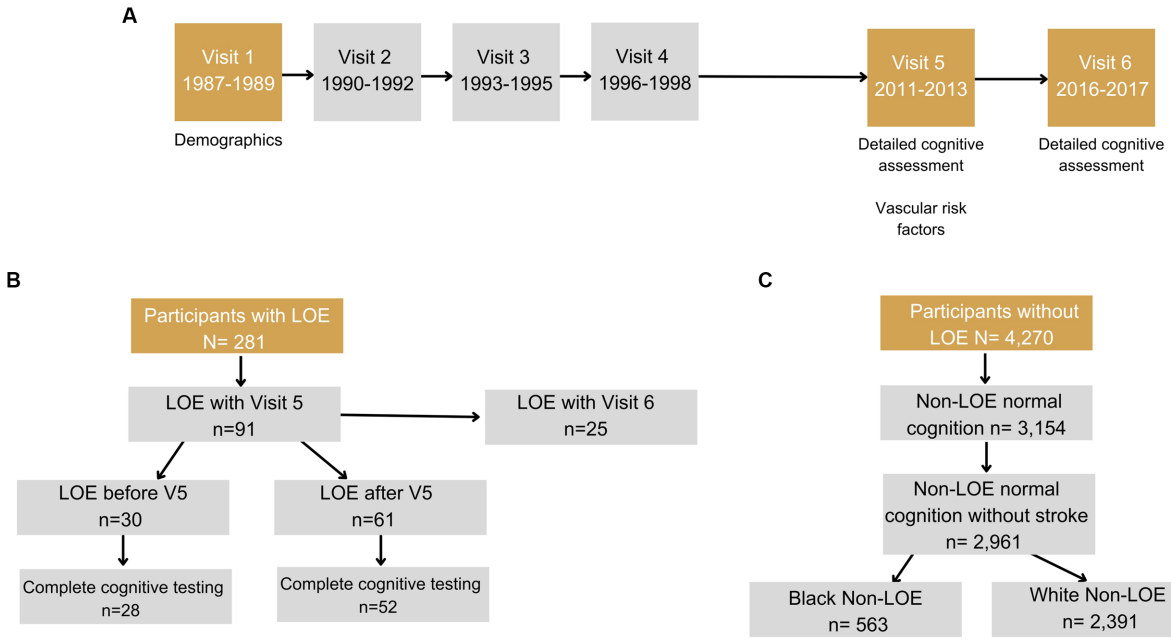


FIGURE 1
Flowcharts shows **(A)** Timeline of visits in ARIC. **(B)** Inclusion/exclusion criteria for participants with late-onset epilepsy (LOE). **(C)** Inclusion/exclusion criteria for participants without LOE and with normal cognition. Visits 2 and 4 include a short battery of cognitive tests, however, these visits were not included in the phenotyping classification.

## 2.3. Demographic, clinical, and vascular risk factors

Demographic variables (i.e., race, sex, education, occupational complexity) were obtained at Visit 1. Occupation was categorized into high (managerial and professional specialty, technical, sales, and administrative support) or low (service, precision production, repair, operators, fabricators, laborers, homemakers) occupational complexities. APOE genotype was ascertained, and participants were classified as having 0, 1, or 2 Apo ε4 alleles (TaqMan assay; Applied Biosystems, Foster City, CA). Age at Visit 5 was used in the analyses. The following vascular risk factors were also ascertained at Visit 5: hypertension, diabetes, hyperlipidemia, body mass index (BMI), and alcohol use and smoking. Hypertension was defined as systolic blood pressure mean ≥ 140 mmHg (mean of second and third measurement), diastolic blood pressure mean ≥ 90 mmHg (mean of second and third measurement), or use of an antihypertensive medication. Diabetes was defined as fasting blood glucose ≥126 mg/dL, non-fasting blood glucose ≥200 mg/dL, use of diabetic medications or insulin, HbA1c > 6.5%, or self-report of physician-diagnosed diabetes. Hyperlipidemia was defined as total cholesterol ≥200 mg/dL. BMI was calculated as weight in kilograms divided by height in meters squared. Obesity defined as a BMI ≥30 was considered a vascular risk factor. Participants self-reported smoking and alcohol use (never, former, current). A burden of vascular risk score was calculated and defined by the number of vascular risk factors present (0, 1, or 2+) which included hypertension, diabetes, hyperlipidemia, obesity, and self-reported smoking. ARIC collected prevalent stroke data at Visit 1 and performs active death and hospital discharge surveillance of all cerebrovascular disease, which is adjudicated via computer algorithm and expert review (63).

## 2.4. Neuropsychological measures

All participants with LOE completed comprehensive neuropsychological testing as part of the ARIC Neurocognitive Study (ARIC-NCS) at Visit 5 and a subset of these participants completed the same battery of tests at a follow-up visit (Visit 6). Although previous ARIC studies have included a three-domain structure that includes Memory, Language and Verbal Fluency, and Sustained Attention and Processing Speed (64), we selected language, learning and memory and processing speed and executive function based on our previous phenotype study in older adults with epilepsy (33). For the purpose of the phenotype approach, processing speed and executive function were combined into one domain. Supplementary Table S1 provides full description of all the tests. Verbal memory was evaluated with the Wechsler Memory Scale-Revised Logical Memory (LM) immediate (LM1) and delayed recall (LM2) (65) and with the delayed word recall test (DWRT) (66, 67). Language ability was evaluated with the Boston Naming Test (BNT) (68), word fluency test (WFT), and animal fluency. Processing speed was assessed with the Trail Making Test condition A (TMT-A) and digit symbol substitution test (DSST) from the Wechsler Adult Intelligence Scale-Revised (69) and mental flexibility/set-shifting was measured with the Trail Making Test B (TMT-B).

## 2.5. ARIC cognitive diagnostic criteria

Mild Cognitive impairment (MCI) and dementia diagnoses in ARIC were based on the following criteria described in Knopman et al. (70); MCI was defined as at least one domain score worse than −1.5 Z, a Clinical Dementia Rating (CDR) sum of boxes >0.5 and ≤3, a Functional

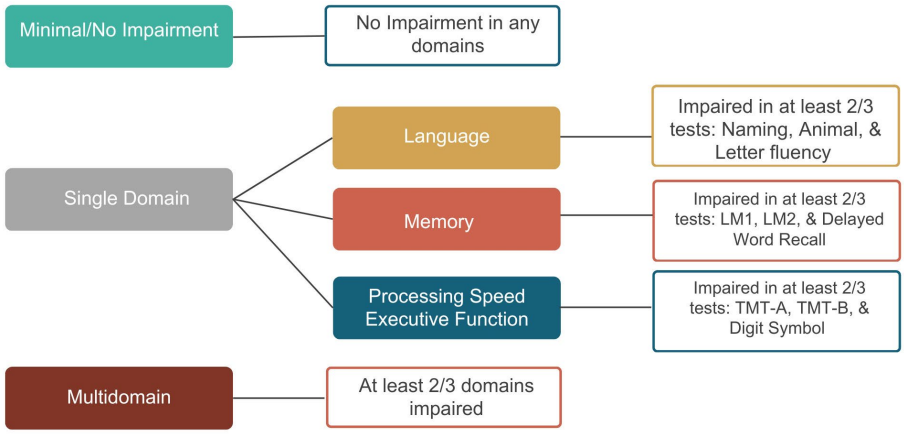


FIGURE 2
Diagnostic classification of cognitive phenotypes. BNT, Boston Naming Test; WFT, Word Fluency Test; LM1, Logical Memory 1; LM2, Logical Memory 2; DWRT, Delayed Word Recall Test; TMT-A, Trail Making Test-A; TMT-B, Trail Making Test-B; DSST, Digit Symbol Substitution Test.

Ability Questionnaire (FAQ) of 5, and decline below the 10 percentile on one test or below the 20th percentile on two tests in the serial ARIC-NCS cognitive battery. Dementia was defined as >1 cognitive domain worse than −1.5 Z, a CDR sum of boxes >3 and FAQ >5, and decline below the 10 percentile on one test or below the 20th percentile on two tests in the serial ARIC-NCS cognitive battery. As described in Knopman et al. (70), cognitive normality required that all ARIC-NCS cognitive domain scores were better than −1.5 Z and that there was an absence of decline below the 10th percentile on one test or below the 20th percentile on two tests in the serial ARIC cognitive battery; and the CDR sum of boxes was required to be ≤ 0.5 and the FAQ ≤ 5.

## 2.6. *Z*-score calculation

Raw scores for all LOE participants were converted into z-scores based on data from a normal control sample stratified by race (i.e., Black and White) and education (i.e., ≤ high school, college, graduate school). The normal control sample for this study consisted of ARIC participants that did not meet criteria for LOE, had no history of stroke, and had normal cognition based on the ARIC cognitive normality definition described above. For measures with significant Shapiro–Wilk test (i.e., $p < 0.05$), extreme outliers defined as observations that fell below Q1–1.5 interquartile range (IQR) or above Q3 + 1.5 IQR were removed from the Non-LOE normal participants. Given the differences in cognitive performance (Supplementary Table S2) between the White and Black Non-LOE participants, z-scores were calculated separately for each racial group.

## 2.7. Base rates of impairment

Rates of impairment at the individual test level were calculated to examine the cognitive processes/tests that were most affected in the ARIC LOE sample. *Z*-scores from Visit 5 were classified as impaired or not impaired using a ≤ −1.0 standard deviation (SD) cutoff. The −1.0 SD was used as the test-level impairment cut-off because this cut-off has been demonstrated to balance sensitivity and stability of impairment when examining profiles of scores (i.e., phenotypes) rather than scores in isolation (71). Base rates were calculated by dividing the number of LOE participants classified as impaired on an individual test to the total number of LOE participants.

## 2.8. Identifying cognitive phenotypes

Cognitive measures were divided into three domains: language, memory, and executive function/processing speed. Figure 2 shows the phenotype classification. Unlike the ARIC diagnostic classification system (described above) which considers change in performance and functional decline in MCI classification, the phenotype classification system is based on cognitive test performance only to allow for the evaluation of profiles and single versus multidomain domain involvement. To be impaired in a domain, at least two tests per domain had to meet the ≤ −1.0SD cutoff. The total number of impaired domains was used to characterize the cognitive phenotypes. Participants were classified as having a multidomain phenotype if at least two out of the three domains were impaired; Single-Domain phenotype was characterized as having one impaired domain; and Minimal/No Impairment was characterized as having no domains impaired.

## 2.9. Longitudinal changes in phenotype membership

The median follow-up time between Visits 5 and 6 was 4 years. There were no differences in the timing of follow up across the cognitive phenotypes $F(2,22) = 0.282$, $p = 0.757$. Twenty-five of the LOE participants with Visit 5 data also had cognitive data at Visit 6 that allowed for longitudinal phenotype characterization. *Z*-scores were also calculated based on the data from Non-LOE normal participants from Visit 5 using methods described above and the cognitive phenotypes were also derived to determine changes in phenotype membership over time. A change in classification was defined as progression to a more impaired phenotype (e.g., from Minimal/No Impairment to Single Domain or Multidomain) or worsening of an already impaired phenotype (e.g., from Single Domain to Multidomain); stable was defined as no change in phenotype

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TABLE 1 Demographics and clinical variables in all LOE sample and normative sample.

| | All LOE | Normative sample | Statistic | *p*-value |
|---|---|---|---|---|
| *N* | 91 | 2,954 | | |
| Age | 77.59 (5.34) | 75.28 (4.98) | **4.35** | **<0.001** |
| Age at first seizure | 79.40 (6.59) | – | – | – |
| LOE diagnosis before V5 | 30 (33%) | – | – | – |
| Sex: Female (%) | 48 (52.7%) | 1767 (59.8%) | – | 0.193 |
| Race: Black (%) | 28 (30.8%) | 564 (19.1%) | – | **<0.001** |
| Education: >HS | 49 (53.8%) | 1,298 (44.1%) | | 0.068 |
| Occupational attainment: High \| Low | 45 (57%) \| 34 (43%) | 1,098 (44.8%) \| 1,353 (55.2%) | – | **0.038** |
| ARIC cognitive diagnosis: Normal \| MCI \| Dementia | 48 (52.7%) \| 29 (31.9%) \| 14 (15.4%) | – | – | – |
| Hypertension (Visit 5) | 74 (82.2%) | 2,155 (73.5%) | – | 0.091 |
| Diabetes (Visit 5) | 36 (39.6%) | 731 (25.5%) | – | **0.002** |
| Hyperlipidemia (Visit 5) | 26 (28.6%) | 2,924 (99%) | – | **<0.001** |
| BMI ≥ 30 (Visit 5) | 20 (22%) | 1,015 (34.4%) | – | **0.013** |
| Stroke* | 18 (19.8%) | – | | |
| Smoking: Current \| Former \| Never \| Not Reported | 4 (4.5%) \| 48 (54.5%) \| 27 (30.7%) \| 9 (10.2%) | 155 (5.4%) \| 1,391 (48.3%) \| 1,126 (39.1%) \| 207 (7.2%) | $X^2 = 3.44$ | 0.329 |
| Alcohol Use: Current \| Former \| Never | 37 (44%) \| 30 (35.7%) \| 17 (20.2%) | 1,534 (54.1%) \| 781 (27.6%) \| 519 (18.3%) | $X^2 = 3.66$ | 0.161 |
| Vascular Risk Burden: 0 factors \| 1 factor \| 2+ factors | 5 (5.6%) \| 33 (36.7%) \| 52 (57.8%) | 2 (<1%)\| 504 (17.1%) \| 2,448 (82.9%) | $X^2 = 140.1$ | **<0.001** |
| APOE4 genotype: 0 allele \| 1 allele \| 2 alleles | 44 (57.9%) \| 29 (38.2%) \| 3 (3.9%) | 2,199 (74.4%) \| 39 (1.3%) \| 716 (24.2%) | $X^2 = 463.2$ | **<0.001** |
| APOE4 genotype: present | 32 (42.1%) | 755 (25.6%) | – | **<0.001** |

*Non-LOE normal participants with a history of stroke were excluded in the current study. LOE, late-onset epilepsy; HS, High school; MCI, mild cognitive impairment; BMI, body mass index; High occupation complexity: managerial and professional specialty, technical, sales, and administrative support; Low occupation complexity: service, precision production, repair, operators, fabricators, laborers, homemakers; Vascular risk burden: number of vascular risk factors present including hypertension, diabetes, hyperlipidemia, obesity, and self-reported smoking.
Bold values means significant with an False Discovery Rate (FDR) correction.

membership; and revert was defined as a change to a less impaired phenotype (e.g., Single Domain to Minimal/No Impairment).

## 2.10. Statistical analyses

Statistical analyses were performed using IBM SPSS Statistics (Version 28). A two-sided corrected value of p of 0.05 was considered statistically significant. Analysis of variance (ANOVA), Fisher–Freeman–Halton exact tests (FE tests), Chi-square tests, and Mann Whitney U tests were used to test for differences in clinical and demographic variables and neuropsychological performance for continuous and categorical variables. When results from the ANOVA were significant, group contrasts were assessed using *post hoc* pairwise tests with Bonferroni correction. Multiple comparisons were corrected using Benjamini-Hochberg false discovery rate for all other statistical tests. For LOE participants with seizure onset prior to cognitive testing, we ran Spearman rho correlations to examine the relationship between age of seizure onset and cognitive performance at Visit 5. Age at Visit 5 testing was first regressed from the cognitive scores and the unstandardized residuals were used in these correlation analyses.

# 3. Results

## 3.1. Clinical and demographic characteristics of LOE participants

Ninety-one LOE participants were included from Visit 5 in the final sample. Demographic and clinical characteristics for the LOE and the Non-LOE normative samples are presented in Table 1. At Visit 5, participants ranged in age from 68 to 88 years, with approximately half of the sample being female and having education greater than a high school degree. More than half of the sample met ARIC normal diagnostic criteria and 95% of the sample had at least one vascular risk factor with hypertension being the most common vascular risk factor; 18 participants had history of stroke. Approximately 33% ($n = 30$) completed Visit 5 after the onset of seizures, and the remainder 67% ($n = 61$) before the first seizure-related code. The average number of years between first seizure and Visit 5 date was 6.44 (SD = 5.22) for those with a seizure onset prior to Visit 5, and 4.52 (SD = 1.36) for those with an onset after Visit 5. There was no statistical difference in age [$t$ (89) = 1.65, $p = 0.102$; Onset before Visit 5 age mean = 78.9, Onset after Visit 5 age mean = 76.95], sex (FE value of $p = 0.824$; Onset before Visit 5 = 50% female, Onset after Visit 5 = 54.1%), or education (FE value of p = 0.824; Onset before Visit 5 = 56.7% education > high school, Onset after Visit 5 = 52.5%) between LOE participants with seizure onset prior to Visit 5 testing and those with onset after testing.

## 3.2. Z-score calculation

A total of 2,954 participants were classified as Non-LOE Normal Participants (Non-LOE NP; Black = 564; White = 2,391). Given that *z*-scores were calculated separately for each racial group, demographic and cognitive data are stratified by race. Supplementary Table S2 shows demographic variables and average cognitive scores across the neuropsychological measures and Supplementary Table S3 shows comparisons of demographic variables between Non-LOE and LOE participants. There were differences in age between both sets of groups,

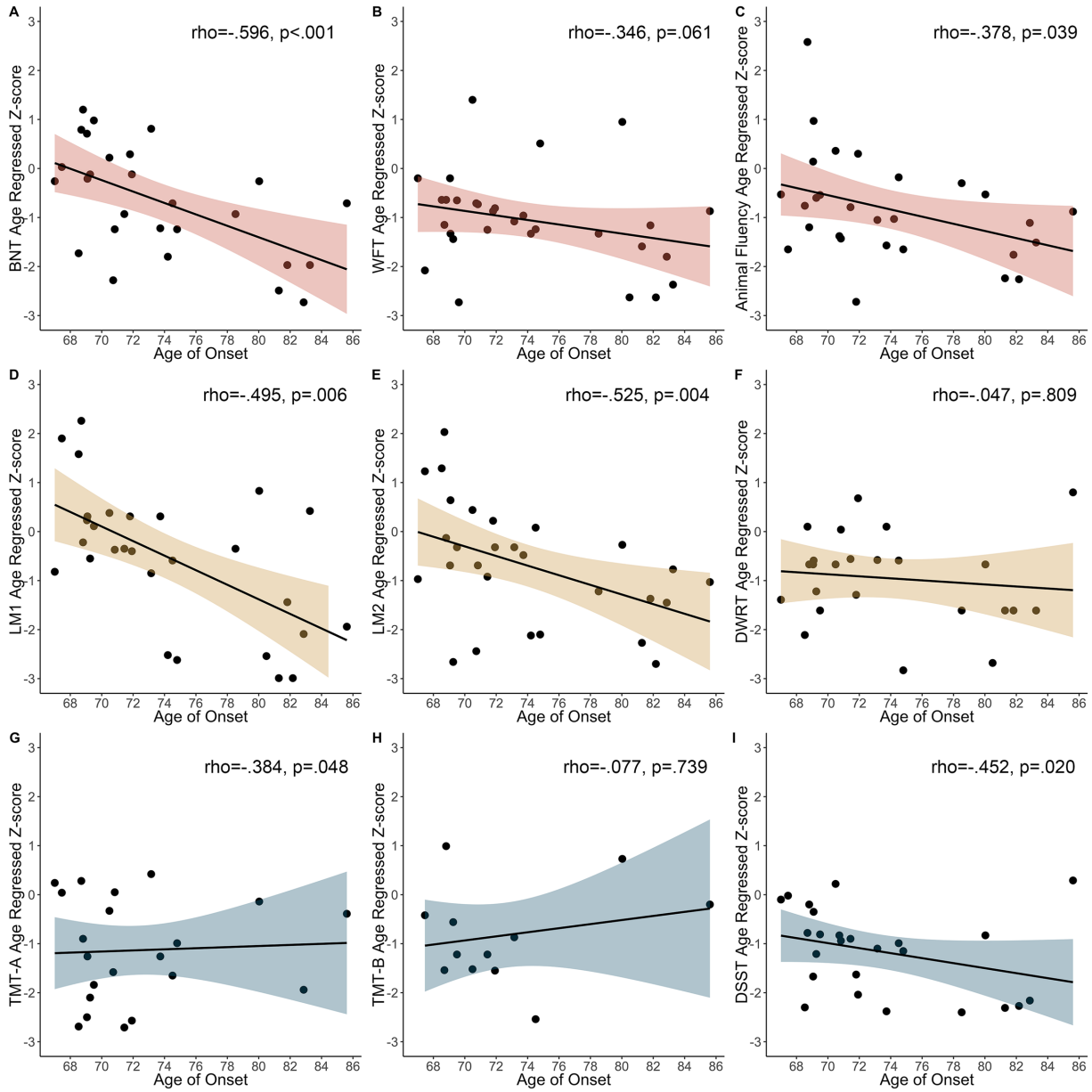


FIGURE 3
Relationship between age of first seizure and age regressed z-scores in participants with seizure onset prior to cognitive testing. **(A)** BNT, Boston Naming Test. **(B)** WFT, Word Fluency Test. **(C)** Animal Fluency. **(D)** LM1, Logical Memory 1. **(E)** LM2, Logical Memory 2. **(F)** DWRT, Delayed Word Recall Test. **(G)** TMT-A, Trail Making Test-A. **(H)** TMT-B, Trail Making Test-B. **(I)** DSST, Digit Symbol Substitution Test.

with the LOE participants being older on average (White LOE = 77.52, Black LOE = 78.13 years versus White Non-LOE = 75.47, Black Non-LOE: 74.46 years). There were no differences in sex or education between the groups.

## 3.3. Relationship between seizure onset and cognitive performance

We observed an inverse relationship between the BNT (rho = −0.596, $p < 0.001$), Animal fluency (rho = −0.378, $p = 0.039$), LM1 (rho = −0.495, $p = 0.006$), LM2 ($r = -0.525$, $p = 0.004$), TMT-A (rho = −0.384, $p = 0.048$), and DSST (rho = −0.452, $p = 0.020$), with an older age of seizure onset associated with worse cognitive performance (Figure 3). There were no other significant correlations (WFT rho = −0.346, $p = 0.061$; DWRT rho = −0.047, $p = 0.809$; TMT-B rho = −0.077, $p = 0.739$).

## 3.4. Rates of impairment in LOE

Figure 4 demonstrates the pattern of impairment across individual measures at Visit 5 using the ≤ −1.0SD cutoff. Rates of impairment in language ranged from 37.6% (BNT) to 46.7% (Animal fluency); impairment rates in memory ranged from 27.4% (LM1) to 47.1% (DWRT); and impairments rates in executive function/processing speed ranged from 44.3% (TMT-A) to 48.5% (TMT-B). At the domain level (i.e.,

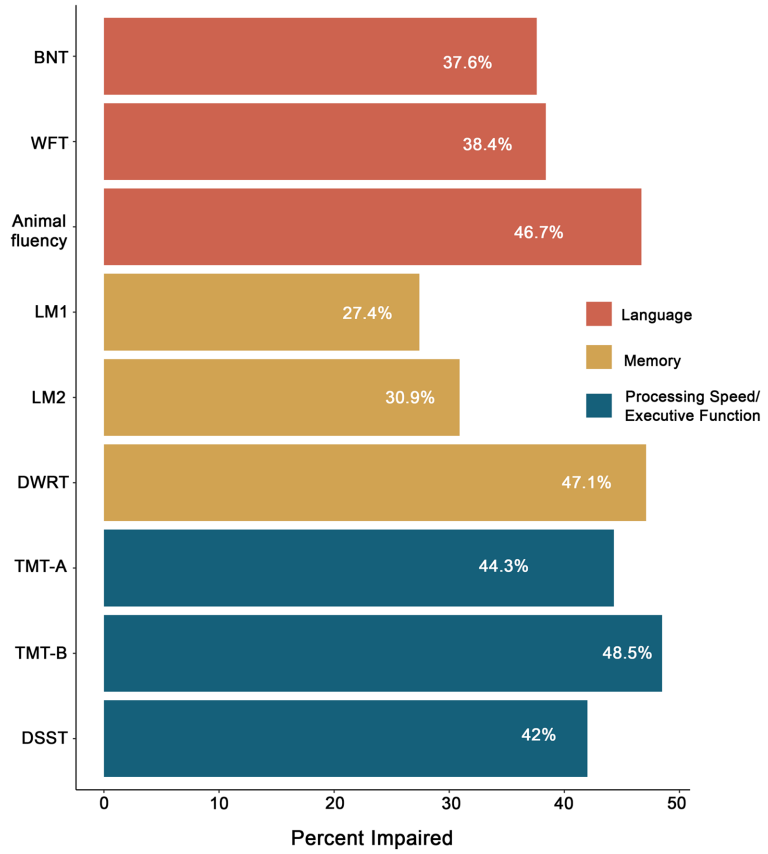


FIGURE 4
Percentage of impairment in LOE across neuropsychological measures based on a ≤ −1.0SD cutoff. BNT, Boston Naming Test; WFT, Word Fluency Test; LM1, Logical Memory 1; LM2, Logical Memory 2; DWRT, Delayed Word Recall Test; TMT-A, Trail Making Test-A; TMT-B, Trail Making Test-B; DSST, Digit Symbol Substitution Test.

two impaired measures within a domain), 39.8% of the total sample was impaired in language, 39.5% in executive function/processing speed, and 29.3% in memory.

## 3.5. Differences in cognitive performance between participants with seizure onset prior to and after cognitive testing

Differences in performance between those with seizure onset prior to and after V5 was significant for WFT [t (84)= 3.04, $p = 0.003$; before V5 mean z-score = −1.05; after V5 mean = −0.331], TMT-A [t (77)=2.07, $p = 0.042$; before V5 mean z-score = −3.24; after V5 mean = −1.26], TMT-B [t (64)=2.89, $p = 0.005$; before V5 mean z-score = −2.32; after V5 mean = −0.976], and Digit Symbol [t (79)=2.23, $p = 0.029$; before V5 mean z-score = −1.25; after V5 mean = −0.700] with those with seizure onset prior to V5 having lower scores. Rates of impairment between the groups differ for TMT-A ($p = 0.019$; Before V5 63% impaired versus 34.6%) and TMT-B ($p = 0.017$; Before V5 71.4% impaired versus 37.8%).

## 3.6. Cognitive phenotypes in LOE

Of the 91 participants, 80 had complete cognitive data and were included in the phenotyping classification. Figure 5A shows the distribution of cognitive phenotypes. Twenty-seven and a half percent of the final LOE sample demonstrated a Multidomain impaired phenotype with impairments in at least two out of three cognitive domains. Within the Multidomain phenotype, 65.2% of participants had impairment in two domains with 53.3% of these participants having impairments in Language and Memory and the remainder 46.7% impairments in executive function/processing speed plus another domain. Thirty-five percent demonstrated a Single-Domain phenotype with 53.6% showing deficits in executive function/processing speed, 35.7% demonstrating language impairments and 10.7% an amnestic profile (i.e., isolated impairments in memory) (Figure 5B). Thirty-six and three tenths demonstrated a Minimal/No Impairment profile with 37.9% of the group showing no impairment in any of the tests and the remainder of the group having impairment on at least one test (34.5% one test, 20.7% two tests, and 6.9% three tests). Figure 6 shows the distribution of z-scores at the individual test level for each cognitive phenotype. Supplementary Table S4 includes average z-scores for each test across the cognitive phenotypes and group comparisons.

The distribution of cognitive phenotypes was different between participants that developed seizures prior to and after Visit 5 cognitive testing (FE = 6.61, $p = 0.037$; Onset before Visit 5: Multidomain = 39.3%, Single = 42.9%, Minimal/No Impairment = 17.9%; Onset after Visit 5: Multidomain = 23.1%, Single = 30.8%, Minimal/No Impairment = 46.2%), with participants with onset after Visit 5 having a greater proportion of participants with Minimal/No Impairment. The majority of the

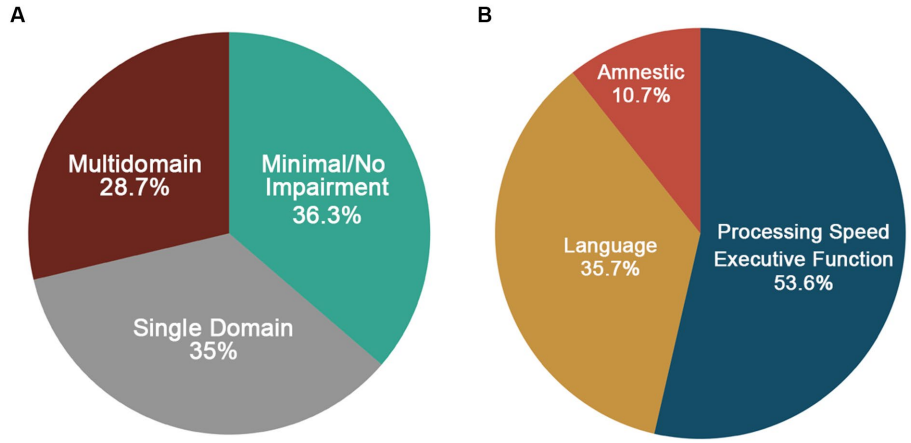


FIGURE 5
**(A)** Distribution of cognitive phenotypes in LOE based on a ≤ −1.0SD cutoff. **(B)** Distribution of domains impaired for the single domain phenotype.

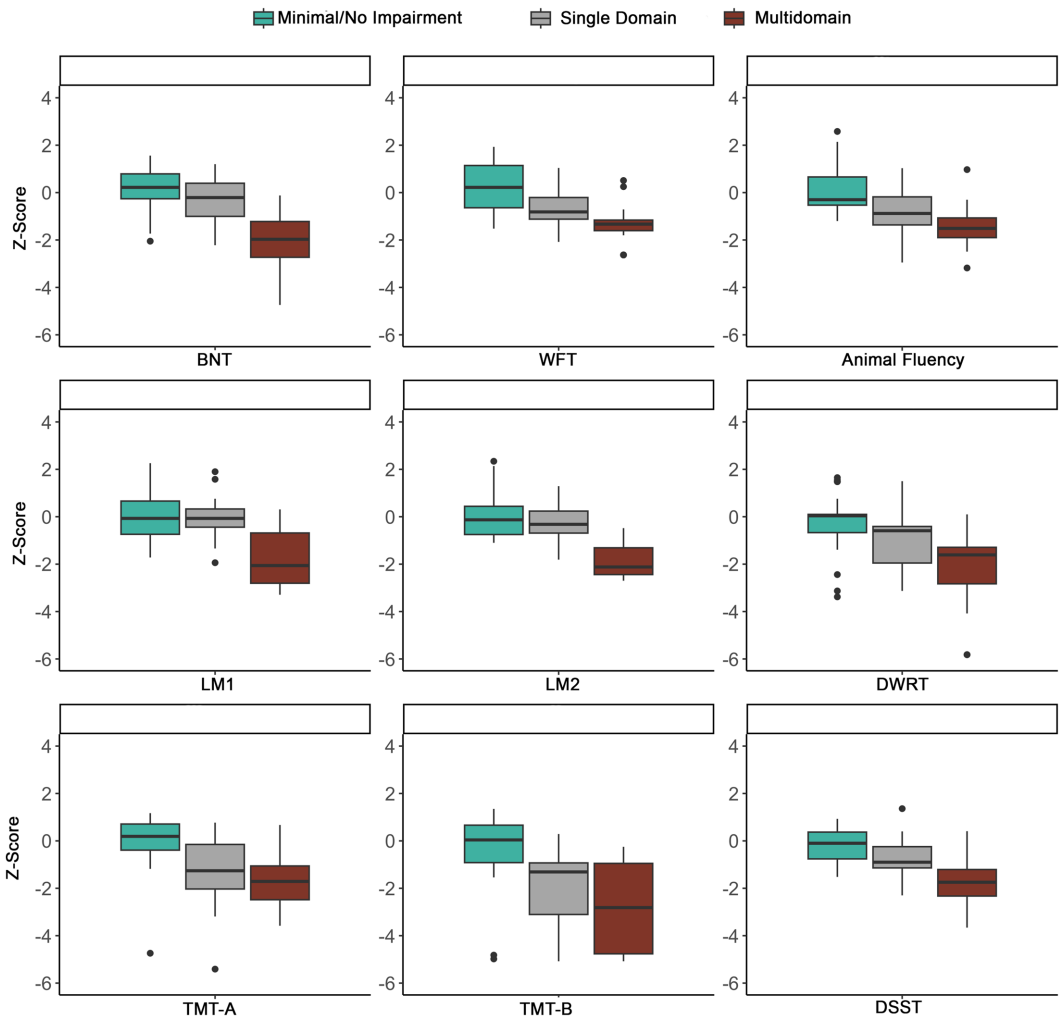


FIGURE 6
Distribution of *z*-scores across phenotypes using the ≤ −1.0SD cutoff. To show the distribution across tests, extreme outliers were removed. BNT, Boston Naming Test; WFT, Word Fluency Test; LM1, Logical Memory 1; LM2, Logical Memory 2; DWRT, Delayed Word Recall Test; TMT-A, Trail Making Test-A; TMT-B, Trail Making Test-B; DSST, Digit Symbol Substitution Test.

TABLE 2 Clinical and demographic characteristics across LOE cognitive phenotypes.

| | Multidomain | Single-domain | Minimal | *F* | *p*-value |
|---|---|---|---|---|---|
| *n* (%)* | 23 (28.7%) | 28 (35%) | 29 (36.3%) | | |
| **Age** | **81.1 (4.76)** | **75.5 (5.54)** | **76.0 (4.36)** | **9.82** | **<0.001** |
| Age at 1st seizure | **81.71 (6.73)** | **75.92 (5.95)** | **79.89 (6.22)** | **5.79** | **0.005** |
| | | | | FE | *p*-value |
| Sex: Female | 14 (60.9%) | 13 (46.4%) | 16 (55.2%) | 1.10 | 0.59 |
| Race: Black | 9 (39.1%) | 7 (25%) | 7 (24.1%) | 1.66 | 0.45 |
| **Education: >HS** | **6 (26.1%)** | **13 (46.4%)** | **23 (79.3%)** | **15.5** | **<0.001** |
| **Occupational complexity: High** | **5 (27.8%)** | **16 (64%)** | **18 (72%)** | **8.83** | **0.008** |
| Antiseizure medications: Yes | 1 (4.3%) | 0 (0%) | 1 (3.4%) | – | – |
| Stroke | 7 (30.4%) | 4 (14.3%) | 7 (24.1%) | 1.99 | 0.39 |
| Hypertension | 18 (78.3%) | 24 (85.7%) | 22 (75.9%) | 0.980 | 0.69 |
| Diabetes | 10 (43.5%) | 13 (46.4%) | 10 (34.5%) | 0.936 | 0.66 |
| BMI ≥ 30 | 5 (21.7%) | 10 (35.7%) | 10 (34.5%) | 1.38 | 0.52 |
| Hyperlipidemia | 5 (21.7%) | 6 (21.4%) | 8 (27.6%) | 0.406 | 0.85 |
| Vascular burden: 2 or more | 13 (56.5%) | 19 (67.9%) | 16 (55.2%) | 1.15 | 0.60 |
| APOE4 genotype: Present | 8 (36.4%) | 8 (38.1%) | 10 (41.7%) | 0.197 | 0.95 |

*Based on the total number of participants with complete cognitive data. LOE, late-onset epilepsy; HS, High school; BMI, body mass index; FE, Fisher–Freeman–Halton exact test. High occupation attainment: managerial and professional specialty, technical, sales, and administrative support. Low occupation attainment: service, precision production, repair, operators, fabricators, laborers, homemakers. Bold: significant with an FDR correction of 0.01.

participants in the Single Domain phenotype had impairments in executive function/processing speed for both groups (Onset before Visit 5: 66.7%; Onset after Visit 5: 43.8%). The distribution of phenotypes without participants with dementia based on the ARIC definition were as followed: Multidomain = 23.6%, Single = 37.5%, and Minimal/No Impairment = 38.9%.

Lastly, we conducted a post-hoc sensitivity analysis to determine the rates of impairment within the Non-LOE normal control sample. We selected 10% of the normal sample and calculated z-scores based on the remainder 90% of the sample. We applied the same phenotype classification described above. The majority of this subsample demonstrated a Minimal/No Impairment profile (78.3%), followed by Single Domain (16.5%) and Multidomain (5.2%). This distribution was significantly different from the LOE phenotype distribution ($\chi^2 = 54.17$, $p < 0.001$).

## 3.7. Demographic and clinical variables across phenotypes

Table 2 includes demographic and clinical factors across cognitive phenotypes. There were differences in age, with the Multidomain phenotype being older compared to the Single Domain phenotype (81.13 years versus 75.54 years, $p < 0.001$) and Minimal/No Impairment (76 years, $p = 0.001$). There were differences in age of seizure onset, with the Multidomain phenotype having an older age of seizure onset compared to the Single Domain phenotype (81.7 years versus 75.92, $p = 0.005$) and there was a trend toward an older age of seizure onset compared to the Minimal/No Impairment (79.89 years, $p = 0.058$). There were differences in education with participants with the Multidomain phenotype having a lower proportion of older adults (26.1%) with an education higher than a high school degree compared to the Single Domain (46.4%) and Minimal/No Impairment (79.3%). Lastly, there were differences in occupational attainment with participants with the Multidomain phenotype having lower occupational complexity relative to the other two groups. Although there were no differences in vascular risk factors across the phenotypes, hypertension was the most common factor with 85.7% of the Single Domain, 78.3% of the Multidomain, and 75.9% of the Minimal/No Impairment phenotypes having hypertension. There were no other differences across phenotype groups.

Seventy-five percent ($n = 6$) of the participants with an ARIC dementia diagnosis demonstrated a Multidomain phenotype, while the remainder two participants had Single Domain or Minimal/No Impairment phenotype. Of those with MCI, 61.5% had a Single phenotype, 26.9% Multidomain, and 11.4% a Minimal/No Impairment phenotype. In those with a Normal Cognition diagnosis based on the ARIC definition, 54.3% had a Minimal/No Impairment phenotype, 23.9% Single Domain, and 21.7% a Multidomain phenotype.

## 3.8. Longitudinal changes in phenotype membership

At ARIC-NCS Visit 6, 25 (27.5%) participants completed testing, 23 (25.3%) were deceased, and the remainder 43 (47.3%) did not complete Visit 6. Out of the 25 participants with LOE and longitudinal data, 12 had a Minimal/No Impairment and 12 had a Single Domain phenotype at Visit 5. The one participant that had a Multidomain phenotype at Visit 5, remained stable at Visit 6 and was not included in additional analyses. Of these 24 participants, 62.5% declined and 37.5% remained stable; no participants reverted. Of those that declined, 46.7% had a Minimal/No Impairment phenotype at Visit 5. Of those that remained stable, 44.4% had a Single Domain and 55.6%

TABLE 3 Clinical and demographic characteristics between stabled and declined participants.

| | Stabled | Decline | Comparison |
|---|---|---|---|
| *n* | 9 (37.5%) | 15 (62.5%) | |
| Age (V5) | 75.56 (3.84) | 74.53 (4.88) | U = 67, $p = 1.00$ |
| Age at 1st seizure | 75.41 (5.83) | 77.53 (4.89) | U = 84, $p = 0.35$ |
| Sex: F (%) | 3 (33.3%) | 8 (53.3%) | U = 0.427, $p = 0.69$ |
| Race: Black (%) | 1 (11.1%) | 5 (33.3%) | FE = 0.906, $p = 0.42$ |
| **Education: > HS** | **9 (100%)** | **6 (40%)** | **FE = 8.64, $p = 0.007$** |
| Vascular Risk (1+ factors) | 9 (100%) | 13 (86.7%) | FE = 1.31, $p = 0.51$ |
| APOE4 Present | 3 (50%) | 3 (25%) | FE = 1.25, $p = 0.34$ |

HS, high school. Bold: significant at $p = 0.05$.

a Minimal/No Impairment phenotype. When comparing demographic and clinical variables between the participants that declined and those that remained stable, the participants that remained stable had higher levels of education (Table 3). Out of the 15 individuals that declined, 3 (20%) met criteria for a new diagnosis of MCI and 3 (20%) a diagnosis of dementia based on the ARIC diagnostic definitions. For those that decline at Visit 6, executive function (50%) was the most commonly impaired domain at Visit 5, followed by language (37.5%), and memory (12.5%).

# 4. Discussion

With a globally aging population and the expected increase in the number of individuals with LOE, it is important to fully characterize the cognitive profiles of older adults with epilepsy to identify those at increased risk for progressive cognitive decline. Here, we show that approximately 63% of older adults who developed LOE demonstrate an impaired cognitive phenotype (i.e., Multidomain or Single Domain phenotype) and that in a sizable subset of individuals, an impaired profile is present prior to the onset of recognized seizures. Further, executive function/processing speed was the most impaired domain in those with isolated impairment and for those patients that declined. We also show that more than half of the participants with longitudinal cognitive data progressed to a more impaired phenotype. Lastly, higher education was associated with minimal or no impairment at our baseline visit (Visit 5) and a lower likelihood of declining over time.

## 4.1. Cognitive impairment in LOE

Given that the small number of studies that have exclusively focused on LOE have included cognitive screeners or had a limited number of neuropsychological tests (22–24, 35–39), the full characterization of cognitive profiles in LOE remains to be examined. In this study, we show that in a population of older adults with LOE who completed a cross-sectional assessment, cognitive impairment is common across a comprehensive battery of tests with rates of impairment ranging from 27% to 48%, with measures of set-shifting, delayed recall, semantic fluency, and processing speed the most prevalent. At the domain level, more than a third of the sample was impaired on at least one domain despite our rather stringent criteria, which required two out of the three measures per domain to be impaired; an approach which has been shown to provide a good balance between sensitivity and specificity for classifying impairment in older adults and which may explain its diagnostic stability across cohorts (71).

In our study, participants with impairments included those with an onset of seizures either prior to or after Visit 5 cognitive testing, and with more than half the sample demonstrating an impaired phenotype. Studies in LOE have reported poorer cognitive outcomes that in some patients are present before the onset of seizures (24, 31, 38). In a larger sample of older adults with LOE from the ARIC study (24), we previously showed that a steeper longitudinal decline in cognitive function occurred prior to the onset of seizures in those who developed LOE versus those who did not, and that this decline in LOE became more rapid after the onset of seizures. Our results support these earlier findings in that in a subset of patients who develop LOE, cognitive dysfunction is present before the onset of seizures with some evidence of progression over time. Noteworthy, the presence of cognitive impairment at the time of an epilepsy diagnosis has been documented across several studies (36, 39, 72–75) implying that cognitive dysfunction may not always be solely caused by the accumulating effects of seizures or the long-term exposure to epilepsy treatment (e.g., antiseizure medications, surgery) but rather may be a result of epileptogenesis or other etiological factors that contribute to the development of seizures later in life (e.g., small vessel disease).

We also show that in those with a seizure onset prior to cognitive testing, an older age of seizure onset was associated with poorer performance on measures of naming, verbal fluency, learning and memory, and processing speed. In contrast, a younger age of seizure onset has been linked to an increased risk for cognitive impairment in early onset epilepsy (40). Thus, developing seizures in older age may accelerate cognitive decline. Interestingly, Liguori and colleagues (38) demonstrated that in a group of older adults with LOE cognitive progression on a global measure of cognitive ability was observed at a 12-month follow-up irrespective of type and number of antiseizure medications. Our findings and that of previous studies highlight that the relationship between classic epilepsy characteristics and cognition may be different for LOE and therefore, more studies are needed to better delineate these associations given their implications for treatment and long-term outcomes.

## 4.2. Cognitive phenotypes in LOE

An important advantage of the phenotyping approach is that it is patient-centered and considers the individual variability within neurological disorders. Traditional approaches to studying neuropsychological syndromes aggregate patients based on the neurological condition (e.g., all patients with epilepsy); however, this approach may obscure important differences across syndrome/disorder subtypes. Further, the phenotype classification reflects the process that clinicians employ which typically consists of examining multiple scores within a domain and base clinical decisions on the pattern of scores rather than isolated impaired scores (71). In the MCI literature, the prodromal phase of AD, identifying cognitive profiles or phenotypes has proven useful for predicting cognitive decline and progression to dementia (42, 76). Specifically, an amnestic, dysnomic, and dysexecutive/mixed phenotypes have been described with unique MRI signatures and differential progression to dementia (41, 42, 76). In our study, a majority of the participants with a dementia diagnosis based on the ARIC definition had a Multidomain phenotype (75%), while those with MCI had a Single Domain (61.5%) phenotype. Although for a large proportion of the participants the pattern of impairment (i.e., phenotype) matched the severity of the diagnosis (e.g., multidomain impairment and a dementia diagnosis), this was not the case for all participants. Given that phenotypes are based on the pattern of cognitive scores alone and do not take into account changes in functional activities of daily living, some individuals may demonstrate a less impaired phenotype but greater functional decline and therefore may meet criteria for dementia. Thus, cognitive phenotypes do not replace diagnostic criteria for MCI and dementia, rather, they help characterize the underlying cognitive profiles within these diagnostic categories. Specifically, an MCI or dementia diagnosis provides information on whether an individual has significant cognitive impairments or has declined from a previous level of functioning, whereas cognitive phenotypes delineate the different patterns of impairment. Therefore, our study provides additional information on the cognitive subtypes associated with LOE, which, when considered in combination with other disease biomarkers, may shed light on differential risk for further cognitive decline.

In our sample, approximately 29% of the older adults demonstrated *global* or Multidomain impairment, which is comparable to rates reported in studies of young-to-middle-aged adults with temporal (40, 50, 51, 53–55) and frontal lobe epilepsy (52). Patients with global impairments are thought to represent a group of patients with potential co-morbid non-epilepsy pathology, elevated health-related risk factors, or sociodemographic factors that may be resulting in greater cognitive dysfunction than expected. Furthermore, these patients demonstrate widespread brain abnormalities that extend beyond the seizure focus potentially explaining the multidomain (i.e., multi lobar) involvement in this impaired profile (40, 49, 51). In our study, individuals with a Multidomain phenotype were older, had fewer years of education, and lower occupational complexity. Fewer years of education has been a consistent finding in patients with epilepsy demonstrating global impairment (40). Thus, these factors may be contributing to their global impaired profile by further exacerbating the effects of epilepsy pathology on cognition. Another possibility is that for some individuals these cognitive deficits were longstanding and therefore may have led to lower educational and occupational attainment. For example, a preexisting learning disability in early childhood may have impacted a person's educational attainment. However, given the nature of our data, we did not have information on early history of cognitive dysfunction and how that may have impacted education/occupational attainment. Although we did not have EEG information on seizure localization/lateralization, the Multidomain phenotype in our study may represent a phenotype with widespread brain anomalies that may be associated with both epilepsy and non-epilepsy pathology.

The Single Domain phenotype was characterized by prominent impairments in executive function/processing speed. By contrast, in younger adults with frontal lobe epilepsy (52) and temporal lobe epilepsy (53), the Single Domain phenotype has been characterized by impairments in language with naming and verbal fluency the most impaired cognitive processes. In fact, in a sample of 1,409 young-to-middle aged adults with temporal lobe epilepsy, 49% of the patients with a Single Domain phenotype had isolated deficits in language (53). In a sample of Spanish-speaking patients with temporal lobe epilepsy, memory was the most commonly impaired domain within the Single Domain phenotype (55). The differences in the nature of the Single Domain phenotype across studies may be due to varying underlying epilepsy etiologies, epilepsy-related clinical factors, sociodemographic characteristics, or differences in brain abnormalities (e.g., lateral versus mesial temporal lobe involvement). Given the nature of epilepsy ascertainment in ARIC, we did not have comprehensive clinical information to examine the epilepsy characteristics associated with these isolated impairments in executive function/processing speed such as brain pathology involving the frontal lobes. However, the high vascular burden in our overall sample may be a contributing factor. Although there were no differences in the number and type of vascular risk factors across the phenotypes, approximately 95% of the sample had at least one vascular risk factor with hypertension being the most common. Elevated vascular risk factors have been associated with the extent of cognitive impairment in patients with epilepsy (77, 78) and with an increased risk of developing LOE (8). In the general population, vascular risk factors, particularly diabetes and hypertension are associated with cognitive decline and dementia (79). Specifically, the presence of vascular risk factors has been implicated in executive dysfunction and slower processing speed given their effects on white matter structures involved in these domains (80, 81). We previously demonstrated an association between increased white matter hyperintensities burden and increased likelihood of developing LOE in a larger sample of participants from ARIC which includes the sample in the current study (61). Given that LOE may be associated with varying etiologies, an executive dysfunction and reduced processing speed phenotype/profile may be indicative of the presence of occult cerebrovascular disease. Notably, impairments in executive function and processing speed (82) may reflect vascular involvement and thus it is possible that a subset of older adults with a dysexecutive and slowed processing speed profile may be at risk for the development of dementia of a mixed or vascular etiology. However, given the small number of LOE participants with dementia in our study, we were not able to delineate differences in profiles.

Lastly, 36.3% of the sample demonstrated a Minimal/No Impairment phenotype which included a subset of participants (37.9%) with no impairment in any of the tests and the majority demonstrating 1–2 impaired scores. Notably, studies have shown that the vast majority of adults demonstrate 1–2 impaired performances across a larger neuropsychological battery (83–85). Thus, an advantage

of the phenotype approach is reducing the likelihood of false positives that may result when examining individual test scores in isolation. The Minimal/No Impairment phenotype in our study was characterized by higher education with more than 65% of the older adults having an advanced degree (i.e., college or graduate degree). Across studies, the rates of this phenotype/profile have ranged from 16% to 54% and have been associated with less disease burden including shorter disease duration, fewer antiseizure medications, and less brain pathology (40). Further, higher education in this group has been a consistent finding across investigations. Higher levels of education and complex occupational attainment have been hypothesized to increase cognitive reserve, a protective mechanism that mitigates the effects of brain pathology on cognition by increasing the cognitive resources available to compensate for cognitive deficiencies. For example, higher levels of education have been associated with a lower risk of developing dementia and/or a delay in the onset of dementia-related symptoms. (86–88). In epilepsy, higher levels of education have been shown to protect against the effects of epilepsy related pathology on cognition, as patients with higher education demonstrate less cognitive impairments despite showing greater disease burden (40, 89). Studies with larger samples examining the clinical and demographic profiles of patients with minimal impairment can help identify protective factors which can inform clinical interventions aimed are reducing cognitive decline.

## 4.3. Cognitive progression

Identifying distinct cognitive phenotypes has been shown to be useful in predicting cognitive progression. In the subset of our sample with longitudinal data, we show that 62.5% of the older adults with either a Single Domain or a Minimal/No phenotype decline (i.e., changed to a more impaired phenotype) at a subsequent visit. Fifty percent of those that decline demonstrated an executive dysfunction profile at baseline and although we were not able to statistically evaluate its predicted value, executive function deficits may be associated with cognitive progression, potentially due to a vascular underlying etiology. Whether epilepsy results in accelerated brain and cognitive aging has been an ongoing debate in the literature. Studies have provided evidence of brain aging in patients with epilepsy that includes both patients with long-standing and newly onset epilepsy (90–92). Importantly, there is evidence of cognitive deterioration regardless of the age of onset (24, 38, 93, 94). In the MCI/AD literature, phenotypes have been shown to have prognostic value improving prediction of clinical course (41). Thus, phenotyping may provide a promising approach to stratifying risk for decline that considers individual variability within patient cohorts and could help identify factors that constitute this group and may buffer against decline (e.g., education).

## 4.4. Limitations

There are several limitations to our study that limit the generalizability of the findings. First, the use of ICD codes to diagnose epilepsy can potentially lead to misclassification of diagnosis. Inherent in this use of code data is the potential to miss cases of childhood epilepsy that have resolved, or to misclassify recurrent provoked seizures as epilepsy if there were multiple hospitalizations for alcohol withdrawal seizures (for example). However, the method (i.e., $\geq 2$ ICD codes) used has been shown to be robust with high sensitivity and specificity (24, 60). Second, our sample size was modest compared to other studies involving cognitive phenotypes in epilepsy. Further, the lack of differences in vascular risk factors across the phenotypes may be explained by the sample size and the fact that most participants in our sample had a high vascular burden and therefore there was less heterogeneity. Studies with larger samples of older adults with LOE are needed in order to replicate our findings and to identify unique vascular and other risk factor profiles associated with each phenotype. Third, although we used a normative sample to account for the effects of age, education, sex, and race on cognitive scores, there were age differences between the normative sample size and the LOE participants. Fourth, we did not have comprehensive epilepsy-related clinical data such as seizure frequency and number of antiseizure medications and type, and therefore could not examine the relationship between phenotype membership and classic epilepsy variables (e.g., antiseizure medications, EEG findings, seizure frequency, epilepsy etiology); full, optimal workup of new-onset epilepsy including lumbar puncture was not available (if performed) (95). Importantly, better epilepsy characterization (i.e., seizure localization/lateralization based on EEG and imaging findings) can help delineate the brain regions associated with different cognitive profiles (i.e., executive function/processing speed = frontal lobe abnormalities; amnestic profile = mesial temporal lobe abnormalities). Based on evidence from several phenotype investigations, two major patterns have emerged with global deficits associated with greater disease burden and elevated risk factors for cognitive impairment while patients with relatively intact profiles demonstrating less disease burden and protective factors. However, these findings have been found primarily in patients with an earlier age of epilepsy onset that have been fully characterized and therefore studies are needed to determine the clinical profiles associated with each phenotype in LOE. Fifth, we only had longitudinal data in a subset of the sample and therefore, longitudinal studies with large samples are needed in order to determine the diagnostic value of the phenotype approach in determining risk of cognitive progression in LOE. Further, selectivity of attrition (e.g., participants returning for cognitive testing due to concerns of decline) could have introduced bias in the longitudinal sample. Interestingly, a study examining differences in cognitive abilities and personality traits between returning and non-returning participants found that returning participants demonstrated higher cognitive abilities and personality traits such as agreeableness and openness which was more apparent in adults older than 50 (96). Thus, it is possible that those participants that returned had better insight into their cognitive abilities and were worried about decline. Lastly, although we used all participants without epilepsy and with normal cognition as our normative sample for determining impairment profiles, future studies comparing the rates and patterns of cognitive impairment (i.e., phenotypes) between LOE participants and Non-LOE participants with other neurological conditions (e.g., MCI, TBI or dementia) can elucidate whether epilepsy is associated with unique patterns of cognitive impairment and/or confers a differential risk for cognitive decline beyond the effects of aging on cognition.

# 5. Conclusion

This study delineates unique cognitive phenotypes in LOE using a large, population-based study cohort. Our findings

demonstrate heterogeneity in cognitive impairment within LOE that can be appreciated by identifying cognitive phenotypes. Thus, the application of this approach may accelerate our understanding of the clinical course of LOE, and guide future interventions aimed at preventing the onset of cognitive dysfunction or reducing the risk of further cognitive decline in older adults.

## Data availability statement

This study analyzed publicly available datasets. These data can be found via application for ARIC Data or through the NIH NHLBI-sponsored Biologic Specimen and Data Repository Information Coordinating Center (BioLINCC) at: https://biolincc.nhlbi.nih.gov/.

## Ethics statement

The studies involving human participants were reviewed and approved by all participating ARIC Institutions Institutional Review Boards. All participants provided written informed consent.

## Author contributions

AR: drafting/revision of the manuscript for content, study concept or design, and analysis or interpretation of data. AS, AK-N, and RG: drafting/revision of the manuscript for content and interpretation of data. EJ and CM: drafting/revision of the manuscript for content, study concept or design, and interpretation of data. All authors contributed to the article and approved the submitted version.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note



## Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2023.1230368/full#supplementary-material

## References

1. Beghi E, Giussani G, Costa C, DiFrancesco JC, Dhakar M, Leppik I, et al. The epidemiology of epilepsy in older adults: a narrative review by the ILAE task force on epilepsy in the elderly. *Epilepsia*. (2023) 64:586–601. doi: 10.1111/epi.17494

2. Brodie MJ, Elder AT, Kwan P. Epilepsy in later life. *Lancet Neurol*. (2009) 8:1019–30. doi: 10.1016/S1474-4422(09)70240-6

3. Sen A, Jette N, Husain M, Sander JW. Epilepsy in older people. *Lancet*. (2020) 395:735–48. doi: 10.1016/S0140-6736(19)33064-8

4. Sen A, Capelli V, Husain M. Cognition and dementia in older patients with epilepsy. *Brain*. (2018) 141:1592–608. doi: 10.1093/brain/awy022

5. Schneider ALC, Gottesman RF, Krauss GL, Gugger J, Diaz-Arrastia R, Kucharska-Newton A, et al. Association of head injury with late-onset epilepsy: results from the atherosclerosis risk in communities cohort. *Neurology*. (2022) 98:e808–17. doi: 10.1212/WNL.0000000000013214

6. Roberts M, Godfrey J, Caird F. Epileptic seizures in the elderly: I. Aetiology and type of seizure. *Age Ageing*. (1982) 11:24–8. doi: 10.1093/ageing/11.1.24

7. Ramsay RE, Macias FM, Rowan AJ. Diagnosing epilepsy in the elderly. *Int Rev Neurobiol*. (2007) 81:129–51. doi: 10.1016/S0074-7742(06)81008-1

8. Johnson EL, Krauss GL, Lee AK, Schneider ALC, Dearborn JL, Kucharska-Newton AM, et al. Association between midlife risk factors and late-onset epilepsy: results from the atherosclerosis risk in communities study. *JAMA Neurol*. (2018) 75:1375–82. doi: 10.1001/jamaneurol.2018.1935

9. Stefanidou M, Himali JJ, Devinsky O, Romero JR, Ikram MA, Beiser AS, et al. Vascular risk factors as predictors of epilepsy in older age: the Framingham Heart Study. *Epilepsia*. (2022) 63:237–43. doi: 10.1111/epi.17108

10. Zhang D, Chen S, Xu S, Wu J, Zhuang Y, Cao W, et al. The clinical correlation between Alzheimer's disease and epilepsy. *Front Neurol*. (2022) 13:922535. doi: 10.3389/fneur.2022.922535

11. Hesdorffer DC, Hauser WA, Annegers JF, Kokmen E, Rocca WA. Dementia and adult-onset unprovoked seizures. *Neurology*. (1996) 46:727–30. doi: 10.1212/WNL.46.3.727

12. Tang T, Zhang R, Pan X. Meta-analysis of the risk of dementia in elderly patients with late-onset epilepsy. *Clin Neurol Neurosurg*. (2022) 223:107499. doi: 10.1016/j.clineuro.2022.107499

13. Huang L, Fu C, Li J, Peng S. Late-onset epilepsy and the risk of dementia: a systematic review and meta-analysis. *Aging Clin Exp Res*. (2022) 34:1771–9. doi: 10.1007/s40520-022-02118-8

14. Ophir K, Ran B, Felix B, Amir G. Ten year cumulative incidence of dementia after late onset epilepsy of unknown etiology. *J Clin Neurosci*. (2021) 86:247–51. doi: 10.1016/j.jocn.2021.01.030

15. Keret O, Hoang TD, Xia F, Rosen HJ, Yaffe K. Association of late-onset unprovoked seizures of unknown etiology with the risk of developing dementia in older veterans. *JAMA Neurol*. (2020) 77:710–5. doi: 10.1001/jamaneurol.2020.0187

16. Johnson EL, Krauss GL, Kucharska-Newton A, Albert MS, Brandt J, Walker KA, et al. Dementia in late-onset epilepsy: the atherosclerosis risk in communities study. *Neurology*. (2020) 95:e3248–56. doi: 10.1212/WNL.0000000000011080

17. Amatniek JC, Hauser WA, DelCastillo-Castaneda C, Jacobs DM, Marder K, Bell K, et al. Incidence and predictors of seizures in patients with Alzheimer's disease. *Epilepsia*. (2006) 47:867–72. doi: 10.1111/j.1528-1167.2006.00554.x

18. Vossel KA, Beagle AJ, Rabinovici GD, Shu H, Lee SE, Naasan G, et al. Seizures and epileptiform activity in the early stages of Alzheimer disease. *JAMA Neurol*. (2013) 70:1158–66. doi: 10.1001/jamaneurol.2013.136

19. Palop JJ, Mucke L. Epilepsy and cognitive impairments in Alzheimer disease. *Arch Neurol*. (2009) 66:435–40. doi: 10.1001/archneurol.2009.15

20. DiFrancesco JC, Tremolizzo L, Polonia V, Giussani G, Bianchi E, Franchi C, et al. Adult-onset epilepsy in presymptomatic Alzheimer's disease: a retrospective study. *J Alzheimers Dis*. (2017) 60:1267–74. doi: 10.3233/JAD-170392

21. Romoli M, Sen A, Parnetti L, Calabresi P, Costa C. Amyloid-β: a potential link between epilepsy and cognitive decline. *Nat Rev Neurol*. (2021) 17:469–85. doi: 10.1038/s41582-021-00505-9

22. Costa C, Romoli M, Liguori C, Farotti L, Eusebi P, Bedetti C, et al. Alzheimer's disease and late-onset epilepsy of unknown origin: two faces of beta amyloid pathology. *Neurobiol Aging*. (2019) 73:61–7. doi: 10.1016/j.neurobiolaging.2018.09.006

23. Fernandes M, Manfredi N, Aluisantonio L, Franchini F, Chiaravalloti A, Izzi F, et al. Cognitive functioning, cerebrospinal fluid Alzheimer's disease biomarkers and cerebral glucose metabolism in late-onset epilepsy of unknown aetiology: a prospective study. *Eur J Neurosci*. (2022) 56:5384–96. doi: 10.1111/ejn.15734

24. Johnson EL, Krauss GL, Walker KA, Brandt J, Kucharska-Newton A, Mosley TH Jr, et al. Late-onset epilepsy and 25-year cognitive change: the atherosclerosis risk in communities (ARIC) study. *Epilepsia*. (2020) 61:1764–73. doi: 10.1111/epi.16616

25. Choi H, Thacker EL, Longstreth WT Jr, Elkind MS, Boehme AK. Cognitive decline in older adults with epilepsy: the cardiovascular health study. *Epilepsia*. (2021) 62:85–97. doi: 10.1111/epi.16748

26. Griffith HR, Martin RC, Bambara JK, Marson DC, Faught E. Older adults with epilepsy demonstrate cognitive impairments compared with patients with amnestic mild cognitive impairment. *Epilepsy Behav*. (2006) 8:161–8. doi: 10.1016/j.yebeh.2005.09.004

27. Miller LA, Galioto R, Tremont G, Davis J, Bryant K, Roth J, et al. Cognitive impairment in older adults with epilepsy: characterization and risk factor analysis. *Epilepsy Behav*. (2016) 56:113–7. doi: 10.1016/j.yebeh.2016.01.011

28. Thompson P, Baxendale S, McEvoy A, Duncan J. Cognitive outcomes of temporal lobe epilepsy surgery in older patients. *Seizure*. (2015) 29:41–5. doi: 10.1016/j.seizure.2015.03.017

29. Galioto R, Blum AS, Tremont G. Subjective cognitive complaints versus objective neuropsychological performance in older adults with epilepsy. *Epilepsy Behav*. (2015) 51:48–52. doi: 10.1016/j.yebeh.2015.06.035

30. Martin RC, Griffith HR, Faught E, Gilliam F, Mackey M, Vogtle L. Cognitive functioning in community dwelling older adults with chronic partial epilepsy. *Epilepsia*. (2005) 46:298–303. doi: 10.1111/j.0013-9580.2005.02104.x

31. Pohlmann-Eden B, Aldenkamp A, Baker G, Brandt C, Cendes F, Coras R, et al. The relevance of neuropsychiatric symptoms and cognitive problems in new-onset epilepsy—current knowledge and understanding. *Epilepsy Behav*. (2015) 51:199–209. doi: 10.1016/j.yebeh.2015.07.005

32. Piazzini A, Canevini MP, Turner K, Chifari R, Canger R. Elderly people and epilepsy: cognitive function. *Epilepsia*. (2006) 47:82–4. doi: 10.1111/j.1528-1167.2006.00884.x

33. Reyes A, Kaestner E, Edmonds EC, Christina Macari A, Wang ZI, Drane DL, et al. Diagnosing cognitive disorders in older adults with epilepsy. *Epilepsia*. (2021) 62:460–71. doi: 10.1111/epi.16780

34. Choi H, Elkind MS, Longstreth W, Boehme AK, Hafen R, Hoyt EJ, et al. Epilepsy, vascular risk factors, and cognitive decline in older adults: the cardiovascular health study. *Neurology*. (2022) 99:e2346–58. doi: 10.1212/WNL.0000000000201187

35. Turon M, Abraira L, Cazorla S, Fonseca E, Quintana M, Toledo M, et al. Vascular risk factors as independent predictors of neurocognitive impairments in patients with late-onset epilepsy who have small-vessel disease. *Epilepsy Behav*. (2020) 104:106443. doi: 10.1016/j.yebeh.2019.106443

36. Witt JA, Werhahn K, Krämer G, Ruckes C, Trinka E, Helmstaedter C. Cognitive-behavioral screening in elderly patients with new-onset epilepsy before treatment. *Acta Neurol Scand*. (2014) 130:172–7. doi: 10.1111/ane.12260

37. Nardi Cesarini E, Babiloni C, Salvadori N, Farotti L, Del Percio C, Pascarelli MT, et al. Late-onset epilepsy with unknown etiology: a pilot study on neuropsychological profile, cerebrospinal fluid biomarkers, and quantitative EEG characteristics. *Front Neurol*. (2020) 11:199. doi: 10.3389/fneur.2020.00199

38. Liguori C, Costa C, Franchini F, Izzi F, Spanetta M, Cesarini EN, et al. Cognitive performances in patients affected by late-onset epilepsy with unknown etiology: a 12-month follow-up study. *Epilepsy Behav*. (2019) 101:106592. doi: 10.1016/j.yebeh.2019.106592

39. DiFrancesco JC, Isella V, Licciardo D, Crivellaro C, Musarra M, Guerra L, et al. Temporal lobe dysfunction in late-onset epilepsy of unknown origin. *Epilepsy Behav*. (2021) 117:107839. doi: 10.1016/j.yebeh.2021.107839

40. Hermann BP, Struck AF, Busch RM, Reyes A, Kaestner E, McDonald CR. Neurobehavioural comorbidities of epilepsy: towards a network-based precision taxonomy. *Nat Rev Neurol*. (2021) 17:731–46. doi: 10.1038/s41582-021-00555-z

41. Edmonds EC, Weigand AJ, Hatton SN, Marshall AJ, Thomas KR, Ayala DA, et al. Patterns of longitudinal cortical atrophy over 3 years in empirically derived MCI subtypes. *Neurology*. (2020) 94:e2532–44. doi: 10.1212/WNL.0000000000009462

42. Edmonds EC, Eppig J, Bondi MW, Leyden KM, Goodwin B, Delano-Wood L, et al. Heterogeneous cortical atrophy patterns in MCI not captured by conventional diagnostic criteria. *Neurology*. (2016) 87:2108–16. doi: 10.1212/WNL.0000000000003326

43. De Meo E, Portaccio E, Giorgio A, Ruano L, Goretti B, Niccolai C, et al. Identifying the distinct cognitive phenotypes in multiple sclerosis. *JAMA Neurol*. (2021) 78:414–25. doi: 10.1001/jamaneurol.2020.4920

44. Hancock LM, Galioto R, Samsonov A, Busch RM, Hermann B, Matias-Guiu JA. A proposed new taxonomy of cognitive phenotypes in multiple sclerosis: the international classification of cognitive disorders in MS (IC-CoDiMS). *Mult Scler J*. (2022) 29:615–27. doi: 10.1177/13524585221127941

45. Barvas E, Mattavelli G, Zappini F, Giardina F, Ottaviani D, Papagno C. Cognitive phenotypes in Parkinson's disease: a latent profile analysis. *Neuropsychology*. (2021) 35:451–9. doi: 10.1037/neu0000737

46. Charman T, Jones CR, Pickles A, Simonoff E, Baird G, Happé F. Defining the cognitive phenotype of autism. *Brain Res*. (2011) 1380:10–21. doi: 10.1016/j.brainres.2010.10.075

47. Matias-Guiu JA, Herrera E, González-Nosti M, Krishnan K, Delgado-Alonso C, Díez-Cirarda M, et al. Development of criteria for cognitive dysfunction in post-COVID syndrome: the IC-CoDi-COVID approach. *Psychiatry Res*. (2023) 319:115006. doi: 10.1016/j.psychres.2022.115006

48. Prabhakaran D, Day GS, Munipalli B, Rush BK, Pudalov L, Niazi SK, et al. Neurophenotypes of COVID-19: risk factors and recovery outcomes. *Brain Behav Immun Health*. (2023) 30:100648. doi: 10.1016/j.bbih.2023.100648

49. Reyes A, Kaestner E, Bahrami N, Balachandra A, Hegde M, Paul BM, et al. Cognitive phenotypes in temporal lobe epilepsy are associated with distinct patterns of white matter network abnormalities. *Neurology*. (2019) 92:e1957–68. doi: 10.1212/WNL.0000000000007370

50. Reyes A, Kaestner E, Ferguson L, Jones JE, Seidenberg M, Barr WB, et al. Cognitive phenotypes in temporal lobe epilepsy utilizing data-and clinically driven approaches: moving toward a new taxonomy. *Epilepsia*. (2020) 61:1211–20. doi: 10.1111/epi.16528

51. Hermann B, Conant LL, Cook CJ, Hwang G, Garcia-Ramos C, Dabbs K, et al. Network, clinical and sociodemographic features of cognitive phenotypes in temporal lobe epilepsy. *Neuroimage Clin*. (2020) 27:102341. doi: 10.1016/j.nicl.2020.102341

52. Arrotta K, Reyes A, Kaestner E, McDonald CR, Hermann BP, Barr WB, et al. Cognitive phenotypes in frontal lobe epilepsy. *Epilepsia*. (2022) 63:1671–81. doi: 10.1111/epi.17260

53. McDonald CR, Busch RM, Reyes A, Arrotta K, Barr W, Block C, et al. Development and application of the international classification of cognitive disorders in epilepsy (IC-CoDE): initial results from a multi-center study of adults with temporal lobe epilepsy. *Neuropsychology*. (2022) 37:301–14. doi: 10.1037/neu0000792

54. Reyes A, Hermann BP, Busch RM, Drane DL, Barr WB, Hamberger MJ, et al. Moving towards a taxonomy of cognitive impairments in epilepsy: application of latent profile analysis to 1178 patients with temporal lobe epilepsy. *Brain Commun*. (2022) 4:fcac289. doi: 10.1093/braincomms/fcac289

55. Reyes A, Salinas L, Hermann BP, Baxendale S, Busch RM, Barr WB, et al. Establishing the cross-cultural applicability of a harmonized approach to cognitive diagnostics in epilepsy: initial results of the international classification of cognitive disorders in epilepsy in a Spanish-speaking sample. *Epilepsia*. (2023) 64:728–41. doi: 10.1111/epi.17501

56. Hermann B, Seidenberg M, Lee E-J, Chan F, Rutecki P. Cognitive phenotypes in temporal lobe epilepsy. *J Int Neuropsychol Soc*. (2007) 13:12–20. doi: 10.1017/S135561770707004X

57. Garcia-Ramos C, Struck AF, Cook C, Prabhakaran V, Nair V, Maganti R, et al. Network topology of the cognitive phenotypes of temporal lobe epilepsy. *Cortex*. (2021) 141:55–65. doi: 10.1016/j.cortex.2021.03.031

58. Baxendale S, Thompson P. The association of cognitive phenotypes with postoperative outcomes after epilepsy surgery in patients with temporal lobe epilepsy. *Epilepsy Behav*. (2020) 112:107386. doi: 10.1016/j.yebeh.2020.107386

59. Wright JD, Folsom AR, Coresh J, Sharrett AR, Couper D, Wagenknecht LE, et al. The ARIC (atherosclerosis risk in communities) study: JACC focus seminar 3/8. *J Am Coll Cardiol*. (2021) 77:2939–59. doi: 10.1016/j.jacc.2021.04.035

60. Reid AY, St Germaine-Smith C, Liu M, Sadiq S, Quan H, Wiebe S, et al. Development and validation of a case definition for epilepsy for use with administrative health data. *Epilepsy Res*. (2012) 102:173–9. doi: 10.1016/j.eplepsyres.2012.05.009

61. Johnson EL, Krauss GL, Lee AK, Schneider ALC, Kucharska-Newton AM, Huang J, et al. Association between white matter hyperintensities, cortical volumes, and late-onset epilepsy. *Neurology*. (2019) 92:e988–95. doi: 10.1212/WNL.0000000000007010

62. Johnson EL, Krauss GL, Kucharska-Newton A, Lam AD, Sarkis R, Gottesman RF. Mortality in patients with late-onset epilepsy: results from the atherosclerosis risk in communities study. *Neurology*. (2021) 97:e1132–40. doi: 10.1212/WNL.0000000000012483

63. Koton S, Schneider AL, Rosamond WD, Shahar E, Sang Y, Gottesman RF, et al. Stroke incidence and mortality trends in US communities, 1987 to 2011. *JAMA*. (2014) 312:259–68. doi: 10.1001/jama.2014.7692

64. Rawlings AM, Bandeen-Roche K, Gross AL, Gottesman RF, Coker LH, Penman AD, et al. Factor structure of the ARIC-NCS neuropsychological battery: an evaluation of invariance across vascular factors and demographic characteristics. *Psychol Assess*. (2016) 28:1674–83. doi: 10.1037/pas0000293

65. Wechsler D. *WMS-R: Wechsler memory scale--revised: manual*. San Antonio, TX: Psychological Corp.: Harcourt brace Jovanovich (1987) viii, 150pp.

66. Schneider AL, Sharrett AR, Gottesman RF, Coresh J, Coker L, Wruck L, et al. Normative data for eight neuropsychological tests in older blacks and whites from the atherosclerosis risk in communities (ARIC) study. *Alzheimer Dis Assoc Disord*. (2015) 29:32–44. doi: 10.1097/WAD.0000000000000042

67. Knopman DS, Ryberg S. A verbal memory test with high predictive accuracy for dementia of the Alzheimer type. *Arch Neurol*. (1989) 46:141–5. doi: 10.1001/archneur.1989.00520380041011

68. Kaplan E, Goodglass H., & Weintraub S. Boston naming test. Pro-ed.; (2001).

69. Wechsler D. *WAIS-R: Wechsler adult intelligence scale-revised*. New York, NY: Psychological Corporation (1981).

70. Knopman DS, Gottesman RF, Sharrett AR, Wruck LM, Windham BG, Coker L, et al. Mild cognitive impairment and dementia prevalence: the atherosclerosis risk in communities neurocognitive study (ARIC-NCS). *Alzheimers Dement (Amst)*. (2016) 2:1–11. doi: 10.1016/j.dadm.2015.12.002

71. Jak AJ, Bondi MW, Delano-Wood L, Wierenga C, Corey-Bloom J, Salmon DP, et al. Quantification of five neuropsychological approaches to defining mild cognitive impairment. *Am J Geriatr Psychiatry*. (2009) 17:368–75. doi: 10.1097/JGP.0b013e31819431d5

72. Äikiä M, Salmenperä T, Partanen K, Kälviäinen R. Verbal memory in newly diagnosed patients and patients with chronic left temporal lobe epilepsy. *Epilepsy Behav*. (2001) 2:20–7. doi: 10.1006/ebeh.2000.0140

73. Pulliainen V, Kuikka P, Jokelainen M. Motor and cognitive functions in newly diagnosed adult seizure patients before antiepileptic medication. *Acta Neurol Scand*. (2000) 101:73–8. doi: 10.1034/j.1600-0404.2000.101002073.x

74. Witt J-A, Helmstaedter C. Should cognition be screened in new-onset epilepsies? A study in 247 untreated patients. *J Neurol*. (2012) 259:1727–31. doi: 10.1007/s00415-012-6526-2

75. Taylor J, Kolamunnage-Dona R, Marson AG, Smith PE, Aldenkamp AP, Baker GA, et al. Patients with epilepsy: cognitively compromised before the start of antiepileptic drug treatment? *Epilepsia*. (2010) 51:48–56. doi: 10.1111/j.1528-1167.2009.02195.x

76. Edmonds EC, McDonald CR, Marshall A, Thomas KR, Eppig J, Weigand AJ, et al. Early versus late MCI: improved MCI staging using a neuropsychological approach. *Alzheimers Dement*. (2019) 15:699–708. doi: 10.1016/j.jalz.2018.12.009

77. Hermann BP, Sager MA, Koscik RL, Young K, Nakamura K. Vascular, inflammatory, and metabolic factors associated with cognition in aging persons with chronic epilepsy. *Epilepsia*. (2017) 58:e152–6. doi: 10.1111/epi.13891

78. Reyes A, Lalani SJ, Kaestner E, Hooper K, Chen A, Macari AC, et al. The impact of cerebrovascular risk factors on postoperative memory decline in patients with left temporal lobe epilepsy. *Epilepsy Behav*. (2020) 102:106558. doi: 10.1016/j.yebeh.2019.106558

79. Kloppenborg RP, van den Berg E, Kappelle LJ, Biessels GJ. Diabetes and other vascular risk factors for dementia: which factor matters most? A systematic review. *Eur J Pharmacol*. (2008) 585:97–108. doi: 10.1016/j.ejphar.2008.02.049

80. Debette S, Seshadri S, Beiser A, Au R, Himali J, Palumbo C, et al. Midlife vascular risk factor exposure accelerates structural brain aging and cognitive decline. *Neurology*. (2011) 77:461–8. doi: 10.1212/WNL.0b013e318227b227

81. Nishtala A, Preis SR, Beiser A, Devine S, Hankee L, Seshadri S, et al. Midlife cardiovascular risk impacts executive function: Framingham offspring study. *Alzheimer Dis Assoc Disord*. (2014) 28:16–22. doi: 10.1097/WAD.0b013e3182a715bc

82. Looi JC, Sachdev PS. Differentiation of vascular dementia from AD on neuropsychological tests. *Neurology*. (1999) 53:670. doi: 10.1212/WNL.53.4.670

83. Palmer BW, Boone KB, Lesser IM, Wohl MA. Base rates of "impaired" neuropsychological test performance among healthy older adults. *Arch Clin Neuropsychol*. (1998) 13:503–11.

84. Axelrod BN, Wall JR. Expectancy of impaired neuropsychological test scores in a non-clinical sample. *Int J Neurosci*. (2007) 117:1591–602. doi: 10.1080/00207450600941189

85. Schretlen DJ, Testa SM, Winicki JM, Pearlson GD, Gordon B. Frequency and bases of abnormal performance by healthy adults on neuropsychological testing. *J Int Neuropsychol Soc*. (2008) 14:436–45. doi: 10.1017/S1355617708080387

86. Roe CM, Xiong C, Miller JP, Morris JC. Education and Alzheimer disease without dementia: support for the cognitive reserve hypothesis. *Neurology*. (2007) 68:223–8. doi: 10.1212/01.wnl.0000251303.50459.8a

87. Whalley LJ, Deary IJ, Appleton CL, Starr JM. Cognitive reserve and the neurobiology of cognitive aging. *Ageing Res Rev*. (2004) 3:369–82. doi: 10.1016/j.arr.2004.05.001

88. Stern Y, Albert S, Tang M-X, Tsai W-Y. Rate of memory decline in AD is related to education and occupation: cognitive reserve? *Neurology*. (1999) 53:1942. doi: 10.1212/WNL.53.9.1942

89. Jokeit H, Ebner A. Long term effects of refractory temporal lobe epilepsy on cognitive abilities: a cross sectional study. *J Neurol Neurosurg Psychiatry*. (1999) 67:44–50. doi: 10.1136/jnnp.67.1.44

90. Pardoe HR, Cole JH, Blackmon K, Thesen T, Kuzniecky R, Investigators HEP. Structural brain changes in medically refractory focal epilepsy resemble premature brain aging. *Epilepsy Res*. (2017) 133:28–32. doi: 10.1016/j.eplepsyres.2017.03.007

91. Hwang G, Hermann B, Nair VA, Conant LL, Dabbs K, Mathis J, et al. Brain aging in temporal lobe epilepsy: chronological, structural, and functional. *NeuroImage Clin*. (2020) 25:102183. doi: 10.1016/j.nicl.2020.102183

92. Galovic M, van Dooren VQ, Postma TS, Vos SB, Caciagli L, Borzì G, et al. Progressive cortical thinning in patients with focal epilepsy. *JAMA Neurol*. (2019) 76:1230–9. doi: 10.1001/jamaneurol.2019.1708

93. Breuer L, Grevers E, Boon P, Bernas A, Bergmans J, Besseling R, et al. Cognitive deterioration in adult epilepsy: clinical characteristics of "accelerated cognitive ageing". *Acta Neurol Scand*. (2017) 136:47–53. doi: 10.1111/ane.12700

94. Breuer LE, Bernas A, Boon P, Besseling RM, Carrette EC, de Louw A, et al. Accelerated cognitive ageing in epilepsy: a neuropsychological evaluation of cognitive deterioration. *Arch Clin Neuropsychol*. (2019) 34:301–9. doi: 10.1093/arclin/acy042

95. DiFrancesco JC, Labate A, Romoli M, Chipi E, Salvadori N, Galimberti CA, et al. Clinical and instrumental characterization of patients with late-onset epilepsy. *Front Neurol*. (2022) 13:851897. doi: 10.3389/fneur.2022.851897

96. Salthouse TA. Selectivity of attrition in longitudinal studies of cognitive functioning. *J Gerontol B Psychol Sci Soc Sci*. (2014) 69:567–74. doi: 10.1093/geronb/gbt046

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# Interictal epileptiform discharges in Alzheimer's disease: prevalence, relevance, and controversies

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Alzheimer's disease (AD) is the most common type of dementia and remains an incurable, progressive disease with limited disease-modifying interventions available. In patients with AD, interictal epileptiform discharges (IEDs) have been identified in up to 54% of combined cohorts of mild cognitive impairment (MCI) or mild dementia and are a marker of a more aggressive disease course. Studies assessing the role of IEDs in AD are limited by the lack of standardization in the definition of IEDs or the different neurophysiologic techniques used to capture them. IEDs are an appealing treatment target given the availability of EEG and anti-seizure medications. There remains uncertainty regarding when to treat IEDs, the optimal drug and dose for treatment, and the impact of treatment on disease course. This review covers the state of knowledge of the field of IEDs in AD, and the steps needed to move the field forward.

KEYWORDS

**Alzheimer's disease, dementia, electroencephalography, interictal discharges, epileptogenesis**

## 1. Introduction

Alzheimer's disease (AD) is the most common type of dementia with devastating effects on cognition in the setting of disrupted synaptic homeostasis, neuronal loss, and impaired neuronal network integrity (1, 2). Clinical studies have suggested a link between AD and epilepsy, given the higher rates of clinical and subclinical seizures in patients with AD (3) and a more aggressive phenotype (early onset and rapid progression) when both disorders are present, or when there is evidence of interictal epileptiform discharges (IEDs) on EEG even in the absence of clinical seizures (4). Seizures can also be one of the first presenting symptoms of AD (4, 5), and an "epileptic variant" of AD is gaining more recognition (6). The accumulation of amyloid-β (a pathogenic hallmark of AD) leads to inhibitory interneuron dysfunction creating a state of network hypersynchrony manifesting as IEDs, clinical and subclinical seizures (7). This raises the appealing prospect of trying to modify the disease course by addressing hypersynchrony with antiseizure medications (ASM).

Electroencephalography (EEG) has proven to be the most accessible and cost-efficient tool to identify epileptiform abnormalities in patients with mild cognitive impairment (MCI) or Alzheimer's dementia (4, 5). Albeit there may be substantial variability in the interpretation and reporting of the data (8). There is a need for clinicians to understand the EEG findings in patients with AD, its role in the pathogenesis and progression of the disease, and when and whether certain findings should be treated. This review will highlight published data regarding IEDs in AD, and discuss study limitations, and controversies regarding treatment.

## 2. Illustrative cases

We present 4 cases with different EEG findings in the setting of non-lesional MRIs and highlight the range of abnormalities that a clinician could face, and the challenge with regards to deciding when to initiate treatment.

Case 1 is a 75-year-old male with a history of mild cognitive impairment and no history of spells concerning for seizures. He had a routine EEG revealing "sharp transients in sleep." An ambulatory EEG showed an isolated left anterior temporal sharp wave in N2 sleep (Figure 1A). Case 2 is a 60-year-old female with a strong family history of AD who presented to the clinic with short-term memory complaints. An ambulatory EEG was obtained revealing occasional periodic left temporal sharp waves in N2 sleep (Figure 1B). Case 3 is an 85-year-old female with a history of mild AD dementia and fluctuating mentation. An ambulatory EEG showed runs of left temporal rhythmic delta activity lasting up to 10 s limited to wakefulness (Figure 1C). Case 4 is a 70-year-old male with a history of mild cognitive impairment and an isolated generalized tonic–clonic seizure; he was maintained on levetiracetam monotherapy. A follow-up ambulatory EEG showed bitemporal independent frequent spike and slow wave discharges in sleep occurring at a frequency of 1/min (Figure 1D).

## 3. EEG findings in AD

Earlier studies in patients with AD suggested that slowing of the occipital peak frequency correlated with the progression of the disease (9). Unfortunately, larger cohort studies failed to confirm this finding (10). Focal slowing on ambulatory EEG is a common finding in older adults in general with a prevalence of up to 63% on ambulatory EEG (11). Focal slowing on a routine EEG is relevant because it is one of the predictors of finding an IED on an ambulatory EEG (11). In the AD literature, the rates of focal slowing range between 44–47% in MCI and mild AD (12), while generalized slowing of the background is seen in 13–30% (3, 12).

More recent studies have highlighted interictal epileptiform discharges (IEDs) as a more relevant electrographic finding in patients with AD or MCI (Table 1); as also previously reviewed by Csernus and colleagues (20). Most of the study findings are limited by the lack of standardization in the definition of IEDs or the variability in the type of study used to capture them (routine vs. long-term vs. ambulatory EEG). An interictal epileptiform abnormality should be defined by at least 4 out of 6 criteria recommended by the international federation for clinical neurophysiology to avoid misinterpretation of normal variants on EEG (19). Common normal variants in older adults that can

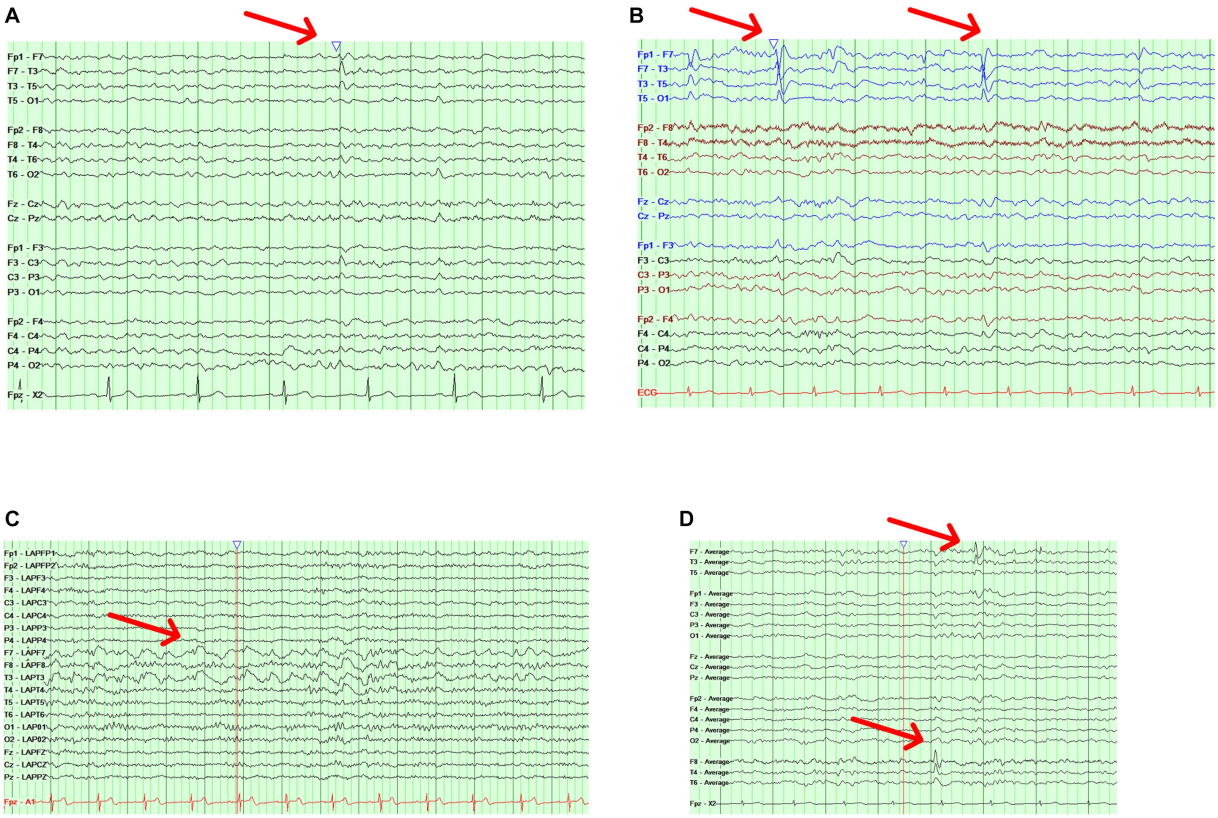


FIGURE 1
Illustrative cases. **(A)** Case 1 with an ambulatory EEG showing a bipolar longitudinal montage with a left temporal sharp wave (red arrow) during stage N2 sleep. **(B)** Case 2 with an ambulatory EEG showing a bipolar longitudinal montage with occasional periodic left temporal sharp waves (red arrow) during stage N2 sleep. **(C)** Case 3 with an ambulatory EEG showing a Laplacian montage with runs of left temporal rhythmic delta activity (red arrow) lasting up to 10 s. **(D)** Case 4 with an ambulatory EEG showing an average referential montage with bitemporal independent frequent spike and slow wave discharges (red arrows).

TABLE 1 Characteristics of IEDs in AD.

| Modality of EEG used/ EEG findings | Incidence; Frequency of IEDs on EEG | AD biomarkers available | Most common EEG scalp localization of IEDs | Most common EEG state for IEDs | Spike detection software used | Inter-rate agreement among EEG raters for IEDs | Number of patients studied | Reference |
|---|---|---|---|---|---|---|---|---|
| Unclear duration of EEG/ Spike or sharp wave discharges | 15 patients (38%); N/A | N/A | Uni or bitemporal | N/A | N/A | N/A | 39 patients with dementia from a registry (74% undergo an EEG) | (13) |
| REEG/ IEDs as per IFCN criteria# | 42 patients (3%); N/A | N/A | Temporal | N/A | N/A | 1 of 3 board-certified clinical neurophysiologists | 1,674 patients attending a memory clinic | (14) |
| LTM and REEG/ Sharp waves, Generalized ED, Focal and diffuse slowing | aMCI or AD evaluated for sz vs. no history of sz: 62% vs. 6%; N/A | 6/54 *2 had autopsy confirmed AD | Temporal (Left) | N/A | N/A | N/A | aMCI + epilepsy: 12 AD plus epilepsy: 35 AD plus IEDs: 7 | (4) |
| LTM and REEG/ Epileptiform abnormalities, focal and generalized slowing | 36% with epileptiform abnormalities; N/A | N/A | Frontal or temporal | N/A | N/A | N/A, retrospective study | 77 patients (88% with possible/ probable/ definite AD) | (5) |
| REEG/ epileptiform discharges (sharp waves or spikes) | 23.1% with epileptiform abnormalities; N/A | 13/13 | Temporal (Left>Right) | N/A | N/A | N/A | 13 patients with AD (MCI) and epilepsy | (6) |
| LTM/ Epileptiform activity+ | 21.2% vs. 0%; 0.03 to 5.18 per hour | 25/33 | Temporal (Left) | Sleep (Stage 2) | SpikeDensityV101 Calculation Engine in Persyst 11 EEG software | Two experienced epileptologists | 33 patients with AD; 19 HC | (3) |
| AEEG/ Epileptiform activity+ | AD vs. HC: 54% vs. 25%; 0.29–6.68 spikes/h | N/A | Temporal (left) | Sleep (Stage 3) | Micromed System PLUS98, Compumedics NeuroScan Curry | Two independent raters | 52 patients with AD 20 HC | (15) |
| Overnight EEG + PSG/ Epileptiform activity* | Probable AD vs. MCI vs. Controls: 6.38% vs. 11.63% vs. 4.54%; N/A | N/A | N/A | N/A | (RembrandtSleep-View, Medcare) | Two trained neurophysiologists | Probable AD: 47 MCI: 43 Controls: 44 | (16) |

*(Continued)*

TABLE 1 (Continued)

| Modality of EEG used/ EEG findings | Incidence; Frequency of IEDs on EEG | AD biomarkers available | Most common EEG scalp localization of IEDs | Most common EEG state for IEDs | Spike detection software used | Inter-rate agreement among EEG raters for IEDs | Number of patients studied | Reference |
|---|---|---|---|---|---|---|---|---|
| AEEG (24 h)/ Epileptiform discharges | AD-no epilepsy vs. AD-epilepsy vs. controls: 22% vs. 53.3% vs. 4.7%; 0 to 0.41/h in AD-no epilepsy 0 to 53.3/h in AD-epilepsy | 18/56 1 autopsy confirmed | Temporal (Left>Right) | Sleep (Stage 2) | Manual; Matlab for revision | Two trained epileptologists screen IEDs followed by a consensus among 9 epileptologists | AD-no epilepsy: 41 (27 MCI) AD-epilepsy: 15 (10 MCI) Controls: 43 | (12) |
| LTM-EEG (24 h)/ Epileptiform activity+ | AD vs. healthy controls: 54% vs. 25%; 0.29–6.68 per hour | N/A | Temporal (Left) | Sleep (stages 2 and 3) | Micromed System PLUS98, Compumedics NeuroScan Curry | Two independent raters | AD: 52; HC: 20 | (17) |
| Ear-EEG^ and 30-min REEG/ ED as per IFCN criteria# | AD vs. HC: 75% vs. 46.7%; mean: 3.03 spikes per 24 h | 20/24 | Set up was limited to Ear-EEG. | At night (64.8%) | N/A | Two experienced clinical neurophysiologists | AD: 25 HC: 15 | (18) |

LTM-EEG, long-term EEG; REEG, routine EEG; AEEG, ambulatory EEG; ED, epileptiform discharges; AD, Alzheimer's disease; aMCI, amnestic mild cognitive impairment; Sz, seizures; IEDs, interictal epileptiform discharges; N/A, not available for review; HC, healthy controls. +, Defined as paroxysmal sharp waveforms 20–200 ms, clearly distinct from ongoing background activity, with an associated subsequent slow wave. *, Defined as paroxysmal EEG sharp grapho elements that disrupted background activity lasting from 20 to 200 ms, with an abrupt change in polarity. #, as defined by the IFCN (19); notably, Ear-EEG cannot identify slow waves as reported by the authors (18). ^ Ear-EEG, defined as electrodes placed inside the ears.

be easily misinterpreted as pathologic include small sharp spikes, wicket rhythms, and wicket spikes (8). Yet, even when these criteria are applied, there may be substantial interrater variability; the inter-rater reliability regarding IEDs is fair at best even among experts (21). This is problematic because AD patients without a history of seizures tend to have only a limited number of discharges on an ambulatory EEG (12) and can be easily misclassified as having IEDs, as shown in the illustrative cases (Figure 1) where the decision to label an EEG as epileptiform or not rests on an isolated discharge.

Notably, only one study has used expert consensus to evaluate the frequency of IEDs in patients with AD (12), while others screened with spike detection software followed by a visual review (Table 1). Recent studies have suggested a higher accuracy in the identification of IEDs for an ambulatory EEG when compared to one or two routine EEGs (22). This could be relevant in the AD population since most IEDs are present in stage 2 sleep (Table 1). The sensitivity of an EEG is correlated with the length of the recording, which explains why a 20–30 min routine EEG may miss IEDs (23), and why there was a delay in appreciating the true burden of IEDs in AD. Other markers of hyperexcitability such as focal rhythmic slowing (24) have only been studied in one cohort (12). A *benign* variant, small sharp spikes (SSS), was seen in a subset of patients with AD; some with a high frequency and unilateral predominance (12), suggesting that these features may also indicate underlying irritability given that they represent outliers and also tended to co-occur in EEGs with IEDs. Most of the studies also reported the temporal lobe as the most frequent region for IEDs (Table 1). The temporal-lobe predominance of IEDs could be due to early seeding by amyloid plaques and hyperphosphorylated tau in the limbic system (25).

It must be kept in mind that surface EEG as a neurophysiologic tool has several limitations including its limited ability to detect deep IEDs such as those located in the hippocampus, or IEDs with a tangential dipole (26). This limitation was highlighted in a study in 2016, where 21% out of 42% of the subjects had MEG-only IEDs with no IEDs noted on EEG (3). Similarly, in a case series of 2 subjects with early onset AD with surface EEG and invasive foramen ovale electrodes, 90–100% of the IEDs noted on the invasive electrodes did not have a surface EEG correlate (27).

As illustrated in Table 1 the subjects examined per study with prolonged EEGs have been limited to date with cohorts often including both MCI and mild dementia patients lumped together. We need more studies to explore whether IEDs vary in prevalence depending on disease stage.

## 4. IEDs and cognition in the epilepsy literature

The association of IEDs on cognition and whether they should be a treatment target has been a matter of debate among epileptologists (28). Transient cognitive impairment secondary to IEDs gained recognition with the advent of computerized testing paradigms. Earlier studies showed that around 50% of subjects with epilepsy exhibited transient impairment coinciding with the

occurrence of an IED, and there was a laterality effect with left-sided discharges affecting verbal tasks while right-sided IEDs affecting visual ones (29, 30). The dysfunction was specifically attributed to the after-going slow wave following the discharge (31). IEDs can also affect cognition when occurring in sleep by affecting sleep-dependent memory consolidation. Sleep is essential in transitioning memories from being hippocampal-dependent into more consolidated memories in widespread cortical networks (32). This process is dependent on NREM sleep with slow oscillations and sleep spindles playing a pivotal role (32). In older adults with epilepsy, the frequency of scalp-detected IEDs in NREM sleep was found to negatively correlate with 24 h recall on a visual memory task (33).

Moving on from surface EEG-based studies, a similar theme also emerges with invasive EEG studies. Hippocampal IEDs detected on depths electrodes were associated with impaired maintenance and retrieval but not encoding on a short-term memory task (34), while the frequency of IEDs detected during sleep was associated with impaired one-week long-term recall (35). IEDs even outside of the epileptogenic zone have also been associated with impaired cognition (36).

Invasive EEG studies have also shed light on how IEDs can disrupt cognitive processes; one mechanism is through a transient decrease in global functional connectivity (37), while another is through the impairment of spindle generation (38) and the induction of pathologic hippocampal-cortical coupling (39). IEDs may also alter the firing of hippocampal neurons leading to a state of transient cognitive impairment (40, 41).

Other markers of epileptogenicity that can be detected using scalp EEG, and have been described in epilepsy patients, include high frequency oscillations (HFOs) (42). They are currently divided into physiologic and pathologic HFOs. Physiologic HFOs have been shown to play a central role in information retrieval and sleep dependent memory consolidation (43, 44). On the other hand, one of the features of pathologic HFOs is that they tend to coincide with IEDs and occur during the earliest stages of non-REM sleep (45). HFOs pose methodological challenges in their recording and detection (46), thus limiting their widespread clinical use in patients with AD; especially since it is difficult to disentangle pathologic from physiologic HFOs.

## 5. IEDs and cognition in the AD literature

Cross-sectional studies of IEDs in AD show a trend for lower mini-mental status exam (MMSE) scores in those with IEDs (14), although this finding was not seen in a study using prolonged ear-EEG recordings (18). Longitudinal studies of AD patients with IEDs have been limited. In a study of 33 patients with AD, those with IEDs had an accelerated decline in their MMSE score and their executive function composite Z-score (a combination of design fluency, information processing speed, and cognitive control from the Stroop test, digit span backward, modified trails and the California verbal learning test) (3). Of note, not all participants had data on the individual tests, and there was no evidence of a decline in the episodic memory, language, or visuospatial function domains (3). The cohort studied predominantly consisted of patients with early-onset AD and 33% with atypical presentations.

In another study, 28 out of 52 AD patients were noted to have IEDs (17). The authors used the cognitive assessments consisting of a Hungarian version of the Addenbrooke Cognitive Examination (ACE); scored from 0–100 and allowing the extraction of MMSE scores, and analysis of the following cognitive subdomains: orientation, attention, memory, verbal fluency, language, and visuospatial ability (17). When compared to AD patients without IEDs, those with IEDs exhibited a faster decline in ACE scores over 3 years (12.15 points per year vs. 8.17 points per year) and on the MMSE (2.71 points per year vs. 2.22 points per year). The study also found a correlation between IED frequency and the rate of decline in the ACE. In comparison, studies evaluating AD patients with comorbid epilepsy treated with anti-seizure medications (ASMs) did not show a change in MMSE scores over at least a 3-year follow-up (47).

## 6. To treat or not to treat: management of IEDs in AD

Although there is mounting evidence regarding the association between IEDs and impaired cognition and accelerated disease course, there are currently no guidelines to screen for IEDs in AD or to treat IEDs. The goal of the treatment is not seizure prevention because there are no currently anti-epileptogenic medications available. Instead, the aim would be to prevent the possible impact of the IEDs on cognition and memory consolidation. In addition, there is also evidence of neuronal hyperactivity (IEDs being one manifestation of this) causing accelerated neurodegeneration by promoting AD pathology (48). The medication that has garnered the most interest has been levetiracetam. Animal AD mouse models exposed to levetiracetam show IED suppression and improvement in cognition (49). In one of the only randomized trials of the treatment of seizures in AD, levetiracetam (dose range 500-2000 mg) was better tolerated when compared to phenobarbital (dose range 50-100 mg) or lamotrigine (dose range 25-100 mg) and was correlated with improved MMSE scores after 1 year (50). Studies evaluating the IED suppression properties of ASMs in epilepsy also show evidence for lamotrigine and topiramate (51). The downside of treatment is that ASMs in general, as a drug class, are commonly associated with cognitive and fatigue side effects (52). While levetiracetam is associated with prominent neuropsychiatric side effects (53), lamotrigine and other sodium channel blockers are risk factors for falls (54). In addition, benzodiazepines are known to increase the risk of cognitive decline and dementia in the elderly (55).

A recent study trying to tackle the balance between IED suppression and adverse effects of ASMs showed that in children undergoing invasive EEG, reaction time improved with IED suppression (with oxcarbazepine) and worsened with increased IED frequency (56). In this study, levetiracetam did not show a clear benefit (56). In a retrospective analysis of older Japanese patients with IEDs on EEG, treatment with various ASMs improved serial 7 scores and MMSE scores in those with IED suppression (57). The first randomized trial for levetiracetam in AD was published in 2021 (58), and several other trials also exploring levetiracetam are

pending. In the trial, 34 patients with AD were treated with levetiracetam at a low dose of 125 mg twice a day vs. placebo and then underwent a washout period and cross-over. Based on overnight EEG and then a 1 h MEG, 13 participants were found to have IEDs. The cognitive battery consisted of the National Institutes of Health Executive Abilities: Measures and Instruments for Neurobehavioral Evaluation and Research (NIH-EXAMINER) which consists of a test measuring executive functions, Stroop color and word test, the Alzheimer's Disease Assessment Scale—Cognitive Subscale (ADAS-Cog), and a virtual route learning test. There was no improvement on the primary endpoints with the medication, however, a subset analysis of those with IEDs showed that they improved on the virtual route learning test and a subscale of the Stroop test. Notably, there was no evidence of IED suppression with the medications (58).

## 7. How to deal with IEDs in AD patients in the clinical practice

Ultimately, the clinician caring for patients with AD is faced with decisions regarding when to order an EEG, how to interpret the data, or when to start an ASM. The other challenge is that diagnosis of epilepsy in an elderly population is challenging, requiring a detailed description of suspected events, consideration of atypical events as seizures (i.e., unexplained falls or brief episodes of confusion), and the need for an expert evaluation (59) (Figure 2). Until we have more evidence from randomized trials that levetiracetam will help AD patients, and more so those with IEDs, routine screening of AD patients with EEG is not recommended. However, one should have a low threshold to screen patients with suspected co-morbid seizures, including those with early onset AD because they are at the highest risk. If an EEG is ordered, it should at least have N2 sleep captured, and that is why 24 h EEGs are preferred over routine EEGs. Interictal discharges as exemplified by the illustrative cases lie along a spectrum, with seizures (clinical and subclinical) occurring at the end of that spectrum and representing the extreme manifestation of network hyperexcitability. Features such as a high IED frequency, periodicity, duration, and perhaps morphological features (spikiness, amplitude) should be considered more concerning and should tip the scale toward treatment (cases 2,3,4). In the absence of more data, isolated and equivocal discharges should not be treated (case 1). When a decision is made to treat, the lowest therapeutic dose should be used to ensure tolerability.

## 8. Conclusion

Network hyperexcitability is a feature of AD, and IEDs are a marker of this phenomenon. They are highly prevalent in AD, are often detected in sleep, and have been linked with deleterious effects on cognition and an accelerated disease course. Limited studies to date

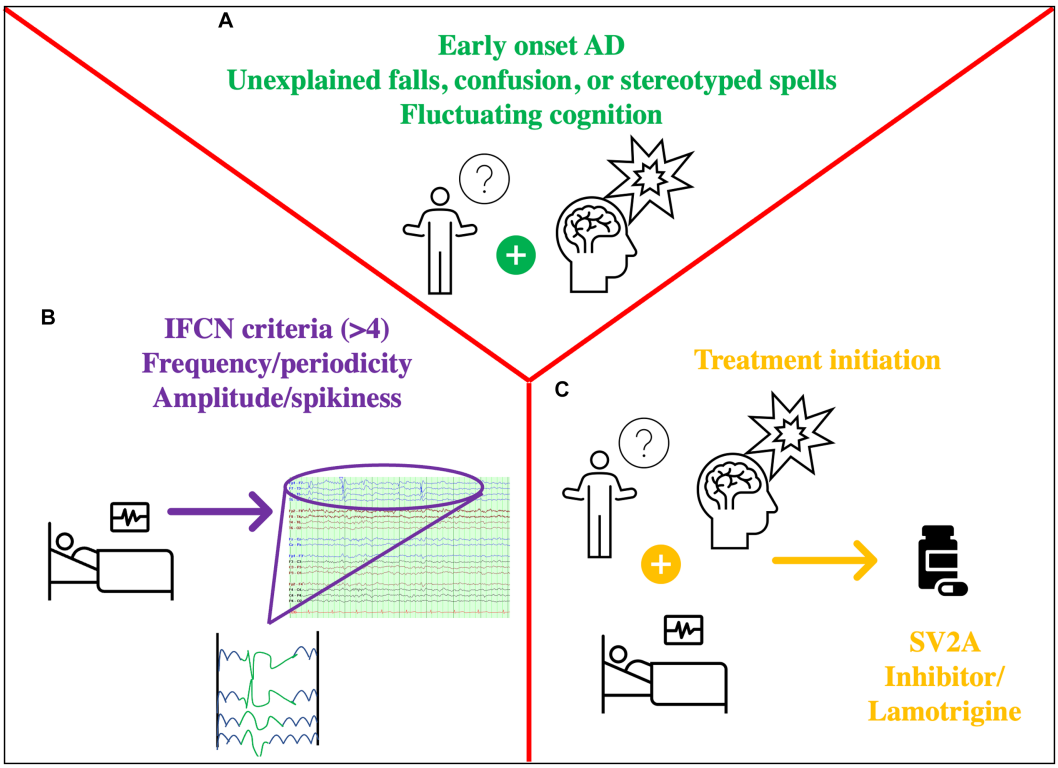


FIGURE 2
Approach to Hyperexcitability in IED. **(A)** History prompting the need for EEG: fluctuating cognition, stereotyped symptoms, distinct confusional spells, (possibly) early-onset AD **(B)** Concerning EEG features: markers of hyperexcitability such as IEDs with >4 out of 6 criteria of the IFCN, unilateral small sharp spikes, temporal rhythmic delta activity. Assess frequency, periodicity, (possibly) amplitude/spikiness. **(C)** Decision to treat based on A + B: consider an SV2A inhibitor such as levetiracetam/(possibly) brivaracetam or lamotrigine.

show some benefit with treatment, however further evidence is needed to determine whether this should become the standard of care.

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## References

1. Querfurth HW, Laferla FM. Alzheimer's disease. *N Engl J Med*. (2010) 362:329–44. doi: 10.1056/NEJMra0909142

2. Knopman DS, Amieva H, Petersen RC, Chételat G, Holtzman DM, Hyman BT, et al. Alzheimer disease. *Nat Rev Dis Primers*. (2021) 7:33. doi: 10.1038/s41572-021-00269-y

3. Vossel KA, Ranasinghe KG, Beagle AJ, Mizuiri D, Honma SM, Dowling AF, et al. Incidence and impact of subclinical epileptiform activity in Alzheimer's disease. *Ann Neurol*. (2016) 80:858–70. doi: 10.1002/ana.24794

4. Vossel KA, Beagle AJ, Rabinovici GD, Shu H, Lee SE, Naasan G, et al. Seizures and epileptiform activity in the early stages of Alzheimer disease. *JAMA Neurol*. (2013) 70:1158–66. doi: 10.1001/jamaneurol.2013.136

5. Sarkis RA, Dickerson BC, Cole AJ, Chemali ZN. Clinical and neurophysiologic characteristics of unprovoked seizures in patients diagnosed with dementia. *J Neuropsychiatry Clin Neurosci*. (2016) 28:56–61. doi: 10.1176/appi.neuropsych.15060143

6. Cretin B, Sellal F, Philippi N, Bousiges O, Di Bitonto L, Martin-Hunyadi C, et al. Epileptic prodromal Alzheimer's disease, a retrospective study of 13 new cases: expanding the Spectrum of Alzheimer's disease to an epileptic variant? *J Alzheimers Dis*. (2016) 52:1125–33. doi: 10.3233/JAD-150096

7. Palop JJ, Mucke L. Amyloid-beta-induced neuronal dysfunction in Alzheimer's disease: from synapses toward neural networks. *Nat Neurosci*. (2010) 13:812–8. doi: 10.1038/nn.2583

8. Amin U, Nascimento F, Karakis I, Schomer D, Benbadis S. Normal variants and artifacts: importance in EEG interpretation. *Epileptic Disord*. (2023). doi: 10.1002/epd2.20040

9. Penttilä M, Partanen JV, Soininen H, Riekkinen PJ. Quantitative analysis of occipital EEG in different stages of Alzheimer's disease. *Electroencephalogr Clin Neurophysiol*. (1985) 60:1–6. doi: 10.1016/0013-4694(85)90942-3

10. Rae-Grant A, Blume W, Lau C, Hachinski VC, Fisman M, Merskey H. The electroencephalogram in Alzheimer-type dementia. A sequential study correlating the electroencephalogram with psychometric and quantitative pathologic data. *Arch Neurol*. (1987) 44:50–4. doi: 10.1001/archneur.1987.00520130042015

11. Tolchin B, Lee JW, Pavlova M, Dworetzky BA, Sarkis RA. Diagnostic yield of ambulatory EEGs in the elderly. *Clin Neurophysiol*. (2017) 128:1350–3. doi: 10.1016/j.clinph.2017.01.005

12. Lam AD, Sarkis RA, Pellerin KR, Jing J, Dworetzky BA, Hoch DB, et al. Association of epileptiform abnormalities and seizures in Alzheimer disease. *Neurology*. (2020) 95:e2259–70. doi: 10.1212/WNL.0000000000010612

13. Rao SC, Dove G, Cascino GD, Petersen RC. Recurrent seizures in patients with dementia: frequency, seizure types, and treatment outcome. *Epilepsy Behav*. (2009) 14:118–20. doi: 10.1016/j.yebeh.2008.08.012

14. Liedorp M, Stam CJ, Van Der Flier WM, Pijnenburg YA, Scheltens P. Prevalence and clinical significance of epileptiform EEG discharges in a large memory clinic cohort. *Dement Geriatr Cogn Disord*. (2010) 29:432–7. doi: 10.1159/000278620

15. Horváth A, Szűcs A, Hidasi Z, Csukly G, Barcs G, Kamondi A. Prevalence, semiology, and risk factors of epilepsy in Alzheimer's disease: an ambulatory EEG study. *J Alzheimers Dis*. (2018) 63:1045–54. doi: 10.3233/JAD-170925

16. Brunetti V, D'atri A, Della Marca G, Vollono C, Marra C, Vita MG, et al. Subclinical epileptiform activity during sleep in Alzheimer's disease and mild cognitive impairment. *Clin Neurophysiol*. (2020) 131:1011–8. doi: 10.1016/j.clinph.2020.02.015

17. Horvath AA, Papp A, Zsuffa J, Szucs A, Luckl J, Radai F, et al. Subclinical epileptiform activity accelerates the progression of Alzheimer's disease: a long-term EEG study. *Clin Neurophysiol*. (2021) 132:1982–9. doi: 10.1016/j.clinph.2021.03.050

18. Musaeus CS, Frederiksen KS, Andersen BB, Høgh P, Kidmose P, Fabricius M, et al. Detection of subclinical epileptiform discharges in Alzheimer's disease using long-term outpatient EEG monitoring. *Neurobiol Dis*. (2023) 183:106149. doi: 10.1016/j.nbd.2023.106149

19. Kural MA, Duez L, Sejer Hansen V, Larsson PG, Rampp S, Schulz R, et al. Criteria for defining interictal epileptiform discharges in EEG: a clinical validation study. *Neurology*. (2020) 94:e2139–47. doi: 10.1212/WNL.0000000000009439

20. Csernus EA, Werber T, Kamondi A, Horvath AA. The significance of subclinical epileptiform activity in Alzheimer's disease: a review. *Front Neurol*. (2022) 13:856500. doi: 10.3389/fneur.2022.856500

21. Jing J, Herlopian A, Karakis I, Ng M, Halford JJ, Lam A, et al. Interrater reliability of experts in identifying Interictal epileptiform discharges in electroencephalograms. *JAMA Neurol*. (2020) 77:49–57. doi: 10.1001/jamaneurol.2019.3531

22. Hernandez-Ronquillo L, Thorpe L, Feng C, Hunter G, Dash D, Hussein T, et al. Diagnostic accuracy of ambulatory EEG vs routine EEG in patients with first single unprovoked seizure. *Neurol Clin Pract*. (2023) 13:e200160. doi: 10.1212/CPJ.0000000000200160

23. Horváth A, Szűcs A, Barcs G, Kamondi A. Sleep EEG detects epileptiform activity in Alzheimer's disease with high sensitivity. *J Alzheimers Dis*. (2017) 56:1175–83. doi: 10.3233/JAD-160994

24. Rodriguez Ruiz A, Vlachy J, Lee JW, Gilmore EJ, Ayer T, Haider HA, et al. Association of Periodic and Rhythmic Electroencephalographic Patterns with Seizures in critically ill patients. *JAMA Neurol*. (2017) 74:181–8. doi: 10.1001/jamaneurol.2016.4990

25. Trejo-Lopez JA, Yachnis AT, Prokop S. Neuropathology of Alzheimer's disease. *Neurotherapeutics*. (2022) 19:173–85. doi: 10.1007/s13311-021-01146-y

26. Beniczky S, Schomer DL. Electroencephalography: basic biophysical and technological aspects important for clinical applications. *Epileptic Disord*. (2020) 22:697–715. doi: 10.1684/epd.2020.1217

27. Lam AD, Deck G, Goldman A, Eskandar EN, Noebels J, Cole AJ. Silent hippocampal seizures and spikes identified by foramen ovale electrodes in Alzheimer's disease. *Nat Med*. (2017) 23:678–80. doi: 10.1038/nm.4330

28. Sánchez Fernández I, Loddenkemper T, Galanopoulou AS, Moshé SL. Should epileptiform discharges be treated? *Epilepsia*. (2015) 56:1492–504. doi: 10.1111/epi.13108

29. Aarts JH, Binnie CD, Smit AM, Wilkins AJ. Selective cognitive impairment during focal and generalized epileptiform EEG activity. *Brain*. (1984) 107:293–308. doi: 10.1093/brain/107.1.293

30. Holmes GL, Lenck-Santini PP. Role of interictal epileptiform abnormalities in cognitive impairment. *Epilepsy Behav*. (2006) 8:504–15. doi: 10.1016/j.yebeh.2005.11.014

31. Shewmon DA, Erwin RJ. Focal spike-induced cerebral dysfunction is related to the after-coming slow wave. *Ann Neurol*. (1988) 23:131–7. doi: 10.1002/ana.410230205

32. Klinzing JG, Niethard N, Born J. Mechanisms of systems memory consolidation during sleep. *Nat Neurosci*. (2019) 22:1598–610. doi: 10.1038/s41593-019-0467-3

33. Sarkis RA, Lam AD, Pavlova M, Locascio JJ, Putta S, Puri N, et al. Epilepsy and sleep characteristics are associated with diminished 24-h memory retention in older adults with epilepsy. *Epilepsia*. (2023). doi: 10.1111/epi.17707

34. Kleen JK, Scott RC, Holmes GL, Roberts DW, Rundle MM, Testorf M, et al. Hippocampal interictal epileptiform activity disrupts cognition in humans. *Neurology*. (2013) 81:18–24. doi: 10.1212/WNL.0b013e318297ee50

35. Lambert I, Tramoni-Negre E, Lagarde S, Roehri N, Giusiano B, Trebuchon-Da Fonseca A, et al. Hippocampal Interictal spikes during sleep impact long-term memory consolidation. *Ann Neurol*. (2020) 87:976–87. doi: 10.1002/ana.25744

36. Ung H, Cazares C, Nanivadekar A, Kini L, Wagenaar J, Becker D, et al. Interictal epileptiform activity outside the seizure onset zone impacts cognition. *Brain*. (2017) 140:2157–68. doi: 10.1093/brain/awx143

37. Bou Assi E, Zerouali Y, Robert M, Lesage F, Pouliot P, Nguyen DK. Large-scale desynchronization during Interictal epileptic discharges recorded with intracranial EEG. *Front Neurol*. (2020) 11:529460. doi: 10.3389/fneur.2020.529460

38. Frauscher B, Bernasconi N, Caldairou B, Von Ellenrieder N, Bernasconi A, Gotman J, et al. Interictal hippocampal spiking influences the occurrence of hippocampal sleep spindles. *Sleep*. (2015) 38:1927–33. doi: 10.5665/sleep.5242

39. Gelinas JN, Khodagholy D, Thesen T, Devinsky O, Buzsáki G. Interictal epileptiform discharges induce hippocampal-cortical coupling in temporal lobe epilepsy. *Nat Med*. (2016) 22:641–8. doi: 10.1038/nm.4084

40. Reed CM, Mosher CP, Chandravadia N, Chung JM, Mamelak AN, Rutishauser U. Extent of single-neuron activity modulation by hippocampal Interictal discharges predicts declarative memory disruption in humans. *J Neurosci*. (2020) 40:682–93. doi: 10.1523/JNEUROSCI.1380-19.2019

41. Landi S, Petrucco L, Sicca F, Ratto GM. Transient Cognitive Impairment in Epilepsy. *Front Mol Neurosci*. (2018) 11:458.

42. Noorlag L, Van Klink NEC, Kobayashi K, Gotman J, Braun KPJ, Zijlmans M. High-frequency oscillations in scalp EEG: a systematic review of methodological choices and clinical findings. *Clin Neurophysiol*. (2022) 137:46–58. doi: 10.1016/j.clinph.2021.12.017

43. Norman Y, Raccah O, Liu S, Parvizi J, Malach R. Hippocampal ripples and their coordinated dialogue with the default mode network during recent and remote recollection. *Neuron*. (2021) 109:2767–2780.e5. doi: 10.1016/j.neuron.2021.06.020

44. Geva-Sagiv M, Mankin EA, Eliashiv D, Epstein S, Cherry N, Kalender G, et al. Augmenting hippocampal-prefrontal neuronal synchrony during sleep enhances memory consolidation in humans. *Nat Neurosci*. (2023) 26:1100–10. doi: 10.1038/s41593-023-01324-5

45. Von Ellenrieder N, Dubeau F, Gotman J, Frauscher B. Physiological and pathological high-frequency oscillations have distinct sleep-homeostatic properties. *Neuroimage Clin*. (2017) 14:566–73. doi: 10.1016/j.nicl.2017.02.018

46. Liu AA, Henin S, Abbaspoor S, Bragin A, Buffalo EA, Farrell JS, et al. A consensus statement on detection of hippocampal sharp wave ripples and differentiation from other fast oscillations. *Nat Commun*. (2022) 13:6000. doi: 10.1038/s41467-022-33536-x

47. Hautecloque-Raysz G, Sellal F, Bousiges O, Phillipi N, Blanc F, Cretin B. Epileptic prodromal Alzheimer's disease treated with Antiseizure medications: medium-term outcome of seizures and cognition. *J Alzheimers Dis*. (2023) 94:1057–74. doi: 10.3233/JAD-221197

48. Targa Dias Anastacio H, Matosin N, Ooi L. Neuronal hyperexcitability in Alzheimer's disease: what are the drivers behind this aberrant phenotype? *Transl Psychiatry*. (2022) 12:257. doi: 10.1038/s41398-022-02024-7

49. Sanchez PE, Zhu L, Verret L, Vossel KA, Orr AG, Cirrito JR, et al. Levetiracetam suppresses neuronal network dysfunction and reverses synaptic and cognitive deficits in an Alzheimer's disease model. *Proc Natl Acad Sci U S A*. (2012) 109:E2895–903. doi: 10.1073/pnas.1121081109

50. Cumbo E, Ligori LD. Levetiracetam, lamotrigine, and phenobarbital in patients with epileptic seizures and Alzheimer's disease. *Epilepsy Behav*. (2010) 17:461–6. doi: 10.1016/j.yebeh.2010.01.015

51. Guida M, Iudice A, Bonanni E, Giorgi FS. Effects of antiepileptic drugs on interictal epileptiform discharges in focal epilepsies: an update on current evidence. *Expert Rev Neurother*. (2015) 15:947–59. doi: 10.1586/14737175.2015.1065180

52. Sarkis RA, Goksen Y, Mu Y, Rosner B, Lee JW. Cognitive and fatigue side effects of anti-epileptic drugs: an analysis of phase III add-on trials. *J Neurol*. (2018) 265:2137–42. doi: 10.1007/s00415-018-8971-z

53. Tao K, Chen H, Chen Y, Gu Y, Wang X. Levetiracetam induces severe psychiatric symptoms in people with epilepsy. *Seizure*. (2022). doi: 10.1016/j.seizure.2022.12.002

54. Marson AG, Kadir ZA, Chadwick DW. New antiepileptic drugs: a systematic review of their efficacy and tolerability. *BMJ*. (1996) 313:1169–74. doi: 10.1136/bmj.313.7066.1169

55. He Q, Chen X, Wu T, Li L, Fei X. Risk of dementia in long-term benzodiazepine users: evidence from a Meta-analysis of observational studies. *J Clin Neurol*. (2019) 15:9–19. doi: 10.3988/jcn.2019.15.1.9

56. Warsi NM, Wong SM, Gorodetsky C, Suresh H, Arski ON, Ebden M, et al. Which is more deleterious to cognitive performance? Interictal epileptiform discharges vs anti-seizure medication. *Epilepsia*. (2023) 64:e75–81. doi: 10.1111/epi.17556

57. Shiozaki K, Kajihara S. Anti-epileptic drugs improved serial 7s scores on the Mini-mental state examination in elderly with cognitive impairment and epileptiform discharge on electroencephalography. *Psychogeriatrics*. (2019) 19:38–45. doi: 10.1111/psyg.12362

58. Vossel K, Ranasinghe KG, Beagle AJ, La A, Ah Pook K, Castro M, et al. Effect of Levetiracetam on cognition in patients with Alzheimer disease with and without epileptiform activity: a randomized clinical trial. *JAMA Neurol*. (2021) 78:1345–54. doi: 10.1001/jamaneurol.2021.3310

59. Lemus HN, Sarkis RA. Epilepsy care in nursing facilities: knowledge gaps and opportunities. *Epilepsy Behav*. (2023) 138:108997. doi: 10.1016/j.yebeh.2022.108997

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# Clinical, imaging, and biomarker evidence of amyloid- and tau-related neurodegeneration in late-onset epilepsy of unknown etiology

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Accumulating evidence suggests amyloid and tau-related neurodegeneration may play a role in development of late-onset epilepsy of unknown etiology (LOEU). In this article, we review recent evidence that epilepsy may be an initial manifestation of an amyloidopathy or tauopathy that precedes development of Alzheimer's disease (AD). Patients with LOEU demonstrate an increased risk of cognitive decline, and patients with AD have increased prevalence of preceding epilepsy. Moreover, investigations of LOEU that use CSF biomarkers and imaging techniques have identified preclinical neurodegeneration with evidence of amyloid and tau deposition. Overall, findings to date suggest a relationship between acquired, non-lesional late-onset epilepsy and amyloid and tau-related neurodegeneration, which supports that preclinical or prodromal AD is a distinct etiology of late-onset epilepsy. We propose criteria for assessing elevated risk of developing dementia in patients with late-onset epilepsy utilizing clinical features, available imaging techniques, and biomarker measurements. Further research is needed to validate these criteria and assess optimal treatment strategies for patients with probable epileptic preclinical AD and epileptic prodromal AD.

KEYWORDS

**late onset epilepsy of unknown etiology, late onset epilepsy, Alzheimer dementia, epileptic prodromal Alzheimer disease, epileptic preclinical Alzheimer disease, late-onset amyloid Beta-related epilepsy, amyloid, tau**

## 1. Introduction

Epilepsy incidence increases with age, with the highest incidence occurring in older adults at almost double the rate observed in young adults (1). A majority of older adults with acquired epilepsy have an underlying cerebrovascular, neoplastic, or other cerebral lesion known to produce seizures (2). However, 25 to 50% of these patients do not have an identifiable etiology of their epilepsy after clinical evaluation and imaging (1, 3–5). This has been named late-onset epilepsy of unknown etiology (LOEU) (6).

Recent research suggests a link between neurodegenerative processes and LOEU. Specifically, amyloid-and tau-related neurodegeneration may contribute to development of some cases of LOEU. Patients with mild cognitive impairment and Alzheimer's disease (AD) have an increased risk of epilepsy, with lifetime seizure risk of up to 20–64% in patients with AD (7). Seizures were previously thought to primarily occur late in the disease course, but it is now recognized that both clinical and subclinical seizures often occur in early stages of AD as well (8, 9). Animal models of amyloidopathy also consistently demonstrate increased frequency of seizures and epileptiform activity (10–12). Similarly, suppression of amyloid-beta precursor protein in animal models reduces epileptiform activity (13) and reduction in endogenous tau confers resistance to induced seizures (14, 15). In addition to direct pro-epileptogenic effects of amyloid and tau pathology, astrocyte-mediated neuroinflammation has been implicated in both preclinical AD and epilepsy and may be a common mechanism of pathogenesis (16, 17). Seizures promoted by neurodegeneration may in turn contribute to further aggregation of amyloid and tau, leading to further cognitive decline (18–20).

Some instances of LOEU may represent a prodrome of AD, with seizures acting as an early marker for impending cognitive decline. Accumulating evidence from epidemiological, neuroimaging, and biomarker investigations of LOEU strengthens this hypothesis. While LOEU likely does not represent a single, homogenous entity, these studies suggest that prodromal AD may produce late-onset epilepsy and can be identified using clinical and biomarker features. This raises the possibility of improving prognostication and providing a potential early window for intervention. In this review, we discuss the evidence that a subset of patients presenting with late-onset epilepsy have prodromal AD, and we propose a classification scheme for use by researchers that, following validation, can be considered for clinical use.

## 2. Evaluation of patients with late-onset epilepsy

Standard literature definitions of late-onset epilepsy of unknown etiology specifies an age of onset cutoff at age 55 or older, without prior history of seizures earlier in life, though some studies use age cutoffs ranging between 40 and 65 (21–23). The evaluation of new-onset epilepsy includes a thorough clinical assessment with detailed neuroimaging, toxic/metabolic laboratory evaluation, and an electroencephalogram (EEG) (24). Aside from an age cutoff, LOEU is otherwise only defined by absence of a clear etiology for developing epilepsy despite completing a standard, comprehensive clinical evaluation. Common causes of acquired, late-onset epilepsy that are important to exclude include ischemic or hemorrhagic cerebral cortical infarction, tumor, post-traumatic encephalomalacia, and preexisting neurodegeneration (Figure 1). Less commonly, late-onset epilepsy can be caused by prior cerebral infection or autoimmune/paraneoplastic disease, and evaluation should be tailored to include cerebrospinal fluid analysis and autoantibody testing when imaging and clinical features suggest an inflammatory process (25).

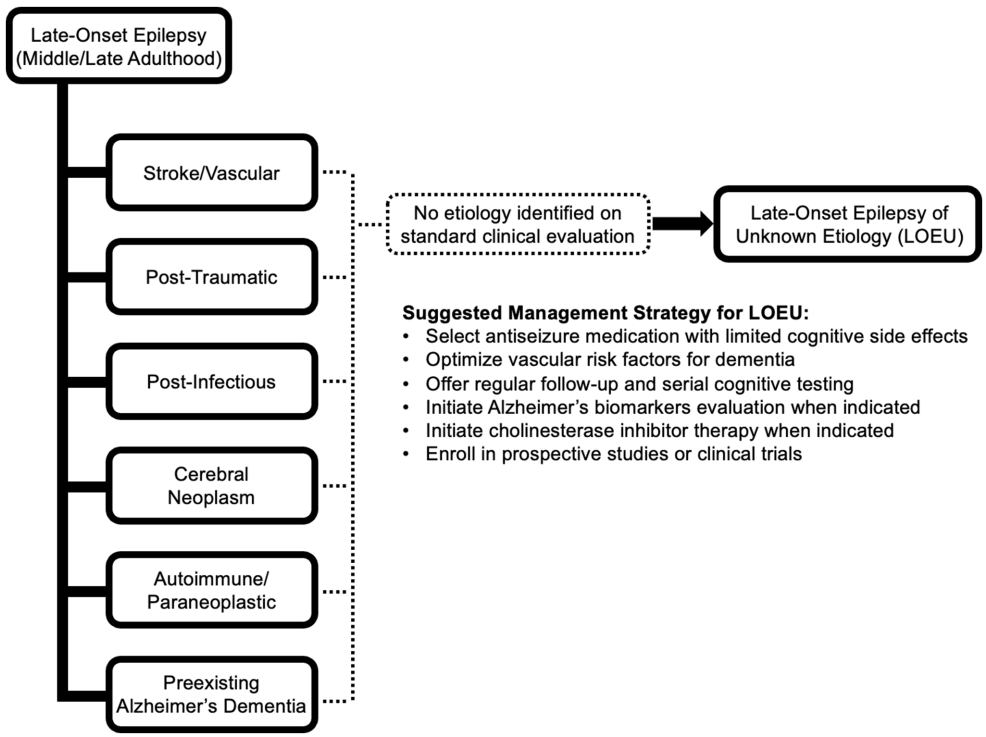


FIGURE 1
Potential etiologies of late-onset epilepsy. If no etiology can be identified after comprehensive standard clinical evaluation including EEG, epilepsy-protocol MRI, and detailed clinical assessment, the presentation is consistent with late-onset epilepsy of unknown etiology (LOEU). Management of LOEU mirrors general management of epilepsy in older adults, with greater emphasis on monitoring for development of cognitive impairment and intervening on comorbid risk factors for cognitive decline.

Given that patients may have structural lesions that are clinically silent aside from producing seizures, high-resolution neuroimaging is an essential tool for evaluating presence of possible structural etiologies. Determination of LOEU should only be made after careful expert review of an epilepsy protocol MRI, preferably obtained using a 3 T MRI machine and including sequences and slice thicknesses recommended by the ILAE Neuroimaging task force for evaluation of epilepsy (26). A detailed susceptibility weighted imaging sequence or gradient echo sequence should also be obtained to detect potentially explanatory cortical microhemorrhages. Though global or focal atrophy is commonly observed, LOEU is defined as "MRI-negative" epilepsy, signifying absence of an explanatory lesion while allowing likely incidental findings. Presence of a lesion likely to produce epilepsy essentially excludes LOEU. However, more reliable determination that a cerebral cortical lesion is unrelated would typically require detailed ictal video-EEG recordings or intracranial EEG recordings, which is not often clinically justifiable, especially when seizure control has been achieved.

Currently, it is unclear whether MRI evidence of hippocampal sclerosis reliably indicates an underlying etiology in LOEU because hippocampal sclerosis is more commonly an etiology of epilepsy with onset earlier in life. Hippocampal sclerosis is less clearly a distinct etiology in late-onset epilepsy, as hippocampal sclerosis in older adults may be produced by multiple pathologic processes, including ischemic injury, AD, and other TDP-43 related diseases. Overall, the presence of hippocampal sclerosis on imaging in LOEU likely does not shed light on a single, unifying etiology (27–29). The difficulty in clinically interpreting hippocampal sclerosis in the older adult has complicated LOEU research, with some studies of LOEU excluding patients with imaging findings of hippocampal sclerosis (30) while others including frank atrophy and sclerosis (21, 31). Further work is needed to assess temporal evolution of hippocampal atrophy and sclerosis in LOEU or if particular imaging or pathologic features of hippocampal sclerosis in LOEU can be connected with specific probable etiologies.

## 3. Common clinical findings in patients with late-onset epilepsy of unknown etiology

Features of seizures in LOEU are largely consistent and are predominantly focal in manifestation. These may be focal with impaired awareness or focal with progression to bilateral tonic–clonic seizures. Focal with intact awareness (an aura in isolation) also may occur. Usually, 15% or fewer patients with LOEU are described as having generalized seizures (32–35). While evaluated as generalized, many of these may be focal onset based on observations that the pathophysiological process producing late-onset epilepsy rarely result in new onset generalized seizures, which typically have onset in childhood to adolescence.

In keeping with a focal onset, the most common EEG abnormalities described in LOEU are focal epileptiform discharges and focal slowing (22, 33, 35). The typical location for epileptiform discharges or slowing is in either unilateral or bilateral temporal lobes. EEG recordings without evidence of epileptiform abnormalities or focal slowing are common, but this may relate to the EEG recording conditions. EEG sensitivity is increased by longer duration of recordings, repeated recordings, and recordings that include sleep. Older adult patients may undergo a limited duration of EEG recording because of lower seizure frequency and rate of medication resistance compared to younger patients. Recordings during sleep significantly increase yield for epileptic abnormalities in LOEU (21) Publications on LOEU rarely describe EEG recording details.

While age 55 is typically used as the age cutoff for categorizing LOEU, the average age for onset of LOEU is often reported to be between 60 and 70 years (32, 35, 36). Patients with LOEU and preexisting mild cognitive impairment (MCI) are older on average than patients with LOEU and without preexisting MCI (35). A large majority of patients with LOEU are reported to respond to initial antiseizure medication treatment and rarely require polytherapy (6, 32–34). Evaluation for surgical treatment due to lack of medication responsiveness is uncommon.

## 4. Epidemiological evidence of an association between LOEU and AD

The most substantial evidence that some cases of LOEU may be a manifestation of preclinical or prodromal AD comes from retrospective epidemiological studies. Separate epidemiological literature on both AD and LOEU support an association (Table 1).

Patients diagnosed with AD have an elevated rate of seizures in the years prior to their diagnosis. In an investigation of patients with early-onset AD (onset before age 65), Samson et al. (37) found that 7% had a history of seizures occurring before AD diagnosis. Similarly, DiFrancesco et al. (38) found that patients with AD had a 17-fold increased risk of preceding LOEU compared to a reference population. AD patients with prior LOEU had onset of epilepsy an average of 4.5 years before AD diagnosis. Vossel et al. (8) investigated patients with diagnosis of AD/aMCI and epilepsy; 83% of patients had onset of epilepsy either preceding or occurring near time of diagnosis, and in 38%, seizures preceded or coincided with onset of cognitive decline. Other studies have confirmed that LOEU may occur before initial cognitive decline in patients who develop AD or MCI. Sarkis et al. (39) investigated patients with both dementia and MRI-negative epilepsy, and found that 8% had onset of epilepsy before documented cognitive decline and 25% had onset prior to a diagnosis of dementia. While this investigation was not limited to AD and did not include subgroup analyses, over 80% of the sample had possible, probable, or autopsy-proven AD. Cretin et al. (32) retrospectively investigated patients at an academic medical center who met criteria for MCI and found that 3.1% of patients with MCI and 5% of patients with amnestic MCI had epilepsy without a defined etiology preceding cognitive complaints. All patients with MCI and preceding LOEU had amnestic MCI, which is associated with an elevated risk of progression to AD (40). Cretin et al. (32) found that these patients developed seizures an average of 2.7 years before self-reported cognitive decline and 6.9 years before a diagnosis of MCI.

Retrospective investigations of LOEU have also identified a subsequent increased risk of developing dementia. Ophir et al. (36) investigated patients with LOEU initially presenting without cognitive symptoms at ages between 55 and 69 found a 10-year cumulative incidence of dementia of 22.2%; mortality at 10 years in this sample was 31%, potentially preventing additional patients from expressing an eventual dementia. Incidence of dementia was

TABLE 1 Epidemiological studies describing an association between LOEU and AD/aMCI.

| Investigations of LOEU | | | | |
|---|---|---|---|---|
| Authors | Year of Publication | Research Design | Average age of epilepsy onset (years) | % LOEU developing dementia |
| Ophir et al. | 2021 | Retrospective | 61 | 22% |
| Kawakami et al. | 2018 | Retrospective | Not reported | 21% |
| Keret et al. | 2020 | Retrospective | Not reported | 8.3% |
| Costa et al. | 2019 | Prospective | Not reported | 25% |
| Johnson et al. | 2020 | Prospective | Not reported (67 or older) | 41.6% |
| **Investigations of AD/aMCI** | | | | |
| Authors | Year of Publication | Research Design | Average age of epilepsy onset (years) | % AD/aMCI with preceding epilepsy |
| Samson et al. | 1996 | Retrospective | Not reported | 7% |
| DiFrancesco et al. | 2017 | Retrospective | 68 | 1.7% |
| Vossel et al. | 2013 | Retrospective | 68 | 3.1% |
| Sarkis et al. | 2016 | Retrospective | 74 | 2.3% |
| Cretin et al. | 2016 | Retrospective | 63 | 3.1% |

higher among patients with LOEU and temporal discharges on baseline EEG, with 10 of 17 developing dementia during the retrospective study period. Kawakami et al. (22) found that patients with LOEU had a 21% cumulative incidence of dementia after 5 years follow-up compared to 4.3% of controls. Utilizing a random sample of patients from the Veterans Health Database, Keret et al. (41) studied patients with onset of epilepsy at age 55 or above who lacked an ICD-9-CM code to explain the cause of their epilepsy. After an average 6.1 years of follow-up, these patients had a hazard ratio of 1.89 (95% CIL 1.62–2.20) for a diagnosis of dementia compared to patients above age 55 who did not develop epilepsy. These investigations did not specifically determine if patients who developed dementia had probable AD versus another etiology.

There are few published prospective studies that follow patients with newly diagnosed LOEU to assess the rate of AD diagnosis. Costa et al. (33) describes prospective follow up of patients with LOEU who were cognitively normal at initial evaluation for up to 5 years. Of these patients, 25% developed dementia with 17.5% meeting criteria for AD during the follow up period. In a prospective study of patients with epilepsy starting at age 67 or later, Johnson et al. (42) found that late-onset epilepsy was associated with an increased risk of subsequent diagnosis of dementia, with an adjusted hazard ratio of 3.05 (95% CI, 2.65–3.51). Median time from diagnosis of epilepsy to diagnosis of dementia was 3.7 years. While this investigation did not solely include patients with LOEU, patients without a history of stroke had an even greater risk of subsequent dementia, with a hazard ratio of 3.39 (95% CI, 2.89–3.97).

Epidemiological associations demonstrate an association between LEOU and increased risk of subsequent stroke (43) and vascular risk factors increase risk of developing late-onset epilepsy (44). This suggests that LOEU is not be a homogenous entity and that previously undetected cerebrovascular disease may be an underlying etiology in some patients. Alternatively, this may reflect shared underlying mechanisms contributing to cerebrovascular disease, AD, and epilepsy.

Existing publications rarely compare risk of cognitive decline in LOEU patients with late-onset epilepsy of known etiology or patients with early-onset epilepsy. Patients with late-onset epilepsy in general demonstrate increased risk of developing dementia (45) potentially related to increased risk of developing vascular dementia in patients with cerebrovascular disease as a cause of epilepsy (46). Epilepsy itself is associated with increased risk of dementia and amyloid pathology (47), even when first diagnosed in early life. In addition to direct structural and functional effects from seizures themselves, potential factors that may impair cognition after development of epilepsy can include traumatic brain injuries from seizure-related accidents (48), medications used to treat epilepsy (49, 50), and epilepsy surgeries (51). Thus, dedicated prospective investigations comparing cognitive decline across all etiologies and ages of onset of epilepsy are warranted to fully identify which patients are at greatest risk of developing AD. As seizure burden is typically low in LOEU and risk of progression to AD appears to be higher specifically in LOEU than in patients with other forms of epilepsy (33, 36, 52), it is likely that the occurrence of seizures in LOEU is reflective of underlying AD pathology, rather than seizures being the major driver of AD onset.

## 5. Quantitative cognitive testing in LOEU

In addition to epidemiological associations between LOEU and development of AD, quantitative cognitive testing has shown impaired cognitive performance in patients with LOEU compared to controls. Fernandes et al. (34) studied patients with LOEU with a Mini-Mental State Examination (MMSE) score greater than 24 and found that patients with LOEU had globally lower cognitive testing performances, including on tests of recall (Rey Auditory Verbal Learning Test), verbal fluency (Phonological Verbal Fluency test, Semantic verbal fluency test), and executive function (Rey–Osterrieth Complex Figure Test) compared to controls. After 12 months, patients with LOEU

showed progressive impairment in the memory domain with lower RALVT-I scores, while controls showed memory improvement with an increase in RAVLT-I scores. Ligori et al. (30) studying patients with LOEU with cutoff MMSE scores above 24 found that patients with LOEU had a statistically significant decline in MMSE scores and word recall at 12 months follow up, though the average decline was small (less than 1 point on average for both tests) and scores on phonological verbal fluency increased. Differences in cognitive score changes between different antiseizure medication regimens were small.

Initial impairment in quantitative cognitive testing is not universally seen; Costa et al. (6, 33) investigated patients with LOEU without dementia and demonstrated no difference in MMSE scores between LOEU and healthy controls at time of enrollment, despite a later 20% rate of progression to dementia after 3 years. Similarly, Nardi Cesarini et al. (35) did not find differences between cognitively normal patients with LOEU and healthy controls. However, patients with LOEU and comorbid MCI had lower average MMSE scores, clock drawing scores, phonemic/letter fluency, and abstract logical reasoning scores than age-matched patients with MCI without epilepsy.

## 6. Genetic markers that confer increased risk of late-onset epilepsy and AD

While genetic investigations have not specifically evaluated LOEU, recent studies have evaluated shared genetic risk factors between late-onset epilepsy and AD. APO-ε4 is a known genetic risk factor for amyloid pathology and AD (53). As part of a prospective cohort study, Johnson et al. (44) found that carrying an APO-ε4 allele was associated with increased risk of developing late-onset epilepsy. This also demonstrated a dose-dependent relationship, with patients carrying two APO-ε4 alleles demonstrating an adjusted hazard ratio of 2.36 (95% CI: 1.65–3.38) and patients carrying a single APO-ε4 demonstrating an adjusted hazard ratio of 1.42 (95% CI: 1.19–1.69). Results held when excluding patients who were diagnosed with stroke or dementia. A subsequent study using the same sample but accounting for additional follow-up time again demonstrated an increased risk of late-onset epilepsy in patients with two APO-ε4 alleles (42). Using a mendelian randomization analysis, Fang et al. (54) found that a genome-wide genetic predisposition to AD was associated with a small but significantly increased risk of both focal epilepsy with hippocampal sclerosis (OR 1.01, 95% CI: 1.004–1.022) and generalized epilepsy (OR 1.05, 95% CI: 1.003–1.105). Genetic predisposition to focal epilepsy with hippocampal sclerosis was also found to be associated with increased risk of AD (OR 3.99).

## 7. Cerebrospinal fluid biomarker evidence of AD pathophysiology in LOEU

With the advent of precision CSF biomarkers and imaging modalities for detecting amyloid and tau pathology, there has been a concerted effort to develop a biomarker-based classification framework for diagnosis of AD. CSF biomarkers are well validated for early, antemortem detection of pathological findings in patients with MCI and AD (55). A NINDS-supported biomarker and neuroimaging-based diagnostic framework utilizes these biomarkers in order to achieve improved diagnostic accuracy, reduce antemortem misdiagnoses, and improve prognostic accuracy of cognitive trajectory (56). This framework is termed the AT(N) classification system, referring to the presence of amyloid, tau, and neurodegeneration in pathologically definite AD. Reduced levels of CSF $A\beta_{1\text{-}42}$ or reduced ratio of $A\beta_{1\text{-}42}$ to $A\beta_{1\text{-}40}$ are indicative of amyloid pathology, including amyloid plaques (56). Increased levels of CSF phosphorylated tau (p-tau) indicate presence of pathologic tau neurofibrillary tangles and increased levels of CSF total tau (t-tau) are correlated with greater neuronal loss and neurodegeneration.

Investigations of patients with LOEU using $A\beta_{1\text{-}42}$ and p-tau CSF biomarkers suggest that some patients have a previously unrecognized amyloidopathy and/or tauopathy. Cretin et al. (32) reported a series of patients with MCI and preceding LOEU. All 13 patients demonstrated either low CSF $A\beta_{1\text{-}42}$ (53.8%) or low CSF $A\beta_{1\text{-}42}/A\beta_{1\text{-}40}$ ratio (46.2%) by the time of MCI diagnosis. Average p-tau levels were found to be elevated. Costa et al. (6, 33) investigated patients with LOEU with MMSE scores greater than 24 and found that 37.5% of patients with LOEU had CSF $A\beta_{1\text{-}42}$ below cutoff pathological levels. As a group, patients with LOEU had lower CSF $A\beta_{1\text{-}42}$ compared to healthy controls, despite similar cognitive performance on tests of recall, attention, and executive function. Patients with LOEU also had significantly greater t-tau levels in CSF, though p-tau levels were not significantly different compared to controls. Importantly, 6 of the 15 patients with LOEU and positive $A\beta_{1\text{-}42}$ biomarker levels developed a clinical dementia and 5 met clinical criteria for AD during an average 3 year follow up period. In contrast, 4 out of 25 patients with LOEU without positive $A\beta_{1\text{-}42}$ biomarker levels developed dementia during the follow up period, 2 of whom met criteria for AD. Fernandes et al. (34) also studied LOEU patients without preexisting diagnosis of MCI and with MMSE greater than 24. Investigators found lower CSF $A\beta_{1\text{-}42}$ and both higher CSF p-tau and t-tau levels compared to controls. Of the sample of 55 patients, 16.4% met cutoff levels of pathologically low CSF $A\beta_{1\text{-}42}$.

Decreased $A\beta_{1\text{-}42}$ is not uniformly observed in LOEU without preexisting MCI. In an investigation by Nardi Cesarini et al. (35) of patients with LOEU with or without comorbid MCI, patients with LOEU without comorbid MCI did not have a significant difference in $A\beta_{1\text{-}42}$ levels compared to controls. Patients with both LOEU and MCI had lower average CSF $A\beta_{1\text{-}42}$ and $A\beta_{1\text{-}42}$/p-tau compared to LOEU without MCI, with 41% demonstrating pathologically low levels. 22.7% of MCI-LOEU had both pathologically decreased amyloid and increased p-tau, meeting both (A+/T+) classification. None of the cognitively normal LOEU patients had positive CSF (A) or (T) classification.

## 8. Imaging findings suggestive of AD pathophysiology in LOEU

By definition, patients with LOEU lack visible structural abnormalities on imaging that are known to produce epilepsy, but visual assessment and volumetric analyses often demonstrate findings suggestive of imaging abnormalities seen in both early AD and occult cerebrovascular disease. Using visual inspection, Nagino et al. (57) found that 58% of patients with LOEU had global atrophy on MRI and 48% had unilateral or bilateral hippocampal atrophy.

The most common imaging abnormality was white matter hyperintensities (a feature of cerebrovascular disease), which was present in 81% of the sample. In a sample of 66 patients with LOEU, Sarkis et al. (21) found that 81% had evidence of temporal atrophy and 21% had moderate or severe hippocampal volume loss assessed using a visual inspection scale validated to predict progression to MCI and AD. In this sample, 34.8% had small-to-large confluent white matter hyperintensities based on visual inspection. In Cretin et al.'s[32] investigation of patients with MCI with preceding LOEU, 12 out of 13 patients demonstrated mild bilateral hippocampal atrophy on visual inspection. In the same study, over two-thirds also had cerebrovascular white matter lesions and over one-third had subcortical lacunes or non-cortical microhemorrhages.

Using quantitative analyses, Hanby et al. (23) also found that patients with LOEU had lower global cortical volume than age-matched controls and had increased burden of white matter hyperintensities. Johnson et al. (58) found that lower total cortical volume was associated with increased likelihood of late-onset epilepsy; while this investigation did not specifically identify patients with LOEU, results held when excluding patients with a diagnosis of stroke or pre-existing dementia. Kaestner et al. (31) performed a detailed investigation of quantitative MRI measures in 23 patients with late-onset TLE, defined in this investigation as patients with onset of TLE after age 50. Compared to healthy controls, patients with late-onset TLE had prominent cortical thinning in mesial temporal lobes, lateral temporal lobes, prefrontal, precentral, and paracentral regions. Directly comparing patients with late-onset TLE to patients with early-onset TLE, patients with LO-TLE had thinner cortex in bilateral fusiform gyri. Of note, this difference in cortical thickness was found even though patients with EO-TLE had approximately 30 years greater duration of epilepsy compared to patients with LO-TLE. Patients with EO-TLE were more likely to have hippocampal sclerosis (58% versus 26%) as assessed by visual inspection.

In addition to MRI evidence of neurodegeneration, FDG-PET also demonstrates evidence of changes suggestive of AD. Using FDG-PET scans, Fernandes et al. (34) found that patients with LOEU had significantly reduced glucose metabolism in the right posterior cingulate cortex and left precuneus compared to controls. Decreased glucose in these regions was correlated with worse recall on both the immediate and delayed Rey Auditory Verbal Learning Test. DiFrancesco et al. (59) also investigated patients using cerebral FDG-PET, revealing temporal lobe hypometabolism in 87% of patients with LOEU. While five patients had multifocal decreased metabolism in temporal lobe structures, the other 15 patients had focal locations of temporal hypometabolism, most commonly in the anterior temporal lobe. Cases without hypometabolism in the temporal lobe had focal hypometabolism in the caudate nucleus. Nearly all patients with lateralized focal slowing or epileptiform discharges on EEG had congruent laterality of hypometabolism.

There have been few investigations using of amyloid or tau specific PET scans to study presence of amyloidopathy and tauopathy in patients with LOEU. Sarkis et al. (60) investigated six patients, ages 69–83, with history of nonlesional epilepsy and cognitive decline consistent with MCI or early dementia using F-18 florbetaben amyloid PET scans. Five of the six patients had seizures preceding cognitive decline; the sixth patient had onset of seizures 2 years following onset of cognitive decline. Four of the six patients had positive amyloid scans assessed by visual inspection. These results and results from CSF studies suggest that amyloid and tau-specific PET scans may be a useful method for assessing patterns of amyloid and tau deposition in patients with LOEU progressing to AD.

## 9. Directions for pathologic studies in LOEU

Despite an existing literature on amyloid and tau pathology in a broad array of patients with epilepsy, there is an absence of publications on pathologic analysis of brain tissue from patients specifically with LOEU. This may be due to both low rates of pharmacoresistant seizures, which obviates a need for surgical resection and therefore reduces the possibility for histopathological review. Enrolling patients into longitudinal studies that include eventual cerebral pathological review after death may reveal distinct patterns of neurodegeneration. In particular, tissue comparisons between patients with LOEU who progress to AD and patients who do not, combined with antemortem EEG or MEG-based localizations of seizure onset zones, may demonstrate molecular and histological findings to explain why some patients demonstrate progressive cognitive decline while others do not. Existing AD tissue banks that include patients who had onset of epilepsy prior to cognitive degeneration may demonstrate distinct patterns of neurodegeneration compared to patients with AD who did not have seizures. Patterns of mesial temporal lobe neurodegeneration may be particularly significant to assess in LOEU given early involvement of the temporal lobe in AD and frequency of temporal EEG and mesial temporal imaging abnormalities in LOEU. TDP-43 protein deposition is often observed in AD with prominent hippocampal sclerosis (28); as CSF biomarker measurement of TDP-43 has limited utility, pathological assessment of TDP-43 in addition to amyloid plaques and tau neurofibrillary tangles should be included in LOEU cases.

As animal models of epilepsy suggest that seizures themselves may induce increased amyloid and tau depositions, pathologic comparisons between patients with LOEU and patients with early onset of epilepsy are also needed to further discern what findings may be a driving factor in producing seizures and what findings are expected as a result of seizures. Existing literature on amyloid and tau pathology in early-onset temporal lobe epilepsy has demonstrated inconsistent results. Resected temporal lobe tissue in a study of patients with pharmacoresistant TLE has demonstrated higher rates of amyloid plaques compared to age-matched autopsy controls (61). In another study, resected tissue demonstrated increased neuronal immunoreactivity for amyloid precursor protein, but did not show higher rates of plaques (62). Tai et al. (63) investigated resected temporal lobe tissue from patients with temporal lobe epilepsy. Typical age of onset of epilepsy occurred in the second decade of life with resections occurring between ages 50–65. 94% of the sample demonstrated evidence of tau neuropathology visualized as neuropil threads, neurofibrillary tangles, or pre-tangles, but prevalence of increased Braak stages (limited to temporal lobe assessment) was not significantly higher in TLE patients than age-match controls. In contrast, in a study of post-mortem pathologic analyses from patients with chronic epilepsy showed increased Braak stages in patients aged 40–65 compared to controls in the same age group, with higher average Braak stages noted in patients with focal onset epilepsy

compared to generalized onset (64). Braak staging did not correlate with presence of hippocampal sclerosis.

## 10. Classification of LOEU as demonstrating epileptic prodromal AD, epileptic preclinical AD, or late-onset Aβ-related epilepsy

In the AT(N) classification scheme, patients with positive amyloid biomarkers are considered to fall on the AD continuum; the presence of a positive amyloid biomarker study alone confers increased risk of cognitive decline in cognitively normal adults (65). It is currently unclear if, given enough time and absence of other causes of mortality, what percentage of patients with positive amyloid biomarkers would eventually develop AD. Presence of both positive amyloid and tau biomarkers establishes particularly elevated risk of progression to AD in MCI and is termed preclinical AD in cognitively normal individuals (66).

Given established epidemiological associations between LOEU and AD, imaging abnormalities in LOEU suggestive of neurodegenerative processes, and results of biomarker testing in LOEU, we propose that amyloid and tau biomarkers can be used to further classify patients with LOEU (Figure 2). Presence of both positive amyloid and tau biomarkers in a patient with late-onset epilepsy with otherwise unknown etiology may be sufficient to categorize patients as demonstrating epileptic preclinical AD or epileptic prodromal AD. In cases without MCI and both positive amyloid and tau neurofibrillary tangle biomarkers, we propose categorizing patients as demonstrating "epileptic preclinical AD," corresponding with terminology proposed for biopathologic preclinical AD (56). In patients with documented MCI, positive amyloid biomarker testing, and positive tau neurofibrillary tangle biomarker testing, we suggest categorization as "epileptic prodromal AD," a term previously proposed by Cretin et al. (32) and again corresponding with existing biopatholic research terminology. While further prospective studies are needed, the presence of LOEU, positive amyloid biomarker testing, and positive tau biomarker testing may each signify independent risk factors for progressive AD-related cognitive decline leading to dementia. A categorization of epileptic preclinical or prodromal AD is significant as it implies that the etiology of epilepsy is no longer unknown but is strongly suspected to be a result of AD pathology.

As patients with LOEU prior to development of MCI/dementia inconsistently demonstrate positive tau biomarkers, but frequently show positive amyloid biomarkers, an additional classification

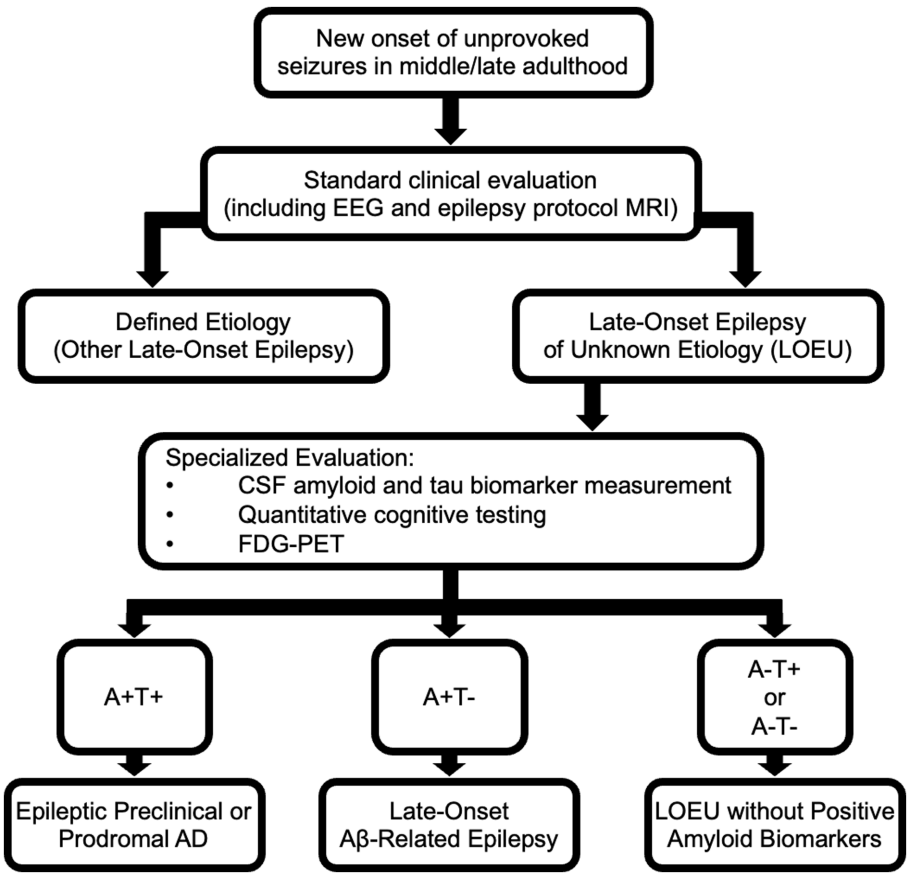


FIGURE 2
Evaluation of late-onset epilepsy of unknown etiology (LOEU) and classification based upon results of biomarker testing and imaging. The AT(N) classification system for AD can be used to classify patients with late-onset epilepsy. "A" refers to amyloid biomarkers; "T" refers to tau biomarkers indicative of neurofibrillary tangles; "N" refers to evidence of neurodegeneration assessed by neuroimaging or biomarkers. PART, Primary Age-Related Tauopathy.

category is needed for patients with positive amyloid but with negative tau markers. These patients may be considered to demonstrate late-onset Aβ-related epilepsy (LAβE), as previously proposed by Romoli et al. (67) This terminology implies that there is some uncertainty regarding eventual development of frank AD and that underlying etiology of epilepsy is not definite, but with suspicion that an amyloidopathy is playing a contributing role.

Studies of biomarkers in LOEU note infrequent instances of patients with pathologic levels of p-tau in CSF, but non-pathologic $A\beta_{1\text{-}42}$ levels (35). CSF biomarker measurements positive for tau pathology but negative for amyloid pathology may seem suggestive of primary age-related tauopathy (PART) (68), but recent research suggests increased soluble CSF p-tau is more closely correlated with amyloid deposition than tau neurofibrillary tangles as measured through PET imaging (69, 70). It is unclear how to classify LOEU with (A−/T+) biomarker profiles at this time. Pathological tau depositions may be a primary driver of seizure activity, occur as a result of seizures stemming from another etiology, or represent an incidental co-occurring process such as from PART. Thus, assessment of patients with LOEU using amyloid and tau PET imaging may clarify (A) and (T) status in patients who demonstrate CSF biomarkers suggestive of (A−/T+). Further research on occurrence rates of preceding seizures in PART may help elucidate a possible relationship between tauopathic processes and epilepsy.

Presence of neurodegeneration on neuroimaging (N+) may be considered supportive of but not necessary for classification of epileptic prodromal AD, epileptic preclinical AD, or LAβE. Supportive patterns of neurodegeneration on MRI or FDG-PET mirror those observed in AD, with features of atrophy or hypometabolism in temporal or parietal structures. It is unclear if substantial burden of white matter disease in LOEU suggests that underlying AD pathology is less likely, or if it reflects common shared factors between AD and cerebrovascular disease. Stratification of patients using novel serum biomarkers of cerebrovascular disease and vascular cognitive impairment may clarify this (71).

The bulk of evidence for proposing these criteria comes from results from CSF amyloid and tau biomarkers in patients with LOEU and from studies of cognitively intact individuals without epilepsy who demonstrate positive biomarkers. Investigations that replicate existing biomarker findings in LOEU and evaluate patients with LOEU longitudinally are needed. While the AT(N) classification scheme for AD also incorporates amyloid and tau PET imaging, there have been few studies utilizing amyloid and tau PET imaging in LOEU. Distinct patterns of deposition may be seen in patients with epileptic preclinical and epileptic prodromal AD that could further understanding why some patients with AD develop seizures at earlier stages of the disease compared to others. Likewise, investigations using plasma-based amyloid and tau biomarkers compared to CSF biomarkers in LOEU have not been systematically performed and are also needed. Normalizing $A\beta_{1\text{-}42}$ levels in comparison to $A\beta_{1\text{-}40}$ (determined through calculating an $A\beta_{1\text{-}42}/A\beta_{1\text{-}40}$ ratio) has been found to increase sensitivity and specificity for discriminating AD from other dementias (72), and this may be useful for assessing for epileptic preclinical or prodromal AD as well. Neurofilament light chain (NFL) may also prove to be elevated in neurodegenerative processes producing LOEU, though this biomarker may have limited specificity for AD (73) and may be impacted by frequency and duration of seizures (74, 75). Other biomarkers undergoing investigation for detecting early preclinical stages of AD, such as the shedded form of platelet-derived growth factor receptor-b and plasma glial fibrillary acidic protein, may supplement existing biomarkers in detecting epileptic preclinical or prodromal AD (16, 76). Particular seizure types, such as temporal lobe seizures, may occur more frequently in epilepsy occurring from early stages of AD and also warrant further study.

Further research may also elucidate important comorbidities that contribute to the observed relationship between LOEU and AD. In addition to both demonstrating increased small vessel cerebrovascular disease, cerebral amyloid angiopathy may contribute to cortical injury and seizures; serial MRI imaging including GRE or SWI may elucidate if suggestive cortical microhemorrhages or siderosis develop over time in LOEU (77). Subclinical seizures and epileptiform activity during sleep may compromise healthy sleep and may play a role in impaired Aβ processing and clearance (78). Traumatic brain injuries occur at an elevated rate in patients with epilepsy and may contribute to development of AD pathology and increased risk of AD (79, 80). AD and epilepsy may both lead to impairments in the blood–brain barrier, contributing to further neuronal dysfunction (81). Lastly, as neurodegenerative processes frequently co-occur, it will be important to continue investigating if co-occurring TDP-43 and alpha-synuclein deposits impact seizure expression.

Currently, categorizing patients as demonstrating epileptic prodromal AD, epileptic preclinical AD, and LAβE is important to recognize clinically, but requires further research to determine an optimal management approach. With further research, these categorizations will be useful for clinicians when counseling patients about prognosis and assessing eligibility for therapies or clinical trials. Phase 3 studies of amyloid lowering antibody therapies excluded patients with recent history of seizures (82, 83). Thus, patients with LOEU may currently have reduced access to anti-amyloid therapies. As the safety of amyloid lowering therapies has not been well investigated in patients with epilepsy, further clinical trials should be performed, including investigating impact on cognitive symptoms, seizure frequency, and development of amyloid related imaging abnormalities. Given recent trial results that found treatment with donanemab had greater benefit when used in early stages of AD (84), it is possible that patients with epileptic preclinical AD without frank cognitive symptoms may be optimal candidates for treatment with anti-amyloid therapies.

At this time, management of epileptic prodromal AD, epileptic preclinical AD, and LAβE should mirror existing strategies for management of late-onset epilepsy and epilepsy in older adults (Figure 1). Avoidance of antiseizure medications with greater cognitive side effects, such as topiramate, zonisamide, and phenobarbital, may be prudent (85). Divalproex sodium has been associated with accelerated cognitive decline and cerebral atrophy in AD; thus treatment with divalproex and other formulations of valproate may not be preferred (86). As levetiracetam has few drug–drug interactions and treatment with low-dose levetiracetam in patients with detectible epileptiform activity has been found to improve spatial memory and executive function (87), levetiracetam or brivaracetam may be preferred initial treatments. Lamotrigine and lacosamide are also well tolerated in older adults, but evidence that these medications and other sodium channel inhibitors slow cardiac conduction is a consideration in patients with existing cardiac comorbidities (88). As causes of dementia are commonly multifactorial, optimization of

cerebrovascular risk factors including hypertension, hyperlipidemia, and diabetes mellitus may slow rates of cognitive decline. Annual or biennial cognitive screening may allow for early detection of progression. Upon meeting criteria for AD, early treatment with anticholinesterase inhibitors may reduce cognitive symptoms. Patients meeting criteria for epileptic prodromal AD, epileptic preclinical AD, or LAβE may eventually be offered enrollment in targeted clinical trials.

## 11. Conclusion

Evidence from recent investigations suggests that LOEU can serve as an early sign of AD. Epidemiological data establish a significant association between LOEU and AD, indicating that individuals with LOEU are at an increased risk of developing AD within years of epilepsy onset. Furthermore, biomarker investigations focusing on amyloid and tau show that biomarker profiles may improve prediction of progression to AD in LOEU patients. Based on this evidence, any older adult presenting with LOEU should have a thorough evaluation for preclinical or prodromal AD. The established relationship between LOEU and AD suggests that patients with LOEU and consistent amyloid and tau biomarkers should be considered for epileptic preclinical or prodromal AD evaluation. To fully understand progression in LOEU, prospective studies that assess changes in imaging features and biomarkers over time are needed. Such studies will not only enhance our understanding of the underlying mechanisms but also improve accuracy in identifying of subgroups of LOEU at the greatest risk of AD.

Future research efforts should explore efficacy of amyloid lowering therapies or other targeted therapeutics in modifying the disease progression of both epilepsy and cognitive dysfunction in LOEU patients. By continuing to elucidate the interplay between LOEU and AD, potential treatment regimens may emerge that address both seizures and cognitive decline, thereby improving overall quality of life for individuals affected by LOEU.

## Author contributions

LH wrote the first draft of the manuscript. All authors contributed to manuscript conceptualization, and manuscript revision. All authors read and approved the submitted version.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note



## References

1. Hauser WA, Annegers JF, Kurland LT. Incidence of epilepsy and unprovoked seizures in Rochester, Minnesota: 1935–1984. *Epilepsia*. (1993) 34:453–8. doi: 10.1111/j.1528-1157.1993.tb02586.x

2. Brodie MJ, Elder AT, Kwan P. Epilepsy in later life. *Lancet Neurol*. (2009) 8:1019–30. doi: 10.1016/S1474-4422(09)70240-6

3. Lühdorf K, Jensen LK, Plesner AM. Etiology of seizures in the elderly. *Epilepsia*. (1986) 27:458–63. doi: 10.1111/j.1528-1157.1986.tb03567.x

4. Tchalla AE, Marin B, Mignard C, Bhalla D, Tabailloux E, Mignard D, et al. Newly diagnosed epileptic seizures: focus on an elderly population on the French island of Réunion in the Southern Indian Ocean. *Epilepsia*. (2011) 52:2203–8. doi: 10.1111/j.1528-1167.2011.03320.x

5. Besocke AG, Rosso B, Cristiano E, Valiensi SM, García MC, Gonorazky SE, et al. Outcome of newly-diagnosed epilepsy in older patients. *Epilepsy Behav*. (2013) 27:29–35. doi: 10.1016/j.yebeh.2012.11.041

6. Costa C, Parnetti L, D'Amelio M, Tozzi A, Tantucci M, Romigi A, et al. Epilepsy, amyloid-β, and D1 dopamine receptors: a possible pathogenetic link? *Neurobiol Aging*. (2016) 48:161–71. doi: 10.1016/j.neurobiolaging.2016.08.025

7. Friedman D, Honig LS, Scarmeas N. Seizures and epilepsy in Alzheimer's disease. *CNS Neurosci Ther*. (2012) 18:285–94. doi: 10.1111/j.1755-5949.2011.00251.x

8. Vossel KA, Beagle AJ, Rabinovici GD, Shu H, Lee SE, Naasan G, et al. Seizures and epileptiform activity in the early stages of Alzheimer disease. *JAMA Neurol*. (2013) 70:1158–66. doi: 10.1001/jamaneurol.2013.136

9. Vossel KA, Tartaglia MC, Nygaard HB, Zeman AZ, Miller BL. Epileptic activity in Alzheimer's disease: causes and clinical relevance. *Lancet Neurol*. (2017) 16:311–22. doi: 10.1016/S1474-4422(17)30044-3

10. Busche MA, Chen X, Henning HA, Reichwald J, Staufenbiel M, Sakmann B, et al. Critical role of soluble amyloid-β for early hippocampal hyperactivity in a mouse model of Alzheimer's disease. *Proc Natl Acad Sci U S A*. (2012) 109:8740–5. doi: 10.1073/pnas.1206171109

11. Paudel YN, Angelopoulou E, Jones NC, O'Brien TJ, Kwan P, Piperi C, et al. Tau related pathways as a connecting link between epilepsy and Alzheimer's disease. *ACS Chem Neurosci*. (2019) 10:4199–212. doi: 10.1021/acschemneuro.9b00460

12. Minkeviciene R, Rheims S, Dobszay MB, Zilberter M, Hartikainen J, Fülöp L, et al. Amyloid beta-induced neuronal hyperexcitability triggers progressive epilepsy. *J Neurosci*. (2009) 29:3453–62. doi: 10.1523/JNEUROSCI.5215-08.2009

13. Born HA, Kim JY, Savjani RR, das P, Dabaghian YA, Guo Q, et al. Genetic suppression of transgenic APP rescues hypersynchronous network activity in a mouse model of Alzeimer's disease. *J Neurosci*. (2014) 34:3826–40. doi: 10.1523/JNEUROSCI.5171-13.2014

14. Roberson ED, Scearce-Levie K, Palop JJ, Yan F, Cheng IH, Wu T, et al. Reducing endogenous tau ameliorates amyloid beta-induced deficits in an Alzheimer's disease mouse model. *Science*. (2007) 316:750–4. doi: 10.1126/science.1141736

15. DeVos SL, Goncharoff DK, Chen G, Kebodeaux CS, Yamada K, Stewart FR, et al. Antisense reduction of tau in adult mice protects against seizures. *J Neurosci*. (2013) 33:12887–97. doi: 10.1523/JNEUROSCI.2107-13.2013

16. Bellaver B, Povala G, Ferreira PCL, Ferrari-Souza JP, Leffa DT, Lussier FZ, et al. Astrocyte reactivity influences amyloid-β effects on tau pathology in preclinical Alzheimer's disease. *Nat Med*. (2023) 29:1775–81. doi: 10.1038/s41591-023-02380-x

17. Seifert G, Carmignoto G, Steinhäuser C. Astrocyte dysfunction in epilepsy. *Brain Res Rev*. (2010) 63:212–21. doi: 10.1016/j.brainresrev.2009.10.004

18. Palop JJ, Mucke L. Network abnormalities and interneuron dysfunction in Alzheimer disease. *Nat Rev Neurosci*. (2016) 17:777–92. doi: 10.1038/nrn.2016.141

19. Alyenbaawi H, Kanyo R, Locskai LF, Kamali-Jamil R, DuVal MG, Bai Q, et al. Seizures are a druggable mechanistic link between TBI and subsequent tauopathy. *elife*. (2021) 10:e58744. doi: 10.7554/eLife.58744

20. Hwang K, Vaknalli RN, Addo-Osafo K, Vicente M, Vossel K. Tauopathy and epilepsy comorbidities and underlying mechanisms. *Front Aging Neurosci*. (2022) 14:903973. doi: 10.3389/fnagi.2022.903973

21. Sarkis RA, Beers L, Farah E, al-Akaidi M, Zhang Y, Locascio JJ, et al. The neurophysiology and seizure outcomes of late onset unexplained epilepsy. *Clin Neurophysiol Off J Int Fed Clin Neurophysiol*. (2020) 131:2667–72. doi: 10.1016/j.clinph.2020.08.014

22. Kawakami O, Koike Y, Ando T, Sugiura M, Kato H, Hiraga K, et al. Incidence of dementia in patients with adult-onset epilepsy of unknown causes. *J Neurol Sci*. (2018) 395:71–6. doi: 10.1016/j.jns.2018.09.010

23. Hanby MF, Al-Bachari S, Makin F, Vidyasagar R, Parkes LM, Emsley HCA. Structural and physiological MRI correlates of occult cerebrovascular disease in late-onset epilepsy. *NeuroImage Clin*. (2015) 9:128–33. doi: 10.1016/j.nicl.2015.07.016

24. Krumholz A, Wiebe S, Gronseth GS, Gloss DS, Sanchez AM, Kabir AA, et al. Evidence-based guideline: management of an unprovoked first seizure in adults: report of the guideline development subcommittee of the American Academy of Neurology and the American Epilepsy Society. *Neurology*. (2015) 84:1705–13. doi: 10.1212/WNL.0000000000001487

25. Lim JA, Lee ST, Moon J, Jun JS, Kim TJ, Shin YW, et al. Development of the clinical assessment scale in autoimmune encephalitis. *Ann Neurol*. (2019) 85:352–8. doi: 10.1002/ana.25421

26. Bernasconi A, Cendes F, Theodore WH, Gill RS, Koepp MJ, Hogan RE, et al. Recommendations for the use of structural magnetic resonance imaging in the care of patients with epilepsy: a consensus report from the International League Against Epilepsy Neuroimaging Task Force. *Epilepsia*. (2019) 60:1054–68. doi: 10.1111/epi.15612

27. Zarow C, Sitzer TE, Chui HC. Understanding hippocampal sclerosis in the elderly: epidemiology, characterization, and diagnostic issues. *Curr Neurol Neurosci Rep*. (2008) 8:363–70. doi: 10.1007/s11910-008-0057-3

28. Amador-Ortiz C, Lin WL, Ahmed Z, Personett D, Davies P, Duara R, et al. TDP-43 immunoreactivity in hippocampal sclerosis and Alzheimer's disease. *Ann Neurol*. (2007) 61:435–45. doi: 10.1002/ana.21154

29. DeGiorgio CM, Tomiyasu U, Gott PS, Treiman DM. Hippocampal pyramidal cell loss in human status epilepticus. *Epilepsia*. (1992) 33:23–7. doi: 10.1111/j.1528-1157.1992.tb02278.x

30. Liguori C, Costa C, Franchini F, Izzi F, Spanetta M, Cesarini EN, et al. Cognitive performances in patients affected by late-onset epilepsy with unknown etiology: a 12-month follow-up study. *Epilepsy Behav EB*. (2019) 101:106592. doi: 10.1016/j.yebeh.2019.106592

31. Kaestner E, Reyes A, Chen A, Rao J, Macari AC, Choi JY, et al. Atrophy and cognitive profiles in older adults with temporal lobe epilepsy are similar to mild cognitive impairment. *Brain J Neurol*. (2021) 144:236–50. doi: 10.1093/brain/awaa397

32. Cretin B, Sellal F, Philippi N, Bousiges O, di Bitonto L, Martin-Hunyadi C, et al. Epileptic prodromal Alzheimer's disease, a retrospective study of 13 new cases: expanding the spectrum of Alzheimer's disease to an epileptic variant? *J Alzheimers Dis*. (2016) 52:1125–33. doi: 10.3233/JAD-150096

33. Costa C, Romoli M, Liguori C, Farotti L, Eusebi P, Bedetti C, et al. Alzheimer's disease and late-onset epilepsy of unknown origin: two faces of beta amyloid pathology. *Neurobiol Aging*. (2019) 73:61–7. doi: 10.1016/j.neurobiolaging.2018.09.006

34. Fernandes M, Manfredi N, Aluisantonio L, Franchini F, Chiaravalloti A, Izzi F, et al. Cognitive functioning, cerebrospinal fluid Alzheimer's disease biomarkers and cerebral glucose metabolism in late-onset epilepsy of unknown aetiology: a prospective study. *Eur J Neurosci*. (2022) 56:5384–96. doi: 10.1111/ejn.15734

35. Nardi Cesarini E, Babiloni C, Salvadori N, Farotti L, del Percio C, Pascarelli MT, et al. Late-onset epilepsy with unknown etiology: a pilot study on neuropsychological profile, cerebrospinal fluid biomarkers, and quantitative EEG characteristics. *Front Neurol*. (2020) 11:199. doi: 10.3389/fneur.2020.00199

36. Ophir K, Ran B, Felix B, Amir G. Ten year cumulative incidence of dementia after late onset epilepsy of unknown etiology. *J Clin Neurosci Off J Neurosurg Soc Australas*. (2021) 86:247–51. doi: 10.1016/j.jocn.2021.01.030

37. Samson WN, van Duijn CM, Hop WC, Hofman A. Clinical features and mortality in patients with early-onset Alzheimer's disease. *Eur Neurol*. (1996) 36:103–6. doi: 10.1159/000117218

38. DiFrancesco JC, Tremolizzo L, Polonia V, Giussani G, Bianchi E, Franchi C, et al. Adult-onset epilepsy in presymptomatic Alzheimer's disease: a retrospective study. *J Alzheimers Dis*. (2017) 60:1267–74. doi: 10.3233/JAD-170392

39. Sarkis RA, Dickerson BC, Cole AJ, Chemali ZN. Clinical and neurophysiologic characteristics of unprovoked seizures in patients diagnosed with dementia. *J Neuropsychiatry Clin Neurosci*. (2016) 28:56–61. doi: 10.1176/appi.neuropsych.15060143

40. Petersen RC, Negash S. Mild cognitive impairment: an overview. *CNS Spectr*. (2008) 13:45–53. doi: 10.1017/s1092852900016151

41. Keret O, Hoang TD, Xia F, Rosen HJ, Yaffe K. Association of late-onset unprovoked seizures of unknown etiology with the risk of developing dementia in older veterans. *JAMA Neurol*. (2020) 77:710–5. doi: 10.1001/jamaneurol.2020.0187

42. Johnson EL, Krauss GL, Kucharska-Newton A, Albert MS, Brandt J, Walker KA, et al. Dementia in late-onset epilepsy: the atherosclerosis risk in communities study. *Neurology*. (2020) 95:e3248–56. doi: 10.1212/WNL.0000000000011080

43. Wall J, Knight J, Emsley HCA. Late-onset epilepsy predicts stroke: systematic review and meta-analysis. *Epilepsy Behav*. (2021) 115:107634. doi: 10.1016/j.yebeh.2020.107634

44. Johnson EL, Krauss GL, Lee AK, Schneider ALC, Dearborn JL, Kucharska-Newton AM, et al. Association between midlife risk factors and late-onset epilepsy: results from the atherosclerosis risk in communities study. *JAMA Neurol*. (2018) 75:1375–82. doi: 10.1001/jamaneurol.2018.1935

45. Huang L, Fu C, Li J, Peng S. Late-onset epilepsy and the risk of dementia: a systematic review and meta-analysis. *Aging Clin Exp Res*. (2022) 34:1771–9. doi: 10.1007/s40520-022-02118-8

46. Sen A, Capelli V, Husain M. Cognition and dementia in older patients with epilepsy. *Brain J Neurol*. (2018) 141:1592–608. doi: 10.1093/brain/awy022

47. Joutsa J, Rinne JO, Hermann B, Karrasch M, Anttinen A, Shinnar S, et al. Association between childhood-onset epilepsy and amyloid burden 5 decades later. *JAMA Neurol*. (2017) 74:583–90. doi: 10.1001/jamaneurol.2016.6091

48. Beghi E, Cornaggia CRESt-1 Group. Morbidity and accidents in patients with epilepsy: results of a European cohort study. *Epilepsia*. (2002) 43:1076–83. doi: 10.1046/j.1528-1157.2002.18701.x

49. Beghi E, Beghi M. Epilepsy, antiepileptic drugs and dementia. *Curr Opin Neurol*. (2020) 33:191–7. doi: 10.1097/WCO.0000000000000802

50. Aldenkamp AP, De Krom M, Reijs R. Newer antiepileptic drugs and cognitive issues. *Epilepsia*. (2003) 44:21–9. doi: 10.1046/j.1528-1157.44.s4.3.x

51. Baxendale S. The impact of epilepsy surgery on cognition and behavior. *Epilepsy Behav*. (2008) 12:592–9. doi: 10.1016/j.yebeh.2007.12.015

52. Breteler MM, de Groot RR, van Romunde LK, Hofman A. Risk of dementia in patients with Parkinson's disease, epilepsy, and severe head trauma: a register-based follow-up study. *Am J Epidemiol*. (1995) 142:1300–5. doi: 10.1093/oxfordjournals.aje.a117597

53. Liu CC, Kanekiyo T, Xu H, Bu G. Apolipoprotein E and Alzheimer disease: risk, mechanisms, and therapy. *Nat Rev Neurol*. (2013) 9:106–18. doi: 10.1038/nrneurol.2012.263

54. Fang Y, Si X, Wang J, Chen Y, Liu Y, Yan Y, et al. Alzheimer disease and epilepsy: a Mendelian randomization study. *Neurology*. (2023) 101:e399–e409. doi: 10.1212/WNL.0000000000207423

55. Blennow K, Zetterberg H. Biomarkers for Alzheimer's disease: current status and prospects for the future. *J Intern Med*. (2018) 284:643–63. doi: 10.1111/joim.12816

56. Jack CR, Bennett DA, Blennow K, Carrillo MC, Feldman HH, Frisoni GB, et al. A/T/N: an unbiased descriptive classification scheme for Alzheimer disease biomarkers. *Neurology*. (2016) 87:539–47. doi: 10.1212/WNL.0000000000002923

57. Nagino N, Kubota Y, Nakamoto H, Miyao S, Kodama T, Ito S, et al. Non-lesional late-onset epilepsy in the elderly Japanese patients: presenting characteristics and seizure outcomes with regard to comorbid dementia. *J Clin Neurosci*. (2022) 103:100–6. doi: 10.1016/j.jocn.2022.05.003

58. Johnson EL, Krauss GL, Lee AK, Schneider ALC, Kucharska-Newton AM, Huang J, et al. Association between white matter hyperintensities, cortical volumes, and late-onset epilepsy. *Neurology*. (2019) 92:e988–95. doi: 10.1212/WNL.0000000000007010

59. DiFrancesco JC, Isella V, Licciardo D, Crivellaro C, Musarra M, Guerra L, et al. Temporal lobe dysfunction in late-onset epilepsy of unknown origin. *Epilepsy Behav*. (2021) 117:107839. doi: 10.1016/j.yebeh.2021.107839

60. Sarkis RA, Gale SA, Yang HS, Lam AD, Singhal T, Cicero S, et al. Utility of amyloid positron emission tomography imaging in older adults with epilepsy and cognitive decline. *Am J Alzheimers Dis Other Dement*. (2023) 38:153331752311600. doi: 10.1177/15333175231160005

61. Mackenzie IR, Miller LA. Senile plaques in temporal lobe epilepsy. *Acta Neuropathol (Berl)*. (1994) 87:504–10. doi: 10.1007/BF00294177

62. Sheng JG, Boop FA, Mrak RE, Griffin WS. Increased neuronal beta-amyloid precursor protein expression in human temporal lobe epilepsy: association with interleukin-1 alpha immunoreactivity. *J Neurochem*. (1994) 63:1872–9. doi: 10.1046/j.1471-4159.1994.63051872.x

63. Tai XY, Koepp M, Duncan JS, Fox N, Thompson P, Baxendale S, et al. Hyperphosphorylated tau in patients with refractory epilepsy correlates with cognitive decline: a study of temporal lobe resections. *Brain J Neurol*. (2016) 139:2441–55. doi: 10.1093/brain/aww187

64. Thom M, Liu JYW, Thompson P, Phadke R, Narkiewicz M, Martinian L, et al. Neurofibrillary tangle pathology and Braak staging in chronic epilepsy in relation to traumatic brain injury and hippocampal sclerosis: a post-mortem study. *Brain J Neurol*. (2011) 134:2969–81. doi: 10.1093/brain/awr209

65. Roe CM, Fagan AM, Grant EA, Hassenstab J, Moulder KL, Maue Dreyfus D, et al. Amyloid imaging and CSF biomarkers in predicting cognitive impairment up to 7.5 years later. *Neurology*. (2013) 80:1784–91. doi: 10.1212/WNL.0b013e3182918ca6

66. Morris JC, Blennow K, Froelich L, Nordberg A, Soininen H, Waldemar G, et al. Harmonized diagnostic criteria for Alzheimer's disease: recommendations. *J Intern Med*. (2014) 275:204–13. doi: 10.1111/joim.12199

67. Romoli M, Sen A, Parnetti L, Calabresi P, Costa C. Amyloid-β: a potential link between epilepsy and cognitive decline. *Nat Rev Neurol*. (2021) 17:469–85. doi: 10.1038/s41582-021-00505-9

68. Crary JF, Trojanowski JQ, Schneider JA, Abisambra JF, Abner EL, Alafuzoff I, et al. Primary age-related tauopathy (PART): a common pathology associated with human aging. *Acta Neuropathol (Berl)*. (2014) 128:755–66. doi: 10.1007/s00401-014-1349-0

69. Therriault J, Vermeiren M, Servaes S, Tissot C, Ashton NJ, Benedet AL, et al. Association of phosphorylated tau biomarkers with amyloid positron emission tomography vs tau positron emission tomography. *JAMA Neurol*. (2023) 80:188–99. doi: 10.1001/jamaneurol.2022.4485

70. Barthélemy NR, Li Y, Joseph-Mathurin N, Gordon BA, Hassenstab J, Benzinger TLS, et al. A soluble phosphorylated tau signature links tau, amyloid and the evolution of stages of dominantly inherited Alzheimer's disease. *Nat Med*. (2020) 26:398–407. doi: 10.1038/s41591-020-0781-z

71. Hinman JD, Elahi F, Chong D, et al. Placental growth factor as a sensitive biomarker for vascular cognitive impairment. *Alzheimers Dement.* (2023) 19:3519–27. doi: 10.1002/alz.12974

72. Janelidze S, Zetterberg H, Mattsson N, Palmqvist S, Vanderstichele H, Lindberg O, et al. CSF Aβ42/Aβ40 and Aβ42/Aβ38 ratios: better diagnostic markers of Alzheimer disease. *Ann Clin Transl Neurol*. (2016) 3:154–65. doi: 10.1002/acn3.274

73. Preische O, Schultz SA, Apel A, Kuhle J, Kaeser SA, Barro C, et al. Serum neurofilament dynamics predicts neurodegeneration and clinical progression in presymptomatic Alzheimer's disease. *Nat Med*. (2019) 25:277–83. doi: 10.1038/s41591-018-0304-3

74. Giovannini G, Bedin R, Ferraro D, Vaudano AE, Mandrioli J, Meletti S. Serum neurofilament light as biomarker of seizure-related neuronal injury in status epilepticus. *Epilepsia*. (2022) 63:e23–9. doi: 10.1111/epi.17132

75. Ouédraogo O, Rébillard RM, Jamann H, Mamane VH, Clénet ML, Daigneault A, et al. Increased frequency of proinflammatory CD4 T cells and pathological levels of serum neurofilament light chain in adult drug-resistant epilepsy. *Epilepsia*. (2021) 62:176–89. doi: 10.1111/epi.16742

76. Nation DA, Sweeney MD, Montagne A, Sagare AP, D'Orazio LM, Pachicano M, et al. Blood-brain barrier breakdown is an early biomarker of human cognitive dysfunction. *Nat Med*. (2019) 25:270–6. doi: 10.1038/s41591-018-0297-y

77. Tabaee Damavandi P, Storti B, Fabin N, Bianchi E, Ferrarese C, DiFrancesco JC. Epilepsy in cerebral amyloid angiopathy: an observational retrospective study of a large population. *Epilepsia*. (2023) 64:500–10. doi: 10.1111/epi.17489

78. Liguori C, Spanetta M, Romoli M, Placidi F, Nardi Cesarini E, Mercuri NB, et al. Sleep disorders and late-onset epilepsy of unknown origin: understanding new trajectories to brain amyloidopathy. *Mech Ageing Dev*. (2021) 194:111434. doi: 10.1016/j.mad.2021.111434

79. Mendez MF. What is the relationship of traumatic brain injury to dementia? *J Alzheimers Dis*. (2017) 57:667–81. doi: 10.3233/JAD-161002

80. Hickman LB, Patel AB, Dubey I, Karimi AH, Zhang X, Janio EA, et al. Self-reported severity and causes of traumatic brain injury in patients with epileptic or functional seizures. *Neurol Clin Pract*. (2022) 12:e189–98. doi: 10.1212/CPJ.0000000000200098

81. Milikovsky DZ, Ofer J, Senatorov VV, Friedman AR, Prager O, Sheintuch L, et al. Paroxysmal slow cortical activity in Alzheimer's disease and epilepsy is associated with blood-brain barrier dysfunction. *Sci Transl Med*. (2019) 11:eaaw8954. doi: 10.1126/scitranslmed.aaw8954

82. Cummings J, Apostolova L, Rabinovici GD, Atri A, Aisen P, Greenberg S, et al. Lecanemab: Appropriate Use Recommendations. *J Prev Alzheimers Dis*. (2023) 10:362–77. doi: 10.14283/jpad.2023.30

83. Salloway S, Chalkias S, Barkhof F, Burkett P, Barakos J, Purcell D, et al. Amyloid-related imaging abnormalities in 2 phase 3 studies evaluating aducanumab in patients with early Alzheimer disease. *JAMA Neurol*. (2022) 79:13–21. doi: 10.1001/jamaneurol.2021.4161

84. Sims JR, Zimmer JA, Evans CD, Lu M, Ardayfio P, Sparks JD, et al. Donanemab in early symptomatic Alzheimer disease: the TRAILBLAZER-ALZ 2 randomized clinical trial. *JAMA*. (2023) 330:e2313239. doi: 10.1001/jama.2023.13239

85. Javed A, Cohen B, Detyniecki K, Hirsch LJ, Legge A, Chen B, et al. Rates and predictors of patient-reported cognitive side effects of antiepileptic drugs: an extended follow-up. *Seizure*. (2015) 29:34–40. doi: 10.1016/j.seizure.2015.03.013

86. Fleisher AS, Truran D, Mai JT, Langbaum JBS, Aisen PS, Cummings JL, et al. Chronic divalproex sodium use and brain atrophy in Alzheimer disease. *Neurology*. (2011) 77:1263–71. doi: 10.1212/WNL.0b013e318230a16c

87. Vossel K, Ranasinghe KG, Beagle AJ, la A, Ah Pook K, Castro M, et al. Effect of levetiracetam on cognition in patients with Alzheimer disease with and without epileptiform activity: a randomized clinical trial. *JAMA Neurol*. (2021) 78:1345–54. doi: 10.1001/jamaneurol.2021.3310

88. French JA, Perucca E, Sander JW, Bergfeldt L, Baulac M, Auerbach DS, et al. FDA safety warning on the cardiac effects of lamotrigine: an advisory from the Ad Hoc ILAE/AES Task Force. *Epilepsia Open*. (2021) 6:45–8. doi: 10.1002/epi4.12475

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# Alzheimer's disease and epilepsy: shared neuropathology guides current and future treatment strategies

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Epilepsy is a cause of profound disability in patients with Alzheimer's disease (AD). The risk of being diagnosed with AD increases the risk for epilepsy, and in parallel, a history of epilepsy increases the likelihood of the development of AD. This bi-directional relationship may be due to underlying shared pathophysiologic hallmarks, including decreased cerebrospinal fluid amyloid beta 42 (Aβ42), increased hyperphosphorylated tau protein, and hippocampal hyperexcitability. Additionally, there are practical treatment considerations in patients with co-morbid AD and epilepsy—namely, there is a higher risk of seizures associated with medications commonly prescribed for Alzheimer's disease patients, including antidepressants and antipsychotics such as trazodone, serotonin norepinephrine reuptake inhibitors (SNRIs), and first-generation neuroleptics. Anti-amyloid antibodies like aducanumab and lecanemab present new and unique considerations in patients with co-morbid AD and epilepsy given the risk of seizures associated with amyloid-related imaging abnormalities (ARIA) seen with this drug class. Finally, we identify and detail five active studies, including two clinical trials of levetiracetam in the respective treatment of cognition and neuropsychiatric features of AD, a study characterizing the prevalence of epilepsy in AD via prolonged EEG monitoring, a study characterizing AD biomarkers in late-onset epilepsy, and a study evaluating hyperexcitability in AD. These ongoing trials may guide future clinical decision-making and the development of novel therapeutics.

KEYWORDS

**Alzheimer's disease, epilepsy, seizures, cortical irritability, epileptiform discharges, management, treatment, therapeutic pipeline**

## Introduction

Alzheimer's disease (AD) and epilepsy exact a profound mental, emotional, and physical toll on patients and caregivers. AD and epilepsy affect 24 million and 50 million people worldwide, respectively (1). As many as 10–22% of patients living with AD will have at least one seizure, and about two-thirds of those patients will have recurrent seizures without a clear acute cause 24 or more hours apart, or epilepsy (2–4). The International League Against Epilepsy (ILAE) includes two other presentations under the umbrella of epilepsy: an unprovoked seizure and a probability of ≥60% of a recurrent seizure or an epilepsy syndrome. The ILAE also has an operational definition

of an epileptic seizure, used in this review, which is a transient manifestation of signs and/or symptoms due to abnormal excessive or synchronous activity of neurons in the brain (5). The National Institute of Aging-Alzheimer's Association workgroups have developed consensus criteria for probable AD dementia with an amnestic presentation, and it is diagnosed when patients: (1) meet criteria for dementia; (2) exhibit insidious onset; (3) have a clear-cut history of declining cognition; and (4) the initial and most prominent cognitive deficit is impairment in learning and recall of information that has been recently learned as well as cognitive dysfunction in one other cognitive domain, and this criteria is used herein. There are also non-amnestic presentations of atypical AD where other cognitive domains are impaired early and prominently (6).

The association between epilepsy and AD appears to be bi-directional, wherein a diagnosis of epilepsy is associated with approximately 2-fold greater odds of both subsequent all-cause dementia and AD based on a systematic review of 20 longitudinal studies (7). Additionally, in the same systematic review, both all-cause dementia and AD were associated with 3-fold greater odds of developing epilepsy (7). Vascular risk factors may modulate this effect; they may slightly decrease the risk of dementia in those with pre-existing seizures and, conversely, increase the risk of the development of seizures in those with a diagnosis of dementia (8). These neurologic conditions have shared pathophysiological features which may underlie this bi-directional association. Additionally, when they occur in concert, there are unique clinical and treatment considerations in these patients that providers need to consider and researchers continue to explore (Table 1).

## Methods

The authors completed a literature review of publications describing the relevant epidemiology, pathophysiology, semiology, and treatment considerations for patients with AD and epilepsy. Research studies were carefully reviewed and selected on the basis of their relevance to this topic (including seizures and Alzheimer's disease, epilepsy and Alzheimer's disease, epileptiform discharges and AD, epileptiform activity and AD, these syndromes and epidemiology, these syndromes and pathophysiology, and these syndromes and treatment). The search was completed using PubMed and Google Scholar with a particular emphasis on articles from the last 5 years and inclusion of older, important studies as well. A search of clinicaltrials.gov for clinical trials in the pipeline was also completed.

## Shared pathology of Alzheimer's disease and epilepsy

### Amyloid

Emerging evidence indicates that AD and epilepsy have shared neuropathological hallmarks. In one study, for example, 40 individuals with a mean age of 70 with late-onset epilepsy of unknown origin (LOEU), which is defined as epilepsy onset over the age of 65 without a clear secondary cause, had significantly lower levels of cerebrospinal fluid amyloid beta 42 (CSF Aβ42) compared to healthy age-matched controls (703.9 ± 388.3 pg./mL in LOEU vs. 975 ± 275 pg./mL in controls, expressed as mean ± standard deviation) (18, 19). Alzheimer's disease is similarly characterized by low levels of Aβ42 in the CSF, which is thought to reflect Aβ42 aggregation in amyloid plaques in the brain (20). In this study, Aβ42 levels lower than 500 pg./mL was considered pathologic (i.e., in the Alzheimer's range), so both those with epilepsy and those without remained, on average, in the normal range (19).

### Tau

In a clinicopathological study, researchers found that hyperphosphorylated tau pathology, as identified by immunohistochemical stains of tissue from temporal lobe resections, was associated with cognitive decline in 31 of 33 individuals with temporal lobe epilepsy (TLE). Hyperphosphorylated tau is the pathological isoform of tau protein that accumulates in the form of neurofibrillary tangles (NFTs) in AD. The participants, all of whom were between ages 50 and 65, underwent temporal lobe resection for refractory TLE. A heavier burden of tau pathology in tissue was associated with a greater decline in verbal learning, verbal recall, and naming, when comparing pre- and post-temporal lobe resection cognitive evaluations 1 year apart. Additionally, higher tau levels in tissue were also associated with a greater likelihood of secondary generalization prior to resection (21, 22). The fact that these individuals had lifelong epilepsy and temporal lobe resections may mean that these results do not generalize to most patients with AD who develop seizures, but the identification of phosphorylated tau in the brain tissue of patients with chronic epilepsy does underscore the potential shared neuropathology between patients with chronic epilepsy and those with AD. Indeed, several studies now suggest that epilepsy in AD and TLE share common neuropathological pathways (23).

In the largest database study to date of biochemical markers of AD and epilepsy, 17,901 patients with CSF tau and a diagnosis of Alzheimer's disease were identified; of these, 851 were also diagnosed with epilepsy. Patients in this study with both epilepsy and AD had higher levels of both total tau and phosphorylated tau in CSF compared with patients with AD who did not have co-morbid epilepsy. Additionally, CSF Aβ42 levels were lower in patients with both diagnoses compared with those with AD alone. These findings strongly suggest that a higher burden of AD pathology is associated with a higher risk of epilepsy and Alzheimer's disease dual diagnoses (24).

In a cohort study of 292 patients with AD and CSF testing who were followed for a mean of 5 years, almost 18% had a first-time seizure. In a univariate analysis, the development of seizures was associated with CSF total tau levels but not with CSF hyperphosphorylated tau or amyloid-β, unlike the database study detailed above. This may suggest greater cortical structural damage in patients with both AD and seizures compared with patients with AD who remain seizure-free. In a Cox regression, the probability of a seizure was associated with CSF total tau but not CSF hyperphosphorylated tau or amyloid-β, which may suggest tau-induced cortical irritability. Of note, this study was completed in a relatively young cohort for typical AD; the mean age of AD onset was 59 in the patients with seizures and almost 65 in those without seizures (25).

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TABLE 1 Summary of high-quality studies related to therapeutic considerations in patients with Alzheimer's disease and co-morbid seizures or epileptiform activity.

| Study | Study type | *N* | Age | *N* (%) epileptiform activity | *N* (%) seizures | Risks and outcomes |
|---|---|---|---|---|---|---|
| Vossel et al. (9) | Phase 2a crossover RCT for levetiracetam | 34 | 62.3 ± 7.7 | Crossover trial: Placebo-levetiracetam: 4 (26.7) Levetiracetam-placebo: 7 (41.2) | 0 | Levetiracetam treatment did not result in improvement in its primary outcome measure (test of executive function). Improved accuracy in exploratory analyses (spatial memory and executive functioning tasks) among participants with epileptiform activity |
| Meador et al. (10) | An RCT assessing the neurocognitive effects of brivaracetam, levetiracetam, and lorazepam in healthy volunteers | 16 | 18–50 | N/A | N/A | Lorazepam adversely affected the CNT score, a composite of EEG, evoked potentials and cognitive tests |
| Taipale et al. (11) | Case-control study to evaluate the association between regular ASM use and incident dementia | 20,325 (dementia of any type) 70,718 (AD) | 75.7 ± 6.7 (dementia of any type) 78.1 ± 7.1 (AD) | N/A | N/A | Regular use of phenobarbital, carbamazepine, phenytoin, valproate, primidone, barbexaclone, ethosuximide, clonazepam, zonisamide, and topiramate was associated with a greater risk of incident dementia and AD, 28 and 15%, respectively compared with controls not on AEDs |
| Cumbo and Ligori (12) | A prospective, randomized, three-arm parallel-group, case–control study for levetiracetam, phenobarbital, and lamotrigine | 95 | 60–90 | N/A | (Average N per month) LEV: 5.52 PB: 5.71 LTG 5.65 | Levetiracetam group exhibited increased MMSE scores and lamotrigine improved mood (Cornell scale for depression) |
| Hill et al. (13) | Cohort study to assess associations between antidepressant and seizures | 238,963 | 20–64 | N/A | 3,325 (1.39) | HR for seizures significantly increased for all antidepressant classes (highest risk: trazodone, lofepramine, and venlafaxine) |
| Chu et al. (14) | A case-control study to investigate the association between exposure to antidepressants and risk of epilepsy | 863 | 48.12 (18.56) (with epilepsy) | N/A | N/A | Patients with depression using SSRIs or SNRIs were two times more likely to develop first-time seizures compared with non-users. Risk of epilepsy increases with longer antidepressant treatment duration |
| Bloechliger et al. (15) | A nested case-control analysis of the association between antipsychotic drug use and the development of first-time seizures in patients with schizophrenia, affective disorders, or dementia | 60,121 | Unknown | N/A | N/A | Patients with dementia had significantly higher incidence rates of first-time seizures, compared with patients with other affective disorders. Drugs such as olanzapine or quetiapine increased risk of seizures |
| Hamberger et al. (16) | An RCT of donepezil to improve memory in epilepsy | 23 (with subjective memory concerns) | 18–55 | N/A | (Average N per month for donepezil) 2.6 (5.7) | Donepezil treatment did not result in improvement in seizure frequency or severity. Treatment also had no significant effect on cognitive scores |
| Ha et al. (17) | A population-based study for incident seizure in dementia (AD or vascular dementia) | 13,767 | 65–95 | N/A | N/A | A slight increase in seizure risk for patients receiving donepezil for 1 year compared to memantine |

RCT, randomized controlled trial; CNT, cognitive neuropsychological test; ASM, antiseizure medication; MMSE, Mini-Mental State Examination; HR, hazard ratio; AD, Alzheimer's disease.

### Brain atrophy

In terms of morphology, a study of 73 participants over the age of 55 with TLE demonstrated a analogous pattern and degree of atrophy of the medial temporal lobes compared to individuals with amnestic mild cognitive impairment (aMCI), an at risk stage for AD dementia (23). Both TLE and aMCI groups showed significant impairment in memory encoding, naming, and category fluency relative to healthy controls (26). These morphologic changes in patients with TLE may lower cognitive reserve and partially explain the increased predisposition of patients with epilepsy to get AD.

Thus, epilepsy, particularly in late life, and AD may share common underlying neuropathology which can serve as a target for therapeutic approaches for both diseases, as detailed in the sections on management below.

## Epileptiform discharges and high frequency oscillations in Alzheimer's disease

### Epileptiform discharges

Subclinical epileptiform activity (SEA) refers to the presence of epileptiform discharges, specifically spikes or sharp waves, in patients without known seizures (27). There is substantial variability in the published prevalence rates of patients living with AD who have SEA (between 3 and 54%) (28). In one study, SEA was assessed in 19 cognitively normal controls and 33 age-matched participants with a mean age of 62 years who met criteria for AD and did not have a history of seizures. SEA was detected in 42% of participants with AD, which was more than four times the detection rate in controls. Of note, 90% of epileptiform discharges in individuals with AD were detected during EEG recordings of sleep. Over an average of 3.3 years, participants with SEA declined significantly on the Mini-Mental State Examination (MMSE) by 3.9 points per year (vs. 1.6 points/year in patients with AD who did not have SEA) and on an executive function composite (29).

A growing body of literature has also evaluated interictal epileptiform activity (IEA), which refers to epileptiform discharges between seizures, and its prevalence in AD. In one study evaluating IEA prevalence, 10 of 48 patients with AD evaluated with 24-h EEG were diagnosed with seizures, and 80% of those patients had IEA (30). In a small case series of two patients with AD who wore foramen ovale electrodes near the temporal lobes, the electrodes detected epileptiform discharges and silent hippocampal seizures during sleep. These patients did not have a clinical history of seizures, and sleep is considered a critical period for memory consolidation. The authors suggest that hippocampal hyperexcitability may contribute to the pathophysiology of AD, but this remains to be proven (31).

It remains unclear whether addressing IEA or SEA may help in the treatment or prevention of cognitive decline in AD, but like the authors of the case series above, some advocate for considering IEA and SEA part of the pathophysiological influences that drive cognitive impairment in AD. Hypothesized mechanisms include a compromised glutamatergic system, excitotoxicity-induced neurodegeneration, accelerated amyloid and tau deposition driven by epileptiform discharges, remodeling due to hyperexcitability resulting in disconnection of functional networks, and alteration of sleep structure, among others (32). Because patients with AD who have SEA experience faster worsening of executive function and global measures of cognition than those without, one phase 2a trial looked at the effect of levetiracetam on 34 participants with probable AD. The study, known as the Levetiracetam for Alzheimer's Disease–Associated Network Hyperexcitability (LEV-AD) trial, failed to meet its primary and secondary endpoints; the former was a test of executive function and the latter were tests of cognition and function. However, in a prespecified exploratory analysis of participants with seizures or SEA, they found that treatment improved two of seven measures tested—a test of executive function (among 9 participants tested) and one of spatial memory (5 participants) (9). Moreover, recent findings indicate that neuronal hyperexcitability in patients with AD may be initiated by suppression of glutamate reuptake, which may suggest a novel therapeutic pathway for SEA in AD (33).

### High frequency oscillations

In addition to SEA and seizures, high frequency oscillations (HFOs) may also be seen on EEG in AD. Most EEG activity falls below a frequency of 30 Hz, but "fast ripples" between 250 and 500 Hz generally only occur at the time of seizure onset in patients with epilepsy—these are termed HFOs (34). In a recent study exploring these phenomena in AD, HFOs were detected in the hippocampi of all 3 AD mouse models evaluated but not in age-matched controls. Although human studies are needed, this novel EEG abnormality in AD may serve as a spatial biomarker for epileptogenicity in patients with AD and may suggest risk of AD development in patients with epilepsy (35).

## Seizure semiology and clinical course in Alzheimer's disease

The predominant seizure type in patients with AD is focal non-motor onset seizures with impaired awareness (29, 36). These seizures may be characterized by an aura (e.g., déjà vu, unexplained emotions, and/or sensory phenomena), impairment in consciousness, and other common seizure semiology (e.g., staring, speech arrest, or memory loss). Patients with AD can also have generalized tonic–clonic seizures, as well. In 10 early onset Alzheimer's disease (EOAD) patients with epilepsy, seizure types included generalized onset tonic-clonic seizures (25%), temporal lobe seizures (25%), myoclonus (25%), focal onset extra-temporal seizures (8%), and other types (17%) (37).

In a study that evaluated National Alzheimer's Coordinating Center (NACC) data to determine the clinical course of seizures in patients with AD, the authors identified a 70% seizure recurrence rate within an average of 8 months of follow-up. Patients with AD and seizures had an earlier onset of cognitive impairment (mean age 65) compared to patients with AD who did not have concomitant seizures (mean age 70). The risk of seizures among patients with AD increased by an average of 0.64% per year (38). A comprehensive review of medical records that identified 1,320 patients with concomitant AD and unprovoked seizures similarly identified an increased seizure risk in patients with an early age of onset of AD and identified this relationship with myoclonus as well. Additionally, the probability of myoclonus increased gradually over time in individuals with AD. Seizures and myoclonus often co-occurred, and the authors

suggest that the presence of myoclonus can guide earlier detection of seizures (39).

## Cognitive impairment due to epilepsy

Cognitive impairment associated with epilepsy can occur independently of AD. This has implications for providers monitoring for cognitive decline in patients with AD who develop epilepsy and in patients with AD where cognitive decline is progressing more steeply than anticipated. Studies show that cognitive scores for 257 participants aged 12–62 years with epilepsy for an average of 7 years were lower for the Montreal Cognitive Assessment (MoCA) and the Clinical Memory Scale compared to healthy controls (40, 41). In a study examining the association between late-onset epilepsy (LOE) and changes in cognitive performance over 25 years, 585 participants (average age 59.4 years) with LOE showed significant cognitive decline in global cognition, verbal memory, executive function, and word fluency compared with healthy non-LOE participants (42). Unsurprisingly, adult patients with temporal lobe epilepsy have more impaired episodic memory compared with those with other regional epilepsy syndromes (43). On the other hand, frontal lobe epilepsy often affects executive function and working memory long-term; additionally, in a systematic review of 35 studies, there was an association between cognitive changes and psychiatric symptoms in nearly 35% of participants with frontal lobe epilepsy (44, 45).

## Use of antiseizure medications in patients with comorbid Alzheimer's disease and epilepsy

Lamotrigine and levetiracetam are both in common use for the treatment of seizures in patients with Alzheimer's disease (46–48). Given promising results in preclinical rodent models, levetiracetam has also been evaluated as a treatment for cognitive impairment in participants with AD in the LEV-AD study detailed above. The study included participants with and without seizures and/or epileptiform discharges. As mentioned, there was no difference between treatment and placebo groups on the primary or secondary outcome measures of cognition and function. Treatment did appear to improve executive function and spatial memory, however, in an exploratory subgroup analysis of those with epileptiform discharges or seizures (9).

Of note, a randomized, double-blind, placebo-controlled study of 16 healthy participants aged 18–50 comparing acute dosing of brivaracetam 10 mg, levetiracetam 500 mg, lorazepam 2 mg, and placebo determined that lorazepam adversely affected the cognitive neurophysiologic test (CNT) score, which is a combination of EEG monitoring, evoked potential recordings, and cognitive performance measures. Brivaracetam did not differ from placebo or levetiracetam on any cognitive measures (10). An important association has been identified in Finnish and German registries between regular use of certain antiseizure medications (ASMs), namely phenobarbital, carbamazepine, phenytoin, valproate, primidone, barbexaclone, ethosuximide, clonazepam, zonisamide, and topiramate, and a greater risk of incident all-cause dementia (28%) and AD (15%). This analysis was adjusted for multiple confounders including a history of epilepsy. When the above medications were compared with a separate set of ASMs, including levetiracetam, oxcarbazepine, lamotrigine, gabapentin, vigabatrin, pregabalin, tiagabine, and lacosamide, the risk of dementia and AD was higher in the former group (11). Additional studies are needed to determine whether a causal inference can be made. In a study of 95 patients with AD and epilepsy, levetiracetam had fewer adverse effects than lamotrigine or phenobarbital. Of note, levetiracetam surprisingly increased MMSE scores (albeit by a modest 0.23 points), and lamotrigine improved mood (12). However, results have varied. A recent retrospective study compared 19 patients with epilepsy and mild cognitive impairment due to AD to 16 patients with MCI due to AD who did not have epilepsy. Nearly 90% of the patients with epilepsy were well-controlled with monotherapy, with seizure control defined as >50% seizure reduction. However, patients required an average of 2 lines of therapy due to adverse events or lack of seizure control. The top two main ASMs based on tolerability and efficacy were lamotrigine in 9 patients and lacosamide in 3 patients. In contrast to the above referenced study, levetiracetam was discontinued in 5 of 5 patients in this group due to adverse events including mood changes, mental slowing, asthenia, apathy, and aggressiveness (49).

## Effect of commonly used treatments in Alzheimer's disease on seizure threshold

### Antidepressants

The prevalence of depression in AD approaches 15%, and antidepressants are commonly prescribed (50). The use of antidepressant drugs may increase risk of epilepsy, and there are unique treatment considerations in patients with co-morbid AD and epilepsy. In a study of 238,963 patients with a diagnosis of depression (age 20–64) taking antidepressants, the hazard ratio for seizures for all antidepressant drug classes significantly increased. Trazodone, lofepramine, and venlafaxine carried the highest risk compared to no treatment (13). A case-control study of 151,005 patients with depression who were prescribed selective serotonin reuptake inhibitors (SSRIs) or selective norepinephrine reuptake inhibitors (SNRIs) were two times more likely to be diagnosed with first-time seizures compared with non-users. Use of low-dose tricyclic antidepressants was not associated with seizures (14). However, tricyclics are often avoided in patients with AD due to their anticholinergic properties. Longer treatment duration with antidepressants is also associated with higher epilepsy risk (14). Thus, in patients with AD, epilepsy, and depression, an SSRI may be the safest pharmacologic option for treatment of depression with careful reassessment of the need for ongoing treatment at regular intervals.

### Antipsychotics

Although there is an FDA black box warning for the use of antipsychotics in patients with AD dementia, they are sometimes necessary when verbal and physical agitation or delusions cannot be redirected, do not respond to alternate therapies, and present a safety concern. Patients with dementia using olanzapine, quetiapine, low-to-medium potency first-generation antipsychotics, and medium-to-high potency first-generation antipsychotics have a higher risk of

seizures compared to their counterparts who are not on these medications. In contrast, the use of amisulpride, aripiprazole, risperidone, or sulpiride does not have an association with increased seizure risk (15). In patients who co-morbid AD and epilepsy who require a medication in the antipsychotic class, these latter options may be optimal; however, the presence of parkinsonism may nonetheless prompt consideration of medications like quetiapine to lower the risk of extrapyramidal side effects.

## Acetylcholinesterase inhibitors

Acetycholinesterase inhibitors, including donepezil, rivastigmine, and galantamine, are commonly prescribed for the treatment of cognitive impairment in mild to severe dementia due to AD. There is a loss of cholinergic innervation from the basal forebrain to the cortex in patients with AD, and this forms the basis of the "cholinergic hypothesis" that some of the cognitive and behavioral symptoms in patients with AD are due to loss of cholinergic inputs that can be ameliorated with acetylcholinesterase inhibitors (51, 52). In a randomized, double-blind, placebo-controlled trial of donepezil to improve memory in epilepsy, 23 patients with epilepsy (ages 18–55) with subjective cognitive impairment were randomized to 3 months of donepezil (10 mg/day) or 3 months of placebo treatment. Each arm then crossed over to the other treatment group. The inclusion criteria included patients with definite epilepsy, the use of ASMs for epilepsy, and reports of memory concerns at the time of enrollment. Donepezil treatment did not result in a change in seizure frequency or severity. Additionally, treatment did not result in any significant changes in memory scores or other cognitive scores (16). In a separate population-based study, 13,767 participants aged 65–95 years who experienced incident seizures with dementia (Alzheimer's dementia or vascular dementia) and prescribed donepezil, rivastigmine, galantamine, or memantine, there was a slight increase in seizure risk for patients receiving donepezil for 1 year compared to memantine. The mechanism is unclear, but off-target reductions in cortical dopamine and serotonin have been proposed (17).

## N-methyl-D-aspartate receptor antagonists

Memantine, an N-methyl-D-aspartate (NMDA) receptor antagonist commonly used in the treatment of moderate to severe dementia due to AD, has previously been shown to reduce seizure severity and duration at certain doses in rodents while inducing seizures in rats with kindled amygdalae at higher doses. As mentioned above, memantine seems to have a better profile in terms of seizure risk compared with donepezil (17).

## Anti-amyloid antibodies

Aducanumab and lecanemab are anti-amyloid antibodies that have both been granted FDA approval for the treatment of Alzheimer's disease; the former was granted accelerated approval, and the latter was granted full approval. In the phase 3 clinical trials EMERGE and ENGAGE for aducanumab, 10.6% of patients who received the high 10 mg/kg dose had recurrent amyloid-related imaging abnormalities (ARIA) most of whom were asymptomatic. Seizures attributed to ARIA were reported in 0.4% of patients treated with the high dose. The overall incidence of seizures was balanced between the aducanumab and placebo groups (53, 54). In a phase 2 study of lecanemab, there was a single case of ARIA with edema (ARIA-E) associated with seizure (55). The phase 3 CLARITY AD trial for lecanemab did not mention seizures (56). Appropriate use recommendations for lecanemab mention that this drug should not be prescribed in patients with seizures since Clarity AD excluded patients who had seizures within the 12 months prior to screening (57). Another anti-amyloid antibody, donanemab, has demonstrated clinical efficacy in AD but is not yet FDA approved. Like aducanemab and lecanemab, donanemab carries the risk of ARIA, which can lead to seizures (58).

## Anti-tau antibodies

Since epileptogenesis in AD is hypothesized to be at least partly tau-mediated, anti-tau monoclonal antibodies hold theoretical promise as a treatment for co-morbid AD and epilepsy. Currently, there are no FDA approved anti-tau antibodies for AD. To date, monoclonal antibodies targeting tau in individuals with AD, including semorinemab, gosuranemab, tilavonemab, and zagotenemab, have failed in major clinical trials (59). In a mouse model of genetic tau reduction in aged mice, tau reduction increased resistance to seizure (60). This work suggests that treatments targeting tau present a critical future direction for research focusing on treatments that dampen hyperexcitability in AD.

# Active clinical studies and future directions

Ongoing trials aim to further explore the effects of levetiracetam on seizures and abnormal discharges in AD. Levetiracetam for Alzheimer's Disease Neuropsychiatric Symptoms Related to Epilepsy Trial (LAPSE) is a phase 2 study wherein investigators intend to recruit 65 participants with probable AD and epileptiform activity identified on EEG and exclude participants previously diagnosed with epilepsy. Participants will take 500 mg twice a day for 1 year and will complete up to 3 serial EEGs. The primary outcome measure for this study is change in the Neuropsychiatric Inventory Score, or NPI (NCT04004702). Another clinical trial in the pipeline is an Investigation of Levetiracetam in Alzheimer's Disease (ILiAD), and the primary outcome is a computerized hippocampus-dependent memory-binding test. This study will randomize 30 participants 50 years and older diagnosed with mild to moderate AD and will exclude participants with a diagnosis of epilepsy. The treatment arm consists of uptitrating levetiracetam by 250 mg at one-week intervals to 1 g twice daily for 4 weeks followed by downtitration over 4 weeks (NCT03489044).

There are a few additional proposed prospective studies that aim to characterize patients with both Alzheimer's disease and epilepsy. The Prevalence of Epilepsy and Sleep Wake Disorders in Alzheimer Disease (PESAD) study aims to perform 48-h ambulatory scalp EEGs and polysomnograms in 100 individuals with AD and 30 gender- and age-matched healthy individuals for early detection of epilepsy and sleep–wake disturbances. Fifteen participants with AD who have epileptic spikes or sleep–wake disorders will undergo invasive EEG

monitoring to evaluate for the presence of hippocampal seizures. Current and future findings may support whether early development of hippocampal hyperexcitability is a precursor to cognitive decline in AD (NCT03617497).

Since there is overlap in AD and epilepsy pathogenesis, studies are also examining the predictive value of biomarkers. One trial examining the profile of CSF biomarkers in AD, which is called the Predictive Value of Biomarkers of Alzheimer's Disease in Elderly Patients with New-onset Epilepsy (BIOMALEPSIE) study, aims to recruit 35 cognitively normal patients older than 60 years with new-onset epilepsy. Investigators hypothesize that elderly participants with new epilepsy diagnoses will have more amyloid pathology than their healthy counterparts (NCT02861846). A final upcoming study aims to explore the prevalence of SEA in the hippocampus in patients with CSF-proven MCI due to AD compared to healthy controls and track its role in clinical progression over 2 years (EADP study, NCT04131491).

## Discussion

Patients diagnosed with AD have a higher risk of seizures compared with counterparts without AD, and patients diagnosed with epilepsy are at increased risk of AD. This bi-directional relationship may be explained by the shared neuropathology of AD and epilepsy, including a decrease in Aβ42 in CSF and an increase in hyperphosphorylated tau protein (19, 21, 22). There is also limited evidence for hippocampal hyperactivity in AD, which may negatively affect memory consolidation (31, 61). Epilepsy is associated with cognitive impairment on its own, and the dual diagnoses of AD and epilepsy may compound the cognitive decline characteristic of AD. Researchers have considered whether cognitive impairment in AD could be treated with ASMs, but a small study of levetiracetam in participants did not result in a cognitive benefit unless participants had epileptiform activity (9). Nonetheless, that study may have been limited by its small size, difficulties with recruitment, and heterogenous patient population in terms of the presence or absence of epileptiform activity. Given these limitations, the door remains open for an antiseizure drug to provide meaningful clinical benefit in patients with AD, and there are currently two active trials examining the effects of levetiracetam on neuropsychiatric features and cognitive impairment in AD via the LAPSE and ILiAD trials (NCT04004702, NCT03489044).

Several medications that are frequently prescribed for patients with Alzheimer's disease can affect seizure threshold, and thus, there are unique treatment considerations in patients with co-morbid epilepsy and AD. Among antidepressants, trazodone, lofepramine, and venlafaxine may be most likely to lower seizure threshold. Among neuroleptics, olanzapine, quetiapine, and first-generation antipsychotic drugs are most likely to increase seizure risk and may need to be avoided in patients with both AD and epilepsy. Also, there is some limited evidence that the rapid withdrawal of acetylcholinesterase inhibitors may lower seizure threshold. Understanding the impact of these commonly used medications on seizure risk can guide clinical decisions for patients with co-morbid AD and epilepsy. Anti-amyloid monoclonal antibodies are not recommended for use in patients with seizure activity, particularly in patients with seizures within the year prior to initiation of therapy, given the risk of seizures associated with ARIA in drugs in this class. However, the study of anti-amyloid antibodies in combination with ASMs represents a potential, as yet unexplored direction for AD with co-morbid epilepsy. Anti-tau antibodies also hold theoretical promise in this patient population.

The ongoing PESAD study seeks to identify the prevalence of epileptiform discharges and seizures in AD via prolonged EEG monitoring, the BIOMALEPSIE trial aims to understand the AD biomarker profile of patients with late-onset epilepsy (NCT03617497, NCT04131491), and the EADP study will characterize SEA in patients with MCI due to AD. These studies are essential given that a better understanding of the underlying shared mechanisms of AD and epilepsy can be used to guide the development of novel therapies in the clinical pipeline.

## Author contributions

OL, TK, and IS-S equally contributed to the drafting and final version of the entire manuscript. All authors contributed to the article and approved the submitted version.

## Conflict of interest

IS-S has received salary support for clinical trials research from Eli Lilly, Eisai, Biogen, Janssen, Novartis, AbbVie, Genentech/Roche, Cortexyme, UCB Biopharma, Alzheon, and Alector.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note



## References

1. Mayeux R, Stern Y. Epidemiology of Alzheimer disease. *Cold Spring Harb Perspect Med*. (2012) 2:a006239. doi: 10.1101/cshperspect.a006239

2. Friedman D, Honig LS, Scarmeas N. Seizures and epilepsy in Alzheimer's disease. *CNS Neurosci Ther*. (2011) 18:285–94. doi: 10.1111/j.1755-5949.2011.00251.x

3. Hauser WA, Morris ML, Heston LL, Anderson VE. Seizures and myoclonus in patients with Alzheimer's disease. *Neurology*. (1986) 36:1226–6. doi: 10.1212/WNL.36.9.1226

4. Mendez MF, Catanzaro P, Doss RC, Arguello R, Frey WH. Seizures in Alzheimer's disease: Clinicopathologic study. *J Geriatr Psychiatry Neurol*. (1994) 7:230–3. doi: 10.1177/089198879400700407

5. Falco-Walter JJ, Scheffer IE, Fisher RS. The new definition and classification of seizures and epilepsy. *Epilepsy Res*. (2018) 139:73–9. doi: 10.1016/j.eplepsyres.2017.11.015

6. McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack CR Jr, Kawas CH, et al. The diagnosis of dementia due to Alzheimer's disease: recommendations from the

National Institute on Aging-Alzheimer's association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement*. (2011) 7:263–9. doi: 10.1016/j.jalz.2011.03.005

7. Dun C, Zhang Y, Yin J, Su B, Peng X, Liu L. Bi-directional associations of epilepsy with dementia and Alzheimer's disease: a systematic review and meta-analysis of longitudinal studies. *Age Ageing*. (2022) 51:afac010. doi: 10.1093/ageing/afac010

8. Pandis D, Scarmeas N. Seizures in Alzheimer disease: clinical and epidemiological data. *Epilepsy Curr*. (2012) 12:184–7. doi: 10.5698/1535-7511-12.5.184

9. Vossel K, Ranasinghe KG, Beagle AJ, La A, Ah Pook K, Castro M, et al. Effect of Levetiracetam on cognition in patients with Alzheimer disease with and without epileptiform activity: a randomized clinical trial. *JAMA Neurol*. (2021) 78:1345–54. doi: 10.1001/jamaneurol.2021.3310

10. Meador KJ, Gevins A, Leese PT, Otoul C, Loring DW. Neurocognitive effects of brivaracetam, levetiracetam, and lorazepam. *Epilepsia*. (2011) 52:264–72. doi: 10.1111/j.1528-1167.2010.02746.x

11. Taipale H, Gomm W, Broich K, Maier W, Tolppanen A-M, Tanskanen A, et al. Use of antiepileptic drugs and dementia risk-an analysis of Finnish health register and German health insurance data. *J Am Geriatr Soc*. (2018) 66:1123–9. doi: 10.1111/jgs.15358

12. Cumbo E, Ligori LD. Levetiracetam, lamotrigine, and phenobarbital in patients with epileptic seizures and Alzheimer's disease. *Epilepsy Behav*. (2010) 17:461–6. doi: 10.1016/j.yebeh.2010.01.015

13. Hill T, Coupland C, Morriss R, Arthur A, Moore M, Hippisley-Cox J. Antidepressant use and risk of epilepsy and seizures in people aged 20 to 64 years: cohort study using a primary care database. *BMC Psychiatry*. (2015) 15:315. doi: 10.1186/s12888-015-0701-9

14. Chu C-S, Lee F-L, Bai Y-M, Su T-P, Tsai S-J, Chen T-J, et al. Antidepressant drugs use and epilepsy risk: a nationwide nested case-control study. *Epilepsy Behav*. (2023) 140:109102. doi: 10.1016/j.yebeh.2023.109102

15. Bloechliger M, Rüegg S, Jick SS, Meier CR, Bodmer M. Antipsychotic drug use and the risk of seizures: follow-up study with a nested case–control analysis. *CNS Drugs*. (2015) 29:591–603. doi: 10.1007/s40263-015-0262-y

16. Hamberger MJ, Palmese CA, Scarmeas N, Weintraub D, Choi H, Hirsch LJ. A randomized, double-blind, placebo-controlled trial of donepezil to improve memory in epilepsy. *Epilepsia*. (2007) 48:1283–91. doi: 10.1111/j.1528-1167.2007.01114.x

17. Ha J, Son N-H, Park YH, Lee E, Kim E, Jung KW. Association of cognitive enhancers and incident seizure risk in dementia: a population-based study. *BMC Geriatr*. (2022) 22:480. doi: 10.1186/s12877-022-03120-5

18. Josephson CB, Engbers JDT, Sajobi TT, Jette N, Agha-Khani Y, Federico P, et al. Towards a clinically informed, data-driven definition of elderly onset epilepsy. *Epilepsia*. (2016) 57:298–305. doi: 10.1111/epi.13266

19. Costa C, Romoli M, Liguori C, Farotti L, Eusebi P, Bedetti C, et al. Alzheimer's disease and late-onset epilepsy of unknown origin: two faces of beta amyloid pathology. *Neurobiol Aging*. (2019) 73:61–7. doi: 10.1016/j.neurobiolaging.2018.09.006

20. Fagan AM, Mintun MA, Mach RH, Lee S-Y, Dence CS, Shah AR, et al. Inverse relation between *in vivo* amyloid imaging load and cerebrospinal fluid Aβ42 in humans. *Ann Neurol*. (2006) 59:512–9. doi: 10.1002/ana.20730

21. Paudel YN, Angelopoulou E, Jones NC, O'Brien TJ, Kwan P, Piperi C, et al. Tau related pathways as a connecting link between epilepsy and Alzheimer's disease. *ACS Chem Neurosci*. (2019) 10:4199–212. doi: 10.1021/acschemneuro.9b00460

22. Tai XY, Koepp M, Duncan JS, Fox N, Thompson P, Baxendale S, et al. Hyperphosphorylated tau in patients with refractory epilepsy correlates with cognitive decline: a study of temporal lobe resections. *Brain*. (2016) 139:2441–55. doi: 10.1093/brain/aww187

23. Noebels J. A perfect storm: converging paths of epilepsy and Alzheimer's dementia intersect in the hippocampal formation. *Epilepsia*. (2011) 52:39–46. doi: 10.1111/j.1528-1167.2010.02909.x

24. Banote RK, Håkansson S, Zetterberg H, Zelano J. CSF biomarkers in patients with epilepsy in Alzheimer's disease: a nation-wide study. Brain. *Communications*. (2022) 4:fcac210. doi: 10.1093/braincomms/fcac210

25. Tábuas-Pereira M, Durães J, Lopes J, Sales F, Bento C, Duro D, et al. Increased CSF tau is associated with a higher risk of seizures in patients with Alzheimer's disease. *Epilepsy Behav*. (2019) 98:207–9. doi: 10.1016/j.yebeh.2019.06.033

26. Kaestner E, Reyes A, Chen A, Rao J, Macari AC, Choi JY, et al. Atrophy and cognitive profiles in older adults with temporal lobe epilepsy are similar to mild cognitive impairment. *Brain*. (2021) 144:236–50. doi: 10.1093/brain/awaa397

27. Zhao B, Shen L-X, Ou Y-N, Ma Y-H, Dong Q, Tan L, et al. Risk of seizures and subclinical epileptiform activity in patients with dementia: a systematic review and meta-analysis. *Ageing Res Rev*. (2021) 72:101478. doi: 10.1016/j.arr.2021.101478

28. Csernus EA, Werber T, Kamondi A, Horvath AA. The significance of subclinical epileptiform activity in Alzheimer's disease: a review. *Front Neurol*. (2022) 13:856500. doi: 10.3389/fneur.2022.856500

29. Vossel KA, Beagle AJ, Rabinovici GD, Shu H, Lee SE, Naasan G, et al. Seizures and epileptiform activity in the early stages of Alzheimer disease. *JAMA Neurol*. (2013) 70:1158–66. doi: 10.1001/jamaneurol.2013.136

30. Horváth A, Szűcs A, Hidasi Z, Csukly G, Barcs G, Kamondi A. Prevalence, semiology, and risk factors of epilepsy in Alzheimer's disease: an ambulatory EEG study. *J Alzheimers Dis*. (2018) 63:1045–54. doi: 10.3233/JAD-170925

31. Lam AD, Deck G, Goldman A, Eskandar EN, Noebels J, Cole AJ. Silent hippocampal seizures and spikes identified by foramen ovale electrodes in Alzheimer's disease. *Nat Med*. (2017) 23:678–80. doi: 10.1038/nm.4330

32. Horvath AA, Csernus EA, Lality S, Kaminski RM, Kamondi A. Inhibiting epileptiform activity in cognitive disorders: possibilities for a novel therapeutic approach. *Front Neurosci*. (2020) 14:557416. doi: 10.3389/fnins.2020.557416

33. Zott B, Simon MM, Hong W, Unger F, Chen-Engerer H-J, Frosch MP, et al. A vicious cycle of β amyloid–dependent neuronal hyperactivation. *Science*. (2019) 365:559–65. doi: 10.1126/science.aay0198

34. Gotman J. High frequency oscillations: the new EEG frontier? *Epilepsia*. (2010) 51:63–5. doi: 10.1111/j.1528-1167.2009.02449.x

35. Lisgaras CP, Scharfman HE. High-frequency oscillations (250–500 Hz) in animal models of Alzheimer's disease and two animal models of epilepsy. *Epilepsia*. (2023) 64:231–46. doi: 10.1111/epi.17462

36. Vossel KA, Tartaglia MC, Nygaard HB, Zeman AZ, Miller BL. Epileptic activity in Alzheimer's disease: causes and clinical relevance. *Lancet Neurol*. (2017) 16:311–22. doi: 10.1016/S1474-4422(17)30044-3

37. Haoudy S, Jonveaux T, Puisieux S, Epstein J, Hopes L, Maillard L, et al. Epilepsy in early onset Alzheimer's disease. *J Alzheimers Dis*. (2022) 85:615–26. doi: 10.3233/JAD-210681

38. Vöglein J, Ricard I, Noachtar S, Kukull WA, Dieterich M, Levin J, et al. Seizures in Alzheimer's disease are highly recurrent and associated with a poor disease course. *J Neurol*. (2020) 267:2941–8. doi: 10.1007/s00415-020-09937-7

39. Beagle AJ, Darwish SM, Ranasinghe KG, La AL, Karageorgiou E, Vossel KA. Relative incidence of seizures and myoclonus in Alzheimer's disease, dementia with Lewy bodies, and frontotemporal dementia. *J Alzheimers Dis*. (2017) 60:211–23. doi: 10.3233/JAD-170031

40. Wang L, Chen S, Liu C, Lin W, Huang H. Factors for cognitive impairment in adult epileptic patients. *Brain Behav*. (2019) 10:e01475. doi: 10.1002/brb3.1475

41. Novak A, Vizjak K, Gacnik A, Rakusa M. Cognitive impairment in people with epilepsy: Montreal cognitive assessment (MoCA) as a screening tool. *Acta Neurol Belg*. (2023) 123:451–6. doi: 10.1007/s13760-022-02046-4

42. Johnson EL, Krauss GL, Walker KA, Brandt J, Kucharska-Newton A, Mosley TH, et al. Late-onset epilepsy and 25-year cognitive change: the atherosclerosis risk in communities (ARIC) study. *Epilepsia*. (2020) 61:1764–73. doi: 10.1111/epi.16616

43. Bell B, Lin JJ, Seidenberg M, Hermann B. The neurobiology of cognitive disorders in temporal lobe epilepsy. *Nat Rev Neurol*. (2011) 7:154–64. doi: 10.1038/nrneurol.2011.3

44. Gold JA, Sher Y, Maldonado JR. Frontal lobe epilepsy: a primer for psychiatrists and a systematic review of psychiatric manifestations. *Psychosomatics*. (2016) 57:445–64. doi: 10.1016/j.psym.2016.05.005

45. Gul A, Ahmad H. Thought suppression predicts task switching deficits in patients with frontal lobe epilepsy. *Neurosci J*. (2015) 20:153–8. doi: 10.17712/nsj.2015.2.20140652

46. Suzuki H, Gen K. Clinical efficacy of lamotrigine and changes in the dosages of concomitantly used psychotropic drugs in Alzheimer's disease with behavioural and psychological symptoms of dementia: a preliminary open-label trial. *Psychogeriatrics*. (2015) 15:32–7. doi: 10.1111/psyg.12085

47. Musaeus CS, Shafi MM, Santarnecchi E, Herman ST, Press DZ. Levetiracetam alters oscillatory connectivity in Alzheimer's disease. *J Alzheimers Dis*. (2017) 58:1065–76. doi: 10.3233/JAD-160742

48. Giorgi FS, Guida M, Vergallo A, Bonuccelli U, Zaccara G. Treatment of epilepsy in patients with Alzheimer's disease. *Expert Rev Neurother*. (2017) 17:309–18. doi: 10.1080/14737175.2017.1243469

49. Hautecloque-Raysz G, Sellal F, Bousiges O, Phillipi N, Blanc F, Cretin B. Epileptic prodromal Alzheimer's disease treated with Antiseizure medications: medium-term outcome of seizures and cognition. *J Alzheimers Dis*. (2023) Preprint:1–18) 94:1057–74. doi: 10.3233/JAD-221197

50. Asmer MS, Kirkham J, Newton H, Ismail Z, Elbayoumi H, Leung RH, et al. Meta-analysis of the prevalence of major depressive disorder among older adults with dementia. *J Clin Psychiatry*. (2018) 79:17r11772. doi: 10.4088/JCP.17r11772

51. Burns A, Rossor M, Hecker J, Gauthier S, Petit H, Möller H-J, et al. The effects of donepezil in Alzheimer's disease – results from a multinational Trial1. *Dement Geriatr Cogn Disord*. (1999) 10:237–44. doi: 10.1159/000017126

52. Seltzer B. Donepezil: a review. *Expert Opin Drug Metab Toxicol*. (2005) 1:527–36. doi: 10.1517/17425255.1.3.527

53. Salloway S, Chalkias S, Barkhof F, Burkett P, Barakos J, Purcell D, et al. Amyloid-related imaging abnormalities in 2 phase 3 studies evaluating Aducanumab in patients with early Alzheimer disease. *JAMA Neurol*. (2022) 79:13–21. doi: 10.1001/jamaneurol.2021.4161

54. Cummings J, Rabinovici GD, Atri A, Aisen P, Apostolova LG, Hendrix S, et al. Aducanumab: appropriate use recommendations update. *J Prev Alzheimers Dis*. (2022) 9:221–30. doi: 10.14283/jpad.2022.34

55. Swanson CJ, Zhang Y, Dhadda S, Wang J, Kaplow J, Lai RYK, et al. A randomized, double-blind, phase 2b proof-of-concept clinical trial in early Alzheimer's disease with lecanemab, an anti-Aβ protofibril antibody. *Alzheimers Res Ther*. (2021) 13:80. doi: 10.1186/s13195-021-00813-8

56. van Dyck CH, Swanson CJ, Aisen P, Bateman RJ, Chen C, Gee M, et al. Lecanemab in early Alzheimer's disease. *N Engl J Med*. (2023) 388:9–21. doi: 10.1056/NEJMoa2212948

57. Cummings J, Apostolova L, Rabinovici GD, Atri A, Aisen P, Greenberg S, et al. Lecanemab: Appropriate Use Recommendations. *J Prev Alzheimers Dis*. (2023) 10:362–77. doi: 10.14283/jpad.2023.30

58. Sims JR, Zimmer JA, Evans CD, Lu M, Ardayfio P, Sparks J, et al. Donanemab in early symptomatic Alzheimer disease: the TRAILBLAZER-ALZ 2 randomized clinical trial. *JAMA*. (2023) 330:512–27. doi: 10.1001/jama.2023.13239

59. Imbimbo BP, Balducci C, Ippati S, Watling M. Initial failures of anti-tau antibodies in Alzheimer's disease are reminiscent of the amyloid-β story. *Neural Regen Res*. (2022) 18:117–8. doi: 10.4103/1673-5374.340409

60. Li Z, Hall AM, Kelinske M, Roberson ED. Seizure resistance without parkinsonism in aged mice after tau reduction. *Neurobiol Aging*. (2014) 35:2617–24. doi: 10.1016/j.neurobiolaging.2014.05.001

61. Bakker A, Krauss GL, Albert MS, Speck CL, Jones LR, Stark CE, et al. Reduction of hippocampal hyperactivity improves cognition in amnestic mild cognitive impairment. *Neuron*. (2012) 74:467–74. doi: 10.1016/j.neuron.2012.03.023

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# Proposed mechanisms of tau: relationships to traumatic brain injury, Alzheimer's disease, and epilepsy

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Traumatic brain injury (TBI), Alzheimer's disease (AD), and epilepsy share proposed mechanisms of injury, including neuronal excitotoxicity, cascade signaling, and activation of protein biomarkers such as tau. Although tau is typically present intracellularly, in tauopathies, phosphorylated (p-) and hyper-phosphorylated (hp-) tau are released extracellularly, the latter leading to decreased neuronal stability and neurofibrillary tangles (NFTs). Tau cleavage at particular sites increases susceptibility to hyper-phosphorylation, NFT formation, and eventual cell death. The relationship between tau and inflammation, however, is unknown. In this review, we present evidence for an imbalanced endoplasmic reticulum (ER) stress response and inflammatory signaling pathways resulting in atypical p-tau, hp-tau and NFT formation. Further, we propose tau as a biomarker for neuronal injury severity in TBI, AD, and epilepsy. We present a hypothesis of tau phosphorylation as an initial acute neuroprotective response to seizures/TBI. However, if the underlying seizure pathology or TBI recurrence is not effectively treated, and the pathway becomes chronically activated, we propose a "tipping point" hypothesis that identifies a transition of tau phosphorylation from neuroprotective to injurious. We outline the role of amyloid beta (Aβ) as a "last ditch effort" to revert the cell to programmed death signaling, that, when fails, transitions the mechanism from injurious to neurodegenerative. Lastly, we discuss targets along these pathways for therapeutic intervention in AD, TBI, and epilepsy.

KEYWORDS

**epilepsy, Alzheimer's disease, tau phosphorylation, amyloid-beta, TBI, endoplasmic reticulum stress**

## 1 Introduction

TBI and CTE are characterized by abnormal tau deposition in brain tissue. Epilepsy can also represent a form of tauopathy, as a result of cellular injury due to repetitive seizures. Seizure-induced injury responses include neuronal excitotoxicity and inflammatory cascades, which can lead to tau deposition and cell death (1–3). Tau is crucial for neuronal structural integrity and intracellular axonal transport (4, 5). Although tau is most commonly present intracellularly, p-tau is also found in the synaptic cleft (6, 7). Hp-tau leads to decreased neuronal stability and extracellular NFT formation, seen in neurodegenerative

disorders including AD, CTE, TBI, and epilepsy. Tau cleaved by caspases, a family of enzymes involved in programmed cell death, is also present in NFTs (8, 9). Tau cleavage at specific sites by caspases increases susceptibility to hyper-phosphorylation and NFT formation, suggesting that cell death pathways contribute to the pathology of tauopathies (9).

The role of inflammation in this cascade, however, is unknown. We briefly outline key inflammatory proteins involved in molecular signaling in TBI, AD, and epilepsy; discuss ER stress and its differing roles in TBI, AD, and epilepsy; and summarize how inflammatory signaling imbalances the ER stress response post-injury. We propose that, in response to acute moderate–severe TBI or single seizures, both inflammatory signaling and an overwhelmed ER stress response activate tau-induced signaling pathways to prevent further cellular dysfunction and restore intracellular homeostasis. Furthermore, we propose that in response to repeated injury, there is chronic activation of pro-inflammatory pathways and continual imbalance of the ER stress response, along with chronic activation of tau-induced signaling pathways.

We discuss three distinct processes, neuroprotection, injury, and degeneration, where injury is potentially reversible, and degeneration represents the spread of toxic effects to neighboring neurons and a lower likelihood of reversibility. We propose pathways by which the neuroinflammatory response to injury (seizures or TBI) contributes to tau hyper-phosphorylation and NFT formation, ultimately presenting our final hypothesis: tau phosphorylation plays a key role in neuroprotection, responding to recurrent seizures/injury, but there is a "tipping point" from neuroprotective to injurious effects – the repeated or sustained induction of an imbalanced ER stress response (specifically, the unfolded protein response [UPR]) and tau phosphorylation/hyper-phosphorylation. The ER stress response stimulates tau phosphorylation and continued tau cleavage; further phosphorylation/hyper-phosphorylation of tau promotes a continued UPR response and promotes neurodegeneration. This chronic dysregulation results in a shift from a tau-induced signaling pathway as a compensatory, neuroprotective response – which once reduced cellular dysfunction and attempted to restore apoptotic-necrotic dynamics and cellular homeostasis – to an injurious mechanism that is unable to maintain intracellular homeostasis, nor dynamically revert to mechanisms of programmed cell death (apoptosis).

Lastly, we propose a role for Aβ and outline its "last ditch effort" to mediate the injurious effects of excitotoxicity and chronic tau pathway activation, reverting the cell to pro-death signaling. However, due to (1) sustained UPR signaling interacting with tau and Aβ (2) the inability of reactive astrocytes and microglia to successfully break down toxic tau and Aβ aggregates, this leads to further tau hyper-phosphorylation resulting in NFT formation, as well as Aβ plaque accumulation – the hallmarks of neurodegeneration seen in AD pathology.

# 2 Injury response: molecular signaling

Inflammatory signaling, excitotoxic propagation, and ER stress play key roles in the atypical activation of cell death cascades and excessive phosphorylation of tau, resulting in downstream toxic tau aggregates and eventual neurodegeneration.

## 2.1 Inflammatory proteins and neurotransmission

Inflammatory proteins, including receptor-interacting kinases (e.g., RIP1/RIP3) and cytokines (e.g., interleukin-1 [IL-1], caspases), modulate inflammatory function and regulate forms of cell death such as necroptosis and apoptosis (10–12). Effects of inflammatory mediators are complex, in that they differ based on injury type, location, and chronicity. Even a single, acute TBI can cause sustained inflammatory signaling, measured by interleukin (IL) protein levels (13). A continued inflammatory response may lead to secondary neuronal injury and a decreased likelihood of spontaneous recovery over time, with persistent neuropsychological deficits. Additional injuries may contribute to chronic functional deficits, due to shortened recovery time between injuries and long-term neurodegeneration.

Neurotransmitters can modulate inflammatory responses in brain injury by disrupting pro-inflammatory cytokines, microglial production, and calcium signaling (14). Glutamate and γ-aminobutyric acid (GABA) are the major excitatory and inhibitory neurotransmitters, respectively. Glutamate release into the synaptic cleft occurs via calcium influx and intracellular calcium-dependent signaling (15). Once glutamate acts upon post-synaptic neurons, astrocytes collect and convert it to glutamine which is transported back to pre-synaptic neurons (16). Neuronal excitotoxicity due to altered glutamate and GABA receptor expression and function is evident in models of TBI (17, 18).

N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) are glutamate receptors responsible for neuronal influx of calcium in post-synaptic neurons. Table 1 summarizes NMDA and AMPA functions during typical neuronal depolarization and action potential propagation. The net effect of selectively activating these receptors and regulating their post-synaptic densities is to potentiate a non-toxic glutamate response, which promotes synaptic plasticity, long-term potentiation, and learning and memory (17, 19, 20). However, if these receptors are unselectively trafficked to/from key synaptic regions in brain injury, the result is an acute disruption of these signaling processes. In mechanical models of injury, down-regulation of the AMPA GluR2 and NMDA N2A receptors, along with up-regulation of the NMDA N2B receptor, lead to atypical calcium influx resulting in acute excitotoxic cell death (21–23).

## 2.2 Molecular signaling in TBI

Although TBI primarily leads to neocortical cell death, hippocampal vulnerability is also apparent. In a controlled cortical impact (CCI) mouse model of moderate TBI, apoptosis of immature hippocampal neurons was observed 24–72 h after injury (24). Limited inflammatory markers may be observed up to 7 days post-CCI, and necrosis of immature hippocampal neurons was evident for at least 14 days post-injury (25, 26). These results demonstrate hippocampal vulnerability in response to TBI that may clinically present as memory complaints.

Both altered excitatory glutamate signaling and reduced GABA-mediated inhibition contribute to excitotoxicity in brain injury (27). In a mouse CCI model, glutamate expression correlated with epileptiform activity within injured and adjacent cortex in the setting of decreased GABAergic interneurons. Further, there was significant

TABLE 1 Typical functionality (during selective activation) of glutamate and GABA receptors.

| Receptor | Subtype | Typical functionality (during selective activation) |
|---|---|---|
| NMDA | NR1 | Glycine-dependent receptor deactivation<br>Localizes with NR2 |
| NMDA | NR2A | Enhancement of excitatory synapses<br>Localizes with NR1<br>Responds and initiates LTP |
| NMDA | NR2B | $Ca^{2+}$ influx mediation<br>Prolongs $Ca^{2+}$ influx<br>Responds and initiates LTD |
| AMPA | GluR1 | Upregulated density in LTP<br>Phosphorylates in LTP<br>Permits $Na^{+}$ and $Ca^{2+}$ permeability |
| AMPA | GluR2 | Restricts $Ca^{2+}$ permeability |
| GABA | A-δ | Inhibition of potentiated response<br>Responds to changes in GABA concentrations |

LTP, long-term potentiation; $Ca^{2+}$, calcium; LTD, long-term depression; $Na^{+}$, sodium.

reduction of the $GABA_A$ γ2-subunit in CCI-injured rats with post-traumatic epilepsy (18). In a mouse CCI model of severe TBI, $GABA_A$ δ and $GABA_B$ B2 receptor subunit expression in dentate gyrus granule cells was reduced by 40–43% (24). In contrast, human studies of chronic, repetitive injuries in athletes (closed head injury [CHI] model) found a compensatory increase in $GABA_B$ receptor expression (28). Decreased $GABA_A$ receptor expression disrupts the inhibitory response (29), while increased $GABA_B$ receptor expression, responsible for membrane hyperpolarization, may serve to avoid further depolarization and excitotoxic effects.

## 2.3 Molecular signaling in AD

AD pathology includes Aβ plaque accumulation and NFT formation, with tau aggregation and hyper-phosphorylation contributing to dysregulated microtubule dynamics and neuronal functioning (30). Necroptosis activation by RIP1/RIP3 kinases was found in postmortem AD brains (31). Elevated levels of inflammatory markers IL-1β, IL-6, and tumor necrosis factor-alpha (TNF-α) were found in postmortem AD and transgenic animal brains, and microglial and astrocytic activation was observed in response to neurotoxic cytokine expression (32–36).

Excitotoxicity due to dysregulated $Ca^{2+}$-mediated NMDA receptor functioning decreases cell survival (37, 38). Aβ regulates synaptic vesicle release and affects NMDA receptor structure, density, and electrophysiology – ultimately affecting glutamate transmission and resulting in cognitive changes (39–43). In AD patients with severe cognitive deterioration, decreased glutamate and GABA levels were noted in temporal cortex and CSF compared to AD patients with mild cognitive deterioration and age-matched controls (44, 45), and decreased concentrations of GABAergic terminals in cortical neurons adjacent to Aβ plaques were found in AD patients and transgenic AD mouse models (46, 47). These findings suggest impaired receptor function and neurotransmission and an imbalance between excitatory and inhibitory activity in AD.

## 2.4 Molecular signaling in epilepsy

Inflammatory responses in epilepsy can contribute to recurrent seizures, secondary neuronal injury, and chronic neurodegeneration (2). During focal to bilateral tonic–clonic seizures, cytokines exert effects through increased AMPA receptor density, NMDA-dependent calcium influx, and reduction of $GABA_A$ receptor density, resulting in greater synaptic glutamate and decreased synaptic GABA concentrations (48–51). Excess glutamate increases the likelihood of neuronal depolarization, excitotoxicity, and eventual cell death (52–54), particularly in models of temporal lobe epilepsy (55). Glia rapidly produce interleukins, particularly interleukin-1 beta (IL-1β), postictally. IL-1β enhances neuronal excitability and sustains inflammatory responses (56, 57). Increased IL-1β activity leads to neuronal degeneration in epileptogenic regions, while astrocytes that express its receptor have neuroprotective functions (1, 58). Astrocytes can mediate the effect of IL-1β on hippocampal neurons, contributing to their likelihood of survival. The presence of astrocytes in epileptogenic regions is a compensatory response to excess synaptic glutamate (59, 60). Increased astrocytes in regions of post-ictal neuronal injury suggest IL-1β involvement in the initiation and continuation of local seizure activity (59).

We propose that during a single seizure and mild TBI (Figure 1), excitotoxic depolarization enhances IL-1β signaling and increases NMDA receptor activity, leading to local propagation of excitotoxic depolarization and extracellular glutamate accumulation. This process, along with increased Aβ and cytokine secretion, recruits astrocytes into the synapse (61) to collect glutamate post-seizure. Excess glutamate also recruits microglia to clear cellular debris, remove excess Aβ, and return to neuronal homeostasis (60, 62, 63). If neuronal homeostasis is not achieved, further excitotoxic injury and cell death signaling can occur.

Neuronal damage in TBI, AD, and epilepsy can result from secondary inflammatory responses and neuronal excitotoxicity. Interleukins, particularly IL-1β, are key modulators of pro-inflammatory responses and apoptosis. Additionally, dysregulation of the glutamate-GABA/excitation-inhibition balance leads to excitotoxic injury and neuronal death.

## 2.5 ER stress and its role in TBI, AD, and epilepsy

ER stress occurs when there is an imbalance between the ability of the ER to fold proteins and the cellular demand for protein folding (64). In response to ER stress, the UPR signals to either (1) protect the cell by correcting the imbalance between folding ability and demand (65) via the protein kinase R-like ER kinase (PERK) pathway or (2) promote programmed cell death. Cell death occurs via C/EBP homologous protein (CHOP) and Apaf-1-dependent apoptosis or via necroptosis involving RIP1/RIP3-activation and rapid ATP depletion (66–68). Acute UPRs are protective to the cell. Sustained UPRs, however, induce caspase-dependent apoptosis (69), deplete intracellular ATP (70), and induce necrosis (70).

ER stress contributes to neuronal loss in TBI (26, 71, 72), AD (73), and epilepsy (74) and correlates with tau phosphorylation in TBI and AD (75, 76). In a CCI rat model, markers of reactive ER stress were associated with increased tau oligomers and tau kinase (GSK-3β) activation (77). To study the relationship between tau phosphorylation

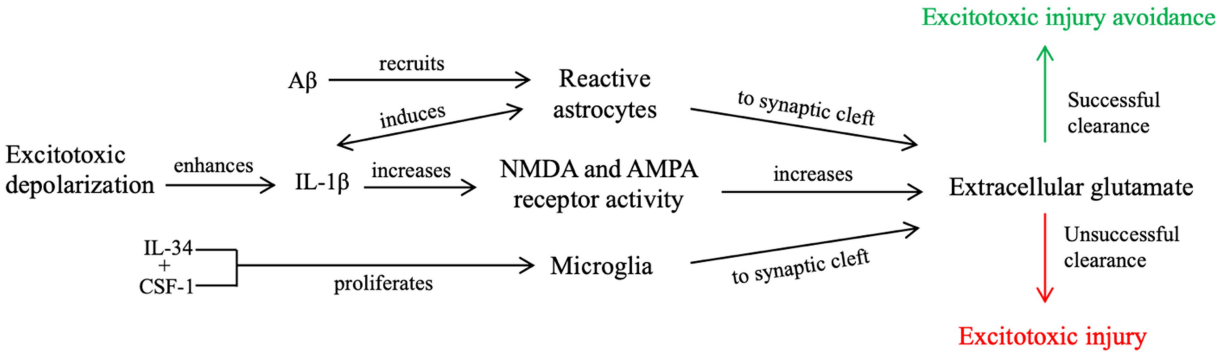


FIGURE 1
Our proposed contributory mechanism of IL-1β signaling during a single, brief seizure or mild TBI. Enhanced IL-1β signaling from excitotoxic depolarization results in increased glutamate receptor activity and further propagation of excitotoxic signaling, resulting in an accumulation of post-synaptic glutamate. Increased neuroinflammatory signaling, including upregulated cytokine and Aβ secretion and increased concentrations of extracellular glutamate, recruit microglia and reactive astrocytes to the post-synaptic cleft. Unsuccessful clearance of extracellular glutamate, cellular debris, and Aβ from the synaptic cleft by reactive astrocytes and microglia leads to further excitotoxic propagation and places the cell at risk for excitotoxic injury. Successful clearance, however, reduces the risk of excitotoxic injury, as it attempts to revert the cell to neuronal homeostasis.

and ER-stress in promoting AD-like pathogenesis, tau phosphorylation was induced in rat cortical neurons, resulting in a UPR response with elevation of p-PERK and other modulator proteins. In the same study, an ER stress inducer enhanced tau phosphorylation at specific sites (75).

In human AD autopsy material, PERK correlated with atypical tau phosphorylation (78), and tau interacted with ER proteins leading to neuronal dysfunction and neurotoxicity (79). In epilepsy, the relationship between ER stress and tau phosphorylation is unknown, although relationships between epilepsy and unfolded proteins have been established. A mouse model of epilepsy suggested that acute, reactive ER stress responses may reduce seizure recurrence or severity (80). In resected tissue from patients with epilepsy due to focal cortical dysplasia, however, there were greater accumulations of unfolded proteins and increased levels of CHOP in patients who were not rendered seizure-free (81). Hence, acute, reactive stress responses may be protective, while chronically increased ER stress may contribute to seizure recurrence.

Aβ can trigger ER stress, just as ER stress can promote Aβ formation, leading to excitotoxicity and apoptosis (82–84). While amyloid precursor protein (APP) increases resistance to ER stress-induced apoptosis in specific cell cultures (85), intracellular Aβ counteracts APP by activating ER stress and pre-disposing cells to other pathways of programmed cell death (86). In brain endothelial cells, Aβ increased concentrations of UPR signaling mediators, increased intracellular $Ca^{2+}$, and upregulated pro-apoptotic transcription factors (87). The relationship between Aβ and excitotoxicity is complex, however, in that Aβ also acts directly on the ER stress response protein XBP1 to reduce intracellular $Ca^{2+}$ concentrations and limit excitotoxic injury (88).

Data suggest initial neuroprotective effects of reactive ER stress, activation of the PERK pathway, and APP (89). However, we postulate that sustained, repeated, or anticipatory (i.e., in the face of chronic injury) induction of the ER stress response may increase atypical tau phosphorylation and Aβ concentrations, with deleterious effects. Aβ has both pro-apoptotic and excitotoxic effects, but to limit neural injury, it acts feeds back on the ER stress response to interrupt it. If Aβ fails to halt its excitotoxic effects, and microglia and reactive astrocytes cannot successfully clear toxic tau and Aβ aggregates, neurodegeneration follows.

## 3 Injury response: the role of tau

Tau plays a key role in ER stress and Aβ pathways. Tau is a neuronal protein that supports axonal transport and microtubule dynamics (4). In neurodegenerative diseases, tau is abnormally present within subcortical neurons, including the hippocampus. Tau hyper-phosphorylation results in deposits of neurofibrillary tangles (NFTs), corresponding with diminished neuronal stability and subsequent aberrant neuronal communication (4, 5). These structural abnormalities lead to cognitive deficits, including memory loss (90–93). Elevated levels of total- (t-), phosphorylated- (p-), and hyperphosphorylated- (hp-) tau are detected in CSF at various time points post-TBI/seizure (91, 94–98). Accumulation and spread of tau aggregates occurs in various cortical and subcortical areas post-injury/seizure and in AD (93, 94, 99–101).

To explain the role of tau in brain injury and its relationship to the above inflammatory and excitotoxic processes, we posit two distinct signaling mechanisms, combining components of various pathways described in the literature: (1) an acute injury response (AIR; Figure 2), and (2) a recurrent injury response (RIR; Figure 3). AIR and RIR propose varying degrees of interleukin, NMDA/AMPA receptor, and $Ca^{2+}$/calmodulin-dependent protein kinase (CaMK) involvement. We also propose a slower neuroprotective tau (NPT) response mechanism shared by acute seizures and TBI. However, with repeated seizures/TBI leading to chronically activated/sustained ER stress responses, the NPT pathway will become dysregulated, resulting in neural injury (Figure 4).

### 3.1 Acute injury response (AIR)

The AIR pathway is a pro-inflammatory mechanism that minimizes the likelihood of acute excitotoxic effects and cell death. In the AIR pathway, an acute TBI or brief seizure leads to IL-1β formation (108–110, 122), which has multiple effects on NMDA and AMPA receptors (Figure 2), including downregulation of NMDA receptors NR1 and NR2B. Unselective CAMK-II

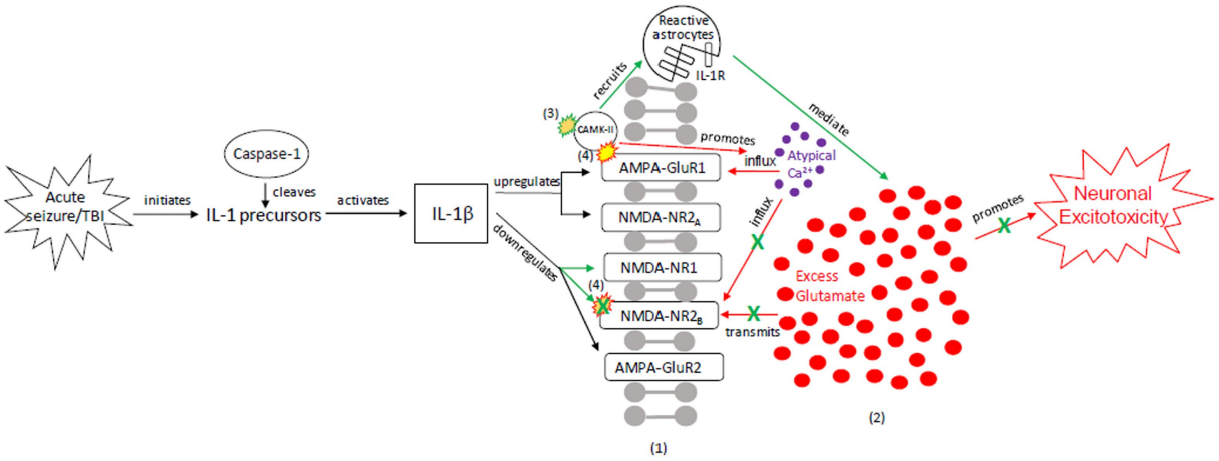


FIGURE 2
Our proposed acute injury response (AIR) mechanism outlining reactive signaling to an acute, brief seizure or acute, mild TBI. This mechanism shunts cellular signaling away from pro-death response pathways and toward cellular protection, with the goals of restoring the balance between glutamate release and reuptake, intracellular $Ca^{2+}$-driven ER stress responses, and apoptotic-necrotic dynamics. In response to acute injury, caspase-1 cleaves IL-1 precursors, resulting in IL-1β formation. IL-1β unselectively up-or down-regulates glutamate receptor subunit densities, resulting in an acute disruption of balanced glutamate release and reuptake. There is increased CaMK-II activation that promotes increased glutamate release (102, 103). Unselective CaMK-II phosphorylation and autophosphorylation occurs at upregulated AMPA-GluR1 (104) but not at down-regulated NMDA-R2B (105), resulting in increased AMPA-GluR1 Ca2+ influx/channel conductance and decreased NMDA-NR2B Ca2+ influx/channel conductance, respectively. However, CaMK-II also recruits astrocytes into the affected region (59, 60, 106, 107). Increased astrocytes/IL-1 receptor density aid in clearing excess glutamate and ILs, inhibiting further glutamate release, thereby limiting excitotoxic propagation. (1) = Neuronal membrane, (2) = Synaptic cleft, (3) = CaMK-II autophosphorylation, (4) = CaMK-II-Glutamate receptor phosphorylation. Red = Excitotoxic signaling, Green = Neuroprotective signaling. X = response reduction/down-regulation.

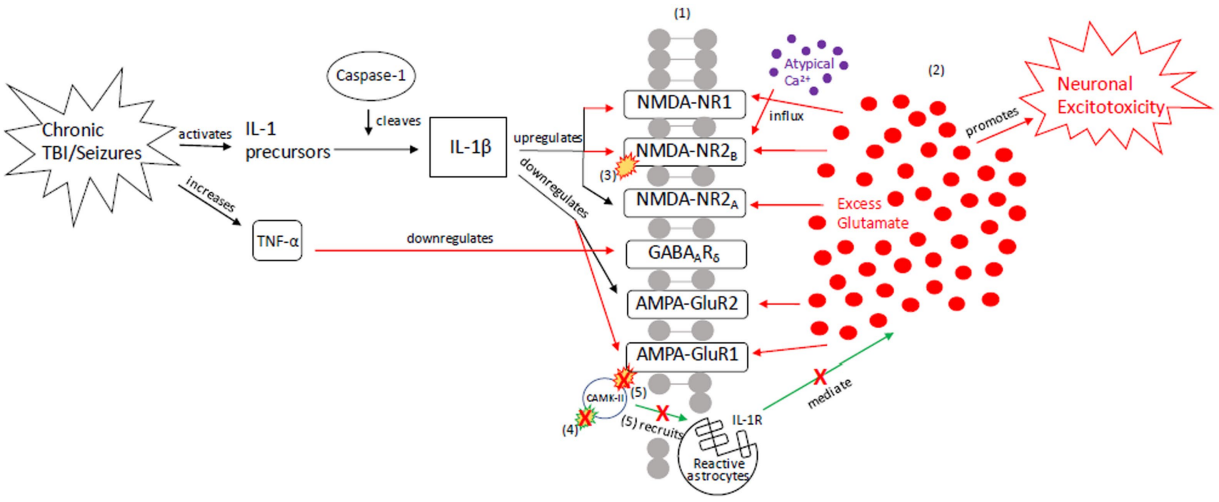


FIGURE 3
Our proposed recurrent injury response (RIR) mechanism outlining reactive signaling to chronic and/or moderate–severe TBI and chronic and/or prolonged seizures. This mechanism shunts cellular signaling toward pro-death response pathways of apoptosis and necrosis due to imbalanced glutamate release and reuptake, $Ca^{2+}$-driven ER stress responses, and apoptotic-necrotic dynamics. In response to chronic injury, caspase-1 cleaves IL-1 precursors, resulting in IL-1β formation, and TNF-α downregulates $GABA_A$ receptors (18, 108–113). However, unlike the AIR mechanism, IL-1β increases NMDA receptor activity via GluNR2B phosphorylation (112). Increased NMDA receptor densities contribute to atypical $Ca^{2+}$ influx and prolonged excitotoxic signaling. Concurrently, AMPA-GluR1 and-GluR2 receptors are down-regulated in response to chronic injury, resulting in dysregulated CaMK-II autophosphorylation and AMPA-GluR1 site phosphorylation (21–23, 49, 114–116). Due to disrupted CaMK-II phosphorylation and autophosphorylation, reactive astrocytes cannot be successfully recruited to the synapse to clear excess glutamate and proteasome recruitment into dendritic spines is impaired, respectively (117). The result is neuronal excitotoxic depolarization and propagation, neurotoxic release of ATP, and preferential apoptotic signaling (118). (1) = Neuronal membrane, (2) = Synaptic cleft, (3) = IL-1β-activated NMDA-NR2B phosphorylation, (4) CaMK-II autophosphorylation, (5) CaMK-II-AMPA-GluR1 phosphorylation. Red = Excitotoxic signaling, Green = Neuroprotective signaling. X = response reduction/down-regulation.

activation, coupled with the IL-1β signaling, promotes atypical calcium influx and excitotoxic glutamate release. As a result, there is an increased probability of cell death unless excess glutamate can be cleared from the synapse. CAMK, however, also recruits astrocytes into the affected region, evidenced by reactive astrocytes and phosphorylated CAMK-II in the hippocampal CA3 region of a kainic acid mouse model (106). The inflow of reactive astrocytes, coupled with increased IL-1 receptor density, clears excess synaptic glutamate (59, 60, 107).

## 3.2 Recurrent injury response (RIR)

The RIR pathway results in excitotoxity and apoptosis (Figure 3). Recurrent TBI activates -IL-1 precursors, which are cleaved into IL-1β by proteases such as caspase-1 (108–110). Similarly, recurrent seizures, through excitotoxic neuronal depolarization, activate caspase-1 and lead to IL-1β signaling (108–111). IL-1β, however, does not down-regulate NMDA receptors as in AIR. Instead, IL-1β hyper-activates NMDA receptors via GluNR2B subunit phosphorylation in

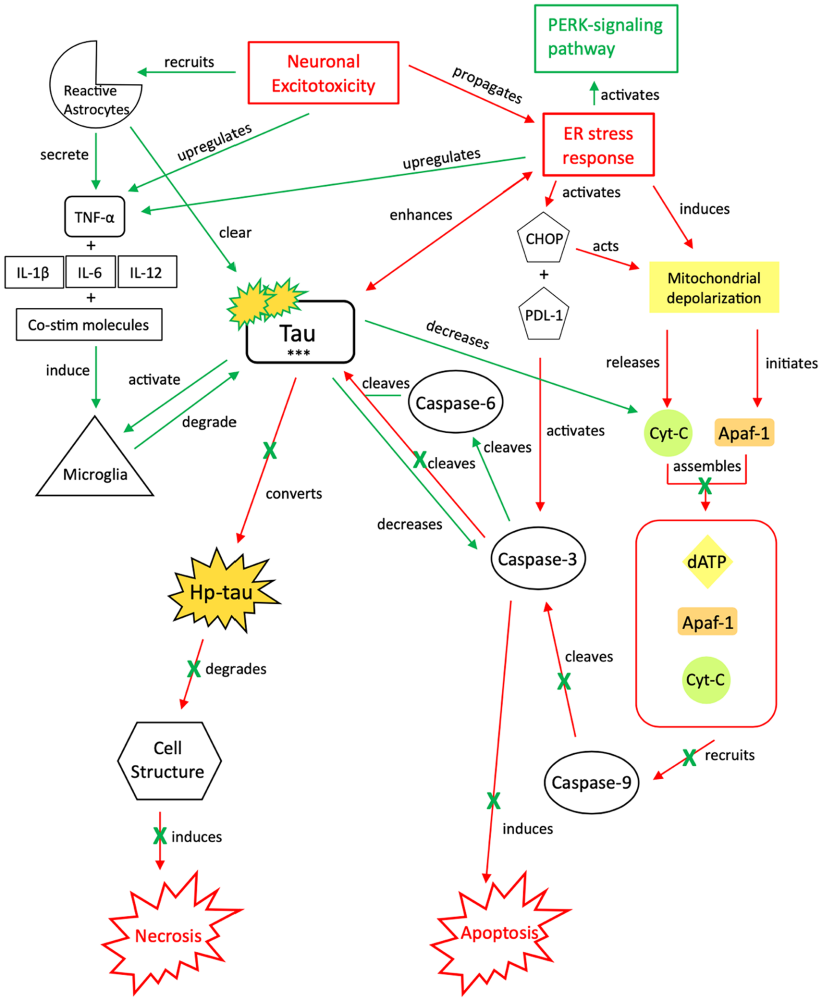


FIGURE 4
Our proposed neuroprotective response mechanism involving tau (NPT). Neuronal excitotoxicity imbalances the ER stress response, which activates two pathways: the PERK pathway, responsible for reverting the cell to homeostasis and preserving its integrity, and pro-cell death signaling cascades via CHOP and rapid mitochondrial depolarization, such as apoptosis. In typical apoptotic signaling, mitochondrial depolarization initiates Apaf-1 and releases cyt-c. Cyt-c, with Apaf-1 and dATP, assembles into an apoptosome complex (119–121). The apoptosome complex recruits caspase-9, caspase-9 cleaves caspase-3, and caspase-3 activates apoptosis (122, 123). Tau preserves cellular integrity and reverts cellular signaling away from pro-cell death signaling cascades. Although reduction of caspase-3 cleavage of tau reverts the cell away from apoptotic signaling, tau is cleaved by additional caspases such as caspase-6, resulting in tau phosphorylation (124–128). The increased presence of p-tau decreases the concentration of cyt-c and caspase-3, thereby further inhibiting apoptotic signaling (122, 129, 130). To avoid additional cell death pathways (i.e., necrosis), increases in cytokine expression, TNF-α, and tau concentrations recruit reactive astrocytes and microglia to break down excess tau into non-toxic components (131–136). Successful breakdown of accumulated tau by microglia and reactive astrocytes downregulates pro-death signaling pathways and restores cellular homeostasis. PERK, protein kinase R-like ER kinase; TNF, tumor necrosis factor; IL, interleukin; Co-stim, co-stimulatory (molecules); cyt-c, cytochrome-c; Apaf-1, apoptotic peptidase activating factor-1; dATP, deoxyadenosine triphosphate; NFTs, neurofibrillary tangles; Red, Pro-death signaling; Green, Neuroprotective signaling; o-tau, tau oligomers; t-tau, total tau; p-tau, phosphorylated tau; ***, O-tau, t-tau, p-tau; X, response reduction/down-regulation.

response to chronic injury (112). The resultant increase in NMDA receptor density contributes to atypical calcium influx, prolongs excitatory synaptic enhancement, and propagates pathologic signaling from excess glutamate.

Further, there is decreased $GABA_A$ receptor density (50) and downregulation of the $GABA_A$ receptor δ-subunit (18, 113), contributing to extracellular glutamate accumulation and excitotoxicity (18, 113). AMPA-GluR1 and GluR2 receptors are also down-regulated in response to injury (21–23, 49, 114–116). As a result of AMPA dysregulation, CAMK-II autophosphorylation is impaired and recruitment of proteasomes – highly active enzyme complexes that play a role in cell-cycle progression – into dendritic spines is blocked, resulting in apoptosis (117). Additionally, subsequent phosphorylation at AMPA receptors also indirectly decreases astrocytic recruitment and clearance of excess glutamate (118).

If the AIR pathway (Figure 2) is unsuccessful in mediating excitotoxicity or if the RIR pathway is activated in chronic injury/seizures (Figure 3), apoptosis (acute programmed cell death) and necrosis (passive cellular degradation and death) result (142). Oxygen free radical production, caspase activation (e.g., caspase-3 and caspase-6), mitochondrial membrane depolarization, and further neurotoxicity occur (143–145). To minimize the possibility of cell death and preserve structural and functional integrity of surrounding neurons, an additional neuro-protective response is needed. We posit that tau signaling pathways first respond to recurrent seizures/injury in attempt to preserve cellular integrity; however, there is a "tipping point" that transitions the mechanism from neuroprotective to injurious – the repeated or sustained induction of an imbalanced ER stress response (specifically, the unfolded protein response [UPR]) and resultant aberrant tau phosphorylation. The ER stress response stimulates tau phosphorylation and continued tau cleavage; further phosphorylation/hyper-phosphorylation of tau promotes a continued UPR response and promotes neurodegeneration. This chronic dysregulation results in a shift from a tau-induced signaling pathway as a compensatory, neuroprotective response – which once reduced cellular dysfunction and restored apoptotic-necrotic dynamics and cellular homeostasis – to an injurious mechanism that is unable to maintain intracellular homeostasis, nor revert to mechanisms of programmed cell death.

## 3.3 Neuroprotective response (NPT): the expression and consumption of tau

In apoptosis, caspase-3 is activated by multiple mechanisms, including inflammatory responses, mitochondrial-based pathways, and an imbalanced ER stress response (119–123) (Figure 4). To divert the cell away from this apoptotic pathway and attempt to restore cellular homeostasis while maintaining structural integrity, caspases and ATP processes that induce apoptosis must be downregulated, TNF-α expression must be promoted, and tau phosphorylation must be induced, in conjunction with ER stress-induced PERK-pathway activation. Decreasing available caspases and apoptotic signaling reduces the likelihood of further neurotoxic depolarization and cell death, while increasing the likelihood that cellular homeostasis is restored (146). Induction of tau phosphorylation via caspase-6 cleavage indirectly reduces apoptotic signaling while preserving cellular integrity; tau also indirectly activates microglia, which are responsible for tau degradation to its non-toxic components.

Both caspase-3 and caspase-6 cleave tau (124–126) at multiple sites, which increases the susceptibility of tau to phosphorylation (9, 126–128). However, increased tau phosphorylation will also decrease caspase-3 activation in a negative feedback loop (122, 129, 130, 147). We posit that although the imbalanced ER stress response induces atypical tau phosphorylation (75), its acute effect is minimal due to this reduction in caspase-3 activation. As caspase-3 activation is required by apoptosis (119–121, 148), we posit that there is a transition from apoptosis to cellular preservation. However, with a halt of apoptotic signaling in the setting of increased tau concentrations, microglial and reactive astrocyte activation via upregulation of TNF-α, pro-inflammatory cytokines (IL-1β, IL-6, IL-12) and enzymes, and co-stimulatory molecules (131, 132) is required to break down tau. Additionally, tau oligomers (o-tau) and aggregates activate microglia to phagocytize tau and process its isoforms into non-toxic components (133–136). The ER stress response also upregulates $Ca^{2+}$-ATPases in microglia, enhancing their capacity for phagocytosis and tau breakdown (149). Tau clearance is crucial to reestablishing cellular homeostasis and re-balancing the ER stress response post-seizure/injury.

## 3.4 Neuro-injurious tau response (NIT): transitioning from neuroprotection to injury

We posit that in an acute, mild TBI or brief seizure, tau will assist the cell in reverting to balanced ER stress response signaling and intracellular homeostasis. However, chronic or sustained activation of tau signaling cascades due to severe and/or recurrent injury will eventually transition this mechanism from neuroprotective to injurious (Figure 5). While tau expression benefits microtubule dynamics, overexpression of phosphorylated, cleaved isoforms disrupts microtubule transport and increases the risk of toxic tau aggregates (150). The overexpression of tau, atypical accumulation of p-tau and hp-tau from caspase-3 cleavage and apoptosis inhibition, and tau deposition due to the inability of microglia to successfully break down toxic tau aggregates, could be a result of the cell's failed attempt to maintain homeostatic microtubule dynamics.

The NPT process depends upon the ability of the cell to revert to balanced ER stress responses, balanced apoptotic-necrotic dynamics, and intracellular homeostasis. Successful reactive astrocytic phagocytosis of tau and microglial clearance of tau play key roles in restoring intracellular dynamics. We posit that in the setting of sustained or recurrent injury, however, the ability of reactive astrocytes and microglia to break down tau becomes dysregulated. A resultant buildup of intra-microglial toxic tau occurs (99), which inhibits microglial and reactive astrocytic phagocytosis, threatens neuronal integrity, and drives expulsion of toxic tau aggregates from the cell via exosomal packaging and secretion. However, these secreted toxic tau aggregates are misfolded (151) and therefore more resistant to microglial break down. These exosomal tau aggregates have injurious effects (99) due to increased likelihood of exosomal leakage and surrounding neuronal uptake (152, 153). Further, the recurrent or sustained activation of the ER stress response reinforces microglial migration and dysregulation and limits the ability of microglia to actively break down tau. The inter-neuronal spread of toxic tau may

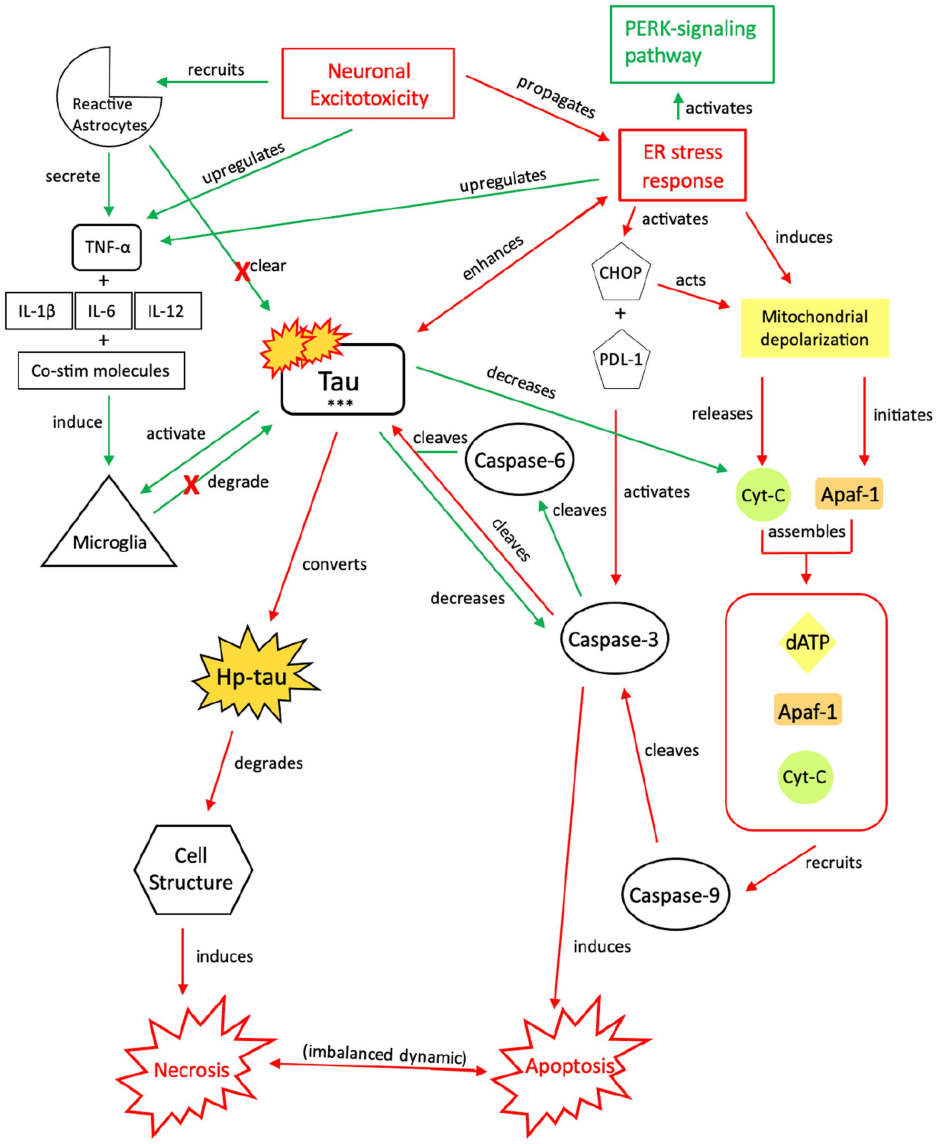


FIGURE 5
Our proposed injurious response mechanism involving tau (NIT) outlining injurious tau signaling and the resulting imbalance in apoptotic-necrotic signaling due to a chronic or sustained injury response from TBI or seizures. Similar to the NPT response, neuronal excitotoxicity imbalances the ER stress response, which activates two pathways: the PERK pathway and pro-cell death signaling pathway. Increased presence of p-tau decreases the concentration of cyt-c and caspase-3, inhibiting apoptotic signaling; although downregulated, caspase-3 still cleaves tau and contributes to tau's toxic effects, which further reverts the cell away from apoptotic signaling toward necrosis (119–121). To compensate for this shift, increased cytokine expression, increased TNF-α, and increased tau concentrations recruit reactive astrocytes and microglia to break down excess tau into non-toxic components (107, 131, 132). However, unsuccessful breakdown of tau by microglia and reactive astrocytes results in a build-up of toxic tau aggregates that are secreted extracellularly (137, 138). Adjacent cells attempt to break down the toxic tau into non-toxic components (99), but chronic activation of the NIT pathway due to recurrent or sustained injury dysregulates this response, resulting in an injurious build-up of toxic levels of tau, hp-tau, and NFTs, which reinforce necrotic signaling (139–141). PERK, protein kinase R-like ER kinase; TNF, tumor necrosis factor; IL, interleukin; Co-stim, co-stimulatory (molecules); cyt-c, cytochrome-c; Apaf-1, apoptotic peptidase activating factor-1; dATP, deoxyadenosine triphosphate; NFTs, neurofibrillary tangles; Red, Pro-death signaling; Green, Neuroprotective signaling; o-tau, tau oligomers; t-tau, total tau; p-tau, phosphorylated tau; ***, O-tau, t-tau, p-tau; X, response reduction/down-regulation.

mark the initial transition from a neuroprotective to a more widespread injurious process.

The NPT response may be an attempt to preserve cellular integrity, by avoiding further injury from apoptosis through tau phosphorylation and limiting effects of necrosis through astrocytic and microglial involvement. Over time, however, the NPT mechanism will still result in cell death if the underlying chronic pathology remains untreated. Further, with recurrent injury (e.g., repetitive seizures, repeated head trauma), the NPT response will become overwhelmed, and an aberrant, injurious process will ensue. Over time, repeated activation of injurious pathways will require a "last ditch effort" to revert the cell to pro-apoptotic signaling cascades and avoid further transition to a widespread neurodegenerative process, which leaves the question – what is the role of Aβ?

## 4 The role of amyloid-β in the transition from neuroprotection to tauopathy

With chronic pathology, Aβ concentrations are also increased by caspase-3-mediated APP cleavage and an imbalanced ER stress response (88, 154, 155). We posit that, in response to recurrent or severe injury, sustained Aβ signaling is a "last ditch effort" by the cell to restore cell death signaling and reduce the injurious effects of an imbalanced ER stress response and atypical tau (Figures 6, 7). Although Aβ induction increases plaque formation, it also has neuroprotective effects, recruiting additional reactive astrocytes and microglia for toxic aggregate breakdown (157, 167, 168, 170). However, if the cell cannot degrade toxic tau and Aβ aggregates and restore cell death signaling, Aβ's relationship with tau further transitions the NPT response to a neurodegenerative process because it prevents tau from appropriately binding to microtubules and induces atypical tau phosphorylation (154, 156, 171, 172) (Figures 8, 9).

In both typical functioning and in response to acute neuronal injury, we postulate that tau and Aβ signaling processes occur in parallel. In acute neuronal injury, however, we propose greater initial reliance on tau signaling in comparison to Aβ signaling, in avoidance of necrotic processes and reorientation toward cellular preservation and stabilization. With recurrent or severe neuronal injury, we postulate that the "last ditch effort" of Aβ indicates a "cellular switch" to greater reliance on Aβ signaling, for the purpose of activating apoptotic signaling and limiting neurotoxic spread. If the underlying injurious pathology is not reduced/halted, the result is a transition of the "at risk" neuroprotective response to one of neurodegeneration.

The ER stress response can induce apoptotic signaling cascades (169) in addition to promoting Aβ formation. Aβ formation comes with several costs, in that Aβ will activate pro-inflammatory responses and caspase-3 activity, in attempts to revert the cell to pro-apoptotic signaling; however, increased caspase-3-selective tau cleavage by Aβ and dysregulated mitochondrial production and recruitment results in further tau-related toxicity and an imbalanced intracellular dynamic (156, 157). While caspase-3 typically promotes tau cleavage and phosphorylation during apoptotic signaling, Aβ increases aberrant caspase-3 activity during necrosis (Figures 6, 7) (155). Hence, Aβ initiates atypical tau cleavage. It renders tau increasingly susceptible to hyperphosphorylation and toxic aggregates, because it atypically alters tau at specific phosphorylation sites (154), ultimately leading to microglial injury and neurotoxicity (Figure 8) (156). In AD, soluble Aβ induces tau hyperphosphorylation in hippocampal neurons, disrupting microtubule stability. De-phosphorylation of Aβ-induced p-tau results in the restoration of tau microtubule binding capacity (154), suggesting that the process is at least partially reversible, and suggests some initial benefit of Aβ formation.

Extracellular insoluble Aβ aggregates, however, are associated with neurotoxicity and degeneration (155). Both soluble and insoluble $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ levels are elevated in patients with AD compared to typical aging brains (174). The soluble forms comprise the greatest proportion of total Aβ in typical aging brains but the lowest in AD brains (174). Acute cell death is highly dependent upon the relationship between soluble Aβ and soluble cytoplasmic tau, which can propagate extracellularly (175). The relationship between Aβ and tau suggests that each can act on the other in a negative feedback loop, triggering the transition from non-toxic to toxic aggregates (175). Therefore, it is possible that soluble Aβ reflects typical brain functioning, but with neuronal injury, neurons are "at-risk" for soluble toxic tau formation and toxic tau/Aβ aggregate propagation extracellularly, resulting in an eventual transition to an insoluble state. This, in turn, reduces the proportion of soluble to insoluble Aβ and soluble phosphorylated to abnormally phosphorylated tau, further transitioning the mechanism to one of eventual degeneration (176).

Toxic Aβ accumulation results from several mechanisms, with prominent roles of microglia and astrocytes. Similar to tau, Aβ clearance requires microglial and reactive astrocytic degradation (Figure 6) (157). Aβ plaques can result from microglial dysregulation and increased Aβ-induced caspase-3 activity, as caspase-3 cleaves APP-β (177). Aβ also activates reactive astrocytes, which cluster around Aβ plaques (Figure 6) (167, 168). The astrocytes secrete interleukins and TNF-α, promoting further inflammation to break down Aβ (167, 168), however, these pro-inflammatory proteins also induce APP-β (178, 179), resulting in increased Aβ concentrations. Further neurodegeneration can also occur due to astrocytic secretion of Aβ (Figure 9) (180). Aβ deposits were found in the hippocampus with progression to the cortex prior to the formation of NFTs in a transgenic AD model, supporting neurodegenerative signaling cascades outlined in Figure 5 (181). Aβ deposits were also found in ~30% of severe TBI cases postmortem (182, 183). This, coupled with Aβ-promoted tau cleavage (9), indicates a relationship between amyloid-β, tau, and NFTs.

*In vitro* and *in vivo*, microglia clear soluble extracellular Aβ via micropinocytosis, in which successful uptake and degradation depends on actin and tubulin dynamics (184). Inflammatory processes promote signaling cascades and the recruitment of microglia to initiate soluble Aβ uptake and degradation (157). In an acute injury model, this process is postulated to be neuroprotective. However, with recurrent or sustained injury, this process may be dysregulated due to unstable actin/tubulin dynamics and imbalanced ATP involvement, leading to further neural injury. The transition from soluble to insoluble Aβ has yet to be fully understood. However, data suggest that a progressive Aβ transition from soluble to insoluble takes place in the ER/intermediate compartment pathway, and that the degree of insolubility correlates with overexpressed APP-β concentration (185). The uptake and degradation of insoluble Aβ, comprised of neurotoxic, soluble Aβ oligomers, occur through different endocytic mechanisms that are microglia and astrocytic receptor mediated (186–188). Further, simultaneous intra-astrocytic accumulation of soluble and neurotoxic Aβ for degradation promotes vesicle-induced neuronal apoptosis (189). Resulting from cell death, cellular contents, including neurotoxic Aβ, are released into cytoplasm and quickly re-phagocytosed by surrounding neurons. In acute injury, this process would be neuroprotective for the prevention of necrosis; however, with recurrent injury, it is a mechanism for further neurotoxic propagation and eventual systemic degradation.

Aβ activity disrupts cellular integrity, but we posit that Aβ attempts to minimize neurodegenerative damage by targeting NMDA/AMPA receptors and mitochondrial membrane potential (MMP) as part of a "last ditch effort" to reactivate apoptotic signaling (Figures 6, 7). Aβ recruits reactive astrocytes to compensate for microglial dysregulation and clear toxic Aβ (Figure 6). However, because shared biochemical mechanisms associated with neuronal homeostasis and cell death are dysregulated, and neuroprotective

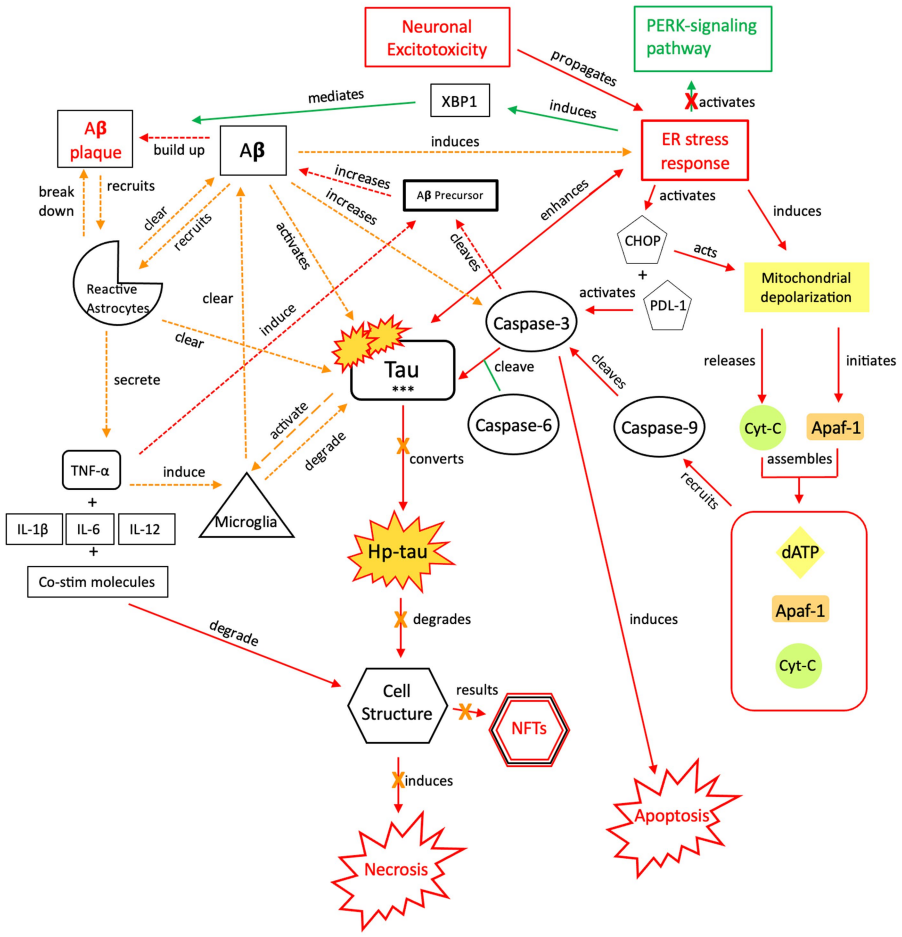


FIGURE 6
The neuroprotective response of Aβ, aka the "last ditch effort" to revert the cell to programmed death signaling and rebalance the apoptotic-necrotic signaling dynamic. In response to a recurrent or sustained ER stress response, imbalanced apoptotic-necrotic signaling dynamic, and atypical tau phosphorylation, Aβ activation both induces the ER stress response and increases caspase-3 cleavage of Aβ precursor protein (155). However, Aβ also recruits microglia and reactive astrocytes in response to excitotoxic signaling and increased tau concentrations (156). Breakdown of toxic tau aggregates and Aβ by microglia and reactive astrocytes mitigates the effect of Aβ-associated tau seeding and propagation (133, 157). As increased microglial trafficking is indirectly induced by the presence of Aβ, this mechanism also has detrimental effects due to shared apolipoprotein E (APOE), amyloidosis, and microglial transcript pathways and sustained neuroinflammation (158). Due to microglial inflammation and activation, reactive astrocytes are upregulated and recruited in attempts to clear toxic tau and Aβ and further orient the cell toward apoptotic signaling (159–162). Ultimately, a reduction in both inflammatory signaling and tau phosphorylation are needed once apoptotic-necrotic signaling dynamics have been reestablished, to prevent transition to an irreversible, degenerative pathway. PERK, protein kinase R-like ER kinase; Aβ, amyloid beta; XBP1, X-box binding protein 1; TNF, tumor necrosis factor; IL, interleukin; Co-stim, co-stimulatory (molecules); Cyt-c, cytochrome-c; Apaf-1, apoptotic peptidase activating factor-1; dATP, deoxyadenosine triphosphate; NFTs, neurofibrillary tangles; Red=Pro-death signaling, Green=Neuroprotective signaling, Orange=Aβ-involved signaling; o-tau, tau oligomers; t-tau, total tau; p-tau, phosphorylated tau; ***=O-tau, t-tau, p-tau. X=reduction/down-regulation. Solid line=signaling cascade induced/propagated by the ER stress response and tau; dashed line=signaling cascades resulting from Aβ involvement.

mechanisms such as reactive astrocytic phagocytosis of Aβ are functioning abnormally (180), these pathways promote further excitotoxic signaling and neurodegeneration (Figures 8, 9). If Aβ is not properly cleared, it can cause further atypical tau hyperphosphorylation, microtubule destabilization, and assembly of tau into filament structures seen in AD (190).

Aβ oligomers preferentially activate NMDA NR1/NR2A receptor subunits, which initiate LTP and regulate NMDA NR2B-mediated calcium influx (191). Aβ oligomers can induce a rapid increase in intracellular $Ca^{2+}$ via NR2B influx and cause mitochondrial damage leading to hippocampal cell death (191). Aβ peptides interfere with CaMK-II activity and decrease AMPA receptor trafficking, leading to atypical synaptic distribution and LTP/LTD disruption (171, 172). The Aβ and NMDA relationships may explain a sustained excitotoxic response seen post-TBI/post-seizure. Due to sustained NR1/NR2A responses to high frequency stimulation, disrupted NR2B-mediated calcium influx, and diminished AMPA receptor activity (171, 172), downstream effects of RIR continue, along with a failure to clear excess synaptic glutamate (Figure 3). AMPA receptors are crucial for synaptic plasticity, learning, and memory (192, 193). Loss of AMPA receptors results in diminished synaptic transmission, long-term depression, and difficulties with learning and memory (193). In both brain tissue from AD patients and Aβ-treated neurons, there are significant decreases in AMPA receptor densities, with higher receptor turnover (194). In the presence of Aβ, decreased AMPA receptor expression and greater receptor turnover may be early indicators of

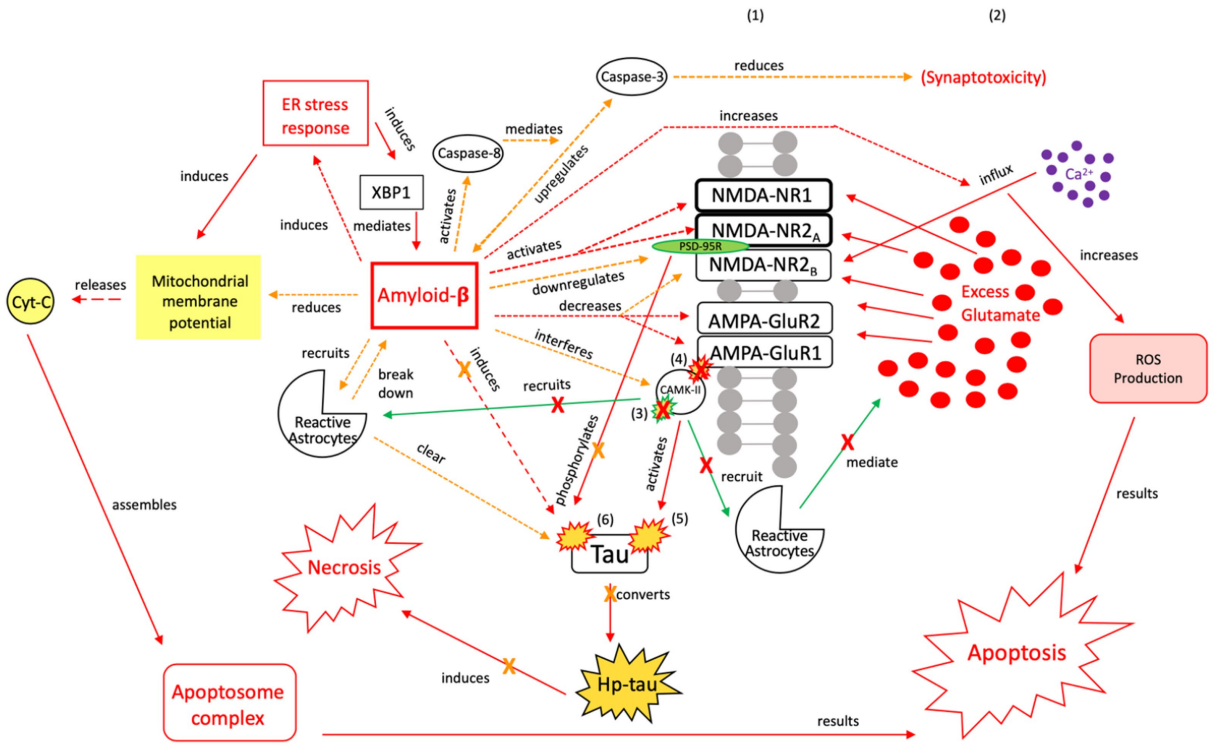


FIGURE 7
The neuroprotective response of Aβ, aka the "last ditch effort" to revert the cell to pro-apoptotic signaling and reduce tau and Aβ toxicity. Selective NMDA regulation, downregulating scaffolding protein PSD-95, and activating caspase-8 reduce the excitotoxic effects of Aβ and atypical tau phosphorylation (154). PSD-95 receptor downregulation results in reduced tau phosphorylation and protection of synapses from the effects of Aβ, while caspase-8 activation indirectly reduces synaptotoxicity by mediating the relationship between Aβ and caspase-3 (163, 164). Aβ also reduces mitochrondrial membrane potential and directly induces the ER stress response, resulting in apoptosome complex formation and eventual ROS-induced apoptosis (165). Reactive astrocytes are recruited to break down Aβ and clear tau aggregates. However, Aβ also has injurious effects, as it increases intracellular $Ca^{2+}$ and ROS production, while also acting directly on tau (154). Therefore, this mechanism is considered a "last ditch effort" to acutely kill the cell via apoptotic signaling to minimize the negative effects from toxic tau, Aβ accumulation, and necrotic signaling. We posit that limiting the effects of tau and Aβ toxicity is predicated on the acute nature of this response and treatment of the underlying pathology to avoid irreversible injury and/or a transition to a more widespread neurodegenerative process. XBP1, X-box binding protein 1; Cyt-c, cytochrome-c; ROS, reactive oxygen species; Red = Pro-death signaling, Green = Neuroprotective signaling, Orange = Aβ-involved signaling; X = reduction/down-regulation. Solid line = signaling cascade induced/propagated by the ER stress response and tau; dashed line = signaling cascades resulting from Aβ involvement. (1) = Neuronal membrane, (2) = Synaptic cleft, (3) = CaMK-II-autophosphorylation, (4) = CaMK-II-Glutamate receptor phosphorylation, (5) = CaMK-II-tau-phosphorylation, (6) = PSD95-NMDA receptor complex-tau phosphorylation.

atypical mechanistic changes associated with AD and resultant cognitive decline. If Aβ is acutely activated, we posit that the cell reorients to apoptotic signaling, minimizing injurious effects of Aβ; however, chronic Aβ activation further imbalances apoptotic-necrotic signaling and initiates a transition of this "last ditch effort" from injurious to neurodegenerative.

Several mechanisms act in concert to increase phosphorylation of tau in the setting of repeated injuries. (1) Aβ induces caspase-3 activation (195) (Figure 5). (2) Aβ-42 reduces MMP in cortical neurons (122, 146, 196), thereby increasing ATP production and cyt-c release. Cyt-c mediates caspase-3 activation that leads to tau cleavage and phosphorylation (197). (3) Endogenous tau interacts with the PSD95-NMDA receptor complex, which selectively phosphorylates tau (198). To efficiently kill the cell via apoptosis, Aβ must activate alternative apoptotic pathways while reducing the tau response. (1) Caspase-8 recruitment by Aβ mediates the relationship between Aβ and caspase-3, resulting in decreased synaptic excitotoxicity and a reorientation toward apoptotic signaling (163). (2) NMDA NR1/NR2A receptor activity affects downstream ROS production resulting in apoptosis (163, 198). (3) Aβ downregulates the PSD95-NMDA receptor complex, decreasing tau phosphorylation. In cultured cells, Aβ-induced apoptosis increased reactive oxygen species (ROS) production but not hp-tau (165). While ROS-produced apoptosis has detrimental effects, as a "last ditch" neuroprotective effort of Aβ, it limits further hp-tau and NFT formation. Limiting the effects of tau and Aβ toxicity is predicated on the acute nature of this response and treatment of the underlying pathology to avoid long-term neurodegeneration.

## 4.1 Summary of NPT, NIT, and Aβ hypotheses

Tau phosphorylation antagonizes apoptotic processes in response to increased ER stress and imbalanced homeostatic dynamics (147). Tau hyperphosphorylation is a reactive response activated when faced with apoptotic cell death (120, 129). The build-up of hp-tau, therefore, could represent a failed neuroprotective mechanism. NFTs, a hallmark

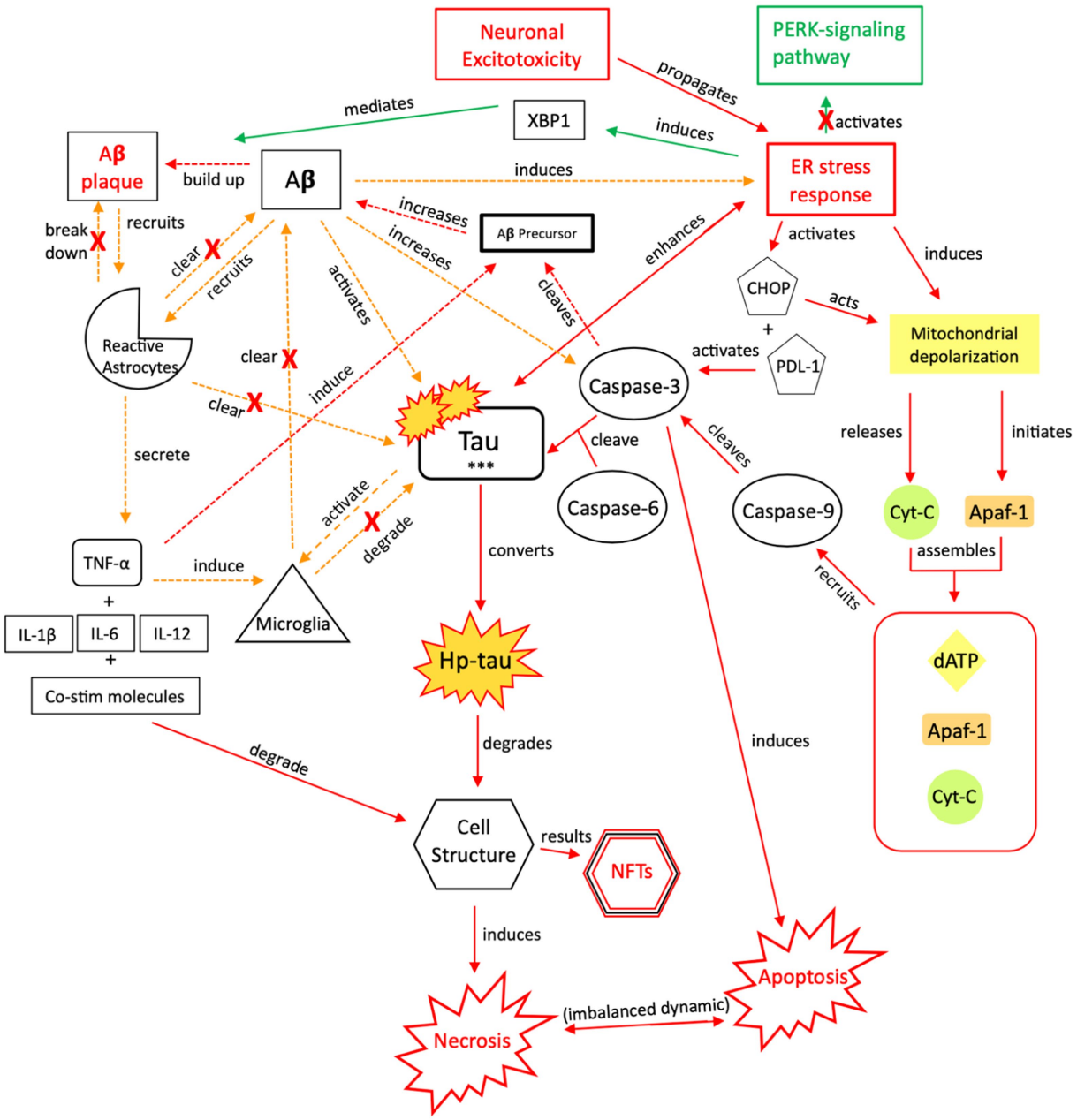


FIGURE 8
The injurious response of Aβ, aka its failed "last ditch effort" to downregulate propagation of toxic tau and rebalance apoptotic-necrotic signaling dynamics. Due to accumulated toxic tau aggregates and dysregulated tau clearance by reactive astrocytes and microglia, pro-death signaling mechanisms become favored over cellular preservation signaling. However, a recurrent, reactive ER stress response leads to an imbalance of apoptotic-necrotic signaling and enhances atypical tau phosphorylation. Further, Aβ precursor protein is cleaved by caspase-3, and Aβ concentrations increase, further propagating the ER stress response (88, 154, 155). Due to the Aβ precursor overexpression and increased Aβ production, defective mitochondria are produced, mitochondrial dynamics are altered, and their trafficking is reduced, leading to further intracellular $Ca^{2+}$ influx and apoptotic-necrotic imbalance (166). However, the ER stress response also has neuroprotective effects, inducing selective transcription factor XBP1, which mediates Aβ plaque formation (88). Simultaneously, Aβ directly recruits reactive astrocytes and indirectly recruits microglia, through TNF-α and pro-inflammatory signaling, which cluster around Aβ plaques to clear them (157, 167, 168). Yet, the induction of pro-inflammatory signaling from astrocytic recruitment further induces Aβ precursor protein; increased Aβ concentrations result in increased atypical tau phosphorylation/hyper-phosphorylation and further ER stress response induction (169). Thus, reactive astrocytes have both neuroprotective and injurious effects (170). Continued apoptotic-necrotic signaling imbalance, degradation in cell structure, and NFT formation results from atypical activation of these pathways. PERK, protein kinase R-like ER kinase; Aβ, amyloid beta; XBP1, X-box binding protein 1; TNF, tumor necrosis factor; IL, interleukin; Co-stim, co-stimulatory (molecules); Cyt-c, cytochrome-c; Apaf-1, apoptotic peptidase activating factor-1; dATP, deoxyadenosine triphosphate; NFTs, neurofibrillary tangles; Red=Pro-death signaling, Green=Neuroprotective signaling, Orange=Aβ-involved signaling; o-tau, tau oligomers; t-tau, total tau; p-tau, phosphorylated tau; ***=O-tau, t-tau, p-tau. X=reduction/down-regulation. Solid line=signaling cascade induced/propagated by the ER stress response and tau; dashed line=signaling cascades resulting from Aβ involvement.

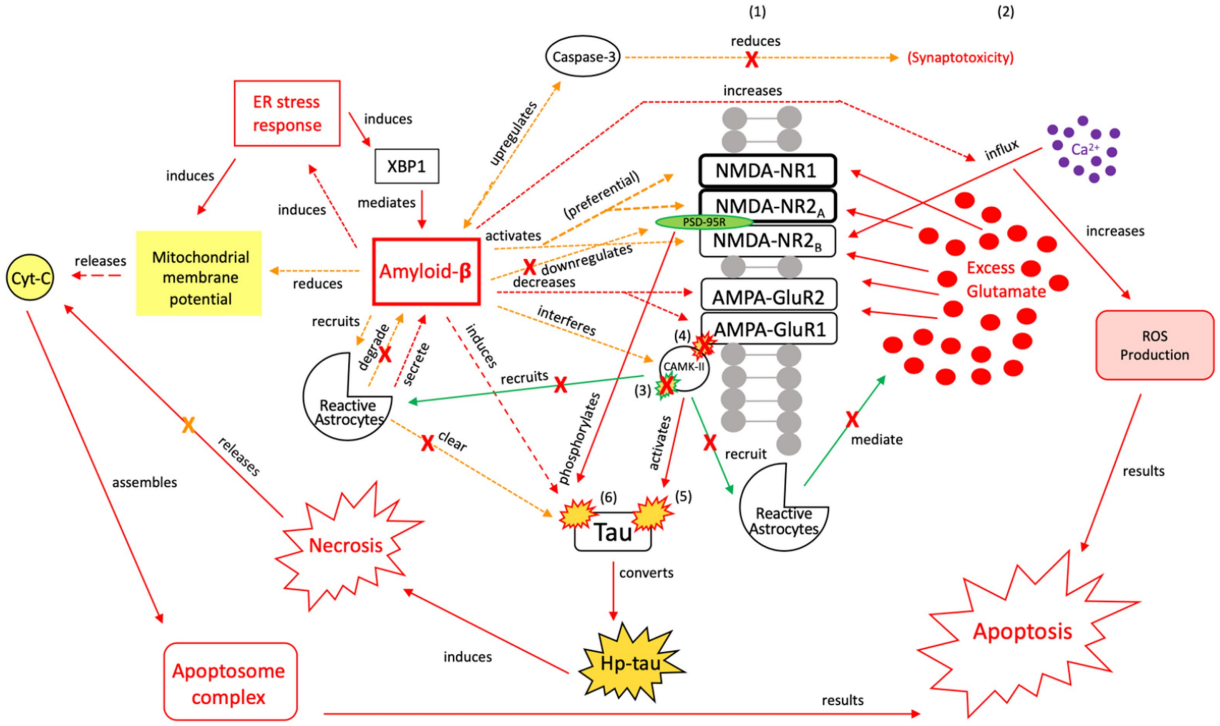


FIGURE 9
The injurious response of Aβ, aka the failed "last ditch effort" to revert the cell to pro-apoptotic signaling and rebalance apoptosis-necrosis, due to recurrent or sustained Aβ signaling. Unlike neuroprotective Aβ responses, preferential activation of NMDA-R1 and-2A/B receptor subunits by Aβ (171, 172), and their increased surface expression regulated by PSD-95, adversely affects channel assembly and conductance (173), promoting further neuroexcitotoxicity, atypical tau phosphorylation, and increased susceptibility to Aβ (164). Unsuccessful toxic tau aggregate and Aβ breakdown by microglia [seen in (A)] propagates the injurious effects of Aβ-associated tau seeding and propagation (133). In the presence of dysregulated tau and Aβ mechanisms, as well as dysregulated microglial and reactive astrocytic clearance, the failure to reduce neuroinflammation and excitotoxic propagation results in a transition from neuroprotection to neurodegeneration. We posit that this point marks the transition from a injurious mechanism to a more widespread neurodegenerative process. XBP1, X-box binding protein 1; Cyt-c, cytochrome-c; ROS, reactive oxygen species; Red=Pro-death signaling, Green=Neuroprotective signaling, Orange=Aβ-involved signaling; X=reduction/down-regulation. Solid line=signaling cascade induced/propagated by the ER stress response and tau; dashed line=signaling cascades resulting from Aβ involvement. (1)=Neuronal membrane, (2)=Synaptic cleft, (3)=CaMK-II-autophosphorylation, (4)=CaMK-II-Glutamate receptor phosphorylation, (5)=CaMK-II-tau-phosphorylation, (6)=PSD95-NMDA receptor complex-tau phosphorylation.

of tauopathies, form as a downstream result of the RIR and NPT pathways. In addition to containing hp-tau, NFTs contain active caspase-6, caspase-6-cleaved tau, and Aβ, further supporting that NFTs are the end result of a neuronal degradation pathway – one that initially includes a neuroprotective pathway preferred by the cell over acute apoptotic death (9), but over time, becomes overwhelmed by the accumulation of repeated injuries.

The NPT response suggests that excess tau is phosphorylated in attempts to preserve cellular integrity in the short-term. In response, microglia and reactive astrocytes are triggered to reinstate homeostasis and break down intracellular tau into non-toxic isoforms. However, in the setting of repeated injury, when excess tau phosphorylation exceeds microglial and astrocytic capacity for tau degradation, toxic tau accumulates. This, along with aberrant tau cleavage, aggregation, and hyper-phosphorylation, propagates a dysregulated microglial response. To combat this, the toxic tau must be expelled from the cell and is done so through exosomal packaging and secretion.

We posit that the response mechanism is neuroprotective to the point of halting apoptosis, phosphorylating tau, and clearing tau via microglia and reactive astrocytes, and that it would continue to be neuroprotective if repetitive seizures or head injuries did not (1) lead to Aβ accumulation and its production of toxic tau and (2) outpace the ability to clear tau. Because epilepsy and repeated TBIs are plagued with recurrent cellular injury and ER response activation, however, a buildup of cleaved, phosphorylated, and hyperphosphorylated tau results in toxic tau aggregates. These toxic aggregates are then propagated to surrounding neurons, adversely affecting these neighboring neurons and increasing the likelihood for localized neuronal degeneration. The extracellular leakage of toxic tau also contributes to NFT formation and induces tauopathy-related necrosis, transitioning the mechanism over time from neuroprotective to neurodegenerative.

The role of Aβ is pivotal in the development of neurodegeneration. Aβ induces tau phosphorylation, contributing to toxic tau aggregates that cannot be cleared by microglia and reactive astrocytes. Additionally, microglia cannot clear the excess Aβ, leading to inflammatory signaling, excitotoxicity, and Aβ plaque accumulation. Microglial and reactive astrocytic dysregulation results in further tau and Aβ leakage that contributes to injury. We posit that, although increased Aβ concentration has a deleterious effect on cellular integrity and microglial functioning, increased Aβ also reactivates preferential apoptotic signaling by

targeting NMDA/AMPA receptor functioning, CaMK-II phosphorylation, astrocytic recruitment, and mitochondrial membrane permeability (Figure 6). Because glutamate transmission, apoptosis, and necrotic signaling share related pathways, this Aβ compensatory mechanism cannot differentiate between typical and atypical activation, such that excitotoxicity continues. Recurrent or sustained activation of these mechanisms results in the necrotic cell death seen in tauopathies.

## 5 Clinical correlations: tau and TBI

Early studies lacked an association between TBI and cerebrospinal fluid (CSF) p-tau levels, likely because of insufficient sensitivity of the assay, requiring development of novel techniques (92, 199). An enhanced immunoassay using multi-arrayed fiber optics (EIMAF) detected acutely increased t-tau and p-tau levels in brain and blood following CCI in rodents and in CSF following severe TBI in humans. T-tau and p-tau levels remained significantly elevated during the chronic stage of CCI in rodents. While t-tau and p-tau levels decreased during the chronic stage of severe TBI in humans, elevated levels were still detected in subsequent months post-injury. T-tau levels approached normal limits approximately one-month post-injury, while p-tau levels remained elevated six months post-injury (200). EIMAF also demonstrated increased p-tau levels, t-tau levels, and p-tau/t-tau ratios in individuals with acute or chronic TBI compared to healthy controls (201). Using a single-molecule enzyme-linked immunosorbent assay (SIMOA), blood t-tau levels were greater in professional hockey players across multiple time points post-head injury (from one to 48 h) compared to preseason (pre-injury) (91). Recent studies have also measured tau within exosomes isolated from plasma (202, 203). This technique has been applied in remote repetitive TBI, with elevated exosomal t-tau and p-tau levels negatively correlating with neuropsychological measures (202, 203).

Tau levels correlate with clinical recovery, with a negative association between CSF tau and clinical improvement (204). Ventricular CSF t-tau concentrations in the setting of severe TBI negatively correlated with clinical improvement over one year (205). Plasma p-and t-tau levels measured in patients ~24-h post-acute head injury were associated with short-and long-term outcomes; p-tau and p-tau/t-tau ratios in blood negatively correlated with recovery in participants with chronic TBI (201). Human data concur with a rat model, in which serum and CSF tau levels positively correlated with traumatic spinal cord injury severity and negatively correlated with locomotor function (206). These results support p-tau as a biomarker that reflects a broad picture of axonal injury, TBI severity, cognitive functioning, and long-term outcomes.

## 6 Clinical correlations: tau and AD

Pathological p-tau aggregation is a biomarker of neurodegeneration in AD. In a transgenic mouse model of AD, microglial activation occurs in a progressive fashion, correlating with increased tau hyper-phosphorylation and Aβ plaque accumulation (207). Human and animal models of AD and other dementias identify atypical tau processes that contribute to increased hyper-phosphorylation, microglial activation, NFT formation, and neurodegeneration (208), including genetic mutations and post-translational modifications (209–214). Atypical tau phosphorylation and APP mutations correlate with NFT formation in animal models and human AD (215, 216). In human AD brain tissue, tau pathology was divided into early and late stages, with tau deposition first observed in entorhinal cortex and hippocampus. Later tau aggregates correlated with cognitive decline (217). In human lateral temporal cortex obtained from late-stage AD brains, increased markers of the ER stress response correlated with decreased post-synaptic PSD-95 markers and increased tau (218).

Increased CSF t-tau levels were also found in patients with AD (219). Elevated CSF tau levels demonstrated a strong association with AD and improved discrimination of AD from other dementias, while Aβ levels failed to improve diagnostic accuracy (220). CSF p-tau181, 217, and 231 concentrations accurately predicted cognitive impairment in patients with AD, but not in patients with other dementias or controls (221). P-tau231 was the earliest detector of increased Aβ in AD pathology, preceding Aβ identification by position emission tomography (PET) (221). Further, increased levels of tau and decreased levels of $A\beta_{1\text{-}42}$ in CSF were reported (222–226), highlighting their contrasting CSF profiles as biomarkers for AD. In plasma, tau levels were significantly higher in patients with AD compared to MCI patients and controls, however, use of plasma tau as a diagnostic test is not yet validated (227).

## 7 Clinical correlations: tau and epilepsy

A link between AD and temporal lobe epilepsy (TLE) is demonstrated by a bidirectional increase in risk, hippocampal damage (228), and cognitive deficits in both disorders, in part due to shared cortical networks, tau deposition, and amyloid pathology. Current research explores the influence of seizure activity on tau levels in brain, CSF, and blood, proposing that epilepsy is a tauopathy like AD and CTE – with proposed mechanisms of tau deposition including production during ictal and interictal activity, axonal sprouting and formation of aberrant connections in response to injury, cell death, physical injury during seizures, and decreased clearance (94). Studying the relationship between tau and epilepsy may address how seizure activity results in neuronal injury.

Limited data are available regarding tau levels in people with epilepsy. Hp-tau deposits were identified in resected temporal lobe tissue from patients with hippocampal sclerosis, evident in nearly 94% of cases and correlating with post-operative declines in verbal memory and naming, though this finding was not seen in all resection studies (94). In late-onset epilepsy of unknown origin, CSF t-tau levels were increased in comparison to controls, with t-tau and p-tau levels predicting onset of dementia (229). Elevated CSF t-tau and p-tau levels were detected in patients with status epilepticus when tested at a median of 72 h from admission (95). In the setting of status, t-tau levels positively correlated with medication resistance, status duration, disability, and development of chronic epilepsy (95). While a transient increase of CSF t-tau was reported within four days of a single, new-onset generalized convulsion, tau elevations in isolated or repeated seizures that

respond promptly to medications are controversial (96, 97). Increased CSF t-tau levels were seen with symptomatic convulsions (of acute or remote etiology), but not in subjects with seizures of idiopathic or cryptogenic cause when levels were obtained within 48 h (98). CSF t-tau levels were decreased, and p-tau unchanged, when CSF was collected at least seven days after the last seizure, but seizure frequency was unknown (230). Blood–brain barrier disruption during seizures may release tau to the periphery, suggested by small, transient elevations of serum T-tau following convulsions (231). Studies of peripheral p-tau and exosomal analyses have not yet been applied to people with epilepsy, and the impact of epilepsy-related factors (e.g., seizure type, epilepsy duration) on peripheral tau levels is unknown.

The relationship between epilepsy and p-tau levels should be explored as a potential marker of neural injury severity and predictor of cognitive function and seizure control. Given the above similarities in injury pathophysiology between AD, TBI, and epilepsy, AD and TBI may serve as guides to identifying overlapping markers of neuronal damage and cognition.

# 8 Treating AD, TBI, and epilepsy: pharmacological interventions

## 8.1 Cytokine targets

A better understanding of tau deposition lends insight into AD, TBI, and epilepsy pathophysiology and presents possible targets for intervention. Potential approaches include neuroprotection, inhibition of inflammatory processes, and disruption of excitotoxic mechanisms. Trials focused on various portions of these pathways. In animal models of TBI, minocycline and statins demonstrate beneficial anti-inflammatory and neuroprotective properties, limit the expression of pro-inflammatory cytokines, and render cell death-associated astrocytes and microglia inactive (232–234). *In vitro* and *in vivo* rat brain TBI and immune system studies identified human-cultured mesenchymal stem cells coupled with purified immune cells as a promising treatment that increases production of anti-inflammatory ILs, while decreasing TNF-α (235–237). IL-34 selectively enhances microglial neuroprotective effects, homeostasis, and neuronal survival by promoting Aβ oligomeric clearance and inducing microglial enzymatic activity. These effects reduce oxidative stress without promoting neurotoxicity (61). Promotion of IL-34 receptor binding or activity may benefit those with recurrent seizures/TBI by enhancing microglial function.

## 8.2 NMDA receptor antagonists

Data regarding NMDA antagonists are mixed. Drugs like amantadine, a weak NMDA antagonist, are commonly used in acute brain injury rehabilitation, although supporting data are limited (238). In some TBI studies, NMDA receptor antagonists lacked efficacy and raised safety concerns (239). A trial of the competitive NMDA antagonist D-CCP-ene for the treatment of intractable focal-onset seizures led to severe adverse events in all eight subjects, including sedation, ataxia, depression, amnesia, and poor concentration (240). Seizure frequency worsened in three subjects and remained unchanged in four subjects; one participant demonstrated improved seizure frequency, yet experienced status epilepticus upon D-CCP-ene withdrawal (240). All subjects withdrew from participation, leading to premature termination of the study. However, in a large, randomized, double-blind, placebo-controlled trial of traxoprodil, an NMDA NR2B subunit antagonist, was found to be well-tolerated in adults with severe TBI; they demonstrated improved Glasgow Coma Scale outcomes 6-months post-injury compared to placebo (241).

In an animal model of hippocampal seizures, MK-801 decreased seizure severity at low doses (242). In 68 patients with super-refractory status epilepticus, ketamine infusions administered for a length of one to four days reduced seizure burden by 50% (243). Upregulated NMDA receptor trafficking in the post-synaptic membrane contributes to super-refractory status epilepticus; NMDA receptor antagonists like MK-801 and ketamine may be effective due to improved penetration of the blood brain barrier and maintain their function even in the presence of increased concentrations of intra-and extra-cellular glutamate (244–246).

Memantine, a low-affinity voltage-dependent uncompetitive NMDA antagonist, approved for use in AD, reduced tau phosphorylation and improved functional outcomes after repetitive mild TBI in adult mice (247). In patients with TLE, memantine improved cognition compared to donepezil (248). In a double-blinded, placebo-controlled trial, once-daily memantine significantly improved episodic memory and quality of life in patients with epilepsy, although confounded by reduced seizure frequency (248, 249). In contrast, in subjects with focal-onset seizures of unchanged frequency, memantine yielded no significant improvement in cognition compared to placebo (250). However, in an open-label extension phase, there were improvements in verbal memory, memory-related quality of life, and executive functioning (250). Overall, NMDA antagonists deserve further study in TBI, AD, and epilepsy (238, 241).

## 8.3 AMPA receptor antagonists

Alternatively, perampanel is highly selective for AMPA receptors and inhibits AMPA-induced calcium influx in rat cortical neurons (251). Pharmacological dampening of AMPA receptor function eliminated interictal-like activity in human lateral amygdala *in vivo*, without reducing AMPA receptor densities observed *in vitro* (252). It is efficacious for treatment of focal-onset seizures with a neutral cognitive profile in adult, geriatric, and pediatric patients (253–255). In a rat CCI model, perampanel preserved neurological function, inhibited apoptosis and microglial activation, reduced brain edema, and preserved blood–brain-barrier functioning post-injury, thereby protecting neuro-vasculature (256). It also reduced brain contusion volume and decreased expression of pro-inflammatory TNF-α and IL-1β (257).

The effects of perampanel on neurological functioning, inflammatory markers, and cognition in patients with AD has yet to be comprehensively studied, outside of isolated case reports. In a case study of an 89 year old woman with severe AD, intractable myoclonic epilepsy, and psychiatric symptoms of circadian rhythm disorder and irritability, perampanel improved both myoclonus and psychiatric symptoms (258). An additional case report demonstrated improved cognitive functioning in a patient with non-convulsive seizures and AD, supporting the case for early administration (259). In transgenic AD mice, inhibition of AMPA

receptors by perampanel reduced hippocampal $A\beta_{40}$ and $A\beta_{42}$ levels and decreased levels of the soluble peptide APPβ by suppressing β-cleavage of APP (260). Further research is needed into the potential effect of perampanel in targeting Aβ pathology by reducing Aβ production in AD.

## 8.4 Metabotropic glutamate receptor treatments

Metabotropic glutamate receptors (mGluR) may also be a target of interest in generalized and focal seizures, as the group II and III mGluR agonists may decrease NMDA receptor function and the risk of excitotoxicity. Animal studies showed anticonvulsant effects of the group II mGluR agonist, DCG-IV, in models of limbic and generalized motor seizures (261–263). Anticonvulsant effects were also noted in DBA/2 rodent models using agonists that target group III $mGluR_8$ and $mGluR_4$, 4A (L-AP4, RS-4-PPG, and ACPT-1) and the antagonist, MPPG. These agents were not found to affect group I, which contribute to epileptogenesis (264, 265). However, mixed responses to mGluR-based treatments have been noted, with proconvulsant effects of group III agonists (L-AP4 and L-SOP) and the MGluR antagonist, MAP4 (266, 267). Further research is needed into safe and effective therapeutic concentrations of mGluR-targeting agents, as well as their role in seizure activity (266–270).

In our proposed RIR model, upregulation of selected NMDA receptors and downregulation of selected AMPA receptors occurs as a result of neuroinflammation in response to sustained or recurrent injury. As a result, there is an increased likelihood of seizure occurrence and atypically high concentrations of intra-and extra-cellular glutamate. At low doses, NMDA receptor antagonists can reduce seizure severity and frequency, but with mixed results. Higher doses, however, risk significant adverse effects. AMPA receptor antagonists, such as perampanel, may show greater promise due to their potential effects on hyperexcitability, underlying pathophysiology of neurodegenerative disorders, and tolerability. MGluR agonists and antagonists showed varied pro-vs. anti-convulsant effects with limited research into safe and effective therapeutic concentrations. Caution in targeting glutamate receptors is warranted.

## 8.5 Monoclonal antibody treatments

Anti-amyloid monoclonal antibodies, such as lecanemab and aducanumab, represent another treatment approach, possibly as maintenance drugs to slow the progression of cognitive decline over the course of the disease. Lecanemab demonstrated high affinity binding to soluble Aβ, and particularly to Aβ soluble protofibrils, which are seen in early AD (271–273). Approved for use in Alzheimer's disease (274), lecanemab reduced Aβ markers and moderately slowed cognitive decline over 18 months compared to placebo (271, 272), although its effectiveness has been questioned. In a transgenic mouse model, aducanumab decreased both soluble and insoluble Aβ in a dose-dependent manner (275). To evaluate the safety and efficacy of aducanumab in reducing cognitive decline in patients with MCI and mild AD, two large, double-blind, placebo-controlled studies were conducted. Results indicated that aducanumab was associated with dose-dependent amyloid related imaging abnormalities (ARIA), with cerebral edema and increased risk of intracerebral hemorrhage, particularly in ApoE-ε4 carriers (276). Infusion-related reactions and other adverse events (including ARIA) make anti-amyloid antibodies a controversial approach in the setting of uncertain benefits. Lecanemab and aducanumab have not yet been tested in patients with TBI or epilepsy, and safety and efficacy clinical trials for both drugs are on-going.

## 8.6 Tau-centric treatments

Reduction of tau levels showed promise in tau-expressing transgenic mice with repetitive mild CHI. Mice were treated with kinase-targeting lithium chloride and R-roscovitine, leading to p-tau reduction that correlated with improved cognition (200, 277). Alternatively, phosphatases dephosphorylate toxic tau into non-toxic isoforms. Phosphatase 2A (PP2A) dephosphorylates hp-tau, but PP2A activity is decreased in AD brain (278, 279). In AD, GSK-3 activation inhibits PP2A (280), and PP2A inhibitory proteins (inhibitor-1 and -2) are upregulated (281). Pharmacological interventions that inhibit GSK-3, such as SAR502250 (282), or support mRNA-based downregulation of PP2A inhibitors-1/2, are promising approaches (281). Drugs for approved for other indications may also be "repurposed" given their effects on tau. Suvorexant, an FDA-approved drug for insomnia, reduces tau phosphorylation at selective sites such as −181 and decreases Aβ concentrations compared to placebo (283); its use should be investigated in other disorders. Angiotensin receptor blockers, FDA-approved for hypertension, have anticonvulsant effects in rats (284–286) and decrease incidence of epilepsy in humans (287), while decreasing CSF t-tau and p-tau in MCI patients (288) and improving cognition in hypertensive older adults with early executive impairment (289) and prodromal AD (290). These results support the need to further investigate the safety and efficacy of tau-targeting drugs in epilepsy.

## 8.7 ER stress response inhibition

Based on our proposed mechanism, drugs that impair the PERK pathway would have injurious effects. In a mouse TBI model, for example, inhibition of the PERK signaling pathway by GSK2606414 exacerbated immature cell loss, dendritic loss, and cell death (26).

Conversely, pharmacological upregulation of the PERK pathway may be an effective treatment target to avoid atypical ER stress response activation, reduce tau phosphorylation by ER stress response signaling (75), and mediate tau hyper-phosphorylation and Aβ neurotoxicity (291).

Drugs that target the ER stress response cell death pathways may also aid in the restoration of intracellular homeostasis, apoptotic-necrotic signaling dynamics, and ER folding capacity. In a rat lateral fluid

percussion model of TBI, administration of the ER stress response inhibitor, salubrinal, 30 min prior to injury significantly reduced the ER stress response, promoted mitochondrial functioning, and inhibited downstream apoptotic signaling (292). In a mouse model of autosomal dominant lateral TLE, 4-phenylbutyric acid restored LGI1 protein function and reduced seizure susceptibility (293). In a mouse model of epilepsy, taurursodiol also reduced seizure susceptibility and mitigated repeated stress-induced neurodegeneration (294). In reducing seizure susceptibility, the likelihood of repeated or chronic activation of the ER stress response and tau-induced pathways decreases. This benefits the cell by favoring restoration of homeostasis, PERK pathway activation, and tau-involvement for maintenance of cellular dynamics; this also reduces the likelihood of repeated/chronic activation of Aβ, resulting in avoidance of irreversible or long-term neurodegeneration.

The numerous proteins and pathways involved in the brain's inflammatory response make it challenging to identify the most appropriate target. Development of inflammatory modulators must also consider that acute inflammation can serve to protect neuronal integrity and avoid cell death, while chronic inflammation may decrease the likelihood of maximal recovery and cell survival. Further research is needed to find preventative and therapeutic agents for AD, TBI, and epilepsy.

## 9 Conclusion

AD, TBI, and epilepsy disrupt neuronal function and promote atypical response signaling. This review examined inflammatory and excitotoxic pathways common to AD, TBI, and epilepsy, the role of the ER stress response in the face of excitotoxicity, and tau and Aβ signaling. We proposed a mechanism by which these pathways can lead to tau deposition. We posit that tau accumulation represents an attempt to shunt the injury response from apoptosis toward neuroprotective signaling that preserves the cell, in attempts to restore homeostasis. This could be viewed as an acute "neuroprotective" response, although, if the underlying pathology is not treated, its recurrent or sustained activation will result in neurodegeneration. Our proposed mechanism supports the case for early intervention. In patients with AD, we must identify risk factors that impact tau and Aβ processes prior to the appearance of cognitive decline. In patients with TBI, this means reducing the likelihood of recurrent injury, reducing injury severity through preventative measures, and providing ample recovery time. In patients with epilepsy, we need to identify the underlying etiologies and reduce seizure frequency and severity. These pathways may present targets for intervention in AD, TBI, and epilepsy. Studies that examine mediators of these signaling cascades are needed.

## Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

## Author contributions

SM: Conceptualization, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. BAL-M: Conceptualization, Funding acquisition, Methodology, Supervision, Visualization, Writing – review & editing.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## References

1. Roth TL, Nayak D, Atanasijevic T, Koretsky AP, Latour LL, McGavern DB. Transcranial amelioration of inflammation and cell death after brain injury. *Nature*. (2014) 505:223–8. doi: 10.1038/nature12808

2. Vezzani A, French J, Bartfai T, Baram TZ. The role of inflammation in epilepsy. *Nat Rev Neurol*. (2011) 7:31–40. doi: 10.1038/nrneurol.2010.178

3. Lucke-Wold BP, Nguyen L, Turner RC, Logsdon AF, Chen YW, Smith KE, et al. Traumatic brain injury and epilepsy: underlying mechanisms leading to seizure. *Seizure*. (2015) 33:13–23. doi: 10.1016/j.seizure.2015.10.002

4. Hernandez F, Avila J. Tauopathies. *Cell Mol Life Sci*. (2007) 64:2219–33. doi: 10.1007/s00018-007-7220-x

5. Johnson GV, Stoothoff WH. Tau phosphorylation in neuronal cell function and dysfunction. *J Cell Sci*. (2004) 117:5721–9. doi: 10.1242/jcs.01558

6. Ittner LM, Ke YD, Delerue F, Bi M, Gladbach A, van Eersel J, et al. Dendritic function of tau mediates amyloid-beta toxicity in Alzheimer's disease mouse models. *Cells*. (2010) 142:387–97. doi: 10.1016/j.cell.2010.06.036

7. Kaech S, Banker G. Culturing hippocampal neurons. *Nat Protoc*. (2006) 1:2406–15. doi: 10.1038/nprot.2006.356

8. Mandelkow E, von Bergen M, Biernat J, Mandelkow EM. Structural principles of tau and the paired helical filaments of Alzheimer's disease. *Brain Pathol*. (2007) 17:83–90. doi: 10.1111/j.1750-3639.2007.00053.x

9. Gamblin TC, Chen F, Zambrano A, Abraha A, Lagalwar S, Guillozet AL, et al. Caspase cleavage of tau: linking amyloid and neurofibrillary tangles in Alzheimer's disease. *Proc Natl Acad Sci U S A*. (2003) 100:10032–7. doi: 10.1073/pnas.1630428100

10. Boraschi D, Italiani P, Weil S, Martin MU. The family of the interleukin-1 receptors. *Immunol Rev*. (2018) 281:197–232. doi: 10.1111/imr.12606

11. Moriwaki K, Chan FK. RIP3: a molecular switch for necrosis and inflammation. *Genes Dev*. (2013) 27:1640–9. doi: 10.1101/gad.223321.113

12. Kelliher MA, Grimm S, Ishida Y, Kuo F, Stanger BZ, Leder P. The death domain kinase RIP mediates the TNF-induced NF-kappaB signal. *Immunity*. (1998) 8:297–303. doi: 10.1016/S1074-7613(00)80535-X

13. Kumar RG, Boles JA, Wagner AK. Chronic inflammation after severe traumatic brain injury: characterization and associations with outcome at 6 and 12 months Postinjury. *J Head Trauma Rehabil*. (2015) 30:369–81. doi: 10.1097/HTR.0000000000000067

14. Hodo TW, de Aquino MTP, Shimamoto A, Shanker A. Critical neurotransmitters in the Neuroimmune network. *Front Immunol*. (2020) 11:1869. doi: 10.3389/fimmu.2020.01869

15. Neher E, Sakaba T. Multiple roles of calcium ions in the regulation of neurotransmitter release. *Neuron*. (2008) 59:861–72. doi: 10.1016/j.neuron.2008.08.019

16. Walls AB, Waagepetersen HS, Bak LK, Schousboe A, Sonnewald U. The glutamine-glutamate/GABA cycle: function, regional differences in glutamate and GABA production and effects of interference with GABA metabolism. *Neurochem Res*. (2015) 40:402–9. doi: 10.1007/s11064-014-1473-1

17. Kumar A, Zou L, Yuan X, Long Y, Yang K. N-methyl-D-aspartate receptors: transient loss of NR1/NR2A/NR2B subunits after traumatic brain injury in a rodent model. *J Neurosci Res*. (2002) 67:781–6. doi: 10.1002/jnr.10181

18. Kharlamov EA, Lepsveridze E, Meparishvili M, Solomonia RO, Lu B, Miller ER, et al. Alterations of GABA(a) and glutamate receptor subunits and heat shock protein in rat hippocampus following traumatic brain injury and in posttraumatic epilepsy. *Epilepsy Res*. (2011) 95:20–34. doi: 10.1016/j.eplepsyres.2011.02.008

19. Chater TE, Goda Y. The role of AMPA receptors in postsynaptic mechanisms of synaptic plasticity. *Front Cell Neurosci*. (2014) 8:401. doi: 10.3389/fncel.2014.00401

20. Atkins CM, Chen S, Alonso OF, Dietrich WD, Hu BR. Activation of calcium/calmodulin-dependent protein kinases after traumatic brain injury. *J Cereb Blood Flow Metab*. (2006) 26:1507–18. doi: 10.1038/sj.jcbfm.9600301

21. Cao F, Zhou Z, Cai S, Xie W, Jia Z. Hippocampal Long-term depression in the presence of calcium-permeable AMPA receptors. *Front Synaptic Neurosci*. (2018) 10:41. doi: 10.3389/fnsyn.2018.00041

22. Patel TP, Ventre SC, Geddes-Klein D, Singh PK, Meaney DF. Single-neuron NMDA receptor phenotype influences neuronal rewiring and reintegration following traumatic injury. *J Neurosci*. (2014) 34:4200–13. doi: 10.1523/JNEUROSCI.4172-13.2014

23. Isaac JT, Ashby MC, McBain CJ. The role of the GluR2 subunit in AMPA receptor function and synaptic plasticity. *Neuron*. (2007) 54:859–71. doi: 10.1016/j.neuron.2007.06.001

24. Parga Becerra A, Logsdon AF, Banks WA, Ransom CB. Traumatic brain injury broadly affects GABAergic signaling in dentate gyrus granule cells. *eNeuro*. (2021) 8:ENEURO.0055–20.2021. doi: 10.1523/ENEURO.0055-20.2021

25. Zhou H, Chen L, Gao X, Luo B, Chen J. Moderate traumatic brain injury triggers rapid necrotic death of immature neurons in the hippocampus. *J Neuropathol Exp Neurol*. (2012) 71:348–59. doi: 10.1097/NEN.0b013e31824ea078

26. Hood KN, Zhao J, Redell JB, Hylin MJ, Harris B, Perez A, et al. Endoplasmic reticulum stress contributes to the loss of newborn hippocampal neurons after traumatic brain injury. *J Neurosci*. (2018) 38:2372–84. doi: 10.1523/JNEUROSCI.1756-17.2018

27. Bao YH, Bramlett HM, Atkins CM, Truettner JS, Lotocki G, Alonso OF, et al. Post-traumatic seizures exacerbate histopathological damage after fluid-percussion brain injury. *J Neurotrauma*. (2011) 28:35–42. doi: 10.1089/neu.2010.1383

28. De Beaumont L, Tremblay S, Poirier J, Lassonde M, Théoret H. Altered bidirectional plasticity and reduced implicit motor learning in concussed athletes. *Cereb Cortex*. (2012) 22:112–21. doi: 10.1093/cercor/bhr096

29. Farrant M, Nusser Z. Variations on an inhibitory theme: phasic and tonic activation of GABA(a) receptors. *Nat Rev Neurosci*. (2005) 6:215–29. doi: 10.1038/nrn1625

30. Mamun AA, Uddin MS, Mathew B, Ashraf GM. Toxic tau: structural origins of tau aggregation in Alzheimer's disease. *Neural Regen Res*. (2020) 15:1417–20. doi: 10.4103/1673-5374.274329

31. Caccamo A, Branca C, Piras IS, Ferreira E, Huentelman MJ, Liang WS, et al. Necroptosis activation in Alzheimer's disease. *Nat Neurosci*. (2017) 20:1236–46. doi: 10.1038/nn.4608

32. Ii M, Sunamoto M, Ohnishi K, Ichimori Y. beta-amyloid protein-dependent nitric oxide production from microglial cells and neurotoxicity. *Brain Res*. (1996) 720:93–100. doi: 10.1016/0006-8993(96)00156-4

33. Griffin WS, Sheng JG, Roberts GW, Mrak RE. Interleukin-1 expression in different plaque types in Alzheimer's disease: significance in plaque evolution. *J Neuropathol Exp Neurol*. (1995) 54:276–81. doi: 10.1097/00005072-199503000-00014

34. Griffin WS, Sheng JG, Royston MC, Gentleman SM, McKenzie JE, Graham DI, et al. Glial-neuronal interactions in Alzheimer's disease: the potential role of a 'cytokine cycle' in disease progression. *Brain Pathol*. (1998) 8:65–72. doi: 10.1111/j.1750-3639.1998.tb00136.x

35. Prehn JH, Bindokas VP, Jordán J, Galindo MF, Ghadge GD, Roos RP, et al. Protective effect of transforming growth factor-beta 1 on beta-amyloid neurotoxicity in rat hippocampal neurons. *Mol Pharmacol*. (1996) 49:319–28.

36. Decourt B, Lahiri DK, Sabbagh MN. Targeting tumor necrosis factor alpha for Alzheimer's disease. *Curr Alzheimer Res*. (2017) 14:412–25. doi: 10.2174/1567205013666160930110551

37. Choi DW. Ionic dependence of glutamate neurotoxicity. *J Neurosci*. (1987) 7:369–79. doi: 10.1523/JNEUROSCI.07-02-00369.1987

38. Choi DW, Koh JY, Peters S. Pharmacology of glutamate neurotoxicity in cortical cell culture: attenuation by NMDA antagonists. *J Neurosci*. (1988) 8:185–96. doi: 10.1523/JNEUROSCI.08-01-00185.1988

39. Abramov E, Dolev I, Fogel H, Ciccotosto GD, Ruff E, Slutsky I. Amyloid-beta as a positive endogenous regulator of release probability at hippocampal synapses. *Nat Neurosci*. (2009) 12:1567–76. doi: 10.1038/nn.2433

40. Domingues A, Almeida S, da Cruz Silva EF, Oliveira CR, Rego AC. Toxicity of beta-amyloid in HEK293 cells expressing NR1/NR2A or NR1/NR2B N-methyl-D-aspartate receptor subunits. *Neurochem Int*. (2007) 50:872–80. doi: 10.1016/j.neuint.2007.03.001

41. Le WD, Colom LV, Xie WJ, Smith RG, Alexianu M, Appel SH. Cell death induced by beta-amyloid 1-40 in MES 23.5 hybrid clone: the role of nitric oxide and NMDA-gated channel activation leading to apoptosis. *Brain Res*. (1995) 686:49–60. doi: 10.1016/0006-8993(95)00450-5

42. De Felice FG, Velasco PT, Lambert MP, Viola K, Fernandez SJ, Ferreira ST, et al. Abeta oligomers induce neuronal oxidative stress through an N-methyl-D-aspartate receptor-dependent mechanism that is blocked by the Alzheimer drug memantine. *J Biol Chem*. (2007) 282:11590–601. doi: 10.1074/jbc.M607483200

43. Lacor PN, Buniel MC, Furlow PW, Sanz Clemente A, Velasco PT, Wood M, et al. Abeta oligomer-induced aberrations in synapse composition, shape, and density provide a molecular basis for loss of connectivity in Alzheimer's disease. *J Neurosci*. (2007) 27:796–807. doi: 10.1523/JNEUROSCI.3501-06.2007

44. Gueli MC, Taibi G. Alzheimer's disease: amino acid levels and brain metabolic status. *Neurol Sci*. (2013) 34:1575–9. doi: 10.1007/s10072-013-1289-9

45. Bareggi SR, Franceschi M, Bonini L, Zecca L, Smirne S. Decreased CSF concentrations of homovanillic acid and gamma-aminobutyric acid in Alzheimer's disease. Age-or disease-related modifications? *Arch Neurol*. (1982) 39:709–12. doi: 10.1001/archneur.1982.00510230035010

46. Garcia-Marin V, Blazquez-Llorca L, Rodriguez J-R, Boluda S, Muntane G, Ferrer I, et al. Diminished perisomatic GABAergic terminals on cortical neurons adjacent to amyloid plaques. *Front Neuroanat*. (2009) 3:28. doi: 10.3389/neuro.05.028.2009

47. Ramos-Miguel A, Hercher C, Beasley CL, Barr AM, Bayer TA, Falkai P, et al. Loss of Munc18-1 long splice variant in GABAergic terminals is associated with cognitive decline and increased risk of dementia in a community sample. *Mol Neurodegener*. (2015) 10:65. doi: 10.1186/s13024-015-0061-4

48. Jara JH, Singh BB, Floden AM, Combs CK. Tumor necrosis factor alpha stimulates NMDA receptor activity in mouse cortical neurons resulting in ERK-dependent death. *J Neurochem*. (2007) 100:1407–20. doi: 10.1111/j.1471-4159.2006.04330.x

49. Stellwagen D, Beattie EC, Seo JY, Malenka RC. Differential regulation of AMPA receptor and GABA receptor trafficking by tumor necrosis factor-alpha. *J Neurosci*. (2005) 25:3219–28. doi: 10.1523/JNEUROSCI.4486-04.2005

50. Vezzani A, Conti M, de Luigi A, Ravizza T, Moneta D, Marchesi F, et al. Interleukin-1beta immunoreactivity and microglia are enhanced in the rat hippocampus by focal kainate application: functional evidence for enhancement of electrographic seizures. *J Neurosci*. (1999) 19:5054–65. doi: 10.1523/JNEUROSCI.19-12-05054.1999

51. During MJ, Spencer DD. Extracellular hippocampal glutamate and spontaneous seizure in the conscious human brain. *Lancet*. (1993) 341:1607–10. doi: 10.1016/0140-6736(93)90754-5

52. Caro-Maldonado A, Tait SWG, Ramírez-Peinado S, Ricci JE, Fabregat I, Green DR, et al. Glucose deprivation induces an atypical form of apoptosis mediated by caspase-8 in Bax-Bak-deficient cells. *Cell Death Differ*. (2010) 17:1335–44. doi: 10.1038/cdd.2010.21

53. Henshall DC, Simon RP. Epilepsy and apoptosis pathways. *J Cereb Blood Flow Metab*. (2005) 25:1557–72. doi: 10.1038/sj.jcbfm.9600149

54. Holmes GL. Seizure-induced neuronal injury: animal data. *Neurology*. (2002) 59:S3–6. doi: 10.1212/WNL.59.9_suppl_5.S3

55. Pitkanen A, Sutula TP. Is epilepsy a progressive disorder? Prospects for new therapeutic approaches in temporal-lobe epilepsy. *Lancet Neurol*. (2002) 1:173–81. doi: 10.1016/S1474-4422(02)00073-X

56. Gahring LC, White HS, Skradski SL, Carlson NG, Rogers SW. Interleukin-1alpha in the brain is induced by audiogenic seizure. *Neurobiol Dis*. (1997) 3:263–9. doi: 10.1006/nbdi.1996.0123

57. Vezzani A, Baram TZ. New roles for interleukin-1 Beta in the mechanisms of epilepsy. *Epilepsy Curr*. (2007) 7:45–50. doi: 10.1111/j.1535-7511.2007.00165.x

58. Johnson VE, Stewart JE, Begbie FD, Trojanowski JQ, Smith DH, Stewart W. Inflammation and white matter degeneration persist for years after a single traumatic brain injury. *Brain*. (2013) 136:28–42. doi: 10.1093/brain/aws322

59. Ravizza T, Vezzani A. Status epilepticus induces time-dependent neuronal and astrocytic expression of interleukin-1 receptor type I in the rat limbic system. *Neuroscience*. (2006) 137:301–8. doi: 10.1016/j.neuroscience.2005.07.063

60. Sul JY, Orosz G, Givens RS, Haydon PG. Astrocytic connectivity in the hippocampus. *Neuron Glia Biol*. (2004) 1:3–11. doi: 10.1017/S1740925X04000031

61. Mizuno T, Doi Y, Mizoguchi H, Jin S, Noda M, Sonobe Y, et al. Interleukin-34 selectively enhances the neuroprotective effects of microglia to attenuate oligomeric amyloid-beta neurotoxicity. *Am J Pathol*. (2011) 179:2016–27. doi: 10.1016/j.ajpath.2011.06.011

62. Czapski GA, Strosznajder JB. Glutamate and GABA in microglia-neuron cross-talk in Alzheimer's disease. *Int J Mol Sci*. (2021) 22:1677. doi: 10.3390/ijms222111677

63. Green KN, Crapser JD, Hohsfield LA. To kill a microglia: a case for CSF1R inhibitors. *Trends Immunol*. (2020) 41:771–84. doi: 10.1016/j.it.2020.07.001

64. Lin JH, Walter P, Yen TS. Endoplasmic reticulum stress in disease pathogenesis. *Annu Rev Pathol*. (2008) 3:399–425. doi: 10.1146/annurev.pathmechdis.3.121806.151434

65. Lindner P, Christensen SB, Nissen P, Møller JV, Engedal N. Cell death induced by the ER stressor thapsigargin involves death receptor 5, a non-autophagic function of MAP1LC3B, and distinct contributions from unfolded protein response components. *Cell Commun Signal*. (2020) 18:12. doi: 10.1186/s12964-019-0499-z

66. Smith MI, Deshmukh M. Endoplasmic reticulum stress-induced apoptosis requires bax for commitment and Apaf-1 for execution in primary neurons. *Cell Death Differ*. (2007) 14:1011–9. doi: 10.1038/sj.cdd.4402089

67. Iurlaro R, Munoz-Pinedo C. Cell death induced by endoplasmic reticulum stress. *FEBS J*. (2016) 283:2640–52. doi: 10.1111/febs.13598

68. Walter F, O'Brien A, Concannon CG, Düssmann H, Prehn JHM. ER stress signaling has an activating transcription factor 6alpha (ATF6)-dependent "off-switch". *J Biol Chem*. (2018) 293:18270–84. doi: 10.1074/jbc.RA118.002121

69. Walter P, Ron D. The unfolded protein response: from stress pathway to homeostatic regulation. *Science*. (2011) 334:1081–6. doi: 10.1126/science.1209038

70. Livezey M, Huang R, Hergenrother PJ, Shapiro DJ. Strong and sustained activation of the anticipatory unfolded protein response induces necrotic cell death. *Cell Death Differ*. (2018) 25:1796–807. doi: 10.1038/s41418-018-0143-2

71. Logsdon AF, Turner RC, Lucke-Wold BP, Robson MJ, Naser ZJ, Smith KE, et al. Altering endoplasmic reticulum stress in a model of blast-induced traumatic brain injury controls cellular fate and ameliorates neuropsychiatric symptoms. *Front Cell Neurosci*. (2014) 8:421. doi: 10.3389/fncel.2014.00421

72. Rubovitch V, Shachar A, Werner H, Pick CG. Does IGF-1 administration after a mild traumatic brain injury in mice activate the adaptive arm of ER stress? *Neurochem Int*. (2011) 58:443–6. doi: 10.1016/j.neuint.2011.01.009

73. West MJ, Coleman PD, Flood DG, Troncoso JC. Differences in the pattern of hippocampal neuronal loss in normal ageing and Alzheimer's disease. *Lancet*. (1994) 344:769–72. doi: 10.1016/S0140-6736(94)92338-8

74. Dam AM. Epilepsy and neuron loss in the Hippocampus. *Epilepsia*. (1980) 21:617–29. doi: 10.1111/j.1528-1157.1980.tb04315.x

75. Ho Y-S, Yang X, Lau JCF, Hung CHL, Wuwongse S, Zhang Q, et al. Endoplasmic reticulum stress induces tau pathology and forms a vicious cycle: implication in Alzheimer's disease pathogenesis. *J Alzheimers Dis*. (2012) 28:839–54. doi: 10.3233/JAD-2011-111037

76. Lucke-Wold BP, Logsdon AF, Turner RC, Huber JD, Rosen CL. Endoplasmic reticulum stress modulation as a target for ameliorating effects of blast induced traumatic brain injury. *J Neurotrauma*. (2017) 34:S-62–70. doi: 10.1089/neu.2016.4680

77. Hylin MJ, Holden RC, Smith AC, Logsdon AF, Qaiser R, Lucke-Wold BP. Juvenile traumatic brain injury results in cognitive deficits associated with impaired endoplasmic reticulum stress and early Tauopathy. *Dev Neurosci*. (2018) 40:175–88. doi: 10.1159/000488343

78. Unterberger U, Höftberger R, Gelpi E, Flicker H, Budka H, Voigtländer T. Endoplasmic reticulum stress features are prominent in Alzheimer disease but not in prion diseases in vivo. *J Neuropathol Exp Neurol*. (2006) 65:348–57. doi: 10.1097/01.jnen.0000218445.30535.6f

79. Meier S, Bell M, Lyons DN, Ingram A, Chen J, Gensel JC, et al. Identification of novel tau interactions with endoplasmic reticulum proteins in Alzheimer's disease brain. *J Alzheimers Dis*. (2015) 48:687–702. doi: 10.3233/JAD-150298

80. Liu DC, Eagleman DE, Tsai NP. Novel roles of ER stress in repressing neural activity and seizures through Mdm2-and p53-dependent protein translation. *PLoS Genet*. (2019) 15:e1008364. doi: 10.1371/journal.pgen.1008364

81. Madhamanchi K, Madhamanchi P, Jayalakshmi S, Panigrahi M, Patil A, Phanithi PB. Endoplasmic reticulum stress and unfolded protein accumulation correlate to seizure recurrence in focal cortical dysplasia patients. *Cell Stress Chaperones*. (2022) 27:633–43. doi: 10.1007/s12192-022-01301-0

82. Hitomi J, Katayama T, Eguchi Y, Kudo T, Taniguchi M, Koyama Y, et al. Involvement of caspase-4 in endoplasmic reticulum stress-induced apoptosis and Abeta-induced cell death. *J Cell Biol*. (2004) 165:347–56. doi: 10.1083/jcb.200310015

83. Ferreiro E, Resende R, Costa R, Oliveira CR, Pereira CMF. An endoplasmic-reticulum-specific apoptotic pathway is involved in prion and amyloid-beta peptides neurotoxicity. *Neurobiol Dis*. (2006) 23:669–78. doi: 10.1016/j.nbd.2006.05.011

84. Salminen A, Kauppinen A, Suuronen T, Kaarniranta K, Ojala J. ER stress in Alzheimer's disease: a novel neuronal trigger for inflammation and Alzheimer's pathology. *J Neuroinflammation*. (2009) 6:41. doi: 10.1186/1742-2094-6-41

85. Kogel D, Schomburg R, Schürmann T, Reimertz C, König H-G, Poppe M, et al. The amyloid precursor protein protects PC12 cells against endoplasmic reticulum stress-induced apoptosis. *J Neurochem*. (2003) 87:248–56. doi: 10.1046/j.1471-4159.2003.02000.x

86. Esposito L, Gan L, Yu GQ, Essrich C, Mucke L. Intracellularly generated amyloid-beta peptide counteracts the antiapoptotic function of its precursor protein and primes proapoptotic pathways for activation by other insults in neuroblastoma cells. *J Neurochem*. (2004) 91:1260–74. doi: 10.1111/j.1471-4159.2004.02816.x

87. Fonseca AC, Ferreiro E, Oliveira CR, Cardoso SM, Pereira CF. Activation of the endoplasmic reticulum stress response by the amyloid-beta 1-40 peptide in brain endothelial cells. *Biochim Biophys Acta*. (2013) 1832:2191–203. doi: 10.1016/j.bbadis.2013.08.007

88. Casas-Tinto S, Zhang Y, Sanchez-Garcia J, Gomez-Velazquez M, Rincon-Limas DE, Fernandez-Funez P. The ER stress factor XBP1s prevents amyloid-beta neurotoxicity. *Hum Mol Genet*. (2011) 20:2144–60. doi: 10.1093/hmg/ddr100

89. Hoozemans JJ, Veerhuis R, Van Haastert ES, Rozemuller JM, Baas F, Eikelenboom P, et al. The unfolded protein response is activated in Alzheimer's disease. *Acta Neuropathol*. (2005) 110:165–72. doi: 10.1007/s00401-005-1038-0

90. Lace G, Savva GM, Forster G, de Silva R, Brayne C, Matthews FE, et al. MRC-CFAS, Hippocampal tau pathology is related to neuroanatomical connections: an ageing population-based study. *Brain*. (2009) 132:1324–34. doi: 10.1093/brain/awp059

91. Shahim P, Tegner Y, Wilson DH, Randall J, Skillbäck T, Pazooki D, et al. Blood biomarkers for brain injury in concussed professional ice hockey players. *JAMA Neurol*. (2014) 71:684–92. doi: 10.1001/jamaneurol.2014.367

92. Zetterberg H, Smith DH, Blennow K. Biomarkers of mild traumatic brain injury in cerebrospinal fluid and blood. *Nat Rev Neurol*. (2013) 9:201–10. doi: 10.1038/nrneurol.2013.9

93. Delacourte A. Pathological tau proteins of Alzheimer's disease as a biochemical marker of neurofibrillary degeneration. *Biomed Pharmacother*. (1994) 48:287–95. doi: 10.1016/0753-3322(94)90174-0

94. Tai XY, Koepp M, Duncan JS, Fox N, Thompson P, Baxendale S, et al. Hyperphosphorylated tau in patients with refractory epilepsy correlates with cognitive decline: a study of temporal lobe resections. *Brain*. (2016) 139:2441–55. doi: 10.1093/brain/aww187

95. Monti G, Tondelli M, Giovannini G, Bedin R, Nichelli PF, Trenti T, et al. Cerebrospinal fluid tau proteins in status epilepticus. *Epilepsy Behav*. (2015) 49:150–4. doi: 10.1016/j.yebeh.2015.04.030

96. Shahim P, Rejdak R, Ksiazek P, Blennow K, Zetterberg H, Mattsson N, et al. Cerebrospinal fluid biomarkers of beta-amyloid metabolism and neuronal damage in epileptic seizures. *Eur J Neurol*. (2014) 21:486–91. doi: 10.1111/ene.12336

97. Matsui T, Maruyama M, Matsushita S, Arai H, Higuchi S, Maruyama K. A transient increase in cerebrospinal fluid tau level after epileptic seizure in an elderly patient. *J Am Geriatr Soc*. (2007) 55:2096–7. doi: 10.1111/j.1532-5415.2007.01440.x

98. Palmio J, Suhonen J, Keränen T, Hulkkonen J, Peltola J, Pirttilä T. Cerebrospinal fluid tau as a marker of neuronal damage after epileptic seizure. *Seizure*. (2009) 18:474–7. doi: 10.1016/j.seizure.2009.04.006

99. Hopp SC, Lin Y, Oakley D, Roe AD, DeVos SL, Hanlon D, et al. The role of microglia in processing and spreading of bioactive tau seeds in Alzheimer's disease. *J Neuroinflammation*. (2018) 15:269. doi: 10.1186/s12974-018-1309-z

100. Edwards G 3rd, Zhao J, Dash PK, Soto C, Moreno-Gonzalez I, et al. Traumatic brain injury induces tau aggregation and spreading. *J Neurotrauma*. (2020) 37:80–92. doi: 10.1089/neu.2018.6348

101. Thom M, Liu JYW, Thompson P, Phadke R, Narkiewicz M, Martinian L, et al. Neurofibrillary tangle pathology and Braak staging in chronic epilepsy in relation to traumatic brain injury and hippocampal sclerosis: a post-mortem study. *Brain*. (2011) 134:2969–81. doi: 10.1093/brain/awr209

102. Kristensen AS, Jenkins MA, Banke TG, Schousboe A, Makino Y, Johnson RC, et al. Mechanism of Ca2+/calmodulin-dependent kinase II regulation of AMPA receptor gating. *Nat Neurosci*. (2011) 14:727–35. doi: 10.1038/nn.2804

103. Barria A, Muller D, Derkach V, Griffith LC, Soderling TR. Regulatory phosphorylation of AMPA-type glutamate receptors by CaM-KII during long-term potentiation. *Science*. (1997) 276:2042–5. doi: 10.1126/science.276.5321.2042

104. Lee HK, Barbarosie M, Kameyama K, Bear MF, Huganir RL. Regulation of distinct AMPA receptor phosphorylation sites during bidirectional synaptic plasticity. *Nature*. (2000) 405:955–9. doi: 10.1038/35016089

105. Mao LM, Jin D-Z, Xue B, Chu X-P, Wang JQ. Phosphorylation and regulation of glutamate receptors by CaMKII. *Sheng Li Xue Bao*. (2014) 66:365–72. doi: 10.13294/j.aps.2014.0044

106. Suh HW, Lee HK, Seo YJ, Kwon MS, Shim EJ, Lee JY, et al. Kainic acid (KA)-induced Ca2+/calmodulin-dependent protein kinase II (CaMK II) expression in the neurons, astrocytes and microglia of the mouse hippocampal CA3 region, and the phosphorylated CaMK II only in the hippocampal neurons. *Neurosci Lett*. (2005) 381:223–7. doi: 10.1016/j.neulet.2005.01.089

107. Moynagh PN. The interleukin-1 signalling pathway in astrocytes: a key contributor to inflammation in the brain. *J Anat*. (2005) 207:265–9. doi: 10.1111/j.1469-7580.2005.00445.x

108. Denes A, Lopez-Castejon G, Brough D. Caspase-1: is IL-1 just the tip of the ICEberg? *Cell Death Dis*. (2012) 3:e338. doi: 10.1038/cddis.2012.86

109. Ghayur T, Banerjee S, Hugunin M, Butler D, Herzog L, Carter A, et al. Caspase-1 processes IFN-gamma-inducing factor and regulates LPS-induced IFN-gamma production. *Nature*. (1997) 386:619–23. doi: 10.1038/386619a0

110. Gu Y, Kuida K, Tsutsui H, Ku G, Hsiao K, Fleming MA, et al. Activation of interferon-gamma inducing factor mediated by interleukin-1beta converting enzyme. *Science*. (1997) 275:206–9. doi: 10.1126/science.275.5297.206

111. Hewett SJ, Jackman NA, Claycomb RJ. Interleukin-1beta in central nervous system injury and repair. *Eur J Neurodegener Dis*. (2012) 1:195–211.

112. Viviani B, Bartesaghi S, Gardoni F, Vezzani A, Behrens MM, Bartfai T, et al. Interleukin-1beta enhances NMDA receptor-mediated intracellular calcium increase through activation of the Src family of kinases. *J Neurosci*. (2003) 23:8692–700. doi: 10.1523/JNEUROSCI.23-25-08692.2003

113. Peng Z, Huang CS, Stell BM, Mody I, Houser CR. Altered expression of the delta subunit of the GABAA receptor in a mouse model of temporal lobe epilepsy. *J Neurosci*. (2004) 24:8629–39. doi: 10.1523/JNEUROSCI.2877-04.2004

114. Gardoni F, Boraso M, Zianni E, Corsini E, Galli CL, Cattabeni F, et al. Distribution of interleukin-1 receptor complex at the synaptic membrane driven by interleukin-1beta and NMDA stimulation. *J Neuroinflammation*. (2011) 8:14. doi: 10.1186/1742-2094-8-14

115. Mohamed NE, Zhao Y, Lee JH, Tan MG, Esiri MM, Wilcock GK, et al. Upregulation of AMPA receptor GluR2 (GluA2) subunits in subcortical ischemic vascular dementia is repressed in the presence of Alzheimer's disease. *Neurochem Int*. (2011) 58:820–5. doi: 10.1016/j.neuint.2011.03.010

116. Bell JD, Park E, Ai J, Baker AJ. PICK1-mediated GluR2 endocytosis contributes to cellular injury after neuronal trauma. *Cell Death Differ*. (2009) 16:1665–80. doi: 10.1038/cdd.2009.106

117. Bingol B, Wang CF, Arnott D, Cheng D, Peng J, Sheng M. Autophosphorylated CaMKIIalpha acts as a scaffold to recruit proteasomes to dendritic spines. *Cells*. (2010) 140:567–78. doi: 10.1016/j.cell.2010.01.024

118. Ashpole NM, Chawla AR, Martin MP, Brustovetsky T, Brustovetsky N, Hudmon A. Loss of calcium/calmodulin-dependent protein kinase II activity in cortical astrocytes decreases glutamate uptake and induces neurotoxic release of ATP. *J Biol Chem*. (2013) 288:14599–611. doi: 10.1074/jbc.M113.466235

119. Kim HE, du F, Fang M, Wang X. Formation of apoptosome is initiated by cytochrome c-induced dATP hydrolysis and subsequent nucleotide exchange on Apaf-1. *Proc Natl Acad Sci U S A*. (2005) 102:17545–50. doi: 10.1073/pnas.0507900102

120. Zeiss CJ. The apoptosis-necrosis continuum: insights from genetically altered mice. *Vet Pathol*. (2003) 40:481–95. doi: 10.1354/vp.40-5-481

121. Zou H, Henzel WJ, Liu X, Lutschg A, Wang X. Apaf-1, a human protein homologous to *C. elegans* CED-4, participates in cytochrome c-dependent activation of caspase-3. *Cells*. (1997) 90:405–13. doi: 10.1016/S0092-8674(00)80501-2

122. Ghavami S, Hashemi M, Ande SR, Yeganeh B, Xiao W, Eshraghi M, et al. Apoptosis and cancer: mutations within caspase genes. *J Med Genet*. (2009) 46:497–510. doi: 10.1136/jmg.2009.066944

123. Salvesen GS. Caspases: opening the boxes and interpreting the arrows. *Cell Death Differ*. (2002) 9:3–5. doi: 10.1038/sj.cdd.4400963

124. Wang Y, Garg S, Mandelkow EM, Mandelkow E. Proteolytic processing of tau. *Biochem Soc Trans*. (2010) 38:955–61. doi: 10.1042/BST0380955

125. Guo H, Albrecht S, Bourdeau M, Petzke T, Bergeron C, LeBlanc AC. Active caspase-6 and caspase-6-cleaved tau in neuropil threads, neuritic plaques, and neurofibrillary tangles of Alzheimer's disease. *Am J Pathol*. (2004) 165:523–31. doi: 10.1016/S0002-9440(10)63317-2

126. Cotman CW, Poon WW, Rissman RA, Blurton-Jones M. The role of caspase cleavage of tau in Alzheimer disease neuropathology. *J Neuropathol Exp Neurol*. (2005) 64:104–12. doi: 10.1093/jnen/64.2.104

127. Horowitz PM, Patterson KR, Guillozet-Bongaarts AL, Reynolds MR, Carroll CA, Weintraub ST, et al. Early N-terminal changes and caspase-6 cleavage of tau in Alzheimer's disease. *J Neurosci*. (2004) 24:7895–902. doi: 10.1523/JNEUROSCI.1988-04.2004

128. Rissman RA, Poon WW, Blurton-Jones M, Oddo S, Torp R, Vitek MP, et al. Caspase-cleavage of tau is an early event in Alzheimer disease tangle pathology. *J Clin Invest*. (2004) 114:121–30. doi: 10.1172/JCI200420640

129. Wang HH, Li HL, Liu R, Zhang Y, Liao K, Wang Q, et al. Tau overexpression inhibits cell apoptosis with the mechanisms involving multiple viability-related factors. *J Alzheimers Dis*. (2010) 21:167–79. doi: 10.3233/JAD-2010-091279

130. Qin H, Srinivasula SM, Wu G, Fernandes-Alnemri T, Alnemri ES, Shi Y. Structural basis of procaspase-9 recruitment by the apoptotic protease-activating factor 1. *Nature*. (1999) 399:549–57. doi: 10.1038/21124

131. Leyns CEG, Holtzman DM. Glial contributions to neurodegeneration in tauopathies. *Mol Neurodegener*. (2017) 12:50. doi: 10.1186/s13024-017-0192-x

132. Pais TF, Figueiredo C, Peixoto R, Braz MH, Chatterjee S. Necrotic neurons enhance microglial neurotoxicity through induction of glutaminase by a MyD88-dependent pathway. *J Neuroinflammation*. (2008) 5:43. doi: 10.1186/1742-2094-5-43

133. Gratuze M, Chen Y, Parhizkar S, Jain N, Strickland MR, Serrano JR, et al. Activated microglia mitigate Abeta-associated tau seeding and spreading. *J Exp Med*. (2021) 218:542. doi: 10.1084/jem.20210542

134. Asai H, Ikezu S, Tsunoda S, Medalla M, Luebke J, Haydar T, et al. Depletion of microglia and inhibition of exosome synthesis halt tau propagation. *Nat Neurosci*. (2015) 18:1584–93. doi: 10.1038/nn.4132

135. Bolós M, Llorens-Martín M, Jurado-Arjona J, Hernández F, Rábano A, Avila J. Direct evidence of internalization of tau by microglia in vitro and in vivo. *J Alzheimers Dis*. (2015) 50:77–87. doi: 10.3233/JAD-150704

136. Luo W, Liu W, Hu X, Hanna M, Caravaca A, Paul SM. Microglial internalization and degradation of pathological tau is enhanced by an anti-tau monoclonal antibody. *Sci Rep*. (2015) 5:11161. doi: 10.1038/srep11161

137. Guo JL, Lee VM. Seeding of normal tau by pathological tau conformers drives pathogenesis of Alzheimer-like tangles. *J Biol Chem*. (2011) 286:15317–31. doi: 10.1074/jbc.M110.209296

138. Frost B, Jacks RL, Diamond MI. Propagation of tau misfolding from the outside to the inside of a cell. *J Biol Chem*. (2009) 284:12845–52. doi: 10.1074/jbc.M808759200

139. DeVos SL, Corjuc BT, Oakley DH, Nobuhara CK, Bannon RN, Chase A, et al. Synaptic tau seeding precedes tau pathology in human Alzheimer's disease brain. *Front Neurosci*. (2018) 12:267. doi: 10.3389/fnins.2018.00267

140. Furman JL, Vaquer-Alicea J, White CL III, Cairns NJ, Nelson PT, Diamond MI. Widespread tau seeding activity at early Braak stages. *Acta Neuropathol*. (2017) 133:91–100. doi: 10.1007/s00401-016-1644-z

141. Kaufman SK, del Tredici K, Thomas TL, Braak H, Diamond MI. Tau seeding activity begins in the transentorhinal/entorhinal regions and anticipates phospho-tau pathology in Alzheimer's disease and PART. *Acta Neuropathol*. (2018) 136:57–67. doi: 10.1007/s00401-018-1855-6

142. Ndountse LT, Chan HM. Role of N-methyl-D-aspartate receptors in polychlorinated biphenyl mediated neurotoxicity. *Toxicol Lett*. (2009) 184:50–5. doi: 10.1016/j.toxlet.2008.10.013

143. Kuhlbrandt W. Structure and function of mitochondrial membrane protein complexes. *BMC Biol*. (2015) 13:89. doi: 10.1186/s12915-015-0201-x

144. Jung KH, Chu K, Lee ST, Park HK, Kim JH, Kang KM, et al. Augmentation of nitrite therapy in cerebral ischemia by NMDA receptor inhibition. *Biochem Biophys Res Commun*. (2009) 378:507–12. doi: 10.1016/j.bbrc.2008.11.081

145. Fan MM, Raymond LA. N-methyl-D-aspartate (NMDA) receptor function and excitotoxicity in Huntington's disease. *Prog Neurobiol*. (2007) 81:272–93. doi: 10.1016/j.pneurobio.2006.11.003

146. Leist M, Single B, Castoldi AF, Kühnle S, Nicotera P. Intracellular adenosine triphosphate (ATP) concentration: a switch in the decision between apoptosis and necrosis. *J Exp Med*. (1997) 185:1481–6. doi: 10.1084/jem.185.8.1481

147. Li HL, Wang HH, Liu SJ, Deng YQ, Zhang YJ, Tian Q, et al. Phosphorylation of tau antagonizes apoptosis by stabilizing beta-catenin, a mechanism involved in Alzheimer's neurodegeneration. *Proc Natl Acad Sci U S A*. (2007) 104:3591–6. doi: 10.1073/pnas.0609303104

148. Fujikawa DG, Ke X, Trinidad RB, Shinmei SS, Wu A. Caspase-3 is not activated in seizure-induced neuronal necrosis with internucleosomal DNA cleavage. *J Neurochem*. (2002) 83:229–40. doi: 10.1046/j.1471-4159.2002.01152.x

149. Morales-Ropero JM, Arroyo-Urea S, Neubrand VE, Martín-Oliva D, Marín-Teva JL, Cuadros MA, et al. The endoplasmic reticulum ca(2+) -ATPase SERCA2b is upregulated in activated microglia and its inhibition causes opposite effects on migration and phagocytosis. *Glia*. (2021) 69:842–57. doi: 10.1002/glia.23931

150. Ebneth A, Godemann R, Stamer K, Illenberger S, Trinczek B, Mandelkow EM, et al. Overexpression of tau protein inhibits kinesin-dependent trafficking of vesicles, mitochondria, and endoplasmic reticulum: implications for Alzheimer's disease. *J Cell Biol*. (1998) 143:777–94. doi: 10.1083/jcb.143.3.777

151. Shi Y, Zhang W, Yang Y, Murzin AG, Falcon B, Kotecha A, et al. Structure-based classification of tauopathies. *Nature*. (2021) 598:359–63. doi: 10.1038/s41586-021-03911-7

152. Wang Y, Balaji V, Kaniyappan S, Krüger L, Irsen S, Tepper K, et al. The release and trans-synaptic transmission of tau via exosomes. *Mol Neurodegener*. (2017) 12:5. doi: 10.1186/s13024-016-0143-y

153. Saman S, Kim WH, Raya M, Visnick Y, Miro S, Saman S, et al. Exosome-associated tau is secreted in tauopathy models and is selectively phosphorylated in cerebrospinal fluid in early Alzheimer disease. *J Biol Chem*. (2012) 287:3842–9. doi: 10.1074/jbc.M111.277061

154. Busciglio J, Lorenzo A, Yeh J, Yankner BA. beta-amyloid fibrils induce tau phosphorylation and loss of microtubule binding. *Neuron*. (1995) 14:879–88. doi: 10.1016/0896-6273(95)90232-5

155. Behl C, Davis JB, Klier FG, Schubert D. Amyloid beta peptide induces necrosis rather than apoptosis. *Brain Res*. (1994) 645:253–64. doi: 10.1016/0006-8993(94)91659-4

156. Garwood CJ, Pooler AM, Atherton J, Hanger DP, Noble W. Astrocytes are important mediators of Abeta-induced neurotoxicity and tau phosphorylation in primary culture. *Cell Death Dis*. (2011) 2:e167. doi: 10.1038/cddis.2011.50

157. Lee CY, Landreth GE. The role of microglia in amyloid clearance from the AD brain. *J Neural Transm (Vienna)*. (2010) 117:949–60. doi: 10.1007/s00702-010-0433-4

158. Kang SS, Ebbert MTW, Baker KE, Cook C, Wang X, Sens JP, et al. Microglial translational profiling reveals a convergent APOE pathway from aging, amyloid, and tau. *J Exp Med*. (2018) 215:2235–45. doi: 10.1084/jem.20180653

159. Lian H, Litvinchuk A, Chiang ACA, Aithmitti N, Jankowsky JL, Zheng H. Astrocyte-microglia cross talk through complement activation modulates amyloid pathology in mouse models of Alzheimer's disease. *J Neurosci*. (2016) 36:577–89. doi: 10.1523/JNEUROSCI.2117-15.2016

160. Lian H, Yang L, Cole A, Sun L, Chiang ACA, Fowler SW, et al. NFκB-activated astroglial release of complement C3 compromises neuronal morphology and function associated with Alzheimer's disease. *Neuron*. (2015) 85:101–15. doi: 10.1016/j.neuron.2014.11.018

161. Litvinchuk A, Wan YW, Swartzlander DB, Chen F, Cole A, Propson NE, et al. Complement C3aR inactivation attenuates tau pathology and reverses an immune network deregulated in Tauopathy models and Alzheimer's disease. *Neuron*. (2018) 100:1337–1353.e5. doi: 10.1016/j.neuron.2018.10.031

162. Liddelow SA, Guttenplan KA, Clarke LE, Bennett FC, Bohlen CJ, Schirmer L, et al. Neurotoxic reactive astrocytes are induced by activated microglia. *Nature*. (2017) 541:481–7. doi: 10.1038/nature21029

163. Liu J, Chang L, Roselli F, Almeida OFX, Gao X, Wang X, et al. Amyloid-β induces caspase-dependent loss of PSD-95 and Synaptophysin through NMDA receptors. *J Alzheimers Dis*. (2010) 22:541–56. doi: 10.3233/JAD-2010-100948

164. Dore K, Carrico Z, Alfonso S, Marino M, Koymans K, Kessels HW, et al. PSD-95 protects synapses from β-amyloid. *Cell Rep*. (2021) 35:109194. doi: 10.1016/j.celrep.2021.109194

165. Ekinci FJ, Linsley MD, Shea TB. Beta-amyloid-induced calcium influx induces apoptosis in culture by oxidative stress rather than tau phosphorylation. *Brain Res Mol Brain Res*. (2000) 76:389–95. doi: 10.1016/S0169-328X(00)00025-5

166. Reddy PH. Amyloid beta, mitochondrial structural and functional dynamics in Alzheimer's disease. *Exp Neurol*. (2009) 218:286–92. doi: 10.1016/j.expneurol.2009.03.042

167. White JA, Manelli AM, Holmberg KH, van Eldik LJ, LaDu MJ. Differential effects of oligomeric and fibrillar amyloid-beta 1-42 on astrocyte-mediated inflammation. *Neurobiol Dis*. (2005) 18:459–65. doi: 10.1016/j.nbd.2004.12.013

168. Wyss-Coray T, Loike JD, Brionne TC, Lu E, Anankov R, Yan F, et al. Adult mouse astrocytes degrade amyloid-beta in vitro and in situ. *Nat Med*. (2003) 9:453–7. doi: 10.1038/nm838

169. Ajoolabady A, Lindholm D, Ren J, Pratico D. ER stress and UPR in Alzheimer's disease: mechanisms, pathogenesis, treatments. *Cell Death Dis*. (2022) 13:706. doi: 10.1038/s41419-022-05153-5

170. Jiwaji Z, Tiwari SS, Avilés-Reyes RX, Hooley M, Hampton D, Torvell M, et al. Reactive astrocytes acquire neuroprotective as well as deleterious signatures in response to tau and Aß pathology. *Nat Commun*. (2022) 13:135. doi: 10.1038/s41467-021-27702-w

171. Gu Z, Liu W, Yan Z. beta-amyloid impairs AMPA receptor trafficking and function by reducing Ca2+/calmodulin-dependent protein kinase II synaptic distribution. *J Biol Chem*. (2009) 284:10639–49. doi: 10.1074/jbc.M806508200

172. Walsh DM, Selkoe DJ. A beta oligomers – a decade of discovery. *J Neurochem*. (2007) 101:1172–84. doi: 10.1111/j.1471-4159.2006.04426.x

173. Lin Y, Skeberdis VA, Francesconi A, Bennett MVL, Zukin RS. Postsynaptic density protein-95 regulates NMDA channel gating and surface expression. *J Neurosci*. (2004) 24:10138–48. doi: 10.1523/JNEUROSCI.3159-04.2004

174. Wang J, Dickson DW, Trojanowski JQ, Lee VMY. The levels of soluble versus insoluble brain Aβ distinguish Alzheimer's disease from Normal and pathologic aging. *Exp Neurol*. (1999) 158:328–37. doi: 10.1006/exnr.1999.7085

175. Bloom GS. Amyloid-β and tau: the trigger and bullet in Alzheimer disease pathogenesis. *JAMA Neurol*. (2014) 71:505–8. doi: 10.1001/jamaneurol.2013.5847

176. Arnsten AFT, Datta D, del Tredici K, Braak H. Hypothesis: tau pathology is an initiating factor in sporadic Alzheimer's disease. *Alzheimers Dement*. (2021) 17:115–24. doi: 10.1002/alz.12192

177. Gervais FG, Xu D, Robertson GS, Vaillancourt JP, Zhu Y, Huang JQ, et al. Involvement of caspases in proteolytic cleavage of Alzheimer's amyloid-beta precursor protein and amyloidogenic a beta peptide formation. *Cells*. (1999) 97:395–406. doi: 10.1016/S0092-8674(00)80748-5

178. Heinrich PC, Castell JV, Andus T. Interleukin-6 and the acute phase response. *Biochem J*. (1990) 265:621–36. doi: 10.1042/bj2650621

179. Goldgaber D, Harris HW, Hla T, Maciag T, Donnelly RJ, Jacobsen JS, et al. Interleukin 1 regulates synthesis of amyloid beta-protein precursor mRNA in human endothelial cells. *Proc Natl Acad Sci U S A*. (1989) 86:7606–10. doi: 10.1073/pnas.86.19.7606

180. Zhao J, O'Connor T, Vassar R. The contribution of activated astrocytes to Abeta production: implications for Alzheimer's disease pathogenesis. *J Neuroinflammation*. (2011) 8:150. doi: 10.1186/1742-2094-8-150

181. Oddo S, Caccamo A, Kitazawa M, Tseng BP, LaFerla FM, et al. Amyloid deposition precedes tangle formation in a triple transgenic model of Alzheimer's disease. *Neurobiol Aging*. (2003) 24:1063–70. doi: 10.1016/j.neurobiolaging.2003.08.012

182. Roberts GW, Gentleman SM, Lynch A, Murray L, Landon M, Graham DI. Beta amyloid protein deposition in the brain after severe head injury: implications for the pathogenesis of Alzheimer's disease. *J Neurol Neurosurg Psychiatry*. (1994) 57:419–25. doi: 10.1136/jnnp.57.4.419

183. Roberts GW, Gentleman SM, Lynch A, Graham DI. beta A4 amyloid protein deposition in brain after head trauma. *Lancet*. (1991) 338:1422–3. doi: 10.1016/0140-6736(91)92724-G

184. Mandrekar S, Jiang Q, Lee CYD, Koenigsknecht-Talboo J, Holtzman DM, Landreth GE. Microglia mediate the clearance of soluble Abeta through fluid phase macropinocytosis. *J Neurosci*. (2009) 29:4252–62. doi: 10.1523/JNEUROSCI.5572-08.2009

185. Skovronsky DM, Doms RW, Lee VMY. Detection of a novel Intraneuronal Pool of insoluble amyloid β protein that accumulates with time in culture. *J Cell Biol*. (1998) 141:1031–9. doi: 10.1083/jcb.141.4.1031

186. Ries M, Sastre M. Mechanisms of Aβ clearance and degradation by glial cells. *Front Aging Neurosci*. (2016) 8:160. doi: 10.3389/fnagi.2016.00160

187. Tolar M, Hey J, Power A, Abushakra S. Neurotoxic soluble amyloid oligomers drive Alzheimer's pathogenesis and represent a clinically validated target for slowing disease progression. *Int J Mol Sci*. (2021) 22:6355. doi: 10.3390/ijms22126355

188. Haass C, Selkoe DJ. Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid β-peptide. *Nat Rev Mol Cell Biol*. (2007) 8:101–12. doi: 10.1038/nrm2101

189. Söllvander S, Nikitidou E, Brolin R, Söderberg L, Sehlin D, Lannfelt L, et al. Accumulation of amyloid-β by astrocytes result in enlarged endosomes and microvesicle-induced apoptosis of neurons. *Mol Neurodegener*. (2016) 11:38. doi: 10.1186/s13024-016-0098-z

190. Wang JZ, Grundke-Iqbal I, Iqbal K. Kinases and phosphatases and tau sites involved in Alzheimer neurofibrillary degeneration. *Eur J Neurosci*. (2007) 25:59–68. doi: 10.1111/j.1460-9568.2006.05226.x

191. Texido L, Martín-Satué M, Alberdi E, Solsona C, Matute C. Amyloid beta peptide oligomers directly activate NMDA receptors. *Cell Calcium*. (2011) 49:184–90. doi: 10.1016/j.ceca.2011.02.001

192. Malinow R, Malenka RC. AMPA receptor trafficking and synaptic plasticity. *Annu Rev Neurosci*. (2002) 25:103–26. doi: 10.1146/annurev.neuro.25.112701.142758

193. Song I, Huganir RL. Regulation of AMPA receptors during synaptic plasticity. *Trends Neurosci*. (2002) 25:578–88. doi: 10.1016/S0166-2236(02)02270-1

194. Zhang Y, Guo O, Huo Y, Wang G, Man HY. Amyloid-β induces AMPA receptor ubiquitination and degradation in primary neurons and human brains of Alzheimer's disease. *J Alzheimers Dis*. (2018) 62:1789–801. doi: 10.3233/JAD-170879

195. Marin N, Romero B, Bosch-Morell F, Llansola M, Felipo V, Romá J, et al. Beta-amyloid-induced activation of caspase-3 in primary cultures of rat neurons. *Mech Ageing Dev*. (2000) 119:63–7. doi: 10.1016/S0047-6374(00)00172-X

196. Han XJ, Hu YY, Yang ZJ, Jiang LP, Shi SL, Li YR, et al. Amyloid beta-42 induces neuronal apoptosis by targeting mitochondria. *Mol Med Rep*. (2017) 16:4521–8. doi: 10.3892/mmr.2017.7203

197. Garrido C, Galluzzi L, Brunet M, Puig PE, Didelot C, Kroemer G. Mechanisms of cytochrome c release from mitochondria. *Cell Death Differ*. (2006) 13:1423–33. doi: 10.1038/sj.cdd.4401950

198. Mondragon-Rodriguez S, Trillaud-Doppia E, Dudilot A, Bourgeois C, Lauzon M, Leclerc N, et al. Interaction of endogenous tau protein with synaptic proteins is regulated by N-methyl-D-aspartate receptor-dependent tau phosphorylation. *J Biol Chem*. (2012) 287:32040–53. doi: 10.1074/jbc.M112.401240

199. Neselius S, Brisby H, Theodorsson A, Blennow K, Zetterberg H, Marcusson J. CSF-biomarkers in Olympic boxing: diagnosis and effects of repetitive head trauma. *PLoS One*. (2012) 7:e33606. doi: 10.1371/journal.pone.0033606

200. Rubenstein R, Chang B, Davies P, Wagner AK, Robertson CS, Wang KKW. A novel, ultrasensitive assay for tau: potential for assessing traumatic brain injury in tissues and biofluids. *J Neurotrauma*. (2015) 32:342–52. doi: 10.1089/neu.2014.3548

201. Rubenstein R, Chang B, Yue JK, Chiu A, Winkler EA, Puccio AM, et al. Comparing plasma Phospho tau, Total tau, and Phospho tau-Total tau ratio as acute and chronic traumatic brain injury biomarkers. *JAMA Neurol*. (2017) 74:1063–72. doi: 10.1001/jamaneurol.2017.0655

202. Kenney K, Qu BX, Lai C, Devoto C, Motamedi V, Walker WC, et al. Higher exosomal phosphorylated tau and total tau among veterans with combat-related repetitive chronic mild traumatic brain injury. *Brain Inj*. (2018) 32:1276–84. doi: 10.1080/02699052.2018.1483530

203. Stern RA, Tripodis Y, Baugh CM, Fritts NG, Martin BM, Chaisson C, et al. Preliminary study of plasma Exosomal tau as a potential biomarker for chronic traumatic encephalopathy. *J Alzheimers Dis*. (2016) 51:1099–109. doi: 10.3233/JAD-151028

204. Zemlan FP, Rosenberg WS, Luebbe PA, Campbell TA, Dean GE, Weiner NE, et al. Quantification of axonal damage in traumatic brain injury: affinity purification and characterization of cerebrospinal fluid tau proteins. *J Neurochem*. (1999) 72:741–50. doi: 10.1046/j.1471-4159.1999.0720741.x

205. Ost M, Nylen K, Csajbok L, Ohrfelt AO, Tullberg M, Wikkelso C, et al. Initial CSF total tau correlates with 1-year outcome in patients with traumatic brain injury. *Neurology*. (2006) 67:1600–4. doi: 10.1212/01.wnl.0000242732.06714.0f

206. Tang Y, Liu HL, Min LX, Yuan HS, Guo L, Han PB, et al. Serum and cerebrospinal fluid tau protein level as biomarkers for evaluating acute spinal cord injury severity and motor function outcome. *Neural Regen Res*. (2019) 14:896–902. doi: 10.4103/1673-5374.249238

207. Kitazawa M, Oddo S, Yamasaki TR, Green KN, LaFerla FM. Lipopolysaccharide-induced inflammation exacerbates tau pathology by a cyclin-dependent kinase 5-mediated pathway in a transgenic model of Alzheimer's disease. *J Neurosci*. (2005) 25:8843–53. doi: 10.1523/JNEUROSCI.2868-05.2005

208. Iqbal K, Liu F, Gong CX, Grundke-Iqbal I. Tau in Alzheimer disease and related tauopathies. *Curr Alzheimer Res*. (2010) 7:656–64. doi: 10.2174/156720510793611592

209. Götz J, Chen F, Barmettler R, Nitsch RM. Tau filament formation in transgenic mice expressing P301L tau *. *J Biol Chem*. (2001) 276:529–34. doi: 10.1074/jbc.M006531200

210. Poorkaj P, Bird TD, Wijsman E, Nemens E, Garruto RM, Anderson L, et al. Tau is a candidate gene for chromosome 17 frontotemporal dementia. *Ann Neurol*. (1998) 43:815–25. doi: 10.1002/ana.410430617

211. Hutton M, Lendon CL, Rizzu P, Baker M, Froelich S, Houlden H, et al. Association of missense and 5′-splice-site mutations in tau with the inherited dementia FTDP-17. *Nature*. (1998) 393:702–5. doi: 10.1038/31508

212. Wesseling H, Mair W, Kumar M, Schlaffner CN, Tang S, Beerepoot P, et al. Tau PTM profiles identify patient heterogeneity and stages of Alzheimer's disease. *Cells*. (2020) 183:1699–1713.e13. doi: 10.1016/j.cell.2020.10.029

213. Baker M, Mackenzie IR, Pickering-Brown SM, Gass J, Rademakers R, Lindholm C, et al. Mutations in progranulin cause tau-negative frontotemporal dementia linked to chromosome 17. *Nature*. (2006) 442:916–9. doi: 10.1038/nature05016

214. Cruts M, Gijselinck I, van der Zee J, Engelborghs S, Wils H, Pirici D, et al. Null mutations in progranulin cause ubiquitin-positive frontotemporal dementia linked to chromosome 17q21. *Nature*. (2006) 442:920–4. doi: 10.1038/nature05017

215. Augustinack JC, Schneider A, Mandelkow EM, Hyman BT. Specific tau phosphorylation sites correlate with severity of neuronal cytopathology in Alzheimer's disease. *Acta Neuropathol*. (2002) 103:26–35. doi: 10.1007/s004010100423

216. Lewis J, Dickson DW, Lin WL, Chisholm L, Corral A, Jones G, et al. Enhanced neurofibrillary degeneration in transgenic mice expressing mutant tau and APP. *Science*. (2001) 293:1487–91. doi: 10.1126/science.1058189

217. Braak H, Braak E. Staging of alzheimer's disease-related neurofibrillary changes. *Neurobiol Aging*. (1995) 16:271–8. doi: 10.1016/0197-4580(95)00021-6

218. Buchanan H, Mackay M, Palmer K, Tothová K, Katsur M, Platt B, et al. Synaptic loss, ER stress and neuro-inflammation emerge late in the lateral temporal cortex and associate with progressive tau pathology in Alzheimer's disease. *Mol Neurobiol*. (2020) 57:3258–72. doi: 10.1007/s12035-020-01950-1

219. Visser PJ, Reus LM, Gobom J, Jansen I, Dicks E, van der Lee SJ, et al. Cerebrospinal fluid tau levels are associated with abnormal neuronal plasticity markers in Alzheimer's disease. *Mol Neurodegener*. (2022) 17:27. doi: 10.1186/s13024-022-00521-3

220. Clark CM, Xie S, Chittams J, Ewbank D, Peskind E, Galasko D, et al. Cerebrospinal fluid tau and β-amyloid: how well do these biomarkers reflect autopsy-confirmed dementia diagnoses? *Arch Neurol*. (2003) 60:1696–702. doi: 10.1001/archneur.60.12.1696

221. Ashton NJ, Benedet AL, Pascoal TA, Karikari TK, Lantero-Rodriguez J, Brum WS, et al. Cerebrospinal fluid p-tau231 as an early indicator of emerging pathology in Alzheimer's disease. *EBioMedicine*. (2022) 76:103836. doi: 10.1016/j.ebiom.2022.103836

222. Andreasen N, Vanmechelen E, van de Voorde A, Davidsson P, Hesse C, Tarvonen S, et al. Cerebrospinal fluid tau protein as a biochemical marker for Alzheimer's disease: a community based follow up study. *J Neurol Neurosurg Psychiatry*. (1998) 64:298–305. doi: 10.1136/jnnp.64.3.298

223. Sunderland T, Linker G, Mirza N, Putnam KT, Friedman DL, Kimmel LH, et al. Decreased β-Amyloid1-42 and increased tau levels in cerebrospinal fluid of patients with Alzheimer disease. *JAMA*. (2003) 289:2094–103. doi: 10.1001/jama.289.16.2094

224. Galasko D, Chang L, Motter R, Clark CM, Kaye J, Knopman D, et al. High cerebrospinal fluid tau and low amyloid β42 levels in the clinical diagnosis of Alzheimer disease and relation to apolipoprotein E genotype. *Arch Neurol*. (1998) 55:937–45. doi: 10.1001/archneur.55.7.937

225. Mehta PD, Pirttilä T, Mehta SP, Sersen EA, Aisen PS, Wisniewski HM. Plasma and cerebrospinal fluid levels of amyloid β proteins 1-40 and 1-42 in Alzheimer disease. *Arch Neurol*. (2000) 57:100–5. doi: 10.1001/archneur.57.1.100

226. Motter R, Vigo-Pelfrey C, Kholodenko D, Barbour R, Johnson-Wood K, Galasko D, et al. Reduction of β-amyloid peptide42 in the cerebrospinal fluid of patients with Alzheimer's disease. *Ann Neurol*. (1995) 38:643–8. doi: 10.1002/ana.410380413

227. Zetterberg H, Wilson D, Andreasson U, Minthon L, Blennow K, Randall J, et al. Plasma tau levels in Alzheimer's disease. *Alzheimers Res Ther*. (2013) 5:9. doi: 10.1186/alzrt163

228. Sanchez MP, García-Cabrero AM, Sánchez-Elexpuru G, Burgos DF, Serratosa JM, et al. Tau-induced pathology in epilepsy and dementia: notions from patients and animal models. *Int J Mol Sci*. (2018) 19:1092. doi: 10.3390/ijms19041092

229. Costa C, Romoli M, Liguori C, Farotti L, Eusebi P, Bedetti C, et al. Alzheimer's disease and late-onset epilepsy of unknown origin: two faces of beta amyloid pathology. *Neurobiol Aging*. (2019) 73:61–7. doi: 10.1016/j.neurobiolaging.2018.09.006

230. Mo L, Ding X, Tan C, Liu X, Wei X, Wang H, et al. Association of cerebrospinal fluid zinc-alpha2-glycoprotein and tau protein with temporal lobe epilepsy and related white matter impairment. *Neuroreport*. (2019) 30:586–91. doi: 10.1097/WNR.0000000000001252

231. Nass RD, Akgün K, Elger C, Reichmann H, Wagner M, Surges R, et al. Serum biomarkers of cerebral cellular stress after self-limiting tonic clonic seizures: an exploratory study. *Seizure*. (2021) 85:1–5. doi: 10.1016/j.seizure.2020.12.009

232. Lozano D, Gonzales-Portillo GS, Acosta S, de la Pena I, Tajiri N, Kaneko Y, et al. Neuroinflammatory responses to traumatic brain injury: etiology, clinical consequences, and therapeutic opportunities. *Neuropsychiatr Dis Treat*. (2015) 11:97–106. doi: 10.2147/NDT.S65815

233. Kovesdi E, Kamnaksh A, Wingo D, Ahmed F, Grunberg NE, Long JB, et al. Acute minocycline treatment mitigates the symptoms of mild blast-induced traumatic brain injury. *Front Neurol*. (2012) 3:111. doi: 10.3389/fneur.2012.00111

234. Homsi S, Federico F, Croci N, Palmier B, Plotkine M, Marchand-Leroux C, et al. Minocycline effects on cerebral edema: relations with inflammatory and oxidative stress markers following traumatic brain injury in mice. *Brain Res*. (2009) 1291:122–32. doi: 10.1016/j.brainres.2009.07.031

235. Lee ST, Chu K, Jung KH, Kim SJ, Kim DH, Kang KM, et al. Anti-inflammatory mechanism of intravascular neural stem cell transplantation in haemorrhagic stroke. *Brain*. (2008) 131:616–29. doi: 10.1093/brain/awm306

236. Aggarwal S, Pittenger MF. Human mesenchymal stem cells modulate allogeneic immune cell responses. *Blood*. (2005) 105:1815–22. doi: 10.1182/blood-2004-04-1559

237. Chen X, Katakowski M, Li Y, Lu D, Wang L, Zhang L, et al. Human bone marrow stromal cell cultures conditioned by traumatic brain tissue extracts: growth factor production. *J Neurosci Res*. (2002) 69:687–91. doi: 10.1002/jnr.10334

238. Ma HM, Zafonte RD. Amantadine and memantine: a comprehensive review for acquired brain injury. *Brain Inj*. (2020) 34:299–315. doi: 10.1080/02699052.2020.1723697

239. Wang KK, Larner SF, Robinson G, Hayes RL. Neuroprotection targets after traumatic brain injury. *Curr Opin Neurol*. (2006) 19:514–9. doi: 10.1097/WCO.0b013e3280102b10

240. Sveinbjornsdottir S, Sander JWAS, Upton D, Thompson PJ, Patsalos PN, Hirt D, et al. The excitatory amino acid antagonist D-CPP-ene (SDZ EAA-494) in patients with epilepsy. *Epilepsy Res*. (1993) 16:165–74. doi: 10.1016/0920-1211(93)90031-2

241. Yurkewicz L, Weaver J, Bullock MR, Marshall LF. The effect of the selective NMDA receptor antagonist traxoprodil in the treatment of traumatic brain injury. *J Neurotrauma*. (2005) 22:1428–43. doi: 10.1089/neu.2005.22.1428

242. Minabe Y, Emori K, Shibata R, Kurachi M. Antiepileptic effects of MK-801, a noncompetitive NMDA-receptor antagonist, in the low-frequency kindling model of epilepsy. *Jpn J Psychiatry Neurol*. (1992) 46:755–61.

243. Alkhachroum A, der-Nigoghossian CA, Mathews E, Massad N, Letchinger R, Doyle K, et al. Ketamine to treat super-refractory status epilepticus. *Neurology*. (2020) 95:e2286–94. doi: 10.1212/WNL.0000000000010611

244. Rogawski MA. Therapeutic potential of excitatory amino acid antagonists: channel blockers and 2,3-benzodiazepines. *Trends Pharmacol Sci*. (1993) 14:325–31. doi: 10.1016/0165-6147(93)90005-5

245. Goodkin HP, Yeh JL, Kapur J. Status epilepticus increases the intracellular accumulation of GABAA receptors. *J Neurosci*. (2005) 25:5511–20. doi: 10.1523/JNEUROSCI.0900-05.2005

246. Naylor DE, Liu H, Niquet J, Wasterlain CG. Rapid surface accumulation of NMDA receptors increases glutamatergic excitation during status epilepticus. *Neurobiol Dis*. (2013) 54:225–38. doi: 10.1016/j.nbd.2012.12.015

247. Mei Z, Qiu J, Alcon S, Hashim J, Rotenberg A, Sun Y, et al. Memantine improves outcomes after repetitive traumatic brain injury. *Behav Brain Res*. (2018) 340:195–204. doi: 10.1016/j.bbr.2017.04.017

248. Oustad M, Najafi M, Mehvari J, Rastgoo A, Mortazavi Z, Rahiminejad M. Effect of donepezil and memantine on improvement of cognitive function in patients with temporal lobe epilepsy. *J Res Med Sci*. (2020) 25:29. doi: 10.4103/jrms.JRMS_209_19

249. Marimuthu P, Varadarajan S, Krishnan M, Shanmugam S, Kunjuraman G, Ravinder JR, et al. Evaluating the efficacy of memantine on improving cognitive functions in epileptic patients receiving anti-epileptic drugs: a double-blind placebo-controlled clinical trial (phase IIIb pilot study). *Ann Indian Acad Neurol*. (2016) 19:344–50. doi: 10.4103/0972-2327.179971

250. Leeman-Markowski BA, Meador KJ, Moo LR, Cole AJ, Hoch DB, Garcia E, et al. Does memantine improve memory in subjects with focal-onset epilepsy and memory dysfunction? A randomized, double-blind, placebo-controlled trial. *Epilepsy Behav*. (2018) 88:315–24. doi: 10.1016/j.yebeh.2018.06.047

251. Bialer M, Johannessen SI, Levy RH, Perucca E, Tomson T, White HS. Progress report on new antiepileptic drugs: a summary of the tenth EILAT conference (EILAT X). *Epilepsy Res*. (2010) 92:89–124. doi: 10.1016/j.eplepsyres.2010.09.001

252. Graebenitz S, Kedo O, Speckmann EJ, Gorji A, Panneck H, Hans V, et al. Interictal-like network activity and receptor expression in the epileptic human lateral amygdala. *Brain*. (2011) 134:2929–47. doi: 10.1093/brain/awr202

253. Chappell AS, Sander JW, Brodie MJ, Chadwick D, Lledo A, Zhang D, et al. A crossover, add-on trial of talampanel in patients with refractory partial seizures. *Neurology*. (2002) 58:1680–2. doi: 10.1212/WNL.58.11.1680

254. Steinhoff BJ, Ben-Menachem E, Ryvlin P, Shorvon S, Kramer L, Satlin A, et al. Efficacy and safety of adjunctive perampanel for the treatment of refractory partial seizures: a pooled analysis of three phase III studies. *Epilepsia*. (2013) 54:1481–9. doi: 10.1111/epi.12212

255. Witt JA, Helmstaedter C. The impact of perampanel on cognition: a systematic review of studies employing standardized tests in patients with epilepsy. *Seizure*. (2022) 94:107–11. doi: 10.1016/j.seizure.2021.12.001

256. Chen T, Liu WB, Qian X, Xie KL, Wang YH. The AMPAR antagonist perampanel protects the neurovascular unit against traumatic injury via regulating Sirt3. *CNS Neurosci Ther*. (2021) 27:134–44. doi: 10.1111/cns.13580

257. Chen T, Dai SH, Jiang ZQ, Luo P, Jiang XF, Fei Z, et al. The AMPAR antagonist Perampanel attenuates traumatic brain injury through anti-oxidative and anti-inflammatory activity. *Cell Mol Neurobiol*. (2017) 37:43–52. doi: 10.1007/s10571-016-0341-8

258. Kumamoto A, Chiba Y, Suda A, Hishimoto A, Kase A. A severe dementia case in end of life care with psychiatric symptoms treated by Perampanel. *J Epilepsy Res*. (2021) 11:93–5. doi: 10.14581/jer.21012

259. Chen YS, Chen TS, Huang CW. Dementia with non-convulsive seizures: a case report. *J Int Med Res*. (2021) 49:3000605211062453. doi: 10.1177/03000605211062453

260. Ueda S, Kuzuya A, Kawata M, Okawa K, Honjo C, Wada T, et al. Acute inhibition of AMPA receptors by perampanel reduces amyloid β-protein levels by suppressing β-cleavage of APP in Alzheimer's disease models. *FASEB J*. (2023) 37:e23252. doi: 10.1096/fj.202300837R

261. Attwell PJE, Singh Kent N, Jane DE, Croucher MJ, Bradford HF. Anticonvulsant and glutamate release-inhibiting properties of the highly potent metabotropic glutamate receptor agonist (2S,2′R,3′R)-2-(2′,3′-dicarboxycyclopropyl)glycine (DCG-IV). *Brain Res*. (1998) 805:138–43. doi: 10.1016/S0006-8993(98)00698-2

262. Kłodzińska A, Bijak M, Chojnacka-Wójcik E, Kroczka B, Świąder M, Czuczwar SJ, et al. Roles of group II metabotropic glutamate receptors in modulation of seizure activity. *Naunyn Schmiedeberg's Arch Pharmacol*. (2000) 361:283–8. doi: 10.1007/s002109900197

263. Miyamoto M, Ishida M, Shinozaki H. Anticonvulsive and neuroprotective actions of a potent agonist (DCG-IV) for group II metabotropic glutamate receptors against intraventricular kainate in the rat. *Neuroscience*. (1997) 77:131–40. doi: 10.1016/S0306-4522(96)00442-3

264. Tang FR, Lee WL, Yang J, Sim MK, Ling EA. Expression of metabotropic glutamate receptor 1alpha in the hippocampus of rat pilocarpine model of status epilepticus. *Epilepsy Res*. (2001) 46:179–89. doi: 10.1016/S0920-1211(01)00276-5

265. Merlin LR, Bergold PJ, Wong RK. Requirement of protein synthesis for group I mGluR-mediated induction of epileptiform discharges. *J Neurophysiol*. (1998) 80:989–93. doi: 10.1152/jn.1998.80.2.989

266. Ghauri M, Chapman AG, Meldrum BS. Convulsant and anticonvulsant actions of agonists and antagonists of group III mGluRs. *Neuroreport*. (1996) 7:1469–74. doi: 10.1097/00001756-199606170-00005

267. Chapman AG, Talebi A, Yip PK, Meldrum BS. Anticonvulsant activity of a mGlu4α receptor selective agonist, (1S,3R,4S)-1-aminocyclopentane-1,2,4-tricarboxylic acid. *Eur J Pharmacol*. (2001) 424:107–13. doi: 10.1016/S0014-2999(01)01013-5

268. Abdul-Ghani A-S, Attwell PJE, Singh Kent N, Bradford HF, Croucher MJ, Jane DE. Anti-epileptogenic and anticonvulsant activity of l-2-amino-4-phosphonobutyrate, a presynaptic glutamate receptor agonist. *Brain Res*. (1997) 755:202–12. doi: 10.1016/S0006-8993(97)00098-X

269. Chapman AG, Nanan K, Yip P, Meldrum BS. Anticonvulsant activity of a metabotropic glutamate receptor 8 preferential agonist, (R,S)-4-phosphonophenylglycine. *Eur J Pharmacol*. (1999) 383:23–7. doi: 10.1016/S0014-2999(99)00615-9

270. Moldrich RX, Beart PM, Jane DE, Chapman AG, Meldrum BS. Anticonvulsant activity of 3,4-dicarboxyphenylglycines in DBA/2 mice. *Neuropharmacology*. (2001) 40:732–5. doi: 10.1016/S0028-3908(01)00002-8

271. van Dyck CH, Swanson CJ, Aisen P, Bateman RJ, Chen C, Gee M, et al. Lecanemab in early Alzheimer's disease. *N Engl J Med*. (2022) 388:9–21. doi: 10.1056/NEJMoa2212948

272. Swanson CJ, Zhang Y, Dhadda S, Wang J, Kaplow J, Lai RYK, et al. A randomized, double-blind, phase 2b proof-of-concept clinical trial in early Alzheimer's disease with lecanemab, an anti-Aβ protofibril antibody. *Alzheimers Res Ther*. (2021) 13:80. doi: 10.1186/s13195-021-00813-8

273. McDade E, Cummings JL, Dhadda S, Swanson CJ, Reyderman L, Kanekiyo M, et al. Lecanemab in patients with early Alzheimer's disease: detailed results on biomarker, cognitive, and clinical effects from the randomized and open-label extension of the phase 2 proof-of-concept study. *Alzheimers Res Ther*. (2022) 14:191. doi: 10.1186/s13195-022-01124-2

274. Hoy SM. Lecanemab: first approval. *Drugs*. (2023) 83:359–65. doi: 10.1007/s40265-023-01851-2

275. Sevigny J, Chiao P, Bussière T, Weinreb PH, Williams L, Maier M, et al. The antibody aducanumab reduces Aβ plaques in Alzheimer's disease. *Nature*. (2016) 537:50–6. doi: 10.1038/nature19323

276. Vaz M, Silva V, Monteiro C, Silvestre S. Role of Aducanumab in the treatment of Alzheimer's disease: challenges and opportunities. *Clin Interv Aging*. (2022) 17:797–810. doi: 10.2147/CIA.S325026

277. Rubenstein R, Sharma DR, Chang B, Oumata N, Cam M, Vaucelle L, et al. Novel mouse Tauopathy model for repetitive mild traumatic brain injury: evaluation of Long-term effects on cognition and biomarker levels after therapeutic inhibition of tau phosphorylation. *Front Neurol*. (2019) 10:124. doi: 10.3389/fneur.2019.00124

278. Wang JZ, Grundke-Iqbal I, Iqbal K. Restoration of biological activity of Alzheimer abnormally phosphorylated tau by dephosphorylation with protein phosphatase-2A, -2B and-1. *Brain Res Mol Brain Res*. (1996) 38:200–8. doi: 10.1016/0169-328X(95)00316-K

279. Gong CX, Shaikh S, Wang JZ, Zaidi T, Grundke-Iqbal I, Iqbal K. Phosphatase activity toward abnormally phosphorylated tau: decrease in Alzheimer disease brain. *J Neurochem*. (1995) 65:732–8. doi: 10.1046/j.1471-4159.1995.65020732.x

280. Liu GP, Zhang Y, Yao XQ, Zhang CE, Fang J, Wang Q, et al. Activation of glycogen synthase kinase-3 inhibits protein phosphatase-2A and the underlying mechanisms. *Neurobiol Aging*. (2008) 29:1348–58. doi: 10.1016/j.neurobiolaging.2007.03.012

281. Tanimukai H, Grundke-Iqbal I, Iqbal K. Up-regulation of inhibitors of protein phosphatase-2A in Alzheimer's disease. *Am J Pathol*. (2005) 166:1761–71. doi: 10.1016/S0002-9440(10)62486-8

282. Griebel G, Stemmelin J, Lopez-Grancha M, Boulay D, Boquet G, Slowinski F, et al. The selective GSK3 inhibitor, SAR502250, displays neuroprotective activity and attenuates behavioral impairments in models of neuropsychiatric symptoms of Alzheimer's disease in rodents. *Sci Rep*. (2019) 9:18045. doi: 10.1038/s41598-019-54557-5

283. Lucey BP, Liu H, Toedebusch CD, Freund D, Redrick T, Chahin SL, et al. Suvorexant acutely decreases tau phosphorylation and Aβ in the human CNS. *Ann Neurol*. (2023) 94:27–40. doi: 10.1002/ana.26641

284. Tchekalarova JD, Ivanova NM, Pechlivanova DM, Atanasova D, Lazarov N, Kortenska L, et al. Antiepileptogenic and neuroprotective effects of losartan in kainate model of temporal lobe epilepsy. *Pharmacol Biochem Behav*. (2014) 127:27–36. doi: 10.1016/j.pbb.2014.10.005

285. Bar-Klein G, Cacheaux LP, Kamintsky L, Prager O, Weissberg I, Schoknecht K, et al. Losartan prevents acquired epilepsy via TGF-β signaling suppression. *Ann Neurol*. (2014) 75:864–75. doi: 10.1002/ana.24147

286. Hong S, JianCheng H, JiaWen W, ShuQin Z, GuiLian Z, HaiQin W, et al. Losartan inhibits development of spontaneous recurrent seizures by preventing astrocyte activation and attenuating blood-brain barrier permeability following pilocarpine-induced status epilepticus. *Brain Res Bull*. (2019) 149:251–9. doi: 10.1016/j.brainresbull.2019.05.002

287. Doege C, Luedde M, Kostev K. Association between angiotensin receptor blocker therapy and incidence of epilepsy in patients with hypertension. *JAMA Neurol*. (2022) 79:1296–302. doi: 10.1001/jamaneurol.2022.3413

288. Hajjar I, Levey A. Association between angiotensin receptor blockers and longitudinal decline in tau in mild cognitive impairment. *JAMA Neurol*. (2015) 72:1069–70. doi: 10.1001/jamaneurol.2015.1001

289. Hajjar I, Brown L, Mack WJ, Chui H. Impact of angiotensin receptor blockers on Alzheimer disease neuropathology in a large brain autopsy series. *Arch Neurol*. (2012) 69:1632–8. doi: 10.1001/archneurol.2012.1010

290. Hajjar I, Okafor M, Wan L, Yang Z, Nye JA, Bohsali A, et al. Safety and biomarker effects of candesartan in non-hypertensive adults with prodromal Alzheimer's disease. *Brain Commun*. (2022) 4:fcac270. doi: 10.1093/braincomms/fcac270

291. Lee DY, Lee KS, Lee HJ, Kim DH, Noh YH, Yu K, et al. Activation of PERK signaling attenuates Abeta-mediated ER stress. *PLoS One*. (2010) 5:e10489. doi: 10.1371/journal.pone.0010489

292. Tan HP, Guo Q, Hua G, Chen JX, Liang JC. Inhibition of endoplasmic reticulum stress alleviates secondary injury after traumatic brain injury. *Neural Regen Res*. (2018) 13:827–36. doi: 10.4103/1673-5374.232477

293. Yokoi N, Fukata Y, Kase D, Miyazaki T, Jaegle M, Ohkawa T, et al. Chemical corrector treatment ameliorates increased seizure susceptibility in a mouse model of familial epilepsy. *Nat Med*. (2015) 21:19–26. doi: 10.1038/nm.3759

294. Zhu X, Dong J, Xia Z, Zhang A, Chao J, Yao H. Repeated restraint stress increases seizure susceptibility by activation of hippocampal endoplasmic reticulum stress. *Neurochem Int*. (2017) 110:25–37. doi: 10.1016/j.neuint.2017.09.002

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# Hippocampal ΔFosB expression is associated with cognitive impairment in a subgroup of patients with childhood epilepsies

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Epilepsy is a chronic neurological disorder characterized by recurrent seizures, and is often comorbid with other neurological and neurodegenerative diseases, such as Alzheimer's disease (AD). Patients with recurrent seizures often present with cognitive impairment. However, it is unclear how seizures, even when infrequent, produce long-lasting deficits in cognition. One mechanism may be seizure-induced expression of ΔFosB, a long-lived transcription factor that persistently regulates expression of plasticity-related genes and drives cognitive dysfunction. We previously found that, compared with cognitively-intact subjects, the activity-dependent expression of ΔFosB in the hippocampal dentate gyrus (DG) was increased in individuals with mild cognitive impairment (MCI) and in individuals with AD. In MCI patients, higher ΔFosB expression corresponded to lower Mini-Mental State Examination scores. Surgically resected DG tissue from patients with temporal lobe epilepsy also showed robust ΔFosB expression; however, it is unclear whether ΔFosB expression also corresponds to cognitive dysfunction in non-AD-related epilepsy. To test whether DG ΔFosB expression is indicative of cognitive impairment in epilepsies with different etiologies, we assessed ΔFosB expression in surgically-resected hippocampal tissue from 33 patients with childhood epilepsies who had undergone Wechsler Intelligence Scale for Children (WISC) testing prior to surgery. We found that ΔFosB expression is inversely correlated with Full-Scale Intelligence Quotient (FSIQ) in patients with mild to severe intellectual disability (FSIQ $< 85$). Our data indicate that ΔFosB expression corresponds to cognitive impairment in epilepsies with different etiologies, supporting the hypothesis that ΔFosB may epigenetically regulate gene expression and impair cognition across a wide range of epilepsy syndromes.

KEYWORDS

**dentate gyrus, Alzheimer's disease, seizures, epigenetic, epilepsy, intellectual disability, deltaFosB, cognition**

# 1 Introduction

Epilepsy is one of the most common neurological diseases and affects people of all ages (1, 2). There is often disrupted consciousness and memory during a seizure, but recurrent seizures can also lead to long-lasting changes in neuronal and network function, and drive chronic impairments in cognition that persist even during seizure-free periods (3–5). Notably, cognitive impairment can develop even with infrequent seizures (6, 7). Seizures are frequently co-morbid with other neurological and neurodegenerative diseases, such as Alzheimer's disease (AD), Down syndrome, autism, Fragile X syndrome, and others, and seizure-induced cognitive dysfunction may also contribute to or exacerbate cognitive deficits observed in those neurological disorders (8–17). Thus, in addition to improving methods of seizure control, it is also critical to understand the molecular and network mechanisms that underlie cognitive impairment in epilepsy, and in particular, long-lasting mechanisms that may be engaged even when seizures are infrequent.

One molecular mechanism that may contribute to such long-lasting effects on cognition is the activity-induced expression of ΔFosB, a highly stable transcription factor in the immediate early gene family, in the hippocampal dentate gyrus (DG). ΔFosB has an unusually long half-life of roughly 8 days *in vivo*, allowing it to accumulate within the nucleus even with relatively infrequent repetitive activation of neurons (18). ΔFosB expression is robustly induced in the nucleus accumbens after exposure to drugs of abuse, and accumulates in the hippocampus following recurrent seizures (19–21). Notably, ΔFosB recruits histone modifying enzymes to epigenetically regulate target gene expression, resulting in long-lasting control of gene expression even after the initial activating stimulus is over (18, 22). In various brain regions, ΔFosB binds to a multitude of gene targets, including those related to neuronal excitability and plasticity (20, 23, 24). Neuronal activity-dependent accumulation of ΔFosB within hippocampal neurons following repeated seizure activity thus chronically alters gene expression and can affect cognitive processes. Indeed, we have previously shown that ΔFosB is robustly induced in dentate granule neurons after seizure activity in mouse models for studying epilepsy or for studying AD, which is accompanied by a high incidence of epilepsy (19, 25). In those studies, ΔFosB expression directly corresponded to cognitive impairment, and inhibition of ΔFosB activity improved cognition (19, 25).

The relevance of ΔFosB to human disease is supported by findings that its expression is increased robustly in the DG of individuals with temporal lobe epilepsy (TLE), AD, or mild cognitive impairment (MCI; often considered prodromal AD) (25). Moreover, in patients with MCI, increasing magnitudes of ΔFosB expression corresponded to poorer performance on the Mini-Mental State Examination (MMSE) test of cognition (25), suggesting that ΔFosB may function similarly in humans as in mouse models of disease.

However, it is unclear whether neuronal activity-dependent ΔFosB expression in the DG also reflects cognitive impairment in patients with epilepsy outside the context of AD, or in patients with epilepsy who develop seizures at younger ages. To assess this possibility, we obtained resected hippocampal DG samples from patients with childhood epilepsies who had undergone neuropsychiatric assessment prior to hippocampectomy, and assessed whether ΔFosB expression in human DG is related to any measures of cognitive function in these patients. We found that DG ΔFosB expression corresponds to decreased Full-Scale Intelligence Quotient (FSIQ), a measure of cognitive ability in children, in patients with borderline to poor intellectual functioning.

# 2 Materials and methods

## 2.1 Human tissue

Fixed DG samples from 33 individuals with childhood epilepsies were obtained from hippocampectomy specimens obtained after surgical resection for treatment of epilepsy at the Children's Hospital of Philadelphia (Philadelphia, PA) between 2000 and 2019. Seven of the 33 samples were obtained from patients who underwent selective hippocampectomies. The remaining 26 samples were obtained from patients who underwent either surgical excision of extra-hippocampal lesions in addition to the hippocampectomy, or temporal lobectomy with the hippocampus being removed as a unique surgical specimen. All samples were formalin-fixed, processed, paraffin-embedded, and sectioned at 5 μm. Clinical information was retrospectively collected from the electronic medical record in accordance with the Children's Hospital of Philadelphia Institutional Review Board (protocol IRB 19-016521).

Fixed DG samples from adult control individuals or individuals with MCI, AD, or TLE were from previously published patient cohorts (25). Briefly, fixed post-mortem DG samples from individuals with AD or MCI and age-matched controls were obtained from the Alzheimer's Disease Research Center at the University of California San Diego (San Diego, CA), and sectioned at 60 μm. Fixed surgically-resected DG samples from individuals with TLE were obtained and used with informed consent under Institutional Review Board protocol H-10255; samples were resection specimens derived from surgery for epilepsy in adult patients treated at Baylor College of Medicine (Houston, TX).

## 2.2 Immunohistochemistry

Fixed DG samples derived from surgical resections of the hippocampus in patients with childhood epilepsies were deparaffinized and rehydrated following a standard procedure: three 5-min rinses in xylenes, two 10-min rinses in 100% ethanol, two 10-min rinses in 95% ethanol, and then two 5-min rinses in distilled water. Sections then underwent alternating rinses with PBS and PBS with 0.5% Triton-X (PBS-Tx-0.5%) in between the following steps: (1) 15-min incubation with endogenous peroxidase blocking solution consisting of 3% hydrogen peroxide, 10% methanol, and PBS; (2) 10-min antigen retrieval with citrate buffer at 85°C; (3) 10-min incubation in 90% formic acid; (4) 60-min incubation with a non-specific blocking solution consisting of 10% normal goat serum (Vector Laboratories, Cat# S-1000, RRID:AB_2336615), 1% blocking grade non-fat dry milk (Bio-Rad, Cat# 1706404), 0.2% gelatin (Sigma-Aldrich, Cat# G2500), and PBS-Tx 0.5%; (5) overnight primary antibody incubation

at 4°C; (6) 60-min secondary antibody incubation; (7) 60-min incubation with avidin-biotin complex (Vectastain, Cat# PK-6100), and (8) 10-min development with diaminobenzidine (Vector Laboratories, Cat# SK-4103, RRID:AB_2336521). The antibody concentrations used were 1:200 for rabbit anti-ΔFosB antibody (Cell Signaling, Cat# 14695, RRID:AB_2798577) and 1:200 for goat anti-rabbit biotinylated antibody (Vector Laboratories, Cat# BA-1000, RRID:AB_2313606).

## 2.3 Imaging and analysis

Immunostained sections were imaged by the RNA *in situ* Hybridization Core facility at Baylor College of Medicine. Analysis was performed using Fiji ImageJ (NIH, RRID:SCR_002285). For quantification of DG ΔFosB expression, images were first converted to 16-bit black and white images. For each patient sample, quantification was performed on 20 randomly selected dentate granule cells following previously published procedures, which we had found allowed for reliable representation of ΔFosB expression in the human DG (25). The mean pixel intensity for each dentate granule cell was measured. The average of the mean pixel intensities of three nearby acellular white matter tract areas was used for background correction. Immunoreactivity (IR) was defined as the average of the mean pixel intensities for the 20 dentate granule cells, corrected for background. Quantification was performed by an experimenter blind to the specific diagnoses and neuropsychiatric testing scores of each patient.

## 2.4 Statistics

Statistical analyses were performed using Prism 10 (GraphPad, RRID:SCR_002798). Differences between two groups were assessed via two-tailed unpaired Student's *t*-tests. Correlations were assessed via simple regression analyses. *P*-value correction for multiple comparisons were performed with the Holm-Sidak *post-hoc* test.

# 3 Results

## 3.1 Patient demographics

We obtained surgically resected hippocampal tissue from 33 patients with childhood epilepsies who had been administered the Wechsler Intelligence Scale for Children, Fourth Edition (WISC-IV) assessment prior to hippocampectomy (Table 1). There were similar numbers of male (48.5%) and female (51.5%) patients, and patient ages ranged from 4.58 to 20.58 years old. All 33 patients were tested prior to hippocampal resection, with the interval between neuropsychiatric assessment and surgery varying from 1 month to almost 5 years.

Of the 33 patients, 23 patients exhibited only focal seizures, six patients exhibited focal seizures with secondary generalization, one patient exhibited only generalized tonic-clonic seizures, and three patients exhibited both focal and generalized seizures. Of the 32 patients who experienced focal seizures, 24 patients had seizures with impaired awareness (complex partial seizures), one patient exhibited focal seizures without impaired awareness (simple partial seizures), and seven patients were unspecified. Four patients had focal seizures secondary to lesions.

Information about seizure history, including age at seizure onset and seizure frequency, was available only for a portion of the patients (19–22 of the 33 patients included in this study). Of the patients with these data available, age at seizure onset was 5.08 ± 3.66 (mean ± SD) years, with variation ranging from within the

TABLE 1 Patient demographic information.

| | Childhood epilepsy cohort |
|---|---|
| **Sex** | **# patients (% patients)** |
| Male | 16 (48.5%) |
| Female | 17 (51.5%) |
| **Age (years)** | **Mean ± SD (range)** |
| At hippocampectomy | 12.74 ± 4.05 (4.58–20.58) |
| At neuropsychiatric testing | 11.71 ± 4.05 (4.50–19.92) |
| Difference | 1.03 ± 1.16 (0.08–4.83) |
| **Seizure onset (22/33 patients)** | **Mean ± SD (range)** |
| Age (years) | 5.08 ± 3.66 (0.00–13.00) |
| Years with seizures prior to hippocampectomy | 7.60 ± 4.06 (1.08–15.92) |
| **Seizure frequency (19/33 patients)** | **Mean ± SD (range)** |
| Seizures per month | 68.7 ± 131.2 (0.25–532) |
| **Neuropathological diagnoses** | **# patients (% patients)** |
| Encephalitis | 5 (15.2%) |
| Tumor | 5 (15.2%) |
| Infarction | 4 (12.1%) |
| Focal cortical dysplasia | 3 (9.1%) |
| Sturge-Weber syndrome | 1 (3%) |
| **Neuropsychiatric diagnoses[#]** | **# patients (% patients)** |
| Attention-deficit/hyperactivity disorder | 8 (24.2%) |
| Asperger's syndrome | 1 (3%) |
| **WISC-IV score** | **Mean ± SD (range)** |
| Full-Scale Intelligence Quotient | 80.4 ± 17.0 (46–105)* |
| General Ability (7/33 patients) | 88.4 ± 15.7 (64–113) |
| Verbal Comprehension (28/33 patients) | 86.7 ± 14.1 (50–116) |
| Perceptual Reasoning (24/33 patients) | 87.4 ± 16.4 (51–112) |
| Working Memory (23/33 patients) | 82.7 ± 16.5 (55–113) |
| Processing Speed (26/33 patients) | 81.3 ± 18.2 (45–119) |

Demographic information regarding patient sex, age, seizure onset, seizure frequency, co-occurrence of other neuropathological and psychiatric diagnoses, and scores on the Wechsler Intelligence Scale for Children-Fourth Edition (WISC-IV) cognitive assessment. For categories in which information is not available for all 33 patients, the number of patients for which the information is available is indicated in parentheses.
[#]Neuropsychiatric diagnoses reflect what was documented in patients' medical records; some terminology may be outdated.
*Full-Scale Intelligence Quotient (FSIQ) of patients in the childhood epilepsy cohort is significantly decreased ($p = 0.0306$, two-tailed unpaired Student's t-test) compared with the general population (mean = 100, SD = 15).
SD, standard deviation.

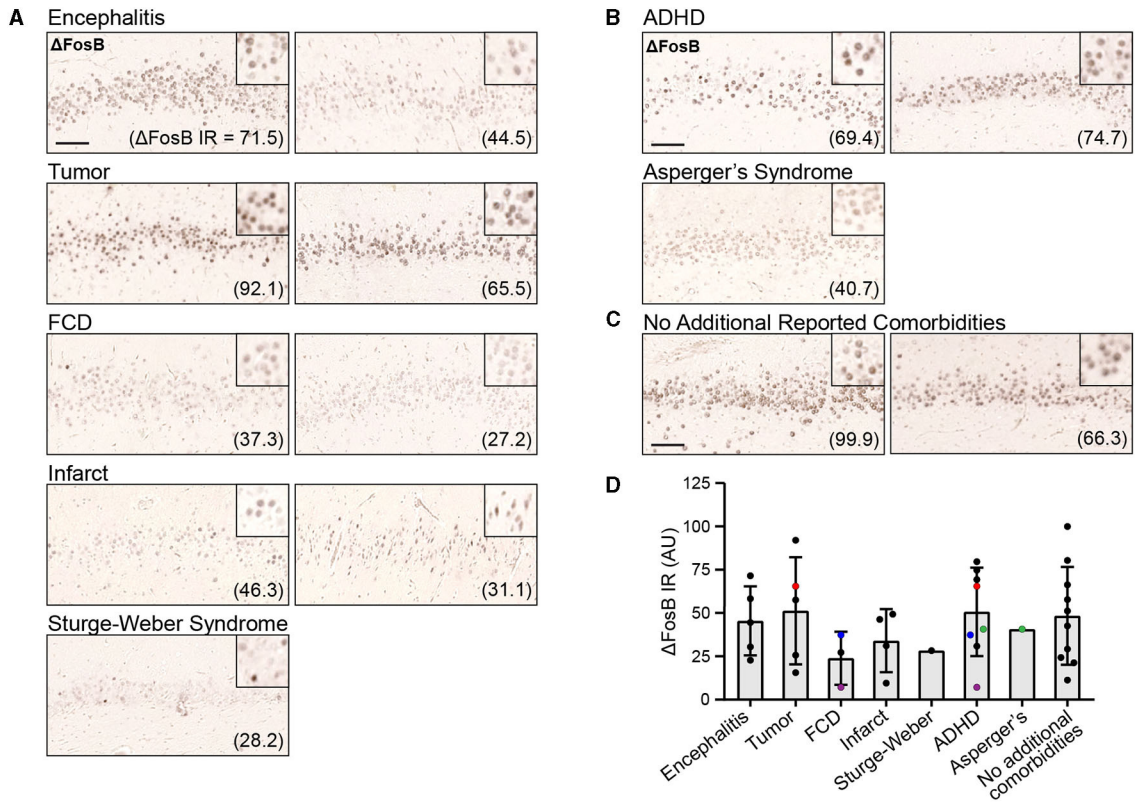


FIGURE 1
Dentate gyrus (DG) ΔFosB immunoreactivity (IR) in patients with childhood epilepsies. **(A, B)** Example images of DG ΔFosB IR in surgically resected hippocampal tissue from patients with childhood epilepsies who presented with additional neuropathological **(A)** and neuropsychiatric diagnoses **(B)**, and from patients without reported comorbidities **(C)**. Quantification of DG ΔFosB IR in arbitrary units (AU) is indicated in parentheses for each patient. **(D)** DG ΔFosB IR quantification for all 33 patients grouped by neuropathological and neuropsychiatric diagnoses. Colored (red, blue, purple, and green) data points indicate patients who had received multiple diagnoses and were therefore represented multiple times in the graph. Scale bar: 100 μm.

1st year of life to 13 years of age. Patients exhibited seizures for 7.6 ± 4.06 (mean ± SD) years prior to resection. The frequency of the seizures that patients presented with ranged from three seizures per year to 15–20 seizures per day.

While etiology of epilepsy was unclear for the majority of cases in this study, there were patients who received clinical diagnoses that have known associations with seizures, including encephalitis (26, 27), tumor (28, 29), infarction (30, 31), focal cortical dysplasia (32, 33), and Sturge-Weber syndrome (34, 35). In addition, 24.2% (8/33) of the patients had psychiatric diagnoses of attention-deficit/hyperactivity disorder (ADHD), with one patient also having Asperger's syndrome, which are comorbidities that have bidirectional relationships with epilepsy (36–39). 30.3% of patients (10/33) did not have additional neuropathological or psychiatric diagnoses.

All patients underwent neuropsychiatric testing prior to hippocampectomy in the form of the WISC-IV. WISC testing is composed of subtests that fall under four broad indices of intellectual functioning, including verbal comprehension, perceptual reasoning, working memory, and processing speed (40). Scores from verbal comprehension and perceptual reasoning subtests constitute the general ability index, while scores from all four indices constitute the Full-Scale Intelligence Quotient (FSIQ) (41, 42). FSIQ is considered a global assessment of cognitive functioning. While documented FSIQ scores were available for all patients in this study, the scores for the individual indices were not available for all patients. The average FSIQ for the general population is 100, with a standard deviation (SD) of 15, and usually ranges from 40 (exceptionally low) to 160 (exceptionally superior) (40). Notably, the average FSIQ of patients with childhood epilepsies included in this study was 80.4 with a SD of 17.0, which is significantly lower than that of the general population (80.4 ± 17 vs. 100 ± 15; $p = 0.031$, two-tailed unpaired Student's $t$-test). Patients who also received an ADHD diagnosis had lower average FSIQ compared with patients who did not receive an ADHD diagnosis (68.38 ± 14.50 vs. 84.28 ± 16.16; $p = 0.0189$, two-tailed unpaired Student's $t$-test), which is consistent with prior findings in the literature (43, 44).

## 3.2 ΔFosB expression in the DG in childhood epilepsy patients is similar to that in patients with TLE, MCI, or AD

To assess whether ΔFosB is expressed in childhood epilepsy syndromes as it is in adult TLE, MCI, and AD, and whether its expression is related to cognitive function in epilepsy, we

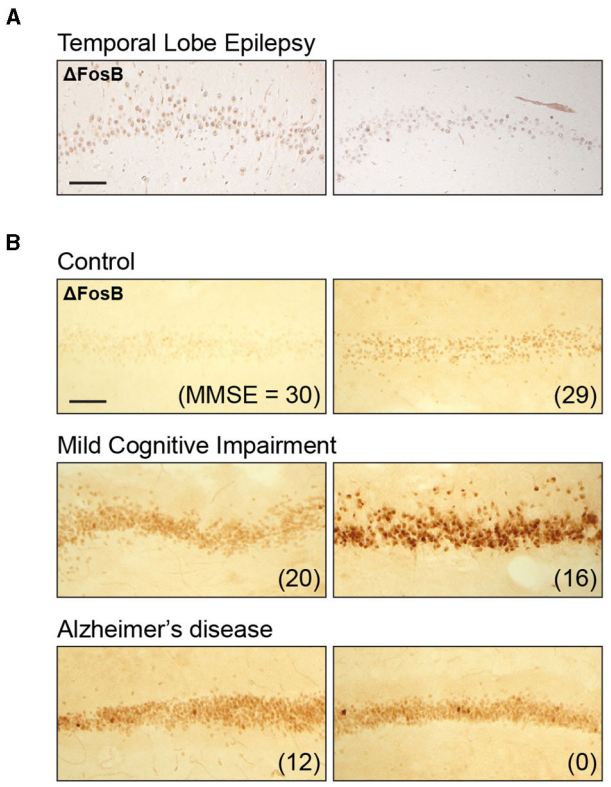


FIGURE 2
Dentate gyrus ΔFosB immunoreactivity in adult individuals with temporal lobe epilepsy (TLE), mild cognitive impairment (MCI), or Alzheimer's disease (AD). **(A)** Example images of dentate gyrus ΔFosB immunoreactivity in surgically resected tissue from two patients with TLE. **(B)** Example images of dentate gyrus ΔFosB immunoreactivity in postmortem samples from control individuals, individuals with MCI, and individuals with AD. Mini-Mental State Examination (MMSE) scores are indicated in parentheses for each patient. Sections from patients included in this figure were stained as part of a previously published study (25); examples shown here are original, previously unpublished images. Scale bar: 100 μm.

first performed immunohistochemistry for ΔFosB on DG samples from these 33 patients (Figure 1; Supplementary Figure 1). We observed distinct nuclear expression of ΔFosB in dentate granule cells, consistent with the pattern observed in animal models with epilepsy and previous studies of human samples (25). We noted that the intensity of ΔFosB expression varied between patients, and this variability was reflected in the quantification of ΔFosB immunoreactivity (indicated by arbitrary units in parentheses; Figure 1D). However, there was no systematic difference in ΔFosB expression between patients with or without additional neuropathological or psychiatric diagnoses in the present dataset (Supplementary Figure 2). In addition, although DG ΔFosB expression in mice corresponds to seizure frequency, DG ΔFosB expression in this cohort of patients with childhood epilepsies did not directly correspond to either seizure frequency ($N = 19$, $R^2 = 0.087$, $p = 0.219$) or number of years patients experienced seizures prior to hippocampectomy ($N = 22$, $R^2 = 0.002$, $p = 0.839$). However, these data were not available for all 33 patients.

To assess whether the DG ΔFosB expression pattern in patients with childhood epilepsies is qualitatively similar to the expression pattern in patients with TLE, we revisited ΔFosB expression patterns in hippocampal resection tissues obtained from adult patients with TLE in a previous study (25). Similar to our findings in patients with childhood epilepsies, ΔFosB expression in adult patients with TLE showed a nuclear pattern, with clearly defined small circular areas of intense staining, particularly in comparison with the diffuse background staining observed in the surrounding brain parenchyma (Figure 2A). This result indicates that DG ΔFosB expression is clearly observed in both childhood and adult epilepsies.

In our previous study demonstrating robust ΔFosB expression in adult TLE, we did not have neuropsychiatric data to assess the relationship between ΔFosB and cognitive function in those individuals. However, we were able to assess the relationship between ΔFosB expression and cognition in individuals with MCI or AD, which is associated with an increase in seizure incidence (45–48). Recent studies demonstrated that seizure activity tends to begin early in disease progression and is associated with earlier and faster rate of cognitive decline (13, 16, 47, 49). In our previous study, we found that ΔFosB expression in the DG was increased in individuals with either MCI or AD compared with control individuals, as shown in Figure 2B. We noted that the staining pattern in the MCI and AD groups was also nuclear, similar to the epilepsy samples (Figure 2B). Of particular relevance to this study, DG ΔFosB expression did not correspond to Mini-Mental State Examination (MMSE) scores in control individuals or in AD patients with severe cognitive impairments, but ΔFosB expression did correspond to MMSE scores in MCI patients, indicating a relationship between DG ΔFosB expression and cognitive dysfunction in earlier or milder stages of AD (25).

## 3.3 ΔFosB expression in the DG of patients with childhood epilepsies corresponds to FSIQ in patients with borderline to poor intellectual functioning

To determine whether DG ΔFosB expression is related to cognitive function in patients with childhood epilepsies, we compared ΔFosB expression levels with FSIQ, a global measure of cognitive functioning. Because we found no relationship between ΔFosB and MMSE scores in control individuals but found a negative relationship in MCI patients in which higher ΔFosB expression reflected poorer cognitive function (25), we divided the childhood epilepsy cohort based on cognitive function, as defined by FSIQ. We used a FSIQ cutoff of 85, above which children are typically considered to have average or above average intellectual functioning, and below which children are considered to have borderline intellectual functioning (FSIQ $>$ 70) or intellectual disability (FSIQ $<$ 70) (50).

We found that in individuals with FSIQ $>$ 85, ΔFosB did not correspond to FSIQ (Figure 3A). However, in individuals with FSIQ $<$ 85, higher levels of ΔFosB expression corresponded to lower FSIQ (Figures 3B, C). There was no significant relationship between any individual index score with ΔFosB in either group, which may in part be due to variable sample sizes since not all index scores were available for every patient (Supplementary Figure 3).

While not statistically significant, we noted that in individuals with FSIQ < 85, the general trend for all indices were negative (i.e., decreased scores with increased ΔFosB; Supplementary Figure 3B), whereas the general trends for individuals with FSIQ > 85 were more mixed (Supplementary Figure 3A). Subdividing patients by sex, time between neuropsychiatric testing and hippocampectomy, and other neuropathological and psychiatric diagnoses did not yield other significant relationships (Supplementary Figure 4). Interestingly, while scores for most indices showed no or negative trends with ΔFosB, the processing speed index score showed positive trends with ΔFosB in several subdivisions of patients (Supplementary Figures 3–5), and the trend was significant in patients whose tissue was found to have hippocampal sclerosis (Supplementary Figure 5F).

## 4 Discussion

In summary, we found that ΔFosB is expressed robustly in the dentate granule cells of patients with childhood epilepsies, similar to adult individuals with TLE, MCI, or AD, and that the magnitude of ΔFosB expression in these cells corresponded to FSIQ in patients whose FSIQ is <85.

Our finding that ΔFosB is expressed similarly in the DG of humans as in that of mice support the potential translatability of the functions and mechanisms of action of ΔFosB that have been uncovered in rodent models of human diseases. An FSIQ of 85 has been used as the cutoff between individuals with average intellectual functioning and those with borderline intellectual functioning (BIF; FSIQ 70–84) or intellectual disability (FSIQ < 70) (50). While BIF is not considered a mental disability in the most recent Diagnostic and Statistical Manual of Mental Disorders (DSM-5), children with BIF have high risk for the same mental, social, and intellectual difficulties as those with intellectual disability (50–54). Our finding that ΔFosB corresponds to FSIQ in this patient subpopulation (FSIQ < 85) suggests that in these individuals, ΔFosB may be engaging mechanisms that negatively affect cognition. It has been shown in rodent models that alterations to ΔFosB expression in the hippocampus in non-disease conditions are sufficient to induce hippocampal-dependent learning and memory deficits, whereas normalizing aberrantly increased ΔFosB activity in disease conditions improves cognition (19, 21, 25, 55). Additionally, due the long half-life ΔFosB, its impact on cognition could persist even during periods in between seizures. Thus, the findings in this study suggest that in patients with FSIQ < 85, achieving seizure control may not be sufficient, and that it may be beneficial to also investigate methods to regulate ΔFosB activity or to manage its downstream effects (21).

ΔFosB did not correspond to FSIQ in patients whose FSIQ is >85, suggesting that it may not closely reflect cognitive function in patients whose cognition scores are considered average or better. It is possible that ΔFosB expression is not sensitive enough to reflect more subtle variations in cognition. Indeed, in our previous study with postmortem tissue, ΔFosB did not correspond to MMSE scores in control individuals, who had average cognition, but did correspond to MMSE scores in MCI individuals, who have below average cognition (25). Similarly, ΔFosB expression corresponded to performance in a hippocampal-dependent memory task in mice used to study AD neuropathology, but not in wildtype control mice (19). Another possibility is that availability of binding partners for ΔFosB may be differentially expressed in the patient subgroups. ΔFosB, like other members of the AP-1 transcription factor family, usually form heterodimers with other AP-1 transcription factors, and the resulting complex regulates gene transcription (18, 56). Future research investigating whether binding partners of ΔFosB are expressed differently in patients with FSIQ above or below 85 may shed light on this possibility.

We also noted that ΔFosB did not correspond to individual WISC index scores, although this may in part be due to reduced power given variable sample sizes, since index scores were not available for all patients in the cohort. Interestingly, while most indices showed no trend or a negative trend with increasing magnitude of ΔFosB expression, the processing speed index instead showed a positive trend in multiple patient subcategories (Supplementary Figures 3–5). Higher processing speed has been hypothesized to reduce the demand on working memory capabilities (57, 58). Therefore, one possibility is that higher processing speed may be a compensatory mechanism engaged as a response to impaired working memory, which may be of interest for future investigations.

There were limitations in this study related to incomplete patient profiles, which may have precluded further insights. Seizure frequency is a critical piece of information that was unavailable for 14 of the 33 total patients investigated in this study. Even for the 19 patients for which this information was available, it is unclear when seizure frequency was assessed relative to when surgical resection of the hippocampus took place. The 8-day *in vivo* half-life of ΔFosB likely limits its ability to reflect seizure history beyond a few weeks prior to sample collection. Thus, ΔFosB may not closely track seizure frequency if that information was obtained too far in advance of the resection. Because it is not possible to obtain similarly processed hippocampal resection tissues from control individuals without a history of seizures, it was also not possible for us to determine the extent to which ΔFosB expression was increased above baseline at the time of surgery. In addition, the interval of time between WISC assessment and surgical resection of the hippocampus varied between patients, which could limit how closely ΔFosB expression (indicative of brain state at time of surgery) reflects cognitive performance (indicative of brain state at time of neuropsychiatric testing). It is also unclear what specific medications or other treatments patients had received prior to neuropsychiatric testing or hippocampectomy. Certain anti-seizure medications have been documented to have side effects on cognition and mood, which could affect performance during neuropsychiatric testing independently of ΔFosB (7, 59–62). Anti-seizure medication may also affect ΔFosB expression by altering seizure frequency (19, 63) or perhaps by direct regulation (21).

There is also limited information available about the etiology of seizures or which brain areas other than the hippocampus were affected by seizure activity, which are factors that can affect the extent and severity of cognitive impairment in epilepsy (7). In the present study, we investigated DG ΔFosB expression, which is indicative of seizure activity in the hippocampus itself, since ΔFosB accumulation occurs in neurons that are (hyper) active. However, ΔFosB in the DG does not regulate all domains of cognitive function, and seizures and lesions present in extra-hippocampal

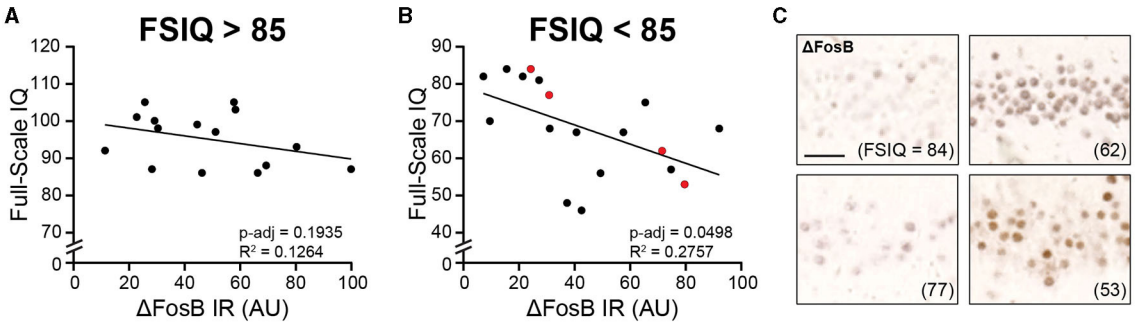


FIGURE 3
Dentate gyrus ΔFosB immunoreactivity corresponds to Full-Scale Intelligence Quotient (FSIQ) in patients with childhood epilepsies and borderline intellectual functioning or intellectual disability. **(A, B)** Regression analyses of dentate gyrus ΔFosB immunoreactivity and FSIQ for patients with FSIQ > 85 **(A)** and patients with FSIQ < 85 **(B)**. **(C)** Representative images of dentate gyrus ΔFosB immunoreactivity of patients indicated in red in **(B)**. FSIQ is indicated in parentheses for each patient. IR, immunoreactivity; AU, arbitrary units. Scale bar: 50 μm.

regions of the brain may also contribute to the variability in neuropsychiatric test performance. Indeed, some patients also had neurological comorbidities that could also impair cognition independently of or concurrently with seizures in the hippocampus. The presence of a tumor, for example, can directly disrupt local neural processing, and treatments for patients with tumors also often have negative effects on cognition (64). Cortical infarct resulting from ischemia can also induce neuronal excitotoxicity and cell death, loss of dendritic spines, alterations in synaptic receptor composition, and long-term potentiation deficits, which can all contribute to cognitive impairment (65). Indeed, there may be pathophysiological mechanisms that both increase seizure propensity and impair cognitive function (7, 66). These factors could obfuscate the relationship between DG ΔFosB expression and cognitive performance.

Despite these limitations, our study demonstrates that robust ΔFosB expression in the DG can be found in individuals of a broad range of ages and with varying medical conditions. Moreover, in specific subsets of those patient populations, DG ΔFosB expression corresponds to aspects of cognitive function, similar to rodent models of the same diseases. These findings suggest that ΔFosB pathways may be important for future studies to further elucidate, as understanding its mechanisms of action has the potential to create new avenues for therapeutic development.

## Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding authors.

## Ethics statement

The studies involving humans were approved by Baylor College of Medicine IRB protocol H-10255 and Children's Hospital of Philadelphia IRB protocol 19-016521. The studies were conducted in accordance with the local legislation and institutional requirements. The human samples used in this study were acquired from a by-product of routine care or industry. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

## Author contributions

C-HF: Conceptualization, Formal analysis, Investigation, Visualization, Writing – original draft, Writing – review & editing. JY: Formal analysis, Funding acquisition, Investigation, Writing – review & editing. CM: Investigation, Writing – review & editing. RR: Investigation, Writing – review & editing. DY: Investigation, Writing – review & editing. AV: Conceptualization, Formal analysis, Investigation, Project administration, Supervision, Writing – original draft, Writing – review & editing. JC: Conceptualization, Funding acquisition, Project administration, Supervision, Writing – original draft, Writing – review & editing.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships

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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.2023.1331194/full#supplementary-material

## References


1. Fiest KM, Sauro KM, Wiebe S, Patten SB, Kwon CS, Dykeman J, et al. Prevalence and incidence of epilepsy: a systematic review and meta-analysis of international studies. *Neurology.* (2017) 88:296–303. doi: 10.1212/WNL.0000000000003509

2. Beghi E. The epidemiology of epilepsy. *Neuroepidemiology.* (2020) 54:185–91. doi: 10.1159/000503831

3. Bell B, Lin JJ, Seidenberg M, Hermann B. The neurobiology of cognitive disorders in temporal lobe epilepsy. *Nat Rev Neurol.* (2011) 7:154–64. doi: 10.1038/nrneurol.2011.3

4. Holmes GL. Cognitive impairment in epilepsy: the role of network abnormalities. *Epileptic Disord.* (2015) 17:101–16. doi: 10.1684/epd.2015.0739

5. Novak A, Vizjak K, Rakusa M. Cognitive impairment in people with epilepsy. *J Clin Med.* (2022) 11:10267. doi: 10.3390/jcm11010267

6. Kleen JK, Scott RC, Lenck-Santini PP, Holmes GL. Cognitive and behavioral co-morbidities of epilepsy. In: Noebels JL, Avoli M, Rogawski MA, Olsen RW, Delgado-Escueta AV, editors. *Jasper's Basic Mechanisms of the Epilepsies.* 4th ed. Bethesda, MD (2012).

7. Lenck-Santini PP, Scott RC. Mechanisms responsible for cognitive impairment in epilepsy. *Cold Spring Harb Perspect Med.* (2015) 5:a022772. doi: 10.1101/cshperspect.a022772

8. Hecht F. Seizure disorders in the fragile X chromosome syndrome. *Am J Med Genet.* (1991) 38:509. doi: 10.1002/ajmg.1320380274

9. Palop JJ, Chin J, Mucke L. A network dysfunction perspective on neurodegenerative diseases. *Nature.* (2006) 443:768–73. doi: 10.1038/nature05289

10. Matsuo M, Maeda T, Sasaki K, Ishii K, Hamasaki Y. Frequent association of autism spectrum disorder in patients with childhood onset epilepsy. *Brain Dev.* (2010) 32:759–63. doi: 10.1016/j.braindev.2010.05.005

11. Lott IT, Doran E, Nguyen VQ, Tournay A, Movsesyan N, Gillen DL. Down syndrome and dementia: seizures and cognitive decline. *J Alzheimers Dis.* (2012) 29:177–85. doi: 10.3233/JAD-2012-111613

12. Chin J, Scharfman HE. Shared cognitive and behavioral impairments in epilepsy and Alzheimer's disease and potential underlying mechanisms. *Epilepsy Behav.* (2013) 26:343–51. doi: 10.1016/j.yebeh.2012.11.040

13. Vossel KA, Beagle AJ, Rabinovici GD, Shu H, Lee SE, Naasan G, et al. Seizures and epileptiform activity in the early stages of Alzheimer disease. *J Am Med Assoc Neurol.* (2013) 70:1158–66. doi: 10.1001/jamaneurol.2013.136

14. El Achkar CM, Spence SJ. Clinical characteristics of children and young adults with co-occurring autism spectrum disorder and epilepsy. *Epilepsy Behav.* (2015) 47:183–90. doi: 10.1016/j.yebeh.2014.12.022

15. Buckley AW, Holmes GL. Epilepsy and autism. *Cold Spring Harb Perspect Med.* (2016) 6:a022749. doi: 10.1101/cshperspect.a022749

16. Vossel KA, Tartaglia MC, Nygaard HB, Zeman AZ, Miller BL. Epileptic activity in Alzheimer's disease: causes and clinical relevance. *Lancet Neurol.* (2017) 16:311–22. doi: 10.1016/S1474-4422(17)30044-3

17. Holmes H, Sawer F, Clark M. Autism spectrum disorders and epilepsy in children: a commentary on the occurrence of autism in epilepsy; how it can present differently and the challenges associated with diagnosis. *Epilepsy Behav.* (2021) 117:107813. doi: 10.1016/j.yebeh.2021.107813

18. Nestler EJ. Review. Transcriptional mechanisms of addiction: role of DeltaFosB. *Philos Trans R Soc Lond B Biol Sci.* (2008) 363:3245–55. doi: 10.1098/rstb.2008.0067

19. Corbett BF, You JC, Zhang X, Pyfer MS, Tosi U, Iascone DM, et al. DeltaFosB regulates gene expression and cognitive dysfunction in a mouse model of Alzheimer's disease. *Cell Rep.* (2017) 20:344–55. doi: 10.1016/j.celrep.2017.06.040

20. Stephens GS, Fu CH, St Romain CP, Zheng Y, Botterill JJ, Scharfman HE, et al. Genes bound by DeltaFosB in different conditions with recurrent seizures regulate similar neuronal functions. *Front Neurosci.* (2020) 14:472. doi: 10.3389/fnins.2020.00472

21. Robison AJ, Nestler EJ. DeltaFOSB: a potentially druggable master orchestrator of activity-dependent gene expression. *ACS Chem Neurosci.* (2022) 13:296–307. doi: 10.1021/acschemneuro.1c00723

22. Mcclung CA, Ulery PG, Perrotti LI, Zachariou V, Berton O, Nestler EJ. DeltaFosB: a molecular switch for long-term adaptation in the brain. *Brain Res Mol Brain Res.* (2004) 132:146–54. doi: 10.1016/j.molbrainres.2004.05.014

23. You JC, Stephens GS, Fu CH, Zhang X, Liu Y, Chin J. Genome-wide profiling reveals functional diversification of ΔFosB gene targets in the hippocampus of an Alzheimer's disease mouse model. *PLoS ONE.* (2018) 13:e0192508. doi: 10.1371/journal.pone.0192508

24. Yeh SY, Estill M, Lardner CK, Browne CJ, Minier-Toribio A, Futamura R, et al. Cell type-specific whole-genome landscape of DeltaFOSB binding in the nucleus accumbens after chronic cocaine exposure. *Biol Psychiatry.* (2023) 94:367–77. doi: 10.1016/j.biopsych.2022.12.021

25. You JC, Muralidharan K, Park JW, Petrof I, Pyfer MS, Corbett BF, et al. Epigenetic suppression of hippocampal calbindin-D28k by DeltaFosB drives seizure-related cognitive deficits. *Nat Med.* (2017) 23:1377–83. doi: 10.1038/nm.4413

26. Vezzani A, French J, Bartfai T, Baram TZ. The role of inflammation in epilepsy. *Nat Rev Neurol.* (2011) 7:31–40. doi: 10.1038/nrneurol.2010.178

27. Spatola M, Dalmau J. Seizures and risk of epilepsy in autoimmune and other inflammatory encephalitis. *Curr Opin Neurol.* (2017) 30:345–53. doi: 10.1097/WCO.0000000000000449

28. Englot DJ, Chang EF, Vecht CJ. Epilepsy and brain tumors. *Handb Clin Neurol.* (2016) 134:267–85. doi: 10.1016/B978-0-12-802997-8.00016-5

29. Goethe EA, Deneen B, Noebels J, Rao G. The role of hyperexcitability in gliomagenesis. *Int J Mol Sci.* (2023) 24:10749. doi: 10.3390/ijms24010749

30. Ferreira-Atuesta C, Dohler N, Erdelyi-Canavese B, Felbecker A, Siebel P, Scherrer N, et al. Seizures after ischemic stroke: a matched multicenter study. *Ann Neurol.* (2021) 90:808–20. doi: 10.1002/ana.26212

31. Galovic M, Ferreira-Atuesta C, Abraira L, Dohler N, Sinka L, Brigo F, et al. Seizures and epilepsy after stroke: epidemiology, biomarkers and management. *Drugs Aging.* (2021) 38:285–99. doi: 10.1007/s40266-021-00837-7

32. Crino PB. Focal cortical dysplasia. *Semin Neurol.* (2015) 35:201–8. doi: 10.1055/s-0035-1552617

33. Guerrini R, Barba C. Focal cortical dysplasia: an update on diagnosis and treatment. *Expert Rev Neurother.* (2021) 21:1213–24. doi: 10.1080/14737175.2021.1915135

34. Smegal LF, Sebold AJ, Hammill AM, Juhasz C, Lo WD, Miles DK, et al. Multicenter research data of epilepsy management in patients with Sturge-Weber syndrome. *Pediatr Neurol.* (2021) 119:3–10. doi: 10.1016/j.pediatrneurol.2021.02.006

35. Sanchez-Espino LF, Ivars M, Antonanzas J, Baselga E. Sturge-Weber syndrome: a review of pathophysiology, genetics, clinical features, and current management approach. *Appl Clin Genet.* (2023) 16:63–81. doi: 10.2147/TACG.S363685

36. Berg AT, Plioplys S, Tuchman R. Risk and correlates of autism spectrum disorder in children with epilepsy: a community-based study. *J Child Neurol.* (2011) 26:540–7. doi: 10.1177/0883073810384869

37. Mouridsen SE, Rich B, Isager T. Epilepsy in individuals with a history of Asperger's syndrome: a Danish nationwide register-based cohort study. *J Autism Dev Disord.* (2013) 43:1308–13. doi: 10.1007/s10803-012-1675-9

38. Fan HC, Chiang KL, Chang KH, Chen CM, Tsai JD. Epilepsy and attention deficit hyperactivity disorder: connection, chance, and challenges. *Int J Mol Sci.* (2023) 24:65270. doi: 10.3390/ijms24065270

39. Uliel-Sibony S, Chernuha V, Tokatly Latzer I, Leitner Y. Epilepsy and attention-deficit/hyperactivity disorder in children and adolescents: an overview of etiology, prevalence, and treatment. *Front Hum Neurosci.* (2023) 17:1021605. doi: 10.3389/fnhum.2023.1021605

40. Wechsler D. *WISC-IV: Wechsler Intelligence Scale for Children*. San Antonio, TX: The Psychological Corporation (2003).

41. Weiss LG. *WISC-IV: Advanced Clinical Interpretation*. Burlington, MA: Academic Press/Elsevier (2006).

42. Lanfranchi S. Is the WISC-IV General Ability Index a useful tool for identifying intellectual disability? *Dev Med Child Neurol.* (2013) 55:782–3. doi: 10.1111/dmcn.12210

43. Voigt RG, Barbaresi WJ, Colligan RC, Weaver AL, Katusic SK. Developmental dissociation, deviance, and delay: occurrence of attention-deficit-hyperactivity disorder in individuals with and without borderline-to-mild intellectual disability. *Dev Med Child Neurol.* (2006) 48:831–5. doi: 10.1111/j.1469-8749.2006.tb01231.x

44. Tallberg P, Rastam M, Perrin S, Hallin AL, Gustafsson P. A longitudinal investigation of cognitive functioning and its relationship to symptom severity and academic functioning in treatment seeking youth with AHDH. *Scand J Child Adolesc Psychiatr Psychol.* (2021) 9:52–63. doi: 10.21307/sjcapp-2021-007

45. Amatniek JC, Hauser WA, Delcastillo-Castaneda C, Jacobs DM, Marder K, Bell K, et al. Incidence and predictors of seizures in patients with Alzheimer's disease. *Epilepsia.* (2006) 47:867–72. doi: 10.1111/j.1528-1167.2006.00554.x

46. Larner AJ. Epileptic seizures in AD patients. *Neuromolecular Med.* (2010) 12:71–7. doi: 10.1007/s12017-009-8076-z

47. Vossel KA, Ranasinghe KG, Beagle AJ, Mizuiri D, Honma SM, Dowling AF, et al. Incidence and impact of subclinical epileptiform activity in Alzheimer's disease. *Ann Neurol.* (2016) 80:858–70. doi: 10.1002/ana.24794

48. Voglein J, Ricard I, Noachtar S, Kukull WA, Dieterich M, Levin J, et al. Seizures in Alzheimer's disease are highly recurrent and associated with a poor disease course. *J Neurol.* (2020) 267:2941–8. doi: 10.1007/s00415-020-09937-7

49. Cretin B, Sellal F, Philippi N, Bousiges O, Di Bitonto L, Martin-Hunyadi C, et al. Epileptic prodromal Alzheimer's disease, a retrospective study of 13 new cases: expanding the spectrum of Alzheimer's disease to an epileptic variant? *J Alzheimer's Dis.* (2016) 52:1125–33. doi: 10.3233/JAD-150096

50. Fernell E, Gillberg C. Borderline intellectual functioning. *Handb Clin Neurol.* (2020) 174:77–81. doi: 10.1016/B978-0-444-64148-9.00006-5

51. Emerson E, Einfeld S, Stancliffe RJ. The mental health of young children with intellectual disabilities or borderline intellectual functioning. *Soc Psychiatry Psychiatr Epidemiol.* (2010) 45:579–87. doi: 10.1007/s00127-009-0100-y

52. Einfeld SL, Ellis LA, Emerson E. Comorbidity of intellectual disability and mental disorder in children and adolescents: a systematic review. *J Intellect Dev Disabil.* (2011) 36:137–43. doi: 10.1080/13668250.2011.572548

53. Melby L, Indredavik MS, Lohaugen G, Brubakk AM, Skranes J, Vik T. Is there an association between full IQ score and mental health problems in young adults? A study with a convenience sample. *BMC Psychol.* (2020) 8:7. doi: 10.1186/s40359-020-0372-2

54. Satila H, Jolma LM, Merilainen-Nipuli M, Koivu-Jolma M. Challenges and neuropsychological functioning in children and adolescents with borderline intellectual functioning. *Children.* (2022) 9:121847. doi: 10.3390/children9121847

55. Eagle AL, Gajewski PA, Yang M, Kechner ME, Al Masraf BS, Kennedy PJ, et al. Experience-dependent induction of hippocampal DeltaFosB controls learning. *J Neurosci.* (2015) 35:13773–83. doi: 10.1523/JNEUROSCI.2083-15.2015

56. Jorissen HJ, Ulery PG, Henry L, Gourneni S, Nestler EJ, Rudenko G. Dimerization and DNA-binding properties of the transcription factor DeltaFosB. *Biochemistry.* (2007) 46:8360–72. doi: 10.1021/bi700494v

57. Portrat S, Camos V, Barrouillet P. Working memory in children: a time-constrained functioning similar to adults. *J Exp Child Psychol.* (2009) 102:368–74. doi: 10.1016/j.jecp.2008.05.005

58. Barrouillet P, Camos V. As time goes by:temporal constraints in working memory. *Curr Dir Psychol Sci.* (2012) 21:413–9. doi: 10.1177/0963721412459513

59. Sankar R, Holmes GL. Mechanisms of action for the commonly used antiepileptic drugs: relevance to antiepileptic drug-associated neurobehavioral adverse effects. *J Child Neurol.* (2004) 19(Suppl.1):S6–14. doi: 10.1177/08830738040190010201

60. Schmitz B. Effects of antiepileptic drugs on mood and behavior. *Epilepsia.* (2006) 47(Suppl.2):28–33. doi: 10.1111/j.1528-1167.2006.00684.x

61. Perucca P, Mula M. Antiepileptic drug effects on mood and behavior: molecular targets. *Epilepsy Behav.* (2013) 26:440–9. doi: 10.1016/j.yebeh.2012.09.018

62. Witt JA, Helmstaedter C. Monitoring the cognitive effects of antiepileptic pharmacotherapy–approaching the individual patient. *Epilepsy Behav.* (2013) 26:450–6. doi: 10.1016/j.yebeh.2012.09.015

63. Fu CH, Iascone DM, Petrof I, Hazra A, Zhang X, Pyfer MS, et al. Early seizure activity accelerates depletion of hippocampal neural stem cells and impairs spatial discrimination in an Alzheimer's disease model. *Cell Rep.* (2019) 27:3741–51 e3744. doi: 10.1016/j.celrep.2019.05.101

64. Coomans MB, Van Der Linden SD, Gehring K, Taphoorn MJB. Treatment of cognitive deficits in brain tumour patients: current status and future directions. *Curr Opin Oncol.* (2019) 31:540–7. doi: 10.1097/CCO.0000000000000581

65. Stradecki-Cohan HM, Cohan CH, Raval AP, Dave KR, Reginensi D, Gittens RA, et al. Cognitive deficits after cerebral ischemia and underlying dysfunctional plasticity: potential targets for recovery of cognition. *J Alzheimer's Dis.* (2017) 60:S87–105. doi: 10.3233/JAD-170057

66. Khalife MR, Scott RC, Hernan AE. Mechanisms for cognitive impairment in epilepsy: moving beyond seizures. *Front Neurol.* (2022) 13:878991. doi: 10.3389/fneur.2022.878991

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# ΔFosB is part of a homeostatic mechanism that protects the epileptic brain from further deterioration

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Activity induced transcription factor ΔFosB plays a key role in different CNS disorders including epilepsy, Alzheimer's disease, and addiction. Recent findings suggest that ΔFosB drives cognitive deficits in epilepsy and together with the emergence of small molecule inhibitors of ΔFosB activity makes it an interesting therapeutic target. However, whether ΔFosB contributes to pathophysiology or provides protection in drug-resistant epilepsy is still unclear. In this study, ΔFosB was specifically downregulated by delivering AAV-shRNA into the hippocampus of chronically epileptic mice using the drug-resistant pilocarpine model of mesial temporal epilepsy (mTLE). Immunohistochemistry analyses showed that prolonged downregulation of ΔFosB led to exacerbation of neuroinflammatory markers of astrogliosis and microgliosis, loss of mossy fibers, and hippocampal granule cell dispersion. Furthermore, prolonged inhibition of ΔFosB using a ΔJunD construct to block ΔFosB signaling in a mouse model of Alzheimer's disease, that exhibits spontaneous recurrent seizures, led to similar findings, with increased neuroinflammation and decreased NPY expression in mossy fibers. Together, these data suggest that seizure-induced ΔFosB, regardless of seizure-etiology, is part of a homeostatic mechanism that protects the epileptic brain from further deterioration.

KEYWORDS

**ΔFosB, granule cell dispersion, neuroinflammation, AAV-shRNA-ΔFosB, mossy fibers, homeostatic mechanism, mouse pilocarpine model of epilepsy**

## Introduction

Epilepsy is a chronic disease affecting more than 70 million individuals worldwide. It is characterized by recurrent unprovoked epileptic seizures (Fisher et al., 2014) that may lead to neurobiological, cognitive, psychological, and social impairments (Fisher et al., 2017; GBD 2016 Neurology Collaborators, 2019). One third of epilepsy patients do not respond to current treatments (symptomatic) (Loscher and Schmidt, 2011), and therefore the need to identify novel therapies that can reverse underlying pathophysiology is high.

Neuronal death, neuronal network rewiring, microgliosis, astrogliosis, alterations in oligodendrocyte functions, and other mechanisms could contribute to drug resistance in epilepsy (Staley, 2015; Vezzani et al., 2019; Sharma et al., 2021; Knowles et al., 2022).

We hypothesized that targeting transcription factors that regulate the expression of many genes at the same time could be an effective strategy to reverse or attenuate one or more of the pathologies associated with epilepsy and may enable seizure suppression and disease modification. In this study, we focused on the transcription factor ΔFosB, whose expression in the hippocampus is robustly increased after seizures, associated with cognitive deficits in mouse models of epilepsy or Alzheimer's disease (AD) neuropathology, and has a long half-life *in vivo* on the order of days, putting it in prime position to exert long-lasting effects on gene expression. We hypothesized that modifying the activity of ΔFosB could have a broad impact on the seizing brain. The ΔFosB protein is encoded by an alternatively spliced variant of the FosB gene, which belongs to Fos protein family of transcription factors. Fos proteins are also called immediate early genes based on their rapid induction in a cell type specific manner including neurons in brain (Choi, 2017). Unlike other Fos proteins, ΔFosB has unusually long half-life that allows it to accumulate and remain in chronically active cells for weeks (Hope et al., 1994; Chen et al., 1997; McClung and Nestler, 2003). Accumulation of ΔFosB has been observed in mouse models of medial temporal lobe epilepsy (mTLE) and in mouse models of AD neuropathology that exhibit spontaneous recurrent seizures (SRSs) (Corbett et al., 2017; You et al., 2017; Stephens et al., 2020). Notably, ΔFosB regulates expression of many genes (McClung and Nestler, 2003; Robison et al., 2013; Lardner et al., 2021) involved in epilepsy-relevant pathways (e.g., excitability and neurotransmission; cellular stress and immunity) (You et al., 2018; Stephens et al., 2020). In addition, ΔFosB activity drives seizure-related cognitive deficits (Eagle et al., 2015; You et al., 2017), one of the major comorbidities of drug-resistant epilepsy (Hermann and Seidenberg, 2007). This combination of unique features (unusually long half-life and epigenetic regulation of many genes) indicates that ΔFosB could serve as a molecular switch that could modify one or more pathological mechanisms associated with epilepsy. Blockade of ΔFosB signaling for several weeks improves cognition in a mouse model of AD neuropathology (Corbett et al., 2017; You et al., 2017); however, whether prolonged blockade of ΔFosB signaling can provide sustained improvement or impact other pathological effects of SRSs is not clear. To address this question, we downregulated ΔFosB expression to inhibit its function in mouse models of mTLE or AD neuropathology over several months. We found that the reduction of ΔFosB signaling for several months exacerbated neuroinflammatory markers and abolished the neuroprotective alterations typically observed in conditions of chronic seizures. These results reveal that ΔFosB plays critical roles in homeostatic mechanisms that protect the epileptic brain from further deterioration.

# Materials and methods

## Adeno-associated viral vector production

Adeno-associated viruses (AAV) were obtained from Vector Biolabs (USA): (1) AAV9-eGFP-U6-m-deltaFosB-shRNA (5′-CACC-GCTGGCCGAGTGAAGTTCAAGT-CTCGAG-ACTTGAACTTCACTCGGCCAG-TTTTT-3′), (2) AAV9-sGFP-U6-Scrmb-shRNA, or were AAV2 constructs that were previously characterized (Zachariou et al., 2006; Robison and Nestler, 2011) and packaged by the University of North Carolina Vector Core: (3) AAV2-CMV-eGFP, or (4) AAV2-CMV-ΔJunD-IRES2-eGFP that acts as an inhibitor of ΔFosB transcriptional activity by binding and preventing dimerization with other AP-1 factors (Brown et al., 1996). These AAV2 constructs are stably expressed throughout the dentate gyrus within 18–22 days of infusion (Corbett et al., 2017; You et al., 2017).

## Animals

Male C57Bl/6 mice (Janvier, France) 15 weeks of age were used in development/validation of AAV9-GFP-U6-m-deltaFosB-shRNA. These experiments were performed at SynapCell and approved by ethical committee of the High Technology Animal Platform (University Grenoble Alpes, France). Animals were housed in cages on wood litter with free access to food and water until surgery. The animal house was maintained under artificial lighting between 7:30 a.m. to 7:30 p.m. in a controlled ambient temperature (22 ± 2°C) and relative humidity. Male NMRI mice (Charles River, France) weighing 28–32 g (5–6 weeks old) were used in the pilocarpine model of mTLE. Mice were housed in a room with controlled environment (temperature 22 ± 2°C, humidity 55 ± 15%, day/night cycle 12/12 h, light on at 6 a.m.) with food and water *ad libitum*. Experiments were conducted in compliance with guidelines issued by the ethic committee for animal experimentation according to Belgian law and in accordance with the European Committee Council directive (2010/63/EU).

For studies involving mouse models of AD neuropathology, we used heterozygous transgenic mice that express human amyloid precursor protein (APP) carrying Swedish (K670N, M671L) and Indiana (V717F) mutations under control of PDGF-β promoter (Line J20; hAPP770) (Mucke et al., 2000). Littermate controls included age- and sex-matched non-transgenic (NTG) mice. Mice were maintained with 12:12 light/dark cycle in cages with corncob bedding and EnviroPak nesting material, with *ad libitum* access to water and LabDiet 5V5R chow. Mice were group-housed 4–5/cage until appropriate ages for studies, and then were singly housed in a quiet environment for 2 days prior to experimentation and/or sacrifice. APP and NTG mice were studied between the ages of 2 and 5.5-months old. This line of APP mice was chosen for these studies because it has been well-characterized for the spontaneous seizures that they exhibit, and for the relationship between seizures and memory deficits (Palop et al., 2007; Sanchez-Varo et al., 2012; Verret et al., 2012; Corbett et al., 2017). We previously demonstrated that in these APP mice, seizures increase ΔFosB accumulation in the dentate gyrus of the hippocampus, where it epigenetically regulates target genes (Corbett et al., 2017; You et al., 2017, 2018; Stephens et al., 2020), making it ideal for these studies. All experiments were performed in accordance with protocols approved by the Institutional Animal Care and Use Committee of Baylor College of Medicine.

## Development of AAV9-GFP-U6-m-deltaFosB-shRNA in naïve mice

Animals received bilateral injection of AAV (total amount of injected AAV particles; low: 1.7E9; or high: 1.7E10) in both hippocampi. AAV9-eGFP-U6-m-deltaFosB-shRNA was injected to the right side and AAV9-eGFP-U6-Scrmb-shRNA was injected to the left side. Mice were anesthetized with isoflurane, placed into a stereotaxic frame and two small holes were bilaterally opened in the skull. A Hamilton syringe was filled with either vehicle (sterile 1x PBS solution with 5% glycerol) or AAV and the needle inserted into the dorsal hippocampus (coordinates from bregma; anteroposterior: −2 mm; mediolateral: ± 1.75 mm; dorsoventral: −2.1 mm). One µl of vector per hemisphere was injected at a speed of 0.10 µl/min. Tissues were collected 4 weeks after AAV injection. Mice were deeply anesthetized with isoflurane and perfused with 1x PBS solution containing heparin, and brains were rapidly removed. Both hippocampi were resected and stored at −80°C.

## Mouse pilocarpine model of mTLE

NMRI mice were intraperitoneally (i.p.) injected with 300 mg/kg of pilocarpine (Sigma-Aldrich), as previously described (Mazzuferi et al., 2012). *N*-methylscopolamine bromide (Sigma-Aldrich, 1 mg/kg, i.p.) was administered 30 min prior to pilocarpine injection to limit peripheral cholinergic effects of pilocarpine. Status epilepticus (SE) typically appeared within the first hour after pilocarpine injection and was characterized by continuous stage 3–5 seizures (continuous tonic-clonic seizures) scored according to the Racine's scale (Racine, 1972) during at least 30 min. Diazepam (10 mg/kg; Roche S.A, Brussels, Belgium) was administered i.p. 3 h after SE onset to reduce the duration of SE. After SE, all animals were intraperitoneally injected with lactated Ringer solution and fed with soaked rodent food. Age-matched and gender-matched control NMRI mice (sham group) were injected i.p. with *N*-methylscopolamine, diazepam and lactated Ringer but received saline instead of pilocarpine.

Mice surviving SE typically showed spontaneous recurrent seizures (SRSs) within days to weeks after SE induction by pilocarpine injection (Mazzuferi et al., 2012). Seizures were monitored using simultaneously video recording and monitoring of locomotor activity with a 3D micro-accelerometer ship secured to the mouse, as previously described (Srivastava et al., 2018). Only secondary generalized seizures Racine's score 3–5 (Racine, 1972) were detected, reviewed and confirmed manually with video by an experienced experimenter blinded to treatment. Seizure duration, severity and frequency were quantified. All mice included in the present study entered SE after pilocarpine injection and developed stage 3–5 SRSs, which were confirmed with video-accelerometry before injecting the virus (see **Supplementary Figures 2A–C**).

## Stereotaxic virus injection

Seven weeks after SE induction, SE mice and control sham mice were anesthetized with 2–3% isoflurane (Oxygen: 1.5 L/min), placed into a stereotaxic frame and two small holes were bilaterally opened in the skull. A Hamilton syringe was filled with either vehicle (sterile 1x PBS solution with 5% glycerol) or AAV (1.7E13 viral particles per ml prepared in 1x PBS solution with 5% glycerol) and the needle inserted into the dorsal hippocampus (coordinates from bregma; anteroposterior: −2 mm; mediolateral: ± 1.75 mm; dorsoventral: −2.1 mm). One µl of vector per hemisphere was injected at a speed of 0.10 µl/min using a microsyringe pump controller. Mice were allowed to recover for 7 days before monitoring seizures with video-accelerometry. All SE mice were monitored with video-accelerometer to quantify the number of SRSs over 14 days before AAV injection. SE mice with comparable number of SRSs before treatment were randomized to receive vehicle, AAV9-eGFP-U6-Scrmb-shRNA (Neg shRNA) or AAV9-GFP-U6-m-deltaFosB-shRNA (ΔFosB shRNA). Control sham mice received vehicle only. For APP and NTG mice (anesthesia was induced by 2–3% isoflurane and maintained using 1–1.5% isoflurane; Oxygen: 1.5 L/min), bilateral DG targeting was achieved by stereotaxic infusion of 1 µl of titer-matched ($\leq 5 \times 10^{12}$) AAV2 solution into the dentate gyrus at rostral (coordinates from bregma; anteroposterior: −1.7 mm; mediolateral: 1.2 mm; dorsoventral: 2 mm) and caudal (coordinates from bregma; anteroposterior: −2.7 mm; mediolateral: 2 mm; dorsoventral: 2.1 mm) coordinates. Mice were allowed to express AAVs for either 4 or 12 weeks, until experimentation and/or euthanasia and brain collection. Virus expression was confirmed using immunohistochemical detection of eGFP or ΔJunD.

## Tissue sampling

Control sham mice and SE mice were deeply anesthetized with 2–3% isoflurane (Oxygen: 1.5 L/min), either 4 or 8 weeks after vehicle or AAV brain injection. They were perfused sequentially via the left ventricle with 30 ml chilled 1x PBS solution containing 10IU/ml heparin, and brains were rapidly removed. The left-brain hemisphere was immersed in 4% paraformaldehyde (PFA) in PBS solution (pH7.4) for 3 h at room temperature, and then soaked in a 15% sucrose solution and stored at 4°C. The dorsal hippocampus was rapidly extracted from the right brain hemisphere, snap frozen in liquid nitrogen, and then stored at −80°C until processing for western blotting and qPCR analyses.

APP and NTG mice were deeply anesthetized using SomnaSol Euthanasia-III solution (390 mg pentobarbital sodium and 50 mg phenytoin sodium; Henry Schein) prior to transcardial perfusion with chilled saline and rapid removal of brains. The right hemibrain was drop-fixed in 4% PFA in PBS solution for 48 h, and then rinsed in PBS for 24 h prior to cryoprotection in 30% sucrose and stored at 4°C. The left hemibrain was snap frozen on dry ice and stored at −80°C until isolation of the hippocampus and processing for qPCR analysis.

## RNA extraction and qPCR

RNA was extracted from the dorsal hippocampus 4 weeks after AAV injection using RNeasy minikit (Qiagen 74134). A total of 500 ng of RNA was reverse transcribed by high-capacity cDNA RT

Kit + RNase Inhibitor (Applied Biosystems cat no 4374966). qPCR was performed using Universal Master Mix (Life Technologies ref 43004437) on CFX384 (BioRad). TaqMan probes (ThermoFisher) were used to detect the gene expression [endogenous controls: Bcl2l13 (Mm00463355_m1)], Brap (Mm00518493_m1); ΔFosB (ARXGTN9); FosB (ARU64JE) and ΔΔCt method was used to determine differential expression.

## Protein extraction and western blotting

Total proteins were extracted from dorsal hippocampus at 4 and 8 weeks after AAV injection using 2X #9803 Cell Lysis Buffer (Cell signaling). Twenty micrograms of total proteins were loaded per well of SDS-PageNovex 8–16% gel, then transferred to a PVDF membrane (Millipore). The membrane was blocked in Odyssey blocking buffer (LI-COR), and ΔFosB was detected using ΔFosB antibody (Cell Signaling #14695S; dilution 1:2000). Actin B was used as loading control (LI-COR; dilution 1:10000). Secondary antibody (LI-COR) was diluted 1:5000. Images were acquired with a LI-COR CLX and visualized using Image Studio.

## Tissue processing and immunohistochemistry

For mTLE model mice and controls, sectioning of the mouse brain hemispheres was performed by Neuroscience Associates (Knoxville, TN, USA) and immunohistochemistry was carried out by indirect immunofluorescence. The list of antibodies used in this study is provided in **Supplementary Table 1**. Free-floating coronal sections (40 μm-thick) were obtained using a cryostat microtome and permeabilized 15 min in Tris-buffered saline (TBS) containing 0.3% Triton X-100 (TBS-T). Then, sections were incubated overnight at room temperature with the primary antibody diluted in TBS-T. After three washes of 5 min in TBS, they were incubated for 1 h at room temperature with secondary antibody and 4′,6-diamidino-2-phenylindole (DAPI, 300 nM) prepared in TBS-T, rinsed 3 times 5 min in TBS, and finally mounted on glass slides using Fluoromount mounting medium (Thermo Fisher Scientific).

For APP and NTG mice, tissue preparation and immunohistochemistry were performed as previously described (Corbett et al., 2017; You et al., 2017; Stephens et al., 2020). Hemibrains were sectioned at 30 μm into ten coronal subseries throughout the rostral-caudal extent of the brain using a freezing, sliding microtome. Sections were stored in cryoprotectant medium (30% glycerol, 30% ethylene glycol, 40% PBS). 3′3-diaminobenzidine (DAB; Sigma-Aldrich) immunolabeling of NPY, Iba1, and GFAP was performed using the primary and biotinylated secondary antibodies indicated in **Supplementary Table 1**.

## Image acquisition and analysis

For mTLE model mice and controls, whole slide imaging was performed using an Axioscan Z1 scanner (Zeiss) with 20x objective. For each experiment, digital acquisitions were performed using consistent exposure parameters, avoiding overexposure, to ensure accurate signal quantification between conditions. Image analysis was performed with VisioPharm 6 software (VisioPharm, Hørsholm, Denmark) as described previously (Albert et al., 2019). Regions of Interest (ROI), such as the whole hippocampus or granular layer of the dentate gyrus, were delineated manually, and automatic quantification of the immunoreactive signal was performed using a linear Bayesian algorithm, providing a value of signal area (marker area in $\mu m^2$). Then, a % marker coverage was calculated (i.e., ratio between the immunoreactivity signal area in $\mu m^2$ and the area of the ROI in $\mu m^2$). In specific cases, number of cells and cell density (number of cells per tissue area) were quantified using Visiopharm. Percent marker coverage, cell number and cell density were quantified in the dorsal and horizontal part of the hippocampus (Bregma −1.34 to −2.54 mm) on 3 to 4 sections per animal using well-defined landmarks based on a mouse brain atlas (Paxinos and Franklin, 2019). All quantifications were done blindly until the end of statistical analysis.

For APP and NTG mice, brightfield microscope images (Zeiss) of coronal brain sections immunostained using DAB as the chromagen were quantified and analyzed using ImageJ. Iba1 and GFAP signal intensity were quantified using the Measure function to calculate the % Area that contained signal intensity above a consistent pre-set threshold within the bounds of the dentate gyrus, averaged from 2 rostral coronal sections. NPY was quantified using the Measure function of ImageJ to measure the "mean gray value" (signal intensity average within a traced ROI) of DAB signal present in the region of mossy fiber tracts projecting to CA3 that are well-known to exhibit robust ectopic NPY expression in mouse models of mTLE and AD. The mean gray values were measured from the mossy fiber ROI and in an adjacent area of similar shape/size in the stratum radiatum immediately superior to the ROI. The average signal measured in the mossy fiber ROI in 2 coronal sections was then divided by the average signal measured in the stratum radiatum of the same sections, and expressed as a fold change relative to NTG-GFP mice.

## ChIP-sequencing and gene ontology network analysis

Chromatin immunoprecipitation and sequencing (ChIP-seq) was performed in samples of whole hippocampus harvested and processed from 2 to 4-month old NTG and APP mice as described in You et al. (2018) and Stephens et al. (2020). The Cytoscape (v3.9.1) platform was used to perform new ClueGO (v2.5.9) gene ontology (GO) analyses (Shannon et al., 2003; Bindea et al., 2009) on the respective sets of all target genes found to be significantly bound by ΔFosB in our published hippocampal ΔFosB ChIP-seq analyses (Stephens et al., 2020) in pilocarpine, vehicle, non-transgenic and APP mice or the set of 442 ΔFosB target genes shared by Pilo and APP mice and not respective controls. Using ClueGO, a two-tailed hypergeometric test with a Benjamini-Hochberg correction (Benjamini and Hochberg, 1995) was used to calculate significant enrichment of Biological Process GO Terms (ontology version: 5/25/2022) with the respective sets of ΔFosB target genes in pilocarpine, vehicle, non-transgenic and APP mice. ClueGO was also used to generate a graphical GO Network from the 442 ΔFosB target genes shared by pilocarpine and APP mice in

which GO Terms enriched with ΔFosB target genes are displayed as functionally grouped nodes. GO Term nodes are connected by lines (edges) indicating that target genes are shared between nodes, and node size increases as a function of GO Term enrichment significance. Once generated from given parameters, GO analyses and networks have been simplified to remove redundant and non-brain organ-specific GO Terms and filtered to only include GO Terms that are related to processes of immunity and/or neuroprotection. GO Network parameters that were changed from ClueGO default settings are as follows: FDR < 0.05 (**Figures 6A, B**) or 0.5 (**Figure 6C**), GO Level range = 3–20, minimum number of genes in GO Term = 1, minimum percentage of genes in GO Term = 0.1%, kappa = 0.62 (**Figures 6A, B**) or 0.67 (**Figure 6C**), and GO Term fusion = TRUE.

### Statistical analysis

GraphPad Prism 9.2.0 software was used to perform all statistical analysis. To determine differences between groups the one-way or two-way ANOVA or the non-parametric Kruskal-Wallis were applied as appropriate. Follow up pairwise comparisons were done using Tukey's *post-hoc* test or Two-stage linear step-up procedure of Benjamini, Krieger and Yekutieli or Benjamini-Hochberg FDR *post-hoc* testing as appropriate. Proportions were compared as appropriate with the Chi-square test. The level of significance was set at $p < 0.05$. Data are presented as mean ± SEM.

## Results

### Development of a new specific inhibitor of ΔFosB

ΔFosB, JunD, and other proteins of the Fos, Jun, ATF, and Maf subfamilies are members of the activator protein-1 (AP-1) complex of transcription factors (Wu et al., 2021) that have a critical function in a wide range of tissues and pathways. A number of studies have demonstrated that ΔFosB plays critical role in epigenetic regulation of gene expression in the brain under physiological conditions (Eagle et al., 2018) and when neuronal activity is chronically stimulated, such as in the nucleus accumbens after drugs of abuse (Robison and Nestler, 2011), and in the dentate gyrus (DG) of the hippocampus in conditions with recurrent seizures (Corbett et al., 2017; You et al., 2017; Stephens et al., 2020). The use of the experimental construct ΔJunD (dominant negative mutant variant of JunD) to block downstream epigenetic actions of ΔFosB (Winstanley et al., 2007) has been instrumental in deciphering the role of ΔFosB in the regulation of neuronal functions, particularly in conditions of chronic activity that lead to accumulation of ΔFosB. However, it is still possible that some of the observed effects are due to binding of ΔJunD to other AP-1 complex members. We therefore developed a shRNA specifically targeting the mRNA of ΔFosB and not its parent transcript FosB (**Supplementary Figure 1A**). To confirm specificity of the newly developed shRNA, AAV-ΔFosB-shRNA was injected into dorsal hippocampus of healthy mice and qPCR (with specific primers for ΔFosB and FosB transcripts) was performed 4 weeks later. We showed that ΔFosB-shRNA can specifically downregulate ΔFosB mRNA in a dose-dependent manner without any effect on FosB mRNA expression (**Supplementary Figures 1B, C**). To further strengthen value of newly developed AAV-ΔFosB-shRNA as specific ΔFosB inhibitor, we assessed if c-Fos protein expression [previously confirmed ΔFosB downstream target (Corbett et al., 2017)] changed when ΔFosB protein is downregulated in mTLE mouse model (described later). We confirmed that subtle decrease of ΔFosB at 4 weeks led to an increase in c-Fos expression in the hippocampus and DG (**Supplementary Figures 3B, C**). Similarly, we observed robust increase in c-Fos protein expression at 8 weeks upon AAV injection (**Supplementary Figure 3B**).

### Administration of an AAV-ΔFosB shRNA led to sustained downregulation of ΔFosB protein in the hippocampus and marked modification of the hippocampal cytoarchitecture of mTLE mouse model

To assess the effects of ΔFosB suppression in the brains of mice in a therapeutically relevant timeframe, AAVs expressing ΔFosB-specific shRNA or negative control (neg) were injected bilaterally in the dorsal hippocampus of chronically epileptic mice displaying spontaneous recurrent seizures (SRSs) 7 weeks after status epilepticus (SE) induced by pilocarpine injection (Experimental design in **Figure 1A**; Seizures in **Supplementary Figures 2A, B**). Due to unusually long half-life of the ΔFosB protein (Hope et al., 1994; Chen et al., 1997; McClung and Nestler, 2003), we assessed its downregulation 4 and 8 weeks after AAV injection by immunohistochemistry (**Figures 1B–E**) and western blotting (**Supplementary Figure 2D**) in the dorsal hippocampus. In vehicle-treated sham animals, ΔFosB was localized in pyramidal neurons of the Ammon's Horn (CA1 to CA3) and granule cells of the DG (**Figures 1B, D**). In vehicle-treated mTLE mice, ΔFosB immunoreactive signal increased significantly, especially in neurons of the CA1 and granule cells of the DG at 4 and 8 weeks (**Figures 1B–E**). Administration of the AAV-Neg shRNA had no impact on ΔFosB levels in mTLE mice at 4 weeks (**Figures 1B, C**) or 8 weeks (**Figures 1D, E**). In contrast, AAV-ΔFosB shRNA injection resulted in a moderate but significant downregulation of ΔFosB at 4 weeks, comparable to physiological levels (sham animals injected with vehicle, **Figures 1B, C**). Strikingly, ΔFosB shRNA led to an almost complete disappearance of the ΔFosB signal in the whole dorsal hippocampus of mTLE mice at 8 weeks, along with a marked modification of the hippocampal cytoarchitecture based on DAPI staining (**Figures 1D, E**). Whereas the CA1 layer of the hippocampus and the granule cell layer of the DG were clearly demarcated in mTLE mice injected with vehicle or AAV-Neg shRNA, these structures were no longer obviously defined at 8 weeks in mTLE mice injected with AAV-ΔFosB shRNA (**Figure 1D**).

To determine whether the downregulation of ΔFosB protein could have an additional impact on the seizure development, epileptic mice were continuously monitored for seizure detection for 14 days beginning at 2 and 6 weeks after AAV injection. The frequency, duration and severity of SRSs remained similar

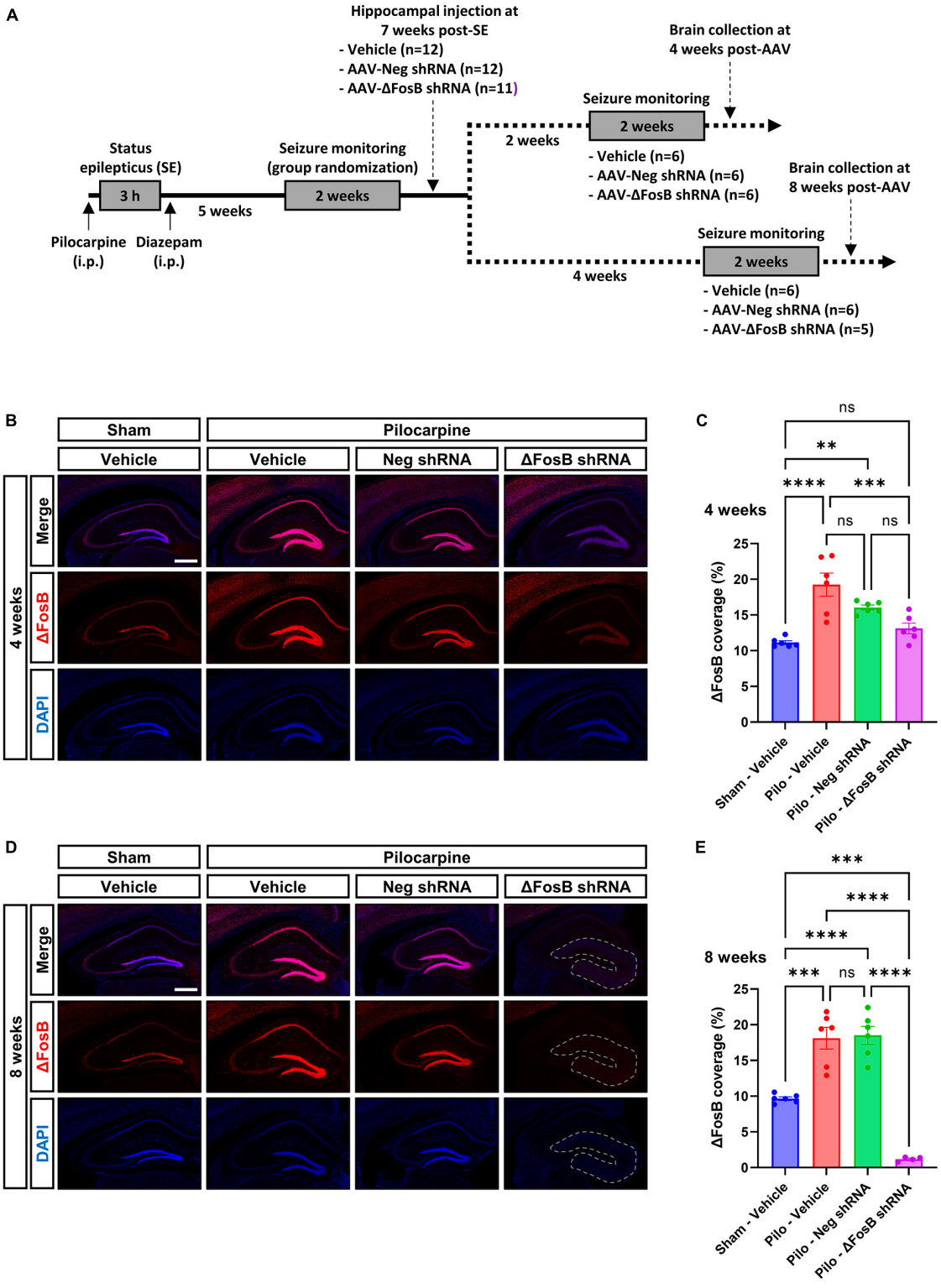


FIGURE 1
Experimental design and validation of ΔFosB knockdown. **(A)** Schematic diagrams showing the experimental design used in the study. Pilocarpine was administered to induce SE followed weeks later by the development of SRSs. SRSs were monitored before AAV administration and SE mice with comparable number of SRSs were randomized to receive vehicle, AAV-Neg shRNA or AAV-ΔFosB shRNA. SRSs were monitored again for 2 weeks starting at 2 weeks or 4 weeks after AAV injection. Terminal collection of the brains was performed at the end of each seizure monitoring phase at 4 weeks or 8 weeks after AAV injection. **(B–E)** Representative hippocampal immunohistochemistry images and quantification of ΔFosB expression levels in the dorsal part of the hippocampus at **(B,C)** 4 weeks and **(D,E)** 8 weeks after vehicle or AAV delivery. 4′,6-diamidino-2-phenylindole (DAPI) was used as a nuclear counterstain. Note that the morphology of the granular layer of the dentate gyrus is markedly affected at 8 weeks with the ΔFosB shRNA, presenting a general size enlargement and decreased DAPI signal intensity (area annotated in D). Data are expressed as mean with standard error of the mean (SEM). Statistical tests: ANOVA followed by Tukey's *post-hoc* test (**$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$; ns: non-significant). Scale bars = 500 μm.

after ΔFosB protein downregulation, suggesting that there was no influence of ΔFosB protein downregulation on seizure development (**Supplementary Figures 2A–C**). It is noteworthy to mention that AAV-ΔFosB shRNA was delivered to the dorsal hippocampus only. TLE is characterized by localization of seizure foci in multiple brain areas including the hippocampus, entorhinal cortex, or amygdala (Bartolomei et al., 2005). Thus, the lack of effect on seizures may be explained by the limited brain coverage we achieved here with the genetic tool (restricted to the dorsal hippocampus), remaining not sufficient to counteract the occurrence of seizures originating from different brain regions. Another explanation could be the low sample size in each group for seizure monitoring ($n$ = 5–6; each group), leading to a statistically underpowered study to capture differences in seizure parameters.

Overall, these observations indicated correct targeting of the dorsal hippocampus with AAVs, and efficient and selective downregulation of ΔFosB protein with the shRNA 8 weeks after viral delivery with concomitant change in hippocampal morphology in mTLE mice.

## Downregulation of ΔFosB induced granule cell dispersion (GCD) in the hippocampus of mTLE mice

We observed that ΔFosB downregulation led to significant histopathological alterations in the hippocampus of mTLE mice, as evidenced by DAPI staining (**Figure 1D**). To further characterize these pathological findings, we analyzed the neuronal architecture using immunohistochemistry with the neuronal marker NeuN. No difference in neuronal cytoarchitecture was observed in the hippocampus of mTLE mice treated with vehicle or AAV-Neg shRNA compared to sham animals treated with vehicle (**Figure 2A**). Furthermore, while ΔFosB knockdown did not alter the hippocampal morphology at 4 weeks (**Figure 2A**; granule cell area measured in **Figure 2C**), significant GCD was observed at 8 weeks as shown by the spread of NeuN staining (**Figure 2A**; granule cell area measured in **Figure 2D**). The granule cell phenotype of the dispersed cells at 8 weeks was corroborated by immunohistochemistry for Prox1, a marker specific to granular cells of the DG (**Supplementary Figure 3A**).

One of the potential mechanisms contributing to the observed GCD in the pilocarpine mouse model involves the disruption of Reelin signaling which plays a pivotal role in modulating neuronal migration and positioning during brain development (Hirota and Nakajima, 2017). In order to explore this hypothesis, we conducted a comprehensive analysis of Reelin expression within the hippocampus utilizing immunohistochemistry. Noteworthy reduction in Reelin protein levels, particularly evident within an area that includes both the lacunosum moleculare layer of the hippocampus and the upper third of the molecular layer of the DG (**Figures 2B–F**), and in the granular layer of the dentate gyrus (**Figures 2B–H**) was observed. This decline was observed at 4 and 8 weeks following the administration of AAV-mediated ΔFosB knockdown.

Our observations suggest that ΔFosB may maintain Reelin signaling and thereby help preserve the positioning of granule cells in conditions with seizure activity.

## Downregulation or inhibition of ΔFosB reverses "protective" adaptations of mossy fibers in the hippocampus of mice with recurrent seizures

It was shown that application of Reelin in older mice or in a model of a neurodevelopmental disorder improves synaptic function (↑ dendrite spine density and LTP) (Rogers et al., 2011) or restores behavioral and morphological deficits (mossy fibers; MF) (Ibi et al., 2020) in the hippocampus. To determine the impact of ΔFosB downregulation on MF we used two axonal markers, NPY (neuropeptide Y) and SV2C (synaptic vesicle glycoprotein 2C), that are typically increased in expression in MF during abnormal structural reorganization of the DG in brains of mTLE patients (Crevecoeur et al., 2014) and animal models (Nadler et al., 2007; Srivastava et al., 2018). In the mTLE mouse model, we confirmed the increase in NPY (**Figures 3A–D**) and SV2C (**Figures 3B–F**) in the MF tracts in the dorsal hippocampus of pilocarpine mice treated with vehicle or AAV-Neg shRNA, compared to sham animals treated with vehicle. Downregulation of ΔFosB reduced NPY at 4 weeks (**Figures 3A–C, E**) and caused expression of both NPY and SV2C to be nearly absent at 8 weeks (**Figures 3A, B, D, F**).

To determine if ΔFosB is required to reverse these MF adaptations across conditions with recurrent seizures regardless of etiology, we performed analogous experiments in which we expressed AAV-GFP or AAV-ΔJunD to block ΔFosB activity for 4 or 12 weeks, and measured induction of NPY-positive MF in transgenic human amyloid precursor protein (APP) mice or non-transgenic (NTG) controls. This APP mouse model of AD neuropathology (Line J20) exhibits recurrent epileptiform spikes and seizure activity beginning in the first months of life (Fu et al., 2019). We have reported that epileptiform activity in pilocarpine or APP mice promotes hippocampal accumulation of ΔFosB and downstream alterations in ΔFosB target gene expression that can impair memory (Corbett et al., 2017; You et al., 2017; Fu et al., 2019). These mice also exhibit increased expression of NPY in MF (Palop et al., 2007; Roberson et al., 2011). Notably, overexpression of ΔJunD reduced NPY signal in APP mice 4 weeks after expression and led to nearly complete reduction by 12 weeks after expression (**Figures 3G–I**). These results suggest that increased expression of ΔFosB is critical for MF adaptive response in the epileptic brain.

## Prolonged downregulation or inhibition of ΔFosB increased neuroinflammation in the hippocampus of seizing mouse brain

Neuroinflammation is another hallmark of recurrent seizures observed in mTLE or AD patients (Kandratavicius et al., 2015; Kinney et al., 2018), and in respective animal models (Zhu et al., 2017; Srivastava et al., 2018). Due to the marked changes in dorsal hippocampus morphology, we hypothesized that ΔFosB protein inhibition would increase neuroinflammation in the brains of mice with recurrent seizures. Microglial cells and astrocytes were analyzed by immunohistochemistry using Iba1 and GFAP

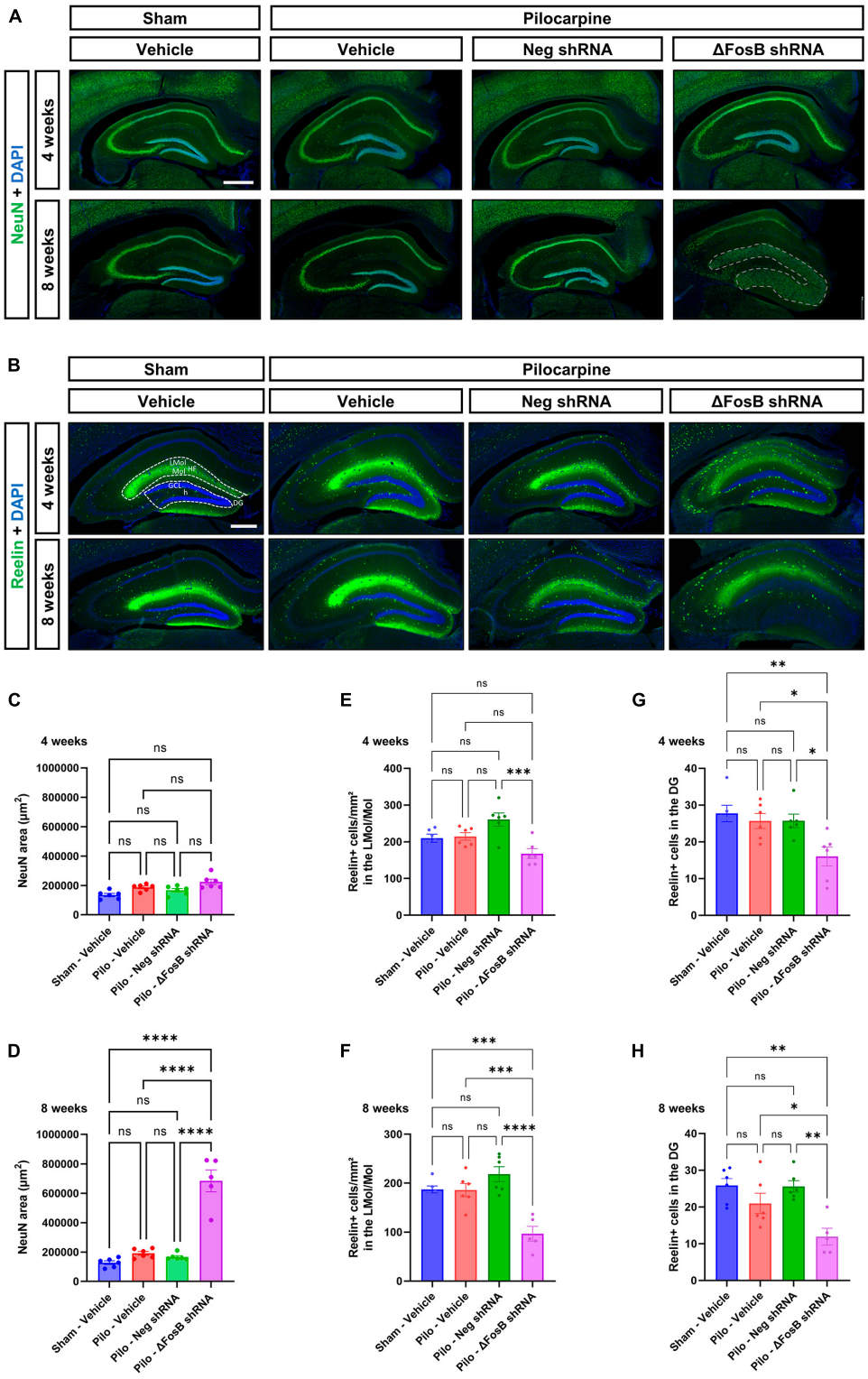


FIGURE 2
ΔFosB downregulation leads to granular cell dispersion and disruption of the Reelin signaling pathway in the dentate gyrus of the mTLE mouse model. **(A)** The neuronal marker NeuN was used to visualize the morphology and cytoarchitecture of the hippocampus. Note that the morphology of the granular layer of the dentate gyrus was markedly affected at 8 weeks with the ΔFosB shRNA [area annotated in **(A)**]. The area of the granular layer was measured at **(C)** 4 weeks and **(D)** 8 weeks. **(B)** The density of Reelin + cells was analyzed by immunohistochemistry in the dorsal part of the hippocampus, **(E,F)** specifically in the area surrounding the hippocampal fissure (HF) [annotated in **(B)**] including the lacunosum moleculare layer of the hippocampus (LMol) and the upper third of the molecular layer of the dentate gyrus (Mol) at 4 weeks and 8 weeks after vehicle or AAV delivery. **(G,H)** In addition, the number of Reelin + cells was quantified in the granule cell layer (GCL) of the dentate gyrus (DG) [including the hilus (h) of the dentate gyrus; annotated in **(B)**]. Data are expressed as mean with standard error of the mean (SEM). Statistical tests: ANOVA followed by Tukey's *post-hoc* test (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$; ns: non-significant). Scale bars = 500 µm.

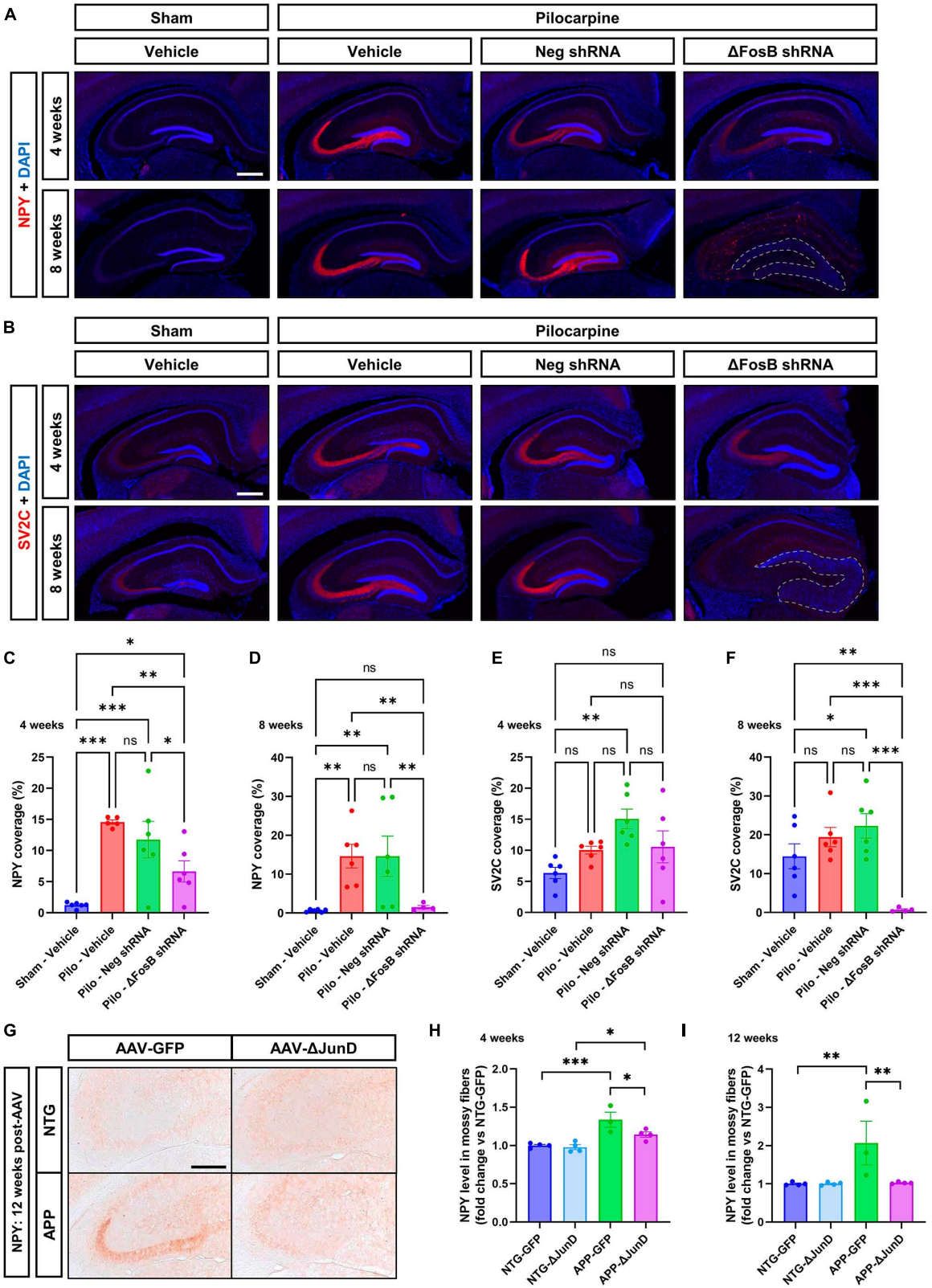


FIGURE 3
ΔFosB inhibition reduces expression of NPY and SV2C in mossy fibers in epileptic brain. **(A)** NPY (neuropeptide Y) and **(B)** SV2C (synaptic vesicle glycoprotein 2C) were used as markers of mossy fibers in the hippocampus. NPY and SV2C immunoreactive signal was quantified in the dorsal part of the hippocampus at **(C,E)** 4 weeks and **(D,F)** 8 weeks after vehicle or AAV delivery. Data are expressed as mean with standard error of the mean (SEM). **(G)** Representative immunostaining of NPY in the dentate gyrus of NTG or APP mice that expressed AAV-GFP or AAV-ΔJunD for 12 weeks. Quantification of NPY expression after **(H)** 4 weeks or **(I)** 12 weeks of AAV expression. Statistical tests: Two-factor ANOVA followed by Two-stage linear step-up procedure of Benjamini, Krieger and Yekutieli **(A–F)** or Benjamini-Hochberg FDR (H-I) *post hoc* testing (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ns: non-significant). Scale bars in **(A,B)** = 500 μm or in **(G)** = 250 μm.

markers, respectively (Becker et al., 2021). Upon vehicle treatment, pilocarpine mice exhibited no significant changes in Iba1 levels at 4 or 8 weeks compared to sham mice (**Figures 4A–D**), while in contrast, GFAP levels were markedly enhanced in the mTLE mouse model at both time points (**Figures 4B–F**). Administration of AAV-ΔFosB shRNA in mTLE mice resulted in a marked increase in Iba1 and GFAP levels notably at 8 weeks in the dorsal hippocampus (compared to AAV-Neg shRNA treatment), suggesting that ΔFosB downregulation exacerbated microglial activation and astrogliosis (**Figures 4A–F**). Further evidence of astrocyte and microglia activation at 4 and 8 weeks following ΔFosB downregulation was suggested by the morphological enlargement of these cells in the hippocampus (High magnification pictures in **Figures 4A, B**).

To determine if ΔFosB is similarly required to suppress immune cell reactivity across conditions with recurrent seizures regardless of etiology, we measured induction of Iba1 and GFAP in APP mice and NTG controls 4 or 12 weeks after infusion with AAV-GFP or AAV-ΔJunD. We found that 4-week expression of AAV-ΔJunD had no effect on Iba1 levels in the dentate gyrus of NTG-ΔJunD or APP-ΔJunD mice compared to respective AAV-GFP controls (**Figures 5A, E**). However, after 12 weeks of ΔFosB blockade, Iba1 levels were robustly increased in NTG-ΔJunD and APP-ΔJunD mice compared to respective AAV-GFP controls (**Figures 5B, F**). Iba1 levels were also significantly higher in APP-ΔJunD than NTG-ΔJunD mice after 12-week ΔFosB blockade (**Figures 5B, F**). GFAP expression did not differ in NTG-ΔJunD or APP-ΔJunD mice compared to respective AAV-GFP controls at 4 weeks post-AAV infusion, although GFAP expression was significantly higher in APP-ΔJunD than NTG-ΔJunD mice after 4-week ΔFosB blockade (**Figures 5C, G**). Similar to Iba1 levels after 12-week ΔFosB blockade, GFAP levels were robustly increased in NTG-ΔJunD and APP-ΔJunD mice compared to respective controls, and GFAP expression was also higher in APP-ΔJunD than NTG-ΔJunD mice (**Figures 5D, H**).

These results indicate that ΔFosB regulates similar functions (e.g., MF adaptive response, neuroinflammation) in brains with recurrent seizures irrespective of seizure etiology.

## A subset of hippocampal ΔFosB target genes in pilocarpine or APP mice are involved in immunity and neuroprotection

To identify novel putative mechanisms by which ΔFosB activity might suppress immune reactivity and pro-inflammatory signaling across conditions with recurrent seizure activity, we performed new Gene Ontology (GO) Biological Process analyses of hippocampal ΔFosB target genes. Using previously published ChIP-seq datasets of hippocampal ΔFosB target genes in pilocarpine and APP mice (Stephens et al., 2020), we performed unfiltered GO Network analyses for respective lists of ΔFosB target genes bound in pilocarpine mice (5880 genes), Vehicle-treated controls (759 genes), APP mice (2839 genes), and NTG controls (1933 genes). GO Networks were then filtered to only include GO Terms (nodes that contain target genes involved in a given process) related to immunity and/or neuroprotection. We found significant enrichment ($p < 0.05$) of GO Terms related to immunity and neuroprotection in both vehicle-treated and pilocarpine mice. However, in pilocarpine mice, enriched GO Terms also included disease-related processes such as responses to amyloid-beta, DNA repair, and calcineurin-mediated signaling (**Figure 6A**). Similar results were obtained when new GO term analyses were performed in APP and NTG mice (**Figure 6B**).

To visualize classes of cellular function by which seizure-induced ΔFosB might suppress neuroinflammation and maintain neuroprotection regardless of seizure etiology, we generated a new GO Network analysis of the GO Terms related to immunity and neuroprotection (filtered as in **Figures 6A, B**) that are enriched by the 442 ΔFosB target genes that are shared by Pilocarpine and APP mice (and are not bound in respective controls). The immune- and neuroprotection-focused GO Network was broadly categorized into five key functional domains: Immune Cell and Cytokine Signaling, Debris and Toxin Clearance, DNA Repair, Cell Death, and Oxidative Stress (**Figure 6C**; gene lists for highlighted GO Terms are in **Table 1**). A full listing of the immunity and neuroprotection-related GO Terms depicted in the GO Network in **Figure 6C** is provided in **Supplementary Table 2**.

# Discussion

In the current study, by using a newly developed specific ΔFosB inhibitor (shRNA) we showed that seizure-induced ΔFosB in the pilocarpine mouse model is part of a homeostatic mechanism that protects the epileptic brain from further deterioration. More specifically, increased ΔFosB activity supports "protective" mossy fiber adaptations, maintains granule cell positioning, and limits neuroinflammatory responses. Furthermore, we recapitulated similar findings (adaptation of mossy fibers and neuroinflammation) in APP mice with recurrent seizures using a previously established inhibitor of ΔFosB activity, a mutant variant of JunD (ΔJunD) that can act in dominant negative fashion. Together, these results demonstrate that ΔFosB exerts critical neuroprotective effects in a seizure etiology-independent manner, indicating that common modes of gene expression can be engaged in distinct neurological disorders accompanied by seizures.

## A balance between neuroprotection and neuroplasticity

We previously demonstrated that seizure-induced ΔFosB accumulation in the DG occurs in both patients and mouse models of Alzheimer's disease, and that the magnitude of ΔFosB expression corresponded with the magnitude of cognitive impairment (Corbett et al., 2017; You et al., 2017). Those results indicated that ΔFosB may drive cognitive decline, particularly because blockade of ΔFosB activity for several weeks improved spatial memory in APP mice. Indeed, we found that ΔFosB bound to a number of plasticity-related genes whose suppression was directly linked to memory deficits, including cFos and calbindin (Corbett et al., 2017; You et al., 2017; Stephens et al., 2020). However, it is notable that for these gene targets, suppression of their expression is not only linked to deficits in hippocampal function, but also with neuroprotective programs that reduce long-term excitotoxicity

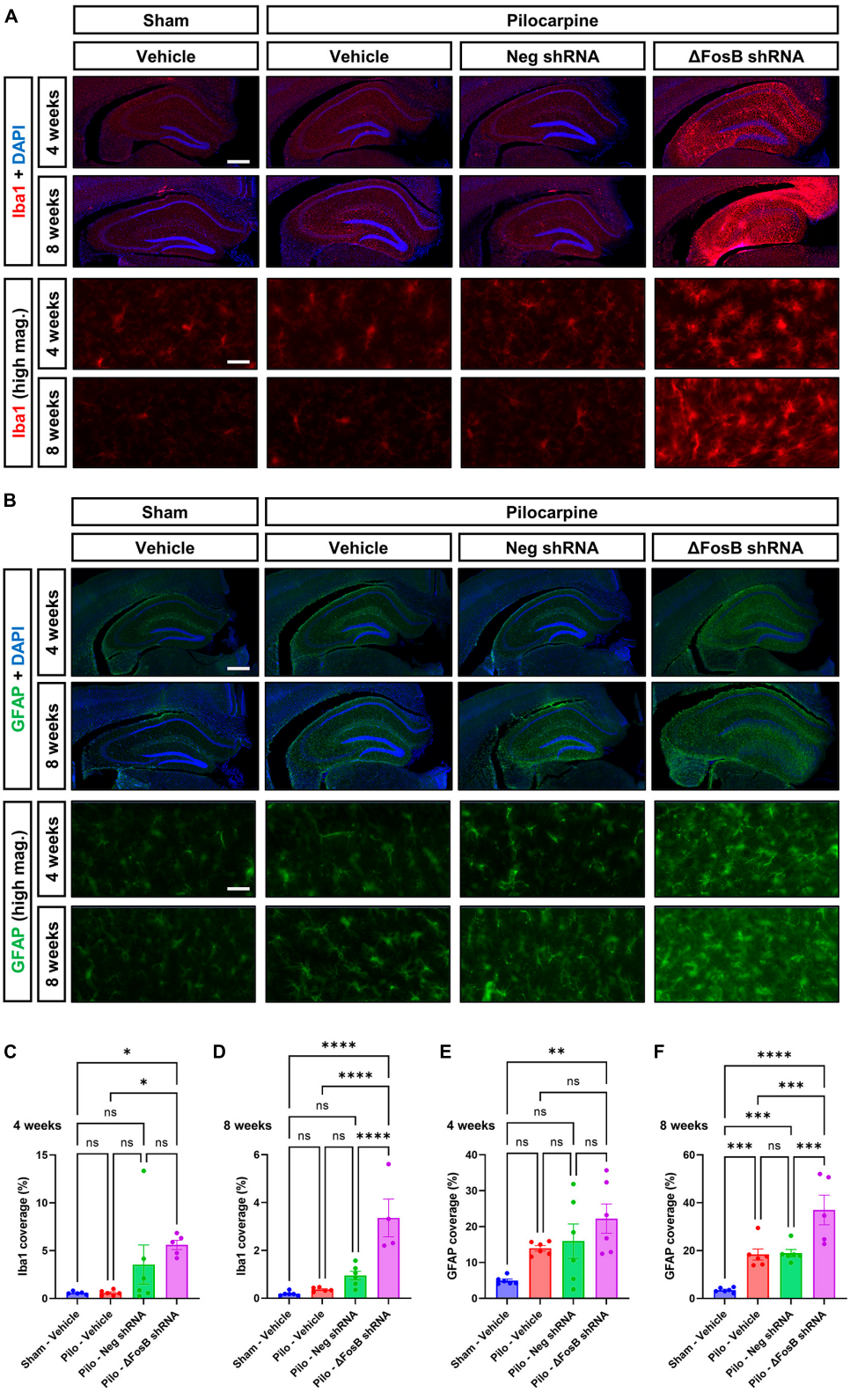


FIGURE 4
Prolonged ΔFosB inhibition exacerbates neuroinflammation in epileptic brain. **(A)** Iba1 (ionized calcium binding adaptor molecule-1) and **(B)** GFAP (glial fibrillary acidic protein) were used as markers of microglia and astrocytes, respectively. High-magnification images (high mag.) in panels **(A,B)** illustrate the morphology of microglia and astrocytes in the hippocampus under the different experimental conditions. Iba1 and GFAP immunoreactive signal was quantified in the dorsal part of the hippocampus at **(C,E)** 4 weeks and **(D,F)** 8 weeks after vehicle or AAV delivery. Data are expressed as mean with standard error of the mean (SEM). Statistical test: ANOVA followed by Two-stage linear step-up procedure of Benjamini, Krieger and Yekutieli *post-hoc* testing (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$; ns: non-significant). Scale bars = 500 μm in low-magnification pictures and 50 μm in high-magnification pictures.

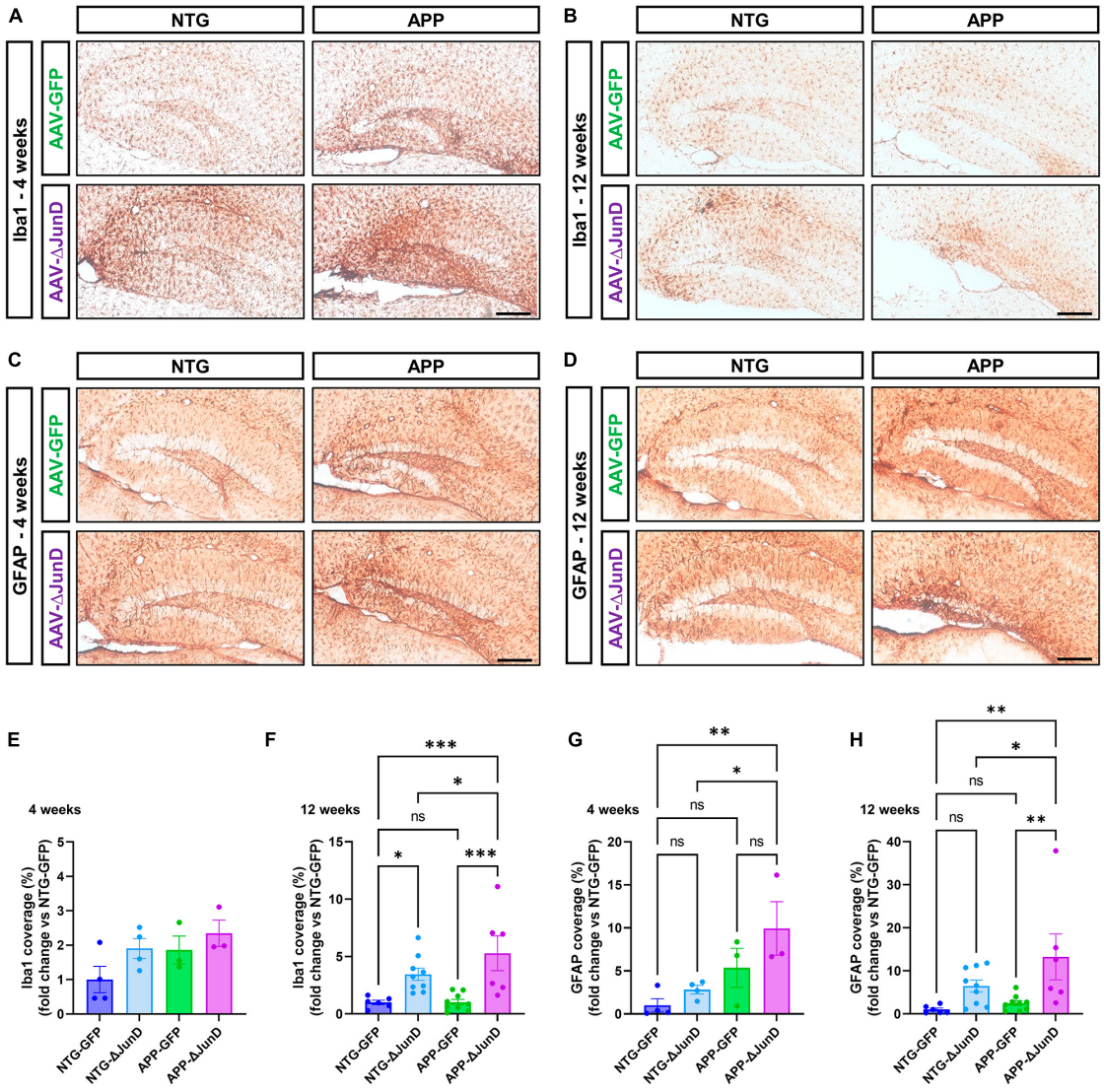


FIGURE 5
Prolonged ΔFosB inhibition exacerbates neuroinflammation in APP mice. **(A,B)** Iba1 and **(C,D)** GFAP were used as markers of microglia and astrocytes, respectively. Iba1 and GFAP immunoreactive signal was quantified in the dentate gyrus of the hippocampus at **(E,G)** 4 weeks and **(F,H)** 12 weeks after AAV delivery. Data are expressed as mean with standard error of the mean (SEM). Statistical tests: Two-factor ANOVA followed by Benjamini-Hochberg FDR *post-hoc* test (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ns: non-significant). Scale bars = 250 μm.

in chronic situations (Molinari et al., 1996; Nagerl et al., 2000; Fleischmann et al., 2003; Palop et al., 2003; Calais et al., 2013), suggesting that the actions of ΔFosB drive neuroprotection in chronic conditions. Indeed, prolonged (>1 month) blockade of ΔFosB in APP mice worsened seizures and memory (unpublished observations), consistent with an overall long-term neuroprotective role for ΔFosB. Our findings therefore suggest that seizure-induced ΔFosB may exert neuroprotection at the cost of limiting plasticity, and highlight possible pathways by which it may do so.

## ΔFosB protein preserves hippocampal architecture in the seizing brain

One of the remarkable observations made in the present study is the hippocampal granule cell dispersion detected in the mouse pilocarpine model of mTLE following down-regulation of ΔFosB. While this phenomenon is part of the histopathological features of patients with intractable TLE (Houser, 1990) and is most prominent in the mouse intrahippocampal kainic acid model of mTLE (Bouilleret et al., 1999; Suzuki et al., 2000), it is rarely described in the rodent pilocarpine model (Jagirdar et al., 2016; Moura et al., 2021). Our results indicate that the downregulation of ΔFosB triggered granule cell dispersion, a morphological rearrangement that is typically not observed in the mouse pilocarpine model, pinpointing a key role for ΔFosB in maintaining the positioning of granule cells within the granule cell layer.

One mechanism by which ΔFosB may exert such strong influence on granule cell position is through epigenetic regulation of *Reln*, the gene that encodes the Reelin protein, which we previously found in a ChIP-seq study to be preferentially bound by ΔFosB in pilocarpine mice (Stephens et al., 2020). Previous

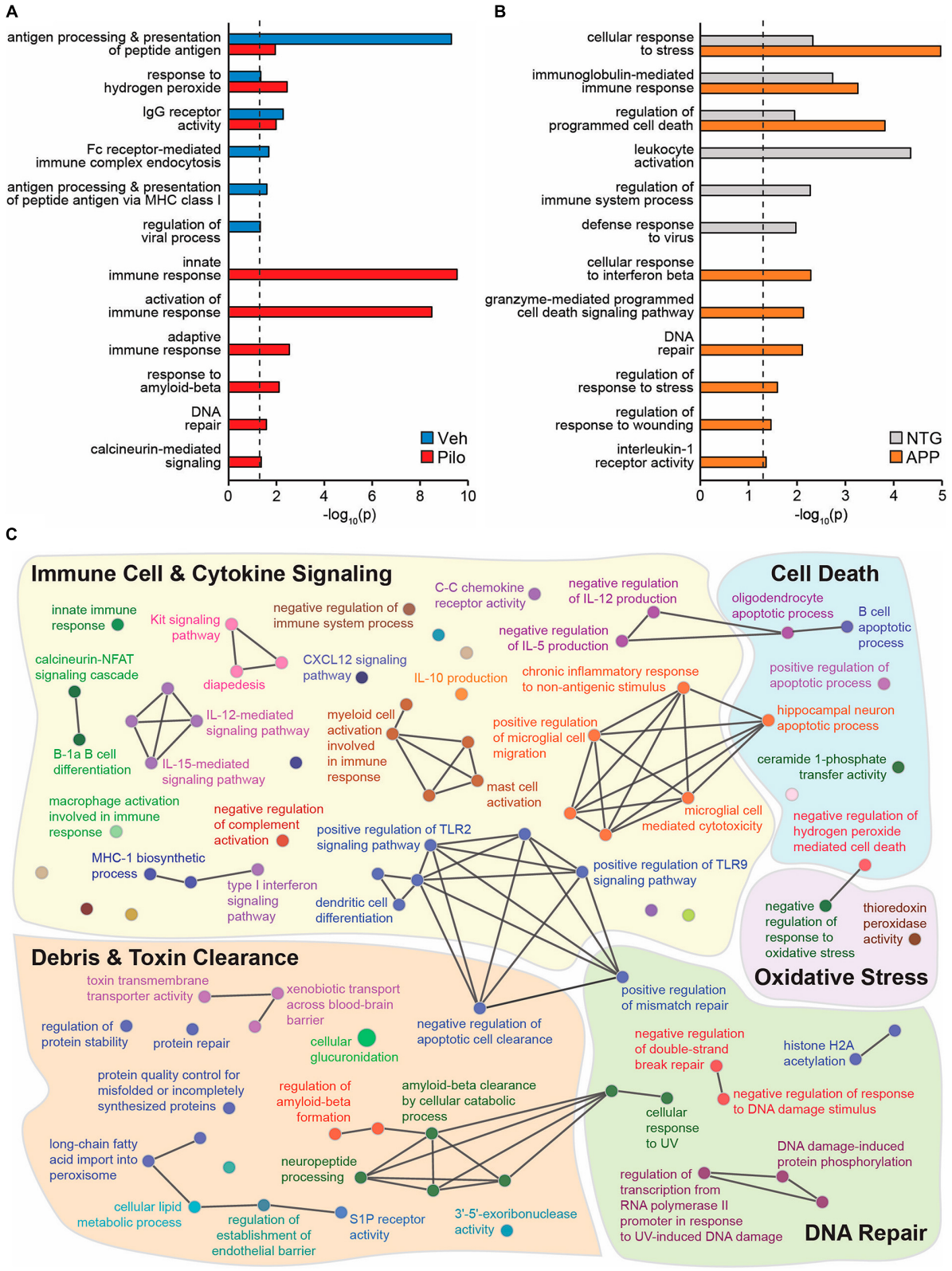


FIGURE 6
Across mouse models with recurrent seizures but not controls, ΔFosB binds to key target genes in the hippocampus involved in immunity and neuroprotection. Hippocampal ΔFosB target genes (Stephens et al., 2020) functionally enrich GO Biological Process Terms related to immunity and/or neuroprotection in **(A)** wildtype mice injected with Vehicle vs. Pilocarpine or in **(B)** NTG vs. APP mice. Enrichment *p*-values for each highlighted GO Term are depicted on the *x*-axis with a $\log_{10}$ scale for ease of comparison. The dashed black line denotes the threshold of significance under two-sided hypergeometric testing with Benjamini-Hochberg FDR = 0.05. **(C)** A GO Biological Process network shows GO Terms related to Immune Cell & Cytokine Signaling (yellow), Cell Death (blue), Oxidative Stress (purple), DNA Repair (green), and Debris & Toxin Clearance (red) that are enriched with the set of 442 hippocampal ΔFosB target genes that are shared between Pilo and APP mice *and not respective control mice*. Nodes indicate individual GO Terms and lines between nodes indicate that connected nodes share genes. Nodes of larger size denote GO Terms enriched at $p = 0.05$ and nodes of smaller size denote GO Terms enriched at $p = 0.5$.

TABLE 1 Gene ontology (GO) biological processes related to immunity and neuroprotection that are enriched in hippocampal ΔFosB target genes in Pilo and APP mice.

| Select gene ontology (GO) terms | | Annotated ΔFosB target genes (of 442 shared by Pilo and APP mice) |
|---|---|---|
| Immune Cell and Cytokine Signaling | Calcineurin-NFAT signaling cascade | *Fhl2, Nfatc1, Rcan1* |
| | Innate immune response | *A2m, Cx3cr1, Defb8, Fer, Hmgb1, Lgr4, Lsm14a, Ly86, Nlrc5, Oxr1, Ppp6c, Rps19, Trim41* |
| | Negative regulation of complement activation | *A2m,* ***Cd46*** |
| | Negative regulation of immune system process | *A2m,* ***Cd46****, Cx3cr1, Cxcl12, Dlg5, Fer,* ***Gal****, Gpr137b, Grem1, Hmgb1,* ***Lrfn5****, Nlrc5,* ***Plcb1****, Rps19, Sox9, Tnfrsf21* |
| | Myeloid cell activation involved in immune response | *Cx3cr1, Fer, Gab2, Hmgb1, Lat, Mrgprb4, Mrgprb5* |
| Debris and Toxin Clearance | Amyloid-beta clearance by cellular catabolic process | *Mme (neprilysin)* |
| | Cellular glucuronidation | *Ugt1a10, Ugt1a6b, Ugt1a7c, Ugt1a9* |
| | Protein quality control for misfolded or incompletely synthesized proteins | *Fbxl17, Ube2w* |
| | Regulation of amyloid-beta formation | *Rtn1, Slc2a13* |
| | Regulation of establishment of endothelial barrier | ***Plcb1****, S1pr2* |
| DNA Repair | Cellular response to UV | *Cdc25a, Chek1, Mme, Usp28* |
| | DNA damage-induced protein phosphorylation | *Chek1* |
| | Histone H2A acetylation | *Epc1, Mbtd1* |
| | Negative regulation of double-strand break repair | *Parpbp, Trip12* |
| | Negative regulation of response to DNA damage stimulus | *Cxcl12, Parpbp, Trip12* |
| Cell Death | B cell apoptotic process | *Slc39a10, Tnfrsf21* |
| | Hippocampal neuron apoptotic process | *Cx3cr1* |
| | Negative regulation of hydrogen peroxide-mediated cell death | *Met* |
| | Oligodendrocyte apoptotic process | *Tnfrsf21* |
| | Positive regulation of apoptotic process | *Bmpr1b,* ***Gal****, Hmgb1, Inhba, Jmy, Melk, Zmat3* |
| Oxidative Stress | Negative regulation of response to oxidative stress | *Met, Oxr1* |
| | Thioredoxin peroxidase activity | *Selenof* |

Bolded gene = implicated in epilepsy (Wang et al., 2017).



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evidence in the literature supports the role of Reelin in granule cell dispersion related to TLE (Leifeld et al., 2022). Reelin is a secreted glycoprotein present in the extracellular matrix that acts as a stop signal for neuronal migration during development (Tissir and Goffinet, 2003) but its function in adult hippocampus is not well studied. Reelin expression decreases after an epileptogenic brain insult and blocking its function in naïve mice promotes granule cell dispersion (Haas et al., 2002; Gong et al., 2007). Furthermore, exogenous supply of Reelin can prevent granule cell dispersion after an epileptogenic brain insult (Haas and Frotscher, 2010). In line with these findings, the granule cell dispersion seen here in the mouse pilocarpine model corresponded with a dramatic decrease in Reelin expression, especially in the areas surrounding the hippocampal fissure including the stratum lacunosum moleculare and the upper third of the molecular layer of the dentate gyrus. The diffuse Reelin immunostaining in the neuropil of these areas support the idea that Reelin may be secreted from terminals of local inhibitory interneurons expressing Reelin or from afferent axon terminals of the perforant pathway that originates from Reelin-expressing neurons of the entorhinal cortex (Pesold et al., 1998). Secreted Reelin may contribute to the formation of neuronal circuits in the adult brain by the use of mechanisms similar to those of embryonic development (Tissir and Goffinet, 2003). Thus, ΔFosB, by maintaining expression of Reelin in the neuropil surrounding the dentate gyrus, might maintain integrity of neuronal circuits by stopping migration of granular cells in a given direction during epileptic conditions.

Notably, although we previously demonstrated that APP mice exhibit reduced Reelin expression in the dentate gyrus (Chin et al., 2007), the magnitude of reduction was less robust than that exhibited in the pilocarpine-treated mice with suppression of ΔFosB in the current study. The subtle reduction in Reelin in APP mice was not associated with granule cell dispersion, which may reflect the observation that the seizures exhibited by APP mice are lower in frequency and severity (1–3 seizures per week, primarily non-convulsive) relative to those induced after pilocarpine induced-SE. These findings support the hypothesis that the magnitude of ΔFosB expression and the neuroprotective pathways engaged in conditions with seizures are calibrated to the level of neuroprotection required.

Another consistent histopathological finding in patients and animal models with TLE is the increased expression of NPY and SV2C in MF (Schmeiser et al., 2017a; Freiman et al., 2021). In our study, NPY and SV2C immunostaining revealed the MF pathway consisting of axons projecting from the granule cell layer of the DG to the CA3 area, several months after pilocarpine induced-SE in mice. Given that NPY inhibits synaptic transmission at mossy fiber synapses on glutamatergic CA3 pyramidal cells (Klapstein and Colmers, 1993), the increased expression of NPY in MF during epileptic conditions may provide an adaptative and protective mechanism against seizure development. This hypothesis is currently used in the field as a basis for exploiting NPY in gene therapy for epilepsy (Cattaneo et al., 2020). Interestingly, we found that downregulation of ΔFosB in the dorsal hippocampus during the chronic phase of epilepsy after pilocarpine-induced SE decreased NPY staining in the dentate gyrus. The concomitant absence of SV2C staining in the same area suggests that MF underwent degeneration. This loss of MF is also observed in mTLE patients and can be driven by neuronal death in CA3 area (Schmeiser et al., 2017b), a phenomenon that we also observe in **Figure 2A** (8 weeks after reduction of ΔFosB). We found a similar attenuation of NPY expression in MF in APP mice in which ΔFosB signaling was blocked, supporting the hypothesis that ΔFosB is required for the protective *de novo* expression of NPY in the MF pathway that occurs in distinct conditions with recurrent seizures. Notably, a decrease in MF density can also be observed in the hippocampus of individuals with schizophrenia and in animal models of schizophrenia, and is believed to contribute to behavioral abnormalities found in the disease (Ibi et al., 2020). The decrease in MF density corresponds with a Reelin deficit in the dentate gyrus of the hippocampus in an animal model of schizophrenia, and both behavioral abnormalities and MF deficits can be rescued by delivery of exogenous Reelin into the dentate gyrus of the hippocampus (Ibi et al., 2020), supporting a role of Reelin in the remodeling of MF during disease progression of neurodevelopmental disorders. It is plausible that a similar pathophysiological mechanism involving a Reelin deficit in the dentate gyrus causes the MF loss that we detect in the epileptic brain following ΔFosB downregulation. This possibility is supported by our present results, in which ΔFosB downregulation induced a striking decrease in Reelin expression in the dentate gyrus of the hippocampus from epileptic mice. Altogether, these results indicate that ΔFosB plays an important role in maintaining the adaptative MF pathway in place under epileptic conditions, perhaps through modulation of the Reelin signaling pathway.

## ΔFosB protein attenuates neuroinflammation in the seizing brain

In this study, consistent with previous reports (Mazzuferi et al., 2012; Clasadonte et al., 2013; Wang et al., 2019), a robust neuroinflammation, characterized by astrocytic activation, was detected in the hippocampus of pilocarpine treated mice. Strikingly, downregulation or inhibition of ΔFosB led to an increase in astrocyte and microglia activation in seizing mice regardless of seizure etiology. From our experimental design it is difficult to address whether increased inflammation precedes (directly related to ΔFosB activity) or is secondary to the dramatic changes in hippocampal cytoarchitecture. In a focal mouse model of mTLE, increased astrogliosis or microgliosis precede granule cell dispersion (Pernot et al., 2011). Furthermore, more progressive granule cell dispersion correlates with increased GFAP-positive fiber density (Heinrich et al., 2006), which is in alignment with findings in mTLE patients (Fahrner et al., 2007). Results from our bioinformatic analysis suggest that ΔFosB via its downstream targets like Cxcl12 (Stephens et al., 2020), a chemokine with confirmed role in epilepsy (Song et al., 2016; Zhou et al., 2017; Xu et al., 2019) could suppress further worsening of neuroinflammation in epileptic brain but we cannot rule out a possibility that increased gliosis is a consequence of mossy fiber degeneration or granule cell dispersion or combination of these processes.

Based on data acquired from pilocarpine mouse models and knowledge from the literature, we propose a molecular model in which ΔFosB directly (Stephens et al., 2020), or indirectly through multiple pathways (McClung and Nestler, 2003; You et al., 2018;

Lardner et al., 2021), regulates expression of Reelin. This regulation, in turn, sustains the protective actions of mossy fibers and maintains granule cells in their correct position in the epileptic brain. In conclusion, our study indicates that ΔFosB protects the brain from further deterioration during seizures, regardless of seizure etiology. Moreover, we have developed a novel ΔFosB-specific inhibitor that can be utilized by the broader scientific community.

## Data availability statement

Publicly available datasets were analyzed in this study. This data can be found here: https://www.ebi.ac.uk/biostudies/arrayexpress/studies/E-MTAB-8954.

## Ethics statement

The animal study was approved by the Ethical Committee of the High technology Animal Platform, University Grenoble Alpes (experiments at SynapCell); local Ethics Committee/according to Belgian law (experiments at UCB Biopharma SRL); Institutional Animal Care and Use Committee of Baylor College of Medicine (experiments at Baylor College of Medicine). The study was conducted in accordance with the local legislation and institutional requirements.

## Author contributions

JCl: Formal analysis, Investigation, Methodology, Project administration, Visualization, Writing – original draft, Writing – review and editing. TD: Investigation, Methodology, Writing – review and editing. GS: Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review and editing. GM-C: Formal analysis, Investigation, Methodology, Project administration, Visualization, Writing – original draft, Writing – review and editing. P-YC: Investigation, Writing – review and editing. MB: Investigation, Methodology, Writing – review and editing. AF: Investigation, Writing – review and editing. JCh: Conceptualization, Funding acquisition, Project administration, Supervision, Writing – original draft, Writing – review and editing. MR: Conceptualization, Formal analysis, Project administration, Supervision, Writing – original draft, Writing – review and editing.

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## Conflict of interest

JCl, GM-C, TD, P-YC, MB, AF, and MR during conducting of the study were employed by UCB Biopharma SRL.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note



## Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnmol.2023.1324922/full#supplementary-material

SUPPLEMENTARY TABLE 1
List of primary and secondary antibodies used for immunohistochemistry.

SUPPLEMENTARY TABLE 2
Full listing of GO Terms represented in network nodes in **Figure 6C**.

SUPPLEMENTARY FIGURE 1
Development of specific ΔFosB inhibitor. **(A)** Selection of ΔFosB and FosB transcripts together with sequences that are targeted by developed shRNA (highlighted in brown and red). As shown, only ΔFosB mRNA can be targeted by developed shRNA. Relative gene expression analysis by qPCR of **(B)** ΔFosB and **(C)** FosB transcripts, 4 weeks after AAV-Neg shRNA or AAV-ΔFosB shRNA were injected in the dorsal hippocampus. Statistical test: ANOVA followed by Tukey's *post-hoc* test (*$p < 0.05$; **$p < 0.01$; ns: non-significant; $n = 6–7$).

SUPPLEMENTARY FIGURE 2
Measuring convulsive seizures in pilocarpine mouse model **(A)** Average number of seizures per day in epileptic mice 2–4 weeks (**left** panel) and from 6 to 8 weeks (**right** panel) after injection of vehicle, AAV-Neg shRNA or AAV-ΔFosB shRNA in the dorsal hippocampus. **Left** panel: $n = 6$ animals per group; non-parametric one-way ANOVA (Kruskal-Wallis test); $p > 0.05$. **Right** panel: $n = 5–6$ animals per group; non-parametric one-way ANOVA (Kruskal-Wallis test); $p > 0.05$). **(B)** Average duration of seizures in epileptic mice 2–4 weeks (**left** panel) and 6–8 weeks (**right** panel) after injection of vehicle, AAV-Neg shRNA or AAV-ΔFosB shRNA in the dorsal hippocampus. **Left** panel: $n = 6$ animals per group; non-parametric one-way ANOVA (Kruskal-Wallis test); $p > 0.05$. **Right** panel: $n = 5–6$ animals per group; non-parametric one-way ANOVA (Kruskal-Wallis test); $p > 0.05$).

**(C)** Proportion of seizures of stage 3, 4, and 5 in epileptic mice 2–4 weeks (**left** panel) and 6–8 weeks (**right** panel) after injection of vehicle, AAV-Neg shRNA or AAV-ΔFosB shRNA in the dorsal hippocampus. **Left** panel: 105 seizures from 6 animals for vehicle group, 71 seizures from 6 animals for AAV-Neg shRNA group, 229 seizures from 6 animals for AAV-ΔFosB shRNA group, chi-square contingency test; $p > 0.05$. **Right** panel: 119 seizures from 6 animals for vehicle group, 171 seizures from 6 animals for AAV-Neg shRNA group, 116 seizures from 5 animals for AAV-ΔFosB shRNA group, chi-square contingency test; $p > 0.05$. Pooled data are shown as mean ± SEM. **(D)** Examples of ΔFosB protein analyses by western blotting from pilocarpine treated mice injected with AAV-Neg shRNA or AAV-ΔFosB shRNA showing ΔFosB protein decrease at 4 and 8 weeks after AAV treatment.

SUPPLEMENTARY FIGURE 3
Immunohistochemistry for Prox1 and c-Fos in the hippocampus of mTLE mouse model. **(A)** Prox1 is primarily expressed in the granule cells of the dentate gyrus and was used to confirm the cellular phenotype of the cells that are dispersed in the hippocampus of the pilocarpine treated mice at 8 weeks. The morphology of the granular layer of the dentate gyrus was markedly affected at 8 weeks with the ΔFosB shRNA. **(B)** c-Fos was used as a marker of neuronal activity in the hippocampus and immunoreactive signal was quantified in the dorsal part of the hippocampus at **(C)** 4 weeks after vehicle or AAV delivery. Data are expressed as mean with standard error of the mean (SEM). Statistical test: ANOVA followed by Tukey's *post-hoc* test (*$p < 0.05$; **$p < 0.01$; ****$p < 0.0001$; ns: non-significant). Scale bars = 500 μ m.

# References


Albert, M., Mairet-Coello, G., Danis, C., Lieger, S., Caillierez, R., Carrier, S., et al. (2019). Prevention of tau seeding and propagation by immunotherapy with a central tau epitope antibody. *Brain* 142, 1736–1750.

Bartolomei, F., Khalil, M., Wendling, F., Sontheimer, A., Regis, J., Ranjeva, J. P., et al. (2005). Entorhinal cortex involvement in human mesial temporal lobe epilepsy: An electrophysiologic and volumetric study. *Epilepsia* 46, 677–687.

Becker, G., Michel, A., Bahri, M. A., Mairet-Coello, G., Lemaire, C., Deprez, T., et al. (2021). Monitoring of a progressive functional dopaminergic deficit in the A53T-AAV synuclein rats by combining 6-[(18)F]fluoro-L-m-tyrosine imaging and motor performances analysis. *Neurobiol. Aging* 107, 142–152.

Benjamini, Y., and Hochberg, Y. (1995). Controlling the false discovery rate: A practical and powerful approach to multiple testing. *J. R. Stat. Soc.* 57, 289–300.

Bindea, G., Mlecnik, B., Hackl, H., Charoentong, P., Tosolini, M., Kirilovsky, A., et al. (2009). ClueGO: A Cytoscape plug-in to decipher functionally grouped gene ontology and pathway annotation networks. *Bioinformatics* 25, 1091–1093.

Bouilleret, V., Ridoux, V., Depaulis, A., Marescaux, C., Nehlig, A., and Le Gal La Salle, G. (1999). Recurrent seizures and hippocampal sclerosis following intrahippocampal kainate injection in adult mice: Electroencephalography, histopathology and synaptic reorganization similar to mesial temporal lobe epilepsy. *Neuroscience* 89, 717–729.

Brown, P. H., Kim, S. H., Wise, S. C., Sabichi, A. L., and Birrer, M. J. (1996). Dominant-negative mutants of cJun inhibit AP-1 activity through multiple mechanisms and with different potencies. *Cell Growth Differ.* 7, 1013–1021.

Calais, J. B., Valvassori, S. S., Resende, W. R., Feier, G., Athie, M. C., Ribeiro, S., et al. (2013). Long-term decrease in immediate early gene expression after electroconvulsive seizures. *J. Neural Transm.* 120, 259–266.

Cattaneo, S., Verlengia, G., Marino, P., Simonato, M., and Bettegazzi, B. (2020). NPY and gene therapy for epilepsy: How, When,. and Y. *Front. Mol. Neurosci.* 13:608001. doi: 10.3389/fnmol.2020.608001

Chen, J., Kelz, M. B., Hope, B. T., Nakabeppu, Y., and Nestler, E. J. (1997). Chronic Fos-related antigens: Stable variants of deltaFosB induced in brain by chronic treatments. *J. Neurosci.* 17, 4933–4941.

Chin, J., Massaro, C. M., Palop, J. J., Thwin, M. T., Yu, G. Q., Bien-Ly, N., et al. (2007). Reelin depletion in the entorhinal cortex of human amyloid precursor protein transgenic mice and humans with Alzheimer's disease. *J. Neurosci.* 27, 2727–2733.

Choi, S. (2017). *Encyclopedia of Signaling Molecules*. New York, NY: Springer.

Clasadonte, J., Dong, J., Hines, D. J., and Haydon, P. G. (2013). Astrocyte control of synaptic NMDA receptors contributes to the progressive development of temporal lobe epilepsy. *Proc. Natl. Acad. Sci. U. S. A.* 110, 17540–17545.

Corbett, B. F., You, J. C., Zhang, X., Pyfer, M. S., Tosi, U., Iascone, D. M., et al. (2017). DeltaFosB regulates gene expression and cognitive dysfunction in a mouse model of Alzheimer's Disease. *Cell Rep.* 20, 344–355.

Crevecoeur, J., Kaminski, R. M., Rogister, B., Foerch, P., Vandenplas, C., Neveux, M., et al. (2014). Expression pattern of synaptic vesicle protein 2 (SV2) isoforms in patients with temporal lobe epilepsy and hippocampal sclerosis. *Neuropathol. Appl. Neurobiol.* 40, 191–204.

Eagle, A. L., Gajewski, P. A., Yang, M., Kechner, M. E., Al Masraf, B. S., Kennedy, P. J., et al. (2015). Experience-dependent induction of hippocampal DeltaFosB controls learning. *J. Neurosci.* 35, 13773–13783.

Eagle, A. L., Williams, E. S., Beatty, J. A., Cox, C. L., and Robison, A. J. (2018). DeltaFosB decreases excitability of dorsal hippocampal CA1 neurons. *eNeuro* 5:ENEURO.0104-18.2018.

Fahrner, A., Kann, G., Flubacher, A., Heinrich, C., Freiman, T. M., Zentner, J., et al. (2007). Granule cell dispersion is not accompanied by enhanced neurogenesis in temporal lobe epilepsy patients. *Exp. Neurol.* 203, 320–332.

Fisher, R. S., Acevedo, C., Arzimanoglou, A., Bogacz, A., Cross, J. H., Elger, C. E., et al. (2014). ILAE official report: A practical clinical definition of epilepsy. *Epilepsia* 55, 475–482.

Fisher, R. S., Cross, J. H., French, J. A., Higurashi, N., Hirsch, E., Jansen, F. E., et al. (2017). Operational classification of seizure types by the International League Against Epilepsy: Position Paper of the ILAE commission for classification and terminology. *Epilepsia* 58, 522–530.

Fleischmann, A., Hvalby, O., Jensen, V., Strekalova, T., Zacher, C., Layer, L. E., et al. (2003). Impaired long-term memory and NR2A-type NMDA receptor-dependent synaptic plasticity in mice lacking c-Fos in the CNS. *J. Neurosci.* 23, 9116–9122.

Freiman, T. M., Haussler, U., Zentner, J., Doostkam, S., Beck, J., Scheiwe, C., et al. (2021). Mossy fiber sprouting into the hippocampal region CA2 in patients with temporal lobe epilepsy. *Hippocampus* 31, 580–592.

Fu, C. H., Iascone, D. M., Petrof, I., Hazra, A., Zhang, X., Pyfer, M. S., et al. (2019). Early seizure activity accelerates depletion of hippocampal neural stem cells and impairs spatial discrimination in an Alzheimer's Disease Model. *Cell Rep.* 27:e4.

GBD 2016 Neurology Collaborators. (2019). Global, regional, and national burden of neurological disorders, 1990-2016: A systematic analysis for the Global Burden of Disease Study 2016. *Lancet Neurol.* 18, 459–480.

Gong, C., Wang, T. W., Huang, H. S., and Parent, J. M. (2007). Reelin regulates neuronal progenitor migration in intact and epileptic hippocampus. *J. Neurosci.* 27, 1803–1811.

Haas, C. A., Dudeck, O., Kirsch, M., Huszka, C., Kann, G., Pollak, S., et al. (2002). Role for reelin in the development of granule cell dispersion in temporal lobe epilepsy. *J. Neurosci.* 22, 5797–5802.

Haas, C. A., and Frotscher, M. (2010). Reelin deficiency causes granule cell dispersion in epilepsy. *Exp. Brain Res.* 200, 141–149.

Heinrich, C., Nitta, N., Flubacher, A., Muller, M., Fahrner, A., Kirsch, M., et al. (2006). Reelin deficiency and displacement of mature neurons, but not neurogenesis, underlie the formation of granule cell dispersion in the epileptic hippocampus. *J. Neurosci.* 26, 4701–4713.

Hermann, B., and Seidenberg, M. (2007). Epilepsy and cognition. *Epilepsy Curr.* 7, 1–6.

Hirota, Y., and Nakajima, K. (2017). Control of neuronal migration and aggregation by reelin signaling in the developing cerebral cortex. *Front. Cell Dev. Biol.* 5:40. doi: 10.3389/fcell.2017.00040

Hope, B. T., Kelz, M. B., Duman, R. S., and Nestler, E. J. (1994). Chronic electroconvulsive seizure (ECS) treatment results in expression of a long-lasting AP-1 complex in brain with altered composition and characteristics. *J. Neurosci.* 14, 4318–4328.

Houser, C. R. (1990). Granule cell dispersion in the dentate gyrus of humans with temporal lobe epilepsy. *Brain Res.* 535, 195–204.

Ibi, D., Nakasai, G., Koide, N., Sawahata, M., Kohno, T., Takaba, R., et al. (2020). Reelin supplementation into the hippocampus rescues abnormal behavior in a mouse model of neurodevelopmental disorders. *Front. Cell Neurosci.* 14:285. doi: 10.3389/fncel.2020.00285

Jagirdar, R., Drexel, M., Bukovac, A., Tasan, R. O., and Sperk, G. (2016). Expression of class II histone deacetylases in two mouse models of temporal lobe epilepsy. *J. Neurochem.* 136, 717–730.

Kandratavicius, L., Peixoto-Santos, J. E., Monteiro, M. R., Scandiuzzi, R. C., Carlotti, C. G., Assirati, J. A. Jr., et al. (2015). Mesial temporal lobe epilepsy

with psychiatric comorbidities: A place for differential neuroinflammatory interplay. *J. Neuroinflammat.* 12:38.

Kinney, J. W., Bemiller, S. M., Murtishaw, A. S., Leisgang, A. M., Salazar, A. M., and Lamb, B. T. (2018). Inflammation as a central mechanism in Alzheimer's disease. *Alzheimers Dement.* 4, 575–590.

Klapstein, G. J., and Colmers, W. F. (1993). On the sites of presynaptic inhibition by neuropeptide Y in rat hippocampus in vitro. *Hippocampus* 3, 103–111.

Knowles, J. K., Xu, H., Soane, C., Batra, A., Saucedo, T., Frost, E., et al. (2022). Maladaptive myelination promotes generalized epilepsy progression. *Nat. Neurosci.* 25, 596–606.

Lardner, C. K., van der Zee, Y., Estill, M. S., Kronman, H. G., Salery, M., Cunningham, A. M., et al. (2021). Gene-Targeted, CREB-Mediated Induction of DeltaFosB controls distinct downstream transcriptional patterns within D1 and D2 medium spiny neurons. *Biol. Psychiatry* 90, 540–549.

Leifeld, J., Forster, E., Reiss, G., and Hamad, M. I. K. (2022). Considering the role of extracellular matrix molecules, in particular reelin, in granule cell dispersion related to temporal lobe epilepsy. *Front. Cell Dev. Biol.* 10:917575. doi: 10.3389/fcell.2022.917575

Loscher, W., and Schmidt, D. (2011). Modern antiepileptic drug development has failed to deliver: Ways out of the current dilemma. *Epilepsia* 52, 657–678.

Mazzuferi, M., Kumar, G., Rospo, C., and Kaminski, R. M. (2012). Rapid epileptogenesis in the mouse pilocarpine model: Video-EEG, pharmacokinetic and histopathological characterization. *Exp. Neurol.* 238, 156–167.

McClung, C. A., and Nestler, E. J. (2003). Regulation of gene expression and cocaine reward by CREB and DeltaFosB. *Nat. Neurosci.* 6, 1208–1215.

Molinari, S., Battini, R., Ferrari, S., Pozzi, L., Killcross, A. S., Robbins, T. W., et al. (1996). Deficits in memory and hippocampal long-term potentiation in mice with reduced calbindin D28K expression. *Proc. Natl. Acad. Sci. U. S. A.* 93, 8028–8033.

Moura, D. M. S., de Sales, I. R. P., Brandao, J. A., Costa, M. R., and Queiroz, C. M. (2021). Disentangling chemical and electrical effects of status epilepticus-induced dentate gyrus abnormalities. *Epilepsy Behav.* 121:106575.

Mucke, L., Masliah, E., Yu, G. Q., Mallory, M., Rockenstein, E. M., Tatsuno, G., et al. (2000). High-level neuronal expression of Aβ1-42 in wild-type human amyloid protein precursor transgenic mice: Synaptotoxicity without plaque formation. *J. Neurosci.* 20, 4050–4058.

Nadler, J. V., Tu, B., Timofeeva, O., Jiao, Y., and Herzog, H. (2007). Neuropeptide Y in the recurrent mossy fiber pathway. *Peptides* 28, 357–364.

Nagerl, U. V., Mody, I., Jeub, M., Lie, A. A., Elger, C. E., and Beck, H. (2000). Surviving granule cells of the sclerotic human hippocampus have reduced Ca(2+) influx because of a loss of calbindin-D(28k) in temporal lobe epilepsy. *J. Neurosci.* 20, 1831–1836.

Palop, J. J., Chin, J., Roberson, E. D., Wang, J., Thwin, M. T., Bien-Ly, N., et al. (2007). Aberrant excitatory neuronal activity and compensatory remodeling of inhibitory hippocampal circuits in mouse models of Alzheimer's disease. *Neuron* 55, 697–711.

Palop, J. J., Jones, B., Kekonius, L., Chin, J., Yu, G. Q., Raber, J., et al. (2003). Neuronal depletion of calcium-dependent proteins in the dentate gyrus is tightly linked to Alzheimer's disease-related cognitive deficits. *Proc. Natl. Acad. Sci. U. S. A.* 100, 9572–9577.

Paxinos, G., and Franklin, K. B. J. (2019). *Paxinos and Franklin's The Mouse Brain in Stereotaxic Coordinates*. London: Academic Press.

Pernot, F., Heinrich, C., Barbier, L., Peinnequin, A., Carpentier, P., Dhote, F., et al. (2011). Inflammatory changes during epileptogenesis and spontaneous seizures in a mouse model of mesiotemporal lobe epilepsy. *Epilepsia* 52, 2315–2325.

Pesold, C., Impagnatiello, F., Pisu, M. G., Uzunov, D. P., Costa, E., Guidotti, A., et al. (1998). Reelin is preferentially expressed in neurons synthesizing gamma-aminobutyric acid in cortex and hippocampus of adult rats. *Proc. Natl. Acad. Sci. U. S. A.* 95, 3221–3226.

Racine, R. J. (1972). Modification of seizure activity by electrical stimulation. II. Motor seizure. *Electroencephalogr. Clin. Neurophysiol.* 32, 281–294.

Roberson, E. D., Halabisky, B., Yoo, J. W., Yao, J., Chin, J., Yan, F., et al. (2011). Amyloid-beta/Fyn-induced synaptic, network, and cognitive impairments depend on tau levels in multiple mouse models of Alzheimer's disease. *J. Neurosci.* 31, 700–711.

Robison, A. J., and Nestler, E. J. (2011). Transcriptional and epigenetic mechanisms of addiction. *Nat. Rev. Neurosci.* 12, 623–637.

Robison, A. J., Vialou, V., Mazei-Robison, M., Feng, J., Kourrich, S., Collins, M., et al. (2013). Behavioral and structural responses to chronic cocaine require a feedforward loop involving DeltaFosB and calcium/calmodulin-dependent protein kinase II in the nucleus accumbens shell. *J. Neurosci.* 33, 4295–4307.

Rogers, J. T., Rusiana, I., Trotter, J., Zhao, L., Donaldson, E., Pak, D. T., et al. (2011). Reelin supplementation enhances cognitive ability, synaptic plasticity, and dendritic spine density. *Learn. Mem.* 18, 558–564.

Sanchez-Varo, R., Trujillo-Estrada, L., Sanchez-Mejias, E., Torres, M., Baglietto-Vargas, D., Moreno-Gonzalez, I., et al. (2012). Abnormal accumulation of autophagic vesicles correlates with axonal and synaptic pathology in young Alzheimer's mice hippocampus. *Acta Neuropathol.* 123, 53–70.

Schmeiser, B., Li, J., Brandt, A., Zentner, J., Doostkam, S., and Freiman, T. M. (2017a). Different mossy fiber sprouting patterns in ILAE hippocampal sclerosis types. *Epilepsy Res.* 136, 115–122.

Schmeiser, B., Zentner, J., Prinz, M., Brandt, A., and Freiman, T. M. (2017b). Extent of mossy fiber sprouting in patients with mesiotemporal lobe epilepsy correlates with neuronal cell loss and granule cell dispersion. *Epilepsy Res.* 129, 51–58.

Shannon, P., Markiel, A., Ozier, O., Baliga, N. S., Wang, J. T., Ramage, D., et al. (2003). Cytoscape: A software environment for integrated models of biomolecular interaction networks. *Genome Res.* 13, 2498–2504.

Sharma, S., Tiarks, G., Haight, J., and Bassuk, A. G. (2021). Neuropathophysiological mechanisms and treatment strategies for post-traumatic epilepsy. *Front. Mol. Neurosci.* 14:612073. doi: 10.3389/fnmol.2021.612073

Song, C., Xu, W., Zhang, X., Wang, S., Zhu, G., Xiao, T., et al. (2016). CXCR4 Antagonist AMD3100 suppresses the long-term abnormal structural changes of newborn neurons in the intraventricular kainic acid model of epilepsy. *Mol. Neurobiol.* 53, 1518–1532.

Srivastava, P. K., van Eyll, J., Godard, P., Mazzuferi, M., Delahaye-Duriez, A., Van Steenwinckel, J., et al. (2018). A systems-level framework for drug discovery identifies Csf1R as an anti-epileptic drug target. *Nat. Commun.* 9:3561.

Staley, K. (2015). Molecular mechanisms of epilepsy. *Nat. Neurosci.* 18, 367–372.

Stephens, G. S., Fu, C. H., St Romain, C. P., Zheng, Y., Botterill, J. J., Scharfman, H. E., et al. (2020). Genes bound by DeltaFosB in different conditions with recurrent seizures regulate similar neuronal functions. *Front. Neurosci.* 14:472. doi: 10.3389/fnins.2020.00472

Suzuki, F., Hirai, H., Onteniente, B., Riban, V., Matsuda, M., and Kurokawa, K. (2000). Long-term increase of GluR2 alpha-amino-3-hydroxy-5-methylisoxazole-4-propionate receptor subunit in the dispersed dentate gyrus after intrahippocampal kainate injection in the mouse. *Neuroscience* 101, 41–50.

Tissir, F., and Goffinet, A. M. (2003). Reelin and brain development. *Nat. Rev. Neurosci.* 4, 496–505.

Verret, L., Mann, E. O., Hang, G. B., Barth, A. M., Cobos, I., Ho, K., et al. (2012). Inhibitory interneuron deficit links altered network activity and cognitive dysfunction in Alzheimer model. *Cell* 149, 708–721.

Vezzani, A., Balosso, S., and Ravizza, T. (2019). Neuroinflammatory pathways as treatment targets and biomarkers in epilepsy. *Nat. Rev. Neurol.* 15, 459–472.

Wang, J., Lin, Z. J., Liu, L., Xu, H. Q., Shi, Y. W., Yi, Y. H., et al. (2017). Epilepsy-associated genes. *Seizure* 44, 11–20.

Wang, Z., Zhou, L., An, D., Xu, W., Wu, C., Sha, S., et al. (2019). Author Correction: TRPV4-induced inflammatory response is involved in neuronal death in pilocarpine model of temporal lobe epilepsy in mice. *Cell Death Dis.* 10:491.

Winstanley, C. A., LaPlant, Q., Theobald, D. E., Green, T. A., Bachtell, R. K., Perrotti, L. I., et al. (2007). DeltaFosB induction in orbitofrontal cortex mediates tolerance to cocaine-induced cognitive dysfunction. *J. Neurosci.* 27, 10497–10507.

Wu, Z., Nicoll, M., and Ingham, R. J. (2021). AP-1 family transcription factors: A diverse family of proteins that regulate varied cellular activities in classical hodgkin lymphoma and ALK+ ALCL. *Exp. Hematol. Oncol.* 10:4.

Xu, T., Yu, X., Deng, J., Ou, S., Liu, X., Wang, T., et al. (2019). CXCR7 regulates epileptic seizures by controlling the synaptic activity of hippocampal granule cells. *Cell Death Dis.* 10:825.

You, J. C., Muralidharan, K., Park, J. W., Petrof, I., Pyfer, M. S., Corbett, B. F., et al. (2017). Epigenetic suppression of hippocampal calbindin-D28k by DeltaFosB drives seizure-related cognitive deficits. *Nat. Med.* 23, 1377–1383.

You, J. C., Stephens, G. S., Fu, C. H., Zhang, X., Liu, Y., and Chin, J. (2018). Genome-wide profiling reveals functional diversification of ΔFosB gene targets in the hippocampus of an Alzheimer's disease mouse model. *PLoS One* 13:e0192508. doi: 10.1371/journal.pone.0192508

Zachariou, V., Bolanos, C. A., Selley, D. E., Theobald, D., Cassidy, M. P., Kelz, M. B., et al. (2006). An essential role for DeltaFosB in the nucleus accumbens in morphine action. *Nat. Neurosci.* 9, 205–211.

Zhou, Z., Liu, T., Sun, X., Mu, X., Zhu, G., Xiao, T., et al. (2017). CXCR4 antagonist AMD3100 reverses the neurogenesis promoted by enriched environment and suppresses long-term seizure activity in adult rats of temporal lobe epilepsy. *Behav. Brain Res.* 322, 83–91.

Zhu, S., Wang, J., Zhang, Y., He, J., Kong, J., Wang, J. F., et al. (2017). The role of neuroinflammation and amyloid in cognitive impairment in an APP/PS1 transgenic mouse model of Alzheimer's disease. *CNS Neurosci. Ther.* 23, 310–320.

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# Latest advances in mechanisms of epileptic activity in Alzheimer's disease and dementia with Lewy Bodies

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Alzheimer's disease (AD) and dementia with Lewy bodies (DLB) stand as the prevailing sources of neurodegenerative dementia, impacting over 55 million individuals across the globe. Patients with AD and DLB exhibit a higher prevalence of epileptic activity compared to those with other forms of dementia. Seizures can accompany AD and DLB in early stages, and the associated epileptic activity can contribute to cognitive symptoms and exacerbate cognitive decline. Aberrant neuronal activity in AD and DLB may be caused by several mechanisms that are not yet understood. Hyperexcitability could be a biomarker for early detection of AD or DLB before the onset of dementia. In this review, we compare and contrast mechanisms of network hyperexcitability in AD and DLB. We examine the contributions of genetic risk factors, $Ca^{2+}$ dysregulation, glutamate, AMPA and NMDA receptors, mTOR, pathological amyloid beta, tau and α-synuclein, altered microglial and astrocytic activity, and impaired inhibitory interneuron function. By gaining a deeper understanding of the molecular mechanisms that cause neuronal hyperexcitability, we might uncover therapeutic approaches to effectively ease symptoms and slow down the advancement of AD and DLB.

KEYWORDS

**Alzheimer's disease, dementia with Lewy bodies, epilepsy, network hyperexcitability, epileptic activity**

## Introduction

Hyperexcitability can be defined as an increased likelihood of firing at the level of the neuron from certain stimuli and/or due to decreased firing thresholds (1). This heightened excitability can clinically present itself as epilepsy. As per the official definition by the International League Against Epilepsy (2), "Epilepsy is characterized by repeated spontaneous bursts of neuronal hyperactivity and high synchronization in the brain." Epilepsy has emerged as a significant global health issue, impacting approximately 70 million individuals worldwide (3–6). Hyperactivity occurs in neuronal populations or brain regions when the frequency of activity is above normal rates. Brain activity is normally regulated with precise timing and regional specificity, however, high synchronization or hypersynchrony denotes an increase in neuronal coordination and cellular firing (7, 8). While epilepsy can manifest in any stage of life, it is notably more common among individuals aged 65 years and older, reaching a prevalence of 5.7% in the Cardiovascular Health Study (9). Increasingly, there is a growing recognition that late-onset epilepsy, starting after age 55, is often not an isolated condition but

is frequently linked to neurodegenerative diseases like Alzheimer's disease (AD) and dementia with Lewy bodies (DLB) (10–12).

AD constitutes 60–70% of all dementia cases and is characterized by a gradual decline in memory and other cognitive functions. At present, there are more than 57 million people globally living with dementia, and this figure is predicted to double every two decades, reaching 74.7 million by 2030 (Alzheimer's Disease International). The buildup of extracellular clusters of amyloid beta (Aβ) plaques and intracellular neurofibrillary tangles (NFTs) consisting of hyperphosphorylated tau protein in the cortical and limbic regions of the human brain signifies the disease's pathological features (13–17). The accumulation of Aβ plaques and NFTs is connected with notable loss of neurons and synapses, along with neuroinflammation (18). In this context, there is a growing number of studies showing that patients with AD exhibit epilepsy, which may be a harbinger or indicator of the disease (11, 19–24). The prevalence of epilepsy in patients with AD is around 10 to 22% (21, 25, 26), while epileptiform activity, with varying characteristics, can be detected in patients with AD and with or without diagnosed epilepsy (23, 27–34). Seizures can begin in preclinical or clinical stages of AD (20, 23, 35, 36). The preponderance of seizures in AD lacks motor characteristics, rendering their diagnosis complex and potentially leading to an underreporting of seizures (23, 36, 37). Some studies suggest seizures can increase the production and deposition of Aβ and hyperphosphorylated tau in the brain and cause a decline in cognition in patients with AD (24, 38–41). Late-onset epilepsy increases risk of AD by around three-fold (12, 42). Notably, AD predisposes patients to develop epilepsy and late-onset epilepsy predisposes patients to develop AD highlighting the bidirectionality between diseases (11, 19).

DLB ranks as the second most frequent neurodegenerative dementia among individuals above the age of 65 (43–45). Clinical criteria encompass cognitive fluctuations, visual hallucinations, rapid eye movement sleep behavior disorder, and parkinsonism (45, 46). The neuropathology of DLB is marked by neuronal Lewy bodies and Lewy neurites, consisting of aggregates of α-synuclein that impact the brainstem along with extensive limbic and neocortical areas (47). This pathology also involves the loss of midbrain dopamine cells and cholinergic neurons in ventral forebrain nuclei, nucleus basalis of Meynert (48, 49). Furthermore, Aβ plaques and NFTs are present in a majority of DLB cases (50, 51). Analogous to AD, individuals with DLB also experience seizures (52). Marawar et al. (53) demonstrated a higher occurrence of seizures in DLB compared to the general population, with a rate of 3.8% in pathologically confirmed DLB across the United States. Meanwhile, Beagle et al. (52) identified a cumulative probability of 14.7% for DLB patients to develop seizures and a 5.1% prevalence of new-onset seizures in a population from the Memory Aging Center at the University of California, San Francisco, while other studies observed a 2–3% seizure prevalence rate in cohorts from Italy, United States, and Sweden (53–55).

In spite of the presence of antiseizure medications, roughly a third of individuals with epilepsy are unable to manage their seizures or develop resistance to the impact of these medications (56–59). This underscores the urgent need to create novel and inventive treatment approaches for epilepsy. Beyond that, therapeutic interventions targeting the molecular mechanisms of neuronal hyperexcitability have promise for treating disorders linked to increased excitability, such as AD and DLB. For example, Vossel et al. (60) showed that low doses of levetiracetam can improve spatial memory and executive function in AD patients with detectable epileptic activity. Levetiracetam also improved attention, oral fluency, and overall cognition in AD patients in a case–control study (61). Also, the clinical trial HOPE4MCI (NCT03486938) uses low dose levetiracetam which has been shown to decrease hippocampal hyperexcitability and attenuate cognitive decline by improving task related memory performance in amnestic mild cognitive impairment (62, 63). These studies show that levetiracetam can improve diverse cognitive functions in various stages of AD, reflecting multiple cortical regions that exhibit hyperexcitability in the disease. As a potential marker of neurodegeneration and pathology progression in AD and DLB, the early detection of cortical hyperexcitability and its mechanistic understanding is instrumental. Hyperexcitability may begin or be a result of neuropathology and may arise due to a number of different factors at varying time points in AD and DLB. Though hyperexcitability has been previously explored in the context of AD (1, 24, 31, 60, 64, 65), the role and mechanisms of hyperexcitability in DLB (66–69), as well as its similarities and differences with AD requires more research. In this review, we explore shared and distinct molecular mechanisms associated with hyperexcitability in AD and DLB, encompassing factors such as genetic risk factors, $Ca^2$ and glutamate contributions, cholinergic pathways, AMPA and NMDA receptors, mTOR, pathological Aβ, tau and α-synuclein, genetic risk factors, altered microglial and astrocytic activity, and impaired inhibitory interneuron function (Figure 1).

## Genetic risk factors

### APOE

The apolipoprotein E (APOE) ε4 allele is implicated in cerebrovascular, mental, and neurological disorders but stands as the primary genetic susceptibility factor for AD, and also increases the severity of neuropathology in DLB (70–75). In the context of hyperexcitability, APOE ε4 (APOE4) has not been associated with early-onset epilepsy, within 12 months of age, (76), but APOE4 has been linked to an increased risk of late-onset epilepsy, starting after age 60, and there exists an allele dose dependence on the incidence of late-onset epilepsy of 2.87, 4.13, and 7.05 per 1,000 person-years for 0, 1, and 2 APOE ε4 alleles, respectively (42, 77). These results persisted when participants with strokes were censored, suggesting that APOE4 confers epilepsy risk through mechanisms beyond its effects on cerebrovascular disease (42). A meta-analysis demonstrated that individuals carrying the APOE4 allele and experiencing temporal lobe epilepsy exhibit seizure onset nearly 4 years earlier than those without the allele (78). Another investigation revealed that individuals with temporal lobe epilepsy and APOE4 have an elevated risk of experiencing verbal learning deficits, particularly among those with a longer epilepsy duration (79). Similarly, mice expressing the human APOE4 allele develop a seizure phenotype that is either absent or less pronounced in mice expressing human APOE2 or APOE3 (80).

The exact mechanisms by which APOE4 promotes heightened neural excitability remain to be fully elucidated. APOE is involved in cholesterol metabolism and transportation, stabilization and solubilization of lipoproteins, and maintaining lipid homeostasis. Additionally, it plays a role in synaptic plasticity, signal transduction, and immunomodulation (81–83). *In vitro* studies utilizing human induced pluripotent stem cell-derived neurons expressing APOE4 demonstrate increased excitability compared to APOE3 isogenic controls. This

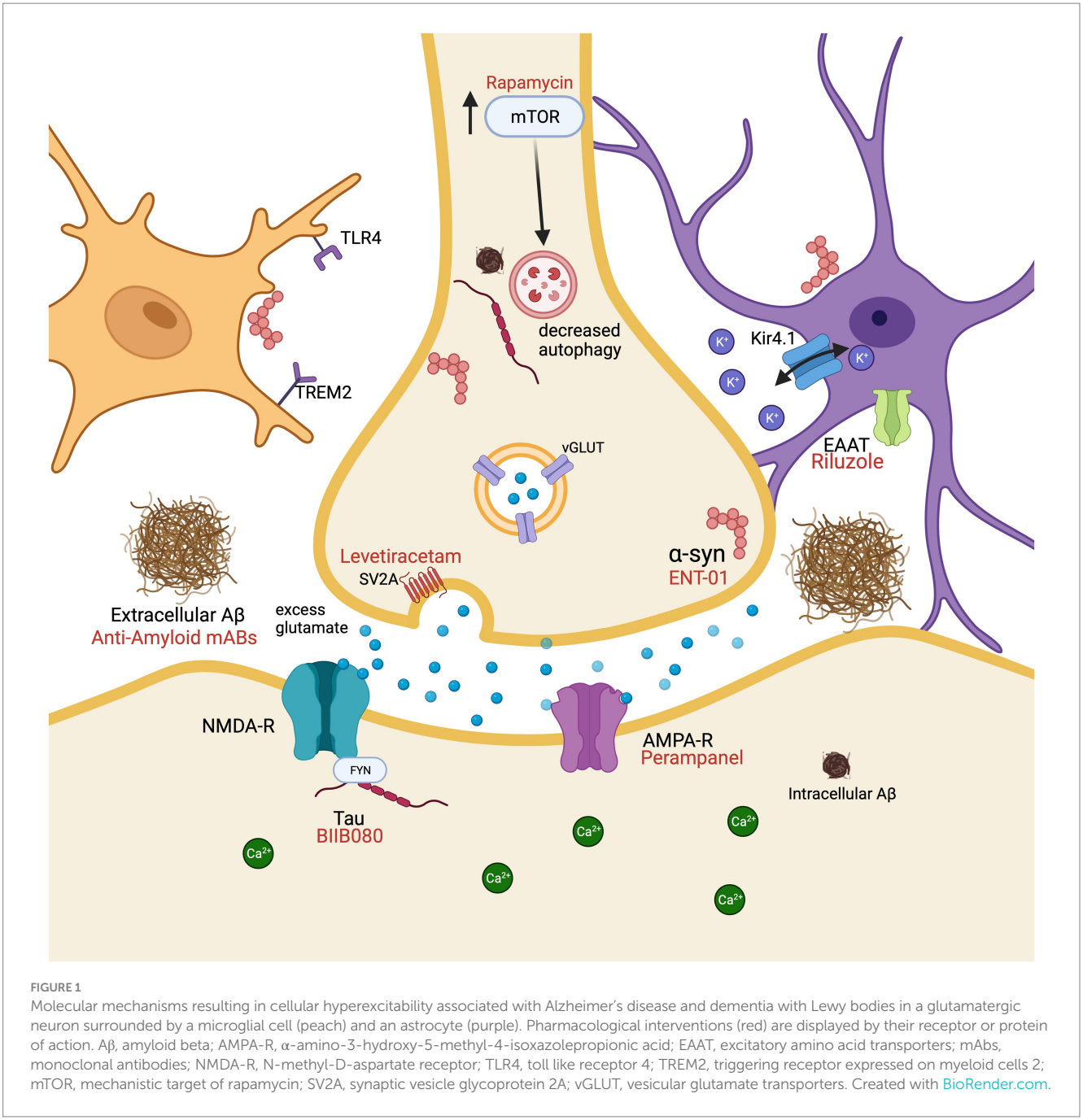


FIGURE 1
Molecular mechanisms resulting in cellular hyperexcitability associated with Alzheimer's disease and dementia with Lewy bodies in a glutamatergic neuron surrounded by a microglial cell (peach) and an astrocyte (purple). Pharmacological interventions (red) are displayed by their receptor or protein of action. Aβ, amyloid beta; AMPA-R, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; EAAT, excitatory amino acid transporters; mAbs, monoclonal antibodies; NMDA-R, N-methyl-D-aspartate receptor; TLR4, toll like receptor 4; TREM2, triggering receptor expressed on myeloid cells 2; mTOR, mechanistic target of rapamycin; SV2A, synaptic vesicle glycoprotein 2A; vGLUT, vesicular glutamate transporters. Created with BioRender.com.

heightened excitability might be attributed to an elevated expression of synaptic proteins like synaptophysin and PSD-95, the upregulation of genes involved in neuronal differentiation, and alterations in cholesterol metabolism (84). Due to the critical importance of APOE in shaping neuronal structure, establishing synapses, and regulating ion channels, changes in cholesterol and lipid concentrations can significantly impact neural excitability (85). For example, in rat hippocampal neurons, changed cholesterol levels differentially affect fast transient currents and delay rectifying currents modulating hyperexcitability (86). A clinical demonstration of importance is evident in Niemann-Pick type C (NPC) disease. In NPC, dysregulation of cholesterol transport and accumulation, can result in an AD-like phenotype, including cortical neurodegeneration, tau hyperphosphorylation, Aβ deposition, and hyperexcitability (87, 88). Vivas et al. (88) has shown that decreased transport of cholesterol from lysosomes disrupts ion channel activity and ultimately results in neuronal hyperexcitability. This mechanism is mediated by the reduction in phosphatidylinositol 4,5-bisphosphate in the plasma membrane resulting in a decrease in KCNQ2/3 current and increased excitability (88). Finally, microglia and astrocytes harboring APOE4 exhibit slowed uptake of extracellular Aβ (84). Consequently, elevated Aβ levels can also lead to increased neural activity. The plethora of physiological functions APOE is involved in results in numerous pathways by which APOE can contribute to hyperexcitability and targeted with therapeutics in AD and DLB (Table 1).

## APP, PSEN1, and PSEN2

Early-onset familial AD, which constitutes less than 1% of cases, can be triggered by highly penetrant mutations in genes encoding

amyloid precursor protein (*APP*) on chromosome 21, presenilin 1 (*PSEN1*) on chromosome 14, and presenilin 2 (*PSEN2*) on chromosome 1 (89–91). Among the roughly 35 distinct *APP* mutations associated with AD pathogenesis are gene locus duplications and point mutations in the coding region, leading to amino acid substitutions. Duplication of the entire gene or locus results in elevated APP and Aβ levels, favoring the formation of Aβ plaques (92, 93). *PSEN1* and *PSEN2* are not only involved in γ-secretase but also in cleaving other type I integral proteins like the Notch receptor (94). Likewise, mutations in *PSEN1* and *PSEN2* hinder γ-secretase activity, causing an imbalance in the $A\beta_{1-42}$ to $A\beta_{1-40}$ ratio due to $A\beta_{1-42}$ overproduction or $A\beta_{1-40}$ underproduction, or a combination thereof. The $A\beta_{1-42}$ to $A\beta_{1-40}$ ratio is significant because an increase in this ratio increases the aggregation and neurotoxicity of the Aβ protein while a decrease in the ratio can decrease deposition (95–98). *APP*, *PSEN1*, and *PSEN2* mutations contribute to neural excitability by activating the mentioned mechanisms via elevated Aβ levels and amyloid plaque formation. It is important to note that early-onset AD is not only related to *APP*, *PSEN1*, and *PSEN2*. Alterations in these three genes only account for 5–10% of early-onset AD with remaining genes and risk factors still to be discovered and studied (89, 99–101). Beyond these known genetic variants causing AD, individuals with Down syndrome possess an extra copy of chromosome 21, housing *APP*, and face an elevated risk of early-onset AD and seizures (102, 103). Estimates indicate that more than 50% of people with Down syndrome will develop Alzheimer's with symptoms emerging in their 50s and 60s (104, 105).

### SNCA

Mutations in the *SNCA* gene, which encodes α-synuclein, lead to parkinsonian disorders, notably including DLB (106–108). Among the numerous mutations, A30P, E46K, G51D, and duplications and triplications of the *SNCA* gene, of specific interest is the A53T point mutation (106, 109, 110). Recent investigations have uncovered that mice expressing human α-synuclein with the A53T mutation manifest a phenotype akin to the human condition (110). They exhibit deficits in long-term potentiation and learning and memory. Furthermore, these mice display a left shift in electroencephalography (EEG) spectral power, mirroring the EEG slowing observed in patients with DLB (110–112). The EEG slowing and shift in spectral power to more delta signifies network dysfunction, a loss of cholinergic neurons, and symptoms of DLB (66, 68). Similarly, Morris et al. (66) demonstrated that neuronal overexpression of wild-type α-synuclein in transgenic mice (Thy1-SYN line 61) also leads to EEG slowing. Both of these models experience seizures and present molecular alterations in the hippocampus that suggest abnormal network excitability, including a depletion of calbindin in the dentate gyrus. These collective findings suggest that higher levels or dysfunction of α-synuclein may contribute to the neuronal hyperactivity found in DLB.

## Degeneration of cholinergic pathways

Acetylcholine is an ester of acetic acid and choline that is released by cholinergic neurons (113, 114). Acetylcholine plays a crucial role as one of the neurotransmitters implicated in cognitive functions like memory and executive function. In both DLB and AD, deficiencies in cholinergic activity are observable (115, 116). These deficiencies manifest as reduced acetylcholine levels and irregularities in the expression of nicotinic and muscarinic receptors. Notably, the extent of cholinergic deficits tends to be more pronounced in DLB when compared to AD, even though DLB typically exhibits less brain volume loss (49, 117). The decline of cholinergic neurons projecting to the cortex contributes to a deceleration of cortical oscillations as seen on EEG, resulting in a shift of spectral power from higher frequency bands (alpha, beta, gamma) to lower ones (delta, theta) (118, 119). DLB patients experience a more significant loss of cholinergic neurons, displaying more pronounced EEG slowing (49). Additionally, DLB patients demonstrate greater clinical improvement with the usage of common acetylcholinesterase inhibitors such as donepezil, rivastigmine, and galantamine compared to AD patients (120, 121). It is unknown whether neurodegeneration of cholinergic neurons contributes to hyperexcitability. However, animal models suggest that early changes in cholinergic tone could contribute to epilepsy in preclinical stages of AD. Interictal spikes have been observed during the rapid eye movement stage of sleep in Tg2576 mice expressing human amyloid precursor protein (APP) at a very young age (5 weeks old), long before the deposition of Aβ (122). After administration of muscarinic cholinergic receptor antagonist, atropine, the investigators observed a reduction in interictal spikes, suggesting that there may be a phase of high cholinergic tone, contributing to epilepsy, prior to reductions in acetylcholine (122). In contrast, donepezil, a cholinesterase inhibitor had no significant effect on interictal spikes (122). Another study using the APPswe/PS1dE9 mouse model presenting with spike–wave discharges (SWDs), showed that donepezil does not have a significant effect on epileptic activity whereas atropine decreases SWDs and results in EEG slowing (123). This information suggests that before the degeneration of cholinergic neurons in AD and DLB, there could be a phase of increased cholinergic tone that contributes to an increase in neuronal activity and epilepsy.

## Glutamate

Glutamate, among the most extensively studied neurotransmitters within the central nervous system, is a non-essential amino acid synthesized within neurons and glial cells using glucose and α-ketoglutarate. It is ubiquitously distributed throughout the brain (124). Glutamate holds significance in cognitive functions like memory and learning, playing a pivotal role in neuronal excitability by expediting swift synaptic activity in neurons—a process regulated by astrocytes and other glial cells (125). The distribution of glutamate across distinct brain compartments is orchestrated by specific transporters and enzymes accountable for its metabolism. Surplus glutamate is eliminated by glial cells through excitatory amino acid transporters (EAAT1, EAAT2) (126). Notably, reduced expression levels of EAAT1 and EAAT2 have been observed in cases of epilepsy (127, 128), while mutations in the *SLC1A3* and *SLC1A2* genes that encodes EAAT1 and EAAT2, can result in episodic ataxia 6, characterized by symptoms of epilepsy, long lasting ataxia attacks and headaches, and epileptic encephalopathies, respectively (129, 130).

Inside astrocytes, glutamate undergoes a transformation into glutamine, subsequently being released and taken up by the neuronal

TABLE 1 Summary of molecular mechanisms cause hyperexcitability and intervention strategies associated with Alzheimer's disease and dementia with Lewy Bodies.

| Molecular mechanisms | Cause of hyperexcitability | Intervention strategies |
|---|---|---|
| Cholinergic pathways | • Increased cholinergic tone before symptom onset | • Cholinergic receptor antagonist during preclinical stages of AD or DLB |
| Excess glutamate | • Excessive $Ca^{2+}$ influx<br>• Overstimulation AMPA and NMDA receptors | • Increase transporters (EAAT1, EAAT2, vGLUT1/vGLUT2) (e.g., riluzole)<br>• Antagonists of ionotropic and metabotropic glutamate receptors (e.g., perampanel)<br>• Antiseizure medications – SV2A mechanism (e.g., leviteracetam, brivaracetam) |
| Overactive mTOR | • Reduced autophagy; buildup of epileptogenic disease proteins | • Inhibition of mTOR (e.g., rapamycin) |
| Higher levels of α-synuclein | • Overactivation of astrocytes and microglia | • Inhibitors of aggregation (e.g., ENT-01)<br>• Inactivation of astrocytes and microglia (e.g., minocycline) |
| Tau protein | • Enables seizures<br>• Can facilitate presynaptic glutamate release | • Tau reduction (e.g., BIIB080) |
| Amyloid beta (Aβ) | • Changes in voltage-dependent channels that maintain neuronal membrane potential<br>• Stimulation of voltage-gated calcium channels<br>• Formation of pores in the membrane thereby increasing $Ca^{+}$ influx | • Antibody-mediated clearance (e.g., anti-amyloid monoclonal antibodies)<br>• Inhibitors of aggregation<br>• Inhibitors of voltage-gated calcium channels |
| Over-stimulation of microglia and astrocytes | • Increases glutamate release<br>• Decreases levels of the astrocytic glutamate transporter EAAT2<br>• Endocytosis of neuronal ionotropic $GABA_A$ receptors<br>• Activation of TLR4 receptors<br>• Increases extracellular K+ levels by astrocytes | • Glial inhibition (e.g., minocycline)<br>• Increase glutamate transporters (e.g., ceftriaxone) |
| GABAergic neuron dysfunction | • Mutations in genes encoding GABA receptor subunits<br>• Decreases voltage-gated sodium channels | • Medications that increaese GABAergic tone (e.g., gabapentin and pregabalin) |
| Genetic risk factors: *APOE* ε4, *APP*, *PSEN1*, *PSEN2*, Trisomy 21, *SNCA* | • Elevated levels of APP and Aβ, and α-synuclein<br>• Impairment in γ-secretase activity | • Gene editing (e.g., CRISPR – in development for humans) |

Aβ, amyloid beta; AD, Alzheimer's Disease; AMPA, receptor, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; APOE, apolipoprotein E; APP, amyloid precursor protein; CRISPR, clustered regularly interspaced short palindromic repeats; DLB, dementia with Lewy Bodies; NMDA receptor, N-methyl-D-aspartate; PSEN, presenilin; SNCA, synuclein alpha; SV2A, synaptic vesicle glycoprotein 2A.

presynaptic compartment. There, it is converted back into glutamate, which then accumulates within synaptic vesicles via vesicular glutamate transporters (vGLUT1/vGLUT2). Although astrocytes are commonly discussed collectively, they are an extremely diverse cell population. A recently described subpopulation of astrocytes specifically mediates the release of glutamate (131). Ultimately, this intricate process facilitates highly efficient neurotransmission within tri or tetrapartite synapses (132, 133).

An imbalance in the expression of vGLUT1 was observed in post-mortem human brain samples at the advanced stages of both AD and DLB (134). Similarly, Liraz et al. (135) discovered reduced levels of vGLUT in the hippocampal neurons of APOE4 mice. Previous research studies have pointed to a decline in the capacity and protein expression of glutamate transporters, as well as a specific loss of vGLUT in AD patients (136–138). A postmortem study showed increases in EAAT1 levels in a subset of pyramidal neurons exhibiting degeneration in the AD brain (139), whereas another postmortem study and *in vitro* assay showed impaired function of EAAT2 in the AD brain (140). Pharmacological administration of riluzole increases glutamate transporter expression, and in the P301L mouse model reverses glutamate related alterations and associated cognitive decline (141). Consequently, elevated levels of glutamate contribute to excitotoxicity and neuronal cell death (142). These findings collectively suggest that as the disease advances, the transporters responsible for glutamate reuptake become less effective, potentially leading to increased neuronal excitability.

Glutamate toxicity primarily arises from an excessive influx of $Ca^{2+}$ (143, 144). Dubinsky (145) demonstrated that hippocampal neurons exposed to toxic levels of glutamate maintained elevated $Ca^{2+}$ levels for around 1 h before returning to baseline levels. As calcium signaling governs a spectrum of cellular processes, the outcome of $Ca^{2+}$ overload entails the activation of catabolic enzymes like calpain I (146), phospholipases, and the release of arachidonic acid (147). This cascade results in an escalation of reactive oxygen and nitrogen species and the eventual collapse of neuronal cells through cytoskeletal degradation and membrane deterioration. Clinically, this associates with the progressive decline in cognition and memory, as well as brain atrophy in AD patients (148, 149). This is further evident in epilepsy

where the seizures ultimately cause excitotoxicity by starting the aforementioned cascade and leading to neuronal cell death and loss (150, 151).

Upon release from synaptic vesicles, glutamate initiates the activation of diverse ionotropic (AMPA, kainate, NMDA) and metabotropic (mGluR1 and mGluR5 in group I, mGluR2 and mGluR3 in group II, and mGluR4,6,8 in group III) glutamate receptors, primarily located in the postsynaptic region (152). The overstimulation of these receptors contributes to the generation of free radicals, possibly as a result of the continued calcium influx, inducing oxidative stress and subsequently disrupting mitochondrial functions (152, 153). This mitochondrial dysfunction plays a role in initiating and advancing epilepsy by triggering sequences of apoptosis (154).

Recent research highlights NMDA receptors (NMDARs) as contributors to neuronal hyperexcitability, suggesting that abnormal activation of these receptors, particularly through $Ca^{2+}$ influx, is implicated in hyperexcitability (155, 156). NMDARs possess a significantly higher permeability for calcium ions compared to other ionotropic glutamate receptors (iGluRs), thus facilitating hyperactivity through calcium influx (155, 156). Memantine, an NMDAR antagonist, has been found to reduce $Ca^{2+}$ influx and improve cognition and behavior in moderate-to-advanced AD (157). On the other hand, direct links between AMPA receptors and epilepsy in AD and DLB are more limited. Elevated levels of AMPA receptors have been observed in the brains of various epilepsy types, in humans and animal models (158, 159), and there is evidence of changes in receptor function through increased levels of AMPA and NMDA receptor subunits in human and mouse epileptic brains (160, 161).

Studies such as that by Teravski et al. (110) involving A53T α-synuclein-expressing neurons have indicated postsynaptic dysfunction, including reduced amplitude of miniature postsynaptic currents and a lower ratio of AMPA to NMDA receptor currents. Such changes coincide with the development of epileptic activity in this model. If the loss of AMPA receptors occurs in GABAergic inhibitory neurons, this could enhance the activity of neurons receiving their projections, potentially leading to neural network hyperactivity in DLB. Further exploration of the roles of NMDA and AMPA receptors in AD and DLB could yield valuable insights into potential treatments for epilepsy associated with these diseases.

## Overactivation of mTOR Pathway

mTOR, mechanistic target of rapamycin, is a highly conserved serine/threonine protein kinase that forms two distinct complexes, mTORC1 and mTORC2. External triggers including energy, oxygen, DNA damage, and amino acids activate the mTOR complexes, and they are implicated in a breadth of physiological functions including cell survival, growth, proliferation, metabolism, protein synthesis and signaling (162). In the brain, mTOR expression is widespread, affecting many neuronal and glial cell types playing a role in axonal development, synaptic plasticity, and neuronal excitability (163). Another crucial role function of mTOR signaling is autophagy, the process of degrading and recycling components of dysfunctional cells and proteins (164). Recently however, the role of autophagy has been expanded and shown to affect neuronal excitability (165). An ATG5 deficient mouse model shows impairment and decreases in protein kinase A (PKA) signaling from the lack of PKA subunit turnover (165). In addition to increased excitatory neurotransmission, alterations in synapses and disruption in AMPA receptor function, seizures also present as a common phenotype in these mice. This further highlights mTOR's myriad functions and its contribution to hyperexcitability and warrants further investigation in the context of neurodegeneration.

Overactivation and dysregulation of mTOR can result in severe pathological changes. mTOR association with hyperexcitability and seizures can be attenuated pharmacologically (125, 166, 167). mTOR hyperactivation is observed in Tuberous Sclerosis Complex (TSC) which presents with epileptic seizures and autism-like traits (162). Loss of the TSC gene in mouse models results in seizures and epilepsy that can be attenuated with the mTOR inhibitor, rapamycin (168). mTOR overactivation can also be activated by seizures evidenced by an increase in phospho-S6 expression in a kainic acid seizure mouse model (169). Inhibition of mTOR and restoration of the excitatory imbalance causing seizures and epilepsy may provide additional benefit for AD and DLB where hyperexcitability may participate in a positive feedback loop (40).

Pertaining to AD and DLB, postmortem examinations revealed increased mTOR activation in AD, DLB, and Parkinson's disease and associations with deficits in autophagy (170–173). Seizures have been shown to both activate mTOR and worsen AD pathology and cognitive deficits. Rapamycin administration can attenuate cognitive deficits in AD models through an increase in autophagy and/or decrease in hyperexcitability, further linking overactivation of mTOR activity and its contribution to AD (174, 175). Within the context of hyperexcitability, mTOR in DLB may be underappreciated and understudied. Since autophagy deficits have been implicated in DLB, hyperexcitability may be a mechanistic link with mTOR. A better understanding of these mechanisms and connection to hyperexcitability in AD and DLB may allow for more targeted therapeutics beyond rapamycin in lessening the overactivation of mTOR and burden of its wide-ranging effects.

## Proteinopathies

### Alpha-synuclein

Alpha-synuclein is a protein composed of 140 amino acids. It was initially identified in association with synaptic vesicles within the presynaptic nerve terminal and has demonstrated interactions with membranes (176, 177). This protein modulates synaptic transmission, influences the density of synaptic vesicles, and contributes to neuronal plasticity (178, 179).

Beyond its synaptic functions, extracellular alpha-synuclein has a pivotal impact on neuroinflammation, neurotoxicity, and the propagation of pathological changes (180). It is transported into the extracellular space following active secretion or release from dying neurons. The exact mechanism behind the secretion of alpha-synuclein is unknown. However, research by Paillusson et al. (181) indicated that enteric neurons can release it via conventional endoplasmic reticulum/Golgi-dependent exocytosis, which is driven by neuronal activity.

Clinical and experimental studies demonstrate that α-synuclein expression participates in epilepsy (182–185). Tweedy et al. (186) demonstrated hippocampal network hyperexcitability in young transgenic mice expressing human mutant alpha-synuclein. Yang et al.

(185) identified anomalous accumulations of this protein in hippocampal samples taken from individuals with mesial temporal lobe epilepsy (MTLE). These deposits were correlated with the loss of neuronal cells and reactive gliosis, indicating a potential link between the presence of the protein and the pathological changes seen in MTLE. Another clinical study in children with epilepsy showed that higher levels of serum α-synuclein correlated with disease severity (182). In the same way that α-synuclein levels are associated with seizures in epilepsy patients, it may also be associated with epileptic events in AD and DLB. A mechanism by which α-synuclein contributes to epilepsy could be activation of astrocytes and microglia, enhancing glial proinflammatory activity cytokines, nitric oxide, and reactive oxygen species (184, 187). More investigations are warranted to determine whether lowering α-synuclein levels or inhibiting its aggregation in the brain modulates epilepsy.

## Tau protein

The microtubule-associated protein tau predominantly resides within axons, where it plays a vital role in assembling microtubules. Tau can also be located in various neuronal compartments, such as somatodendritic regions and nuclei, and it is even detectible within glial cells (188, 189). In cases of pathology, tau undergoes hyperphosphorylation within neurons, diminishing its affinity for tubulin. This leads to the aggregation of tau into neuropil filaments or NFTs, giving rise to tauopathies (190–192).

Brain aggregates of hyperphosphorylated tau have been noted in patients with epilepsy as well as various models of epilepsy (40, 68, 193). This indicates that the abnormal aggregation of phosphorylated tau might play a role in the pathogenesis of epilepsy. Additionally, the tau protein seems to contribute to the development of epilepsy in the context of AD and DLB. Referencing Hwang et al. (40), endogenous tau acts as an enabler of hyperexcitability and seizures and, within the context of epilepsy and AD, a complex balance may occur in an attempt to decrease hyperexcitability. Total tau is reduced after 2 months in a status epilepticus (SE) model of epilepsy (194). After 4 months, tau levels return to normal while phosphorylation at tau sites S202/T205 is reduced by about 50%. This may highlight how tau changes in response to hyperexcitability over time in an attempt to reach homeostasis.

Numerous investigations have demonstrated that genetically altering tau or diminishing tau levels can result in an increase or decrease in seizures and epileptic activity across different animal models. Ablating both tau and Fyn in a mouse model shows robust neuroprotection from pentylenetetrazol, including increased seizure latency, reduced seizure stage, and reduced gliosis (195). Roberson et al. (196) demonstrated that reducing normal tau prevents the occurrence of spontaneous epileptiform activity across multiple lines of transgenic mice expressing human APP. Conversely, transgenic mice that overexpressed wild-type human tau or tau with an A152T mutation exhibit epileptiform activity and heightened susceptibility to seizures (197). The A152T tau mutation induces more pronounced network hyperexcitability compared to wild-type tau (197). *In vitro* studies using the rTg4510 mouse model, which features mutant (P301L) human tau, revealed increased neuronal excitability in the cortex's layer 3 even before the formation of NFTs. In the CA1 region of the hippocampus, pyramidal neurons display heightened firing, while inhibitory interneurons exhibit reduced activity, indicating a breakdown in inhibitory synaptic transmission (198, 199).

Recent investigations involving mice expressing human α-synuclein with the A53T mutation highlighted that endogenous tau contributes to hyperexcitability and that epileptic activity diminishes in the absence of tau (68). Delving deeper into the pathways influenced by tau, Decker et al. (200) demonstrated that hyperphosphorylated tau could stimulate presynaptic glutamate release, resulting in hyperexcitability. The toxicity of glutamate has been linked to tau-mediated neuronal cell death and behavioral deficits in drosophila (201).

The observation that physiological endogenous tau levels in adult mice impact seizure susceptibility suggests that similar relationships might exist in humans, potentially influencing the risk of developing seizures. This supports the notion that reducing tau could contribute to preventing seizures (202) and offers an opportunity for pharmacological intervention targeting tau.

## Amyloid beta (Aβ)

Amyloid precursor protein (APP) is a transmembrane protein encompassing a sizable extracellular domain and a smaller intracellular segment. Amyloid-beta (Aβ) peptides stem from the proteolytic cleavage of APP, sequentially catalyzed by β-secretase and γ-secretase. In pathological conditions, Aβ peptides amass into dense fibrillary plaques. Aβ has been demonstrated to incite network dysregulation, culminating in heightened synchronicity and seizures. This increased neuronal activity, in turn, exacerbates neurodegeneration (203). Recent investigations indicate that Aβ possesses epileptogenic properties and can significantly influence the trajectory of cognitive decline (12, 204). Ovsepian and O'Leary (205) proposed that seizures might foster the deposition of Aβ plaques. This epileptogenic potential of Aβ was validated in the APP/PS1 model, where neurons exhibiting epileptic discharges were found to colocalize with Aβ plaques (206). Evidence also suggests that Aβ could have epileptogenic effects even during pre-plaque stages. Hyperactivity among hippocampal neurons during the initial phases of Aβ pathology, when Aβ fibrils remain soluble, has been observed in APP/PS1 mice (122, 207). APP/PS1 mice also present with an increase in soluble and insoluble $A\beta_{1\text{-}42}$ and an increase in seizure susceptibility with corneal kindling (208). Aβ oligomers, being synaptotoxic, might trigger epileptic discharges prior to plaque deposition (209). Exposure to Aβ oligomers can also lead to spontaneous neuronal firing in hippocampal neurons (210).

Studies indicate that Aβ-triggered neuronal epileptic activity is tied to alterations in voltage-dependent channels that regulate the neuronal membrane potential. In a drosophila model expressing human Aβ42, Ping et al. (211) demonstrated that fewer Kv4 channels in neurons promote hyperexcitability, while Kv2 and Kv3 channels remained unaffected. Other research has shown that Aβ can perturb calcium homeostasis by either stimulating voltage-gated calcium channels or creating membrane pores, thereby augmenting calcium influx (212). Additionally, Aβ can influence glutamate release. Talantova et al. (213) illustrated that Aβ interacts with α7 nicotinic acetylcholine receptors, leading to the release of astrocytic glutamate, which subsequently activates extrasynaptic NMDA receptors on neurons. Similarly, Zott et al. (214) employed Aβ-amyloidosis models to reveal that hyperactivity is initiated by dampening glutamate reuptake. Soluble Aβ oligomers hinder the uptake of glutamate and intensify extrasynaptic NMDAR activation. Thus, Aβ can trigger a sequence of molecular events culminating in neural hyperexcitability.

## Glia and neuroinflammation

Glial cells are brain defense cells comprising microglia, astrocytes, and oligodendrocytes. When stimulated, microglia and reactive astrocytes release modulators to facilitate the recovery of the tissue from damage (215, 216). However, the continuous stimulation of the glial network causes a cascade of molecular events leading to neuroinflammation (215, 217). Investigators have previously proposed that neuroinflammation stimulates heightened neuronal activity and seizures, and the disruption of glial immunoinflammatory function is considered a factor that could predispose to or play a role in the emergence of seizures (218, 219). Therefore, inflammatory mediators and epileptic seizures form a vicious positive feedback loop, reinforcing each other (220). This vicious cycle can be found in diseases with neuroinflammatory conditions such as AD and DLB and to be responsible for epileptogenesis.

Elevated concentrations of pro-inflammatory cytokines, notably interleukin-1β (IL-1β), IL-6, and tumor necrosis factor-α (TNF-α), have been linked to epileptic seizures (221). The increased concentration of pro-inflammatory mediators can participate in hyperexcitability by increasing glutamate release via decreasing levels of the astrocytic glutamate transporter EAAT2 (excitatory amino acid transporter 2) (222, 223). Before and after seizures, there is an increase in the levels of pro-inflammatory cytokines and the expression of their receptors in both glial cells and neurons (221). In epileptic and AD patients, TNFα levels are elevated in the brain (224, 225), and TNFα increases the sensitivity of AMPA and NMDA glutamatergic receptors in the postsynaptic neuron, leading to excitotoxicity (223, 226). TNFα also induces endocytosis of neuronal ionotropic $GABA_A$ receptors, so that neurotransmission becomes more excitatory, leading to epilepsy (227). Furthermore, Xiaoqin et al. (85) found that the intracerebroventricular injection of IL-1β in rats leads to a reduction in cortical and hippocampal GABA concentration, while simultaneously increasing glutamate release. This alteration in neurotransmitter balance enhances the brain's vulnerability to seizures.

Another potential mechanism underlying seizure activation is the engagement of TLR4 receptors (228). TLR4 acts as the primary receptor for the proinflammatory mediator High Mobility Group Box 1 (HMGB1). Activation of TLR4 via HMGB1 sets off seizures by initiating a $Ca^{2+}$ influx subsequent to the phosphorylation of the NR2B subunit of the NMDAR. In support of this, Maroso et al. (229) revealed an elevation in TLR4 expression in hippocampal samples from individuals with drug-resistant temporal lobe epilepsy compared to control subjects. Furthermore, inflammation triggers the release of reactive oxygen species and reactive nitrogen species, thereby heightening susceptibility to seizures and intensifying the inflammatory milieu in the brain (230). This inflammatory environment gives rise to mediators like pro-inflammatory cytokines, transforming growth factor-β, and prostaglandin E2 (231). These mediators stimulate astrocytes and impact glutamate release, culminating in hyperexcitability.

Triggering receptor expressed on myeloid cells 2 (TREM2) are primarily expressed by microglia and play a role in the immune response. TREM2 expression in the brain has been found to be increased in AD and thought to provide an adaptive response to AD pathology, while reduction in TREM2 and mutant variants increases susceptibility to hyperexcitability and epileptic activity (232, 233). In regards to DLB, results are mixed as to whether soluble TREM2 is increased, and more research is needed to determine TREM2's influence on DLB-related hyperexcitability (234–236).

In addition to releasing inflammatory mediators, glial cells, particularly astrocytes, play a role in maintaining ion balance by clearing extracellular potassium (K+) during neuronal repolarization. Wang et al. (237) demonstrated that the onset of seizures is linked to elevated extracellular K+ levels due to astrocytic activity. Notably, the protein expression of the astrocytic potassium channel Kir4.1 is diminished in both a mouse model of AD and in the brains of AD patients (238).

Astrocytes often undergo reactive changes, termed reactive astrocytosis, characterized by increased astrocyte size and number. These changes are frequently observed alongside neuronal loss and synaptic reorganization (239). Reactive astrocytosis is present in conditions such as epilepsy, AD, and DLB, and it might contribute to neural hyperexcitability by influencing the function of astrocytic membrane K+ channels (240–242). In light of these insights, understanding the underlying mechanisms of inflammation in the development of epilepsy could pave the way for the discovery of promising antiseizure medications.

## GABAergic dysfunction

Gamma-aminobutyric acid (GABA) serves as the primary inhibitory neurotransmitter within the central nervous system. It is synthesized by the enzyme glutamic acid decarboxylase (240). This neurotransmitter is primarily found in interneurons that establish synapses on cell bodies and nearby axon segments. Released into the synaptic cleft, GABA exerts its influence through activation of $GABA_A$ and $GABA_B$ receptors. $GABA_A$ receptors function as ligand-gated ion channels, promptly inducing inhibition by enhancing chloride influx into cells. In the context of AD, studies have shown moderate reductions in $GABA_A$ receptors within the brain (243, 244). $GABA_B$ receptors, on the other hand, are G protein-coupled ion channels that augment extracellular potassium transport while concurrently decreasing calcium influx. Mutations in genes encoding GABA receptor subunits have been linked to a range of epileptic disorders (245).

Investigations point to a potential mechanism involving GABAergic dysfunction contributing to hyperexcitability by influencing voltage-gated sodium channels. Studies by Verret et al. (246) and Hamm et al. (247) demonstrated variable decreases in Nav1.1 and Nav1.6 within hippocampus and somatosensory cortex mouse models of AD. These channels enhance gamma oscillations during exploration, which can help suppress epileptiform discharges. Consequently, Nav1.1 and Nav1.6 hold potential as targets for addressing epileptic seizures in the context of AD.

# Conclusion

In conclusion, hyperexcitability in AD and DLB arises from a combination of multiple factors working together to disrupt regulation of neuronal excitability. In this context, we observe that genetic predispositions, initial elevations in cholinergic activity, excessive calcium influx causing glutamate toxicity, heightened NMDA and AMPA receptor sensitivity, overactivation of mTOR, disruptions in calcium homeostasis due to Aβ, tau, and α-synuclein, hyperstimulation

of microglia and astrocytes, and GABA dysfunction collectively contribute to the promotion of hyperexcitability in AD and DLB. By understanding the specific dysfunctions within these pathways, it becomes possible to develop targeted therapeutic strategies aimed at restoring proper neuronal excitability. Such interventions hold the potential to alleviate the symptoms associated with these neurodegenerative disorders, offering hope for improved treatments and better quality of life for affected individuals.

## Author contributions

MV: Writing – review & editing. KA-O: Writing – review & editing. KV: Writing – review & editing.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note



## References

1. Targa Dias Anastacio H, Matosin N, Ooi L. Neuronal hyperexcitability in Alzheimer's disease: what are the drivers behind this aberrant phenotype? *Transl Psychiatry*. (2022) 12:257. doi: 10.1038/s41398-022-02024-7

2. Scheffer IE, Berkovic S, Capovilla G, Connolly MB, French J, Guilhoto L, et al. ILAE classification of the epilepsies: position paper of the ILAE Commission for Classification and Terminology. *Epilepsia*. (2017) 58:512–21. doi: 10.1111/epi.13709

3. Collaborators GBDN. Global, regional, and national burden of neurological disorders, 1990-2016: a systematic analysis for the global burden of disease study 2016. *Lancet Neurol*. (2019) 18:459–80. doi: 10.1016/S1474-4422(18)30499-X

4. Falco-Walter J. Epilepsy-definition, classification, pathophysiology, and epidemiology. *Semin Neurol*. (2020) 40:617–23. doi: 10.1055/s-0040-1718719

5. Fiest KM, Sauro KM, Wiebe S, Patten SB, Kwon CS, Dykeman J, et al. Prevalence and incidence of epilepsy: A systematic review and meta-analysis of international studies. *Neurology*. (2017) 88:296–303. doi: 10.1212/WNL.0000000000003509

6. Thijs RD, Surges R, O'brien TJ, Sander JW. Epilepsy in adults. *Lancet*. (2019) 393:689–701. doi: 10.1016/S0140-6736(18)32596-0

7. Jiruska P, De Curtis M, Jefferys JG, Schevon CA, Schiff SJ, Schindler K. Synchronization and desynchronization in epilepsy: controversies and hypotheses. *J Physiol*. (2013) 591:787–97. doi: 10.1113/jphysiol.2012.239590

8. Margineanu DG. Epileptic hypersynchrony revisited. *Neuroreport*. (2010) 21:963–7. doi: 10.1097/WNR.0b013e32833ed111

9. Choi H, Pack A, Elkind MS, Longstreth WT Jr, Ton TG, Onchiri F. Predictors of incident epilepsy in older adults: the cardiovascular health study. *Neurology*. (2017) 88:870–7. doi: 10.1212/WNL.0000000000003662

10. Costa C, Romoli M, Liguori C, Farotti L, Eusebi P, Bedetti C, et al. Alzheimer's disease and late-onset epilepsy of unknown origin: two faces of beta amyloid pathology. *Neurobiol Aging*. (2019) 73:61–7. doi: 10.1016/j.neurobiolaging.2018.09.006

11. Johnson EL, Krauss GL, Kucharska-Newton A, Albert MS, Brandt J, Walker KA, et al. Dementia in late-onset epilepsy: the atherosclerosis risk in communities study. *Neurology*. (2020) 95:e3248–56. doi: 10.1212/WNL.0000000000011080

12. Keret O, Hoang TD, Xia F, Rosen HJ, Yaffe K. Association of Late-Onset Unprovoked Seizures of unknown etiology with the risk of developing dementia in older veterans. *JAMA Neurol*. (2020) 77:710–5. doi: 10.1001/jamaneurol.2020.0187

13. Deture MA, Dickson DW. The neuropathological diagnosis of Alzheimer's disease. *Mol Neurodegener*. (2019) 14:32. doi: 10.1186/s13024-019-0333-5

14. Fan L, Mao C, Hu X, Zhang S, Yang Z, Hu Z, et al. New insights into the pathogenesis of Alzheimer's disease. *Front Neurol*. (2019) 10:1312. doi: 10.3389/fneur.2019.01312

15. Kinney JW, Bemiller SM, Murtishaw AS, Leisgang AM, Salazar AM, Lamb BT. Inflammation as a central mechanism in Alzheimer's disease. *Alzheimers Dement*. (2018) 4:575–90. doi: 10.1016/j.trci.2018.06.014

16. Perl DP. Neuropathology of Alzheimer's disease. *Mt Sinai J Med*. (2010) 77:32–42. doi: 10.1002/msj.20157

17. Soria Lopez JA, Gonzalez HM, Leger GC. Alzheimer's disease. *Handb Clin Neurol*. (2019) 167:231–55. doi: 10.1016/B978-0-12-804766-8.00013-3

18. Mangalmurti A, Lukens JR. How neurons die in Alzheimer's disease: implications for neuroinflammation. *Curr Opin Neurobiol*. (2022) 75:102575. doi: 10.1016/j.conb.2022.102575

19. Dun C, Zhang Y, Yin J, Su B, Peng X, Liu L. Bi-directional associations of epilepsy with dementia and Alzheimer's disease: a systematic review and meta-analysis of longitudinal studies. *Age Ageing*. (2022) 51:afac010. doi: 10.1093/ageing/afac010

20. Fang Y, Si X, Wang J, Wang Z, Chen Y, Liu Y, et al. Alzheimer disease and epilepsy: A Mendelian randomization study. *Neurology*. (2023) 101:e399–409. doi: 10.1212/WNL.0000000000207423

21. Friedman D, Honig LS, Scarmeas N. Seizures and epilepsy in Alzheimer's disease. *CNS Neurosci Ther*. (2012) 18:285–94. doi: 10.1111/j.1755-5949.2011.00251.x

22. Giorgi FS, Saccaro LF, Busceti CL, Biagioni F, Fornai F. Epilepsy and Alzheimer's disease: potential mechanisms for an association. *Brain Res Bull*. (2020) 160:107–20. doi: 10.1016/j.brainresbull.2020.04.009

23. Vossel KA, Beagle AJ, Rabinovici GD, Shu H, Lee SE, Naasan G, et al. Seizures and epileptiform activity in the early stages of Alzheimer disease. *JAMA Neurol*. (2013) 70:1158–66. doi: 10.1001/jamaneurol.2013.136

24. Vossel KA, Tartaglia MC, Nygaard HB, Zeman AZ, Miller BL. Epileptic activity in Alzheimer's disease: causes and clinical relevance. *Lancet Neurol*. (2017) 16:311–22. doi: 10.1016/S1474-4422(17)30044-3

25. Mendez M, Lim G. Seizures in elderly patients with dementia: epidemiology and management. *Drugs Aging*. (2003) 20:791–803. doi: 10.2165/00002512-200320110-00001

26. Miranda DDC, Brucki SMD. Epilepsy in patients with Alzheimer's disease: A systematic review. *Dement Neuropsychol*. (2014) 8:66–71. doi: 10.1590/S1980-57642014DN81000010

27. Ciliento R, Gjini K, Dabbs K, Hermann B, Riedner B, Jones S, et al. Prevalence and localization of nocturnal epileptiform discharges in mild cognitive impairment. *Brain Commun*. (2023) 5:fcad302. doi: 10.1093/braincomms/fcad302

28. Devulder A, Macea J, Kalkanis A, De Winter FL, Vandenbulcke M, Vandenberghe R, et al. Subclinical epileptiform activity and sleep disturbances in Alzheimer's disease. *Brain Behav*. (2023) 13:e3306. doi: 10.1002/brb3.3306

29. Horvath AA, Papp A, Zsuffa J, Szucs A, Luckl J, Radai F, et al. Subclinical epileptiform activity accelerates the progression of Alzheimer's disease: A long-term EEG study. *Clin Neurophysiol*. (2021) 132:1982–9. doi: 10.1016/j.clinph.2021.03.050

30. Lam AD, Deck G, Goldman A, Eskandar EN, Noebels J, Cole AJ. Silent hippocampal seizures and spikes identified by foramen ovale electrodes in Alzheimer's disease. *Nat Med*. (2017) 23:678–80. doi: 10.1038/nm.4330

31. Lam AD, Sarkis RA, Pellerin KR, Jing J, Dworetzky BA, Hoch DB, et al. Association of epileptiform abnormalities and seizures in Alzheimer disease. *Neurology*. (2020) 95:e2259–70. doi: 10.1212/wnl.0000000000010612

32. Musaeus CS, Frederiksen KS, Andersen BB, Hogh P, Kidmose P, Fabricius M, et al. Detection of subclinical epileptiform discharges in Alzheimer's disease using long-term outpatient EEG monitoring. *Neurobiol Dis*. (2023) 183:106149. doi: 10.1016/j.nbd.2023.106149

33. Vossel KA, Ranasinghe KG, Beagle AJ, Mizuiri D, Honma SM, Dowling AF, et al. Incidence and impact of subclinical epileptiform activity in Alzheimer's disease. *Ann Neurol*. (2016) 80:858–70. doi: 10.1002/ana.24794

34. Yeh WC, Hsu CY, Li KY, Chien CF, Huang LC, Yang YH. Association between subclinical epileptiform discharge and the severity of cognitive decline in Alzheimer's disease: A longitudinal cohort study. *J Alzheimers Dis*. (2022) 90:305–12. doi: 10.3233/JAD-220567

35. Amatniek JC, Hauser WA, Delcastillo-Castaneda C, Jacobs DM, Marder K, Bell K, et al. Incidence and predictors of seizures in patients with Alzheimer's disease. *Epilepsia*. (2006) 47:867–72. doi: 10.1111/j.1528-1167.2006.00554.x

36. Sarkis RA, Dickerson BC, Cole AJ, Chemali ZN. Clinical and neurophysiologic characteristics of unprovoked seizures in patients diagnosed with dementia. *J Neuropsychiatry Clin Neurosci*. (2016) 28:56–61. doi: 10.1176/appi.neuropsych.15060143

37. Horvath A, Szucs A, Hidasi Z, Csukly G, Barcs G, Kamondi A. Prevalence, semiology, and risk factors of epilepsy in Alzheimer's disease: An ambulatory EEG study. *J Alzheimers Dis*. (2018) 63:1045–54. doi: 10.3233/JAD-170925

38. Canet G, Zub E, Zussy C, Hernandez C, Blaquiere M, Garcia V, et al. Seizure activity triggers tau hyperphosphorylation and amyloidogenic pathways. *Epilepsia*. (2022) 63:919–35. doi: 10.1111/epi.17186

39. Dolev I, Fogel H, Milshtein H, Berdichevsky Y, Lipstein N, Brose N, et al. Spike bursts increase amyloid-beta 40/42 ratio by inducing a presenilin-1 conformational change. *Nat Neurosci*. (2013) 16:587–95. doi: 10.1038/nn.3376

40. Hwang K, Vaknalli RN, Addo-Osafo K, Vicente M, Vossel K. Tauopathy and epilepsy comorbidities and underlying mechanisms. *Front Aging Neurosci*. (2022) 14:903973. doi: 10.3389/fnagi.2022.903973

41. Joutsa J, Rinne JO, Hermann B, Karrasch M, Anttinen A, Shinnar S, et al. Association between childhood-onset epilepsy and amyloid burden 5 decades later. *JAMA Neurol*. (2017) 74:583–90. doi: 10.1001/jamaneurol.2016.6091

42. Johnson EL, Krauss GL, Lee AK, Schneider ALC, Dearborn JL, Kucharska-Newton AM, et al. Association between midlife risk factors and late-onset epilepsy: results from the atherosclerosis risk in communities study. *JAMA Neurol*. (2018) 75:1375–82. doi: 10.1001/jamaneurol.2018.1935

43. Hogan DB, Fiest KM, Roberts JI, Maxwell CJ, Dykeman J, Pringsheim T, et al. The prevalence and incidence of dementia with Lewy bodies: a systematic review. *Can J Neurol Sci*. (2016) 43:S83–95. doi: 10.1017/cjn.2016.2

44. Kane JPM, Surendranathan A, Bentley A, Barker SAH, Taylor JP, Thomas AJ, et al. Clinical prevalence of Lewy body dementia. *Alzheimers Res Ther*. (2018) 10:19. doi: 10.1186/s13195-018-0350-6

45. Mckeith IG, Boeve BF, Dickson DW, Halliday G, Taylor JP, Weintraub D, et al. Diagnosis and management of dementia with Lewy bodies: fourth consensus report of the DLB consortium. *Neurology*. (2017) 89:88–100. doi: 10.1212/WNL.0000000000004058

46. Yamada M, Komatsu J, Nakamura K, Sakai K, Samuraki-Yokohama M, Nakajima K, et al. Diagnostic criteria for dementia with Lewy bodies: updates and future directions. *J Mov Disord*. (2020) 13:1–10. doi: 10.14802/jmd.19052

47. Goedert M, Spillantini MG, Del Tredici K, Braak H. 100 years of Lewy pathology. *Nat Rev Neurol*. (2013) 9:13–24. doi: 10.1038/nrneurol.2012.242

48. Harding AJ, Halliday GM. Cortical Lewy body pathology in the diagnosis of dementia. *Acta Neuropathol*. (2001) 102:355–63. doi: 10.1007/s004010100390

49. Tiraboschi P, Hansen LA, Alford M, Sabbagh MN, Schoos B, Masliah E, et al. Cholinergic dysfunction in diseases with Lewy bodies. *Neurology*. (2000) 54:407–11. doi: 10.1212/WNL.54.2.407

50. Mattila PM, Roytta M, Torikka H, Dickson DW, Rinne JO. Cortical Lewy bodies and Alzheimer-type changes in patients with Parkinson's disease. *Acta Neuropathol*. (1998) 95:576–82. doi: 10.1007/s004010050843

51. Schneider JA, Arvanitakis Z, Bang W, Bennett DA. Mixed brain pathologies account for most dementia cases in community-dwelling older persons. *Neurology*. (2007) 69:2197–204. doi: 10.1212/01.wnl.0000271090.28148.24

52. Beagle AJ, Darwish SM, Ranasinghe KG, La AL, Karageorgiou E, Vossel KA. Relative incidence of seizures and myoclonus in Alzheimer's disease, dementia with Lewy bodies, and frontotemporal dementia. *J Alzheimers Dis*. (2017) 60:211–23. doi: 10.3233/JAD-170031

53. Marawar R, Wakim N, Albin RL, Dodge H. Seizure occurrence and related mortality in dementia with Lewy bodies. *Epilepsy Behav*. (2020) 111:107311. doi: 10.1016/j.yebeh.2020.107311

54. Arnaldi D, Donniaquio A, Mattioli P, Massa F, Grazzini M, Meli R, et al. Epilepsy in neurodegenerative dementias: A clinical, epidemiological, and EEG study. *J Alzheimers Dis*. (2020) 74:865–74. doi: 10.3233/JAD-191315

55. Zelano J, Brigo F, Garcia-Patek S. Increased risk of epilepsy in patients registered in the Swedish dementia registry. *Eur J Neurol*. (2020) 27:129–35. doi: 10.1111/ene.14043

56. Ali A. Global Health: epilepsy. *Semin Neurol*. (2018) 38:191–9. doi: 10.1055/s-0038-1646947

57. Engel J Jr. Approaches to refractory epilepsy. *Ann Indian Acad Neurol*. (2014) 17:S12–7. doi: 10.4103/0972-2327.128644

58. Kwan P, Schachter SC, Brodie MJ. Drug-resistant epilepsy. *N Engl J Med*. (2011) 365:919–26. doi: 10.1056/NEJMra1004418

59. Stafstrom CE, Carmant L. Seizures and epilepsy: an overview for neuroscientists. *Cold Spring Harb Perspect Med*. (2015) 5:a022426. doi: 10.1101/cshperspect.a022426

60. Vossel K, Ranasinghe KG, Beagle AJ, La A, Ah Pook K, Castro M, et al. Effect of Levetiracetam on cognition in patients with Alzheimer disease with and without epileptiform activity: A randomized clinical trial. *JAMA Neurol*. (2021) 78:1345–54. doi: 10.1001/jamaneurol.2021.3310

61. Cumbo E, Ligori LD. Levetiracetam, lamotrigine, and phenobarbital in patients with epileptic seizures and Alzheimer's disease. *Epilepsy Behav*. (2010) 17:461–6. doi: 10.1016/j.yebeh.2010.01.015

62. Bakker A, Krauss GL, Albert MS, Speck CL, Jones LR, Stark CE, et al. Reduction of hippocampal hyperactivity improves cognition in amnestic mild cognitive impairment. *Neuron*. (2012) 74:467–74. doi: 10.1016/j.neuron.2012.03.023

63. Rosenzweig-Lipson S, Barton R, Gallagher M, Mohs R. HOPE4MCI trial: first trial targeting reduction of hippocampal overactivity to treat mild cognitive impairment due to Alzheimer's disease with AGB101. *Alzheimers Dement*. (2021) 17:e057813. doi: 10.1002/alz.057813

64. Ranasinghe KG, Kudo K, Hinkley L, Beagle A, Lerner H, Mizuiri D, et al. Neuronal synchrony abnormalities associated with subclinical epileptiform activity in early-onset Alzheimer's disease. *Brain*. (2022) 145:744–53. doi: 10.1093/brain/awab442

65. Toniolo S, Sen A, Husain M. Modulation of brain Hyperexcitability: potential new therapeutic approaches in Alzheimer's disease. *Int J Mol Sci*. (2020) 21:9318. doi: 10.3390/ijms21239318

66. Morris M, Sanchez PE, Verret L, Beagle AJ, Guo W, Dubal D, et al. Network dysfunction in alpha-synuclein transgenic mice and human Lewy body dementia. *Ann Clin Transl Neurol*. (2015) 2:1012–28. doi: 10.1002/acn3.257

67. Musaeus CS, Kjaer TW, Cacic Hribljan M, Andersen BB, Hogh P, Kidmose P, et al. Subclinical epileptiform activity in dementia with Lewy bodies. *Mov Disord*. (2023) 38:1861–70. doi: 10.1002/mds.29531

68. Peters ST, Fahrenkopf A, Choquette JM, Vermilyea SC, Lee MK, Vossel K. Ablating tau reduces Hyperexcitability and moderates electroencephalographic slowing in transgenic mice expressing A53T human alpha-Synuclein. *Front Neurol*. (2020) 11:563. doi: 10.3389/fneur.2020.00563

69. Zhao B, Shen LX, Ou YN, Ma YH, Dong Q, Tan L, et al. Risk of seizures and subclinical epileptiform activity in patients with dementia: A systematic review and meta-analysis. *Ageing Res Rev*. (2021) 72:101478. doi: 10.1016/j.arr.2021.101478

70. Borroni B, Grassi M, Costanzi C, Archetti S, Caimi L, Padovani A. APOE genotype and cholesterol levels in lewy body dementia and Alzheimer disease: investigating genotype-phenotype effect on disease risk. *Am J Geriatr Psychiatry*. (2006) 14:1022–31. doi: 10.1097/01.JGP.0000225088.29353.08

71. Dickson DW, Heckman MG, Murray ME, Soto AI, Walton RL, Diehl NN, et al. APOE epsilon4 is associated with severity of Lewy body pathology independent of Alzheimer pathology. *Neurology*. (2018) 91:e1182–95. doi: 10.1212/WNL.0000000000006212

72. Koutsodendris N, Nelson MR, Rao A, Huang Y. Apolipoprotein E and Alzheimer's disease: findings, hypotheses, and potential mechanisms. *Annu Rev Pathol*. (2022) 17:73–99. doi: 10.1146/annurev-pathmechdis-030421-112756

73. Lumsden AL, Mulugeta A, Zhou A, Hypponen E. Apolipoprotein E (APOE) genotype-associated disease risks: a phenome-wide, registry-based, case-control study utilising the UK biobank. *EBioMedicine*. (2020) 59:102954. doi: 10.1016/j.ebiom.2020.102954

74. Saunders AM, Strittmatter WJ, Schmechel D, George-Hyslop PH, Pericak-Vance MA, Joo SH, et al. Association of apolipoprotein E allele epsilon 4 with late-onset familial and sporadic Alzheimer's disease. *Neurology*. (1993) 43:1467–72. doi: 10.1212/WNL.43.8.1467

75. Sun YY, Wang Z, Huang HC. Roles of ApoE4 on the pathogenesis in Alzheimer's disease and the potential therapeutic approaches. *Cell Mol Neurobiol*. (2023) 43:3115–36. doi: 10.1007/s10571-023-01365-1

76. Blumcke I, Brockhaus A, Scheiwe C, Rollbrocker B, Wolf HK, Elger CE, et al. The apolipoprotein E epsilon 4 allele is not associated with early onset temporal lobe epilepsy. *Neuroreport*. (1997) 8:1235–7. doi: 10.1097/00001756-199703240-00035

77. Liang Y, Zhou Z, Wang H, Cheng X, Zhong S, Zhao C. Association of apolipoprotein E genotypes with epilepsy risk: A systematic review and meta-analysis. *Epilepsy Behav*. (2019) 98:27–35. doi: 10.1016/j.yebeh.2019.06.015

78. Kauffman MA, Consalvo D, Moron DG, Lereis VP, Kochen S. ApoE epsilon4 genotype and the age at onset of temporal lobe epilepsy: a case-control study and meta-analysis. *Epilepsy Res*. (2010) 90:234–9. doi: 10.1016/j.eplepsyres.2010.05.007

79. Gambardella A, Aguglia U, Chifari R, Labate A, Manna I, Serra P, et al. ApoE epsilon4 allele and disease duration affect verbal learning in mild temporal lobe epilepsy. *Epilepsia*. (2005) 46:110–7. doi: 10.1111/j.0013-9580.2005.15804.x

80. Hunter JM, Cirrito JR, Restivo JL, Kinley RD, Sullivan PM, Holtzman DM, et al. Emergence of a seizure phenotype in aged apolipoprotein epsilon 4 targeted replacement mice. *Brain Res*. (2012) 1467:120–32. doi: 10.1016/j.brainres.2012.05.048

81. Chernick D, Ortiz-Valle S, Jeong A, Qu W, Li L. Peripheral versus central nervous system APOE in Alzheimer's disease: interplay across the blood-brain barrier. *Neurosci Lett*. (2019) 708:134306. doi: 10.1016/j.neulet.2019.134306

82. Eichner JE, Dunn ST, Perveen G, Thompson DM, Stewart KE, Stroehla BC. Apolipoprotein E polymorphism and cardiovascular disease: a HuGE review. *Am J Epidemiol*. (2002) 155:487–95. doi: 10.1093/aje/155.6.487

83. Martins IJ, Hone E, Foster JK, Sunram-Lea SI, Gnjec A, Fuller SJ, et al. Apolipoprotein E, cholesterol metabolism, diabetes, and the convergence of risk factors for Alzheimer's disease and cardiovascular disease. *Mol Psychiatry*. (2006) 11:721–36. doi: 10.1038/sj.mp.4001854

84. Lin YT, Seo J, Gao F, Feldman HM, Wen HL, Penney J, et al. APOE4 causes widespread molecular and cellular alterations associated with Alzheimer's disease phenotypes in human iPSC-derived brain cell types. *Neuron*. (2018) 98:1294. doi: 10.1016/j.neuron.2018.06.011

85. Xiaoqin Z, Zhengli L, Changgeng Z, Xiaojing W, Li L. Changes in behavior and amino acid neurotransmitters in the brain of rats with seizure induced by IL-1beta or IL-6. *J Huazhong Univ Sci Technolog Med Sci*. (2005) 25:236–9. doi: 10.1007/BF02828129

86. Guo J, Chi S, Xu H, Jin G, Qi Z. Effects of cholesterol levels on the excitability of rat hippocampal neurons. *Mol Membr Biol*. (2008) 25:216–23. doi: 10.1080/09687680701805541

87. Malnar M, Hecimovic S, Mattsson N, Zetterberg H. Bidirectional links between Alzheimer's disease and Niemann-pick type C disease. *Neurobiol Dis*. (2014) 72:37–47. doi: 10.1016/j.nbd.2014.05.033

88. Vivas O, Tiscione SA, Dixon RE, Ory DS, Dickson EJ. Niemann-pick type C disease reveals a link between lysosomal cholesterol and PtdIns (4, 5) P (2) that regulates neuronal excitability. *Cell Rep*. (2019) 27:2636–2648 e 2634. doi: 10.1016/j.celrep.2019.04.099

89. Dai MH, Zheng H, Zeng LD, Zhang Y. The genes associated with early-onset Alzheimer's disease. *Oncotarget*. (2018) 9:15132–43. doi: 10.18632/oncotarget.23738

90. Kim DH, Yeo SH, Park JM, Choi JY, Lee TH, Park SY, et al. Genetic markers for diagnosis and pathogenesis of Alzheimer's disease. *Gene*. (2014) 545:185–93. doi: 10.1016/j.gene.2014.05.031

91. Lanoiselee HM, Nicolas G, Wallon D, Rovelet-Lecrux A, Lacour M, Rousseau S, et al. APP, PSEN1, and PSEN2 mutations in early-onset Alzheimer disease: A genetic screening study of familial and sporadic cases. *PLoS Med*. (2017) 14:e1002270. doi: 10.1371/journal.pmed.1002270

92. Ovchinnikov DA, Korn O, Virshup I, Wells CA, Wolvetang EJ. The impact of APP on Alzheimer-like pathogenesis and gene expression in down syndrome iPSC-derived neurons. *Stem Cell Rep*. (2018) 11:32–42. doi: 10.1016/j.stemcr.2018.05.004

93. Sleegers K, Brouwers N, Gijselinck I, Theuns J, Goossens D, Wauters J, et al. APP duplication is sufficient to cause early onset Alzheimer's dementia with cerebral amyloid angiopathy. *Brain*. (2006) 129:2977–83. doi: 10.1093/brain/awl203

94. De Strooper B, Iwatsubo T, Wolfe MS. Presenilins and gamma-secretase: structure, function, and role in Alzheimer disease. *Cold Spring Harb Perspect Med*. (2012) 2:a006304. doi: 10.1101/cshperspect.a006304

95. Gu L, Guo Z. Alzheimer's Abeta42 and Abeta40 peptides form interlaced amyloid fibrils. *J Neurochem*. (2013) 126:305–11. doi: 10.1111/jnc.12202

96. Kim J, Onstead L, Randle S, Price R, Smithson L, Zwizinski C, et al. Abeta40 inhibits amyloid deposition in vivo. *J Neurosci*. (2007) 27:627–33. doi: 10.1523/JNEUROSCI.4849-06.2007

97. Kuperstein I, Broersen K, Benilova I, Rozenski J, Jonckheere W, Debulpaep M, et al. Neurotoxicity of Alzheimer's disease Abeta peptides is induced by small changes in the Abeta42 to Abeta40 ratio. *EMBO J*. (2010) 29:3408–20. doi: 10.1038/emboj.2010.211

98. Pauwels K, Williams TL, Morris KL, Jonckheere W, Vandersteen A, Kelly G, et al. Structural basis for increased toxicity of pathological abeta42: abeta 40 ratios in Alzheimer disease. *J Biol Chem*. (2012) 287:5650–60. doi: 10.1074/jbc.M111.264473

99. Brouwers N, Sleegers K, Van Broeckhoven C. Molecular genetics of Alzheimer's disease: an update. *Ann Med*. (2008) 40:562–83. doi: 10.1080/07853890802186905

100. Mol MO, van der Lee SJ, Hulsman M, Pijnenburg YAL, Scheltens P, Netherlands Brain Bank, et al. Mapping the genetic landscape of early-onset Alzheimer's disease in a cohort of 36 families. *Alzheimers Res Ther*. (2022) 14:77. doi: 10.1186/s13195-022-01018-3

101. Wingo TS, Lah JJ, Levey AI, Cutler DJ. Autosomal recessive causes likely in early-onset Alzheimer disease. *Arch Neurol*. (2012) 69:59–64. doi: 10.1001/archneurol.2011.221

102. Rahman MM, Fatema K. Seizures in down syndrome: An update. *Mymensingh Med J*. (2019) 28:712–5.

103. Salehi A, Ashford JW, Mufson EJ. The link between Alzheimer's disease and down syndrome. A historical perspective. *Curr Alzheimer Res*. (2016) 13:2–6. doi: 10.2174/1567205012999151021102914

104. Fortea J, Zaman SH, Hartley S, Rafii MS, Head E, Carmona-Iragui M. Alzheimer's disease associated with down syndrome: a genetic form of dementia. *Lancet Neurol*. (2021) 20:930–42. doi: 10.1016/S1474-4422(21)00245-3

105. Lott IT, Head E. Dementia in down syndrome: unique insights for Alzheimer disease research. *Nat Rev Neurol*. (2019) 15:135–47. doi: 10.1038/s41582-018-0132-6

106. Kruger R, Kuhn W, Muller T, Woitalla D, Graeber M, Kosel S, et al. Ala30Pro mutation in the gene encoding alpha-synuclein in Parkinson's disease. *Nat Genet*. (1998) 18:106–8. doi: 10.1038/ng0298-106

107. Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, et al. Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. *Science*. (1997) 276:2045–7. doi: 10.1126/science.276.5321.2045

108. Zarranz JJ, Alegre J, Gomez-Esteban JC, Lezcano E, Ros R, Ampuero I, et al. The new mutation, E46K, of alpha-synuclein causes Parkinson and Lewy body dementia. *Ann Neurol*. (2004) 55:164–73. doi: 10.1002/ana.10795

109. Mehra S, Sahay S, Maji SK. Alpha-Synuclein misfolding and aggregation: implications in Parkinson's disease pathogenesis. *Biochim Biophys Acta Proteins Proteom*. (2019) 1867:890–908. doi: 10.1016/j.bbapap.2019.03.001

110. Teravskis PJ, Covelo A, Miller EC, Singh B, Martell-Martinez HA, Benneyworth MA, et al. A53T mutant alpha-Synuclein induces tau-dependent postsynaptic impairment independently of neurodegenerative changes. *J Neurosci*. (2018) 38:9754–67. doi: 10.1523/jneurosci.0344-18.2018

111. Duda JE, Giasson BI, Mabon ME, Miller DC, Golbe LI, Lee VM, et al. Concurrence of alpha-synuclein and tau brain pathology in the Contursi kindred. *Acta Neuropathol*. (2002) 104:7–11. doi: 10.1007/s00401-002-0563-3

112. Lee MK, Stirling W, Xu Y, Xu X, Qui D, Mandir AS, et al. Human α-synuclein-harboring familial Parkinson's disease-linked ala-53 → Thr mutation causes neurodegenerative disease with α-synuclein aggregation in transgenic mice. *Proc Natl Acad Sci U S A*. (2002) 99:8968–73. doi: 10.1073/pnas.132197599

113. Gigout S, Wierschke S, Lehmann TN, Horn P, Dehnicke C, Deisz RA. Muscarinic acetylcholine receptor-mediated effects in slices from human epileptogenic cortex. *Neuroscience*. (2012) 223:399–411. doi: 10.1016/j.neuroscience.2012.07.044

114. Picciotto MR, Higley MJ, Mineur YS. Acetylcholine as a neuromodulator: cholinergic signaling shapes nervous system function and behavior. *Neuron*. (2012) 76:116–29. doi: 10.1016/j.neuron.2012.08.036

115. Chen ZR, Huang JB, Yang SL, Hong FF. Role of cholinergic signaling in Alzheimer's disease. *Molecules*. (2022) 27:1816. doi: 10.3390/molecules27061816

116. Duda JE. Pathology and neurotransmitter abnormalities of dementia with Lewy bodies. *Dement Geriatr Cogn Disord*. (2004) 17:3–14. doi: 10.1159/000074677

117. Barber R, Ballard C, Mckeith IG, Gholkar A, O'brien JT. MRI volumetric study of dementia with Lewy bodies: a comparison with AD and vascular dementia. *Neurology*. (2000) 54:1304–9. doi: 10.1212/WNL.54.6.1304

118. Andersson M, Hansson O, Minthon L, Rosen I, Londos E. Electroencephalogram variability in dementia with lewy bodies, Alzheimer's disease and controls. *Dement Geriatr Cogn Disord*. (2008) 26:284–90. doi: 10.1159/000160962

119. Bonanni L, Thomas A, Tiraboschi P, Perfetti B, Varanese S, Onofrj M. EEG comparisons in early Alzheimer's disease, dementia with Lewy bodies and Parkinson's disease with dementia patients with a 2-year follow-up. *Brain*. (2008) 131:690–705. doi: 10.1093/brain/awm322

120. Lam B, Hollingdrake E, Kennedy JL, Black SE, Masellis M. Cholinesterase inhibitors in Alzheimer's disease and Lewy body spectrum disorders: the emerging pharmacogenetic story. *Hum Genomics*. (2009) 4:91–106. doi: 10.1186/1479-7364-4-2-91

121. Simard M, Van Reekum R. The acetylcholinesterase inhibitors for treatment of cognitive and behavioral symptoms in dementia with Lewy bodies. *J Neuropsychiatry Clin Neurosci*. (2004) 16:409–25. doi: 10.1176/jnp.16.4.409

122. Kam K, Duffy AM, Moretto J, Lafrancois JJ, Scharfman HE. Interictal spikes during sleep are an early defect in the Tg2576 mouse model of beta-amyloid neuropathology. *Sci Rep*. (2016) 6:20119. doi: 10.1038/srep20119

123. Jin N, Ziyatdinova S, Gureviciene I, Tanila H. Response of spike-wave discharges in aged APP/PS1 Alzheimer model mice to antiepileptic, metabolic and cholinergic drugs. *Sci Rep*. (2020) 10:11851. doi: 10.1038/s41598-020-68845-y

124. Schousboe A, Scafidi S, Bak LK, Waagepetersen HS, Mckenna MC. Glutamate metabolism in the brain focusing on astrocytes. *Adv Neurobiol*. (2014) 11:13–30. doi: 10.1007/978-3-319-08894-5_2

125. Albrecht J, Zielinska M. Mechanisms of excessive extracellular glutamate accumulation in temporal lobe epilepsy. *Neurochem Res*. (2017) 42:1724–34. doi: 10.1007/s11064-016-2105-8

126. Czapski GA, Strosznajder JB. Glutamate and GABA in microglia-neuron cross-talk in Alzheimer's disease. *Int J Mol Sci*. (2021) 22:11677. doi: 10.3390/ijms222111677

127. Sarac S, Afzal S, Broholm H, Madsen FF, Ploug T, Laursen H. Excitatory amino acid transporters EAAT-1 and EAAT-2 in temporal lobe and hippocampus in intractable temporal lobe epilepsy. *APMIS*. (2009) 117:291–301. doi: 10.1111/j.1600-0463.2009.02443.x

128. Soni N, Reddy BV, Kumar P. GLT-1 transporter: an effective pharmacological target for various neurological disorders. *Pharmacol Biochem Behav*. (2014) 127:70–81. doi: 10.1016/j.pbb.2014.10.001

129. Chivukula AS, Suslova M, Kortzak D, Kovermann P, Fahlke C. Functional consequences of SLC1A3 mutations associated with episodic ataxia 6. *Hum Mutat*. (2020) 41:1892–905. doi: 10.1002/humu.24089

130. Kovermann P, Kolobkova Y, Franzen A, Fahlke C. Mutations associated with epileptic encephalopathy modify EAAT2 anion channel function. *Epilepsia*. (2022) 63:388–401. doi: 10.1111/epi.17154

131. De Ceglia R, Ledonne A, Litvin DG, Lind BL, Carriero G, Latagliata EC, et al. Specialized astrocytes mediate glutamatergic gliotransmission in the CNS. *Nature*. (2023) 622:120–9. doi: 10.1038/s41586-023-06502-w

132. Mahmoud S, Gharagozloo M, Simard C, Gris D. Astrocytes maintain glutamate homeostasis in the CNS by controlling the balance between glutamate uptake and release. *Cell*. (2019) 8:184. doi: 10.3390/cells8020184

133. Robinson MB. The family of sodium-dependent glutamate transporters: a focus on the GLT-1/EAAT2 subtype. *Neurochem Int*. (1998) 33:479–91. doi: 10.1016/S0197-0186(98)00055-2

134. Garcia-Esparcia P, Diaz-Lucena D, Ainciburu M, Torrejon-Escribano B, Carmona M, Llorens F, et al. Glutamate transporter GLT1 expression in Alzheimer disease and dementia with Lewy bodies. *Front Aging Neurosci*. (2018) 10:122. doi: 10.3389/fnagi.2018.00122

135. Liraz O, Boehm-Cagan A, Michaelson DM. ApoE4 induces Abeta42, tau, and neuronal pathology in the hippocampus of young targeted replacement apoE4 mice. *Mol Neurodegener*. (2013) 8:16.

136. Kirvell SL, Esiri M, Francis PT. Down-regulation of vesicular glutamate transporters precedes cell loss and pathology in Alzheimer's disease. *J Neurochem*. (2006) 98:939–50. doi: 10.1111/j.1471-4159.2006.03935.x

137. Takahashi K, Foster JB, Lin CL. Glutamate transporter EAAT2: regulation, function, and potential as a therapeutic target for neurological and psychiatric disease. *Cell Mol Life Sci*. (2015) 72:3489–506. doi: 10.1007/s00018-015-1937-8

138. Takahashi K, Kong Q, Lin Y, Stouffer N, Schulte DA, Lai L, et al. Restored glial glutamate transporter EAAT2 function as a potential therapeutic approach for Alzheimer's disease. *J Exp Med*. (2015) 212:319–32. doi: 10.1084/jem.20140413

139. Scott HL, Pow DV, Tannenberg AE, Dodd PR. Aberrant expression of the glutamate transporter excitatory amino acid transporter 1 (EAAT1) in Alzheimer's disease. *J Neurosci*. (2002) 22:RC206. doi: 10.1523/JNEUROSCI.22-03-j0004.2002

140. Scott HA, Gebhardt FM, Mitrovic AD, Vandenberg RJ, Dodd PR. Glutamate transporter variants reduce glutamate uptake in Alzheimer's disease. *Neurobiol Aging*. (2011) 32:553.e1–553.e11. doi: 10.1016/j.neurobiolaging.2010.03.008

141. Hunsberger HC, Weitzner DS, Rudy CC, Hickman JE, Libell EM, Speer RR, et al. Riluzole rescues glutamate alterations, cognitive deficits, and tau pathology associated with P301L tau expression. *J Neurochem*. (2015) 135:381–94. doi: 10.1111/jnc.13230

142. Alijanpour S, Miryounesi M, Ghafouri-Fard S. The role of excitatory amino acid transporter 2 (EAAT2) in epilepsy and other neurological disorders. *Metab Brain Dis*. (2023) 38:1–16. doi: 10.1007/s11011-022-01091-5

143. Mattson MP. Glutamate and neurotrophic factors in neuronal plasticity and disease. *Ann N Y Acad Sci*. (2008) 1144:97–112. doi: 10.1196/annals.1418.005

144. Tymianski M, Charlton MP, Carlen PL, Tator CH. Source specificity of early calcium neurotoxicity in cultured embryonic spinal neurons. *J Neurosci*. (1993) 13:2085–104. doi: 10.1523/JNEUROSCI.13-05-02085.1993

145. Dubinsky JM. Intracellular calcium levels during the period of delayed excitotoxicity. *J Neurosci*. (1993) 13:623–31. doi: 10.1523/JNEUROSCI.13-02-00623.1993

146. Siman R, Noszek JC, Kegerise C. Calpain I activation is specifically related to excitatory amino acid induction of hippocampal damage. *J Neurosci*. (1989) 9:1579–90. doi: 10.1523/JNEUROSCI.09-05-01579.1989

147. Lazarewicz JW, Wroblewski JT, Costa E. N-methyl-D-aspartate-sensitive glutamate receptors induce calcium-mediated arachidonic acid release in primary cultures of cerebellar granule cells. *J Neurochem*. (1990) 55:1875–81. doi: 10.1111/j.1471-4159.1990.tb05771.x

148. Danysz W, Parsons CG, Mobius HJ, Stoffler A, Quack G. Neuroprotective and symptomatological action of memantine relevant for alzheimer's disease — a unified glutamatergic hypothesis on the mechanism of action. *Neurotox Res*. (2000) 2:85–97. doi: 10.1007/BF03033787

149. Wenk GL. Neuropathologic changes in Alzheimer's disease: potential targets for treatment. *J Clin Psychiatry*. (2006) 67:3–7.

150. Barker-Haliski M, White HS. Glutamatergic mechanisms associated with seizures and epilepsy. *Cold Spring Harb Perspect Med*. (2015) 5:a022863. doi: 10.1101/cshperspect.a022863

151. Green JL, Dos Santos WF, Fontana ACK. Role of glutamate excitotoxicity and glutamate transporter EAAT2 in epilepsy: opportunities for novel therapeutics development. *Biochem Pharmacol*. (2021) 193:114786. doi: 10.1016/j.bcp.2021.114786

152. Hanada T. Ionotropic glutamate receptors in epilepsy: A review focusing on AMPA and NMDA receptors. *Biomol Ther*. (2020) 10:464. doi: 10.3390/biom10030464

153. Srivastava A, Das B, Yao AY, Yan R. Metabotropic glutamate receptors in Alzheimer's disease synaptic dysfunction: therapeutic opportunities and Hope for the future. *J Alzheimers Dis*. (2020) 78:1345–61. doi: 10.3233/jad-201146

154. Mendez-Armenta M, Nava-Ruiz C, Juarez-Rebollar D, Rodriguez-Martinez E, Gomez PY. Oxidative stress associated with neuronal apoptosis in experimental models of epilepsy. *Oxidative Med Cell Longev*. (2014) 2014:293689:1–12. doi: 10.1155/2014/293689

155. Hanson JE, Ma K, Elstrott J, Weber M, Saillet S, Khan AS, et al. GluN2A NMDA receptor enhancement improves brain oscillations, synchrony, and cognitive functions in Dravet syndrome and Alzheimer's disease models. *Cell Rep*. (2020) 30:e384:381–396.e4. doi: 10.1016/j.celrep.2019.12.030

156. Lerdkrai C, Asavapanumas N, Brawek B, Kovalchuk Y, Mojtahedi N, Olmedillas Del Moral M, et al. Intracellular ca(2+) stores control in vivo neuronal hyperactivity in a mouse model of Alzheimer's disease. *Proc Natl Acad Sci U S A*. (2018) 115:E1279–88. doi: 10.1073/pnas.1714409115

157. Matsunaga S, Kishi T, Iwata N. Memantine monotherapy for Alzheimer's disease: a systematic review and meta-analysis. *PLoS One*. (2015) 10:e0123289. doi: 10.1371/journal.pone.0123289

158. Brines ML, Sundaresan S, Spencer DD, De Lanerolle NC. Quantitative autoradiographic analysis of ionotropic glutamate receptor subtypes in human temporal lobe epilepsy: up-regulation in reorganized epileptogenic hippocampus. *Eur J Neurosci*. (1997) 9:2035–44. doi: 10.1111/j.1460-9568.1997.tb01371.x

159. Graebenitz S, Kedo O, Speckmann EJ, Gorji A, Panneck H, Hans V, et al. Interictal-like network activity and receptor expression in the epileptic human lateral amygdala. *Brain*. (2011) 134:2929–47. doi: 10.1093/brain/awr202

160. Mathern GW, Pretorius JK, Leite JP, Kornblum HI, Mendoza D, Lozada A, et al. Hippocampal AMPA and NMDA mRNA levels and subunit immunoreactivity in human temporal lobe epilepsy patients and a rodent model of chronic mesial limbic epilepsy. *Epilepsy Res*. (1998) 32:154–71. doi: 10.1016/S0920-1211(98)00048-5

161. Ying Z, Babb TL, Comair YG, Bushey M, Touhalisky K. Increased densities of AMPA Glu R1 subunit proteins and presynaptic mossy fiber sprouting in the fascia dentata of human hippocampal epilepsy. *Brain Res*. (1998) 798:239–46. doi: 10.1016/S0006-8993(98)00421-1

162. Saxton RA, Sabatini DM. mTOR signaling in growth, metabolism, and disease. *Cell*. (2017) 169:361–71. doi: 10.1016/j.cell.2017.03.035

163. Bockaert J, Marin P. mTOR in brain physiology and pathologies. *Physiol Rev*. (2015) 95:1157–87. doi: 10.1152/physrev.00038.2014

164. Stavoe AKH, Holzbaur ELF. Autophagy in neurons. *Annu Rev Cell Dev Biol*. (2019) 35:477–500. doi: 10.1146/annurev-cellbio-100818-125242

165. Overhoff M, Tellkamp F, Hess S, Tolve M, Tutas J, Faerfers M, et al. Autophagy regulates neuronal excitability by controlling cAMP/protein kinase A signaling at the synapse. *EMBO J*. (2022) 41:e110963. doi: 10.15252/embj.2022110963

166. Butler CR, Boychuk JA, Smith BN. Effects of rapamycin treatment on neurogenesis and synaptic reorganization in the dentate gyrus after controlled cortical impact injury in mice. *Front Syst Neurosci*. (2015) 9:163. doi: 10.3389/fnsys.2015.00163

167. Citraro R, Leo A, Constanti A, Russo E, De Sarro G. mTOR pathway inhibition as a new therapeutic strategy in epilepsy and epileptogenesis. *Pharmacol Res*. (2016) 107:333–43. doi: 10.1016/j.phrs.2016.03.039

168. Zeng LH, Xu L, Gutmann DH, Wong M. Rapamycin prevents epilepsy in a mouse model of tuberous sclerosis complex. *Ann Neurol*. (2008) 63:444–53. doi: 10.1002/ana.21331

169. Zeng LH, Rensing NR, Wong M. The mammalian target of rapamycin signaling pathway mediates epileptogenesis in a model of temporal lobe epilepsy. *J Neurosci*. (2009) 29:6964–72. doi: 10.1523/JNEUROSCI.0066-09.2009

170. An WL, Cowburn RF, Li L, Braak H, Alafuzoff I, Iqbal K, et al. Up-regulation of phosphorylated/activated p 70 S6 kinase and its relationship to neurofibrillary pathology in Alzheimer's disease. *Am J Pathol*. (2003) 163:591–607. doi: 10.1016/S0002-9440(10)63687-5

171. Crews L, Spencer B, Desplats P, Patrick C, Paulino A, Rockenstein E, et al. Selective molecular alterations in the autophagy pathway in patients with Lewy body disease and in models of alpha-synucleinopathy. *PLoS One*. (2010) 5:e9313. doi: 10.1371/journal.pone.0009313

172. Hou X, Watzlawik JO, Fiesel FC, Springer W. Autophagy in Parkinson's disease. *J Mol Biol*. (2020) 432:2651–72. doi: 10.1016/j.jmb.2020.01.037

173. Li X, Alafuzoff I, Soininen H, Winblad B, Pei JJ. Levels of mTOR and its downstream targets 4E-BP1, eEF2, and eEF2 kinase in relationships with tau in Alzheimer's disease brain. *FEBS J*. (2005) 272:4211–20. doi: 10.1111/j.1742-4658.2005.04833.x

174. Gourmaud S, Stewart DA, Irwin DJ, Roberts N, Barbour AJ, Eberwine G, et al. The role of mTORC1 activation in seizure-induced exacerbation of Alzheimer's disease. *Brain*. (2022) 145:324–39. doi: 10.1093/brain/awab268

175. Spilman P, Podlutskaya N, Hart MJ, Debnath J, Gorostiza O, Bredesen D, et al. Inhibition of mTOR by rapamycin abolishes cognitive deficits and reduces amyloid-beta levels in a mouse model of Alzheimer's disease. *PLoS One*. (2010) 5:e9979. doi: 10.1371/journal.pone.0009979

176. Fortin DL, Troyer MD, Nakamura K, Kubo S, Anthony MD, Edwards RH. Lipid rafts mediate the synaptic localization of alpha-synuclein. *J Neurosci*. (2004) 24:6715–23. doi: 10.1523/JNEUROSCI.1594-04.2004

177. Maroteaux L, Campanelli JT, Scheller RH. Synuclein: a neuron-specific protein localized to the nucleus and presynaptic nerve terminal. *J Neurosci*. (1988) 8:2804–15. doi: 10.1523/JNEUROSCI.08-08-02804.1988

178. Cabin DE, Shimazu K, Murphy D, Cole NB, Gottschalk W, Mcilwain KL, et al. Synaptic vesicle depletion correlates with attenuated synaptic responses to prolonged

repetitive stimulation in mice lacking alpha-synuclein. *J Neurosci*. (2002) 22:8797–807. doi: 10.1523/JNEUROSCI.22-20-08797.2002

179. Cheng F, Vivacqua G, Yu S. The role of alpha-synuclein in neurotransmission and synaptic plasticity. *J Chem Neuroanat*. (2011) 42:242–8. doi: 10.1016/j.jchemneu.2010.12.001

180. Lee HJ, Bae EJ, Lee SJ. Extracellular α-synuclein—a novel and crucial factor in Lewy body diseases. *Nat Rev Neurol*. (2014) 10:92–8. doi: 10.1038/nrneurol.2013.275

181. Paillusson S, Clairembault T, Biraud M, Neunlist M, Derkinderen P. Activity-dependent secretion of alpha-synuclein by enteric neurons. *J Neurochem*. (2013) 125:512–7. doi: 10.1111/jnc.12131

182. Choi J, Kim SY, Kim H, Lim BC, Hwang H, Chae JH, et al. Serum alpha-synuclein and IL-1beta are increased and correlated with measures of disease severity in children with epilepsy: potential prognostic biomarkers? *BMC Neurol*. (2020) 20:85. doi: 10.1186/s12883-020-01662-y

183. Hussein AM, Eldosoky M, El-Shafey M, El-Mesery M, Ali AN, Abbas KM, et al. Effects of metformin on apoptosis and alpha-synuclein in a rat model of pentylenetetrazole-induced epilepsy. *Can J Physiol Pharmacol*. (2019) 97:37–46. doi: 10.1139/cjpp-2018-0266

184. Li A, Choi YS, Dziema H, Cao R, Cho HY, Jung YJ, et al. Proteomic profiling of the epileptic dentate gyrus. *Brain Pathol*. (2010) 20:1077–89. doi: 10.1111/j.1750-3639.2010.00414.x

185. Yang JW, Czech T, Felizardo M, Baumgartner C, Lubec G. Aberrant expression of cytoskeleton proteins in hippocampus from patients with mesial temporal lobe epilepsy. *Amino Acids*. (2006) 30:477–93. doi: 10.1007/s00726-005-0281-y

186. Tweedy C, Kindred N, Curry J, Williams C, Taylor JP, Atkinson P, et al. Hippocampal network hyperexcitability in young transgenic mice expressing human mutant alpha-synuclein. *Neurobiol Dis*. (2021) 149:105226. doi: 10.1016/j.nbd.2020.105226

187. Marques O, Outeiro TF. Alpha-synuclein: from secretion to dysfunction and death. *Cell Death Dis*. (2012) 3:e350. doi: 10.1038/cddis.2012.94

188. Gotz J, Probst A, Spillantini MG, Schafer T, Jakes R, Burki K, et al. Somatodendritic localization and hyperphosphorylation of tau protein in transgenic mice expressing the longest human brain tau isoform. *EMBO J*. (1995) 14:1304–13. doi: 10.1002/j.1460-2075.1995.tb07116.x

189. Papasozomenos SC, Binder LI. Phosphorylation determines two distinct species of tau in the central nervous system. *Cell Motil Cytoskeleton*. (1987) 8:210–26. doi: 10.1002/cm.970080303

190. Kovacs GG. Tauopathies. *Handb Clin Neurol*. (2017) 145:355–68. doi: 10.1016/B978-0-12-802395-2.00025-0

191. Meng JX, Zhang Y, Saman D, Haider AM, De S, Sang JC, et al. Hyperphosphorylated tau self-assembles into amorphous aggregates eliciting TLR4-dependent responses. *Nat Commun*. (2022) 13:2692. doi: 10.1038/s41467-022-30461-x

192. Xia Y, Prokop S, Gorion KM, Kim JD, Sorrentino ZA, Bell BM, et al. Tau Ser 208 phosphorylation promotes aggregation and reveals neuropathologic diversity in Alzheimer's disease and other tauopathies. *Acta Neuropathol Commun*. (2020) 8:88. doi: 10.1186/s40478-020-00967-w

193. Thom M, Liu JY, Thompson P, Phadke R, Narkiewicz M, Martinian L, et al. Neurofibrillary tangle pathology and Braak staging in chronic epilepsy in relation to traumatic brain injury and hippocampal sclerosis: a post-mortem study. *Brain*. (2011) 134:2969–81. doi: 10.1093/brain/awr209

194. Concepcion FA, Ekstrom NA, Khan MN, Estes OO, Poolos NP. Progressive dysregulation of tau phosphorylation in an animal model of temporal lobe epilepsy. *Neuroscience*. (2023) 522:42–56. doi: 10.1016/j.neuroscience.2023.04.020

195. Putra M, Puttachary S, Liu G, Lee G, Thippeswamy T. Fyn-tau ablation modifies PTZ-induced seizures and post-seizure hallmarks of early Epileptogenesis. *Front Cell Neurosci*. (2020) 14:592374. doi: 10.3389/fncel.2020.592374

196. Roberson ED, Halabisky B, Yoo JW, Yao J, Chin J, Yan F, et al. Amyloid-beta/Fyn-induced synaptic, network, and cognitive impairments depend on tau levels in multiple mouse models of Alzheimer's disease. *J Neurosci*. (2011) 31:700–11. doi: 10.1523/JNEUROSCI.4152-10.2011

197. Maeda S, Djukic B, Taneja P, Yu GQ, Lo I, Davis A, et al. Expression of A152T human tau causes age-dependent neuronal dysfunction and loss in transgenic mice. *EMBO Rep*. (2016) 17:530–51. doi: 10.15252/embr.201541438

198. Crimins JL, Rocher AB, Luebke JI. Electrophysiological changes precede morphological changes to frontal cortical pyramidal neurons in the rTg4510 mouse model of progressive tauopathy. *Acta Neuropathol*. (2012) 124:777–95. doi: 10.1007/s00401-012-1038-9

199. Witton J, Staniaszek LE, Bartsch U, Randall AD, Jones MW, Brown JT. Disrupted hippocampal sharp-wave ripple-associated spike dynamics in a transgenic mouse model of dementia. *J Physiol*. (2016) 594:4615–30. doi: 10.1113/jphysiol.2014.282889

200. Decker JM, Kruger L, Sydow A, Dennissen FJ, Siskova Z, Mandelkow E, et al. The tau/A152T mutation, a risk factor for frontotemporal-spectrum disorders, leads to NR2B receptor-mediated excitotoxicity. *EMBO Rep*. (2016) 17:552–69. doi: 10.15252/embr.201541439

201. Kilian JG, Hsu HW, Mata K, Wolf FW, Kitazawa M. Astrocyte transport of glutamate and neuronal activity reciprocally modulate tau pathology in Drosophila. *Neuroscience*. (2017) 348:191–200. doi: 10.1016/j.neuroscience.2017.02.011

202. Devos SL, Goncharoff DK, Chen G, Kebodeaux CS, Yamada K, Stewart FR, et al. Antisense reduction of tau in adult mice protects against seizures. *J Neurosci*. (2013) 33:12887–97. doi: 10.1523/JNEUROSCI.2107-13.2013

203. Palop JJ, Mucke L. Network abnormalities and interneuron dysfunction in Alzheimer disease. *Nat Rev Neurosci*. (2016) 17:777–92. doi: 10.1038/nrn.2016.141

204. Costa C, Romoli M, Calabresi P. Late onset epilepsy and Alzheimer's disease: exploring the dual pathogenic role of amyloid-beta. *Brain*. (2018) 141:e60. doi: 10.1093/brain/awy162

205. Ovsepian SV, O'leary VB. Neuronal activity and amyloid plaque pathology: an update. *J Alzheimers Dis*. (2016) 49:13–9. doi: 10.3233/JAD-150544

206. Busche MA, Eichhoff G, Adelsberger H, Abramowski D, Wiederhold KH, Haass C, et al. Clusters of hyperactive neurons near amyloid plaques in a mouse model of Alzheimer's disease. *Science*. (2008) 321:1686–9. doi: 10.1126/science.1162844

207. Minkeviciene R, Rheims S, Dobszay MB, Zilberter M, Hartikainen J, Fulop L, et al. Amyloid beta-induced neuronal hyperexcitability triggers progressive epilepsy. *J Neurosci*. (2009) 29:3453–62. doi: 10.1523/JNEUROSCI.5215-08.2009

208. Vande Vyver M, Barker-Haliski M, Aourz N, Nagels G, Bjerke M, Engelborghs S, et al. Higher susceptibility to 6 Hz corneal kindling and lower responsiveness to antiseizure drugs in mouse models of Alzheimer's disease. *Epilepsia*. (2022) 63:2703–15. doi: 10.1111/epi.17355

209. Ziyatdinova S, Ronnback A, Gurevicius K, Miszczuk D, Graff C, Winblad B, et al. Increased epileptiform EEG activity and decreased seizure threshold in Arctic APP transgenic mouse model of Alzheimer's disease. *Curr Alzheimer Res*. (2016) 13:817–30. doi: 10.2174/1567205013666160129095508

210. Shankar GM, Li S, Mehta TH, Garcia-Munoz A, Shepardson NE, Smith I, et al. Amyloid-beta protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. *Nat Med*. (2008) 14:837–42. doi: 10.1038/nm1782

211. Ping Y, Hahm ET, Waro G, Song Q, Vo-Ba DA, Licursi A, et al. Linking abeta42-induced hyperexcitability to neurodegeneration, learning and motor deficits, and a shorter lifespan in an Alzheimer's model. *PLoS Genet*. (2015) 11:e1005025. doi: 10.1371/journal.pgen.1005025

212. Ho R, Ortiz D, Shea TB. Amyloid-beta promotes calcium influx and neurodegeneration via stimulation of L voltage-sensitive calcium channels rather than NMDA channels in cultured neurons. *J Alzheimers Dis*. (2001) 3:479–83. doi: 10.3233/JAD-2001-3507

213. Talantova M, Sanz-Blasco S, Zhang X, Xia P, Akhtar MW, Okamoto S, et al. Abeta induces astrocytic glutamate release, extrasynaptic NMDA receptor activation, and synaptic loss. *Proc Natl Acad Sci U S A*. (2013) 110:E2518–27. doi: 10.1073/pnas.1306832110

214. Zott B, Simon MM, Hong W, Unger F, Chen-Engerer HJ, Frosch MP, et al. A vicious cycle of beta amyloid-dependent neuronal hyperactivation. *Science*. (2019) 365:559–65. doi: 10.1126/science.aay0198

215. Glass CK, Saijo K, Winner B, Marchetto MC, Gage FH. Mechanisms underlying inflammation in neurodegeneration. *Cell*. (2010) 140:918–34. doi: 10.1016/j.cell.2010.02.016

216. Patani R, Hardingham GE, Liddelow SA. Functional roles of reactive astrocytes in neuroinflammation and neurodegeneration. *Nat Rev Neurol*. (2023) 19:395–409. doi: 10.1038/s41582-023-00822-1

217. Bernaus A, Blanco S, Sevilla A. Glia crosstalk in Neuroinflammatory diseases. *Front Cell Neurosci*. (2020) 14:209. doi: 10.3389/fncel.2020.00209

218. Devinsky O, Vezzani A, Najjar S, De Lanerolle NC, Rogawski MA. Glia and epilepsy: excitability and inflammation. *Trends Neurosci*. (2013) 36:174–84. doi: 10.1016/j.tins.2012.11.008

219. Pracucci E, Pillai V, Lamers D, Parra R, Landi S. Neuroinflammation: A signature or a cause of epilepsy? *Int J Mol Sci*. (2021) 22:6981. doi: 10.3390/ijms22136981

220. Sanz P, Garcia-Gimeno MA. Reactive glia inflammatory signaling pathways and epilepsy. *Int J Mol Sci*. (2020) 21:4096. doi: 10.3390/ijms21114096

221. De Vries EE, Van Den Munckhof B, Braun KP, Van Royen-Kerkhof A, De Jager W, Jansen FE. Inflammatory mediators in human epilepsy: A systematic review and meta-analysis. *Neurosci Biobehav Rev*. (2016) 63:177–90. doi: 10.1016/j.neubiorev.2016.02.007

222. Bedner P, Steinhauser C. TNFalpha-driven astrocyte purinergic signaling during Epileptogenesis. *Trends Mol Med*. (2019) 25:70–2. doi: 10.1016/j.molmed.2018.12.001

223. Clark IA, Vissel B. Excess cerebral TNF causing glutamate excitotoxicity rationalizes treatment of neurodegenerative diseases and neurogenic pain by anti-TNF agents. *J Neuroinflammation*. (2016) 13:236. doi: 10.1186/s12974-016-0708-2

224. Kaur D, Sharma V, Deshmukh R. Activation of microglia and astrocytes: a roadway to neuroinflammation and Alzheimer's disease. *Inflammopharmacology*. (2019) 27:663–77. doi: 10.1007/s10787-019-00580-x

225. Vargas-Sanchez K, Mogilevskaya M, Rodriguez-Perez J, Rubiano MG, Javela JJ, Gonzalez-Reyes RE. Astroglial role in the pathophysiology of status epilepticus: an overview. *Oncotarget*. (2018) 9:26954–76. doi: 10.18632/oncotarget.25485

226. Beattie EC, Stellwagen D, Morishita W, Bresnahan JC, Ha BK, Von Zastrow M, et al. Control of synaptic strength by glial TNFalpha. *Science*. (2002) 295:2282–5. doi: 10.1126/science.1067859

227. Henstridge CM, Tzioras M, Paolicelli RC. Glial contribution to excitatory and inhibitory synapse loss in neurodegeneration. *Front Cell Neurosci*. (2019) 13:63. doi: 10.3389/fncel.2019.00063

228. Balosso S, Liu J, Bianchi ME, Vezzani A. Disulfide-containing high mobility group box-1 promotes N-methyl-D-aspartate receptor function and excitotoxicity by activating toll-like receptor 4-dependent signaling in hippocampal neurons. *Antioxid Redox Signal*. (2014) 21:1726–40. doi: 10.1089/ars.2013.5349

229. Maroso M, Balosso S, Ravizza T, Liu J, Aronica E, Iyer AM, et al. Toll-like receptor 4 and high-mobility group box-1 are involved in ictogenesis and can be targeted to reduce seizures. *Nat Med*. (2010) 16:413–9. doi: 10.1038/nm.2127

230. Terrone G, Balosso S, Pauletti A, Ravizza T, Vezzani A. Inflammation and reactive oxygen species as disease modifiers in epilepsy. *Neuropharmacology*. (2020) 167:107742. doi: 10.1016/j.neuropharm.2019.107742

231. Terrone G, Salamone A, Vezzani A. Inflammation and epilepsy: preclinical findings and potential clinical translation. *Curr Pharm Des*. (2017) 23:5569–76. doi: 10.2174/1381612823666170926113754

232. Das M, Mao W, Shao E, Tamhankar S, Yu GQ, Yu X, et al. Interdependence of neural network dysfunction and microglial alterations in Alzheimer's disease-related models. *iScience*. (2021) 24:103245. doi: 10.1016/j.isci.2021.103245

233. Das M, Mao W, Voskobiynyk Y, Necula D, Lew I, Petersen C, et al. Alzheimer risk-increasing TREM2 variant causes aberrant cortical synapse density and promotes network hyperexcitability in mouse models. *Neurobiol Dis*. (2023) 186:106263. doi: 10.1016/j.nbd.2023.106263

234. Morenas-Rodriguez E, Alcolea D, Suarez-Calvet M, Munoz-Llahuna L, Vilaplana E, Sala I, et al. Different pattern of CSF glial markers between dementia with Lewy bodies and Alzheimer's disease. *Sci Rep*. (2019) 9:7803. doi: 10.1038/s41598-019-44173-8

235. Wilson EN, Swarovski MS, Linortner P, Shahid M, Zuckerman AJ, Wang Q, et al. Soluble TREM2 is elevated in Parkinson's disease subgroups with increased CSF tau. *Brain*. (2020) 143:932–43. doi: 10.1093/brain/awaa021

236. Zhou W., Zhou Y., Li J. (2023). Association between cerebrospinal fluid soluble TREM2, Alzheimer's Disease and Other Neurodegenerative Diseases. *J Clin Med* 12:3589. doi: 10.3390/jcm12103589

237. Wang F, Qi X, Zhang J, Huang JH. Astrocytic modulation of potassium under seizures. *Neural Regen Res*. (2020) 15:980–987.

238. Wilcock DM, Vitek MP, Colton CA. Vascular amyloid alters astrocytic water and potassium channels in mouse models and humans with Alzheimer's disease. *Neuroscience*. (2009) 159:1055–69. doi: 10.1016/j.neuroscience.2009.01.023

239. Shapiro LA, Wang L, Ribak CE. Rapid astrocyte and microglial activation following pilocarpine-induced seizures in rats. *Epilepsia*. (2008) 49:33–41. doi: 10.1111/j.1528-1167.2008.01491.x

240. Cohen-Gadol AA, Pan JW, Kim JH, Spencer DD, Hetherington HH. Mesial temporal lobe epilepsy: a proton magnetic resonance spectroscopy study and a histopathological analysis. *J Neurosurg*. (2004) 101:613–20. doi: 10.3171/jns.2004.101.4.0613

241. De Sousa RAL. Reactive gliosis in Alzheimer's disease: a crucial role for cognitive impairment and memory loss. *Metab Brain Dis*. (2022) 37:851–7. doi: 10.1007/s11011-022-00953-2

242. Lopez-Valdes HE, Martinez-Coria H. The role of Neuroinflammation in age-related dementias. *Rev Investig Clin*. (2016) 68:40–8.

243. Govindpani K, Turner C, Waldvogel HJ, Faull RLM, Kwakowsky A. Impaired expression of GABA signaling components in the Alzheimer's disease middle temporal gyrus. *Int J Mol Sci*. (2020) 21:8704. doi: 10.3390/ijms21228704

244. Limon A, Reyes-Ruiz JM, Miledi R. Loss of functional GABA(A) receptors in the Alzheimer diseased brain. *Proc Natl Acad Sci U S A*. (2012) 109:10071–6. doi: 10.1073/pnas.1204606109

245. Fu X, Wang YJ, Kang JQ, Mu TW. GABA(A) receptor variants in epilepsy In: SJ Czuczwar, editor. *epilepsy*. Brisbane, AU: (2022)

246. Verret L, Mann EO, Hang GB, Barth AM, Cobos I, Ho K, et al. Inhibitory interneuron deficit links altered network activity and cognitive dysfunction in Alzheimer model. *Cell*. (2012) 149:708–21. doi: 10.1016/j.cell.2012.02.046

247. Hamm V, Heraud C, Bott JB, Herbeaux K, Strittmatter C, Mathis C, et al. Differential contribution of APP metabolites to early cognitive deficits in a TgCRND8 mouse model of Alzheimer's disease. *Sci Adv*. (2017) 3:e1601068. doi: 10.1126/sciadv.1601068

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# Overlapping and distinct phenotypic profiles in Alzheimer's disease and late onset epilepsy: a biologically-based approach

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Due to shared hippocampal dysfunction, patients with Alzheimer's dementia and late-onset epilepsy (LOE) report memory decline. Multiple studies have described the epidemiological, pathological, neurophysiological, and behavioral overlap between Alzheimer's Disease and LOE, implying a bi-directional relationship. We describe the neurobiological decline occurring at different spatial in AD and LOE patients, which may explain why their phenotypes overlap and differ. We provide suggestions for clinical recognition of dual presentation and novel approaches for behavioral testing that reflect an "inside-out," or biologically-based approach to testing memory. New memory and language assessments could detect—and treat—memory impairment in AD and LOE at an earlier, actionable stage.

KEYWORDS

**late onset epilepsy, Alzheimer's disease, neuropsychology, mild cognitive impairment, memory, language, hippocampus, interictal epileptiform discharge**

## 1 Introduction

Both patients with Alzheimer's disease and epilepsy patients report difficulty with episodic memory, or remembering autobiographical events (see Box 1) (1, 2). Common complaints including forgetting conversations, losing personal items, or repeating questions or stories. Besides similarity in clinical presentation, the epidemiological, pathological, and neurophysiological overlap between Alzheimer's Disease and late-onset epilepsy (LOE, first seizure after the age of 60) has been well-described (3–5) (see Box 1). Epidemiologically, AD patients have a seizure incidence of 12%–28% (6), while patients with LOE have a 3-fold higher risk of developing dementia (3). After a diagnosis of LOE, patients have a median time of 3.66 years to dementia ascertainment (2).

When should clinicians suspect AD pathology in the older patient presenting with their first lifetime seizure, and seizures in dementia patients? The authors propose that understanding the shared and distinct biology between AD and LOE will improve diagnosis and management, especially during early stages of each condition.

In this review, we will describe the pathological, neurophysiological, and neuroimaging overlap between AD and LOE. With this biological foundation, we review their cognitive phenotypes as revealed in neuropsychological testing and suggest a few diagnostic approaches. Finally, we propose new behavioral assays that reflect an "inside-out," or biologically-based approach to testing memory. New memory assessments could be used to detect—and

treat—memory impairment in AD and LOE at an earlier, actionable stage (11–13).

Search terms used in PubMed for this review include Alzheimer's disease; late-onset epilepsy; early-onset epilepsy; Alzheimer's Disease pathology; late-onset epilepsy; early onset-Alzheimer's; late-onset epilepsy clinical presentation; neuropsychology and epilepsy; neuropsychology and Alzheimer's disease; cognitive and epilepsy; cognitive and Alzheimer's disease; cognitive phenotype and epilepsy; cognitive phenotype and Alzheimer's disease; memory and epilepsy; memory and Alzheimer's disease; naming and epilepsy; naming and Alzheimer's disease; language and epilepsy; language and Alzheimer's disease; executive functions and epilepsy; executive functions and Alzheimer's disease; Natural Language Processing and Epilepsy; Natural Language Processing and Alzheimer's Disease; Automated Speech Analysis and Alzheimer's Disease; and Eye tracking and Memory.

The authors acknowledge the heterogeneity in AD presentation and etiology but will focus this review on typical AD, which presents with memory dysfunction as a chief complaint. AD includes both early and late onset AD, which share pathology and clinical features. Atypical presentations of AD, or "non-amnestic" AD have been estimated to comprise less than one-third of young AD patients (<65 years) (14), and thus only 6%–7% of the total AD cohort. While atypical AD is a rare but important condition to recognize, we will focus on typical AD and its overlap with LOE. Likewise, familial (i.e., genetic etiology due to APP, PSEN1, or PSEN2 mutations) and sporadic AD share similar neuropathology and clinical features, but familial AD presents earlier. Because familial AD is relatively rare (5% of total AD prevalence), we will not treat familial AD separately from sporadic AD (1).

Furthermore, the terminology LOE will be used in this paper to include both known and unknown causes of seizures in older age. AD pathology can co-exist with other known structural causes in older age, especially vascular etiologies such as stroke or microvascular disease. AD pathology may also comprise a meaningful portion of the one-third of older patients with epilepsy of unknown cause. Of note, TLE is the dominant cohort of focal epilepsy patients and represents the largest cohort of LOE cases (15). The grouping of late-onset temporal lobe epilepsy (TLE) and TLE has been used in neuroimaging and neuropsychology, and thus will be used in this review.

# 2 Shared pathological processes in AD and LOE

AD and LOE mainly affect temporal lobe and specifically hippocampus (5, 15, 16) at early stages. Several MRI, pathological, neurophysiological, and behavioral studies demonstrate the pathophysiological overlap between the AD and LOE pathways, which may explain similar cognitive presentations (17, 18).

## 2.1 Pathological amyloid and tau accumulation

Similar accumulation patterns of extracellular amyloid-beta peptides (A-beta) and intracellular tau tangles (Box 1) (8, 19) that have been well described in the AD population with recent rodent and human work suggesting a similar process in LOE patients (19). Amyloid precursor protein (APP) is an essential membrane glycoprotein that supports numerous physiological functions, including neuronal development, signaling, and intracellular transport (20). Normally, APP cleavage results in several types of a-beta peptides. An imbalance between a-beta production and degradation and clearance leads to extracellular accumulation in hippocampus, neocortex, and the cerebral vasculature, likely initiating AD (20–22). a-beta accumulation outside of neurons blocks cell to cell signaling in the brain and triggers microglial activation. Chronic low-level inflammation characterizes AD, can overwhelm the glial response, and leads to brain atrophy. A-beta's role in contributing to hyperexcitability and seizures has recently been reported (8, 13). Pathologically high CSF a-beta levels are measured in 37.5% of LOE patients compared to healthy age-matched controls and are associated with a 3.4-fold higher risk of progression to dementia (23).

Tau is an intracellular micro-tubule associated protein whose pathologic accumulation results in impairment of intracellular function including glucose transport and direct neural degeneration. Phosphorylated tau (p-tau) is seen multiple degenerative and epilepsy conditions, including AD, movement disorders, temporal lobe epilepsy, post-traumatic epilepsy, autism, Dravet's syndrome, focal cortical dysplasia, and tuberous sclerosis (9). Examination of resected temporal lobe tissue in a cohort of older TLE patients ($n = 33$, age 50–65) revealed excess tau pathology in 94% of samples (24). Tau burden correlated with the degree of cognitive impairment (24) (Figure 1).

## 2.2 Brain atrophy

Both amnestic mild cognitive impairment (aMCI), the precursor to AD, and LOE patients show atrophy of the bilateral medial temporal lobe structures—including entorhinal, parahippocampal, hippocampal, temporal pole, and fusiform regions (18, 25). LOE patients posssess greater left entorhinal and temporal pole thinning, while patients with amnestic MCI show greater thinning of the bilateral middle temporal cortex and right inferior temporal cortex (Figure 1). Patients with LOE show thinner motor cortex compared to healthy controls (HCs) and amnestic MCI subjects. There has been recent interest in the piriform cortex, a small region sitting adjacent to MTL that supports olfaction and memory and contributes to seizure kindling (26, 27). The piriform cortex is bilaterally atrophied in patients in MCI and AD, and unilaterally atrophied on the side of mesial temporal sclerosis in epilepsy (26).

What is the effect of epilepsy duration? Lifetime seizure frequency may not be the sole driver of cortical thinning (28), as pathological decline can start earlier than clinical presentation in LO-TLE patients (2, 18). Slightly different patterns emerge in early and late onset epilepsy. Patients with LOE demonstrate greater atrophy of the fusiform gyri and similar cognitive profiles compared to patients with early-onset TLE (EO-TLE), even though the latter group endured over 30 years of seizures. As would be expected from this shared pattern of brain atrophy, both LOE and aMCI patients demonstrate memory impairment compared to HCs (17, 18).

BOX 1 Definitions

**Episodic Memory** is memory for personally-experienced events, for example, what one ate for lunch, a conversation with a friend, a movie narrative, and or the birth of one's child. These memories may include people, context, perceptual detail, timing and sequence, emotion, and meaning. Episodic memory includes 3 phases: encoding, consolidation, and retrieval. The hippocampus is thought to be critically involved in these three stages during waking and sleep. Patients with hippocampal dysfunction, such as patients with Alzheimer's Disease, traumatic brain injury, and temporal lobe epilepsy commonly report memory impairment as a cognitive comorbidity.

**Alzheimer's Disease** (AD) is the most common neurodegenerative disorder in the US, affecting 1 in 9 people aged 65 or older in the US (6.7 million). Age is the greatest risk factor for AD: 13.1% of people ages 75 to 84, and 33.3% of people age 85 or older have AD (1). Classically, AD presents as impairment in episodic memory function, then language and executive function. While there are no effective cures, there are medications which can slow cognitive decline or address comorbid symptoms. Recent work suggests that between 12% and 28% of patients with AD have seizures arising from the mesial temporal lobe (7).

**Early onset Alzheimer's Disease (EOAD)** is the clinical presentation of Alzheimer's Disease before the age of 65. Clinical features and pathology are similar to late-onset Alzheimer's disease (LOAD).

**Amyloid Beta**. Accumulation of the protein beta-amyloid outside neurons defines early pathophysiological changes in AD. Extracellular a-beta accumulation is associated with neuronal cell dysfunction, inflammation, and cell death (8).

**Tau**. Tau is an intracellular microtubule associated protein whose pathologic accumulation impairs intracellular function, including glucose transport, and contributes to direct neural degeneration. Phosphorylated tau (p-tau) is seen multiple degenerative and epilepsy conditions, including AD, movement disorders, temporal lobe epilepsy, post-traumatic epilepsy, autism, Dravet's syndrome, focal cortical dysplasia, and tuberous sclerosis (9).

**Seizures** are events of abnormal sustained electrical activity in the brain that manifest silently or as sudden changes in awareness, sensation, movement, or behavior. Seizures can be provoked by transient medical factors such as excessive alcohol, recreational drug use, or infection. Seizures can also recurrent event arising from abnormal brain activity.

**Epilepsy** is a neurological disease defined by the potential for recurrent seizures, and may be treated by medications, devices, or surgery. Epilepsy can be caused through many mechanisms, such as genetics, developmental malformations, traumatic brain injury, or stroke. A Many patients with epilepsy do not have a known cause to seizures, and are classified as "cryptogenic-onset epilepsy".

**Late-Onset Epilepsy (LOE)** is presentation of first seizure in an epilepsy patient after the age of 60. In two thirds of cases of LOE, a structural cause can be determined, such as cerebrovascular disease (stroke, 30%–50%), neurodegenerative disease (10%–20%), traumatic brain injury (TBI, ≲25%) and brain tumors (10%–30%). Other less common causes of seizures are infection, drug and alcohol toxicity and withdrawal, and autoimmune encephalitis (10).

**Late-Onset Epilepsy of Unknown Etiology (LOUE)**. For the one-third of patients without an identified structural cause, or cryptogenic epilepsy, occult cerebrovascular disease and/or prodromal neurodegenerative disease, are highly suspected.

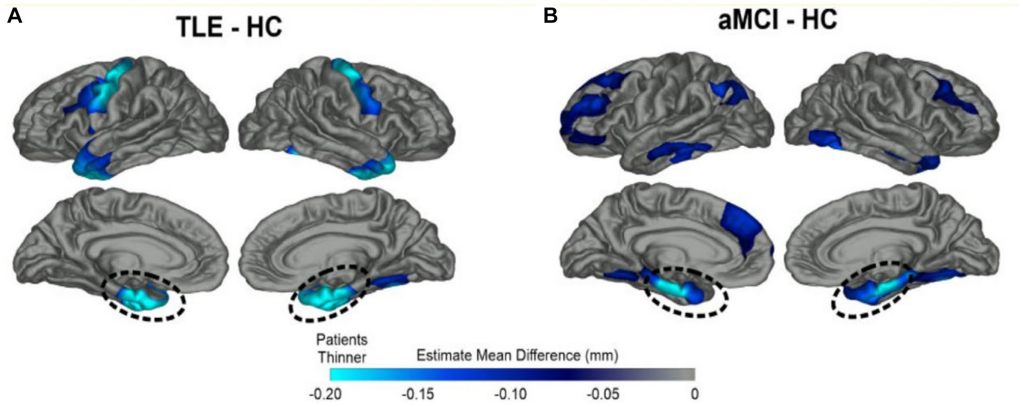


FIGURE 1
Overlapping patterns of MTL cortical atrophy in TLE and amnestic MCI. Patterns of cortical thinning for **(A)** TLE and **(B)** amnestic MCI patients relative to healthy elderly control subjects (HC). Dark blue represents cortex thinner than healthy controls while turquoise regions demonstrate the most thinning. Both patient groups showed prominent cortical thinning in bilateral medial temporal lobe (MTL) regions highlighted by dashed lines; TLE patients also showed thinning of the primary motor cortex compared to HCs. From Kaestner (2020) with permission (18).

## 2.3 Patterns of hippocampal subfield dysfunction

The hippocampus and adjacent medial temporal lobe structures are affected in AD and temporal lobe epilepsy, as well as several cognitive and psychiatric disorders such as vascular disease, schizophrenia, depression, and PTSD (29). Our understanding of the heterogeneity of cell types, gene expression profiles, and related function has been studied across the long and transverse axes within the hippocampus. The entorhinal cortex (EC) is considered a gateway to the hippocampus, receiving monosynaptic input from various cortical regions, including the perirhinal cortex (the "what" pathway), the parahippocampal cortex (the "where" pathway), the amygdala, and the sensory cortex (Figure 2A). The EC relays this topographically organized information to the hippocampus. Anterior structures such as the amygdala have direct and indirect (via EC) connections to the anterior hippocampus, or head. Conversely, posterior structures such as visual and association cortex have extensive direct and indirect connections with the posterior hippocampus, or tail (29). Along the transverse axis, the entorhinal cortex connects with the dentate gyrus, CA3, CA1, and the subiculum. In the tri-synaptic pathway, information from EC is delivered to DG → CA3 → CA1→ subiculum (Figure 2B). Within CA3, there are auto-association fibers with extensive connections along the hippocampal long axis. Information largely flows out through CA1 and subiculum to be delivered directly or indirectly to cortext through EC, in a topographically preserved manner (29).

High-resolution structural and functional MRI, CT perfusion, and post-mortem studies suggest that hippocampal subfields along the anterior and posterior hippocampus are differentially vulnerable in the spectrum of neuropsychiatric disorders, likely due to differential gene expression profiles (29). A functional differentiation of hippocampal subfields has been proposed (Figure 3), which may be useful to distinguish patient groups. Because each subfield serves as a conduit of information flow, upstream injury will impair downstream functioning and result in more severe memory deficits.

For example, imaging and pathology studies show that the dentate gyrus (DG) is particularly important in "pattern separation," or representation of similar events as distinct and non-overlapping items (Figure 3) shown in rodents and humans (30–34). DG is particularly vulnerable to aging across species (35). Behaviorally, aged rats and humans have difficulty in distinguishing between similar contexts (36).

AD involves early cell loss in entorhinal cortex (EC) which affects downstream structures such as DG, CA3, CA1, and subiculum (Figure 4), the primary outflow tracts (25, 37). AD patients therefore present with difficulty in all stages of memory, including maintaining information over brief delays (e.g., delayed match to sample tasks, pattern separation deficits, consolidation, and retrieval). In contrast, temporal lobe epilepsy begins with cell loss in dentate gyrus and CA3/CA1, with relatively preserved subiculum, CA2 and EC entorhinal cortex (Figure 4) (38). Therefore, one may expect that TLE patients have difficulty with separation of details (DG), association and consolidation between present and past (CA3/CA1), but less difficulty with forming and retrieving memories *per se*. Both pathological patterns differ from the decline of dentate gyrus function seen in normal aging (35, 39).

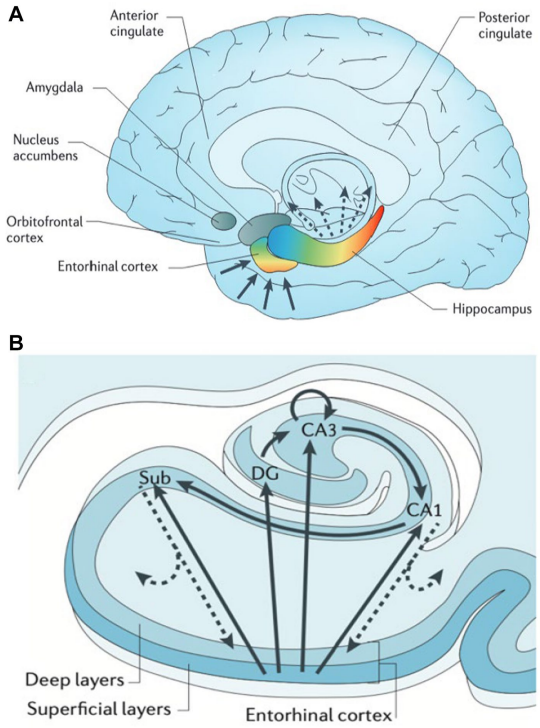


FIGURE 2
Hippocampal functional organization. **(A)** The hippocampus receives and delivers input from cortex in a topographically organized manner. Anterior cortical regions such as amygdala and frontal lobe relay information directly and indirectly (via entorhinal cortex, EC) to anterior hippocampus (head) and amygdala. Likewise posterior regions such as occipital cortex connect directly and indirectly to posterior hippocampus (tail). **(B)** The hippocampal transverse axis shows how input received by EC is processed within the hippocampus, then delivered back to cortex directly via CA1/subiculum and indirectly through EC with a preserved topographical gradient. From Small (2013) with permission (29).

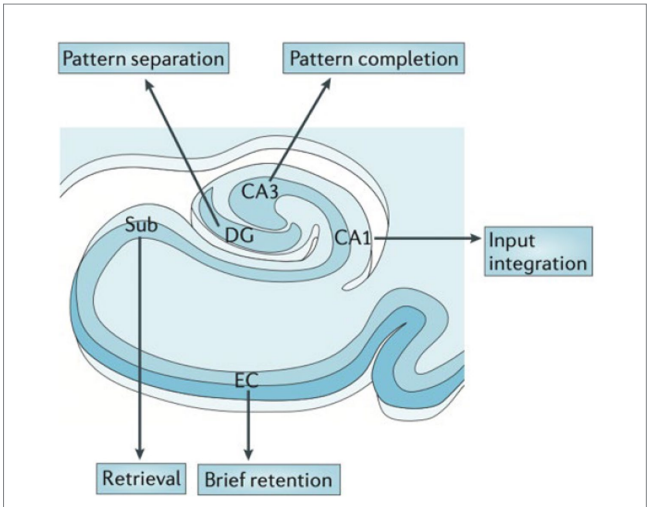


FIGURE 3
Hypothesized functional organization of the hippocampal transverse axis. Rodent and human studies suggest a functional specialization between the hippocampal subfields. From Small (2023) with permission (29).

Indeed, patients with amnestic MCI have demonstrated poorer delayed memory performance relative to late onset TLE (18, 40). Knowledge of differing subfield patterns of early stages of AD and TLE could be used to design more behaviorally specific tasks to aid in diagnosis and to provide a benchmark for performance (29). Of course, as each of these disease progresses, pathology spreads to nearby regions.

## 2.4 Interictal epileptiform discharges

Interictal epileptiform discharges (IEDs) are pathological bursts of neuronal activity suggestive of cortical hyperexcitability. These subclinical epileptiform events have been observed in 20–50% of AD patients (5, 16, 41), and are associated with accelerated cognitive decline (16). A-beta's role in contributing to hyperexcitability and seizures has been reported (8, 13). Converging evidence demonstrates that IEDs impair encoding, maintenance, consolidation, and retrieval of verbal material (42–47). Left temporal and parietal neocortical IEDs are associated with impaired memory for word list items and word pairs (44, 47). IEDs outside the seizure onset zone (SOZ) in higher order visual processing regions have been associated with impaired encoding and retrieval performance for words (47). Hippocampal IEDs during encoding of a face-profession pair can reduce odds of recall by 15%; IEDs during recall can reduce odds of recall by 25%, potentially by acutely decreasing hippocampal sharp wave ripples (SWRs). Hippocampal IEDs during sleep impair long-term memory consolidation of verbal and visual material (48). We hypothesize that hippocampal IEDs, prevalent in AD and LOE, can occur during critical memory stages during wake and sleep states to directly compete with physiological processes (49). These interactions contribute to dynamic fluctuations in memory function, and a potential target for closed loop neurostimulation protocols to remediate memory function.

# 3 Characterizing AD and LOE cognitive phenotypes

While neuroimaging and neurophysiology (i.e., EEG) play an essential role in making the clinical diagnosis of AD or LOE, neuropsychological assessment is the gold standard for the assessment, characterization, and tracking of cognitive impairment.

The impairments arising in these conditions differ in the onset, severity, and course from the decline seen in normal aging, including decreased processing speed, memory, language, and executive function (50). Episodic memory and language are the neurocognitive domains most affected in typical AD and TLE and emphasized during neuropsychological testing in both clinical and research settings. Episodic memory has been evaluated by using list-learning, story recall, and figural reproduction tasks (e.g., Rey Auditory Verbal Learning Test & Wechsler Memory Scale) (51). Language tasks have included measures of picture naming (e.g., Boston Naming Test) and verbal fluency (e.g., letter & category). Measures of executive functioning, including tests of rapid mental tracking and problem solving (e.g., Trail Making Test & Wisconsin Cart Sorting Test) are also used in patients with other etiologies such as frontotemporal or vascular dementia. The neuropsychological tests used today for these purposes are criticized for using outdated methodology and for extensive time required to administer and score the tests (52).

Patients with AD and TLE both exhibit deficits on neuropsychological tests requiring recall of newly learned material after a delay period of 20-min or more (53, 54). Results from neuropsychological studies show subtle but important differences in the cognitive presentation between these two groups. Deficits in episodic memory, or difficulty remembering personally experienced events, are commonly the first manifestation of AD. This deficit may involve a combination of reduced encoding of new information and a disturbance of the ability to transfer that information into long-term storage, or a consolidation deficit (54). These cognitive deficits may be due to early involvement of entorhinal cortex (hippocampal input and output, short term retention), (Table 1; Figures 3,4).

The memory deficits seen in TLE are believed to result from difficulty consolidating newly learned information. On testing, this is displayed as rapid forgetting (58). These could be secondary to early involvement of dentate gyrus/CA3 and CA1, subfields responsible for pattern separation and integration (Figures 3,4; Table 1) (29, 35). Furthermore, difficulty with long term consolidation could be secondary to increased frequency of interictal discharges during NREM sleep (59, 60) or decreased spindle activity seen during

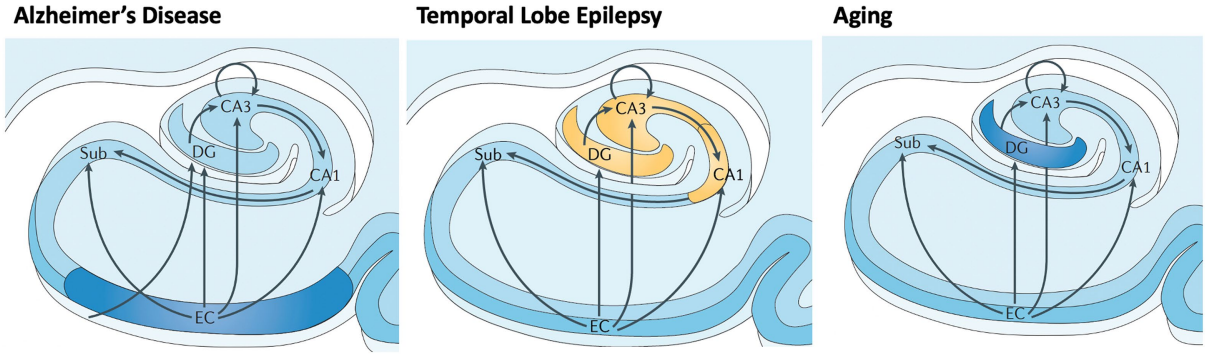


FIGURE 4
Differential vulnerability of hippocampal subfields in early AD, TLE, and normal aging. Subfields include EC: entorhinal cortex; Sub: subiculurm; DG: dentate gyrus; CA3; CA1. Early involvement of subfields varies between neurological disorders and normal aging, causing local and downstream cognitive dysfunction. Adapted from Small et al. (2013) (29).

NREM sleep (61). Additionally, TLE patients with unilateral onset of left or right hemisphere seizures may exhibit a material-specific impairment in memory for verbal or nonverbal material (62). Finally, there is converging evidence suggesting that subclinical discharges may disrupt consolidation processes causing accelerated rates of forgetting in both conditions (48, 63).

Patients with AD are believed to progress to more widespread and profound declines in language and other domains as the neuropathology spreads from medial temporal lobe structures to association cortices of the temporal, frontal, and parietal lobes (55). Deficits in confrontation naming and semantic fluency (i.e., number of words generated within a category such as animals or fruits) result from loss of semantic knowledge stores. In contrast, naming deficits present when seizures arise from the language dominant hemisphere and are characterized by a deficit in semantic retrieval (64). Executive dysfunction can be identified in early and later stages of both conditions, but is milder than the executive dysfunction associated with other variants of dementia and epilepsy, including frontotemporal dementia (FTD) and frontal lobe epilepsy (FLE) (65, 66).

The profiles of neurocognitive disturbance in AD and TLE are generally studied at the group level. Individual patients exhibit a more heterogenous profile of deficits in episodic memory, language, and executive function than what is reported in group studies. Neuropsychology has recently transitioned to a more empirical approach to identify cognitive phenotypes associated with AD and TLE. Using data science methods, studies of AD have identified a number of phenotypes presenting with generalized cognitive deficits or focal profiles of impairment in memory, language, or other function. These phenotypes may differ in rates of progression and can be distinguished by unique genetic and biomarker profiles (67, 68). A similar literature in TLE has yielded a set of 3–4 cognitive phenotypes initially identified by Hermann and colleagues (55) and replicated, and differ in rates of cognitive decline and brain atrophy (69, 70).

Historically, study of neurocognitive impairment in TLE has focused on children and younger adults. Attention is shifting to older patients with longstanding epilepsy (EOE) and/or those with LOE to better understand how decades of seizures contribute to cognitive decline (71). Surprisingly, patients with EOE demonstrate a pattern of impairment on neuropsychological tests analogous to the decline seen in LOE to and aMCI patients (17). However, direct comparisons of MCI and TLE groups find that MCI patients exhibit greater impairment on tests of delayed memory while LOE patients have a more widespread profile of deficits in language, executive dysfunction, and visuospatial skills (18, 72, 73).

Several studies have demonstrated accelerated cognitive decline in patients with epilepsy, correlating with findings of increased atrophy on neuroimaging (2, 74). Research progress has been hindered over the years by a lack of an accepted taxonomy to classify cognitive disorders in patients with epilepsy across the lifespan (75). Studies using methods for diagnosing MCI in non-epileptic populations have found that approximately 60% of older individuals with epilepsy would meet diagnostic criteria for MCI (72, 73). Questions have arisen whether these findings are reflective of the effects of early cognitive deficits interacting with effects of normal aging, an accelerated form of aging, or chronic accumulation of environmental and health-related factors that reduce cognitive reserve (76).

While there is significant overlap in AD and TLE cognitive profiles, subtle differences exist. Patients with neurodegenerative conditions would be expected to decline over time while patients with well controlled epilepsy may not have significant memory decline. A more sophisticated understanding of their pathological, anatomical, and neurophysiological profiles could guide clinical phenotyping and diagnosis, especially at early stages of cognitive decline or seizure presentation.

## 4 Clinical diagnosis and differentiation

For guidance on diagnosing Alzheimer's Disease and late-onset epilepsy, we refer readers to published guidelines (77, 78). However, even clinicians who diagnose and manage dementia or epilepsy may have difficulty recognizing seizures in AD patients or vice versa, especially at initial presentation. We offer several recommendations based on this review of the literature and our clinical experience with both populations.

1 *A careful medical and family history should be taken* to identify vascular causes which can cause cognitive decline or contribute to accelerated AD; sleep apnea; alcohol and drug use; family history of early onset dementia and

TABLE 1 Patterns of cognitive impairment seen in Alzheimer's disease and temporal lobe epilepsy using traditional neuropsychological testing.

| Neuropsychological domain | Representative neuropsychological tests | Alzheimer's disease | Temporal lobe epilepsy |
|---|---|---|---|
| Episodic memory | List learning (e.g., RAVLT) | Reduced encoding of new information and consolidation information into long term storage (55). | Intact encoding; Disruption in consolidation of newly learned information (56). Material specific impairments in verbal and visual memory in lateralized cases (57). |
| | Story recall (e.g., WMS LM) | | |
| | Figural reproduction (e.g., RCFT) | | |
| Language | Picture naming (e.g., BNT) | Deficits in confrontation naming and verbal fluency secondary to a loss of semantic knowledge stores (55). | Intact knowledge stores with a primary difficulty in retrieving lexical and semantic information (61). |
| | Letter fluency (e.g., COWAT) | | |
| | Category fluency (e.g., Animal naming) | | |
| Executive functions | Trail making test (TMT) | Mild deficits that do not extend to the severity observed in cases of frontotemporal dementia FTD (62). | Mild deficits that do not extend to the severity observed in cases of frontal lobe epilepsy (63). |
| | Sorting tests (e.g., WCST) | | |
| | Planning tests (e.g., TOL) | | |

traumatic brain injury (which causes both cognitive decline and seizures).

2 *Patients with amnestic MCI or early AD often have difficulty with both information encoding and retrieval, whereas patients with LOE primarily have impairments in retrieval, especially during delayed recall.* This observation is consistent with the concept of differential subfield vulnerability at early stages (29). This subtle difference can be assessed during the MMSE or MOCA during the clinical visit. During verbal encoding of 3 or 5 words, patients with aMCI or AD struggle to learn words, require multiple registration trials, and demonstrate difficulty with retrieval. LOE patients show more selective weakness in delayed retrieval. Neuropsychological evaluation should focus on whether memory impairment is isolated to difficulty with consolidation (more likely to be TLE only) or difficulty with all stages of memory function, including encoding and retrieval (more likely to be AD); other cognitive domains such as language and executive function are often more affected in neurodegenerative conditions than in isolated LOE.

3 *Clinical follow-up is important.* The degree and pace of cognitive decline is often faster in patients with aMCI or AD than in LOE patients (18). Patients with well controlled LOE are more likely to remain cognitively stable over time if their seizures and other medical issues are well controlled.

4 *When patients with aMCI and AD report a history of fluctuating mental status, or discrete episodes of altered awareness, agitation, or psychosis, seizures should be suspected.* Mesial temporal lobe onset seizures (mTLE) can present with psychic auras of anxiety and déjà vu, or viscero-sensory sensations with nausea, "butterflies," and epigastric rising. Seizures can occur with or without alteration in awareness (focal impaired aware or focal unimpaired aware) and result in behavioral arrest. Seizures typically last from seconds to a minute, and rarely continue past 1–2 min. Seizures involving mTLE can progress to include obvious motor signs, such as posturing, repetitive clonic jerking (unilateral or bilateral, or tonic stiffening). Patients may be lethargic, confused, agitated, or even psychotic after seizures. Occult seizures should be suspected when MCI and AD patients become suddenly agitated or psychotic, or demonstrate fluctuating mental status (although Lewy Body Dementia could also be in the differential). Nocturnal seizures should be suspected when the patient wakes up confused or disoriented.

5 *We recommend an MRI Brain* for all patients who report memory or cognitive issues, and patients presenting with late-onset epilepsy. Besides obvious structural abnormalities, special attention should be paid to hippocampal volumes, white matter disease, lobar specific atrophy, or generalized atrophy that could point to underlying AD (bilateral hippocampal/temporal; parietal) or another neurodegenerative pathology. When the MRI Brain is structurally normal, and a neurodegenerative condition is strongly suspected, we recommend a PET MRI Brain to assess for lobar-specific dysfunction.

6 *We recommend ambulatory EEG (24 h) in all patients*, given that interictal epileptiform discharges (IEDs), especially from temporal lobe, are facilitated during non-REM Sleep (60, 79). Patients with temporal lobe epilepsy typically have unilateral spikes, whereas patients with aMCI or AD have bilateral hyperexcitability (16). AD patients with clinical seizures have characteristic IEDs seen on ambulatory EEG—discharges are bilateral, small and spiky in appearance, frequent, and occurring in wakefulness and REM sleep (16). Even with a normal ambulatory EEG, there may undetected interictal epileptiform discharges and silent seizures in AD patients that are only detected with invasive recordings (7). Besides IEDs, other EEG changes are apparent in aMCI and early AD. Bilateral frontotemporal slowing and mild slowing and desynchronization in the posterior dominant rhythm have been linked to the degree of amyloid and tau deposition, respectively (19). Sleep EEG in patients with temporal lobe epilepsy shows decreased spindle density (61). While mesial temporal IEDs can be difficult to detect with conventional scalp EEG, our center has had improved sensitivity with adding subtemporal (T1/T2) leads.

Taking the next step will require development of more sensitive and automated cognitive assays. Current neuropsychological tests are reliant on outdated models of cognitive functioning and depend on paper-pencil format (52, 80, 81). There is a clear need to "update testing" to reflect more contemporary cognitive models of development and administration through digital formats such as computers or smartphones, and could facilitate more frequent testing. Empirical study of cognitive phenotypes using network and artificial intelligence (AI) approaches can be used to efficiently and objectively analyze test findings (82, 83).

## 5 Novel directions in memory assessment

An ideal clinical assessment could (1) differentiate between early stages of AD and LOE, (2) detect early and subtle forms of memory impairment and precisely measure cognitive performance over time, and (3) be scaled to widespread patient populations. Testing would capture clinically meaningful memory behaviors, disambiguate language from memory, measure these behaviors sensitively and objectively, and allow serial assessment over time (84).

Subjective memory impairment, which correlates with initial amyloid accumulation in the brain, can precede AD dementia ascertainment by up to 18 years (56). Yet these subtle declines may not be detected by current neuropsychological testing methods. Many of the current cognitive assessments have been criticized as labor-intensive, subjective, and data-poor estimates of human behavior (52, 85, 86). In contrast to current practice of testing word lists, pairs, paragraphs, and abstract drawings, patients report difficulty with episodic memory, or remembrance of personally experienced events. Episodic memory under real-world circumstances binds perceptual details, spatial context, and temporal order to specific events (87), which may be missed in standard neuropsychological tests.

For example, development of tasks that measure subfield-specific functions could be useful for early diagnosis, phenotyping, and tracking. Given the differing cell types and differential functions of the hippocampal subfields, and differential patterns of decline in early AD, LO-TLE, and normal aging, some have proposed a functional map of the hippocampus, along the transverse (subfield-level) and longitudinal (anterior–posterior) axes (Figures 3, 4) (29). Combining these behavioral insights with automated segmentation of

hippocampal subfields allows more precise functional-anatomical correlations (88). For example, CA3 is thought to be responsible for pattern integration (29), while dentate gyrus performs pattern separation (i.e., distinction between similar features or events). Some studies demonstrate decreased pattern separation in older individuals related to DG dysfunction with aging (30).

Future assessments could embrace more complex, naturalistic memory paradigms and computational analysis to make scoring more objective, sensitive, and quantifiable. Moreover, ideal testing could disambiguate language from memory function. Here, we highlight two promising directions in cognitive testing.

## 5.1 Eye tracking

Eye tracking, or the measurement of saccades, fixations, and pupillometry with high spatiotemporal precision, is an ideal method to readout brain-behavior relationships (57, 89). While rodents use mainly olfaction and locomotion to explore their environment, humans and other primates primarily depend on vision to extract and remember information about the world. Eye movements shape what is encoded – by chunking a continuous visual stream of information to deliver to widespread brain regions, including the hippocampus. Eye movement can track hippocampal activity at the millisecond time scale, as demonstrated by recent studies combining oculomotor measurements and hippocampal depth recordings in surgical epilepsy patients (90).

When scanning the environment, eye movements rapidly switch between saccades and fixations. Saccades are sudden, ballistic eye movements between objects or features in the environment, while fixations are prolonged gaze on attended objects. Eye movements are not random but influenced by visual properties of the object (e.g., color, contrast) and past experience (i.e., episodic and semantic memories). For example, monkeys, human infants, and healthy adults prefer looking at novel vs. familiar objects (91–94). More gaze fixations occur within new vs. repeated viewing (Figure 5A) or within manipulated sections of the scene (Figure 5B), even if the subject is unaware of the manipulation (95–97). In contrast, patients with hippocampal damage have impaired novelty preference, manifest as equal time spent looking at new and old objects (98–100).

Besides novelty detection, eye movements reveal relational memory between objects (99, 101–103) and temporal sequences (104). Finally, eye tracking could provide a means to disambiguate language from memory testing. The strong preference for novelty manifested through gaze preference has been proposed to be a useful means of tracking memory changes in the preclinical and clinical AD populations (92, 105).

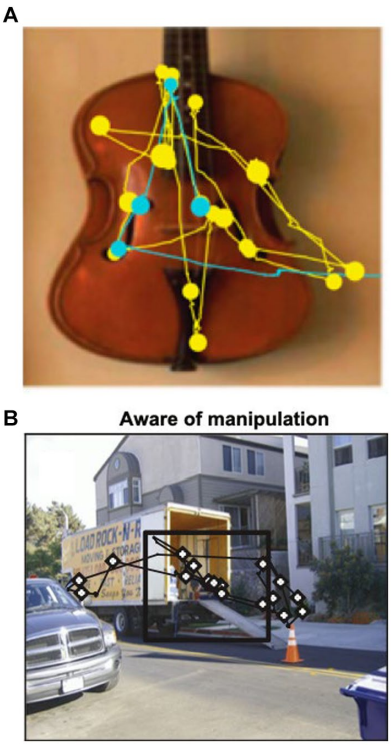


FIGURE 5
**(A)** Representative scan path showing that a macaque spends more time looking at the image during the first viewing (yellow) compared to second viewing (blue). Circles represent fixations; lines represent saccades. Adapted from Jutras et al. (89). **(B)** Macaques fixate more frequently on a manipulated (novel) scene area (inside black square). From Smith et al. (90).

## 5.2 Spontaneous recall and natural language processing

While memory impairment is characteristic of AD presentation, patients may also present with subtle language decline. Word-finding difficulties and a restricted lexicon result in "empty speech" or verbose but incoherent speech (106–109).

Machine and deep learning methods applied to patient spontaneous speech have been applied to help in diagnosis of psychosis (107) and schizophrenia, and could be useful for AD diagnosis can aid in AD diagnosis (109–111). Several studies have leveraged publicly available speech samples. The DementiaBank corpus of speech samples was collected between 1983 and 1988 from healthy and cognitively impaired patients at the University of Pittsburgh (111). Clinical information including MMSE, neuropsychological and physical assessment, and clinical records were used to classify patients as possible or probable AD (167 participants). Control subjects ($n$ = 167) were also included. The Cookie Theft picture description task from the Boston Diagnostic Aphasia Examination was used to elicit spontaneous speech, then transcribed at the word level, segmented into utterances, and annotated with pauses, paraphasias, and unintelligible words. Several of the studies using automatic, natural language processing-based features extracted from DementiaBank samples are summarized in Table 2.

AD patients typically demonstrate slowed speech rate, word finding, and word retrieval difficulty (111, 112). One study using natural language processing (NLP) analyzed speech samples of 99 patients with probable AD to 99 healthy controls (108). Low-level features such as simpler syntactic structure (i.e., arrangement of words and phrases to create meaningful sentences) and decreased use of lexical components [i.e., autonomous units of language, such as words, prefixes (pre-, post-), suffixes (-s, -ing)] could differentiate AD patients from healthy controls (108). Another study found that linguistic features of descriptive speech (Cookie Theft task) in AD patients showed acoustic differences and semantic, syntactic, and informational

TABLE 2 Examples of automated speech analysis in Alzheimer's disease.

| Publication | Patient groups | Main finding | Methods |
|---|---|---|---|
| Orimaye et al. (2017) (108) | Probable AD ($n = 99$)<br>Healthy Controls ($n = 99$) | • Probable AD group had less use of syntactical components and greater use of lexical components in language compared to Healthy Controls (HCs).<br>• Less use of n-grams (combinations or sequences of words that create a unit of meaning) in probable AD group than in HCs. | • DementiaBank language transcript clinical dataset (111).<br>• Automatic extraction of lexical, syntactic, and n-gram features of transcripts. |
| Yeung et al. (2021) (114) | Healthy controls ($n = 10$)<br>MCI ($n = 10$)<br>AD ($n = 10$) | • Greater severity in word-finding difficulty and incoherence in MCI and AD compared to controls.<br>• Automatically extracted features such as decreased word length and speech rate and increased pause frequency and length most strongly correlated with clinician ratings of WFD. | • DementiaBank speech samples (111).<br>• 5 clinicians blindly rated each speech sample on word finding difficulty, incoherence, perseveration, and speech errors, on a Likert scale from zero (nL) to 3 (severe impairment).<br>• Automatic extraction of lexical, syntactic, semantic, and acoustic properties. |
| Fraser et al. (JAD, 2016) (115) | Healthy controls ($n = 97$)<br>Possible and Probable AD ($n = 167$) | • Built a model which discriminates between HCs and possible/probable AD with 81% accuracy.<br>• Semantic impairment, acoustic abnormality, syntactic impairment, and information impairment predict dementia diagnosis. | • DementiaBank speech samples (111).<br>• Considered 370 features including syntactic complexity, grammar, vocabulary richness, lexical content, repetitiveness, and acoustic. |
| Beltrami (Front. Aging Neurosci 2018) (116) | Cognitively Impaired ($n = 48$: 32 MCI, 16 early dementia)<br>Healthy Controls ($n = 48$) | • Acoustic features most altered in the patients compared to controls (including speech rate and pauses, and spectral properties).<br>• Lexical features differentiate early dementia patients (e.g., fewer content words and modifiers).<br>• Syntactic features (e.g., sentence complexity, fewer embedded phrases) decreased in early dementia and MCI patients. | • Prospective study of spontaneous speech during description of a picture, typical working day, and last remembered dream.<br>• Automatic extraction of lexical, rhythmic, acoustic, and syntactic features of speech. |

differences, compared to healthy elderly (113). NLP methods applied to natural speech demonstrate that syntactic complexity combined with traditional neuropsychological test scores can differentiate between healthy elderly and MCI with high accuracy (>80%) (117).

To our knowledge, there have been no automated analyses of speech from epilepsy patients to detect cognitive changes. Given the widespread cognitive effects that have been discovered in patients with TLE (55), especially arising from the dominant lobe (62), word finding and speech changes would be expected. One study combined a questionnaire survey with NLP analysis of patients' descriptions of their most recent description of transient loss of consciousness could predict a seizure or non-epileptic event with 85.5% accuracy ($n = 21$ epilepsy patients, $n = 24$ non-epileptic patients) (118).

Existing linguistic tools and insights into AD decline could be leveraged to quantify memory impairment. For example, the face-name task is an ecologically valid behavioral task that correlates with degree of amyloid burden in anterior hippocampus and limbic regions in healthy elderly individuals (119). However, to our knowledge, there are no existing tools to assess memory impairment using automated methods, that are both sensitive and scalable.

## 6 Conclusion

Results of MRI, pathological, neurophysiological, and behavioral studies demonstrate significant overlap between AD and LOE. Understanding the pathophysiological profiles of each disease can aid clinical detection at early disease stages, or once a primary diagnosis is made, recognize the presentation of a second diagnosis. We highlight the cognitive differences between early AD and LOE, but emphasize the need for new testing approaches, including those utilizing eye tracking and natural language processing, to measure subtle changes in memory at the preclinical or early clinical stages.

## Author contributions

AL: Conceptualization, Writing – original draft, Writing – review & editing. WB: Conceptualization, Writing – original draft, Writing – review & editing.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note



## References

1. 2023 Alzheimer's disease facts and figures. *Alzheimers Dement*. (2023) 19:1598–695. doi: 10.1002/alz.13016

2. Sen, A, Capelli, V, and Husain, M. Cognition and dementia in older patients with epilepsy. *Brain*. (2018) 141:1592–608. doi: 10.1093/brain/awy022

3. Johnson, EL, Krauss, GL, Kucharska-Newton, A, Albert, MS, Brandt, J, Walker, KA, et al. Dementia in late-onset epilepsy: The Atherosclerosis Risk in Communities study. *Neurology*. (2020) 95:e3248–56. doi: 10.1212/WNL.0000000000011080

4. Stefanidou, M, Beiser, AS, Himali, JJ, Peng, TJ, Devinsky, O, Seshadri, S, et al. Bi-directional association between epilepsy and dementia: The Framingham Heart Study. *Neurology*. (2020) 95:e3241–7. doi: 10.1212/WNL.0000000000011077

5. Vossel, KA, Beagle, AJ, Rabinovici, GD, Shu, H, Lee, SE, Naasan, G, et al. Seizures and epileptiform activity in the early stages of Alzheimer disease. *JAMA Neurol*. (2013) 70:1158–66. doi: 10.1001/jamaneurol.2013.136

6. Baker, J, Libretto, T, Henley, W, and Zeman, A. A Longitudinal Study of Epileptic Seizures in Alzheimer's Disease. *Front Neurol*. (2019) 10:1266. doi: 10.3389/fneur.2019.01266

7. Lam, AD, Deck, G, Goldman, A, Eskandar, EN, Noebels, J, and Cole, AJ. Silent hippocampal seizures and spikes identified by foramen ovale electrodes in Alzheimer's disease. *Nat Med*. (2017) 23:678–80. doi: 10.1038/nm.4330

8. Romoli, M, Sen, A, Parnetti, L, Calabresi, P, and Costa, C. Amyloid-beta: a potential link between epilepsy and cognitive decline. *Nat Rev Neurol*. (2021) 17:469–85. doi: 10.1038/s41582-021-00505-9

9. Hwang, K, Vaknalli, RN, Addo-Osafo, K, Vicente, M, and Vossel, K. Tauopathy and Epilepsy Comorbidities and Underlying Mechanisms. *Front Aging Neurosci*. (2022) 14:903973. doi: 10.3389/fnagi.2022.903973

10. Sarkis, RA, Willment, KC, Pennell, PB, and Marshall, G. Late-onset unexplained epilepsy: What are we missing? *Epilepsy Behav*. (2019) 99:106478. doi: 10.1016/j.yebeh.2019.106478

11. van Dyck, CH, Swanson, CJ, Aisen, P, Bateman, RJ, Chen, C, Gee, M, et al. Lecanemab in Early Alzheimer's Disease. *N Engl J Med*. (2023) 388:9–21. doi: 10.1056/NEJMoa2212948

12. Vossel, K, Ranasinghe, KG, Beagle, AJ, La, A, Ah Pook, K, Castro, M, et al. Effect of Levetiracetam on Cognition in Patients With Alzheimer Disease With and Without Epileptiform Activity: A Randomized Clinical Trial. *JAMA Neurol*. (2021) 78:1345–54. doi: 10.1001/jamaneurol.2021.3310

13. Vossel, KA, Tartaglia, MC, Nygaard, HB, Zeman, AZ, and Miller, BL. Epileptic activity in Alzheimer's disease: causes and clinical relevance. *Lancet Neurol*. (2017) 16:311–22. doi: 10.1016/S1474-4422(17)30044-3

14. Graff-Radford, J, Yong, KXX, Apostolova, LG, Bouwman, FH, Carrillo, M, Dickerson, BC, et al. New insights into atypical Alzheimer's disease in the era of biomarkers. *Lancet Neurol*. (2021) 20:222–34. doi: 10.1016/S1474-4422(20)30440-3

15. Sarkis, RA, Beers, L, Farah, E, Al-Akaidi, M, Zhang, Y, Locascio, JJ, et al. The neurophysiology and seizure outcomes of late onset unexplained epilepsy. *Clin Neurophysiol*. (2020) 131:2667–72. doi: 10.1016/j.clinph.2020.08.014

16. Lam, AD, Sarkis, RA, Pellerin, KR, Jing, J, Dworetzky, BA, Hoch, DB, et al. Association of epileptiform abnormalities and seizures in Alzheimer disease. *Neurology*. (2020) 95:e2259–70. doi: 10.1212/WNL.0000000000010612

17. Griffith, HR, Martin, RC, Bambara, JK, Marson, DC, and Faught, E. Older adults with epilepsy demonstrate cognitive impairments compared with patients with amnestic mild cognitive impairment. *Epilepsy Behav*. (2006) 8:161–8. doi: 10.1016/j.yebeh.2005.09.004

18. Kaestner, E, Reyes, A, Chen, A, Rao, J, Macari, AC, Choi, JY, et al. Atrophy and cognitive profiles in older adults with temporal lobe epilepsy are similar to mild cognitive impairment. *Brain*. (2021) 144:236–50. doi: 10.1093/brain/awaa397

19. Ranasinghe, KG, Cha, J, Iaccarino, L, Hinkley, LB, Beagle, AJ, Pham, J, et al. Neurophysiological signatures in Alzheimer's disease are distinctly associated with TAU, amyloid-beta accumulation, and cognitive decline. *Sci Transl Med*. (2020) 12:4069. doi: 10.1126/scitranslmed.aaz4069

20. Chen, GF, Xu, TH, Yan, Y, Zhou, YR, Jiang, Y, Melcher, K, et al. Amyloid beta: structure, biology and structure-based therapeutic development. *Acta Pharmacol Sin*. (2017) 38:1205–35. doi: 10.1038/aps.2017.28

21. Murphy, MP, and LeVine, H 3rd. Alzheimer's disease and the amyloid-beta peptide. *J Alzheimers Dis*. (2010) 19:311–23. doi: 10.3233/JAD-2010-1221

22. Selkoe, DJ, and Hardy, J. The amyloid hypothesis of Alzheimer's disease at 25 years. *EMBO Mol Med*. (2016) 8:595–608. doi: 10.15252/emmm.201606210

23. Costa, C, Romoli, M, Liguori, C, Farotti, L, Eusebi, P, Bedetti, C, et al. Alzheimer's disease and late-onset epilepsy of unknown origin: two faces of beta amyloid pathology. *Neurobiol Aging*. (2019) 73:61–7. doi: 10.1016/j.neurobiolaging.2018.09.006

24. Tai, XY, Koepp, M, Duncan, JS, Fox, N, Thompson, P, Baxendale, S, et al. Hyperphosphorylated tau in patients with refractory epilepsy correlates with cognitive decline: a study of temporal lobe resections. *Brain*. (2016) 139:2441–55. doi: 10.1093/brain/aww187

25. Mueller, SG, Schuff, N, Yaffe, K, Madison, C, Miller, B, and Weiner, MW. Hippocampal atrophy patterns in mild cognitive impairment and Alzheimer's disease. *Hum Brain Mapp*. (2010) 31:1339–47. doi: 10.1002/hbm.20934

26. Steinbart, D, Yaakub, SN, Steinbrenner, M, Guldin, LS, Holtkamp, M, Keller, SS, et al. Automatic and manual segmentation of the piriform cortex: Method development and validation in patients with temporal lobe epilepsy and Alzheimer's disease. *Hum Brain Mapp*. (2023) 44:3196–209. doi: 10.1002/hbm.26274

27. Vaughan, DN, and Jackson, GD. The piriform cortex and human focal epilepsy. *Front Neurol*. (2014) 5:259. doi: 10.3389/fneur.2014.00259

28. Coan, AC, Appenzeller, S, Bonilha, L, Li, M, and Cendes, F. Seizure frequency and lateralization affect progression of atrophy in temporal lobe epilepsy. *Neurology*. (2009) 17:834–42. doi: 10.1212/WNL.0b013e3181b783dd

29. Small, SA, Schobel, SA, Buxton, RB, Witter, MP, and Barnes, CA. A pathophysiological framework of hippocampal dysfunction in ageing and disease. *Nat Rev Neurosci*. (2011) 12:585–601. doi: 10.1038/nrn3085

30. Bakker, A, Kirwan, CB, Miller, M, and Stark, CE. Pattern separation in the human hippocampal CA3 and dentate gyrus. *Science*. (2008) 319:1640–2. doi: 10.1126/science.1152882

31. Clelland, CD, Choi, M, Romberg, C, Clemenson, GD Jr, Fragniere, A, Tyers, P, et al. A functional role for adult hippocampal neurogenesis in spatial pattern separation. *Science*. (2009) 325:210–3. doi: 10.1126/science.1173215

32. Leutgeb, JK, Leutgeb, S, Moser, MB, and Moser, EI. Pattern separation in the dentate gyrus and CA3 of the hippocampus. *Science*. (2007) 315:961–6. doi: 10.1126/science.1135801

33. Marr, D. Simple memory: a theory for archicortex. *Philos Trans R Soc Lond Ser B Biol Sci*. (1971) 262:23–81. doi: 10.1098/rstb.1971.0078

34. McHugh, TJ, Jones, MW, Quinn, JJ, Balthasar, N, Coppari, R, Elmquist, JK, et al. Dentate gyrus NMDA receptors mediate rapid pattern separation in the hippocampal network. *Science*. (2007) 317:94–9. doi: 10.1126/science.1140263

35. Small, SA, Chawla, MK, Buonocore, M, Rapp, PR, and Barnes, CA. Imaging correlates of brain function in monkeys and rats isolates a hippocampal subregion differentially vulnerable to aging. *Proc Natl Acad Sci USA*. (2004) 101:7181–6. doi: 10.1073/pnas.0400285101

36. Yassa, MA, Stark, SM, Bakker, A, Albert, MS, Gallagher, M, and Stark, CE. High-resolution structural and functional MRI of hippocampal CA3 and dentate gyrus in patients with amnestic Mild Cognitive Impairment. *NeuroImage*. (2010) 51:1242–52. doi: 10.1016/j.neuroimage.2010.03.040

37. Mueller, SG, and Weiner, MW. Selective effect of age, Apo e4, and Alzheimer's disease on hippocampal subfields. *Hippocampus*. (2009) 19:558–64. doi: 10.1002/hipo.20614

38. Mueller, SG, Laxer, KD, Scanlon, C, Garcia, P, McMullen, WJ, Loring, DW, et al. Different structural correlates for verbal memory impairment in temporal lobe epilepsy with and without mesial temporal lobe sclerosis [Research Support, N.I.H., Extramural]. *Hum Brain Mapp*. (2012) 33:489–99. doi: 10.1002/hbm.21226

39. Small, SA, Tsai, WY, DeLaPaz, R, Mayeux, R, and Stern, Y. Imaging hippocampal function across the human life span: is memory decline normal or not? *Ann Neurol*. (2002) 51:290–5. doi: 10.1002/ana.10105

40. Hermann, B, Conant, LL, Cook, CJ, Hwang, G, Garcia-Ramos, C, Dabbs, K, et al. Network, clinical and sociodemographic features of cognitive phenotypes in temporal lobe epilepsy. *Neuroimage Clin*. (2020) 27:102341. doi: 10.1016/j.nicl.2020.102341

41. Vossel, KA, Ranasinghe, KG, Beagle, AJ, Mizuiri, D, Honma, SM, Dowling, AF, et al. Incidence and impact of subclinical epileptiform activity in Alzheimer's disease. *Ann Neurol*. (2016) 80:858–70. doi: 10.1002/ana.24794

42. Aarts, JH, Binnie, CD, Smit, AM, and Wilkins, AJ. Selective cognitive impairment during focal and generalized epileptiform EEG activity. *Brain*. (1984) 107:293–308.

43. Gelinas, JN, Khodagholy, D, Thesen, T, Devinsky, O, and Buzsaki, G. Interictal epileptiform discharges induce hippocampal-cortical coupling in temporal lobe epilepsy. *Nat Med*. (2016) 22:641–8. doi: 10.1038/nm.4084

44. Horak, PC, Meisenhelter, S, Song, Y, Testorf, ME, Kahana, MJ, Viles, WD, et al. Interictal epileptiform discharges impair word recall in multiple brain areas. *Epilepsia*. (2016) 58:373. doi: 10.1111/epi.13633

45. Krauss, GL, Summerfield, M, Brandt, J, Breiter, S, and Ruchkin, D. Mesial temporal spikes interfere with working memory. *Neurology*. (1997) 49:975–80. doi: 10.1212/WNL.49.4.975

46. Stemmer, N, Strekalova, E, Djogo, N, Ploger, F, Loers, G, Lutz, D, et al. Generation of amyloid-beta is reduced by the interaction of calreticulin with amyloid precursor protein, presenilin and nicastrin. *PLoS One*. (2013) 8:e61299. doi: 10.1371/journal.pone.0061299

47. Ung, H, Cazares, C, Nanivadekar, A, Kini, L, Wagenaar, J, Becker, D, et al. Interictal epileptiform activity outside the seizure onset zone impacts cognition. *Brain*. (2017) 140:2157–68. doi: 10.1093/brain/awx143

48. Lambert, I, Tramoni-Negre, E, Lagarde, S, Roehri, N, Giusiano, B, Trebuchon-Da Fonseca, A, et al. Hippocampal Interictal Spikes during Sleep Impact Long-Term Memory Consolidation. *Ann Neurol*. (2020) 87:976–87. doi: 10.1002/ana.25744

49. Henin, S, Shankar, A, Borges, H, Flinker, A, Doyle, W, Friedman, D, et al. Spatiotemporal dynamics between interictal epileptiform discharges and ripples during associative memory processing. *Brain*. (2021) 144:1590–1602. doi: 10.1093/brain/awab044

50. Harada, CN, Natelson Love, MC, and Triebel, KL. Normal cognitive aging. *Clin Geriatr Med*. (2013) 29:737–52. doi: 10.1016/j.cger.2013.07.002

51. Lezak, MD, Howieson, DB, Bigler, ED, and Tranel, D. *Neuropsychological Assessment. 5th* ed. New York: Oxford University Press. (2012).

52. Miller, JB, and Barr, WB. The Technology Crisis in Neuropsychology. *Arch Clin Neuropsychol*. (2017) 32:541–54. doi: 10.1093/arclin/acx050

53. Kalak, N, Gerber, M, Kirov, R, Mikoteit, T, Yordanova, J, Puhse, U, et al. Daily morning running for 3 weeks improved sleep and psychological functioning in healthy adolescents compared with controls [Controlled Clinical Trial]. *J Adolesc Health*. (2012) 51:615–22. doi: 10.1016/j.jadohealth.2012.02.020

54. Salmon, DP, and Bondi, MW. Neuropsychological assessment of dementia. *Annu Rev Psychol*. (2009) 60:257–82. doi: 10.1146/annurev.psych.57.102904.190024

55. Hermann, B, Seidenberg, M, Lee, EJ, Chan, F, and Rutecki, P. Cognitive phenotypes in temporal lobe epilepsy. *J Int Neuropsychol Soc*. (2007) 13:12–20. doi: 10.1017/S135561770707004X

56. Kaup, AR, Nettiksimmons, J, LeBlanc, ES, and Yaffe, K. Memory complaints and risk of cognitive impairment after nearly 2 decades among older women. *Neurology*. (2015) 85:1852–8. doi: 10.1212/WNL.0000000000002153

57. Kragel, JE, and Voss, JL. Looking for the neural basis of memory. *Trends Cogn Sci*. (2022) 26:53–65. doi: 10.1016/j.tics.2021.10.010

58. Rodini, M, De Simone, MS, Caltagirone, C, and Carlesimo, GA. Accelerated long-term forgetting in neurodegenerative disorders: A systematic review of the literature. *Neurosci Biobehav Rev*. (2022) 141:104815. doi: 10.1016/j.neubiorev.2022.104815

59. Loddenkemper, T, Lockley, SW, Kaleyias, J, and Kothare, SV. Chronobiology of epilepsy: diagnostic and therapeutic implications of chrono-epileptology. *J Clin Neurophysiol*. (2011) 28:146–53. doi: 10.1097/WNP.0b013e31821213d4

60. Malow, BA, Kushwaha, R, Lin, X, Morton, KJ, and Aldrich, MS. Relationship of interictal epileptiform discharges to sleep depth in partial epilepsy [Research Support, U.S. Gov't, P.H.S.]. *Electroencephalogr Clin Neurophysiol*. (1997) 102:20–6. doi: 10.1016/S0013-4694(96)96028-9

61. Bender, AC, Jaleel, A, Pellerin, KR, Moguilner, S, Sarkis, R, Cash, SS, et al. Altered Sleep Micro-architecture and Cognitive Impairment in Patients With Temporal Lobe Epilepsy. *Neurology*. (2023) 101:e2376. doi: 10.1212/WNL.0000000000207942

62. Milner, B. In: Brain mechanisms suggested by studies of temporal lobes. *Brain Mechanisms Underlying Speech and Language*. Eds. C. H. Millikan and F. L. Darley. New York: Grune & Stratton (1967).

63. Szabo, B, Cretin, B, Gérard, F, Curot, J, Barbeau, E, Pariente, J, et al. Sleep: The Tip of the Iceberg in the Bidirectional Link Between Alzheimer's Disease and Epilepsy [Review]. *Front Neurol*. (2022) 13:292. doi: 10.3389/fneur.2022.836292

64. Bartha-Doering, L, and Trinka, E. The interictal language profile in adult epilepsy. *Epilepsia*. (2014) 55:1512–25. doi: 10.1111/epi.12743

65. Harciarek, M, and Jodzio, K. Neuropsychological differences between frontotemporal dementia and Alzheimer's disease: a review. *Neuropsychol Rev*. (2005) 15:131–45. doi: 10.1007/s11065-005-7093-4

66. Patrikelis, P, Angelakis, E, and Gatzonis, S. Neurocognitive and behavioral functioning in frontal lobe epilepsy: a review. *Epilepsy Behav*. (2009) 14:19–26. doi: 10.1016/j.yebeh.2008.09.013

67. Bondi, MW, Edmonds, EC, Jak, AJ, Clark, LR, Delano-Wood, L, McDonald, CR, et al. Neuropsychological criteria for mild cognitive impairment improves diagnostic precision, biomarker associations, and progression rates. *J Alzheimers Dis*. (2014) 42:275–89. doi: 10.3233/JAD-140276

68. Vardy, ER, Brown, K, Stopford, CL, Thompson, JC, Richardson, AM, Neary, D, et al. Cognitive phenotypes in Alzheimer's disease and genetic variants in ACE and IDE. *Neurobiol Aging*. (2012) 33:1486.e1481–2. doi: 10.1016/j.neurobiolaging.2010.11.003

69. McDonald, CR, Busch, RM, Reyes, A, Arrotta, K, Barr, W, Block, C, et al. Development and application of the International Classification of Cognitive Disorders in Epilepsy (IC-CoDE): Initial results from a multi-center study of adults with temporal lobe epilepsy. *Neuropsychology*. (2023) 37:301–14. doi: 10.1037/neu0000792

70. Reyes, A, Kaestner, E, Ferguson, L, Jones, JE, Seidenberg, M, Barr, WB, et al. Cognitive phenotypes in temporal lobe epilepsy utilizing data-and clinically driven approaches: Moving toward a new taxonomy. *Epilepsia*. (2020) 61:1211–20. doi: 10.1111/epi.16528

71. Hermann, B, Seidenberg, M, Sager, M, Carlsson, C, Gidal, B, Sheth, R, et al. Growing old with epilepsy: the neglected issue of cognitive and brain health in aging and elder persons with chronic epilepsy. *Epilepsia*. (2008) 49:731–40. doi: 10.1111/j.1528-1167.2007.01435.x

72. Nardi Cesarini, E, Babiloni, C, Salvadori, N, Farotti, L, Del Percio, C, Pascarelli, MT, et al. Late-Onset Epilepsy With Unknown Etiology: A Pilot Study on Neuropsychological Profile, Cerebrospinal Fluid Biomarkers, and Quantitative EEG Characteristics [Original Research]. *Front Neurol*. (2020) 11:199. doi: 10.3389/fneur.2020.00199

73. Reyes, A, Kaestner, E, Edmonds, EC, Christina Macari, A, Wang, ZI, Drane, DL, et al. Diagnosing cognitive disorders in older adults with epilepsy. *Epilepsia*. (2021) 62:460–71. doi: 10.1111/epi.16780

74. Jokeit, H, and Ebner, A. Long term effects of refractory temporal lobe epilepsy on cognitive abilities: a cross sectional study. *J Neurol Neurosurg Psychiatry*. (1999) 67:44–50. doi: 10.1136/jnnp.67.1.44

75. Norman, M, Wilson, SJ, Baxendale, S, Barr, W, Block, C, Busch, RM, et al. Addressing neuropsychological diagnostics in adults with epilepsy: Introducing the International Classification of Cognitive Disorders in Epilepsy: The IC CODE Initiative. *Epilepsia Open*. (2021) 6:266–75. doi: 10.1002/epi4.12478

76. Breuer, LE, Boon, P, Bergmans, JW, Mess, WH, Besseling, RM, de Louw, A, et al. Cognitive deterioration in adult epilepsy: Does accelerated cognitive ageing exist? *Neurosci Biobehav Rev*. (2016) 64:1–11. doi: 10.1016/j.neubiorev.2016.02.004

77. Dam, AM, Fuglsang-Frederiksen, A, Svarre-Olsen, U, and Dam, M. Late-onset epilepsy: etiologies, types of seizure, and value of clinical investigation, EEG, and computerized tomography scan. *Epilepsia*. (1985) 26:227–31. doi: 10.1111/j.1528-1157.1985.tb05410.x

78. Jack, CR Jr, Albert, MS, Knopman, DS, McKhann, GM, Sperling, RA, Carrillo, MC, et al. Introduction to the recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement*. (2011) 7:257–62. doi: 10.1016/j.jalz.2011.03.004

79. Malow, BA, Lin, X, Kushwaha, R, and Aldrich, MS. Interictal spiking increases with sleep depth in temporal lobe epilepsy. *Epilepsia*. (1998) 39:1309–16. doi: 10.1111/j.1528-1157.1998.tb01329.x

80. Bilder, RM. Neuropsychology 3.0: evidence-based science and practice. *J Int Neuropsychol Soc*. (2011) 17:7–13. doi: 10.1017/s1355617710001396

81. Parsons, T, and Duffield, T. Paradigm Shift Toward Digital Neuropsychology and High-Dimensional Neuropsychological Assessments: Review [Viewpoint]. *J Med Internet Res*. (2020) 22:e23777. doi: 10.2196/23777

82. Battista, P, Salvatore, C, Berlingeri, M, Cerasa, A, and Castiglioni, I. Artificial intelligence and neuropsychological measures: The case of Alzheimer's disease. *Neurosci Biobehav Rev*. (2020) 114:211–28. doi: 10.1016/j.neubiorev.2020.04.026

83. Frank, B, Hurley, L, Scott, TM, Olsen, P, Dugan, P, and Barr, WB. Machine learning as a new paradigm for characterizing localization and lateralization of neuropsychological test data in temporal lobe epilepsy. *Epilepsy Behav*. (2018) 86:58–65. doi: 10.1016/j.yebeh.2018.07.006

84. Kessels, RPC. Improving precision in neuropsychological assessment: Bridging the gap between classic paper-and-pencil tests and paradigms from cognitive neuroscience. *Clin Neuropsychol*. (2019) 33:357–68. doi: 10.1080/13854046.2018.1518489

85. Collins, FS, and Riley, WT. NIH's transformative opportunities for the behavioral and social sciences. *Sci Transl Med*. (2016) 8:366ed314. doi: 10.1126/scitranslmed.aai9374

86. Leurent, C, and Ehlers, MD. Digital technologies for cognitive assessment to accelerate drug development in Alzheimer's disease. *Clin Pharmacol Ther*. (2015) 98:475–6. doi: 10.1002/cpt.212

87. Dede, AJ, Frascino, JC, Wixted, JT, and Squire, LR. Learning and remembering real-world events after medial temporal lobe damage. *Proc Natl Acad Sci USA*. (2016) 113:13480–5. doi: 10.1073/pnas.1617025113

88. Van Leemput, K, Bakkour, A, Benner, T, Wiggins, G, Wald, LL, Augustinack, J, et al. Automated segmentation of hippocampal subfields from ultra-high resolution in vivo MRI. *Hippocampus*. (2009) 19:549–57. doi: 10.1002/hipo.20615

89. Jutras, MJ, Fries, P, Buffalo, EA. Oscillatory activity in the monkey hippocampus during visual exploration and memory formation. *Proc Natl Acad Sci USA*. (2013) 110:13144–9. doi: 10.1073/pnas.1302351110

90. Smith, CN, Hopkins, RO, and Squire, LR. Experience-dependent eye movements, awareness, and hippocampus-dependent memory. *J Neurosci*. (2006) 26:11304–12. doi: 10.1523/JNEUROSCI.3071-06.2006

91. Meister, MLR, and Buffalo, EA. Getting directions from the hippocampus: The neural connection between looking and memory. *Neurobiol Learn Mem*. (2016) 134:135–44. doi: 10.1016/j.nlm.2015.12.004

92. Kragel, JE, Van Haerents, S, Templer, JW, Schuele, S, Rosenow, JM, Nilakantan, AS, et al. Hippocampal theta coordinates memory processing during visual exploration. *elife*. (2020) 9:52108. doi: 10.7554/eLife.52108

93. Crutcher, MD, Calhoun-Haney, R, Manzanares, CM, Lah, JJ, Levey, AI, and Zola, SM. Eye tracking during a visual paired comparison task as a predictor of early dementia. *Am J Alzheimers Dis Other Dement*. (2009) 24:258–66. doi: 10.1177/1533317509332093

94. Reynolds, GD. Infant visual attention and object recognition. *Behav Brain Res*. (2015) 285:34–43. doi: 10.1016/j.bbr.2015.01.015

95. Zola, SM, Squire, LR, Teng, E, Stefanacci, L, Buffalo, EA, and Clark, RE. Impaired recognition memory in monkeys after damage limited to the hippocampal region. *J Neurosci*. (2000) 20:451–63. doi: 10.1523/JNEUROSCI.20-01-00451.2000

96. Althoff, RR, and Cohen, NJ. Eye-movement-based memory effect: a reprocessing effect in face perception. *J Exp Psychol Learn Mem Cogn*. (1999) 25:997–1010. doi: 10.1037/0278-7393.25.4.997

97. Hannula, DE, Althoff, RR, Warren, DE, Riggs, L, Cohen, NJ, and Ryan, JD. Worth a glance: using eye movements to investigate the cognitive neuroscience of memory. *Front Hum Neurosci*. (2010) 4:166. doi: 10.3389/fnhum.2010.00166

98. Smith, CN, and Squire, LR. Experience-dependent eye movements reflect hippocampus-dependent (aware) memory. *J Neurosci*. (2008) 28:12825–33. doi: 10.1523/JNEUROSCI.4542-08.2008

99. McKee, RD, and Squire, LR. On the development of declarative memory. *J Exp Psychol Learn Mem Cogn*. (1993) 19:397–404. doi: 10.1037/0278-7393.19.2.397

100. Ryan, JD, Althoff, RR, Whitlow, S, and Cohen, NJ. Amnesia is a deficit in relational memory. *Psychol Sci*. (2000) 11:454–61. doi: 10.1111/1467-9280.00288

101. Zola, SM, Manzanares, CM, Clopton, P, Lah, JJ, and Levey, AI. A behavioral task predicts conversion to mild cognitive impairment and Alzheimer's disease. *Am J Alzheimers Dis Other Dement*. (2013) 28:179–84. doi: 10.1177/1533317512470484

102. Hannula, DE, and Ranganath, C. The eyes have it: hippocampal activity predicts expression of memory in eye movements. *Neuron*. (2009) 63:592–9. doi: 10.1016/j.neuron.2009.08.025

103. Hannula, DE, Ryan, JD, Tranel, D, and Cohen, NJ. Rapid onset relational memory effects are evident in eye movement behavior, but not in hippocampal amnesia. *J Cogn Neurosci*. (2007) 19:1690–705. doi: 10.1162/jocn.2007.19.10.1690

104. Ryan, JD, and Cohen, NJ. Processing and short-term retention of relational information in amnesia. *Neuropsychologia*. (2004) 42:497–511. doi: 10.1016/j.neuropsychologia.2003.08.011

105. Ryan, JD, Moses, SN, and Villate, C. Impaired relational organization of propositions, but intact transitive inference, in aging: Implications for understanding underlying neural integrity. *Neuropsychologia*. (2009) 47:338–53. doi: 10.1016/j.neuropsychologia.2008.09.006

106. Bott, NT, Lange, A, Rentz, D, Buffalo, E, Clopton, P, and Zola, S. Web Camera Based Eye Tracking to Assess Visual Memory on a Visual Paired Comparison Task. *Front Neurosci*. (2017) 11:370. doi: 10.3389/fnins.2017.00370

107. Bedi, G, Carrillo, F, Cecchi, G. A., Slezak, D. F., Sigman, M, Mota, N. B., et al. Automated analysis of free speech predicts psychosis onset in high-risk youths. *npj Schizophrenia*. (2015) 1:15030. doi: 10.1038/npjschz.2015.30

108. Orimaye, SO, Wong, JS, Golden, KJ, Wong, CP, and Soyiri, IN. Predicting probable Alzheimer's disease using linguistic deficits and biomarkers. *BMC Bioinformatics*. (2017) 18:34. doi: 10.1186/s12859-016-1456-0

109. Vigo, I, Coelho, L, and Reis, S. Speech-and Language-Based Classification of Alzheimer's Disease: A Systematic Review. *Bioengineering*. (2022) 9:10027. doi: 10.3390/bioengineering9010027

110. Yang, Q, Li, X, Ding, X, Xu, F, and Ling, Z. Deep learning-based speech analysis for Alzheimer's disease detection: a literature review. *Alzheimers Res Ther*. (2022) 14:186. doi: 10.1186/s13195-022-01131-3

111. Becker, J. T., Boller, F, Lopez, O. L., Saxton, J, and McGonigle, K. L. The natural history of Alzheimer's disease: description of study cohort and accuracy of diagnosis. *Arch. Neurol*. (1994) 51:585–594.

112. Croisile, BBM, Carmoi, T, Lepage, Y, and Aimard, G. Comparison Between Oral and Written Spelling in Alzheimer's Disease. *Brain Lang*. (1996) 54:361–87. doi: 10.1006/brln.1996.0081

113. Croisile, BSB, Brabant, MJ, Duchene, A, Lepage, Y, Aimard, G, and Trillet, M. Comparative Study of Oral and Written Picture Description in Patients with Alzheimer's Disease. *Brain Lang*. (1996) 53:1–19. doi: 10.1006/brln.1996.0033

114. Yeung, A, Iaboni, A, Rochon, E, Lavoie, M, Santiago, C, Yancheva, M, et al. Correlating natural language processing and automated speech analysis with clinician assessment to quantify speech-language changes in mild cognitive impairment and Alzheimer's dementia. *Alzheimers Res Ther*. (2021) 13:109. doi: 10.1186/s13195-021-00848-x

115. Fraser, KC, and Rudzicz, F. Linguistic Features Identify Alzheimer's Disease in Narrative Speech. *J Alzheimers Dis*. (2016) 49:407–22. doi: 10.3233/JAD-150520

116. Beltrami, D, Favretti, R, Favretti, RR, Ghidoni, E, Tamburini, F, and Calza, L. Speech Analysis by Natural Language Processing Techniques: A Possible Tool for Very Early Detection of Cognitive Decline? *Front Aging Neurosci*. (2018) 10:369. doi: 10.3389/fnagi.2018.00369

117. Pevy, N, Christensen, H, Walker, T, and Reuber, M. Predicting the cause of seizures using features extracted from interactions with a virtual agent. *Seizure*. (2024) 114:84–89. doi: 10.1016/j.seizure.2023.11.022

118. Rentz, DM, Amariglio, RE, Becker, JA, Frey, M, Olson, LE, Frishe, K, et al. Face-name associative memory performance is related to amyloid burden in normal elderly. *Neuropsychologia*. (2011) 49:2776–83. doi: 10.1016/j.neuropsychologia.2011.06.006

119. Sperling, RA, Bates, JF, Cocchiarella, AJ, Schacter, DL, Rosen, BR, and Albert, MS. Encoding novel face-name associations: a functional MRI study. *Hum Brain Mapp*. (2001) 14:129–39. doi: 10.1002/hbm.1047

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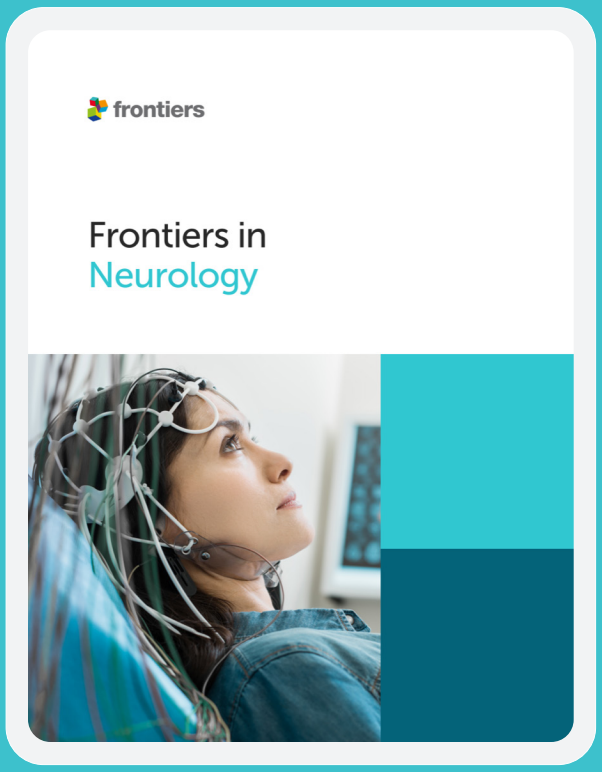


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