Silent hippocampal seizures and spikes identified by foramen ovale electrodes in Alzheimer’s disease

Alice D Lam1, Gina Deck1, Alica Goldman2, Emad N Eskandar3, Jeffrey Noebels2 & Andrew J Cole1

We directly assessed mesial temporal activity using intracranial foramen ovale electrodes in two patients with Alzheimer’s disease (AD) without a history or EEG evidence of seizures. We detected clinically silent hippocampal seizures and epileptiform spikes during sleep, a period when these abnormalities were most likely to interfere with memory consolidation. The findings in these index cases support a model in which early development of occult hippocampal hyperexcitability may contribute to the pathogenesis of AD.

One century ago, microscopic analysis of brain tissue from a single patient, Auguste Deter, established a link between amyloid plaques, neurofibrillary tangles, and severe memory loss1. Amyloid plaques were first described by Blocq and Marinesco in 1892 in patients with epilepsy2. AD and epilepsy both impair cognition and manifest overlapping patterns of cellular neurodegeneration and hypometabolism in the temporal lobe3. Despite extraordinary advances in understanding of the molecular pathology of AD4, the structural basis for the early fluctuations of cognitive decline in AD remains elusive and cannot be explained from purely microscopic or macroscopic pathological standpoints. Correlation of cognitive deficits with amyloid plaque load is weak5, comparable brain atrophy can be seen in cognitively normal adults6, and those with non-dementing diseases (including epilepsy)7, and a fluctuating disease course is not easily explained by a monotonic process of progressive cell death. Interneurons, however, are among the first to die in AD mesial temporal cortex8, and the ensuing degradation of synaptic connectivity with circuit remodeling could disrupt network oscillations that contribute to memory storage and retrieval. Intermittent temporal lobe dysrhythmia could therefore account for the early fluctuations in cognition in individuals with AD. Here we used intracranial electrodes inserted through the foramen ovale (FO) and positioned adjacent to the mesial temporal lobe (mTL)9 to, for the first time to our knowledge, directly assess mTL activity in two patients with AD and fluctuating cognition.

The first patient examined was a 67-year-old woman with no seizure history who over 1 year developed cognitive decline that was punctuated by confusional episodes described as hours of repetitive questioning and garbled speech that required days for full recovery. Neuropsychological testing demonstrated amnestic mild cognitive impairment. Additional clinical details are reported in the Supplementary Note.

Brain magnetic resonance imaging (MRI) showed diffuse atrophy, and 2-[(18)F]fluoro-2-deoxyglucose positron emission topography ((18)FDG-PET) demonstrated left temporoparietal hypometabolism (Supplementary Fig. 1a,b). A 35-min scalp electroencephalogram (EEG) obtained during sleep showed normal sleep architecture, including spindles and K-complexes, and no evidence of focal slowing or epileptiform discharges. Cerebrospinal fluid (CSF) analysis showed an amyloid–tau index of 0.44 (an index of <1.0 is abnormal) and a phosphorylated tau level of 95.9 pg/ml (a level of >61 pg/ml is abnormal), which are consistent with a diagnosis of AD. Genetic testing revealed APOE3 and APOE4 alleles of APOE but no exome variants in three known familial AD-associated genes (Supplementary Fig. 2).

On continuous video-EEG monitoring, left temporal sharp waves were seen at a rate of ~2 waves/h during wakefulness and ~40–70 waves/h during sleep. Rare right temporal sharp waves also occurred during sleep (~5 waves/h). On the basis of a high index of suspicion for occult seizures, the patient was implanted with bilateral FO electrodes targeting the mTL (Fig. 1a). Intracranial recordings from these electrodes revealed abundant mTL spiking (~400 spikes/h during wakefulness and up to ~850 spikes/h during sleep), with 85% of these spikes arising from the left mTL. The majority (95%) of the spikes detected on the FO electrodes were not evident on the scalp EEG (Fig. 1b). During the first 12 h of recording, the patient had three subclinical seizures arising from the left mTL, all of which occurred during sleep (Fig. 1c). Scalp EEG during these seizures showed no ictal activity. One seizure was associated with awakening from sleep, whereas the others had no overt clinical manifestations. The patient was treated with levetiracetam, a common antiepileptic drug that has been shown to reduce abnormal spiking activity in animal models of AD10 and to reduce hippocampal hyperactivity in humans with amnestic mild cognitive impairment11. Levetiracetam binds to SV2A12, a synaptic vesicle protein that regulates neurotransmitter release, although the exact mechanism of levetiracetam’s anticonvulsant effect is unknown.

After the start of levetiracetam (1,500 mg/d), no further seizures were captured on the FO electrodes over the following 48 h before their removal, and the spike frequency was reduced by 65%. Twelve months later, the patient reported one spell of confusion following several consecutively missed doses of levetiracetam. Repeat neuropsychological testing showed mild progression of her cognitive deficits.

The second patient included in this study was initially evaluated at age 58 for gradual cognitive decline, including repetitive questioning, misplacing objects, and social withdrawal. She had no prior history of seizures. Within 5 years, she had severe dementia (additional clinical details are reported in the Supplementary Note). Brain MRI demonstrated diffuse atrophy (Supplementary Fig. 1c), and CSF analysis showed an amyloid–tau index of 0.26 and a phosphorylated tau level...
of 73.2 pg/ml, which are consistent with a diagnosis of early-onset AD. There was no reported history of early-onset dementia in this patient's family (although genetic analysis was not obtained). At age 63, dramatic fluctuations in anxiety prompted further evaluation. Continuous video-EEG monitoring demonstrated rare multifocal epileptiform discharges occurring independently over the right temporal, left temporal, and bifrontal regions (~2 discharges/h during wakefulness and ~12 discharges/h during sleep). Bilateral FO electrode L temp R temp L paras R paras Vertex Coronal LFO RFO L hemi R hemi Vertex LFO RFO © 2017 Nature America, Inc., part of Springer Nature. All rights reserved.

Figure 1 Subclinical mTL seizures and spikes captured with FO electrodes in two patients with AD. (a–c) Patient 1. (a) Skull X-rays showing radiopaque FO electrodes with lateral (left) and anterior–posterior (right) views. (b) Left mTL spikes (arrowheads) without a scalp EEG correlate. Calibration scale: 200 µV, 1 s. (c) Electrographic seizure from the left mTL (arrow) without a scalp EEG ictal correlate. Panels show continuous EEG spanning 60 s. Calibration scale: 150 µV, 1 s. (d,e) Patient 2. (d) Right mTL spikes (arrowheads) without a scalp EEG correlate. Calibration scale: 200 µV, 1 s. (e) Three types of epileptiform discharges. Left, right mTL spike (arrowhead) with a scalp EEG correlate that resembles a benign epileptiform transient of sleep (box). Middle, bifrontal spike wave on scalp EEG (box) without an FO correlate. Right, left temporal sharp wave on scalp EEG (box) without an FO correlate. Calibration scale: 200 µV, 1 s. An anterior–posterior bipolar montage is shown in b–d. L temp, left temporal (Fp1–F7, F7–T3, T3–T5, T5–O1); R temp, right temporal (Fp2–F8, F8–T4, T4–T6, T6–O2); L paras, left parasagittal (Fp1–F3, F3–C3, C3–P3, P3–O1); R paras, right parasagittal (Fp2–F4, F4–C4, C4–P4, P4–O2); vertex (Fz–Cz, Cz–Pz); coronal, coronal ring (T1–T3, T3–C3, C3–Cz, Cz–C4, C4–T4, T4–T2, T2–T1); LFO, left FO (LFO1–LFO2, LFO2–LFO3, LFO3–LFO4); RFO, right FO (RFO1–RFO2, RFO2–RFO3, RFO3–RFO4). A referential montage (C2 reference) is shown in e. L hemi, left hemisphere (Fp1, F7, T1, T3, T5, F3, C3, P3, O1); R hemi, right hemisphere (Fp2, F8, T2, T4, T6, F4, C4, P4, O2); LFO, left FO (LFO1, LFO2, LFO3, LFO4); RFO, right FO (RFO1, RFO2, RFO3, RFO4). Contact 1 is deepest on FOs.
**BRIEF COMMUNICATIONS**

**Fig. 2** mTL spikes detected on FO electrodes are absent from scalp EEG recordings. Quantification and comparison of spike frequencies simultaneously observed on scalp EEG (SE) and FO electrodes during wakefulness and sleep for patients 1 and 2.

**METHODS**

Methods, including statements of data availability and any associated accession codes and references, are available in the online version of the paper.

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**AUTHOR CONTRIBUTIONS**

A.D.L., G.D., A.G., E.N.E., J.N., and A.J.C. drafted and edited the manuscript. A.G. prepared the figures. A.D.L. performed the spike quantification. A.G. performed the genetic analysis. A.J.C. and J.N. conceived the study.

**COMPETING FINANCIAL INTERESTS**

The authors declare no competing financial interests.
**ONLINE METHODS**

**FO electrode placement.** After explaining the risks and benefits of the procedure, consent for FO electrode placement was obtained from either the patient (patient 1 had the capacity to provide consent) or the patient's healthcare proxy (patient 2). Placement of FO electrodes\(^{14}\) was performed in the operating room under sterile conditions, with the patient under general endotracheal anesthesia and positioned supine with the neck extended and the head turned away from the site of insertion. Prior to insertion, each cheek was prepped with chlorhexidine, and 1% lidocaine was injected at a site 2.5 cm lateral to the labial fissure. With the fluoroscope C-arm angled 45° from vertical, the FO was visualized. A 16-gauge needle was initially used to pass through the skin, and a 10-gauge needle with stylet (Ad-Tech, Racine, WI) was advanced toward the FO under fluoroscopic guidance. The needle tip was placed at a site approximately 2 mm anterior to the clival line, and a lateral fluoroscopic image was taken to confirm the needle's position. The stylet was then removed, and a four-contact FO electrode (Ad-Tech, Racine, WI) was advanced through the needle under fluoroscopic guidance so that all electrode contacts were positioned beyond the tip of the needle. A lateral fluoroscopic image was taken to confirm electrode position, and the needle was gently withdrawn. The electrode was sutured to the cheek with 0 silk sutures. The image was taken to confirm electrode position, and the needle was gently withdrawn. The electrode was sutured to the cheek with 0 silk sutures. The same procedure was repeated to place the FO electrode on the contralateral side. After placement of both FO electrodes, anteroposterior and lateral fluoroscopic images were taken to confirm final electrode position, and sterile dressings were placed over the electrode exit site on each cheek. The patient was awakened from general anesthesia and observed in the post-anesthesia recovery room for 1 h. A post-procedure head computed tomography (CT) scan was obtained, and the patient was brought to the inpatient Epilepsy Monitoring Unit for further monitoring. While FO electrodes were in place, the patient remained on vancomycin and ceftriaxone antimicrobial prophylaxis. At the end of the investigation, prophylactic anticoagulation was held for 24 h, and the FO electrodes were removed at the bedside.

**Scalp EEG and FO electrode recordings.** Scalp electrodes were placed using the International 10–20 system with anterior temporal electrodes (T1, T2). All recordings were acquired using Xtek hardware (Natus Medical, Pleasanton, CA) with data sampled at 1,024 Hz. Scalp EEG and FO electrode recordings were visually interpreted as per routine clinical practice. Spike quantification was performed manually on randomly sampled 30- to 60-min segments of uninterrupted awake or asleep states.

**Cognitive testing.** The Blessed Information–Memory–Concentration test\(^{26}\) and Montreal Cognitive Assessment\(^{27}\) were administered by physicians and/or other trained personnel during Memory Disorders Unit and/or Epilepsy Clinic visits. Clinical dementia rating (CDR) scales were assessed at each Memory Disorders Unit visit.

**Laboratory testing.** CSF amyloid and tau analysis was performed by Athena Diagnostics, Marlborough, MA (ADmark Alzheimer's Evaluation).

**Imaging.** For patient 1, MR brain images were obtained with and without gadolinium contrast medium on a Siemens 3T Trio scanner, with the following sequences: sagittal FLAIR, sagittal T1, axial FLAIR, axial T2, coronal FLAIR, coronal T2, axial SWI, and axial DWI. \(^{18}\)FDG-PET images were obtained on a Siemens Biograph PET/CT scanner 45 min after injection with 5.5 mCi of \(^{18}\)Ffluoro-deoxyglucose. For patient 2, 3T MR brain images were obtained without contrast on a GE 3T 12.0 HD scanner with the following sequences: sagittal FLAIR, sagittal T1, axial FLAIR, axial T2, coronal T1, axial GRE, and axial DWI. All scans were performed for clinical purposes and interpreted by staff neuroradiologists.

**Genetic analysis.** Informed consent was obtained from all subjects undergoing genetic analysis. Five of six siblings of patient 1 were enrolled in an IRB-approved neurogenetics project at Baylor College of Medicine (H-15798) with informed consent. As described previously\(^{28}\), DNA extracted from peripheral blood samples was submitted for whole-exome sequencing (Genet, Gaithersburg, MD). Sequencing produced more than 120× average depth of coverage per exome (median coverage = 115×) per sample with >90% of the captured bases at >20× coverage. Raw sequencing reads were processed using the Codified (https://www.scienceexchange.com/labs/codified-genomics) annotation platform\(^{29}\). Alleles with >20× coverage and 40% allele fraction were considered for further analysis. The similarity score (the proportion of the rare variants with a minor allele frequency (MAF) of <0.5 that were shared among siblings was as expected, averaging 46% and ranging between 42% and 49%). We identified, on average, 707 total variants per exome (range 694–739) and an average of 131 variants per exome in known disease-associated genes (range 124–138).

**Data availability.** The data that support the findings of this study are available from the corresponding author upon reasonable request.

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