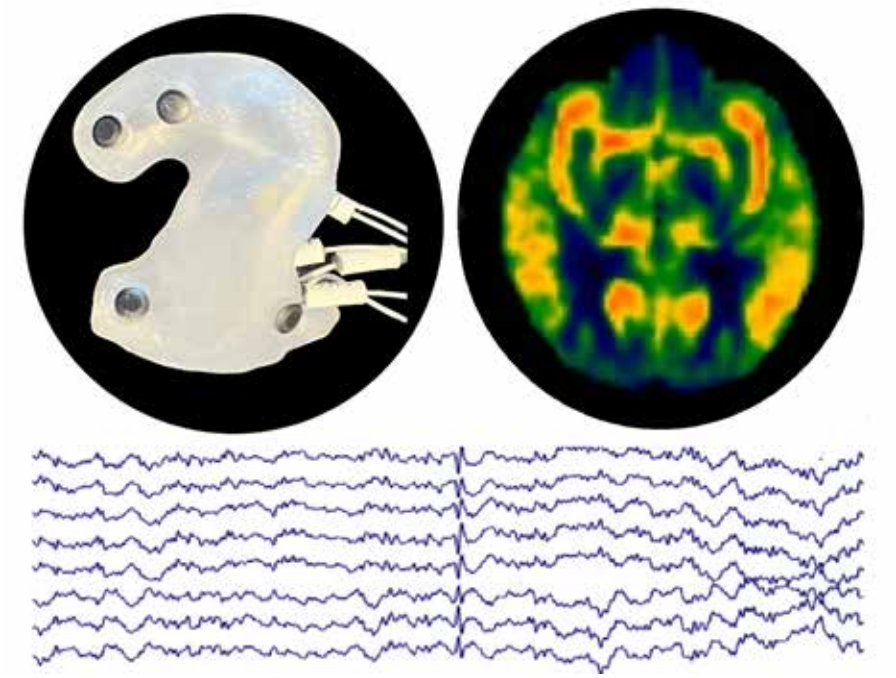
UNIVERSITY OF COPENHAGEN
FACULTY OF HEALTH AND MEDICAL SCIENCES

Long-term outpatient ear-EEG monitoring for detection of epileptiform discharges in patients with Alzheimer's disease and Lewy body dementia



PhD thesis

Christian Sandøe Musaeus

DANISH DEMENTIA RESEARCH CENTRE

REGION H Rigshospitalet

This thesis was submitted to the Graduate School of Health and Medical Sciences, University of Copenhagen on 24th of April 2023

PhD Thesis

Long-term outpatient ear-EEG monitoring for detection of epileptiform discharges in patients with Alzheimer's disease and Lewy body dementia

Author Christian Sandøe Musaeus
Danish Dementia Research Centre
Department of Neurology, Rigshospitalet,
University of Copenhagen
Copenhagen, Denmark

This thesis has been submitted to the Graduate School of Health and Medical Sciences, University of Copenhagen on 24th of April 2023

Supervisors

Principal supervisor Professor Gunhild Waldemar
Danish Dementia Research Centre
Department of Neurology, Rigshospitalet, and
Department of Clinical Medicine, University of Copenhagen
Copenhagen, Denmark

Primary co-supervisor Kristian Steen Frederiksen, MD, PhD
Danish Dementia Research Centre
Department of Neurology, Rigshospitalet,
University of Copenhagen
Copenhagen, Denmark

Assessment committee

Chairperson

Professor, Rigmor Højland Jensen, MD, DMSc
Department of Neurology
University of Copenhagen – Rigshospitalet
Copenhagen, Denmark

Assessor

Professor Sándor Beniczky, MD, PhD
Department of Clinical Neurophysiology
Aarhus University Hospital and Institute of Clinical Medicine, Aarhus University
Aarhus, Denmark

Assessor

Assistant Professor Alice D. Lam, MD, PhD
Harvard Medical School and Massachusetts General Hospital
Department of Neurology, Epilepsy Service
Boston, USA

Preface

This PhD project took place at the Danish Dementia Research Centre and the Memory Clinic at Rigshospitalet in close collaboration with Regional Dementia Research Center and the Neurophysiology Research Unit at Zealand University Hospital – Roskilde, the Functional Imaging Unit at Rigshospitalet (Glostrup), T&W Engineering, Center for Ear-EEG at Aarhus University, and Department of Clinical Neurophysiology at Rigshospitalet. The project was supported with grants from the Alzheimer Research Foundation, Toyota Foundation, Axel Juul Muusfeldts Foundation, Ellen Mørchs foundation, Rigshospitalet Research Foundation, T&W Engineering, and the Lundbeck Foundation.

First, I would like to express my heartfelt thanks to all the patients and volunteers that took part in the studies and to their families. Participation in this research project was complex and I can only admire your commitment and support for our research, and I appreciate all the valuable inputs that you have given me throughout this process.

Further, I would like to express my deep gratitude to my supervisors Gunhild Waldemar and Kristian Steen Frederiksen for their support throughout my PhD. Their guidance, and expertise have been invaluable in helping me achieving my goals. I especially appreciate how Gunhild has helped and guided me during challenging times, which I would not have been able to handle without her, and how Kristian's guidance on navigating everything from helping me deal with my own insecurities in the research process to providing me with valuable advice on my career, has made a significant positive impact on me. Their support has not only shaped my research but also helped me grow as an individual. I would also like to extend my heartfelt thanks to Troels Wesenberg Kjær for his invaluable assistance and support throughout my PhD. Furthermore, his encouraging talks and discussions throughout numerous hours of reviewing epileptiform discharges provided me with much needed motivation.

I also want to express my appreciation to all the study partners for their invaluable support and contributions throughout this project. From recruiting participants in Roskilde, analyzing and help with acquisition of conventional EEG recordings at the units working in neurophysiology, helping with acquiring and subsequent analyzing MRI at the Functional Imaging Unit, and the unwavering commitment to solve technical challenges related to the ear-EEG with the partners at both T&W engineering and Aarhus University. Specifically, I would like to thank Susanne Kristiansen and Oda Jakobsen for their assistance with recruiting participants and with study visits, Peter Høgh and Birgitte Bo Andersen for the help related to the patient visits and always helpful talks, Melita Cacic Hribljan and Martin Fabricius for analyzing the EEG data and insightful discussions, Ulrich Lindberg for sitting with me week after week and helping analyzing our ASL-MRI data, Henrik Bo Wiberg Larsson for his comments and suggestions during the analysis of the data, Mark Bitsch Vestergaard for his support in data analysis and help in Python, and Preben Kidmose and the whole team at T&W engineering (Richard Tøpholm, Mike Lind Rank, Martin Christian Hemmsen, Chris Henriksen and Ken Bliesmann) for always being available with any assistance related to the acquisition, delivering, reparation or analyzing the ear-EEG. I am grateful for our collaboration and honored to have had the opportunity to work with such a talented and dedicated group of people.

A special thanks to Daniel Zvi Press for scientific inspiration and collaboration throughout the last several years. His enthusiasm for science and his passion for sharing his knowledge and experience have been a constant source of inspiration and motivation for me. The opportunity to collaborate with him in person during my stay at Beth Israel Deaconess Medical Center was inspirational.

I want to express my gratefulness to the researchers and the clinical staff at the Danish Dementia Research Centre and the memory clinic at Rigshospitalet as well as my fellow PhD-students at both Righospitalet and Zealand University Hospital. Thank you for interesting discussions and help

throughout the work leading to this thesis. Specifically, I would like to thank Anja Hviid Simonsen, and Jørgen Erik Nielsen for your advice and support throughout the last several years.

Lastly, I would like to thank my wife, my two children, my brother, and my parents for all their support during the past four years.

Studies

This thesis is based on four original studies:

Study I **Musaeus CS**, Waldemar G, Andersen BB, Høgh P, Kidmose P, Hemmsen MC, Rank ML, Kjær TW, Frederiksen KS. Long-Term EEG Monitoring in Patients with Alzheimer's Disease Using Ear-EEG: A Feasibility Study. J Alzheimers Dis. 2022;90(4):1713-1723. doi: 10.3233/JAD-220491.

Study II **Musaeus CS**, Frederiksen KS, Andersen BB, Høgh P, Kidmose P, Fabricius M, Hribljan MC, Hemmsen MC, Rank ML, Waldemar G, Kjær TW. Detection of subclinical epileptiform discharges in Alzheimer's disease using long-term outpatient EEG monitoring. Neurobiol Dis. 2023 Jul;183:106149. doi: 10.1016/j.nbd.2023.106149. Epub 2023 May 15.

Study III **Musaeus CS**, Kjær TW, Andersen BB, Høgh P, Kidmose P, Fabricius M, Hribljan MC, Hemmsen MC, Rank ML, Waldemar G, Frederiksen KS. Subclinical epileptiform activity in dementia with Lewy bodies. Mov Disord. 2023 Jul 11. doi: 10.1002/mds.29531. Online ahead of print.

Study IV **Musaeus CS**, Kjær TW, Lindberg U, Vestergaard MB, Larsson HBW, Press DZ, Andersen BB, Høgh P, Kidmose P, Hemmsen MC, Rank ML, Hasselbalch SG, Waldemar G, Frederiksen KS. Subclinical epileptiform discharges in Alzheimer's disease are associated with increased hippocampal blood flow (submitted).

List of Abbreviations

Abbreviation	Definition
AD	Alzheimer's disease
AE	Adverse events
ANOVA	Analysis of variance
ASL-MRI	Arterial spin-labeling magnetic resonance imaging
CCMT	Category Cued Memory Test
DLB	Dementia with Lewy bodies
Epileptiform discharges	Spikes or sharp waves
eTIV	The estimated total intracranial volume
FAQ IADL	Functional Assessment Questionnaire Instrumental Activities of Daily Living
ICV	Intracranial volume
IFCN	International Federation of Clinical Neurophysiology
M0	Equilibrium magnetization scan
MMSE	Mini-mental state examination
NPI	Neuropsychiatric inventory
PCASL sequence	A pseudo continuous ASL sequence
PCM	Phase contrast mapping
rCBF	Regional cerebral blood flow

SAE	Serious adverse events
T1-MPRAGE	T1-weighted magnetization prepared rapid acquisition gradient echo
TE	The echo time
TR	The repetition time

Contents

Summary

Patients with dementia have an increased risk of seizures. An indicator of seizures are spikes or sharp waves (epileptiform discharges) identified with EEG. In preclinical studies, both amyloid and α-synuclein depositions have shown to induce epileptiform discharges. Studies in patients with Alzheimer's disease (AD) using long-term EEG monitoring for 24 hours found that up to half showed epileptiform discharges. However, these studies were limited to only 24-hour recordings and a novel technique, called ear-EEG, has been shown to be able to detect epileptiform discharges in patients with epilepsy. In epilepsy, ictal activity has shown to lead to increased cerebral perfusion, but no studies have so far investigated if a similar hemodynamic response exists for epileptiform discharges in patients with dementia.

The overarching aim of this project was to investigate the prevalence and clinical relevance of epileptiform discharges in patients with AD and dementia with Lewy bodies (DLB) using long-term outpatient monitoring with ear-EEG.

In this longitudinal observational study, 25 patients with AD, 10 patients with DLB, and 15 healthy controls underwent up to three ear-EEG recordings, each lasting up to two days, over a period of 6 months. The study also included measurement of regional cerebral blood flow (rCBF) of the hippocampus as measured with arterial spin-labeling magnetic resonance imaging (ASL-MRI) scans performed close in time with the first ear-EEG assessment.

The study found that ear-EEG is a feasible and safe technique for long-term outpatient monitoring of patients with AD and DLB. At baseline, 75% of patients with AD, 80% of patients with DLB, and 46.7% of healthy controls had epileptiform discharges. Most epileptiform discharges occurred at night, and a significantly higher spike frequency was found in patients with AD and DLB compared to healthy controls. Considerable variability between ear-EEG recordings was found across multiple

recordings. A significant linear association between spike frequency and normalized rCBF in the hippocampus was found for patients with AD.

Since ear-EEG is safe and feasible for long-term EEG monitoring, this technique can be used more widely in this patient population. The ear-EEG findings suggests that epileptiform discharges are common in neurodegenerative disorders. As epileptiform discharges are seen in both AD and DLB, this suggests that they are an unspecific marker of neurodegeneration or associated with the underlying neuropathological depositions of amyloid or α-synuclein. With repeated and long-term EEG recordings, most patients showed epileptiform discharges, suggesting that spike frequency, as compared to simply the presence of epileptiform discharges, is a more relevant marker of hyperexcitability when investigating the effect of anti-seizure medication. The association between rCBF and spike frequency in AD suggests a similar hemodynamic response to the one seen in patients with epilepsy. Future research should focus on investigating the effect of levetiracetam on spike frequency as measured with ear-EEG to fully understand the clinical relevance of long-term ear-EEG recordings.

Summary in Danish

Patienter med demens har en øget risiko for epileptiske anfald. En indikator for anfald er epileptiforme udladninger identificeret med EEG. I prækliniske studier har både amyloid- og α-synuclein-aflejringer vist sig at inducere epileptiforme udladninger. Tidligere undersøgelser med EEG-monitorering i et døgn har vist, at op mod halvdelen af patienterne med Alzheimers sygdom (AD) har epileptiforme udladninger. Disse undersøgelser var dog begrænset til 24-timers optagelser, og en ny teknik, såkaldt øre-EEG, har vist sig at kunne påvise epileptiforme udladninger hos patienter med epilepsi. Desuden er der ved epilepsi fundet en sammenhæng mellem iktal aktivitet og øget blodgennemstrømningen i hjernen, men det er ikke blevet undersøgt, om der eksisterer et lignende sammenhæng mellem epileptiforme udladninger og blodgennemstrømningen hos patienter med AD.

Det overordnede formål med dette projekt var at undersøge prævalensen og den kliniske relevans af epileptiforme udledninger hos patienter med AD og Lewy body demens (DLB) ved brug af langtids ambulant monitorering med øre-EEG.

I dette longitudinelle observationsstudie gennemgik 25 patienter med AD, 10 patienter med DLB og 15 raske kontrolpersoner op til tre øre-EEG-optagelser, som hver varede op til to dage over en periode på 6 måneder. Foruden dette fik alle foretaget en måling af den regionale cerebrale blodgennemstrømning (rCBF) i hippocampus målt med arteriel spin-labeling magnetic resonance imaging (ASL-MRI). Dette blev gjort tæt på tidspunktet for første øre-EEG-undersøgelse.

I denne afhandling fandt vi, at øre-EEG er en gennemførlig og sikker teknik til langvarig ambulant monitorering af patienter med AD og DLB. Ved baseline besøget blev epileptiforme udladninger påvist hos 75 % af patienterne med AD, 80 % af patienterne med DLB og 46,7 % af de raske kontrolpersoner. De fleste epileptiforme udladninger forekom om natten, og spikefrekvensen (epileptiforme udladninger/døgn) var signifikant højere hos patienter med AD og DLB sammenlignet

med raske kontrolpersoner. Der blev fundet betydelig variation mellem øre-EEG-optagelser på tværs af optagelserne. En signifikant lineær sammenhæng mellem spikefrekvensen og den normaliserede rCBF i hippocampus blev fundet for patienter med AD.

Disse resultater tyder på, at epileptiforme udladninger er almindeligt forekommende ved neurodegenerative lidelser. Da epileptiforme udledninger ses i både AD og DLB kunne det tyde på, at de er en uspecifik markør for neurodegeneration, eller at de muligvis er forbundet med de underliggende neuropatologiske aflejringer af amyloid eller α-synuclein. Med gentagne og langvarige EEG-optagelser fandtes, at de fleste patienter havde epileptiforme udladninger, hvilket tyder på, at spike frekvensen er en mere relevant markør for hyperexcitabilitet end blot tilstedeværelsen af epileptiforme udladninger, når man vil undersøge effekten af antiepileptiske præparater. Forbindelsen mellem rCBF og spike frekvensen i AD tyder på et lignende hæmodynamisk respons som set hos patienter med epilepsi. Fremtidig forskning bør fokusere på at undersøge effekten af levetiracetam på spike frekvensen målt med øre-EEG for fuldt ud at forstå den kliniske relevans af long-term øre-EEG-optagelser.

Background

Prevalence of epilepsy in dementia

Epilepsy is a clinical diagnosis and has been defined as the patient experiencing at least two seizures or with the revised criteria having one seizure with the expectation that another will occur [1]. In general, epilepsy can occur in any brain disorder and the risk rates vary depending on the etiology but has been linked to increased disease burden [2]. In patients with dementia of different etiologies, studies have found an increased risk of developing epilepsy [3,4] with a two- to three-fold increase as compared to the general population. This could indicate that the neurodegeneration predisposes the patient to developing seizures.

The most common type of dementia is Alzheimer's disease (AD), which is a neurodegenerative condition characterized by depositions of amyloid and tau in the brain that lead to neurodegeneration and thereby cognitive impairment [5]. In AD, multiple studies have demonstrated an increased risk of developing epileptic seizures [6–11] with varying prevalence. In the cohort studies, the prevalence of seizures ranged from 1.5% [6] to 9.7% [9] in patients with AD. Furthermore, one study followed the patients with AD until death and found that seizures were present in 64% of patients with AD, which can be attributed increased prevalence of seizures in the later stages of the disease [11]. This underlines that the risk of developing epilepsy increases with the disease burden [12]. The most common type of seizure in patients with AD is focal impaired awareness seizures [10,12], which may be difficult to detect due to the symptoms of AD can resemble the symptoms of a focal impaired awareness seizure.

The second most common type of neurodegenerative dementia is dementia with Lewy bodies (DLB), which is neuropathologically characterized by accumulation of α-synuclein protein in Lewy bodies [13]. Clinically, DLB is characterized by progressive cognitive decline and include symptoms of

cognitive fluctuations, visual hallucinations, and parkinsonism [14]. Although there is an increased risk of developing seizures due to the progressive neurodegeneration, the number of studies investigating the link between epilepsy and DLB is sparse. One study found a higher risk of developing seizures than in the general population [15] and case reports have shown patients with DLB presenting with epileptic seizures [16,17] or a patient with epilepsy presenting symptoms mimicking DLB [18]. Due to DLB being a progressive neurodegenerative disease with symptoms of cognitive fluctuations, it is likely that epilepsy is more prevalent than previously expected due to the findings in patients with AD.

As recently documented by a systematic review, only one randomized trial has investigated the efficacy of different types of anti-seizure medication in patients with dementia and epilepsy [19]. In this study investigating patients with AD, no significant difference was found for efficacy between levetiracetam, lamotrigine, or phenobarbital but levetiracetam led to improved cognitive function in patients with AD [20]. This finding suggests that there may also be more general cognitive benefits from threating seizures in patients with dementia.

Epileptiform discharges in patients with Alzheimer's disease

An important tool for seizure characterization is electroencephalography (EEG). EEG can be used to identify spikes/sharp waves (epileptiform discharges), which can be an indicator of seizures. By applying short-term conventional EEG recordings (< 1 hour) in a large mixed memory clinic cohort, only 3% of participants had epileptiform discharges [21]. However, in studies using long-term in-patient EEG recordings for 24 hours, 22%-54% of patients with AD showed epileptiform discharges without clinical seizures [22–24], which correlated with a more rapid rate of progression [22,24]. Although the majority of studies found a significant increase in epileptiform discharges, one study

that performed a single full night polysomnography recording did not find a significant difference between AD and HC [25]. Due to the discrepancy in the number of epileptiform discharges between short-term and long-term EEG monitoring, it suggests that short-term EEG monitoring is not sensitive for detecting epileptiform discharges in patients with AD. This is supported by studies showing an association between recording length and number of epileptiform discharges in patients with AD [26,27]. Although this is expected due to an increased risk of epileptiform discharges, it is important to perform long-term EEG recordings to measure the occurrence of epileptiform discharges in patients with dementia.

Long-term EEG monitoring

Long-term in-patient EEG monitoring is ideal for investigating epileptiform discharges, but it can be challenging for patients with dementia since they must be hospitalized throughout the period. Therefore, there is a need for accessible continuous EEG monitoring for outpatient recordings. Although long-term EEG monitoring has been used in patients with epilepsy, modalities for long-term outpatient EEG monitoring in patients with dementia have not been investigated yet. So far, multiple types of wearable EEG methods exist such as EEG electrodes around the ear (cEEGrid) [28] or ear-EEG [29] but none have been used in patients with neurodegenerative diseases.

Validity of ear-EEG

To use ear-EEG for detection of epileptiform discharges, it is crucial to understand its current validity. There are three important aspects:

1. Signal quality validation: how the ear-EEG device's signal quality compares to traditional scalp EEG through validation studies.

2. Utilization in settings and populations: the settings and populations where the ear-EEG device has found application, shedding light on its versatility.
3. Performance characteristics in epileptiform discharge and seizure detection: the device's performance characteristics, particularly its efficacy in detecting epileptiform discharges and seizures.

Signal quality validation

Studies have found that ear-EEG is able to detect similar signal responses to different paradigms as compared to scalp EEG in a laboratory setting with healthy young participants [29,30]. Specifically, a study using wet ear-EEG electrodes investigated different paradigms such as auditory steady-state response with white noise as well as alpha activity related to open/closed eyes [30]. Here, the study found that ear-EEG shows similar performance as scalp EEG. In terms of dry electrodes, which has been used in the current study, a similar study has been conducted using the same paradigms including alpha-band modulation comparing ear-EEG to scalp EEG [29]. This study found that for the between ear measurement of dry ear-EEG electrodes, a similar pattern was found with scalp EEG, but with lower amplitude. When using dry electrodes for out-patient monitoring, it is important to consider the limitations and whether it outweighs the disadvantages of using wet electrodes, which include having to apply gel and checking whether the gel is not drying up after longer recordings. So far, no studies have investigated the quality of ear-EEG recordings in an in-home setting. It is likely that the signal quality in general may be lower compared to the laboratory setting due to challenges associated with placement of the electrodes and increased number of artefacts from for example muscle activity.

Utilization in settings and populations

The adoption of ear-EEG in clinical studies has been very limited with most studies primarily focusing on young and healthy participants. However, these investigations have consistently demonstrated the reliable performance of dry electrode ear-EEG systems [31] and has demonstrated that participants can use the equipment in a home setting without supervision [32]. So far, the only clinical applicability of ear-EEG has been in patients with epilepsy [33–35]. Here, ear-EEG has primarily been employed for monitoring seizure activity in patients suffering from epilepsy in an in-patient environment [33,35]. To our knowledge, no studies have investigated the feasibility of utilizing ear-EEG for out-patient monitoring in a clinical population. Since out-patient monitoring has never been performed in a patient population, it is necessary to investigate its feasibility.

Performance characteristics in epileptiform discharge and seizure detection

In terms of capability of detecting EEG seizure activity, a few studies have shown that in-ear-EEG [33], behind-the-ear EEG [36] or ear-EEG being a part of a multi-modal detection system [34] is able to detect seizures with a good sensitivity [33,34,36,37]. All the recordings were performed on patients who were hospitalized, and not in the out-patient setting. A single study showed that ear-EEG is able to detect epileptiform discharges co-registered with scalp EEG in patients (n=15, age range: 18-60) with temporal lobe epilepsy [33], who were hospitalized in an epilepsy monitoring unit. Although this is an important finding, no studies have so far made a direct comparison between ear-EEG and scalp EEG in detection of epileptiform discharges (i.e., sensitivity and specificity of the ear-EEG).

In conclusion, ear-EEG has not yet been applied in patients with neurodegenerative diseases and there has been no studies on the sensitivity of ear-EEG in detecting epileptiform discharges. However, since it is possible to detect epileptiform discharges using wet-electrode ear-EEG and dry-electrode

ear-EEG can detect a similar signal as scalp EEG (although smaller amplitude), it indicates that ear-EEG may be a potentially suitable device for long-term monitoring in patients with AD.

Hypothesis of hyperexcitability in dementia

Hyperexcitability can be defined as an increased probability of neuronal activation by a certain stimulus and is associated with epileptiform discharges. In preclinical models of AD, multiple studies report a hyperexcitability in the hippocampus in animal models as reported in a recent review [38]. As for a link between hyperexcitability and the pathological changes in patients with AD, studies have found that increased neuronal activation cause increased amyloid depositions [39] and leads to the release of tau [40]. Furthermore, a study found that reducing neuronal excitability leads to decreased amyloid depositions [41]. These findings support a link between increased epileptiform discharges and the neuropathology seen in Alzheimer's disease.

In patients with AD, neurodegeneration is pronounced in the temporal lobes with atrophy in several temporal lobe structures [42,43]. Due to this location, it would be of importance to understand if mechanisms related to hyperexcitability, and subsequent epileptiform discharges resemble the mechanism seen in temporal lobe epilepsy. When investigating the response to ictal activity in patients with temporal lobe epilepsy, studies have found an ictal hyperperfusion [44–46] whereas hypoperfusion is present in the interictal periods [47–49], which has been hypothesized to be related to less active brain tissue. In patients with AD, studies have shown a decreased regional cerebral blood flow (rCBF) in the regions affected by neurodegeneration during rest, including the hippocampus [50–53]. However, a few studies found increased rCBF in the temporal lobes including the hippocampus but did not monitor the neurophysiological changes [54,55]. In the context of epileptiform discharges, it is possible that the increased rCBF in patients with AD represent a state of

hyperexcitability, which would result in epileptiform discharges. Although the epileptiform discharges seen in AD only involve spikes/sharp waves and not ictal activity [22–24], it is possible that a similar increase in rCBF may underly epileptiform discharges due to hyperexcitability but this has not been investigated so far.

In DLB, only a few studies have investigated the association between the neuropathological changes and epileptiform discharges in preclinical models. In DLB mouse models, depositions of α-synuclein have been shown to induce epileptiform discharges that can escalate into seizure activity [56] during non-REM sleep [57]. Since REM sleep has been shown to possibly inhibit epileptiform discharges [58,59] and REM sleep becomes altered in patients with DLB [60], the fragmented REM sleep in DLB could increase the probability of epileptiform discharges during the neurodegenerative processes. Furthermore, studies have also found depositions of amyloid in the brain in patients with DLB [61,62] and a similar underlying mechanism of epileptiform discharges is therefore possible in AD and DLB.

Potential clinical relevance of epileptiform discharges

Due to the findings of brain hyperexcitability, suppressing epileptiform discharges may represent a novel therapeutic target in AD and DLB. This is even more relevant due to the findings of epileptiform discharges leading to a more rapid progression in patients with AD [22,24] and in mouse models of AD [63,64]. To further explore these findings, a few studies have investigated the effect of levetiracetam in patients with AD [65–67]. One study investigating levetiracetam in mild cognitive impairment, found a normalization of the hippocampal hyperactivity on bold fMRI and an improvement in memory [65] while another found a possible normalization of the EEG abnormalities found in patients with AD [67]. More recently a study showed improvement in executive function in

patients with AD who were given low-dose levetiracetam, and the improvement was restricted to patients with epileptiform discharges on EEG [66]. However, none of the studies performed long-term EEG monitoring, and it is possible that the cognitive improvement after anti-seizure medication is restricted to only patients with substantial epileptiform discharges. Another important aspect is the variability in the number of epileptiform discharges between different recordings. That is, if the epileptiform discharges only occur during specific periods as has been seen with electrographic seizures in patients with epilepsy [68], it may be difficult to determine if epileptiform discharges are present or not with a single long-term in-patient recording. Hence, long-term outpatient recordings over multiple days or repeated recordings may be better suited to investigate the number of epileptiform discharges.

Aims

The overarching aim of this research project was to investigate the prevalence and potential clinical significance of epileptiform discharges using long-term EEG monitoring in patients with Alzheimer's disease and Lewy body dementia.

Study objectives

Objective 1: Investigate the feasibility of ear EEG for long-term monitoring in patients with mild to moderate Alzheimer's disease

Hypothesis: Ear-EEG for long-term EEG monitoring is feasible and safe in patients with Alzheimer's disease

Objective 2: Investigate the prevalence of epileptiform discharges in patients with Alzheimer's disease and Lewy Body dementia as measured with ear EEG.

Hypothesis: Subclinical epileptiform discharges are significantly more prevalent in Alzheimer's disease and Lewy body dementia as compared to healthy controls.

Objective 3: Investigate the association between epileptiform discharges as measured by ear EEG and cerebral blood flow (rCBF) in the hippocampus in patients with Alzheimer's disease.

Hypothesis: In Alzheimer's disease, increased epileptiform discharges as measured by ear EEG are associated with an increased rCBF in the hippocampus.

Methods

To ensure precision, large parts of the text in the method section was reproduced verbatim from the four manuscripts within the present thesis [27,69–71].

Participants

The description of the participants has to a large extent been reproduced from studies included in this PhD-thesis [27,69–71]. All patients with AD and DLB who had mild to moderate dementia were recruited from the Memory Clinic at Copenhagen University Hospital – Rigshospitalet, Copenhagen and the Memory Clinic at Zealand University Hospital, Roskilde. The patients with AD met the NIA-AA criteria for probable AD with amnestic presentation [72] and the patients with DLB met the Consortium criteria for DLB [73] both determined based on a consensus conference. This consensus conference was conducted after clinical evaluation and evaluated the clinical findings including structural imaging and, in most instances, 18F-flourdeoxyglucose positron emission tomography and neuropsychological evaluation. For a full description of the diagnostic evaluation see the attached manuscripts [27,69]. The inclusion and exclusion criteria for patients with AD [27,70] and DLB [69] as well as the HC [27,69] can be found in the attached manuscripts [27,69–71].

Ethical considerations

The description of the ethical considerations has been reproduced from studies included in this PhD-thesis [27,69–71]. The study was approved by the Ethical Committee if the Capital Region of Denmark (H-17035751), by the Danish Medicines Agency (2017112288), and by the Data Protection

Agency (P-2021-866). Written informed consent was given by all participants before entering the study. The study was registered at clinicaltrials.gov (NCT04436341).

Study design

In this longitudinal study, a total of eight visits were planned over a six-month period for patients with AD and DLB, and a total of four visits for the HC, see Figure 1. The program for the first four visits for HC was like the program for AD/DLB patients except that the FAQ IADL and the NPI were not administered.

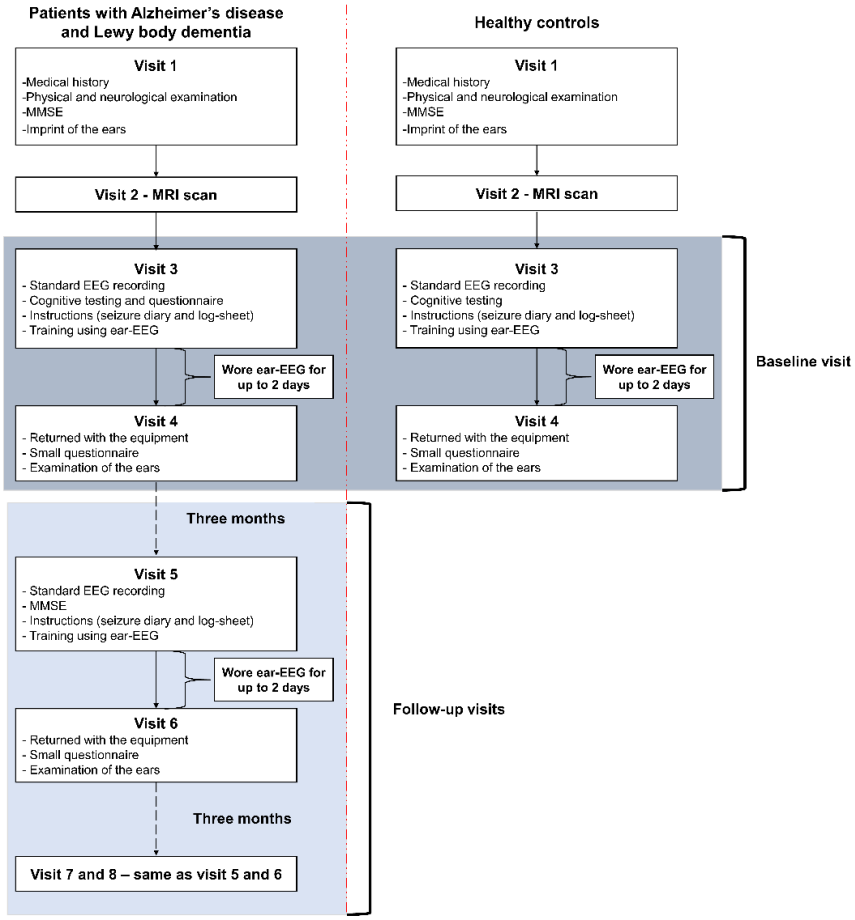


Figure 1: Study design. All participants underwent the first session of ear-EEG recording (baseline) with the participants with AD, and DLB also undergoing up to two follow-up visits**.** The figure was reproduced from [27,69,70].

The following description of the study design has been reproduced from studies included in this PhD-thesis [27,69–71] since the participants underwent the same procedures. The three two-day ear-EEG recordings for patients with AD and DLB were planned three months apart, see Figure 1. At the first visit (baseline), informed consent was obtained followed by a physical and neurological examination, the MMSE as well as an assessment of medical history. Furthermore, an imprint of the ears was performed using an ear impression silicone (Otoform A Soft X (Dreve, Germany)). As has been reported in the attached manuscripts [27,69,70], the patient underwent the following: visit 2) MRI scan, visit 3) standard scalp EEG recording together with ear-EEG, Functional Assessment Questionnaire IADL (FAQ IADL) [74], neuropsychiatric inventory (NPI) [75], and the Category Cued Memory Test (CCMT) [76].. At visit 3, the patient and the caregiver were informed about seizures and introduced to a seizure diary where they could note any symptoms of seizures. In addition, the patient and caregiver were also informed and trained in how to use the ear-EEG at home. This information included a log-sheet to take notes on when the participant wore the ear-EEG. After having the ear-EEG at home for approximately 48 hours (visit 4) the patient and/or the caregiver returned with the equipment. At this visit, the patient filled a questionnaire (see below), and the external ear was examined for any lesions and participants were asked about any adverse events. Visit 5 and 6, and visits 7 and 8 were similar to visits 3 and 4 except that MMSE was performed at visit 5, and 7 [27,69,70]. In the study investigating the association between epileptiform discharges and hippocampal rCBF, only the first ear-EEG assessment was used [71].

The following description of the questionnaire has been reproduced from studies included in this PhD-thesis [27,69,70]. A short questionnaire about the use of the ear-EEG was administered to the participants after wearing the ear-EEG for 2 days, which was conducted at visit 4, 6, and 8. This questionnaire included: a) how did it feel to wear the ear-EEG, b) did you have any problems sleeping with the equipment, c) did the ear-EEG ever fall out of the ear, and d) do you have any suggestions

for improvement [27,69,70]. To understand if the participant had experienced any adverse events, questions a) and b) were evaluated in combination with the examination of the ears.

Assessment of feasibility

The following description of the assessment of feasibility has been reproduced from the first study in the Ph.D.-thesis [70]. In the current thesis, feasibility was assessed on the ability of the patients to wear the ear-EEG. This included responses to the questionnaire, data quality, and safety. Here, safety was assessed based on the occurrence of adverse events (AE) including serious adverse events (SAE) related to the ear-EEG.

Feasibility criteria: More than 80% of the patients with AD wore the ear-EEG for at least 24 hours and at least one night over the recording period (2 days) [70].

Ear-EEG recording, pre-processing and data quality

The following description of the ear-EEG recording, pre-processing and data quality has been reproduced from studies included in this PhD-thesis [27,69–71] . The current ear-EEG setup is a method using six dry-electrodes mounted in earpieces. The earpieces are custom-made with 3D-printing of the ear imprint and worn in both ears. For an image of the current ear-EEG setup, see figure in Musaeus et al. [70]. The electrodes of the ear-EEG was labelled according to a previous description [77]. During the recording, the ground electrode was placed approximately 2 cm under the midline of the clavicle on the left or right side. The recordings were performed with the TMSi Mobita EEG amplifier (TMSi systems), which was either borne in a small bag around the neck or in a belt bag. A sampling rate of 1000 Hz was chosen. For the pre-processing, the data were loaded into

MATLAB (v2020b) and the pre-processing is based on an artefact rejection pipeline that has previously been used in other studies [31]. The pre-processing pipeline has been described in detail in the feasibility study [70].

The amount of data after the pre-processing (pre-processed data), which is the amount of data (in time) when at least one electrode in each ear were present was assessed. Lastly, the amount of pre-processed data during the night (10 pm-8am) and day (8am-10pm) was calculated. The patients' reported start time of recording was used [27,78].

Ear-EEG data review

The following description of the ear-EEG data review has been reproduced from studies included in this PhD-thesis [27,69–71]. Visual review and annotation of the ear-EEG recordings was performed by the PhD-student using the EEGLAB toolbox (v13.6.5b). During the review, the average referenced montage was used and 10 second epochs were inspected at a time with ±30 μV amplitude range. In this montage, the first 12 channels were filtered data whereas the following 12 channels were after pre-processing. The subsequent 72 channels were the comparisons between electrodes, see attached manuscripts for a full description [27,69]. The removed segments after the pre-processing pipeline and segments with data from only one ear were not inspected.

As reported in the manuscripts [27,69], a sharp asymmetric negative potential of 20-200 ms duration was considered an epileptiform discharge (spike/sharp wave) if it met the International Federation of Clinical Neurophysiology (IFCN) criteria [79]. The IFCN criteria consists of the epileptiform discharges being: 1) di- or tri-phasic with a pointed peak with 2) a different wave duration than the background activity, 3) asymmetry of the waveform, 4) background activity was disrupted by the presence of the epileptiform discharge and is most cases the 5) followed by a slow after-wave [79].

Due to the low spatial resolution of ear-EEG, the spatial distribution of spikes/sharp waves could not be investigated (IFCN criteria 6). However, all epileptiform discharges fulfilled at least four out of the six IFCN criteria. The annotations underwent review by a board-certified clinical neurophysiologist (TWK), who made the final ruling. Both the PhD-student and TWK were blinded to the diagnosis when reviewing the EEGs [27,69].

Conventional EEG

The scalp EEGs was recorded from 19 electrodes and consisted of 10 minutes of alternating eyes open, and eyes closed followed by 20 minutes of sleep. A full description of the conventional EEGs can be found in the studies included in this PhD-thesis [27,69].

All scalp EEGs were assessed by a clinical neurophysiologist (either TWK or MCH). This led to the EEGs being classified as 1) Normal EEG, 2) Slowing (subclassification into focal slowing as defined by intermittent or persistent low frequency theta or delta and/or diffuse slowing as defined by a posterior dominant rhythm below 7 Hz) and 3) epileptiform discharges [27,69].

Adverse events

The following description of adverse events has been reproduced from studies included in this PhD-thesis [27,69,70]. Adverse events were part of the evaluation of the feasibility of ear-EEG. To monitor possible adverse events, the investigators were automatically notified of any hospitalization. A serious adverse event (SAE) was defined according to the international criteria (Good Clinical Practice) [70].

Magnetic resonance imaging

All data was recorded on a 3T Achieva dStream (Philips, Best, The Netherlands) with a 32-channel head receive coil. The description of the MRI acquisition and processing has been reproduced from a study included in this PhD-thesis [71].

Structural image analysis

A sagittal 3D T1-weighted magnetization prepared rapid acquisition gradient echo (T1-MPRAGE) was recorded with the following acquisition parameters: repetition time (TR) 6.9 ms, echo time (TE) 2.82 ms, flip angle 9, matrix size 256x255, 155 slices, voxel size $1.1\times1.1\times1.1$ mm^3, The T1-weighted images were segmented using Freesurfer (version 7.2.0, https://surfer.nmr.mgh.harvard.edu/) and the volumes for each hippocampus and the inferior lateral ventricles were obtained. Both were normalized to the intracranial volume (ICV). The estimated total intracranial volume (eTIV) generated by FreeSurfer was used as an estimate for ICV in this study as has previously been shown [80].

Phase contrast mapping

The mean global cerebral blood flow (CBF) was obtained using velocity sensitive phase contrast mapping (PCM) MRI [81,82]. Blood velocity contrast maps were acquired by a turbo field echo sequence. Measurements were acquired from an imaging plane perpendicular to the carotid arteries and one perpendicular to the basilar artery. Here, the blood flows were calculated in both internal carotids and the basilar artery by multiplying the mean blood velocity by the cross-sectional area from regions of interest defining each vessel [83]. To calculate the global mean CBF, the total blood flow

from each artery was normalized to the total brain weight, which was estimated from the segmentation of the structural MRI images with an assumed brain density of 1.05 g/mL Calculations were performed using a custom-built script in Python.

Arterial spin labeling MRI analysis

A pseudo continuous ASL (PCASL) sequence with Look-Locker Echo Planar Imaging was chosen. The labeling plane was placed across the neck 9 cm beneath the center of the imaging slab and the labeling duration was 1650 ms. The acquisition parameters were: 13 slices, TE 10.8 ms, voxel size 3.44x3.44x6.6 mm^3, FoV 220x220x85 mm^3, TR was 300 ms, Look-Locker Flip-Angle 40^o, slice acquisition duration 22 ms, SENSE factor 2.3, The post-labeling delays were set at [100, 400, 700, 1000, 1300, 1600, 1900 ms]. Each ASL pair (label and control) took 8 seconds. In the current study, the region of interest was the hippocampus, which resulted in the acquisition plane being placed parallel to the inferior lateral ventricles. A single equilibrium magnetization scan (M0) was acquired after each ASL scan using the same parameters as for the ASL images except for a 10,000 ms TR.

ASL images were quantified using BASIL in FSL (FMRIB software library, version 6.0.5.1, www.fmrib.ox.ac.uk) with quantification [84] and fitting of the macrovascular compartment [85]. The initial prior of bolus arrival time was adjusted to account for the delayed arrival as seen in the current sample, which resulted in a selected arrival time prior of 1.6 s. Lastly, the ASL data were registered to the T1wscan. We did not obtain individual hematocrit values for the participants.

To quantify rCBF in the hippocampus, we first extracted all the values and then removed the voxels with zero values as these were assumed to be contaminated by CSF. Afterwards, any values more than two standard deviations above the mean were removed since they were assumed most likely to

represent arteries. Finally, the median value of CBF was extracted for each hippocampus and normalized to the global mean CBF as measured with PCM and the mean of the two values were computed. The same approach was used for the precuneus.

Statistics

The following description of the statistics has in part been reproduced from studies included in this PhD-thesis [27,69–71]. The analyses were performed in RStudio (v1.2.1335). When comparing age, education, and cognitive scores, we performed an ANOVA across the three groups (AD, DLB, and HC). Chi-squared tests was used performed for comparing sex.

To compare the number of participants in each group with spike/sharp waves at baseline and the number of participants with slowing, as well as the number of spikes per night and spikes per day, we performed either a Chi-squared test or Fisher's exact test (in cases where one input variable had a value of 5 or less) [27,69]. When comparing the spike frequency (number of spikes or sharp waves per 24 hours) between HC and individuals with AD or DLB at baseline, we calculated the rate ratio using the *rateratio* function from the *epitools* toolbox [27,69].

Spearman's correlation was used to examine the relationship between spike frequency across all recordings and cognitive test scores. For assessing changes in spikes or sharp waves between visits for in individuals with AD or DLB, a generalized linear mixed-effect model was used. This model included visit number (categorical variable) and log-transformed the amount of pre-processed EEG (in minutes) as the offset variable with a person specific random intercept in a Poisson regression model using the *lme4* package. To evaluate changes in Mini-Mental State Examination (MMSE) scores across visits, we employed a linear mixed model. This model included visit number as a fixed effect and assumed an unstructured covariance pattern to account for repeated measurements on the

same subjects. We used the *LMMStar* package for this analysis [27,69]. When comparing the time difference between the ear-EEG recording and MRI scan, the structural MRI results, global blood flow, and rCBF in the hippocampus or precuneus, we performed t-tests between AD and HC. Simple linear regression was used to test if spike frequency significantly predicted the normalized mean rCBF in the hippocampus in patients with AD as well in HC.

Result Summary

Patient characteristics

In this thesis, a total of 25 patients with AD, 10 patients with DLB and 15 HC were included. See Table 1 for participant characteristics. One patient with AD had less than one hour of ear-EEG data after pre-processing and was not included in the ear-EEG analyses. The investigation of the feasibility of the ear-EEG was conducted on the first 10 patients with AD who were included in the study.

Table 1: Characteristics of the participants.

	Healthy controls	Alzheimer's disease	Lewy body dementia	p-value
Number of participants	15	25	10	
Age, mean (SD)	69.5 (7.93)	70.3 (7.79)	69.9 (8.49)	0.959
Males/females	8/7	15/10	10/0	0.036
Education, mean (SD)	15.2 (2.04)	14.2 (3.08)	14.3 (2.91)	0.534
Cholinesterase inhibitor, n (%)	0	24 (96%)	9 (90%)	
Levodopa, n (%)	0	0	3 (30%)	
SSRI/SNRI, n (%)	1 (7%)	4 (16%)	2 (20%)	
MMSE, mean (SD)	29.3 (0.88)	23.4 (3.29)	26.7 (1.83)	<0.001
CCMT, immediate recall	38.1 (5)	18.2 (5.69)	26.8 (7.64)	<0.001
CCMT, delayed recall	37.2 (5.67)	12.8 (5.35)	24.3 (9.43)	<0.001
CCMT, recognition	47.5 (0.64)	37.38 (5.87)	44.5 (4.48)	<0.001
NPI		4.83 (4.06)	7.67 (7.19)	
ADL		14.28 (5.78)	12.67 (5.79)	
Amyloid positive, n/total*		18/20	4/5	
Phosphorylated tau, mean (SD)#		82.41 (31.19)	39.2 (10.08)	
Total tau, mean (SD)#		568.88 (242.34)	222.8 (92.07)	

SSRI/SNRI: Selective Serotonin Reuptake Inhibitor/ Serotonin and Noradrenaline Reuptake Inhibitor, MMSE: Mini-Mental State Examination, CCMT: Category Cued Memory Test, ADL: Activities of Daily Living, Time in days refers to the number of days between first day of ear-EEG recording and MRI scan in patients who were included in the regression analysis, NPI: Neuropsychiatric Inventory, SD: Standard deviation, ICV: intracranial volume, * Amyloid positive was defined by either a value below the year-corrected cut-off using CSF amyloid or positive amyloid PIB-PET at the time of diagnosis in patients who had undergone testing in either one of these modalities (AD n = 20, DLB n = 5), # Both phosphorylated tau (cut-off <60) and total tau (cut-off <400) was measured as part of the initial diagnosis. The table was in part reproduced from studies included in this PhD-thesis [27,69–71].

Feasibility of ear-EEG

See table 2 for patient characteristics of the 10 patients with AD from the feasibility part of the thesis.

Table 2: Patient characteristics (feasibility study)

	Alzheimer's disease
Number of participants	10
Age, mean (SD)	74.7 (6.0)
Males/females	6/4
Education, mean (SD)	13.5 (2.99)
MMSE	23.7 (4.0)
NPI	3.8 (2.7)
ADL	14.2 (5.8)

MMSE: Mini-Mental State Examination, ADL: Activities of Daily Living, NPI: Neuropsychiatric Inventory, SD: Standard deviation. The table was in part reproduced from the first study in this PhD-thesis [70].

All patients in the feasibility study (n=10) wore the ear-EEG for at least 24 hours and at least one full night. The responses to the questionnaire administered after wearing the ear-EEG can be seen in Table 3. All patients in the feasibility study needed their caregiver's help. This was help with adjusting the earpiece into the ear and/or starting the recording.

Table 3: Answers to questionnaire after ear-EEG recording [70].

	Yes/Total included
Difficulties wearing the equipment	5/10
EEG recorder was uncomfortable	4/10
Mild tenderness in or around the ear	7/10
Mild redness	3/10
Small abrasions of the skin	2/10

In the patients with AD, one had abrasions in the ear and later developed pain and swelling of the left chin. This could be indicative of a mild skin infection. One of the adverse events led to medical

contact but none of them lasted more than a week [70]. None of the AEs found in the studies were classified as SAEs.

In the feasibility study, six participants dropped out of the study. As reported in the feasibility study, the main reason was not wanting to wear the ear-EEG anymore due to adverse events (n = 3), not wanting to participate during the COVID-19 pandemic (n = 1), personal reasons (n = 1) and moved to a nursing home (n = 1) [70].

Epileptiform discharges in patients with Alzheimer's disease

Prevalence of epileptiform discharges with ear-EEG at baseline

During the baseline ear-EEG assessment, it was found that 75.0% (18 out of 24) of patients with AD and 46.7% (7 out of 15) of HC exhibited epileptiform discharges (p-value = 0.073, as illustrated in Figure 2A). In patients with AD, the spike frequency was significantly higher, ranging from 0 to 13.04 (with a mean of 3.03 spikes per 24 hours), in contrast to HC, which showed a range of 0 to 6.66 (with a mean of 1.04 spikes per 24 hours). The risk ratio was 2.9 (confidence interval: 1.77-5.01), with a p-value of less than 0.001, as shown in Figure 2B for a representation of spike distribution. Figures 2C and 2D provide an overview of the 24-hour distribution of epileptiform discharges for both AD and HC. A statistically significant difference was seen in the occurrence of epileptiform discharges during the nighttime (p-value=0.014), with 64.8% in patients with AD compared to 31.3% in HC [27].

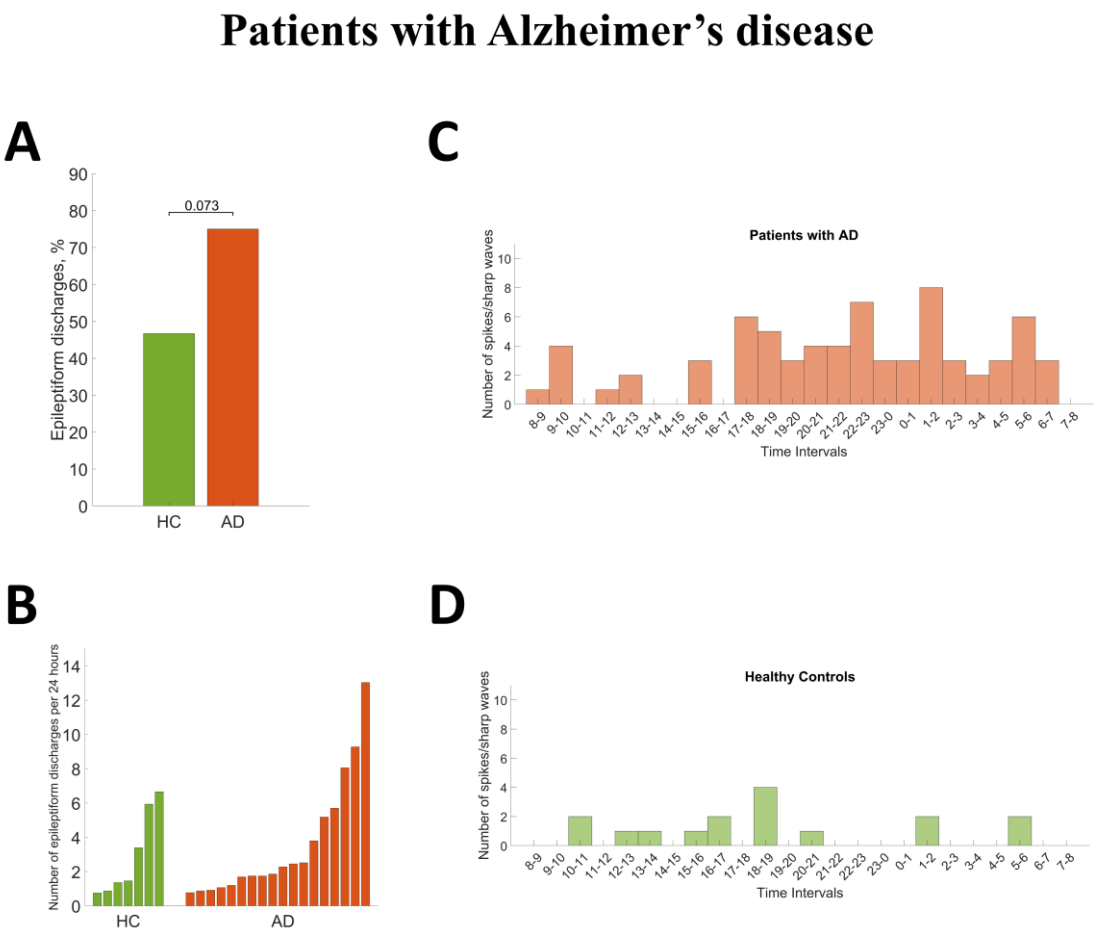


Figure 2: (A) The proportion of patients with AD and HC with epileptiform discharges. (B) Spike frequency (spikes/sharp waves per 24 hours) in each patient with AD and HC with epileptiform discharges at baseline. Distribution of epileptiform discharges by time intervals for (C) for patients with AD. (D) show the distribution of epileptiform discharges by time interval for HC. The figure was in part reproduced from a study included in this PhD-thesis [27] .

Variability in epileptiform discharges across recordings

There were no statistically significant differences in spike frequency between the baseline and the follow-up visits (for visit 1-2: estimate 1.34, p-value = 0.239, and for visit 1-3: estimate 1.24, p-value = 0.417), as seen in Table 4. When considering all the recordings collectively, it was found that

epileptiform discharges were present in 83.3% of patients after the second recording and in 91.7% after the third recording. For patients with AD, the combined data from all recordings showed a spike frequency of 2.94 spikes per 24 hours. Only two of these patients exhibited epileptiform discharges in all ear-EEG recordings. No significant differences were identified in the MMSE scores across visits (for visit 1-2: estimate -0.6, p-value = 0.082, and for visit 1-3: estimate -1.08, p-value = 0.209) [27].

Table 4: Epileptiform discharges across ear-EEG recordings in patients with AD.

Patients with Alzheimer's disease					
	1st ear-EEG recording	2nd ear-EEG recording	p-value (1st vs 2nd)	3nd ear-EEG recording	p-value (1st vs 3rd)
Number of patients, n	15	15		11	
Number with ED, %	73.33%	46.67%		63.64%	
Spikes/24 hours	1.96	2.89	0.239	2.78	0.417
Spikes, n	31	36		30	
Clean data, mean hours (SD)	25.4 (6.3)	19.9 (10.6)		23.5 (7.32)	
MMSE, mean (SD)	23.73 (3.37)	23.13 (3.62)	0.082	23.55 (3.72)	0.209
Time since last recording in days, mean (SD)		171 (86.5)		144 (92.2)	

ED: Epileptiform discharges, MMSE: Mini-Mental State Examination. The table was reproduced from a study included in this PhD-thesis [27] .

Epileptiform discharges in patients with Lewy body dementia

Prevalence of epileptiform discharges with ear-EEG at baseline

In the baseline assessment, it was found that epileptiform discharges were present in 80% (8 out of 10) of patients with DLB and 46.7% (7 out of 15) of HC (p-value = 0.210, as depicted in Figure 3A). Furthermore, at baseline, patients with DLB had a significantly higher spike frequency (ranging from 0 to 5.97, with a mean of 2.63 spikes per 24 hours) compared to HC (ranging from 0 to 6.66, with a mean of 1.04 spikes per 24 hours), with a risk ratio of 2.52 (confidence interval: 1.42-4.61) and a p-

value of 0.001 (Figure 3B for a graphical representation of spike distribution). Within the DLB patient group, 59.3% of spikes were detected at night (Figure 3C), whereas this was the case for 31.3% of HC (Figure 3D). However, this was not statistically significant between the two groups, with a p-value of 0.076 [69].

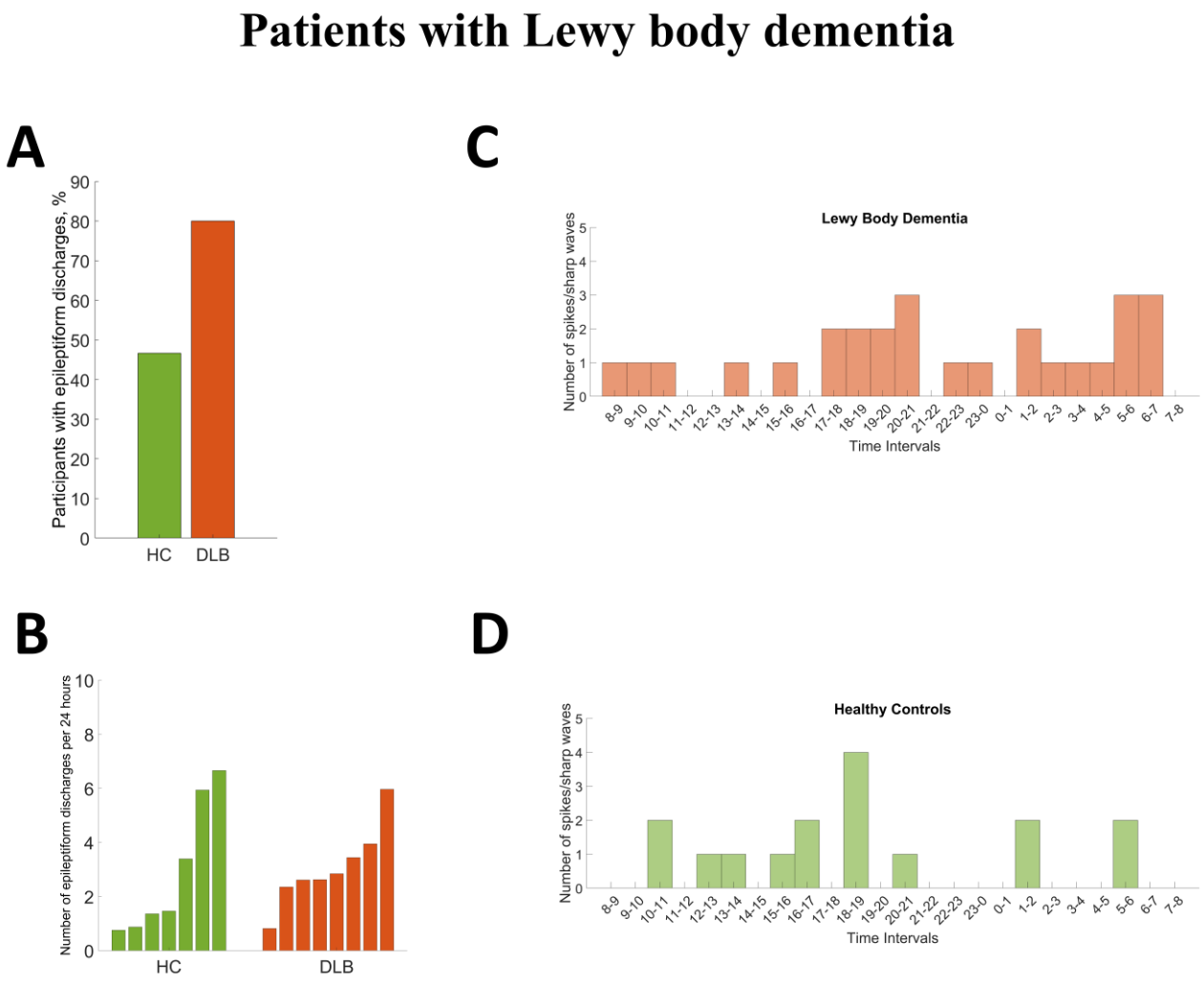


Figure 3: (A) The proportion of patients with DLB and HC with epileptiform discharges. (B) Spike frequency (spikes/sharp waves per 24 hours) in each patient with DLB and HC with epileptiform discharges at baseline. Distribution of epileptiform discharges by time intervals for (C) for patients with DLB. (D) show the distribution of epileptiform discharges by time interval for HC. The figure was in part reproduced from a study included in this PhD-thesis [69] .

Variability in epileptiform discharges across recordings

There was a considerable variability in the number of epileptiform discharges across recording sessions with a significant difference in spike frequency between the baseline and the follow-up visits (for visit 1-2: estimate 0.33, p-value = 0.029, and for visit 1-3: estimate 2.45, p-value = 0.002), as detailed in Table 5. Notably, at visit 3, one patient had a high number of 22 epileptiform discharges. When this outlier was excluded, there were no significant differences between visit 1 and visit 3. When considering all the recordings, it was found that 90% of DLB patients exhibited epileptiform discharges. In patients with DLB, the combined spike frequency from all recordings amounted to 2.75 spikes per 24 hours, with a total of 70 epileptiform discharges detected over 25.45 days of pre-processed data. No significant differences were identified in MMSE scores across visits (for visit 1-2: estimate 0.88, p-value = 0.809, and for visit 1-3: estimate 1.34, p-value = 0.588), as presented in Table 5 [69].

Table 5: Epileptiform discharges across ear-EEG recordings in patients with DLB.

Patients with dementia due to Lewy bodies					
	1st ear-EEG recording	2nd ear-EEG recording	p-value (1st vs 2nd)	3nd ear-EEG recording	p-value (1st vs 3rd)
Number of patients, n	10	8		7	
Number with ED, %	80%	38%		71%	
Spikes/24 hours	2.63	0.69	0.029	5.32	0.002
Spikes, n	31	5		34	
Clean data, mean hours (SD)	28.24 (10.06)	21.88 (9.79)		21.92 (8.07)	
MMSE, mean (SD)	26.7 (1.83)	27 (3.02)	0.809	27.43 (3.05)	0.588
Time since last recording, mean (SD)		115.13 (44.71)		96.71 (37.01)	

ED: Epileptiform discharges, MMSE: Mini-Mental State Examination, SD: Standard deviation. The table was reproduced from a study included in this PhD-thesis [69].

Association between epileptiform discharges and cognition in dementia

Between the spike frequency in patients with AD and the baseline MMSE, immediate recall or delayed recall, no significant correlations were found. In patients with DLB, a significant correlation was found between the spike frequency and the baseline MMSE (rho = -798, p-value = 0.006). No significant correlations were found for immediate recall and delayed recall.

Conventional EEG and patient diaries

In AD, 29.2% (7/24) had slowing in the EEG as compared to 6.7% (1/15) of the HC (p-value = 0.121) while this was 80% (8/10) in DLB, which was significantly different than in HC (p-value = <0.001). No epileptiform discharges were found in any of the conventional EEG recordings.

In AD, one patient reported (1/65 recordings) a sense of déjà vu in the patient diaries [27]. In the patient diaries from patients with DLB, three separate patients/caregiver reported the following: 1) a caregiver reported minor jerks of the patients' body during sleep lasting only a few seconds, 2) a patient reported visual hallucinations at night and 3) the caregiver reported three instances of suspected symptoms: a) a major jerk of the body in the morning, b) a caregiver reported that the patient reported seeing family members who were not present and that the caregiver interpreted as hallucinations and c) the patient reported an unfamiliar taste in the mouth [69].

Data quality and adverse events

Data quality at baseline

The amount of pre-processed data was lower in AD with 23.7 hours (SD: 7.6 hours, range: 3.1-33.7 hours) as compared to both HC with 27.7 hours and DLB with 28.2 hours (SD: 10.1 hours, range:

10.2-45.7 hours) at baseline. In all three groups, more pre-processed data was available at night (AD: 80.1%, DLB: 81.8%, HC: 78.7%) as compared to the daytime (AD: 61.1%, DLB: 66.1%, HC: 63.9%)[27,69] . The amount of pre-processed data for the follow-up visits for AD and DLB can be seen in Table 4 and Table 5.

Adverse events at baseline

A larger proportion of patients with AD (79.2%) had adverse events related to the ear-EEG as compared to both DLB (70%) and HC (60%). Over half the participants had problems sleeping with the ear-EEG. One of the participants with abrasions developed pain and swelling as reported under the feasibility study [70]. None of the AE related to ear-EEG were classified as serious AEs at baseline or follow-up [27,69,70].

MRI results

See table 6 for an overview of the time between MRI scan and the baseline ear-EEG recording as well as the results for the hippocampus volume between AD and HC.

Table 6: Structural MRI results and time between MRI and ear-EEG results.

	Healthy controls	Alzheimer's disease	p-value
Time in days between MRI and ear-EEG, mean (SD)	21.4 (12.46)	17.8 (10.26)	0.332
Left hippocampus volume, % of ICV, mean (SD)	0.26 (0.03)	0.21 (0.03)	<0.001
Right hippocampus volume, % of ICV, mean (SD)	0.26 (0.3)	0.21 (0.03)	<0.001

SD: Standard deviation. Table was reproduced from a study included in this PhD-thesis [71].

Global and regional blood flow

Global cerebral blood flow was not significantly different (p-value = 0.872, t-value = 0.16, df =38) between AD (mean (SD): 43.23 (12.28)) and HC (mean (SD): 42.60 (11.01)).

No significant differences were found for the normalized rCBF value in the hippocampus between the AD, and HC for left hippocampus (p-value = 0.367, t-value = -0.91, df = 38), right hippocampus (p-value = 0.092, t-value = -1.73, df = 38), or mean hippocampus (p-value = 0.118, t-value = -1.60, df = 38), see Figure 4 [71] .

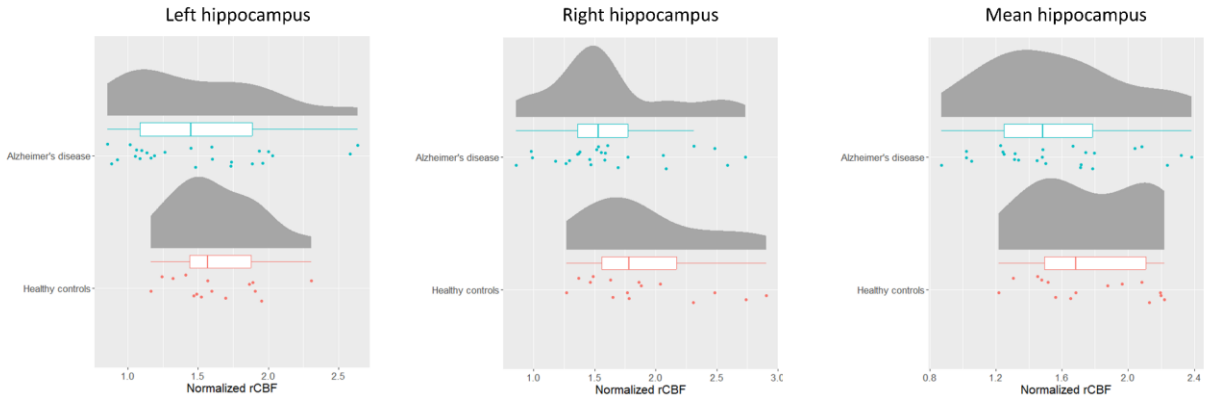


Figure 4: Raincloud plots showing mean normalized rCBF. No significant differences were found between HC, and AD for left hippocampus, right hippocampus, or mean hippocampus. Figure was reproduced from a study included in this PhD-thesis [71].

Association between ASL and spike frequency

A significant linear association between spike frequency and normalized rCBF in the hippocampus was found for AD. An outlier was found and removed from the final analysis (estimate: 0.109, t-value

= 4.03, p-value <0.001) [71] . See Figure 5. No significant association between rCBF and spike frequency was found for the HC (estimate: 0.021, t-value = 0.491, p-value = 0.632)

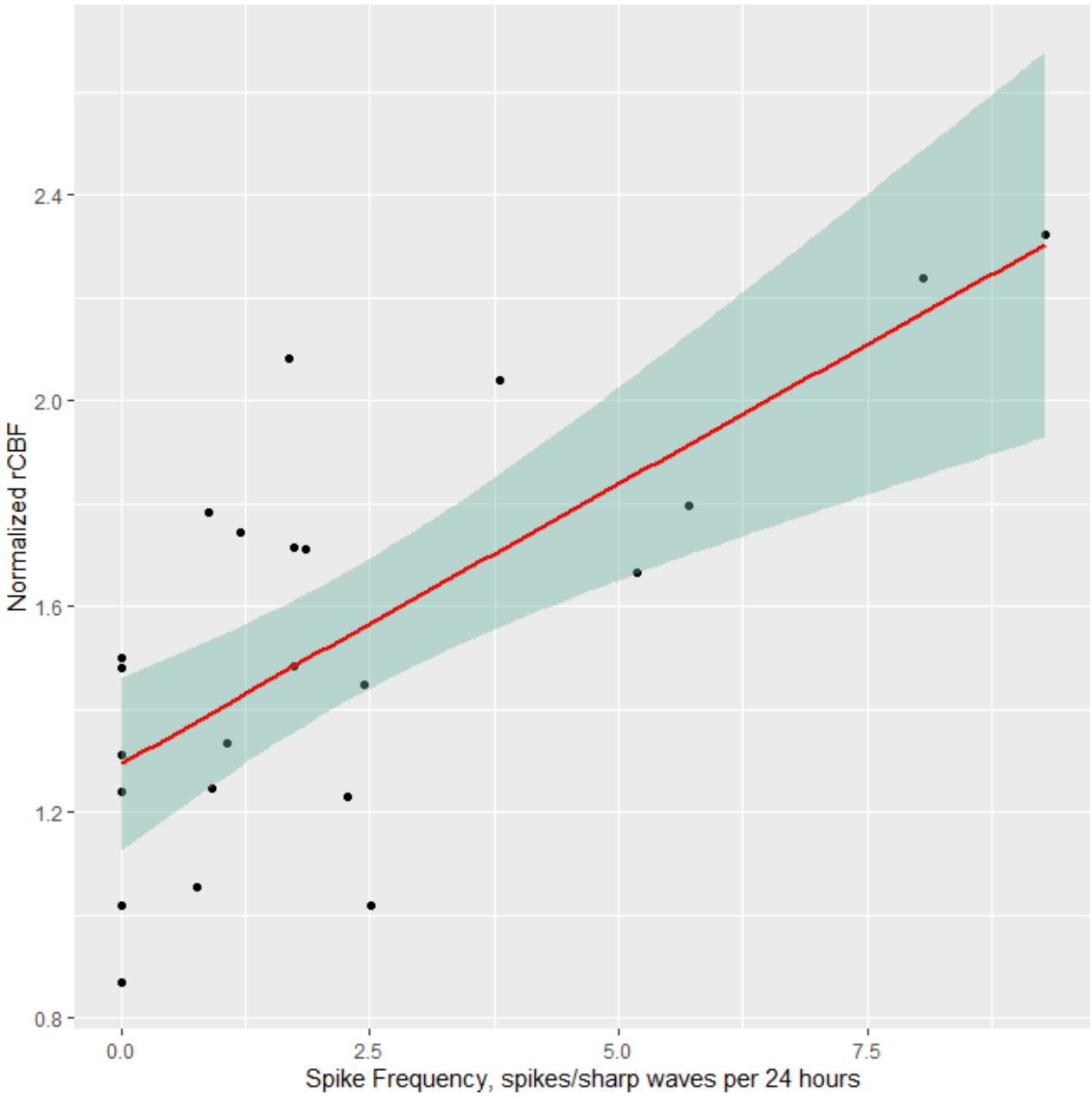


Figure 5: Association between normalized rCBF in hippocampus and spike frequency as measured with ear-EEG. Simple linear regression was applied to test if spike frequency was associated with the normalized mean rCBF in the hippocampus in patients with AD (p<0.001). Figure was reproduced from a study included in this PhD-thesis [71].

Discussion

In this PhD-thesis, the prevalence and clinical relevance of epileptiform discharges in patients with AD and DLB were investigated. Firstly, we found that outpatient long-term EEG monitoring with ear-EEG was feasible in patients with AD. Due to the ear-EEG being feasible and safe, we were able to investigate the prevalence of epileptiform discharges. A significantly higher spike frequency was observed in patients with AD and DLB as compared to HC without any clinical manifestations of the epileptiform discharges. Across multiple recordings, a large variance in the epileptiform discharges were found. As hypothesized, the increased epileptiform discharges as measured by ear EEG were associated with an increased rCBF in the hippocampus in patients with AD.

Feasibility of ear-EEG

So far, no studies have investigated long-term outpatient EEG monitoring in patients with AD, but this has been investigated in patients with epilepsy. As expected, multiple factors affect the patients' engagement in long-term recordings. In previous studies, this included that the participant was undisturbed by the device during daily activities and sleep [86,87] as well as the devices' appearance [87,88].

In the current ear-EEG setup used in this study, the TMSi amplifier interfered with daily activities due to its weight and carrying it in a bag around the neck, which may have limited the patients' daily activities. To partly overcome this issue, a belt bag was introduced after the feasibility study. This led to the participants not having to carry the TMSi amplifier around their neck and possibly being more comfortable. However, the concealment of the device posed a challenge, possibly preventing certain patients from using it in outdoor settings. In the feasibility study, half the patients experienced

problems with wearing the device at night, which most likely led to worse quality of sleep. This was similar for all the patients recruited as part of this thesis. As for improving the comfortability during sleep, future prototypes of the ear-EEG should address this issue to ensure that it is easier for patients to wear the equipment at night.

For most participants in the feasibility study as well as the participants included later in the study, it was common to experience soreness in the ears. In a previous study using a different ear-EEG prototype in patients with temporal lobe epilepsy, the majority of patients experienced some degree of soreness but discontinuation of the use of the ear-EEG only occurred for two of the participants (13.3%) [33]. The large difference as compared to the findings in the current thesis may be explained by several factors including younger participants and being hospitalized at the time during ear-EEG recordings [33]. However, based on the experience gained in the feasibility study, breaks were allowed during the recording sessions, during which participants were given the opportunity to temporarily remove the ear-EEG device (especially during meals) but were encouraged to wear the ear-EEG at night due to the previous finding of a higher prevalence of epileptiform discharges during sleep [23,24]. However, we still experienced many dropouts from the study with 54% of patients with AD and 30% of patients with DLB, which in most part was due to difficulty wearing the ear-EEG. This suggests that changes in the equipment is needed to improve comfortability of the ear-EEG.

Epileptiform discharges in patients with dementia

Proportion of patients with epileptiform discharges

There was a trend towards a higher proportion of patients with epileptiform discharges at baseline in the AD and DLB group (75% and 80%) compared to the HC group (46%). Although epileptiform

discharges have not been investigated in DLB with long-term EEG, this has been conducted in patients with AD. The results are in line with earlier studies, indicating that a higher proportion of patients with AD without epilepsy (ranging from 22% to 54%) showed epileptiform discharges in 24-hour EEG recordings when compared to HC. [22–24]. As compared to the long-term EEG recordings, no epileptiform discharges were found using conventional EEG (30 minutes) in the current study, which is in line with a previous study in a large mixed memory clinic cohort where only 3% of participants had epileptiform discharges using short-term conventional EEG recordings [21]. This association between recording length and number of epileptiform discharges is not surprising and has previously been shown in patients with AD [26] and can be seen here with >90% of patients with AD showing epileptiform discharges if all three ear-EEG recordings were considered. Furthermore, the number of hours needed before a 95% probability for detecting an epileptiform discharge can be computed based on the observed probability in the current thesis. Specifically, using a geometric distribution we can compute the probability of observing at least one epileptiform discharge given a specific probability of occurrence. Since the occurrence of epileptiform discharges is higher at night, the computation violates the assumption of a constant probability but simply illustrates the average number of recording hours needed to find one epileptiform discharge. As the recording time increases, the probability of observing at least one epileptiform discharge also increases (see Figure 6). Therefore, with longer recording times with for example long-term ear-EEG recordings, the classification of either having or not having epileptiform discharges is not appropriate and other measures like spike frequency should be considered.

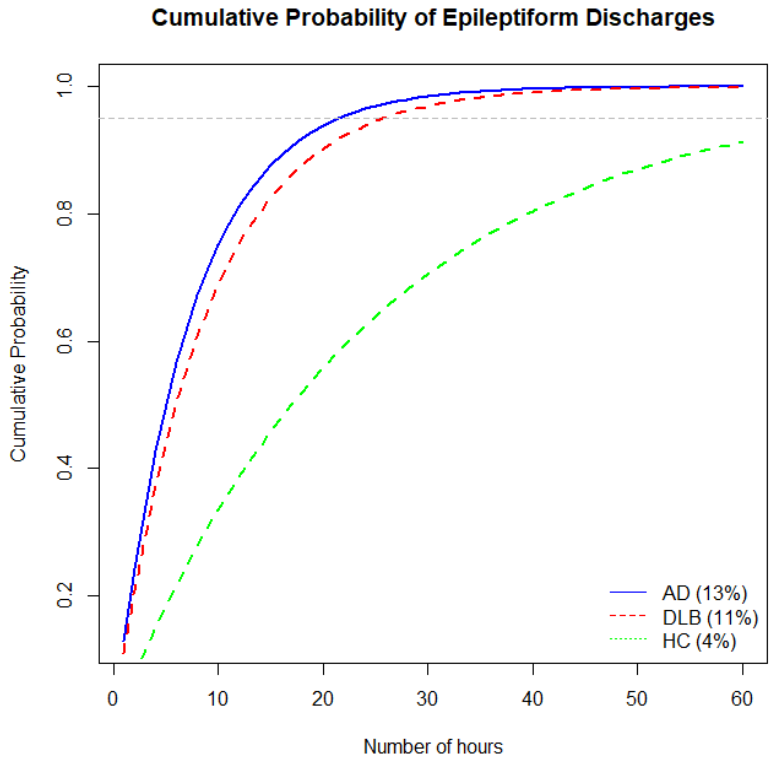


Figure 6. The cumulative probability of observing at least one epileptiform discharge. This is shown between 1-60 hours of recording for three groups labeled as AD, DLB, and HC. The blue line represents the cumulative probability for the patients with AD, while the red and green lines represent the cumulative probabilities for the DLB and HC, respectively. The plot shows that the probability of observing at least one epileptiform discharge increases with more recorded hours for all three groups, but the probabilities are lower for the HC compared to both AD, and DLB. The horizontal grey line indicates 95% probability of having at least one epileptiform discharge. [1]

Nonetheless, the findings that nearly half of the HC showed epileptiform discharges using ear-EEG was still unexpected and cannot solely be attributed to the recording time. It is possible that the ear-EEG detects more false positive epileptiform discharges as compared to scalp EEG. Specifically, due

[1] In patients with AD: 3.03/24 = 0.126 ≈ 0.13
R code: p <- 0.13
prob <- (1 - p) ^ (1:60 - 1) * p
cum_prob <- cumsum(prob)

to the low spatial resolution of the ear-EEG it is not possible to evaluate all IFCN criteria [79] for an epileptiform discharge. In addition, due to only one rater evaluating the epileptiform discharges, there may be some false positives, but this should be similar between the groups due to the blinding of the rater. Although a previous study using conventional scalp EEG found a fair inter-rater reliability of interictal epileptiform discharges [89], it would be of interest to conduct a similar study due to the limited spatial resolution of ear-EEG. Although speculative, another explanation for a higher number of HC showing epileptiform discharges could be underlying AD pathology without clinical correlate that led to an increased number of epileptiform discharges in some of the HC as AD pathology is common in this age group [90]. Studies are needed to investigate if underlying AD pathology in persons without cognitive symptoms leads to an increased number of epileptiform discharges.

Spike frequency in patients with dementia

In patients with AD and DLB the spike frequency was significantly higher as compared to HC. While there is no consensus on the relationship between epileptiform discharges and seizures [91], studies have shown that the spike frequency is linked to the seizure frequency in persons with temporal lobe epilepsy [92]. Studies in patients with AD have shown that the spike frequency can vary significantly in long-term EEG studies (24 hours), ranging from a median frequency of 3 per 24 hours [23] to an average of 2 spikes per hour in the patients with epileptiform discharges [22]. The reason for the lower number of epileptiform discharges in the current study could be due to multiple factors. First, the variation in spike frequency observed across studies with 24-hour recordings may be attributable to differences in disease severity, given that studies reporting higher spike frequencies tended to have included patients with lower MMSE scores [22–24]. Secondly, previous studies have for the most part found epileptiform discharges in the temporal region but some do occur in the frontal region in

patients with AD [22–24,93,94] and with the suboptimal spatial resolution of the ear-EEG, this could account for the lower spike frequency in the current study. Although the spike frequency may have been higher if recorded with scalp EEG, it was still possible to observe epileptiform discharges. The reason for the ear-EEG being able to capture epileptiform discharges could in large part be the location of neurodegeneration in patients with AD. Specifically, the presence of increased temporal lobe atrophy in the early stages of AD [95,96] in combination with deposition of amyloid [38] may have been the main reason for epileptiform discharges.

In contrast, DLB patients tend to have less atrophy in their temporal lobes [97] but patients with DLB had a significantly higher number of epileptiform discharges as compared to HC. One possible explanation for the increased epileptiform discharges observed in patients with DLB could be the presence of α-synuclein depositions, which have been demonstrated to promote network excitability and potentially lead to seizure activity [56]. As for being able to observe epileptiform discharges in the temporal lobes, studies that have found associations between the densities of Lewy bodies in the temporal lobes and visual hallucinations, as well as a link between visual hallucinations and epileptiform discharges in the temporal lobes [98,99]. However, due to a high frequency of amyloid deposition in DLB [100,101] and present findings of amyloid pathology in 4 of the patients (5/10 underwent a lumbar puncture), it is also possible that AD related pathology leads to epileptiform discharges in DLB patients. Due to the limited spatial resolution of ear-EEG, performing EEG monitoring over the parietal or occipital cortex may result in the detection of a higher number of epileptiform discharges in patients with DLB. However, studies using long-term scalp EEG in patients with DLB is needed.

Occurrence of epileptiform discharges at night

In both patients with AD and DLB in the current study, most epileptiform discharges occurred at night, which has previously been reported in long-term EEG studies in AD [22–24]. Although previous studies have reported a higher spike frequency during sleep in patients with AD compared to HC, a recent study using full-night polysomnography did not find a significant difference in spike frequency between the two groups. [25]. Although there is not a consensus on whether patients with AD show an increased spike frequency at night, most studies support the current findings.

The pathological mechanism behind epileptiform discharges occurring during sleep is unknown but has been shown to be common in patients with temporal lobe epilepsy [102]. In a mouse model of beta-amyloid neuropathology, epileptiform discharges have been shown to be common during REM-sleep [63,103]. However, this is in contrast to studies in patients with AD where the vast majority of epileptiform discharges occurred during N2 sleep [23] or both N2 and N3 [22]. Furthermore, a different pattern is seen in mouse models with α-synucleinopathy where a study found increasing epileptiform discharges during non-REM sleep [57]. Due to only a few studies having investigated whether epileptiform discharges occur in REM or non-REM sleep, no definite conclusion can be draw from the existing literature. However, due to the finding of epileptiform discharges occurring during non-REM sleep in patients with AD, it seems possible that patients with neurodegenerative diseases experience a higher incidence of epileptiform discharges during non-REM sleep. This is supported by a study suggesting that REM sleep may be able to inhibit epileptiform discharges [58,59]. Furthermore, in patients with DLB, REM sleep behavior disorder is common [60] with sleep fragmentation and shortened REM time [104]. Due to the disruption of REM sleep in patients with DLB, it is likely to be a contributing factor that could worsen or possibly lead to epileptiform discharges. However, sleep staging of the acquired ear-EEG recordings is needed to better understand the relationship between epileptiform discharges and sleep.

Spike frequency across repeated recordings

This is the first study to investigate the spike frequency using repeated long-term EEG recordings in patients with dementia. We found that the spike frequency varied considerably between visits for both AD and DLB. One explanation is natural variability, which has been seen with ultra long-term EEG recordings for detection of EEG seizures in patients with epilepsy. Here, the study found that there may be longer periods without epileptiform discharges [68]. Another reason could be specific triggers that may lead to an increased hyperexcitability of the temporal lobes like progression of the disease with increased neurodegeneration. Furthermore, this could in part also be due to the recording length since the total amount of pre-processed data in the second recording was lower than the first recording, which may account for some of the variance. Another explanation could be a due to fluctuations in cognition, but this study was not specifically designed to investigate a possible clinical correlate to variability in spike frequency. However, future studies in this field could explore potential links between cognitive fluctuation and atrophy of specific brain regions such as the anterior cingulate cortex and bilateral insular regions, which have been hypothesized to play a role in cognitive fluctuations in DLB [105,106]. One way to investigate the association between spike frequency and cognition could be digital cognitive testing in combination with long-term ear-EEG monitoring. As for the clinical relevance of epileptiform discharges, the large variance further supports that the spike frequency may be a more suitable measure of hyperexcitability. However, ultra long-term EEG monitoring is necessary to fully understand the variability over longer periods of time.

Association between epileptiform discharges and cerebral blood flow

In the current study, we found an association between spike frequency and rCBF in AD but not in HC, which suggest a that these physiological responses are connected in AD. Similarly, in patients

with epilepsy, an increased rCBF in the epileptic focus has been found during the ictal phase [44–46]. Even though only spikes/sharp waves were observed and not ictal activity, the results indicate a shared hemodynamic response to epileptiform discharges in epilepsy and AD. As a result of this similarity to epilepsy, it is probable that the elevated rCBF in the hippocampus is associated with an increased metabolic demand [107], which again could lead to neurodegeneration.

In a study using a more sensitive measuring technique for detecting epileptiform discharges in patients with AD with electrodes in foramen ovale, it was found that these discharges are much more common in the hippocampus than detected with scalp/ear-EEG and this could indicate a hyperexcitable state [108]. This hyperexcitable state may also be less transient than usually associated with epilepsy, which could explain the positive association between the spike frequency and rCBF in hippocampus although the ASL acquisition and ear-EEG recording were not conducted simultaneously. Based on the current findings, it is therefore likely that at least some of the patients with AD show an underlying hyperexcitable state in the hippocampus.

Although we did not find a significant difference in mean rCBF between AD and HC, previously studies have for the most part observed decreased rCBF in the temporal lobes [109,110] in patients with AD. In context of epileptiform discharges, it could be hypothesized that the decreased rCBF could be caused by similar mechanisms as seen in epilepsy with enhanced inhibitory GABAergic transmission [111] to lower the risk of transitioning to ictal activity [112] and possibly inhibiting the propagation of epileptiform discharges. This inhibition would lead to both a lower hippocampal rCBF and spike frequency (see Figure 7) and could potentially lead to worse cognitive performance due to GABAergic inhibition of neuronal functioning outside the epileptic focus. However, this is speculative and studies investigating this in patients with AD is needed.

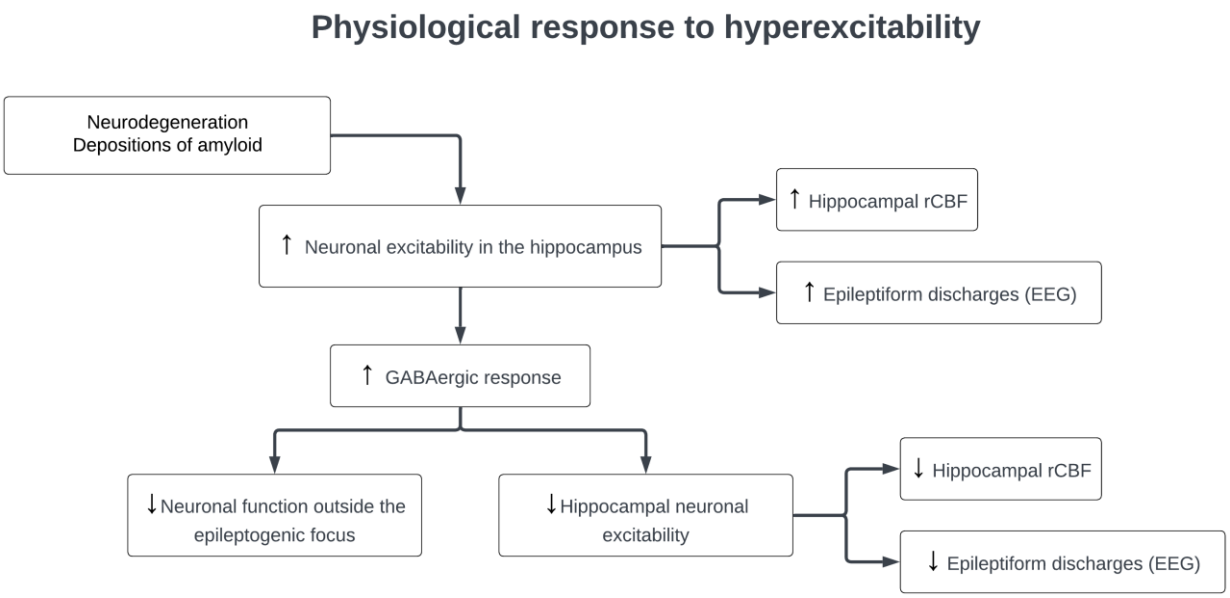


Figure 7: Hypothesis of the physiological response to hyperexcitability.

One way to understand the association between hyperexcitability and rCBF is by investigating the changes in rCBF in patients with AD after administration of levetiracetam. Here, one study found an increased hippocampal rCBF as measured with ASL-MRI [113]. This is in contrast with the findings as a decrease in rCBF would be expected (i.e., a normalization) since patients with higher occurrence of epileptiform discharges had an increased rCBF. However, more studies are needed to fully understand the effect of levetiracetam on rCBF as well as understanding the possible role of GABAergic inhibition on epileptiform discharges in patients with AD.

Strengths

The strength of the studies in this thesis is that they represent the first demonstration of the feasibility of ear-EEG monitoring in a home setting in a group of patients with dementia where hospitalization may be challenging. The dry electrodes exhibit significantly higher electrode-skin impedance

compared to wet electrodes [114] and have not previously been applied in any clinical setting. However, the high impedance can be seen as a problem in determining the quality of the recordings. Technically, overcoming this challenge requires amplifiers with very high input impedance and low electrical noise when recording ear-EEG. This was addressed by the choice of amplifier, which arguable made impedance of the electrodes a relatively minor concern.

Limitations

The studies conducted as part of this PhD thesis also have several limitations, with the most important being insufficient length of concurrent scalp and ear-EEG recordings, sample size, and time interval between ear-EEG and MRI acquisition.

A previous study found that ear-EEG is able to detect epileptiform discharges in focal temporal lobe seizures [33] but this was not systematically compared to scalp EEG. In the current study, we did not record simultaneous ear-EEG and scalp EEG, which would have been preferable due to the lack of studies comparing ear-EEG and scalp EEG in detection of epileptiform discharges. Specifically, it is not possible to ascertain whether the IFCN criteria for spatial distribution of epileptiform discharges is fulfilled with ear-EEG recordings due to low spatial resolution. It is therefore possible that benign variants may be misinterpreted as epileptiform discharges. Thus, benign epileptiform transients of sleep could be misinterpreted as epileptiform discharges. However, benign epileptiform variants are usually distinguishable from epileptiform discharges as they do not disrupt the background activity [115,116], which is one of the criteria for defining an epileptiform discharge according to the IFCN criteria [79]. This diminishes the risk of misinterpretation, but to ascertain this with more certainty, simultaneous recordings would have been necessary. Besides benign epileptiform transients of sleep, other types of benign variants exist, such as wicket spikes, but since these occur in short runs, they

would be distinguishable from single epileptiform discharges. Therefore, studies comparing ear-EEG and conventional EEG are still needed but would require hospitalization.

The number of participants in the studies was relatively small. The small sample size may explain the high variability in the results leading to an underestimation of the difference in prevalence of epileptiform discharges between AD and HC. In addition, the HC group had more pre-processed data compared to the AD group (not the DLB group), which may have overinflated the prevalence of epileptiform discharges in HC. However, this discrepancy was accounted for in the analysis. In addition, we only conducted a single session of ear-EEG for the HC group and conducting repeated ear-EEG measurements could have offered additional insights into the within-subject variability of epileptiform discharges, which was found for both AD and DLB.

An important limitation when investigating the association between epileptiform discharges and hippocampal rCBF is that the MRI acquisition was not performed on the same day as the initiation of the ear-EEG recording. This may have led to an underestimation of the association due to variability in the measured epileptiform discharges. Furthermore, we did not acquire ASL images from the whole brain due to the focus on hippocampus, which limits further exploration into the possible association between epileptiform discharges and other brain regions.

Possible clinical relevance of epileptiform discharges

Only very few patients reported symptoms in the patient diaries and most symptoms could be related to the underlying neurodegenerative disease. In general, due to the design of the current study with remote monitoring we can neither confirm or reject the possibility that some of the participants were suffering from or would develop seizures. This would require a more extensive questionnaire of possible symptoms of seizures as well as a longer period of follow-up. Although no seizures were

found, the findings in the current thesis and in previous studies in patients with AD showing epileptiform discharges to be a prognostic marker [22,24] suggest that epileptiform discharges may be a therapeutic target. Nevertheless, if we were to adopt this perspective, it would entail a change in the current perception regarding the appropriate timing for administering anti-seizure treatment, as seizures are currently considered crucial in determining the need for treatment. To understand this, a recent clinical trial investigated the effect of levetiracetam in patients with AD. Here, the study found improvement in cognitive function but only in the patients who had epileptiform discharges [66]. However, based on the current findings, there are several issues that should be considered when investigating the potential effect of anti-seizure medication in patients with AD with epileptiform discharges. First, as longer EEG recording times are conducted (e.g., with ear-EEG), it is not relevant to simply consider whether epileptiform discharges are present. Instead, higher spike frequency should be used a marker for initiating treatment with anti-seizure medication. However, the precise cut-off for spike frequency that warrants treatment is not understood and larger studies are needed to understand the normal range in older persons without evidence of a neurodegenerative disorder. One way would be to perform digital cognitive assessment during long-term EEG monitoring to possible increase our knowledge on the clinical correlate since digital cognitive testing can be performed multiple times during the recording period.

Conclusions and future perspectives

Ear-EEG is a feasible technique for long-term outpatient monitoring of possible epileptiform discharges in patients with mild to moderate AD with only mild adverse events for the participants. By using long-term outpatient EEG monitoring, it was seen that most patient with AD and DLB showed epileptiform discharges, the majority of which occurred at night. The epileptiform discharges

may in large part originate from the temporal lobes in AD and may be associated with the insular region in DLB, but more studies are needed to understand the origins of the epileptiform discharges. As for the epileptogenic factor, since epileptiform discharges can be found in both AD, and DLB, it is possible that epileptiform discharges are an unspecific marker of neurodegeneration. Alternatively, that they are a more specific phenomenon associated with depositions of amyloid and α-synuclein. With repeated and long-term EEG recordings, most patients showed epileptiform discharges. This suggests that considering spike frequency as a marker of hyperexcitability it is more relevant than simply whether spikes are present or not in studies investigating the effect of anti-seizure medication. As expected, increased spike frequency was accompanied by an increased blood flow in the hippocampus in patients with AD. The coupling between epileptiform discharges and blood flow has previously been found in patients with epilepsy and may be a shared hemodynamic response across pathologies.

Although a previous study comparing ear-EEG with scalp EEG monitoring has shown that ear-EEG is able to detect interictal spike morphology [33] and a good correspondence between ear-EEG and scalp EEG has been found [30,31], new studies are needed to compare detection of epileptiform discharges measured with ear-EEG and conventional long-term EEG recordings in patients with AD. Thus, it would be of interest to directly compare conventional EEG with dry-electrodes ear-EEG since there may be difference in the amplitude of the signal, affecting the findings. Due to previous findings of epileptiform discharges leading to a more rapid progression in patients with AD [22,24], it would be important to investigate if anti-seizure medication may be a therapeutic target. A study investigating the effect of levetiracetam on spike frequency as measured with ear-EEG is needed to fully appreciate the clinical implication of long-term ear-EEG recordings. In the current study, we found an association between rCBF and spike frequency, but more studies are needed to understand

the relationship between spike frequency and markers of neuronal damage (e.g., neurofilament light chain) or possibly product of glycolysis (e.g., lactate) to fully elucidate this relationship.

The current study demonstrated that outpatient long-term EEG monitoring with ear-EEG is feasible and can detect subclinical epileptiform discharges in both patients with AD and DLB with an increased spike frequency as compared to HC. The association between spike frequency and increased perfusion in hippocampus in AD suggests a similar mechanism as seen in epilepsy.

References

[1] Fisher RS, Acevedo C, Arzimanoglou A, Bogacz A, Cross JH, Elger CE, Engel Jr J, Forsgren L, French JA, Glynn M, Hesdorffer DC, Lee BI, Mathern GW, Moshé SL, Perucca E, Scheffer IE, Tomson T, Watanabe M, Wiebe S (2014) ILAE Official Report: A practical clinical definition of epilepsy. *Epilepsia* **55**, 475–482.

[2] Burman J, Zelano J (2017) Epilepsy in multiple sclerosis: A nationwide population-based register study. *Neurology* **89**, 2462–2468.

[3] Zelano J, Brigo F, Garcia-Patek S (2019) Increased risk of epilepsy in patients registered in the Swedish Dementia Registry. *Eur J Neurol* **27**, 129–135.

[4] Subota A, Pham T, Jetté N, Sauro K, Lorenzetti D, Holroyd-Leduc J (2017) The association between dementia and epilepsy: A systematic review and meta-analysis. *Epilepsia* **58**, 962–972.

[5] Knopman DS, Amieva H, Petersen RC, Chételat G, Holtzman DM, Hyman BT, Nixon RA, Jones DT (2021) Alzheimer disease. *Nat Rev Dis Prim* **7**, 33.

[6] Scarmeas N, Honig LS, Choi H, Cantero J, Brandt J, Blacker D, Albert M, Amatniek JC, Marder K, Bell K, Hauser WA, Stern Y (2009) Seizures in Alzheimer Disease. *Arch Neurol* **66**,.

[7] Amatniek JC, Hauser WA, DelCastillo-Castaneda C, Jacobs DM, Marder K, Bell K, Albert M, Brandt J, Stern Y (2006) Incidence and Predictors of Seizures in Patients with Alzheimer's Disease. *Epilepsia* **47**, 867–872.

[8] Lozsadi DA, Larner AJ (2006) Prevalence and Causes of Seizures at the Time of Diagnosis of Probable Alzheimer's Disease. *Dement Geriatr Cogn Disord* **22**, 121–124.

[9] Bernardi S, Scaldaferri N, Vanacore N, Trebbastoni A, Francia A, D'Amico A, Prencipe M (2010) Seizures in Alzheimer's disease: a retrospective study of a cohort of outpatients. *Epileptic Disord* **12**, 16–21.

[10] Rao SC, Dove G, Cascino GD, Petersen RC (2009) Recurrent seizures in patients with dementia: Frequency, seizure types, and treatment outcome. *Epilepsy Behav* **14**, 118–120.

[11] Risse SC, Lampe TH, Bird TD, Nochlin D, Sumi SM, Keenan T, Cubberley L, Peskind E, Raskind MA (1990) Myoclonus, Seizures, and Paratonia in Alzheimer Disease. *Alzheimer Dis Assoc Disord* **4**, 217–225.

[12] Pandis D, Scarmeas N (2012) Seizures in Alzheimer Disease: Clinical and Epidemiological Data. *Epilepsy Curr* **12**, 184–187.

[13] Outeiro TF, Koss DJ, Erskine D, Walker L, Kurzawa-Akanbi M, Burn D, Donaghy P, Morris C, Taylor J-P, Thomas A, Attems J, McKeith I (2019) Dementia with Lewy bodies: an update and outlook. *Mol Neurodegener* **14**, 5.

[14] Yamada M, Komatsu J, Nakamura K, Sakai K, Samuraki-Yokohama M, Nakajima K, Yoshita M (2020) Diagnostic Criteria for Dementia with Lewy Bodies: Updates and Future Directions. *J Mov Disord* **13**, 1–10.

[15] Beagle AJ, Darwish SM, Ranasinghe KG, La AL, Karageorgiou E, Vossel KA (2017) Relative Incidence of Seizures and Myoclonus in Alzheimer's Disease, Dementia with Lewy Bodies, and Frontotemporal Dementia. *J Alzheimers Dis* **60**, 211–223.

[16] Sun L, Cao J, Chu F na, Wang Z, Lv Y (2014) Dementia with Lewy bodies versus nonconvulsive status epilepticus in the diagnosis of a patient with cognitive dysfunction, complex visual hallucinations and periodic abnormal waves in EEG: a case report. *BMC Neurol* **14**,.

[17] Tun MZ, Soo WK, Wu K, Kane R (2017) Dementia with Lewy bodies presenting as probable epileptic seizure. *BMJ Case Rep* **2017**,.

[18] Park IS, Yoo SW, Lee K-S, Kim J-S (2014) Epileptic seizure presenting as dementia with Lewy bodies. *Gen Hosp Psychiatry* **36**, 230.e3-230.e5.

[19] Musaeus CS, Nilsson C, Cooper C, Kramberger MG, Verdelho A, Stefanova E, Religa D, Waldemar G, Frederiksen KS (2021) Pharmacological Medical Treatment of Epilepsy in Patients with Dementia: A Systematic Review. *Curr Alzheimer Res* **18**, 689–694.

[20] Cumbo E, Ligori LD (2010) Levetiracetam, lamotrigine, and phenobarbital in patients with epileptic seizures and Alzheimer's disease. *Epilepsy Behav* **17**, 461–466.

[21] Liedorp M, Stam CJ, van der Flier WM, Pijnenburg YAL, Scheltens P (2010) Prevalence and Clinical Significance of Epileptiform EEG Discharges in a Large Memory Clinic Cohort. *Dement Geriatr Cogn Disord* **29**, 432–437.

[22] Horvath AA, Papp A, Zsuffa J, Szucs A, Luckl J, Radai F, Nagy F, Hidasi Z, Csukly G, Barcs G, Kamondi A (2021) Subclinical epileptiform activity accelerates the progression of Alzheimer's disease: A long-term EEG study. *Clin Neurophysiol Off J Int Fed Clin Neurophysiol* **132**, 1982–1989.

[23] Lam AD, Sarkis RA, Pellerin KR, Jing J, Dworetzky BA, Hoch DB, Jacobs CS, Lee JW, Weisholtz DS, Zepeda R, Westover MB, Cole AJ, Cash SS (2020) Association of epileptiform abnormalities and seizures in Alzheimer disease. *Neurology* **95**, e2259–e2270.

[24] Vossel KA, Ranasinghe KG, Beagle AJ, Mizuiri D, Honma SM, Dowling AF, Darwish SM, Van Berlo V, Barnes DE, Mantle M, Karydas AM, Coppola G, Roberson ED, Miller BL, Garcia PA, Kirsch HE, Mucke L, Nagarajan SS (2016) Incidence and impact of subclinical epileptiform activity in Alzheimer's disease. *Ann Neurol* **80**, 858–870.

[25] Brunetti V, D'Atri A, Della Marca G, Vollono C, Marra C, Vita MG, Scarpelli S, De Gennaro L, Rossini PM (2020) Subclinical epileptiform activity during sleep in Alzheimer's disease and mild cognitive impairment. *Clin Neurophysiol* **131**, 1011–1018.

[26] Horváth A, Szűcs A, Barcs G, Kamondi A (2017) Sleep EEG Detects Epileptiform Activity in Alzheimer's Disease with High Sensitivity. *J Alzheimers Dis* **56**, 1175–1183.

[27] Musaeus CS, Frederiksen KS, Andersen BB, Høgh P, Kidmose P, Fabricius M, Hribljan MC, Hemmsen MC, Rank ML, Waldemar G, Kjær TW (2023) Detection of subclinical epileptiform discharges in Alzheimer's disease using long-term outpatient EEG monitoring. *Neurobiol Dis* **183**, 106149.

[28] Bleichner MG, Debener S (2017) Concealed, Unobtrusive Ear-Centered EEG Acquisition: cEEGrids for Transparent EEG. *Front Hum Neurosci* **11**, 163.

[29] Kappel SL, Rank ML, Toft HO, Andersen M, Kidmose P (2019) Dry-Contact Electrode Ear-EEG. *IEEE Trans Biomed Eng* **66**, 150–158.

[30] Mikkelsen KB, Kappel SL, Mandic DP, Kidmose P (2015) EEG Recorded from the Ear: Characterizing the Ear-EEG Method. *Front Neurosci* **9**,.

[31] Mikkelsen KB, Tabar YR, Kappel SL, Christensen CB, Toft HO, Hemmsen MC, Rank ML, Otto M, Kidmose P (2019) Accurate whole-night sleep monitoring with dry-contact ear-EEG. *Sci Rep* **9**,.

[32] Tabar YR, Mikkelsen KB, Shenton N, Kappel SL, Bertelsen AR, Nikbakht R, Toft HO, Henriksen CH, Hemmsen MC, Rank ML, Otto M, Kidmose P (2023) At-home sleep monitoring using generic ear-EEG. *Front Neurosci* **17**, 987578.

[33] Zibrandtsen IC, Kidmose P, Christensen CB, Kjaer TW (2017) Ear-EEG detects ictal and interictal abnormalities in focal and generalized epilepsy - A comparison with scalp EEG monitoring. *Clin Neurophysiol* **128**, 2454–2461.

[34] Munch Nielsen J, Zibrandtsen IC, Masulli P, Lykke Sørensen T, Andersen TS, Wesenberg Kjær T (2022) Towards a wearable multi-modal seizure detection system in epilepsy: A pilot study. *Clin Neurophysiol Off J Int Fed Clin Neurophysiol* **136**, 40–48.

[35] Jørgensen SD, Zibrandtsen IC, Kjaer TW (2020) Ear-EEG-based sleep scoring in epilepsy: A comparison with scalp-EEG. *J Sleep Res* **29**, e12921.

[36] You S, Hwan Cho B, Shon Y-M, Seo D-W, Kim IY (2022) Semi-supervised automatic seizure detection using personalized anomaly detecting variational autoencoder with behind-the-ear EEG. *Comput Methods Programs Biomed* **213**, 106542.

[37] Zibrandtsen IC, Kidmose P, Kjaer TW (2018) Detection of generalized tonic-clonic seizures from ear-EEG based on EMG analysis. *Seizure* **59**, 54–59.

[38] Targa Dias Anastacio H, Matosin N, Ooi L (2022) Neuronal hyperexcitability in Alzheimer's disease: what are the drivers behind this aberrant phenotype? *Transl Psychiatry* **12**, 257.

[39] Yamamoto K, Tanei Z-I, Hashimoto T, Wakabayashi T, Okuno H, Naka Y, Yizhar O, Fenno LE, Fukayama M, Bito H, Cirrito JR, Holtzman DM, Deisseroth K, Iwatsubo T (2015) Chronic optogenetic activation augments aβ pathology in a mouse model of Alzheimer disease. *Cell Rep* **11**, 859–865.

[40] Pooler AM, Phillips EC, Lau DHW, Noble W, Hanger DP (2013) Physiological release of endogenous tau is stimulated by neuronal activity. *EMBO Rep* **14**, 389–394.

[41] Yuan P, Grutzendler J (2016) Attenuation of β-Amyloid Deposition and Neurotoxicity by Chemogenetic Modulation of Neural Activity. *J Neurosci Off J Soc Neurosci* **36**, 632–641.

[42] Cohen AD, Klunk WE (2014) Early detection of Alzheimer's disease using PiB and FDG PET. *Neurobiol Dis* **72 Pt A**, 117–122.

[43] Whitwell JL, Przybelski SA, Weigand SD, Knopman DS, Boeve BF, Petersen RC, Jack Jr CR (2007) 3D maps from multiple MRI illustrate changing atrophy patterns as subjects progress from mild cognitive impairment to Alzheimer's disease. *Brain* **130**, 1777–1786.

[44] Duncan R (1992) Epilepsy, cerebral blood flow, and cerebral metabolic rate. *Cerebrovasc Brain Metab Rev* **4**, 105–121.

[45] Dupont P, Zaknun JJ, Maes A, Tepmongkol S, Vasquez S, Bal CS, Van Paesschen W, Carpintiero S, Locharernkul C, Dondi M (2009) Dynamic perfusion patterns in temporal lobe epilepsy. *Eur J Nucl Med Mol Imaging* **36**, 823–830.

[46] Nguyen D, Kapina V, Seeck M, Viallon M, Fedespiel A, Lovblad KO (2010) Ictal hyperperfusion demonstrated by arterial spin-labeling MRI in status epilepticus. *J Neuroradiol = J Neuroradiol* **37**, 250–251.

[47] Pendse N, Wissmeyer M, Altrichter S, Vargas M, Delavelle J, Viallon M, Federspiel A, Seeck M, Schaller K, Lövblad KO (2010) Interictal arterial spin-labeling MRI perfusion in intractable epilepsy. *J Neuroradiol = J Neuroradiol* **37**, 60–63.

[48] Lim Y-M, Cho Y-W, Shamim S, Solomon J, Birn R, Luh WM, Gaillard WD, Ritzl EK, Theodore WH (2008) Usefulness of pulsed arterial spin labeling MR imaging in mesial temporal lobe epilepsy. *Epilepsy Res* **82**, 183–189.

[49] Haller S, Zaharchuk G, Thomas DL, Lovblad K-O, Barkhof F, Golay X (2016) Arterial Spin Labeling Perfusion of the Brain: Emerging Clinical Applications. *Radiology* **281**, 337–356.

[50] Roquet D, Sourty M, Botzung A, Armspach J-P, Blanc F (2016) Brain perfusion in dementia with Lewy bodies and Alzheimer's disease: an arterial spin labeling MRI study on prodromal and mild dementia stages. *Alzheimers Res Ther* **8**,.

[51] Johnson NA, Jahng G-H, Weiner MW, Miller BL, Chui HC, Jagust WJ, Gorno-Tempini ML, Schuff N (2005) Pattern of Cerebral Hypoperfusion in Alzheimer Disease and Mild Cognitive Impairment Measured with Arterial Spin-labeling MR Imaging: Initial Experience. *Radiology* **234**, 851–859.

[52] Yoshiura T, Hiwatashi A, Yamashita K, Ohyagi Y, Monji A, Takayama Y, Nagao E, Kamano H, Noguchi T, Honda H (2009) Simultaneous Measurement of Arterial Transit Time, Arterial Blood Volume, and Cerebral Blood Flow Using Arterial Spin-Labeling in Patients with Alzheimer Disease. *Am J Neuroradiol* **30**, 1388–1393.

[53] Wolk DA, Detre JA (2012) Arterial spin labeling MRI: an emerging biomarker for Alzheimer's disease and other neurodegenerative conditions. *Curr Opin Neurol* **25**, 421–428.

[54] Alsop DC, Casement M, de Bazelaire C, Fong T, Press DZ (2008) Hippocampal hyperperfusion in Alzheimer's disease. *Neuroimage* **42**, 1267–1274.

[55] Fleisher AS, Podraza KM, Bangen KJ, Taylor C, Sherzai A, Sidhar K, Liu TT, Dale AM, Buxton RB (2009) Cerebral perfusion and oxygenation differences in Alzheimer's disease risk. *Neurobiol Aging* **30**, 1737–1748.

[56] Morris M, Sanchez PE, Verret L, Beagle AJ, Guo W, Dubal D, Ranasinghe KG, Koyama A, Ho K, Yu G-Q, Vossel KA, Mucke L (2015) Network dysfunction in α-synuclein transgenic mice and human Lewy body dementia. *Ann Clin Transl Neurol* **2**, 1012–1028.

[57] Peters ST, Fahrenkopf A, Choquette JM, Vermilyea SC, Lee MK, Vossel K (2020) Ablating Tau Reduces Hyperexcitability and Moderates Electroencephalographic Slowing in Transgenic Mice Expressing A53T Human α-Synuclein . *Front Neurol* **11**,.

[58] Frauscher B, von Ellenrieder N, Dubeau F, Gotman J (2016) EEG desynchronization during phasic REM sleep suppresses interictal epileptic activity in humans. *Epilepsia* **57**, 879–888.

[59] Campana C, Zubler F, Gibbs S, de Carli F, Proserpio P, Rubino A, Cossu M, Tassi L, Schindler K, Nobili L (2017) Suppression of interictal spikes during phasic rapid eye movement sleep: a quantitative stereo-electroencephalography study. *J Sleep Res* **26**, 606–613.

[60] Chan P-C, Lee H-H, Hong C-T, Hu C-J, Wu D (2018) REM Sleep Behavior Disorder (RBD) in Dementia with Lewy Bodies (DLB). *Behav Neurol* **2018**, 9421098.

[61] Gomperts SN, Rentz DM, Moran E, Becker JA, Locascio JJ, Klunk WE, Mathis CA, Elmaleh DR, Shoup T, Fischman AJ, Hyman BT, Growdon JH, Johnson KA (2008) Imaging amyloid deposition in Lewy body diseases. *Neurology* **71**, 903–910.

[62] Biundo R, Weis L, Fiorenzato E, Pistonesi F, Cagnin A, Bertoldo A, Anglani M, Cecchin D, Antonini A (2021) The contribution of beta-amyloid to dementia in Lewy body diseases: a 1-year follow-up study. *Brain Commun* **3**, fcab180.

[63] Kam K, Duffy ÁM, Moretto J, LaFrancois JJ, Scharfman HE (2016) Interictal spikes during sleep are an early defect in the Tg2576 mouse model of β-amyloid neuropathology. *Sci Rep* **6**, 20119.

[64] Bezzina C, Verret L, Juan C, Remaud J, Halley H, Rampon C, Dahan L (2015) Early Onset of Hypersynchronous Network Activity and Expression of a Marker of Chronic Seizures in the Tg2576 Mouse Model of Alzheimer's Disease. *PLoS One* **10**, e0119910.

[65] Bakker A, Krauss GL, Albert MS, Speck CL, Jones LR, Stark CE, Yassa MA, Bassett SS, Shelton AL, Gallagher M (2012) Reduction of Hippocampal Hyperactivity Improves Cognition in Amnestic Mild Cognitive Impairment. *Neuron* **74**, 467–474.

[66] Vossel K, Ranasinghe KG, Beagle AJ, La A, Ah Pook K, Castro M, Mizuiri D, Honma SM, Venkateswaran N, Koestler M, Zhang W, Mucke L, Howell MJ, Possin KL, Kramer JH, Boxer AL, Miller BL, Nagarajan SS, Kirsch HE (2021) Effect of Levetiracetam on Cognition in Patients With Alzheimer Disease With and Without Epileptiform Activity: A Randomized Clinical Trial. *JAMA Neurol* **78**, 1345–1354.

[67] Musaeus CS, Shafi MM, Santarnecchi E, Herman ST, Press DZ (2017) Levetiracetam Alters Oscillatory Connectivity in Alzheimer's Disease. *J Alzheimer's Dis* **58**,.

[68] Weisdorf S, Duun-Henriksen J, Kjeldsen MJ, Poulsen FR, Gangstad SW, Kjær TW (2019) Ultra-long-term subcutaneous home monitoring of epilepsy-490 days of EEG from nine patients. *Epilepsia* **60**, 2204–2214.

[69] Musaeus CS, Kjær TW, Cacic Hribljan M, Andersen BB, Høgh P, Kidmose P, Fabricius M, Hemmsen MC, Rank ML, Waldemar G, Frederiksen KS (2023) Subclinical Epileptiform Activity in Dementia with Lewy Bodies. *Mov Disord*.

[70] Musaeus CS, Waldemar G, Andersen BB, Høgh P, Kidmose P, Hemmsen MC, Rank ML, Kjær TW, Frederiksen KS (2022) Long-Term EEG Monitoring in Patients with Alzheimer's Disease Using Ear-EEG: A Feasibility Study. *J Alzheimer's Dis* **90**, 1713–1723.

[71] Musaeus CS, Kjær TW, Lindberg U, Vestergaard MB, Larsson HBW, Press DZ, Andersen BB, Høgh P, Kidmose P, Hemmsen MC, Rank ML, Hasselbalch SG, Waldemar G, Frederiksen KS Subclinical epileptiform discharges in Alzheimer's disease are associated with increased hippocampal blood flow. *Submitted*.

[72] McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack CR, Kawas CH, Klunk WE, Koroshetz WJ, Manly JJ, Mayeux R, Mohs RC, Morris JC, Rossor MN, Scheltens P, Carrillo MC, Thies B, Weintraub S, Phelps CH (2011) 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* **7**, 263–269.

[73] McKeith IG, Dickson DW, Lowe J, Emre M, O'Brien JT, Feldman H, Cummings J, Duda JE, Lippa C, Perry EK, Aarsland D, Arai H, Ballard CG, Boeve B, Burn DJ, Costa D, Del Ser T, Dubois B, Galasko D, Gauthier S, Goetz CG, Gomez-Tortosa E, Halliday G, Hansen LA, Hardy J, Iwatsubo T, Kalaria RN, Kaufer D, Kenny RA, Korczyn A, Kosaka K, Lee VMY, Lees A, Litvan I, Londos E, Lopez OL, Minoshima S, Mizuno Y, Molina JA, Mukaetova-Ladinska EB, Pasquier F, Perry RH, Schulz JB, Trojanowski JQ, Yamada M (2005) Diagnosis and management of dementia with Lewy bodies. *Neurology* **65**, 1863 LP – 1872.

[74] Pfeffer RI, Kurosaki TT, Harrah CH, Chance JM, Filos S (1982) Measurement of Functional Activities in Older Adults in the Community. *J Gerontol* **37**, 323–329.

[75] Cummings JL, Mega M, Gray K, Rosenberg-Thompson S, Carusi DA, Gornbein J (1994) The Neuropsychiatric Inventory: Comprehensive assessment of psychopathology in dementia. *Neurology* **44**, 2308.

[76] Vogel A, Stokholm J, Andreasen R, Henriksen BD, Brønniche V, Madsen GJ, Gustafsson M, Overgaard S, Guldberg A-M, Jørgensen K (2018) Psychometric properties and reference data for Danish versions of Free and Cued Selective Reminding Test, Category Cued Memory Test and Logical Memory. **59**, 496–502.

[77] Kidmose P, Looney D, Mandic DP (2012) Auditory evoked responses from Ear-EEG recordings. In *2012 Annual International Conference of the IEEE engineering in Medicine and Biology Society* IEEE.

[78] Press DZ, Musaeus CS, Zhao L, Breton JM, Shafi MM, Dai W, Alsop DC (2023) Levetiracetam Increases Hippocampal Blood Flow in Alzheimer's Disease as Measured by Arterial Spin Labelling MRI. *J Alzheimers Dis* **93**, 939–948.

[79] Kural MA, Duez L, Sejer Hansen V, Larsson PG, Rampp S, Schulz R, Tankisi H, Wennberg R, Bibby BM, Scherg M, Beniczky S (2020) Criteria for defining interictal epileptiform discharges in EEG. *Neurology* **94**, e2139 LP-e2147.

[80] Voevodskaya O, Simmons A, Nordenskjöld R, Kullberg J, Ahlström H, Lind L, Wahlund L-O, Larsson E-M, Westman E (2014) The effects of intracranial volume adjustment approaches on multiple regional MRI volumes in healthy aging and Alzheimer's disease . *Front Aging Neurosci* **6**,.

[81] Bakker CJ, Hartkamp MJ, Mali WP (1996) Measuring blood flow by nontriggered 2D phase-contrast MR angiography. *Magn Reson Imaging* **14**, 609–614.

[82] Vestergaard MB, Lindberg U, Aachmann-Andersen NJ, Lisbjerg K, Christensen SJ, Rasmussen P, Olsen NV, Law I, Larsson HBW, Henriksen OM (2017) Comparison of global cerebral blood flow measured by phase-contrast mapping MRI with (15) O-H(2) O positron emission tomography. *J Magn Reson Imaging* **45**, 692–699.

[83] Vestergaard MB, Ghanizada H, Lindberg U, Arngrim N, Paulson OB, Gjedde A, Ashina M, Larsson HBW (2022) Human Cerebral Perfusion, Oxygen Consumption, and Lactate Production in Response to Hypoxic Exposure. *Cereb Cortex* **32**, 1295–1306.

[84] Chappell MA, Groves AR, Whitcher B, Woolrich MW (2009) Variational Bayesian Inference for a Nonlinear Forward Model. *Trans Sig Proc* **57**, 223–236.

[85] Chappell MA, MacIntosh BJ, Donahue MJ, Günther M, Jezzard P, Woolrich MW (2010) Separation of macrovascular signal in multi-inversion time arterial spin labelling MRI. *Magn Reson Med* **63**, 1357–1365.

[86] Van de Vel A, Smets K, Wouters K, Ceulemans B (2016) Automated non-EEG based seizure detection: Do users have a say? *Epilepsy Behav* **62**, 121–128.

[87] Hoppe C, Feldmann M, Blachut B, Surges R, Elger CE, Helmstaedter C (2015) Novel techniques for automated seizure registration: Patients' wants and needs. *Epilepsy Behav* **52**, 1–7.

[88] Simblett SK, Biondi A, Bruno E, Ballard D, Stoneman A, Lees S, Richardson MP, Wykes T (2020) Patients' experience of wearing multimodal sensor devices intended to detect epileptic seizures: A qualitative analysis. *Epilepsy Behav* **102**, 106717.

[89] Jing J, Herlopian A, Karakis I, Ng M, Halford JJ, Lam A, Maus D, Chan F, Dolatshahi M, Muniz CF, Chu C, Sacca V, Pathmanathan J, Ge W, Sun H, Dauwels J, Cole AJ, Hoch DB, Cash SS, Westover MB

(2020) Interrater Reliability of Experts in Identifying Interictal Epileptiform Discharges in Electroencephalograms. *JAMA Neurol* **77**, 49–57.

[90] Jansen WJ, Ossenkoppele R, Knol DL, Tijms BM, Scheltens P, Verhey FRJ, Visser PJ, Aalten P, Aarsland D, Alcolea D, Alexander M, Almdahl IS, Arnold SE, Baldeiras I, Barthel H, van Berckel BNM, Bibeau K, Blennow K, Brooks DJ, van Buchem MA, Camus V, Cavedo E, Chen K, Chetelat G, Cohen AD, Drzezga A, Engelborghs S, Fagan AM, Fladby T, Fleisher AS, van der Flier WM, Ford L, Förster S, Fortea J, Foskett N, Frederiksen KS, Freund-Levi Y, Frisoni GB, Froelich L, Gabryelewicz T, Gill KD, Gkatzima O, Gómez-Tortosa E, Gordon MF, Grimmer T, Hampel H, Hausner L, Hellwig S, Herukka S-K, Hildebrandt H, Ishihara L, Ivanoiu A, Jagust WJ, Johannsen P, Kandimalla R, Kapaki E, Klimkowicz-Mrowiec A, Klunk WE, Köhler S, Koglin N, Kornhuber J, Kramberger MG, Van Laere K, Landau SM, Lee DY, de Leon M, Lisetti V, Lleó A, Madsen K, Maier W, Marcusson J, Mattsson N, de Mendonça A, Meulenbroek O, Meyer PT, Mintun MA, Mok V, Molinuevo JL, Møllergård HM, Morris JC, Mroczko B, Van der Mussele S, Na DL, Newberg A, Nordberg A, Nordlund A, Novak GP, Paraskevas GP, Parnetti L, Perera G, Peters O, Popp J, Prabhakar S, Rabinovici GD, Ramakers IHGB, Rami L, Resende de Oliveira C, Rinne JO, Rodrigue KM, Rodríguez-Rodríguez E, Roe CM, Rot U, Rowe CC, Rüther E, Sabri O, Sanchez-Juan P, Santana I, Sarazin M, Schröder J, Schütte C, Seo SW, Soetewey F, Soininen H, Spiru L, Struyfs H, Teunissen CE, Tsolaki M, Vandenberghe R, Verbeek MM, Villemagne VL, Vos SJB, van Waalwijk van Doorn LJC, Waldemar G, Wallin A, Wallin ÅK, Wiltfang J, Wolk DA, Zboch M, Zetterberg H (2015) Prevalence of cerebral amyloid pathology in persons without dementia: a meta-analysis. *JAMA* **313**, 1924–1938.

[91] Karoly PJ, Freestone DR, Boston R, Grayden DB, Himes D, Leyde K, Seneviratne U, Berkovic S, O'Brien T, Cook MJ (2016) Interictal spikes and epileptic seizures: their relationship and underlying rhythmicity. *Brain* **139**, 1066–1078.

[92] Janszky J, Hoppe M, Clemens Z, Janszky I, Gyimesi C, Schulz R, Ebner A (2005) Spike frequency is dependent on epilepsy duration and seizure frequency in temporal lobe epilepsy. *Epileptic Disord* **7**, 355–359.

[93] Sarkis RA, Dickerson BC, Cole AJ, Chemali ZN (2016) Clinical and Neurophysiologic Characteristics of Unprovoked Seizures in Patients Diagnosed With Dementia. *J Neuropsychiatry Clin Neurosci* **28**, 56–61.

[94] Cretin B, Sellal F, Philippi N, Bousiges O, Di Bitonto L, Martin-Hunyadi C, Blanc F (2016) 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* **52**, 1125–1133.

[95] Ossenkoppele R, Cohn-Sheehy BI, La Joie R, Vogel JW, Möller C, Lehmann M, van Berckel BNM, Seeley WW, Pijnenburg YA, Gorno-Tempini ML, Kramer JH, Barkhof F, Rosen HJ, van der Flier WM, Jagust WJ, Miller BL, Scheltens P, Rabinovici GD (2015) Atrophy patterns in early clinical stages across distinct phenotypes of Alzheimer's disease. *Hum Brain Mapp* **36**, 4421–4437.

[96] Harper L, Bouwman F, Burton EJ, Barkhof F, Scheltens P, O'Brien JT, Fox NC, Ridgway GR, Schott JM (2017) Patterns of atrophy in pathologically confirmed dementias: a voxelwise analysis. *J Neurol Neurosurg Psychiatry* **88**, 908–916.

[97] Mak E, Su L, Williams GB, O'Brien JT (2014) Neuroimaging characteristics of dementia with Lewy bodies. *Alzheimers Res Ther* **6**, 18.

[98] Harding AJ, Broe GA, Halliday GM (2002) Visual hallucinations in Lewy body disease relate to Lewy bodies in the temporal lobe. *Brain* **125**, 391–403.

[99] Fry A, Singh D, Manganas L, Gordon ML, Christodoulou C, Leung H-C, Schwartz GJ (2022) Parkinson's

Disease With Visual Hallucinations Is Associated With Epileptiform Activity on EEG . *Front Neurol* **12**,.

[100] Nelson PT, Jicha GA, Kryscio RJ, Abner EL, Schmitt FA, Cooper G, Xu LO, Smith CD, Markesbery WR (2010) Low sensitivity in clinical diagnoses of dementia with Lewy bodies. *J Neurol* **257**, 359–366.

[101] Kim WS, Kågedal K, Halliday GM (2014) Alpha-synuclein biology in Lewy body diseases. *Alzheimers Res Ther* **6**, 73.

[102] Sammaritano M, Gigli GL, Gotman J (1991) Interictal spiking during wakefulness and sleep and the localization of foci in temporal lobe epilepsy. *Neurology* **41**, 290 LP – 290.

[103] B Szabo A, Cattaud V, Bezzina C, Dard RF, Sayegh F, Gauzin S, Lejards C, Valton L, Rampon C, Verret L, Dahan L (2023) Neuronal hyperexcitability in the Tg2576 mouse model of Alzheimer's disease - the influence of sleep and noradrenergic transmission. *Neurobiol Aging* **123**, 35–48.

[104] Bugalho P, Salavisa M, Marto JP, Borbinha C, Alves L (2019) Polysomnographic data in Dementia with Lewy Bodies: correlation with clinical symptoms and comparison with other α-synucleinopathies. *Sleep Med* **55**, 62–68.

[105] O'Dowd S, Schumacher J, Burn DJ, Bonanni L, Onofrj M, Thomas A, Taylor J-P (2019) Fluctuating cognition in the Lewy body dementias. *Brain* **142**, 3338–3350.

[106] Cauda F, Torta DME, Sacco K, D'Agata F, Geda E, Duca S, Geminiani G, Vercelli A (2013) Functional anatomy of cortical areas characterized by Von Economo neurons. *Brain Struct Funct* **218**, 1–20.

[107] Alavi A, Yakir S, Newberg AB (2011) Positron emission tomography in seizure disorders. *Ann N Y Acad Sci* **1228**, E1–E12.

[108] Lam AD, Deck G, Goldman A, Eskandar EN, Noebels J, Cole AJ (2017) Silent hippocampal seizures and spikes identified by foramen ovale electrodes in Alzheimer's disease. *Nat Med* **23**, 678–680.

[109] Asllani I, Habeck C, Scarmeas N, Borogovac A, Brown TR, Stern Y (2007) Multivariate and Univariate Analysis of Continuous Arterial Spin Labeling Perfusion MRI in Alzheimer's Disease. *J Cereb Blood Flow Metab* **28**, 725–736.

[110] Bron EE, Steketee RME, Houston GC, Oliver RA, Achterberg HC, Loog M, van Swieten JC, Hammers A, Niessen WJ, Smits M, Klein S (2014) Diagnostic classification of arterial spin labeling and structural MRI in presenile early stage dementia. *Hum Brain Mapp* **35**, 4916–4931.

[111] Muldoon SF, Villette V, Tressard T, Malvache A, Reichinnek S, Bartolomei F, Cossart R (2015) GABAergic inhibition shapes interictal dynamics in awake epileptic mice. *Brain* **138**, 2875–2890.

[112] Chang Y-Y, Gong X-W, Gong H-Q, Liang P-J, Zhang P-M, Lu Q-C (2018) GABAA Receptor Activity Suppresses the Transition from Inter-ictal to Ictal Epileptiform Discharges in Juvenile Mouse Hippocampus. *Neurosci Bull* **34**, 1007–1016.

[113] Musaeus CS, Zhao L, Dai W, Breton J, Shafi M, Alsop DC, Press DZ (2020) A randomized controlled trial measuring changes in cerebral blood flow after levetiracetam in patients with Alzheimer's disease. *Alzheimer's Dement* **16**, e045476.

[114] Kappel SL, Kidmose P (2015) Study of impedance spectra for dry and wet EarEEG electrodes. *Annu Int Conf IEEE Eng Med Biol Soc IEEE Eng Med Biol Soc Annu Int Conf* **2015**, 3161–3164.

[115] Britton JW, Frey LC, Hopp JL, Korb P, Koubeissi MZ, Lievens WE, Pestana-Knight EM, St. Louis EK (2016) *Electroencephalography (EEG): An Introductory Text and Atlas of Normal and Abnormal*

Findings in Adults, Children, and Infants [Internet], , Chicago.

[116] Wüstenhagen S, Terney D, Gardella E, Meritam Larsen P, Rømer C, Aurlien H, Beniczky S (2022) EEG normal variants: A prospective study using the SCORE system. *Clin Neurophysiol Pract* **7**, 183–200.

Appendix

Manuscript I

Long-Term EEG Monitoring in Patients with Alzheimer's Disease Using Ear-EEG: A Feasibility Study

The publication is available at IOS Press through http://dx.doi.org/10.3233/JAD-220491

Journal of Alzheimer's Disease 90 (2022) 1713–1723
DOI 10.3233/JAD-220491
IOS Press

Long-Term EEG Monitoring in Patients with Alzheimer's Disease Using Ear-EEG: A Feasibility Study

Christian Sandøe Musaeus[a,*], Gunhild Waldemar[a,b], Birgitte Bo Andersen[a], Peter Høgh[c,b], Preben Kidmose[d], Martin Christian Hemmsen[f], Mike Lind Rank[f], Troels Wesenberg Kjær[g,b] and Kristian Steen Frederiksen[a]

[a]*Department of Neurology, Danish Dementia Research Centre (DDRC), Rigshospitalet, University of Copenhagen, Denmark*
[b]*Department of Clinical Medicine, University of Copenhagen, Denmark*
[c]*Department of Neurology, Regional Dementia Research Centre, Zealand University Hospital, Roskilde, Denmark*
[d]*Department of Electrical and Computer Engineering, Aarhus University, Aarhus N, Denmark*
[e]*Department of Clinical Neurophysiology, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark*
[f]*T&W Engineering, Lillerød, Denmark*
[g]*Department of Neurology, Zealand University Hospital, Roskilde, Denmark*

Accepted 24 September 2022
Pre-press 29 October 2022

Abstract.
Background: Previous studies have reported that epileptiform activity may be detectible in nearly half of patients with Alzheimer's disease (AD) on long-term electroencephalographic (EEG) recordings. However, such recordings can be uncomfortable, expensive, and difficult. Ear-EEG has shown promising results for long-term EEG monitoring, but it has not been used in patients with AD.
Objective: To investigate if ear-EEG is a feasible method for long-term EEG monitoring in patients with AD.
Methods: In this longitudinal, single-group feasibility study, ten patients with mild to moderate AD were recruited. A total of three ear-EEG recordings of up to 48 hours three months apart for six months were planned.
Results: All patients managed to wear the ear-EEG for at least 24 hours and at least one full night. A total of 19 ear-EEG recordings were performed (self-reported recording, mean: 37.15 hours (SD: 8.96 hours)). After automatic pre-processing, a mean of 27.37 hours (SD: 7.19 hours) of data with acceptable quality in at least one electrode in each ear was found. Seven out of ten participants experienced mild adverse events. Six of the patients did not complete the study with three patients not wanting to wear the ear-EEG anymore due to adverse events.
Conclusion: It is feasible and safe to use ear-EEG for long-term EEG monitoring in patients with AD. Minor adjustments to the equipment may improve the comfort for the participants.

Keywords: Alzheimer's disease, ear-EEG, electroencephalography, long-term EEG, wearable

INTRODUCTION

Patients with Alzheimer's disease (AD) are at an increased risk of developing epileptic seizures [1–6], which may increase with the severity of AD [7, 8]

*Correspondence to: Christian Sandøe Musaeus, Rigshospitalet, University of Copenhagen Inge Lehmanns Vej 8, 2100 Copenhagen, Denmark. E-mail: Christian.sandoee.musaeus@regionh.dk.

and may be detrimental for their functional abilities. An indicator of seizures is epileptiform activity in the electroencephalography (EEG). Epileptiform activity may not be accompanied by clinical manifestations. Furthermore, the most prevalent type of seizure in patients with AD is focal impaired awareness seizures [5, 9], which may mimic symptoms of AD and may go unnoticed by patient and caregiver. Therefore, seizures may be much more common in patients with AD than previously reported. This has been underscored in a study showing that epileptiform activity without clinical seizure phenomena is present in 42.4% of patients with AD [10]. However, although in-patient EEG monitoring over longer periods may be more effective, such in-patient monitoring can be difficult for the patients with dementia, is expensive and not possible for extended periods. This implies the importance of both recognizing epileptiform activity in patients with AD and the need for easy and inexpensive continuous monitoring.

In recent years, methods for out-patient assessment of a variety of biometric data in AD have been investigated [11]. However, no devices have so far been applied for continuous EEG recordings in AD. In patients with epilepsy, several methods for long-term EEG [12] as well as other wearable devices [13] have been studied including ear-EEG [14] and subcutaneous electrodes [15]. Ear-EEG is a method where EEG signals are acquired from electrodes placed within a customized earpiece inserted into each ear canal. The ear-EEG has several advantages including that the patients can stay in a familiar environment with minimal interference of everyday life while undergoing long-term EEG monitoring and are able to easily remove and insert the device back into the ear canal [16–18]. However, the EEG coverage of the brain using ear-EEG involves mainly the temporal lobes. This may be less of a disadvantage when examining patients with AD since the majority of epileptiform activity arise from the temporal lobes [10, 19]. Also, previous studies have shown a good correspondence between ear-EEG and scalp EEG [20, 21]. In addition, a recent study found that ear-EEG can be used to detect EEG patterns associated with focal temporal lobe seizure [14]. Other studies have shown that ear-EEG performs well in sleep scoring [21–24]. Therefore, ear-EEG may be a suitable tool to investigate whether patients with AD develop subclinical epileptiform activity.

Since out-patient long-term EEG monitoring of patients with AD may pose other challenges than in cognitively perserved persons, it is relevant to examine the feasibility of such methods, and to determine the best tradeoffs between both data quality and compliance. Specifically, patients with AD suffer from memory problems and other cognitive deficits like executive functions [25], which may give rise to problems planning the recordings, setting up the equipment, or remembering the instructions. This may lead to lower compliance and quality of the recordings. Furthermore, patients with AD may have a different tolerance to pain [26, 27], or may not respond adequately to other safety issues.

In the present feasibility study, we therefore aimed to investigate if ear-EEG is a feasible and safe method for long-term EEG monitoring of patients with AD.

MATERIALS AND METHODS

Patients

Patients with mild to moderate AD were recruited from either the Memory Clinic at Rigshospitalet, Copenhagen or the Memory Clinic at Zealand University Hospital, Roskilde. All patients met the NIA-AA criteria for probable AD with amnestic presentation [28] and the diagnosis was determined based on a consensus conference. Inclusion criteria were 1) age between 50-90 years, 2) a Mini-Mental Status Examination (MMSE) [29] score between 16-28, 3) native Danish speaker, 4) at least 7 years of education, 5) hearing and vision sufficient for neuropsychological examination, 6) the general health conditions of the patient allowed participation in the study (as judged by the principal investigator), 7) did not suffer from any psychiatric (except mild depression) or neurological conditions that affects the brain except AD, 8) any treatment with either anti-dementia medication or selective serotonin reuptake inhibitors (if relevant) were given in a stable regimen within the last three months up to inclusion, 9) no alcohol or drug abuse within the last two years, 10) an MRI or CT scan that supported the diagnosis of AD, 11) no contraindications for MRI, and 12) lived with a caregiver who were able to assist the patient with the home recordings.

The following exclusion criteria were applied: 1) epilepsy prior to the diagnosis of AD, 2) focal pathology (except AD related atrophy) in the hippocampus, i.e., hippocampal sclerosis, 3) living with relative with serious illness or impaired activities of daily living, 4) living in a nursing home, 5) treated with anti-epileptic medication, tricyclic antidepressants or antipsychotics, 6) daily or almost daily administra-

tion of medication with known anticholinergic or adrenergic effect, which may affect cognitive abilities or EEG, 7) large cerebral infarctions or more than four lacunar infarctions on MRI, 8) suffering from facial tics/facial hyperkinetic disorders, or 9) daily use of hearing aids.

The study was approved by the Capital Region Ethics Committee (H-17035751) and by the Danish Medicines Agency (2017112288). All participants gave their written informed consent before participating in the study. The study is registered at clinicaltrials.gov (NCT04436341).

Study design

In this longitudinal single-group feasibility study, a total of 8 visits including an MRI scan was planned over a 6-month period (see Fig. 1). The three two-day ear-EEG recordings were performed three months apart, i.e., at the beginning of the study, at three months and at 6 months follow-up. At visit 1 (baseline), informed consent was obtained followed by assessment of medical history, a physical and neurological examination, the MMSE (to assess global cognition) and an imprint of the ears using Otoform a soft ear impression silicone. Subsequently, the patient underwent the following: visit 2) MRI scan, visit 3) included standard EEG recording together with ear-EEG, Functional Assessment Questionnaire IADL (FAQ IADL) [30] (to assess everyday function) and neuropsychiatric inventory (NPI) [31] (to assess behavioral and psychological symptoms). Further, the patient and the caregiver were briefly instructed about seizures and introduced to a seizure dairy to take home, and information and training on how to use the ear-EEG at home. This included a log-sheet for the patient or caregiver to take notes on when the patient wore the ear-EEG. After approximately 48 hours (visit 4) the patient and/or the caregiver returned with the equipment and a questionnaire was used, see below. For most of the visit, the outer ear was examined for any minor lesions. Visit 5 and 6 and visit 7 and 8 were the same as visit 3 and 4 (except MMSE).

Due to the COVID pandemic, the time between visits was prolonged including the time between visit 4 and 5, and visit 6 and 7.

A short questionnaire was administered by the investigator when the patient and/or the caregiver returned the ear-EEG (visit 4, 6, and 8). The questionnaire included the following questions: a) how did it feel to wear the ear-EEG, b) did you have any problems sleeping with the equipment, c) did the ear-EEG ever fall out of the ear, and d) do you have any suggestions for improvement.

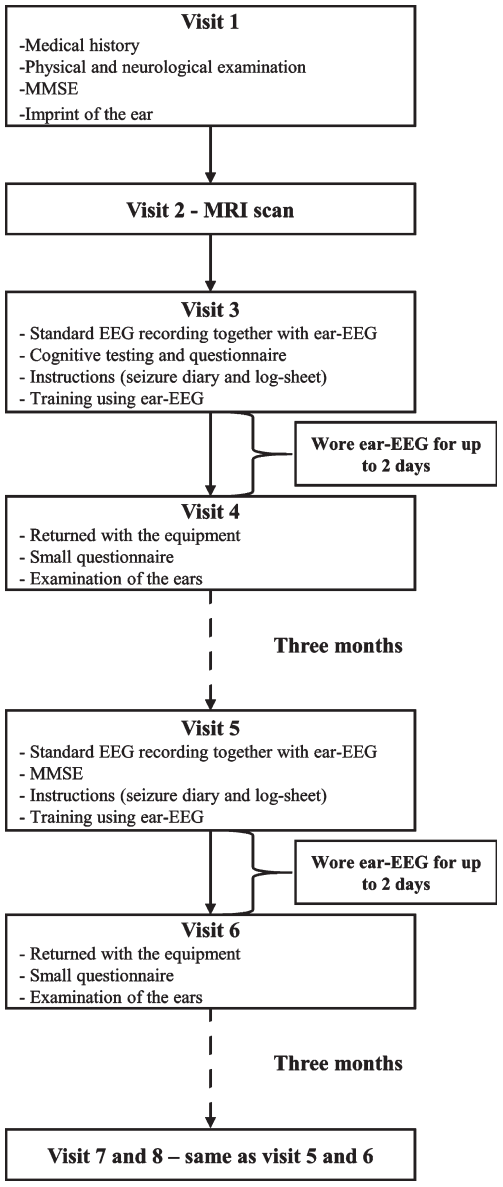


Fig. 1. Study design. Firstly, the patients were included in the study after written informed consent. The figure shows the following visits with visit 5 and 6 being three months after the visit 4 and visit 7 and 8 being three months after visit 5.

Ear-EEG

The ear-EEG consists of six dry-electrodes mounted in earpieces which are custom-made using ear imprints and 3D-printing and therefore fitted indi-

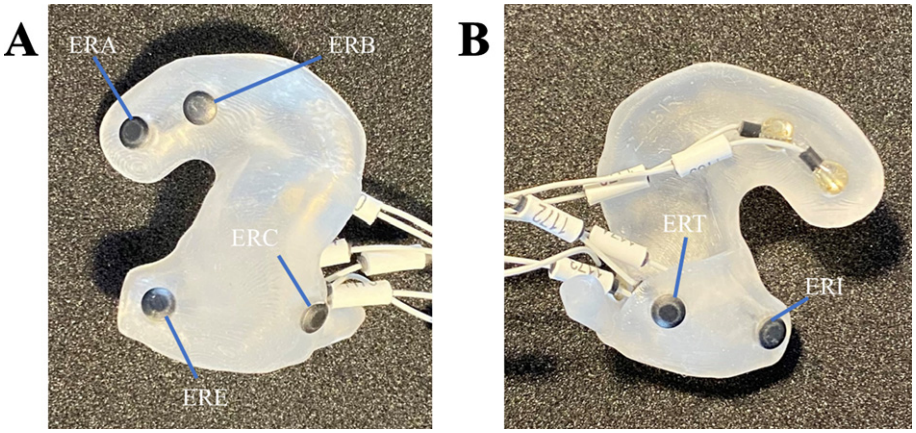


Fig. 2. Figure showing the two side of the ear-EEG (A and B). The electrodes are marked with the respective names.

vidually to each participant. The ear-piece was placed inside the ear canal and in the concha (Fig. 2A) [20]. Earpieces were worn in both ears. The labeling convention of the ear-EEG electrodes has previously been described [32], and the labeling in the current study can be seen in Fig. 2A and B. The ground electrode was placed approximately 2 cm under the midline of the clavicle on either the right or left side. The ear-EEG recordings were performed using the TMSi Mobita EEG amplifier (TMSi systems), which was borne in a small bag around the neck and connected to the electrodes through individual wires. For the recordings, a sampling rate of 1000 Hz was chosen.

Data quality

The EEG data were loaded into MATLAB (v2020b) using a custom script for loading *poly5* files. Afterwards, the EEGs were bandpass filtered from 0.5-70 Hz and subsequently notch filtered from 49-51 Hz and 99-101 Hz using the *pop_eegfiltnew* function from EEGLAB [33]. Next, the following artefact rejection pipeline, which has previously been used in other studies [21], was used. Firstly, if a channel were outside the dynamical range of the amplifier, which is a typical indication of an electrode with poor contact to the body, the reading was replaced with a 'NaN'-value, and hence automatically discarded upon loading. The discarded data was considered in the subsequent analysis of data quality. Afterwards, large amplitude samples isolated to a single channel were labelled as artifacts. Lastly, movement or muscle activation, which may also result in large amplitude deviations across multiple channels were labelled as artifacts. The Matlab scripts for preprocessing can be found in the Supplementary Material.

To assess the amount of data after the preprocessing, the amount of time when data from at least one electrode in each ear were present ((ELA or ALB or ELC or ELE or ELI or ELT) and (ERA or ARB or ERC or ERE or ERI or ERT)) was calculated. In addition, the amount of available data for each electrode separately were also examined. Lastly, the amount of time where data from at least one electrode on each ear were present during the night (10 pm-8am) and day (8am-10pm) was examined. Here, we only used the patients' reported start time of recording due to the discrepancy between amount of data recorded on the EEG recorder and self-reported recording times. The code for the calculations can be found in Supplementary Material.

Assessment of feasibility

Feasibility was assessed based on the overall ability of conducting the study including safety, acceptability, improvements in physical capacity, and the participants' ability to complete the physical tests. Safety was assessed based on the occurrence of adverse events (AE) and serious adverse events (SAE, i.e., AEs leading to, e.g., hospitalization and death). Acceptability was assessed on the basis of attendance rate, level of training intensity achieved (% of heart rate reserve and perceived exertion), number of drop-outs, and self-report. Furthermore, the ability of the clinical outcome measures to assess change

over time (i.e., absence of floor or ceiling effects) was evaluated.

Feasibility was assessed based on the overall ability of the patients to wear the ear-EEG including responses to questionnaire, data quality and the amount of data recorded, and safety. Safety was assessed based on the occurrence of adverse events (AE) including serious adverse events (SAE) (e.g., events leading to hospitalization). The duration of time that the patient wore the ear-EEG was based on the log-sheet that the patient and/or caregiver filled out. In addition, we evaluated the amount of data of acceptable quality after pre-processing. This included the useable data at night (10pm – 8am) as compared to daytime (8am – 10pm). Two of the 19 recordings were not included in the night/day calculations due to either only having recorded during the daytime ($n = 1$) or did not fill out the log-sheet as instructed ($n = 1$).

The predetermined criteria to test whether ear-EEG is a feasible tool for long-term EEG monitoring was that at least 80% of the participants wore the ear-EEG for at least 24 h and at least one night over each 2-day period completed. The predetermined threshold of 24 h was used as previous studies using conventional long-term EEG to examine epileptiform activity in patients with AD had recorded for up to 24 h.

A time-frequency representation, a so-called spectrogram, was used to visualize the data for a single subject over the whole recording. Here, the pre-processed signal was averaged to a single channel for the left side and all NaN values were substituted with zeros. The spectrogram was calculated using a 5000-point discrete-time Fourier transform with a Hamming window, 10% overlap (equal to 500) and 5000 frequency points. The mean frequency was calculated between 1 and 70 Hz. The displayed times are from the noted start times of recordings performed by the patient and caregiver. Due to average referencing of the signal, the amplitude of the signal on the left side will be equal to the right side, which means that we did not perform a spectrogram for both left and right side. The full code for creating and visualizing the spectrogram and mean frequency can be found in the supplementary material.

To visualize the data, we showed 10 s of data with artefacts that would be removed using the pre-processing script as well as 10 s of data without artefacts recorded at night. In addition, we showed the data in the frequency domain using the EEGLAB *spectopo* function between all available channels and visualized from 0-60 Hz.

Table 1
Baseline demographics and clinical scales

Gender, female/male	4/6
Age, mean (SD)	74.7 (6.0)
Education in years, mean (SD)	13.5 (2.99)
Symptom onset in years, mean (SD)	2.92 (1.6)
MMSE, mean (SD)	23.7 (4.0)
ADL, mean (SD)	14.2 (5.8)
NPI, mean (SD)	3.8 (2.7)

MMSE, Mini-Mental State Examination; CCMT, Category Cued Memory Test; ADL, Activities of Daily Living; NPI, Neuropsychiatric Inventory; SD, standard deviation.

Adverse events

Adverse events were recorded throughout the whole study period. In addition, the investigators were automatically notified of any hospitalization during the time the patients were enrolled in the study. An SAE was defined according to the international criteria (Good Clinical Practice).

RESULTS

A total of 10 patients with AD were eligible for inclusion and gave informed consent. Baseline characteristic including results from cognitive tests can be seen in Table 1.

Feasibility

All patients wore the ear-EEG for at least 24 h and at least one full night. A total of 19 ear-EEG recordings were performed out of a possible total of 30. Mean self-reported length of the first ear-EEG recording was 36.97 h (SD: 3.93 h, Range: 28.75 h – 42.58 h). The mean self-reported length for all 19 recordings were 37.15 h (SD: 8.96 h, range: 9.25 h – 58.5 h). Recording duration, MMSE, and days since last ear-EEG recording for the patients who participated for more than one ear-EEG session can be seen in Table 2. When interviewed, half the patients described difficulties putting on and wearing the equipment. All participants needed their caregiver's help to either adjust the earpiece into the ear or start the recording. Four of the participants described that the EEG recorder was too heavy or uncomfortable for wearing around the neck during the day.

A total of seven participants developed mild tenderness in or around the ear. This included three incidences of mild redness of the skin on the external ear and two incidences where participants developed small abrasions of the skin. One of the participants

Table 2
Participants with more than one ear-EEG session

	First ear-EEG recording ($n=5$)	Second ear-EEG recording ($n=5$)	Third ear-EEG recording ($n=4$)
Ear-EEG recording duration, mean	38.32 h	36.88 h	37.93 h
MMSE, mean (SD)	24.2 (4.0)	23.2 (4.8)	22.8 (3.7)
Days since last ear-EEG recording		248.4 (89.8)	179 (124.5)

MMSE, Mini-Mental State Examination.

with abrasions developed pain and swelling of the left chin indicative of a mild skin infection. Three patients did not show any discomfort or side-effects to wearing the ear-EEG. Only one of the adverse events led to medical contact and none of them lasted more than a week. None were classified as SAEs.

Six participants dropped out after the first ($n=5$) or second time ($n=1$) that they wore the ear-EEG. Four participants completed all visits. The reasons for dropping out were: not wanting to wear the ear-EEG anymore due to adverse events ($n=3$), not wanting to participate during the COVID-19 pandemic ($n=1$), personal reasons ($n=1$), and moved to a nursing home ($n=1$).

Data quality

Plots of 10-s segments of artefactual and acceptable-quality data in both time and frequency domain can be found in Fig. 3. The mean number of recorded hours as recorded on the EEG recorder were 38.42 (SD: 9.44 h). When investigating the amount of time where at least one electrode had sufficient artifact free data on each side, an average of 11.05 h (SD: 5.61 h) were removed. This resulted in a mean number of 27.37 h (SD: 7.19 h) of at least one-electrode data corresponding to total 71.24% of the data being of acceptable quality.

Data quality for each of the channels are reported in Table 3. Overall, the channels with the least discarded data were ELC, ELE, ERC, ERE, and ERI.

Regarding the distribution of data quality across the day, we found that during the nighttime 84.64% of the data remained after preprocessing as compared to 60.77% of daytime data.

Spectrogram and mean frequency for the ear-EEG signal can be seen in Fig. 4. As can be seen on the figure, there is a decrease in high-frequency activity and a lower mean frequency at night, which is probably due to the patient sleeping.

DISCUSSION

This is the first study to provide data on the feasibility of out-patient long-term EEG monitoring in patients with mild to moderate AD. All patients were able to wear the device for at least one recording (>24 h) and thus, according to our predefined threshold, long-term EEG recording is feasible in AD patients. Data quality was acceptable and nighttime recording yielded a markedly higher proportion of data with acceptable quality. Although 7 out of 10 participants developed mild discomfort while wearing the device, there were no SAEs, and therefore it may be considered safe to use ear-EEG for long-term out-patient monitoring in patients with AD.

To our knowledge, long-term out-patient EEG monitoring with a wearable device has never been performed in patients with AD but has been used in patients with epilepsy. Here, studies have shown that multiple factors affect long-term engagement including the patient being undisturbed by the device during daily activities and sleep [34, 35] as well as the appearance of the device [35, 36]. In the current prototype of the ear-EEG setup, the TMSi amplifier was heavy to carry around the neck in a bag and interfered with daily activities. A solution was to change to a belt bag strapped around the waist that could store the TMSi amplifier, which makes it more comfortable and easier for the participants to perform daily activities. In addition, the device was difficult to conceal, which may have prevented some of the patients from wearing it outside of the house or limited the activities patients engaged in during the recording. Furthermore, half of the patients had trouble wearing it at night, which led to lower quality of sleep. One contributing factor could be that if patients slept on the side, the earpieces may have caused pressure on the ear. Development of devices for long-term EEG recordings in patients with AD should have an increased focus on comfort during sleep in future prototypes of the ear-EEG device or a solution to support the head when sleeping, i.e., a C-

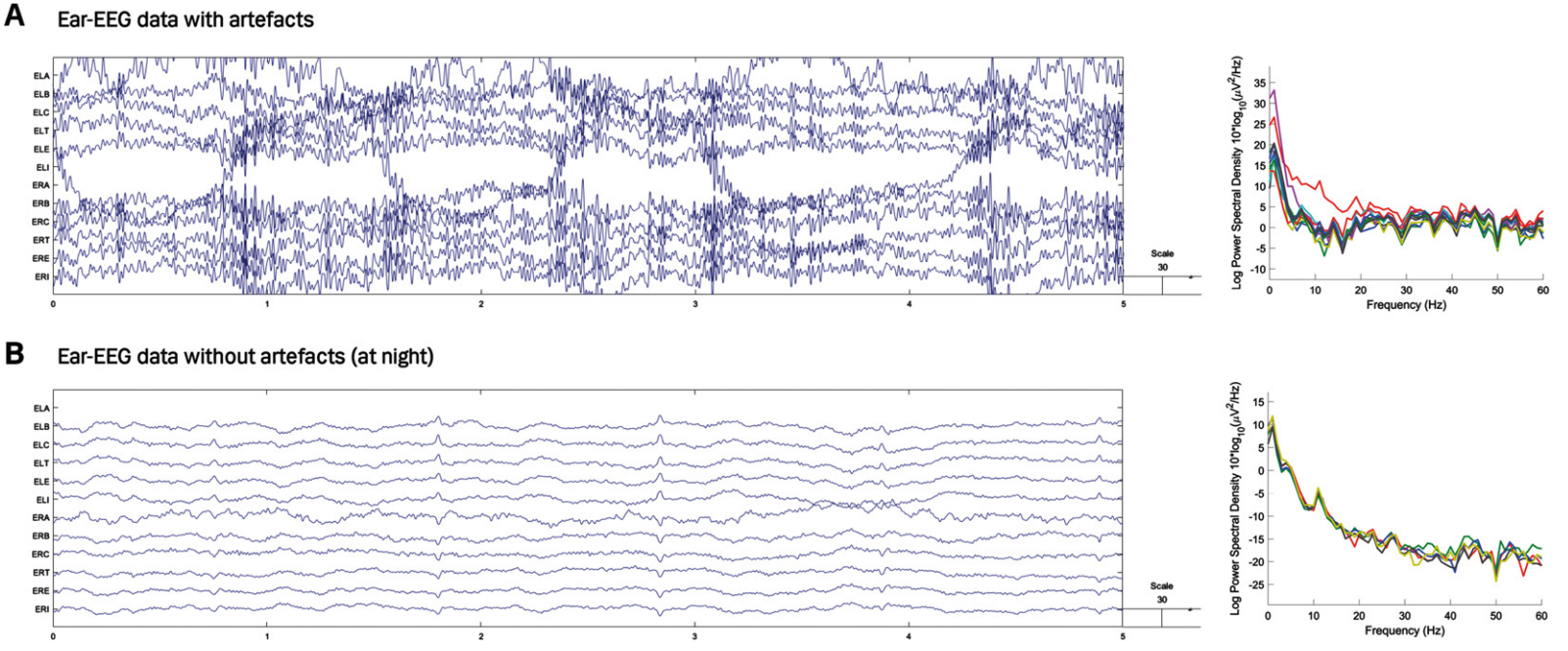


Fig. 3. Figure showing a 10-s ear-EEG segment with A) artefacts or B) without artefacts in both time and frequency domain. Pulse artefacts are visible in B). In the frequency domain, it can be observed that much more high frequency activity (>30 Hz) is present in A as compared to B.

shaped pillow to decrease the pressure on the ear. In a previous study investigating the ability of ear-EEG to detect the seizures in patients with suspected temporal lobe epilepsy ($n = 15$), the majority of patients experienced some degree of soreness but this only led to discontinuation of the use of the ear-EEG for two of the participants (13.3%) [14], which is less than in the current study with discontinuation of three participants (30%). However, the patients with epilepsy were for the most part younger and were hospitalized at the time of EEG recordings, which made it easier to seek help. Furthermore, less electrodes were used (three on each side) as compared to the present study. The higher number of electrodes and thus closer proximity of the electrodes to each other may have been the cause of pain. Specifically, ELE and ELI, and ERE and ERI electrodes, may have been placed too close within the ear canal, which led to increased pressure. The positioning of the two electrodes in the ear canal should be adjusted. An overview of the suggestions for improving the ear-EEG recordings can be found in Table 4.

A study has previously looked at data quality using the same ear-EEG setup as presented here with dry electrodes and investigated out-patient sleep monitoring. The study included young healthy participants (mean age: 25.9 years) and showed very good data quality with 91% accepted data with overall good data quality across the electrodes [21]. We found that 71.24% of the data was acceptable data quality with recordings at night (10 pm–8 am) yielding the most acceptable data quality (84.18% compared to 61.15% during the day). Compared to the study in a younger population, nighttime recordings in terms of the amount of data of acceptable data quality was similar. As compared to the study investigating out-patient sleep monitoring [21], we found large differences in the data quality between electrodes, which may be due to participants/caregiver had to place them in the patients ears themselves. Specifically, it may be difficult to adjust the A and B electrodes (see Fig. 2) within the concha cymba. To increase the likelihood that the caregivers can use the equipment correctly, an instruction sheet could be helpful and has been implemented in future out-patient ear-EEG recordings.

When plotting a spectrogram from an ear-EEG recording, we found that more activity was present in 7-9 Hz during the day as compared to the night (11pm-10am) as well as a lower mean-frequency at night. We hypothesize that this is due to the patient sleeping since a previous study comparing changes in the frequency domain has found slowing of activity during sleep as compare to wakefulness [23]. Another explanation for lower mean frequency during sleep could be less artefacts due to muscle activity. To further explore the changes at night, it would be of interest to apply methods developed for automatic sleep staging using ear-EEG [21], but the methods have not been validated in older participants or patients with AD.

Most participants in the study ($n = 7$) reported mild tenderness in or around the ear after wearing the ear-EEG. In comparison, another long-term ear-EEG study [21] found occasional soreness and only one incident of skin irritation ($n = 20$). However, an important difference is that in this study the EEG was measured for a long continuous period, whereas in [21] it was only measured at night with breaks during the day. In a study investigating ear-EEG in patients with temporal lobe epilepsy the majority of participants experienced soreness, which increased as more time went on [14]. However, this study used another prototype of the ear-EEG and applied gel instead of dry electrodes, which makes the comparison between the current study more difficult. Therefore, future studies using ear-EEG for long-term out-patient recordings should consider breaks throughout the day especially in patients with dementia. These breaks could conveniently be placed during meals since chewing leads to considerable artefacts in the ear-EEG. Furthermore, two participants developed small abrasions, which we hypothesize could be due to either the edges of the electrodes, the caregiver inserting the ear-EEG or adjustment of the ear-EEG throughout the recording by the participant. In one of these cases, we found that the participant developed pain and swelling of the left cheek, which may have been caused by an infection. The patient was completely recovered after five days. This type of adverse event has not previously been reported using ear-EEG but should be considered when informing the participants of the possible risk. Overall, the adverse events were considered mild, and no SAEs were found, and it therefore seems safe to apply ear-EEG in this patient population. So far, no other wearable modalities for long-term EEG monitoring in patients with AD have been investigated. Methods like sheet-type EEG [37] or around the ear (cEEGrid) [38] may lead to less side effects in patients with AD but studies using other wearable EEG devices for long-term out-patient recordings in AD are needed.

The study has some limitations. First, the number of subjects is low due to the nature of a feasibility

Table 3
Data for each electrode after pre-processing

	Percentage of data
ELA	44.33
ELB	41.84
ELC	64.46
ELT	49.17
ELE	65.96
ELI	55.15
ERA	53.54
ERB	49.01
ERC	64.52
ERT	50.41
ERE	68.89
ERI	61.63

The Ear-EEG electrodes are labelled by 3-letter acronyms: E stands for ear followed by letter L for left or R for right and the third letter corresponds to a specific electrode position, see Fig. 2.

study but a more representative sample of patients with AD may have given rise to different considerations regarding the use of ear-EEG. Furthermore, the quality of the ear imprints may have varied due to either errors in the process or the patient moving while the imprint was made. This may have led to inaccuracies of the imprint, which in turn may have caused tenderness in the ear. We did not implement different recording lengths (i.e., one long recording compared to multiple shorter recordings) for the participants, which may have given a better understanding on how to use ear-EEG for long-term monitoring in patients with AD. Furthermore, the TMSi amplifiers does not record time during the EEG recordings, which made the subsequent analysis highly dependent on the log-sheets and was therefore not possible for one of the recordings. Lastly, a more comprehensive questionnaire to understand the comfortability of ear-EEG should be administered in future studies.

Conclusion

Ear-EEG is a feasible technique for long-term outpatient monitoring of patients with mild to moderate AD with only mild adverse events for the participants. The overall data quality was good with an excellent quality during the night, which is optimal since a study found that most of the reported epileptiform activity in patients with AD were present at night [19]. In addition, it was possible by visualizing the ear-EEG using a spectrogram to see changes between day and night. The electrodes located within the ear canal (ELE/ELI, and ERE/ERI) showed the best data quality. This may be due to these electrodes being easier

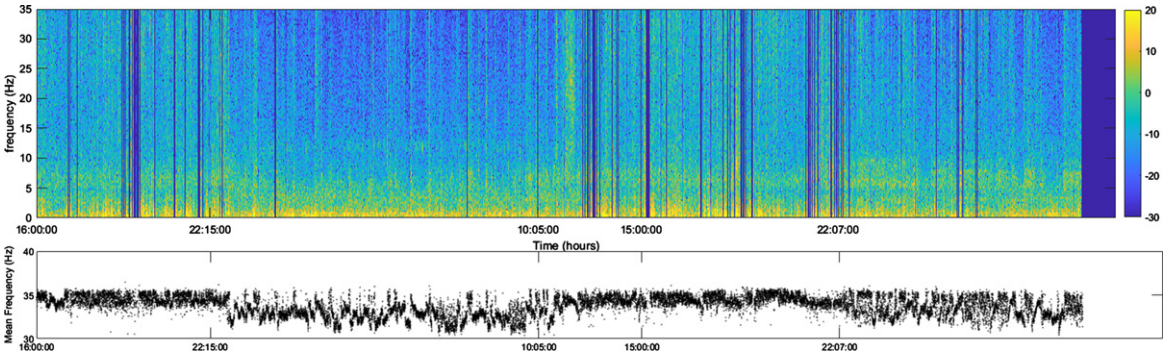


Fig. 4. Spectrogram for the mean ear-EEG signal and mean frequency from the left ear-EEG. As can be seen on the figure, there is a decrease in high-frequency activity and a lower mean frequency as night, which could be due to the patient sleeping. The displayed times are from the noted start times of recordings performed by the patient and caregiver.

Table 4
Table showing the challenges during the ear-EEG recordings as well as suggested improvement for long-term ear-EEG recordings in patients with AD

#	Patient reported discomfort	Mitigation strategies
1	Mild tenderness of the ear	• Breaks throughout the recording, i.e., during meals
2	Minor abrasions in the ears	• Less sharp electrode edges
3	Disturbed sleep due to ear-EEG	• Improved comfort when laying on the side for the ear-EEG • Support for the head, i.e., C-shaped pillow around the ear
4	Amplifier too heavy and interferes with daily activities	• Belt bag strapped around the waist • Smaller amplifier
5	Unwanted attention to the device in public	• Developing a smaller amplifier and less wires from the ear-EEG

to correctly place within the ear or that the electrodes within the ear have lower impedance due to a moister environment [16]. Minor adjustments may increase the comfort for the participants. Future studies using more participants will show whether ear-EEG is able to detect epileptiform activity in patients with AD.

ACKNOWLEDGMENTS

The study was funded by the Alzheimer Research Foundation (grant number: 181003), Toyota Foundation (KJ/BG-9171 F), Axel Juul Muusfeldts Foundation (2016-527), Ellen Mørchs foundation (J.nr. 32491419), Rigshospitalet Research Foundation and T&W Engineering. None of the funding parties had a role in the collection, analysis, and interpretation of data or in the writing of the manuscript or whether to publish the results of the study. Two authors (MLR, MCH) who are employees of T&W Engineering contributed to the interpretation and writing of the report.

We would like to thank Susanne Kristiansen and Oda Jakobsen for their help recruiting participants and helping during study visits.

Authors' disclosures available online (https://www.j-alz.com/manuscript-disclosures/22-0491r2).

SUPPLEMENTARY MATERIAL

The supplementary material is available in the electronic version of this article: https://dx.doi.org/10.3233/JAD-220491.

REFERENCES

[1] Scarmeas N, Honig LS, Choi H, Cantero J, Brandt J, Blacker D, Albert M, Amatniek JC, Marder K, Bell K, Hauser WA, Stern Y (2009) Seizures in Alzheimer disease. *Arch Neurol* **66**, 992-997.

[2] Amatniek JC, Hauser WA, DelCastillo-Castaneda C, Jacobs DM, Marder K, Bell K, Albert M, Brandt J, Stern Y (2006) Incidence and predictors of seizures in patients with Alzheimer's disease. *Epilepsia* **47**, 867-872.

[3] Lozsadi DA, Larner AJ (2006) Prevalence and causes of seizures at the time of diagnosis of probable Alzheimer's disease. *Dement Geriatr Cogn Disord* **22**, 121-124.

[4] Bernardi S, Scaldaferri N, Vanacore N, Trebbastoni A, Francia A, D'Amico A, Prencipe M (2010) Seizures in Alzheimer's disease: A retrospective study of a cohort of outpatients. *Epileptic Disord* **12**, 16-21.

[5] Rao SC, Dove G, Cascino GD, Petersen RC (2009) Recurrent seizures in patients with dementia: Frequency, seizure types, and treatment outcome. *Epilepsy Behav* **14**, 118-120.

[6] Risse SC, Lampe TH, Bird TD, Nochlin D, Sumi SM, Keenan T, Cubberley L, Peskind E, Raskind MA (1990) Myoclonus, seizures, and paratonia in Alzheimer disease. *Alzheimer Dis Assoc Disord* **4**, 217-225.

[7] Romanelli MF, Morris JC, Ashkin K, Coben LA (1990) Advanced Alzheimer's disease is a risk factor for late-onset seizures. *Arch Neurol* **47**, 847-850.

[8] Förstl H (1992) Neurologic signs in Alzheimer's disease. *Arch Neurol* **49**, 1038.

[9] Pandis D, Scarmeas N (2012) Seizures in Alzheimer disease: Clinical and epidemiological data. *Epilepsy Curr* **12**, 184-187.

[10] Vossel KA, Ranasinghe KG, Beagle AJ, Mizuiri D, Honma SM, Dowling AF, Darwish SM, Van Berlo V, Barnes DE, Mantle M, Karydas AM, Coppola G, Roberson ED, Miller BL, Garcia PA, Kirsch HE, Mucke L, Nagarajan SS (2016) Incidence and impact of subclinical epileptiform activity in Alzheimer's disease. *Ann Neurol* **80**, 858-870.

[11] Kourtis LC, Regele OB, Wright JM, Jones GB (2019) Digital biomarkers for Alzheimer's disease: The mobile/wearable devices opportunity. *NPJ Digit Med* **2**, 9.

[12] Casson A, Yates D, Smith S, Duncan J, Rodriguez-Villegas E (2010) Wearable electroencephalography. *IEEE Eng Med Biol Mag* **29**, 44-56.

[13] Brinkmann BH, Karoly PJ, Nurse ES, Dumanis SB, Nasseri M, Viana PF, Schulze-Bonhage A, Freestone DR, Worrell G, Richardson MP, Cook MJ (2021) Seizure diaries and forecasting with wearables: Epilepsy monitoring outside the clinic. *Front Neurol* **12**, 690404.

[14] Zibrandtsen IC, Kidmose P, Christensen CB, Kjaer TW (2017) Ear-EEG detects ictal and interictal abnormalities in focal and generalized epilepsy - A comparison with scalp EEG monitoring. *Clin Neurophysiol* **128**, 2454-2461.

[15] Weisdorf S, Duun-Henriksen J, Kjeldsen MJ, Poulsen FR, Gangstad SW, Kjær TW (2019) Ultra-long-term subcutaneous home monitoring of epilepsy-490 days of EEG from nine patients. *Epilepsia* **60**, 2204-2214.

[16] Kappel SL, Rank ML, Toft HO, Andersen M, Kidmose P (2019) Dry-contact electrode ear-EEG. *IEEE Trans Biomed Eng* **66**, 150-158.

[17] Looney D, Kidmose P, Park C, Ungstrup M, Rank M, Rosenkranz K, Mandic D (2012) The in-the-ear recording concept: User-centered and wearable brain monitoring. *IEEE Pulse* **3**, 32-42.

[18] Looney D, Park C, Kidmose P, Rank ML, Ungstrup M, Rosenkranz K, Mandic DP (2011) An in-the-ear platform for recording electroencephalogram. In *2011 Annual International Conference of the IEEE Engineering in Medicine and Biology Society* IEEE.

[19] Lam AD, Sarkis RA, Pellerin KR, Jing J, Dworetzky BA, Hoch DB, Jacobs CS, Lee JW, Weisholtz DS, Zepeda R, Westover MB, Cole AJ, Cash SS (2020) Association of epileptiform abnormalities and seizures in Alzheimer disease. *Neurology* **95**, e2259-e2270.

[20] Mikkelsen KB, Kappel SL, Mandic DP, Kidmose P (2015) EEG recorded from the ear: Characterizing the ear-EEG method. *Front Neurosci* **9**, 438.

[21] Mikkelsen KB, Tabar YR, Kappel SL, Christensen CB, Toft HO, Hemmsen MC, Rank ML, Otto M, Kidmose P (2019) Accurate whole-night sleep monitoring with dry-contact ear-EEG. *Sci Rep* **9**, 16824.

[22] Nakamura T, Alqurashi YD, Morrell MJ, Mandic DP (2020) Hearables: Automatic overnight sleep monitoring with standardized in-ear EEG sensor. *IEEE Trans Biomed Eng* **67**, 203-212.

[23] Zibrandtsen I, Kidmose P, Otto M, Ibsen J, Kjaer TW (2016) Case comparison of sleep features from ear-EEG and scalp-EEG. *Sleep Sci* **9**, 69-72.

[24] Jørgensen SD, Zibrandtsen IC, Kjaer TW (2019) Ear-EEG-based sleep scoring in epilepsy: A comparison with scalp-EEG. *J Sleep Res* **29**, e12921.

[25] Guarino A, Favieri F, Boncompagni I, Agostini F, Cantone M, Casagrande M (2019) Executive functions in Alzheimer disease: A systematic review. *Front Aging Neurosci* **10**, 437.

[26] Benedetti F, Vighetti S, Ricco C, Lagna E, Bergamasco B, Pinessi L, Rainero I (1999) Pain threshold and tolerance in Alzheimer's disease. *Pain* **80**, 377-382.

[27] Defrin R, Amanzio M, de Tommaso M, Dimova V, Filipovic S, Finn DP, Gimenez-Llort L, Invitto S, Jensen-Dahm C, Lautenbacher S, Oosterman JM, Petrini L, Pick CG, Pickering G, Vase L, Kunz M (2015) Experimental pain processing in individuals with cognitive impairment: Current state of the science. *Pain* **156**, 1396-1408.

[28] McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack CR, Kawas CH, Klunk WE, Koroshetz WJ, Manly JJ, Mayeux R, Mohs RC, Morris JC, Rossor MN, Scheltens P, Carrillo MC, Thies B, Weintraub S, Phelps CH (2011) 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* **7**, 263-269.

[29] Folstein MF, Folstein SE, McHugh PR (1975) "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* **12**, 189-198.

[30] Pfeffer RI, Kurosaki TT, Harrah CH, Chance JM, Filos S (1982) Measurement of functional activities in older adults in the community. *J Gerontol* **37**, 323-329.

[31] Cummings JL, Mega M, Gray K, Rosenberg-Thompson S, Carusi DA, Gornbein J (1994) The Neuropsychiatric Inventory: Comprehensive assessment of psychopathology in dementia. *Neurology* **44**, 2308.

[32] Kidmose P, Looney D, Mandic DP (2012) Auditory evoked responses from Ear-EEG recordings. In *2012 Annual International Conference of the IEEE engineering in Medicine and Biology Society* IEEE.

[33] Delorme A, Makeig S (2004) EEGLAB: An open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. *J Neurosci Methods* **134**, 9-21.

[34] Van de Vel A, Smets K, Wouters K, Ceulemans B (2016) Automated non-EEG based seizure detection: Do users have a say? *Epilepsy Behav* **62**, 121-128.

[35] Hoppe C, Feldmann M, Blachut B, Surges R, Elger CE, Helmstaedter C (2015) Novel techniques for automated seizure registration: Patients' wants and needs. *Epilepsy Behav* **52**, 1-7.

[36] Simblett SK, Biondi A, Bruno E, Ballard D, Stoneman A, Lees S, Richardson MP, Wykes T (2020) Patients' experience of wearing multimodal sensor devices intended to detect epileptic seizures: A qualitative analysis. *Epilepsy Behav* **102**, 106717.

[37] Li F, Egawa N, Yoshimoto S, Mizutani H, Kobayashi K, Tachibana N, Takahashi R (2019) Potential clinical applications and future prospect of wireless and mobile electroencephalography on the assessment of cognitive impairment. *Bioelectricity* **1**, 105-112.

[38] Bleichner MG, Debener S (2017) Concealed, unobtrusive ear-centered EEG acquisition: cEEGrids for transparent EEG. *Front Hum Neurosci* **11**, 163.

Manuscript II

Detection of subclinical epileptiform discharges in Alzheimer's disease using long-term outpatient EEG monitoring

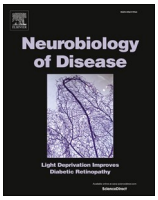
Musaeus CS, Frederiksen KS, Andersen BB, Høgh P, Kidmose P, Fabricius M, Hribljan MC, Hemmsen MC, Rank ML, Waldemar G, Kjær TW. Detection of subclinical epileptiform discharges in Alzheimer's disease using long-term outpatient EEG monitoring. Neurobiol Dis. 2023 Jul;183:106149. doi: 10.1016/j.nbd.2023.106149. Epub 2023 May 15.

Neurobiology of Disease 183 (2023) 106149

Contents lists available at ScienceDirect

Neurobiology of Disease

journal homepage: www.elsevier.com/locate/ynbdi



Detection of subclinical epileptiform discharges in Alzheimer's disease using long-term outpatient EEG monitoring

Christian Sandøe Musaeus [a,*], Kristian Steen Frederiksen [a], Birgitte Bo Andersen [a], Peter Høgh [b,c], Preben Kidmose [d], Martin Fabricius [e], Melita Cacic Hribljan [e], Martin Christian Hemmsen [f], Mike Lind Rank [f], Gunhild Waldemar [a,c], Troels Wesenberg Kjær [a]

[a] Danish Dementia Research Centre, Department of Neurology, Copenhagen University Hospital – Rigshospitalet, Copenhagen, Denmark
[b] Regional Dementia Research Centre, Department of Neurology, Zealand University Hospital, Roskilde, Denmark
[c] Department of Clinical Medicine, University of Copenhagen, Denmark
[d] Department of Electrical and Computer Engineering, Aarhus University, Aarhus, Denmark
[e] Department of Clinical Neurophysiology, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark
[f] T&W Engineering, Lillerød, Denmark

ARTICLE INFO

Keywords:
Subclinical epileptiform discharges
Alzheimer's disease
EEG
Long-term EEG
Ear-EEG
Wearable
Device

ABSTRACT

Background: In patients with Alzheimer's disease (AD) without clinical seizures, up to half have epileptiform discharges on long-term in-patient electroencephalography (EEG) recordings. Long-term in-patient monitoring is obtrusive, and expensive as compared to outpatient monitoring. No studies have so far investigated if long-term outpatient EEG monitoring is able to identify epileptiform discharges in AD. Our aim is to investigate if epileptiform discharges as measured with ear-EEG are more common in patients with AD compared to healthy elderly controls (HC).

Methods: In this longitudinal observational study, 24 patients with mild to moderate AD and 15 age-matched HC were included in the analysis. Patients with AD underwent up to three ear-EEG recordings, each lasting up to two days, within 6 months.

Results: The first recording was defined as the baseline recording. At baseline, epileptiform discharges were detected in 75.0% of patients with AD and in 46.7% of HC (p-value = 0.073). The spike frequency (spikes or sharp waves/24 h) was significantly higher in patients with AD as compared to HC with a risk ratio of 2.90 (CI: 1.77–5.01, $p < 0.001$). Most patients with AD (91.7%) showed epileptiform discharges when combining all ear-EEG recordings.

Conclusions: Long-term ear-EEG monitoring detects epileptiform discharges in most patients with AD with a three-fold increased spike frequency compared to HC, which most likely originates from the temporal lobes. Since most patients showed epileptiform discharges with multiple recordings, elevated spike frequency should be considered a marker of hyperexcitability in AD.

1. Introduction

Multiple studies have demonstrated that patients with Alzheimer's disease (AD) are at increased risk of developing epileptic seizures (Scarmeas et al., 2009; Amatniek et al., 2006; Lozsadi and Larner, 2006; Bernardi et al., 2010; Rao et al., 2009; Risse et al., 1990). In addition, the epileptic seizures may mimic symptoms of AD since they often present as focal impaired awareness seizures (Rao et al., 2009; Pandis and Scarmeas, 2012). A common indicator of seizures are epileptiform discharges identified with electroencephalography (EEG). Recently, studies using long-term EEG recordings for 24 h have found that 22%–54% of patients with AD without clinical seizures have epileptiform discharges (Horvath et al., 2021; Lam et al., 2020; Vossel et al., 2016) with a significant increase in epileptiform discharges. However, a single study using over-night polysomnography did not find a significant difference between AD and HC (Pfeffer et al., 1982). In some of these studies, a correlation between epileptiform discharges and a more rapid rate of progression has been found (Horvath et al., 2021; Vossel et al.,

* Corresponding author at: Rigshospitalet, University of Copenhagen, Inge Lehmanns Vej 8, 2100 Copenhagen, Denmark.
E-mail address: Christian.sandoee.musaeus@regionh.dk (C.S. Musaeus).

https://doi.org/10.1016/j.nbd.2023.106149
Received 17 March 2023; Received in revised form 26 April 2023; Accepted 9 May 2023
Available online 15 May 2023

2016). This may suggest that their suppression could have clinical benefits, which has been indicated in randomized clinical trials suggesting both cognitive and neurophysiological beneficial effects of the anti-seizure medication, levetiracetam in patients with AD (Musaeus et al., 2017; Bakker et al., 2012; Vossel et al., 2021).

Although long-term in-patient EEG monitoring can detect epileptiform discharges in patients with AD, it can be difficult for patients with dementia to be hospitalized, moreover, it is expensive and not possible for extended periods. Due to the clinical relevance of detecting epileptiform discharges, there is a need for accessible and dependable continuous EEG monitoring in an outpatient setting. One method is ear-EEG (Kappel et al., 2019), which in a recent study was found to be a feasible approach for long-term EEG monitoring in patients with AD (Musaeus et al., 2022). Ear-EEG is a method where EEG signals are recorded from electrodes placed within a customized earpiece inserted into each ear and is associated with little interference of everyday life (Kappel et al., 2019; Looney et al., 2012; Looney et al., 2011). Previous studies have shown a good correspondence between ear-EEG and scalp EEG (Mikkelsen et al., 2015; Mikkelsen et al., 2019) and ear-EEG has been used for detection of epileptiform discharges in focal temporal lobe seizures (Zibrandtsen et al., 2017), which makes it a prime candidate method.

In the current study, we investigated if epileptiform discharges as measured with ear-EEG occurs with a higher frequency in patients with AD compared to age- and gender-matched healthy controls. To understand the relationship between epileptiform discharges and AD, we examined the variability across time and the relationship with cognitive function.

2. Materials and methods

2.1. Participants

Patients with mild to moderate AD were recruited from the Memory Clinic at Copenhagen University Hospital – Rigshospitalet, Copenhagen and the Memory Clinic at Zealand University Hospital, Roskilde. Ten of the patients have also participated in the feasibility study (Musaeus et al., 2022). All patients met the NIA-AA criteria for probable AD with amnestic presentation (McKhann et al., 2011). The diagnosis was determined based on a consensus conference after clinical evaluation, which included structural imaging and, in most instances, 18F-flourdeoxyglucose positron emission tomography. Some patients underwent lumbar puncture ($n = 18$) with evaluation of amyloid-β42, phosphorylated tau, and total tau while some underwent a (11)C-labelled Pittsburgh Compound-B-PET scan ($n = 2$) or both ($n = 3$). The threshold for phosphorylated tau and total tau were 80 pg/ml and 400 pg/ml respectively. For Amyloid beta 1–42 (Aβ42), an upwards drift in the CSF levels has been observed (Simonsen et al., 2021) and a year-specific threshold was applied. Inclusion criteria were 1) a mini-mental state examination (MMSE) (Folstein et al., 1975) score between 16 and 28, 2) age between 50 and 90 years, 3) native Danish speaker, 4) at least 7 years of education, 5) hearing and vision sufficient for neuropsychological examination, 6) no alcohol or drug abuse within the last two years, 7) no contraindications for MRI, 8) an MRI or CT scan that supported the diagnosis of AD, 9) the general health conditions of the patient allowed participation in the study (as judged by the principal investigator), and 10) living with a caregiver who was able to assist the patient with the home recordings.

The following exclusion criteria were applied: 1) psychiatric (except mild depression) or neurological conditions that affects the brain except AD, 2) epilepsy prior to the diagnosis of AD (no patients were diagnosed with epilepsy before entering the study), 3) focal pathology (except AD related atrophy) in the hippocampus, i.e. hippocampal sclerosis, 4) living with a relative with serious illness or impaired activities of daily living, 5) living in a nursing home, 6) currently treated with anti-epileptic medication, tricyclic antidepressants or antipsychotics, 7) daily or almost daily administration of medication with known anti-cholinergic or adrenergic effect, which may affect cognitive abilities or EEG, 8) large cerebral infarctions or more than four lacunar infarctions on MRI, 9) suffering from facial tics/facial hyperkinetic disorders or 10) daily use of hearing aids.

The healthy controls (HC) had all been participants in other studies and had expressed interest in participating in new studies. The inclusion criteria were 1) normal cognition as judged by the principal investigator. This judgement was based on the totality of the data available to the principal investigator, which included medical history, clinical examination, and cognitive testing, 2) the general health allowed participation in the study, 3) did not suffer from any psychiatric (except mild depressive symptoms or mild depression) or neurological conditions that affect the brain as well as criteria 2–7 as applied in patients with AD. The following exclusion criteria were applied: 1) diagnosed with epilepsy, 2) focal pathology in the brain (except mild hippocampal atrophy as a smaller hippocampal volume is seen with increasing age (Nobis et al., 2019) and was therefore not considered a marker of disease if it was judged to be appropriate for the age of the HC) as well as exclusion criteria 6–10 as applied in patients with AD.

The study was approved by the Capital Region Ethics Committee (H-17035751), by the Danish Medicines Agency (2017112288), and by the Data Protection Agency (P-2021-866). All participants gave their written informed consent before participating in the study. The study is registered at clinicaltrials.gov (NCT04436341).

2.2. Study design

In this longitudinal study, a total of eight visits was planned over a 6-month period for patients with AD and a total of four visits for the HC (Fig. 1).

In patients with AD, the three two-day ear-EEG recordings were planned three months apart, i.e., at the beginning of the study (baseline), at three months and at 6 months follow-up. At visit 1 (baseline), informed consent was obtained followed by assessment of medical history, a physical and neurological examination, the MMSE and an imprint of the ears using Otoform A Soft X (Dreve, Germany), a soft ear impression silicone. Subsequently, the patient underwent the following: visit 2) MRI scan to evaluate exclusion criteria, visit 3) standard EEG recording together with ear-EEG, Functional Assessment Questionnaire IADL (FAQ IADL) (Pfeffer et al., 1982) (to assess everyday function), neuropsychiatric inventory (NPI) (Cummings et al., 1994) (to assess behavioral and psychological symptoms), and the Category Cued Memory Test (CCMT) (Vogel et al., 2018) to assess memory function. Further, the patient and the caregiver were briefly instructed about seizures and introduced to a seizure diary to take home, and information and training on how to use the ear-EEG at home was conducted. This included a log-sheet to take notes on when the patient wore the ear-EEG. After approximately 48 h (visit 4) the patient and/or the caregiver returned with the equipment and the patient filled a questionnaire. The external ear was examined for any minor lesions when the patient was present to return the equipment (visit 4, 6, and 8) and participants were queried regarding any possible adverse events. The visit 5 and 6, and visit 7 and 8 were similar to visit 3 and 4 except that MMSE was performed at visit 5, and 7. Based on the experiences gathered in the feasibility study (Musaeus et al., 2022), breaks were permitted throughout the recording periods where the participants were allowed to take out the ear-EEG. The focus was on the participants performed ear-EEG recordings at night, therefore, the participants were encouraged to wear the ear-EEG device particularly during nighttime. A short questionnaire about the use of the ear-EEG was administered to AD patients at visit 4, 6, and 8. See supplementary material.

2.2.1. Ear-EEG recording

The ear-EEG earpieces were custom-made in silicone and mounted with six dry-electrodes (Kappel et al., 2019) (T&W engineering,

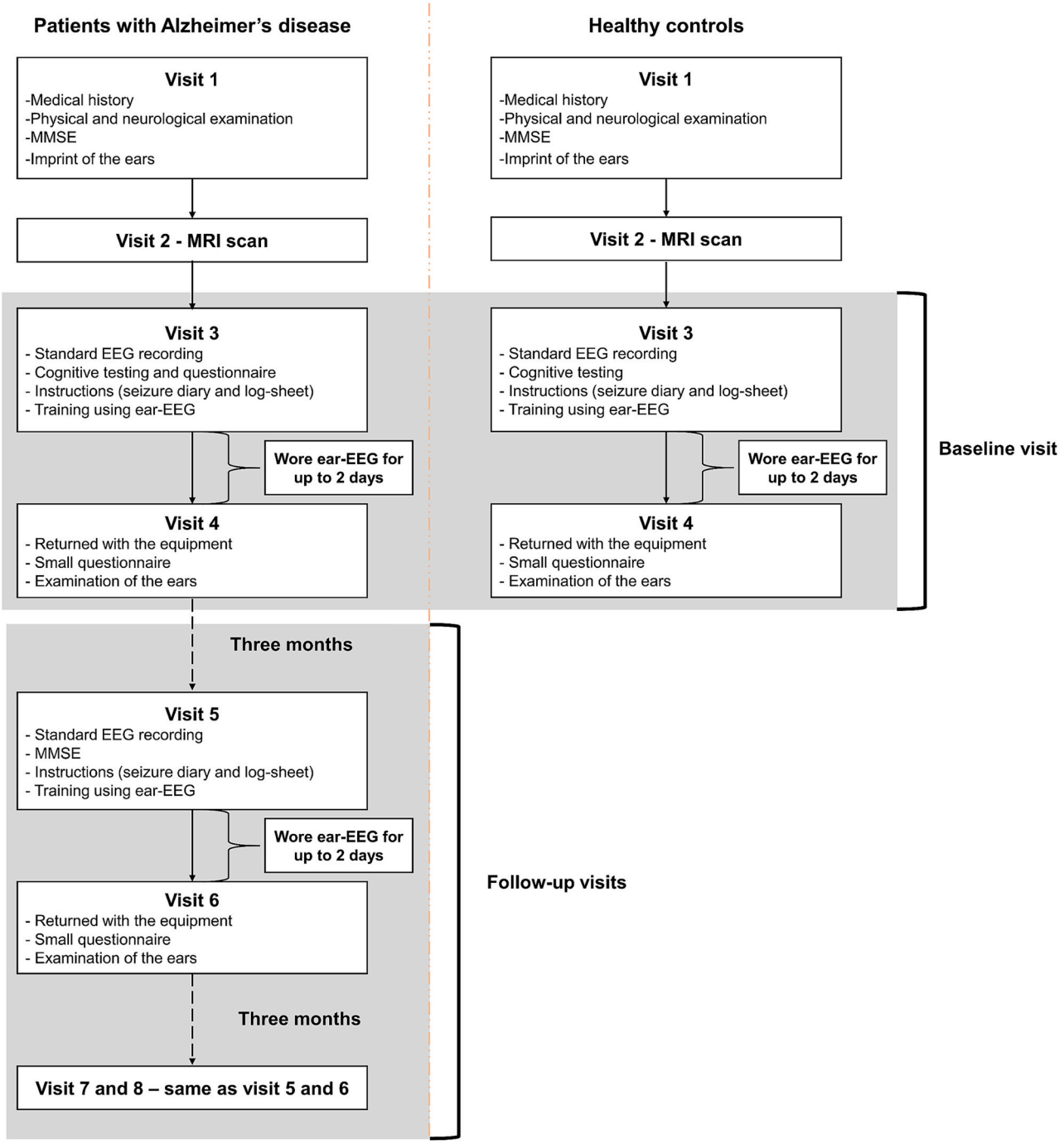


Fig. 1. Study design. Firstly, the patients with AD and healthy controls were included in the study after written informed consent. Both patients with AD and HC underwent the first session of ear-EEG recording (baseline) with the patients with AD also undergoing up to two follow-up visits.

Denmark) and placed inside the ear canal and concha. The ear-piece was placed inside the ear canal and in the concha (Mikkelsen et al., 2015) in both ears with the labeling of the ear-EEG electrodes being previously described (Kidmose et al., 2012). The ground electrode was placed approximately 2 cm under the midline of the clavicle on either one side. The ear-EEG recordings were performed using the TMSi Mobita EEG amplifier (TMSi systems), which was worn in either a small bag around the neck or in a belt bag and connected to the electrodes. Impedance was not checked during the recording. The sampling rate was 1000 Hz.

Before visual inspection, the ear-EEG data was pre-processed. This was performed to ease the visual inspection and to compare the number of artifacts in the recordings conducted in patients with AD as compared to the HC. The EEG data were loaded into MATLAB (v2020b) using a custom script for loading poly5 files. Afterwards, the EEGs were band-pass filtered from 0.5 to 70 Hz and subsequently notch filtered from 49 to 51 Hz and 99–101 Hz using the pop_eegfiltnew function from EEGLAB (Cretin et al., 2016). Next, the following artefact rejection pipeline, which has previously been used in other studies, (McKhann et al., 2011) was used. Firstly, if a channel were outside the dynamical range of the amplifier, which is a typical indication of an electrodes with poor contact to the body, the reading was replaced with a 'NaN'-value, and hence automatically discarded upon loading. The discarded data was considered in the subsequent analysis of data quality. Afterwards, large amplitude samples isolated to a single channel were labelled as artifacts.

Lastly, movement or muscle activation, which may also result in large amplitude deviations across multiple channels were labelled as artifacts.

The amount of time when data from at least one electrode in each ear were present was calculated (afterwards called pre-processed data). The amount of data for each individual electrode and the amount of pre-processed data during the night (10 pm - 8 am) and day (8 am - 10 pm) was examined.

2.2.2. Standard EEG recordings

We performed one 30-min 19-channel EEG recording at visit 4, 6, and 8. This consisted of 10 min of alternating eyes open, and eyes closed (30 s each) followed by 20 min of sleep. If the participants got drowsy, the length of the eyes open/closed segments were adjusted. The EEGs were recorded using Nicolet One EEG (Nervus) recording software 5.82 (Natus) with a standard 44-channel headbox. Each subject was fitted with a cap using silver-silver-chloride-coated electrodes. The EEGs was recorded from 19 electrodes positioned according to the International 10–20 system. For impedance, the aim was to reach below 10 kΩ for all electrodes during the recordings.

The EEGs were assessed by experienced clinical neurophysiologists (TWK and MCH). The results from the EEGs were either classified as 1) Normal EEG, 2) Slowing with subclassification into focal slowing as defined by intermittent or persistent low frequency theta or delta and/or diffuse slowing as defined by a posterior dominant rhythm below 7 Hz and 3) epileptiform discharges.

A comparison of the signal between ear-EEG and scalp-EEG can be found in supplementary material.

2.2.3. Ear-EEG review and annotation

Ear-EEG recordings were visually reviewed and annotated by CSM using the EEGLAB toolbox (v13.6.5b) implemented in MATLAB (MathWorks, 2020b). The average referenced montage was used and 10 s epochs were inspected at a time with ±30 μV amplitude range. In this montage, the first 12 channels were low-pass filtered at 70 Hz, high-pass filtered at 1 Hz, and notch filtered at 50 Hz. The next 12 channels were after pre-processing. The subsequent 36 channels were the comparisons between electrodes with the active electrodes being on the left (e.g., ELA-ERA, ELA-ERB (Kidmose et al., 2012)) and the last 36 channels were created using the opposite laterality. The segments disregarded by the pre-processing pipeline and segments only containing recordings from one ear were not inspected.

A sharp asymmetric negative potential of 20–200 ms duration was considered an epileptiform discharge (spike (20–70 ms)/sharp wave (70–200 ms)) if it met the IFCN criteria (Kural et al., 2020), which compromised of 1) di- or tri-phasic with a pointed peak with 2) a different wave duration than the background activity, 3) asymmetry of the waveform, 4) background activity was disrupted by the presence of the epileptiform discharge and in most cases was 5) followed by a slow after-wave. Due to the nature of ear-EEG, we could not investigate the spatial distribution of the spikes/sharp waves (IFCN criteria 6). Practically, this led to all epileptiform discharges fulfilling at least four out of the six IFCN criteria. Epileptiform discharges were not considered if it was only present in a single electrode. All annotations performed by CSM underwent review by a board-certified clinical neurophysiologist (TWK), who made the final ruling. When we use the term epileptiform discharges it covers both spikes and sharp waves and assumes an underlying irritative process like what is seen in epilepsy, even if this cannot be stated with absolute certainty. Both CSM and TWK were blinded to the diagnosis when reviewing the EEGs.

2.3. Statistics

All statistics were performed in RStudio (v1.2.1335). When comparing age, education, cognitive scores, and the length of ear-EEG recordings between AD (at baseline) and HC, we performed two-sample *t*-tests. The distribution of the data as well as the variance was checked. Chi-squared test or Fisher's exact test (if one input variable being ≤5) was performed for comparing sex, co-morbidities, the number of participants in each group with spike/sharp waves at baseline, the number of participants with slowing and the number of spikes/night and spikes/day between AD and HC. A comparison of participant characteristics of the HC with and without epileptiform discharges was performed using two-sample Wilcoxon test for continuous variables and Fisher's exact test for categorical data (see Supplementary material).

When comparing the number of spikes or sharp waves/24 h (spike frequency) between HC, and AD at baseline, we calculated the rate ratio using the function *rateratio* from the *epitools* toolbox.

A delineation plot showing an analysis of the classification accuracy between AD and HC based on the spike frequency using three different thresholds (1.5, 4.5, and 8) was conducted. See supplementary material.

Spearman's correlation between the spike frequency across all recordings and cognitive tests was performed.

Changes in spikes or sharp waves between visits were analyzed using a generalized linear mixed-effect model including visit number (categorical variable) and log-transformed the amount of pre-processed EEG (in minutes) as the offset variable with a person specific random intercept in a Poisson regression model using the *lme4* package. Changes in MMSE over visits were analyzed using a linear mixed model including visit number (categorical) as fixed effect and further assuming an unstructured covariance pattern to account for repeated measurements on the same subjects with the *LMMStar* package.

The R code and output from the subsequent analyses can be found in the supplementary material.

3. Results

3.1. Demographics

A total of 25 patients with AD and 15 HC were eligible for inclusion and gave informed consent. One patient with AD was excluded due to having less than one hour of data after pre-processing. For the follow-up visits, 15 patients with AD participated in two ear-EEG recordings and 11 in all three visits. The patients were followed in the project for an average of 276 days (SD: 118, range: 56–468 days). See Table 1 for demographics, cognitive scores, co-morbidities, and AD markers at time of diagnosis.

3.2. Prevalence of epileptiform discharges with ear-EEG at baseline

See Fig. 2A for examples of epileptiform discharges. Epileptiform discharges were detected in 75.0% (18/24) of patients with AD and in 46.7% (7/15) of HC at baseline (p-value = 0.073) (Fig. 2B). At baseline, the spike frequency was significantly higher in patients with AD and epileptiform discharges (range: 0–13.04 (mean: 3.03 spikes/24 h) as compared to HC (range: 0–6.66 (mean: 1.04 spikes/24 h) with a risk ratio of 2.9 (CI: 1.77–5.01, p-value ≤0.001). See Fig. 2C for an overview of the distribution of spikes. A delineation plot of the spike frequency can be found in the supplementary material (Supplementary Fig. 4).

See Fig. 2D for distribution of epileptiform discharges over 24 h. A significant different in epileptiform discharges at night (p-value = 0.014) in AD (64.8%) as compared to HC (31.3%). Two epileptiform discharges that occurred in a patient with AD and a HC were not noted on the log-sheet and therefore not included in this analysis. Furthermore, one patient with AD and one HC did not sleep with the equipment.

3.3. Variability in epileptiform discharges across recordings

No significant difference in spike frequency between the baseline and the follow-up visits (visit 1–2: estimate: 1.34, p-value = 0.239, visit 1–3: estimate: 1.24, p-value = 0.417) were observed. See Table 2 for the patients that completed at least two ear-EEG recordings. A large variance between the number of spikes across recordings for each subject

Table 1
Baseline demographics, medication, neuropsychological test scores, and questionnaire scores.

	Healthy controls	Alzheimer's diseae	p-value
Number of participants	15	24	
Age, mean (SD)	69.5 (7.93)	70.3 (7.79)	0.8
Males/females	8/7	14/10	0.8
Education, mean (SD)	15.2 (2.04)	14.2 (3.08)	0.3
MMSE, mean (SD)	29.3 (0.88)	23.5 (3.32)	<0.001
CCMT, immediate recall	38.1 (5)	18.4 (5.66)	<0.001
CCMT, delayed recall	37.2 (5.67)	13.2 (5.19)	<0.001
CCMT, recognition	47.5 (0.64)	38.1 (4.66)	<0.001
NPI		4.25 (2.72)	
ADL		14.5 (5.84)	
Hypercholesterolemia, n (%)	3 (20%)	7 (29%)	0.7
Hypertension, n (%)	5 (33%)	12 (50%)	0.3
Arrhythmia, n (%)	3 (20%)	2 (8%)	0.4
Mild depression, n (%)	1 (7%)	4 (17%)	0.6
Cholinesterase inhibitor, n (%)		23 (96%)	
Memantine, n (%)		5 (21%)	
SSRI/SNRI, n (%)	1 (7%)	4 (17%)	
Years from diagnosis, mean (SD)		1.96 (1.74)	
Amyloid positive, n (%)*		18 (90%)	
Phosphorylated tau, mean (SD) #		82.41 (31.19)	
Total tau, mean (SD)#		568.88 (242.34)	

SSRI/SNRI: Selective Serotonin Reuptake Inhibitor/ Serotonin and Noradrenaline Reuptake Inhibitor, MMSE: Mini-Mental State Examination, CCMT: Category Cued Memory Test, ADL: Activities of Daily Living, NPI: Neuropsychiatric Inventory, SD: Standard deviation, * Amyloid positive was defined by either a value below the year-corrected cut-off using CSF amyloid or positive amyloid PIB-PET at the time of diagnosis in patients who had undergone testing in either one of these modalities ($n = 20$), # Both phosphorylated tau (cut-off <60) and total tau (cut-off <400) was measured as part of the initial diagnosis ($n = 18$).

was seen (Supplementary Fig. 1). Combining all the recordings, we found that epileptiform discharges were found in 83.3% after the second and in 91.7% after the third recording. The two participants that did not show epileptiform discharges only had a single ear-EEG recording performed. The spike frequency with all recordings combined in patients with AD, we found a spike frequency of 2.94 spikes/24 h. Only two showed epileptiform discharges at all ear-EEG recordings. No significant differences were found for MMSE (Supplementary Fig. 2) across visits (visit1–2: estimate: −0.6, p-value = 0.082, visit1–3: estimate: −1.08, p-value = 0.209).

Table 2
Description of epileptiform discharges in the patients with more than one ear-EEG recording.

	1st ear-EEG recording	2nd ear-EEG recording	p-value (1st vs 2nd)	3nd ear-EEG recording	p-value (1st vs 3rd)
Number of patients, n	15	15		11	
Number with ED, %	73.33%	46.67%		63.64%	
Spikes/24 h	1.96	2.89	0.239	2.78	0.417
Spikes, n	31	36		30	
Clean data, mean hours (SD)	25.4 (6.3)	19.9 (10.6)		23.5 (7.32)	
MMSE, mean (SD)	23.73 (3.37)	23.13 (3.62)	0.082	23.55 (3.72)	0.209
Time since last recording in days, mean (SD)		171 (86.5)		144 (92.2)	

ED: Epileptiform discharges, MMSE: Mini-Mental State Examination.

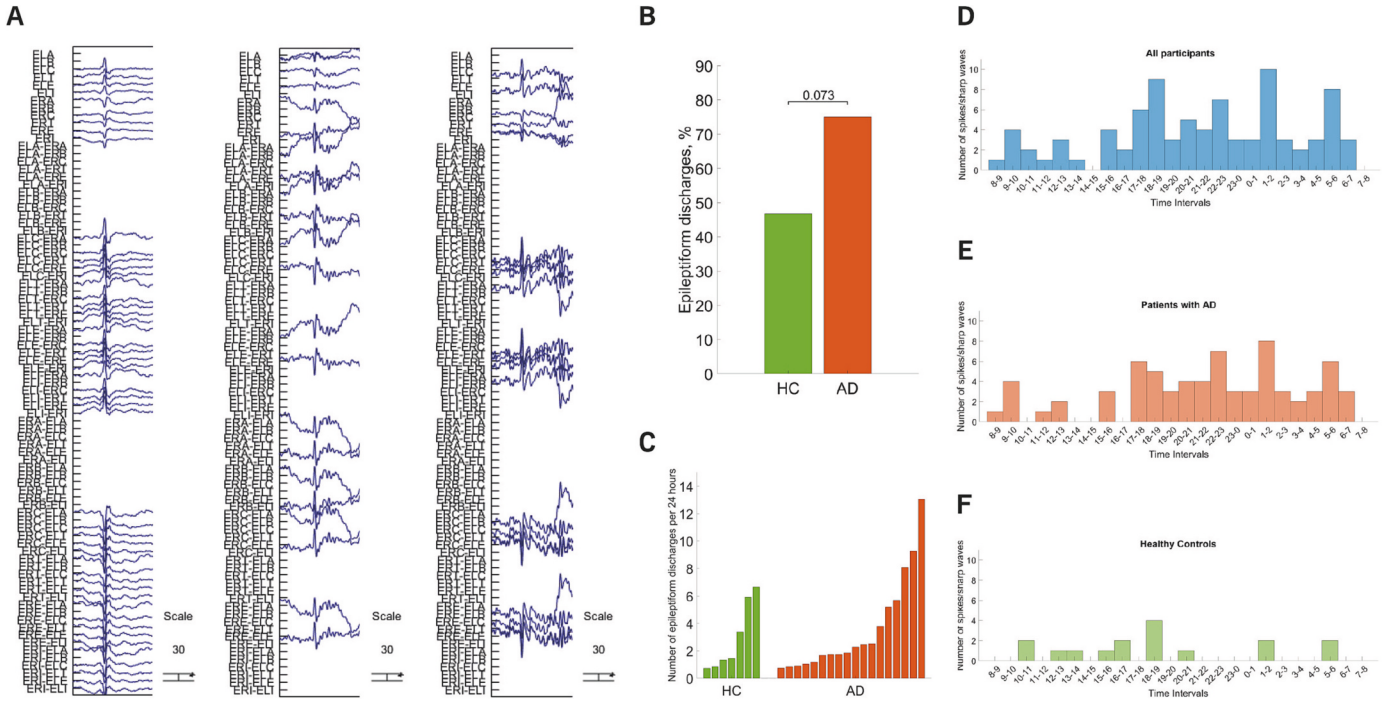


Fig. 2. Characterization of spikes/sharp waves in patients with AD compared to HC at baseline (first ear-EEG recording). (A) Shows examples of epileptiform discharges. (B) The proportion of patients with AD and HC with epileptiform discharges. (C) Spike frequency (spikes/sharp waves per 24 h) in each patient with AD or HC with epileptiform discharges at baseline. (D) Distribution of the total number epileptiform discharges by time intervals for all participants for the baseline recording with (E) for patients with AD and (F) for HC.

3.4. Association between memory function and epileptiform discharges

No significant correlations were found between the spike frequency in patients with AD and the baseline MMSE, immediate recall or delayed recall (Supplementary Fig. 3). See supplementary material for comparison of patients with and without epileptiform discharges.

3.5. Conventional EEG and patient diaries

In AD, 29.2% (7/24) had slowing in the EEG as compared to 6.7% (1/15) of the HC (p-value = 0.121). Location of slowing was mostly temporal. More details can be seen in the supplementary material. No electrographic seizures were found. One patient reported (1/65 recordings) a sense of déjà vu in the patient diaries.

3.6. Data quality

At baseline, the mean number of recorded hours were 34.6 h (SD: 10.1 h) in patients with AD as compared to 40.9 h (SD: 9.5) in HC. The amount of pre-processed data was lower in AD with 23.7 h (SD: 7.6 h, range: 3.1–33.7 h) as compared to HC with 27.7 h (SD: 8.0 h, range: 12.1–37.2 h) but not significant (p-value = 0.1, t-value = −2, df = 37) (Fig. 3C). During the nighttime, 80.1% of the data in patients with AD and 78.7% of data in HC remained after preprocessing as compared to the daytime with 61.1% of data in patients with AD and 63.9% of the data in HC. See Fig. 3 for data quality for each electrode.

3.7. Adverse events at baseline

A larger proportion of patients with AD (79.2%) had adverse events

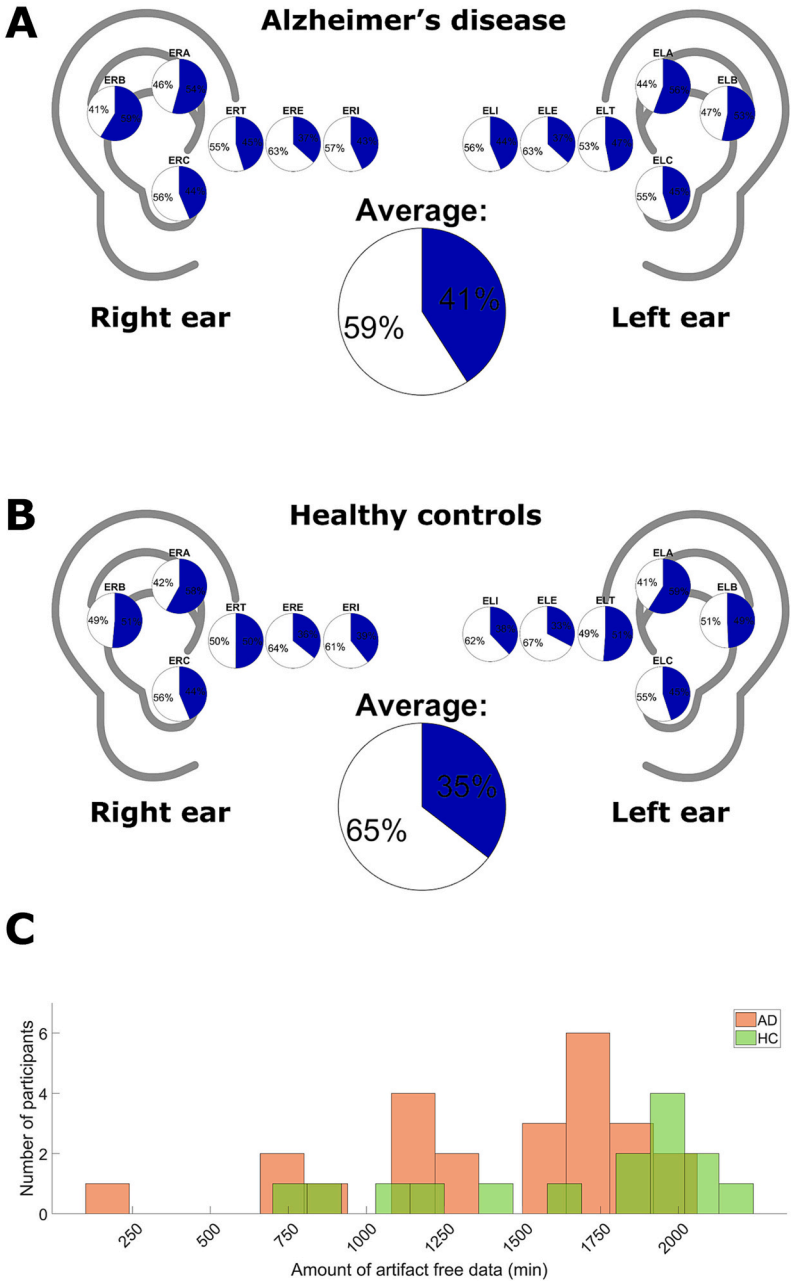


Fig. 3. Data quality for the specific electrodes and length of EEG recordings at baseline. (A) shows the percentage of data kept (white) and removed (blue) for each specific electrode and as an average across electrodes after pre-processing in patients with AD. (B) displays the same for HC. (C) histogram of the distribution of EEG length for AD and HC. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

related to the ear-EEG as compared to HC (60%). In addition, 59.5% of the participants who slept with ear-EEG experienced some difficulty sleeping during the baseline recording. One of the participants with abrasions developed pain and swelling as has previously been reported (Musaeus et al., 2022). None of the AE related to ear-EEG were classified as serious AEs.

The reasons for dropping out of the study were mostly discomfort associated with wearing the ear-EEG ($n = 8$). A full description can be found in the supplementary material.

4. Discussion

This is the first study to provide data on the prevalence of epileptiform discharges using outpatient long-term EEG monitoring in patients with AD. At baseline, 75% of patients with AD had epileptiform discharges with a three-fold higher spike frequency as compared to HC. Most epileptiform discharges occurred at night in AD. The spike frequency showed considerable variability across recordings with a total of 91.7% of patients showing epileptiform discharges when examining all recordings. A negative correlation between cognitive test scores and spike frequency was found but was not significant. The data quality of the ear-EEG was acceptable for application as a method for detection of epileptiform discharges and participants only experienced mild adverse events.

In the current study, there was a trend towards a higher fraction of patients with AD with epileptiform discharges at baseline (75%) compared to HC (46.7%). This is in line with previous studies showing that a larger percentage of patients with AD without epilepsy (22–54%) had epileptiform discharges on 24-h EEG recordings compared to HC (4.7%–25%) (Horvath et al., 2021; Lam et al., 2020; Vossel et al., 2016). The more epileptiform discharges could be explained by longer recordings including up to two nights, which is supported by a study showing a significant correlation between the sensitivity of EEG and the length of recordings (Horváth et al., 2017). No epileptiform discharges were found using conventional EEG recordings, which is in line with a study showing epileptiform discharges being present in only 2% of patients with AD (Liedorp et al., 2010). The length of the recording combined with the majority of epileptiform discharges in AD occurring during sleep (Horvath et al., 2021; Lam et al., 2020; Vossel et al., 2016), likely explain the disparity between ear-EEG and conventional EEG. In addition, we found that more patients showed epileptiform activity if the recording was repeated, which suggests that more recordings are needed to detect epileptiform discharges in AD patients. Taken together, these highlights both the importance and the timing of recordings to capture epileptiform discharges in AD. The fact that almost half the HC had epileptiform discharges using ear-EEG is surprising but may in large part be due to the long recordings and to a lesser extent possible insidious AD pathology without clinical correlate or other comorbidities (e.g., vascular lesions) but this was not investigated. Furthermore, no significant differences were found for the participant characteristics for HC with and without epileptiform discharges, but this may be due to the low sample size. Moreover, the ratio of patients with detected epileptiform discharges varied among the baseline, second, and third recordings (73.33%, 46.67%, and 63.64%, respectively). Therefore, it is possible that the difference in number of epileptiform discharges between the two groups with almost half the HC having epileptiform discharges was affected by this variability. Repeated long-term EEG monitoring in HC is needed to fully understand this aspect. Furthermore, a comparison between ear-EEG and long-term scalp EEG monitoring is needed.

The frequency of epileptiform discharges has previously varied considerably between studies from a median frequency of 3 per 24 h (Lam et al., 2020) to 2.02 spikes/h (Horvath et al., 2021). Although to some degree speculative, the studies showing higher spike frequency included patients with lower MMSE score (mean: 20–22) as compared to the study showing the lowest spike frequency (mean: 26) (Horvath et al., 2021; Lam et al., 2020; Vossel et al., 2016) indicating an association with severity of disease and spike frequency. In support of this, a study showed that seizures became more prevalent as the disease progressed (Amatniek et al., 2006). Since our AD cohort had an average MMSE of 23.5 and due to the low spatial resolution of the ear-EEG and being strongly affected by muscle activity, the lower spike frequency could in part be due to these methodological shortcomings. Conversely, the vast majority of epileptiform discharges appear in the frontotemporal regions in AD (Horvath et al., 2021; Lam et al., 2020; Vossel et al., 2016; Sarkis et al., 2016; Cretin et al., 2016), which is the area best covered by ear-EEG. Combined with studies showing atrophy of the temporal lobes in the early stages of the disease (Ossenkoppele et al., 2015; Harper et al., 2017), this suggest that the epileptiform discharges are associated with neuropathological mechanisms in AD. However, studies are needed to understand if epileptiform discharges appear in other regions as the disease progresses. Another possibility is that hyperexcitability in the temporal lobe is restricted to only a limited number of the patients as suggested by the classification accuracy between AD, and HC based on the spike frequency (Supplementary Fig. 4). Furthermore, it is necessary to investigate if anti-seizure medication should be administered if epileptiform discharges are present as previously suggested (Vossel et al., 2021) or that a specific threshold for the spike frequency warrants treatment with anti-seizure medication.

So far, no studies have investigated changes in the spike frequency across repeated recordings in patients with AD. No significant difference in spike frequency between the baseline and the follow-up visits was found, which may be due to considerable variability between visits. One explanation is that there may be longer periods of time without epileptiform discharges as seen with ultra long-term EEG recordings detecting EEG seizures in patients with epilepsy (Weisdorf et al., 2019). This is supported by epileptiform discharges being present in 91.67% of patients with AD if all recordings were combined. The spike frequency may be a more suitable measure when investigating epileptiform discharges in AD. Although there is no clear consensus on the link between interictal spikes and seizures (Karoly et al., 2016), the spike frequency has been associated with seizure frequency in patients with temporal lobe epilepsy (Janszky et al., 2005), and patient with temporal lobe epilepsy show cognitive impairment (Hermann et al., 1997). In transgenic mice, a study has suggested that the hyperexcitability leads to development of compensatory inhibitory mechanisms that reduce function of specific circuits (Palop et al., 2007). In addition, a clinical trial investigating the effect of levetiracetam in patients with AD only found improvement in patients who had epileptiform discharges (Vossel et al., 2021). This supports the idea that higher spike frequency may warrant treatment with anti-seizure medication. Due to the short intervals between visits, we did not examine the association with cognitive changes, which has previously been shown in both mice models (Kam et al., 2016; Bezzina et al., 2015) and patients with AD (Horvath et al., 2021; Vossel et al., 2016; Høgh et al., 2002).

An interesting finding was that most spikes occurred at night in patients with AD (64.79%) (Fig. 2E). This has previously been reported in long-term EEG studies, (Horvath et al., 2021; Lam et al., 2020; Vossel et al., 2016) but the underlying pathological reason is unknown. However, interictal epileptiform discharges more often occur during sleep in patients with temporal lobe epilepsy (Sammaritano et al., 1991) and hippocampal interictal spikes were negatively correlated with long-term memory consolidation (Lambert et al., 2020). In support of this, a studies in transgenic mice model of beta-amyloid neuropathology found that interictal spikes mostly occurred during REM-sleep (Kam et al., 2016; Szabo et al., 2023) Combined, this indicates that epileptiform discharges during sleep may be associated with memory dysfunction in patients with AD.

Our study has several limitations. First, we did not record simultaneous scalp EEG, which would be preferable when validating the epileptiform discharges. However, ear-EEG has been used for detection of epileptiform discharges in focal temporal lobe seizures (Zibrandtsen et al., 2017). The number of participants was lower than similar studies,

but we were able to conduct longer outpatient EEG recordings using ear-EEG. The HC had more pre-processed data than AD, but this was adjusted for in the analyses. However, we only recorded one session of ear-EEG for the HC and repeated ear-EEG measurement may have provided more information on the variability of epileptiform discharges. The number of patients that dropped-out was similar to the feasibility study, which calls for adjustments to the ear-EEG to increase the comfort. In terms of recording, the TMSi amplifier does not note time-of-day during recordings, which means that the participant/caregiver must do so, but this was rarely a problem (two epileptiform discharges). Another aspect is the lack of characterization of sleep stages, which may have improved the interpretation of the results but so far automatic sleep staging tools for ear-EEG have only been tested in healthy young participants (Mikkelsen et al., 2019). Furthermore, due to the low spatial resolution of the ear-EEG and muscle artifacts, it gives rise to some uncertainty during the annotation of epileptiform discharges, which called for a more restrictive approach. Lastly, we did not perform any measurement of AD biomarkers in the HC, which may have explained the large number of HC with epileptiform discharges.

5. Conclusion

Using long-term outpatient EEG monitoring, most patient with AD showed epileptiform discharges occurring at night, which most likely originated in the temporal lobes. We hypothesize that it is related to disrupted memory consolidation as supported by lower cognitive test performance scores sensitive to temporal lobe function. Larger studies are needed to confirm and further elucidate this possible relationship. Repeated and long-term EEG recordings are necessary to capture all epileptiform activity based on the high degree of variability in spike frequency between recordings. Since most patients showed epileptiform discharges with multiple recordings and almost half the HCs had epileptiform discharges, it is more relevant to consider spike frequency as a marker of hyperexcitability than simply whether spikes are present. Therefore, our findings support using long-term outpatient monitoring of spike frequency when investigating anti-seizure medication in the treatment approach to AD.

Funding

The study was funded by the Alzheimer Research Foundation (grant numer: 181003), Toyota Foundation (KJ/BG-9171 F), Axel Juul Muusfeldts Foundation (2016–527), Ellen Mørchs foundation (J.nr. 32491419), Rigshospitalet Research Foundation and T&W Engineering. None of the funding parties had a role in the collection, analysis, and interpretation of data or in the writing of the manuscript or whether to publish the results of the study.

CRediT authorship contribution statement

Christian Sandøe Musaeus: Conceptualization, Methodology, Software, Formal analysis, Investigation, Data curation, Writing – original draft, Visualization, Project administration, Funding acquisition. **Kristian Steen Frederiksen:** Conceptualization, Methodology, Investigation, Writing – review & editing, Supervision, Project administration. **Birgitte Bo Andersen:** Conceptualization, Investigation, Resources, Writing – review & editing. **Peter Høgh:** Conceptualization, Investigation, Resources, Writing – review & editing. **Preben Kidmose:** Conceptualization, Methodology, Software, Writing – review & editing, Visualization. **Martin Fabricius:** Conceptualization, Resources, Writing – review & editing, Formal analysis. **Melita Cacic Hribljan:** Conceptualization, Formal analysis, Resources, Writing – review & editing. **Martin Christian Hemmsen:** Conceptualization, Methodology, Resources, Writing – review & editing. **Mike Lind Rank:** Conceptualization, Methodology, Resources, Writing – review & editing. **Gunhild Waldemar:** Conceptualization, Methodology, Resources, Investigation, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Troels Wesenberg Kjær:** Conceptualization, Methodology, Formal analysis, Resources, Writing – review & editing, Visualization, Supervision, Project administration.

Declaration of Competing Interest

Christian Sandøe Musaeus received an unrestricted grant from T&W engineering. Two authors (Mike Lind Rank, and Martin Christian Hemmsen) who are employees of T&W Engineering contributed to the interpretation and writing of the manuscript. Troels Wesenberg Kjær consults for T&W Engineering.

Data availability

Data will be made available on request.

Acknowledgements

We would like to thank Susanne Kristiansen and Oda Jakobsen for their assistance in recruiting participants and helping during study visits.

References

Amatniek, J.C., Hauser, W.A., DelCastillo-Castaneda, C., Jacobs, D.M., Marder, K., Bell, K., Albert, M., Brandt, J., Stern, Y., 2006. Incidence and predictors of seizures in patients with Alzheimer's disease. Epilepsia 47, 867–872.

Bakker, A., Krauss, G.L., Albert, M.S., Speck, C.L., Jones, L.R., Stark, C.E., Yassa, M.A., Bassett, S.S., Shelton, A.L., Gallagher, M., 2012. Reduction of hippocampal hyperactivity improves cognition in amnestic mild cognitive impairment. Neuron 74, 467–474.

Bernardi, S., Scaldaferri, N., Vanacore, N., Trebbastoni, A., Francia, A., D'Amico, A., Prencipe, M., 2010. Seizures in Alzheimer's disease: a retrospective study of a cohort of outpatients. Epileptic Disord. 12, 16–21.

Bezzina, C., Verret, L., Juan, C., Remaud, J., Halley, H., Rampon, C., Dahan, L., 2015. Early onset of hypersynchronous network activity and expression of a marker of chronic seizures in the Tg2576 mouse model of Alzheimer's disease. PLoS One 10, e0119910.

Cretin, B., Sellal, F., Philippi, N., Bousiges, O., Di Bitonto, L., Martin-Hunyadi, C., Blanc, F., 2016. 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. 52, 1125–1133.

Cummings, J.L., Mega, M., Gray, K., Rosenberg-Thompson, S., Carusi, D.A., Gornbein, J., 1994. The neuropsychiatric inventory: comprehensive assessment of psychopathology in dementia. Neurology 44, 2308.

Folstein, M.F., Folstein, S.E., McHugh, P.R., 1975. "Mini-mental state." A practical method for grading the cognitive state of patients for the clinician. J. Psychiatr. Res. 12, 189–198.

Harper, L., Bouwman, F., Burton, E.J., Barkhof, F., Scheltens, P., O'Brien, J.T., Fox, N.C., Ridgway, G.R., Schott, J.M., 2017. Patterns of atrophy in pathologically confirmed dementias: a voxelwise analysis. J. Neurol. Neurosurg. Psychiatry 88, 908–916.

Hermann, B.P., Seidenberg, M., Schoenfeld, J., Davies, K., 1997. Neuropsychological characteristics of the syndrome of mesial temporal lobe epilepsy. Arch. Neurol. 54, 369–376.

Høgh, P., Smith, S.J., Scahill, R.I., Chan, D., Harvey, R.J., Fox, N.C., Rossor, M.N., 2002. Epilepsy presenting as AD: neuroimaging, electroclinical features, and response to treatment. Neurology 58, 298–301.

Horváth, A., Szűcs, A., Barcs, G., Kamondi, A., 2017. Sleep EEG detects epileptiform activity in Alzheimer's disease with high sensitivity. J. Alzheimers Dis. 56, 1175–1183.

Horvath, A.A., Papp, A., Zsuffa, J., Szucs, A., Luckl, J., Radai, F., Nagy, F., Hidasi, Z., Csukly, G., Barcs, G., Kamondi, A., 2021. Subclinical epileptiform activity accelerates the progression of Alzheimer's disease: a long-term EEG study. Clin. Neurophysiol. 132, 1982–1989.

Janszky, J., Hoppe, M., Clemens, Z., Janszky, I., Gyimesi, C., Schulz, R., Ebner, A., 2005. Spike frequency is dependent on epilepsy duration and seizure frequency in temporal lobe epilepsy. Epileptic Disord. 7, 355–359.

Kam, K., Duffy, Á.M., Moretto, J., LaFrancois, J.J., Scharfman, H.E., 2016. Interictal spikes during sleep are an early defect in the Tg2576 mouse model of β-amyloid neuropathology. Sci. Rep. 6, 20119.

Kappel, S.L., Rank, M.L., Toft, H.O., Andersen, M., Kidmose, P., 2019. Dry-contact electrode ear-EEG. IEEE Trans. Biomed. Eng. 66, 150–158.

Karoly, P.J., Freestone, D.R., Boston, R., Grayden, D.B., Himes, D., Leyde, K., Seneviratne, U., Berkovic, S., O'Brien, T., Cook, M.J., 2016. Interictal spikes and epileptic seizures: their relationship and underlying rhythmicity. Brain 139, 1066–1078.

Kidmose, P., Looney, D., Mandic, D.P., 2012. Auditory evoked responses from ear-EEG recordings. In: 2012 Annual International Conference of the IEEE engineering in Medicine and Biology Society IEEE.
Kural, M.A., Duez, L., Sejer Hansen, V., Larsson, P.G., Rampp, S., Schulz, R., Tankisi, H., Wennberg, R., Bibby, B.M., Scherg, M., Beniczky, S., 2020. Criteria for defining interictal epileptiform discharges in EEG. Neurology 94 (e2139 LP-e2147).
Lam, A.D., Sarkis, R.A., Pellerin, K.R., Jing, J., Dworetzky, B.A., Hoch, D.B., Jacobs, C.S., Lee, J.W., Weisholtz, D.S., Zepeda, R., Westover, M.B., Cole, A.J., Cash, S.S., 2020. Association of epileptiform abnormalities and seizures in Alzheimer disease. Neurology 95, e2259–e2270.
Lambert, I., Tramoni-Negre, E., Lagarde, S., Roehri, N., Giusiano, B., Trebuchon-Da Fonseca, A., Carron, R., Benar, C.-G., Felician, O., Bartolomei, F., 2020. Hippocampal Interictal spikes during sleep impact long-term memory consolidation. Ann. Neurol. 87, 976–987.
Liedorp, M., Stam, C.J., van der Flier, W.M., Pijnenburg, Y.A.L., Scheltens, P., 2010. Prevalence and clinical significance of epileptiform EEG discharges in a large memory clinic cohort. Dement. Geriatr. Cogn. Disord. 29, 432–437.
Looney, D., Park, C., Kidmose, P., Rank, M.L., Ungstrup, M., Rosenkranz, K., Mandic, D. P., 2011. An in-the-ear platform for recording electroencephalogram. In: 2011 Annual International Conference of the IEEE Engineering in Medicine and Biology Society IEEE.
Looney, D., Kidmose, P., Park, C., Ungstrup, M., Rank, M., Rosenkranz, K., Mandic, D., 2012. The in-the-ear recording concept: user-centered and wearable brain monitoring. IEEE Pulse 3, 32–42.
Lozsadi, D.A., Larner, A.J., 2006. Prevalence and causes of seizures at the time of diagnosis of probable Alzheimer's disease. Dement. Geriatr. Cogn. Disord. 22, 121–124.
McKhann, G.M., Knopman, D.S., Chertkow, H., Hyman, B.T., Jack, C.R., Kawas, C.H., Klunk, W.E., Koroshetz, W.J., Manly, J.J., Mayeux, R., Mohs, R.C., Morris, J.C., Rossor, M.N., Scheltens, P., Carrillo, M.C., Thies, B., Weintraub, S., Phelps, C.H., 2011. 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. 7, 263–269.
Mikkelsen, K.B., Kappel, S.L., Mandic, D.P., Kidmose, P., 2015. EEG recorded from the ear: characterizing the ear-EEG method. Front. Neurosci. 9.
Mikkelsen, K.B., Tabar, Y.R., Kappel, S.L., Christensen, C.B., Toft, H.O., Hemmsen, M.C., Rank, M.L., Otto, M., Kidmose, P., 2019. Accurate whole-night sleep monitoring with dry-contact ear-EEG. Sci. Rep. 9.
Musaeus, C.S., Shafi, M.M., Santarnecchi, E., Herman, S.T., Press, D.Z., 2017. Levetiracetam alters oscillatory connectivity in Alzheimer's disease. J. Alzheimers Dis. 58.
Musaeus, C.S., Waldemar, G., Andersen, B.B., Høgh, P., Kidmose, P., Hemmsen, M.C., Rank, M.L., Kjær, T.W., Frederiksen, K.S., 2022. Long-Term EEG Monitoring in Patients with Alzheimer's Disease Using Ear-EEG: A Feasibility Study. J Alzheimer's Dis (In Press).
Nobis, L., Manohar, S.G., Smith, S.M., Alfaro-Almagro, F., Jenkinson, M., Mackay, C.E., Husain, M., 2019. Hippocampal volume across age: nomograms derived from over 19,700 people in UK biobank. NeuroImage Clin. 23, 101904.
Ossenkoppele, R., Cohn-Sheehy, B.I., La Joie, R., Vogel, J.W., Möller, C., Lehmann, M., van Berckel, B.N.M., Seeley, W.W., Pijnenburg, Y.A., Gorno-Tempini, M.L., Kramer, J.H., Barkhof, F., Rosen, H.J., van der Flier, W.M., Jagust, W.J., Miller, B.L., Scheltens, P., Rabinovici, G.D., 2015. Atrophy patterns in early clinical stages across distinct phenotypes of Alzheimer's disease. Hum. Brain Mapp. 36, 4421–4437.
Palop, J.J., Chin, J., Roberson, E.D., Wang, J., Thwin, M.T., Bien-Ly, N., Yoo, J., Ho, K.O., Yu, G.-Q., Kreitzer, A., Finkbeiner, S., Noebels, J.L., Mucke, L., 2007. Aberrant excitatory neuronal activity and compensatory remodeling of inhibitory hippocampal circuits in mouse models of Alzheimer's disease. Neuron 55, 697–711.
Pandis, D., Scarmeas, N., 2012. Seizures in Alzheimer disease: clinical and epidemiological data. Epilepsy Curr. 12, 184–187.
Pfeffer, R.I., Kurosaki, T.T., Harrah, C.H., Chance, J.M., Filos, S., 1982. Measurement of functional activities in older adults in the community. J. Gerontol. 37, 323–329.
Rao, S.C., Dove, G., Cascino, G.D., Petersen, R.C., 2009. Recurrent seizures in patients with dementia: frequency, seizure types, and treatment outcome. Epilepsy Behav. 14, 118–120.
Risse, S.C., Lampe, T.H., Bird, T.D., Nochlin, D., Sumi, S.M., Keenan, T., Cubberley, L., Peskind, E., Raskind, M.A., 1990. Myoclonus, seizures, and Paratonia in Alzheimer disease. Alzheimer Dis. Assoc. Disord. 4, 217–225.
Sammaritano, M., Gigli, G.L., Gotman, J., 1991. Interictal spiking during wakefulness and sleep and the localization of foci in temporal lobe epilepsy. Neurology 41, 290 (LP – 290).
Sarkis, R.A., Dickerson, B.C., Cole, A.J., Chemali, Z.N., 2016. Clinical and neurophysiologic characteristics of unprovoked seizures in patients diagnosed with dementia. J. Neuropsychiatr. Clin. Neurosci. 28, 56–61.
Scarmeas, N., Honig, L.S., Choi, H., Cantero, J., Brandt, J., Blacker, D., Albert, M., Amatniek, J.C., Marder, K., Bell, K., Hauser, W.A., Stern, Y., 2009. Seizures in Alzheimer Disease. Arch. Neurol. 66.
Simonsen, A.H., Musaeus, C.S., Christensen, G.L., Hasselbalch, S.G., Waldemar, G., 2021. Upwards drift of cerebrospinal fluid amyloid-β 42 over twelve years in a consecutive clinical cohort. J. Alzheimers Dis. 81, 1369–1373.
Szabo, A., Cattaud, V., Bezzina, C., Dard, R.F., Sayegh, F., Gauzin, S., Lejards, C., Valton, L., Rampon, C., Verret, L., Dahan, L., 2023. Neuronal hyperexcitability in the Tg2576 mouse model of Alzheimer's disease - the influence of sleep and noradrenergic transmission. Neurobiol. Aging 123, 35–48.
Vogel, A., Stokholm, J., Andreasen, R., Henriksen, B.D., Brønniche, V., Madsen, G.J., Gustafsson, M., Overgaard, S., Guldberg, A.-M., Jørgensen, K., 2018. Psychometric properties and reference data for Danish versions of free and cued selective reminding test. Category Cued Memory Test Logical Memory 59, 496–502.
Vossel, K.A., Ranasinghe, K.G., Beagle, A.J., Mizuiri, D., Honma, S.M., Dowling, A.F., Darwish, S.M., Van Berlo, V., Barnes, D.E., Mantle, M., Karydas, A.M., Coppola, G., Roberson, E.D., Miller, B.L., Garcia, P.A., Kirsch, H.E., Mucke, L., Nagarajan, S.S., 2016. Incidence and impact of subclinical epileptiform activity in Alzheimer's disease. Ann. Neurol. 80, 858–870.
Vossel, K., Ranasinghe, K.G., Beagle, A.J., La, A., Ah Pook, K., Castro, M., Mizuiri, D., Honma, S.M., Venkateswaran, N., Koestler, M., Zhang, W., Mucke, L., Howell, M.J., Possin, K.L., Kramer, J.H., Boxer, A.L., Miller, B.L., Nagarajan, S.S., Kirsch, H.E., 2021. Effect of Levetiracetam on cognition in patients with Alzheimer disease with and without epileptiform activity: a randomized clinical trial. JAMA Neurol. 78, 1345–1354.
Weisdorf, S., Duun-Henriksen, J., Kjeldsen, M.J., Poulsen, F.R., Gangstad, S.W., Kjær, T. W., 2019. Ultra-long-term subcutaneous home monitoring of epilepsy-490 days of EEG from nine patients. Epilepsia 60, 2204–2214.
Zibrandtsen, I.C., Kidmose, P., Christensen, C.B., Kjaer, T.W., 2017. Ear-EEG detects ictal and interictal abnormalities in focal and generalized epilepsy - a comparison with scalp EEG monitoring. Clin. Neurophysiol. 128, 2454–2461.

Supplementary materials to: "Subclinical epileptiform discharges in Alzheimer's disease using long-term outpatient EEG monitoring"

Reason for dropping out of the study:

- Discomfort with ear-EEG (n = 8)
- Not wanting to participate during the COVID-19 pandemic (n = 1)
- Wanted instead to participate in randomized controlled trial (n = 1)
- Did not cooperate with study procedures (n=1)
- Personal reasons (n = 1)
- Moved to another region (n=1)
- Moved to a nursing home (n = 1).

The questionnaire included the following questions:

a) how did it feel to wear the ear-EEG

b) did you have any problems sleeping with the equipment

c) did the ear-EEG ever fall out of the ear

d) do you have any suggestions for improvement.

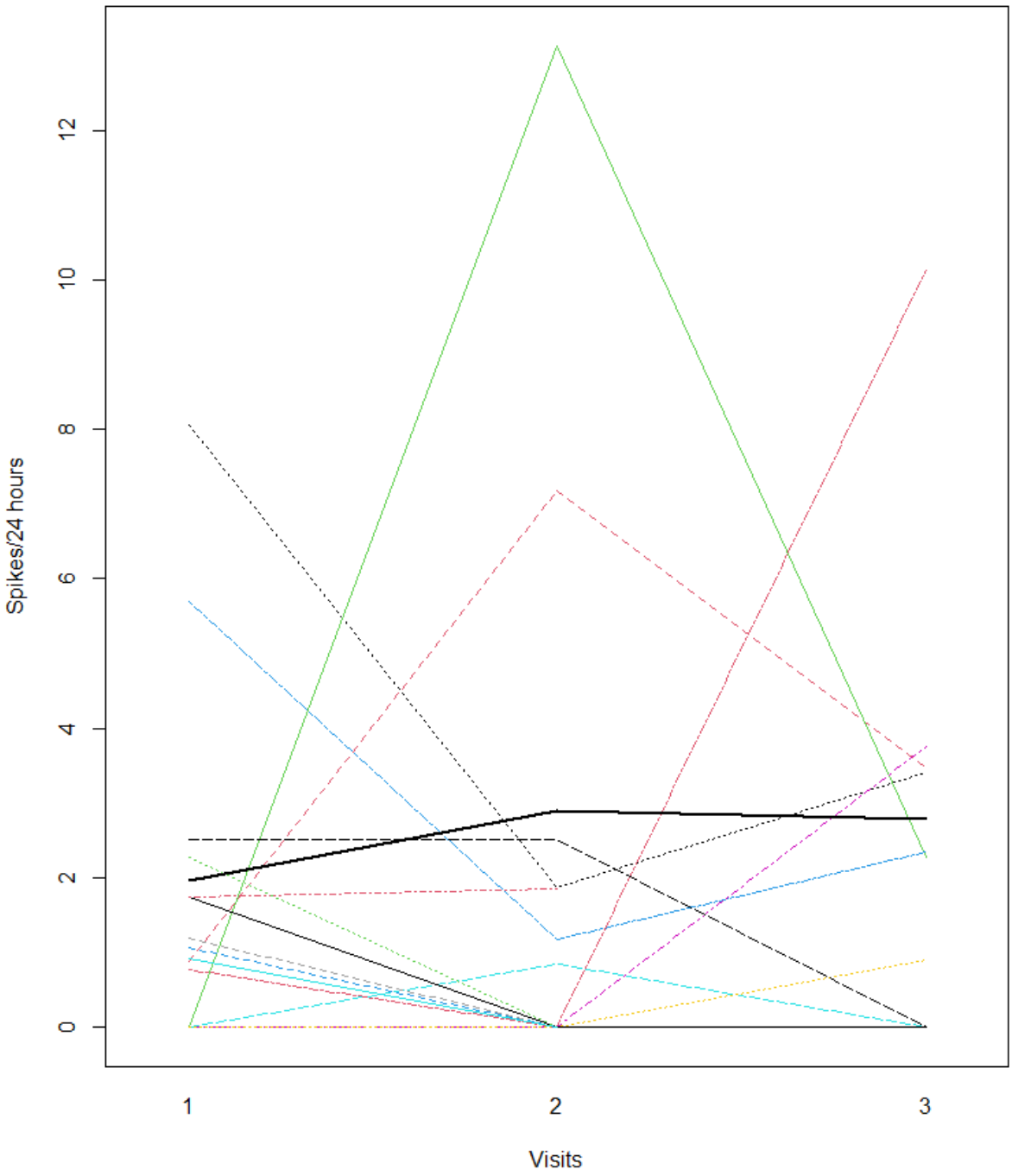
Question a) and b) was used to evaluate if the participant had experienced any adverse events together with the examination of the ears.

Location of conventional EEG slowing

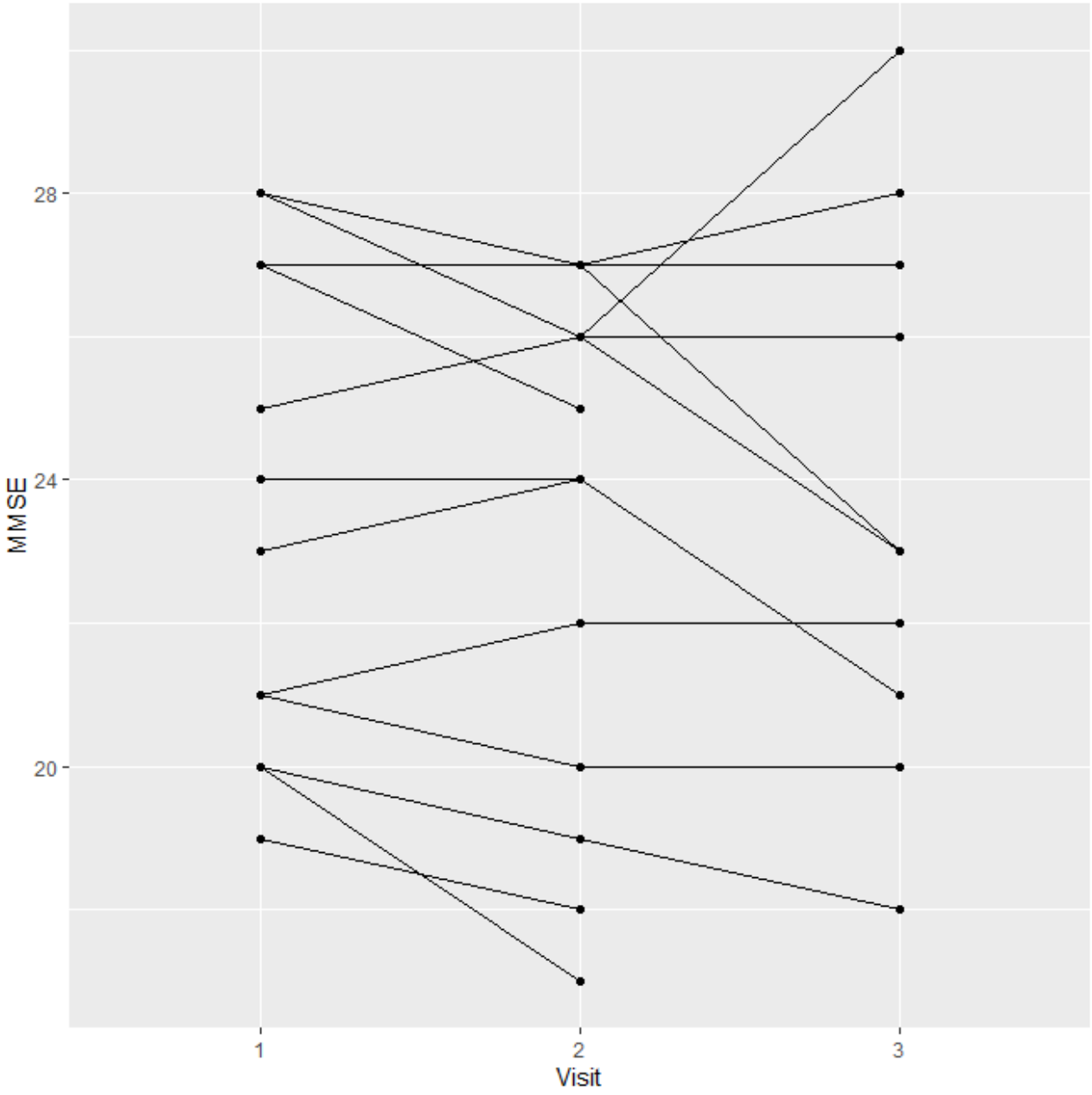
In patients with Alzheimer's disease:

- Four had focal slowing being either temporal or both temporal and frontal
- Two had slowing of the background activity
- One showed both.

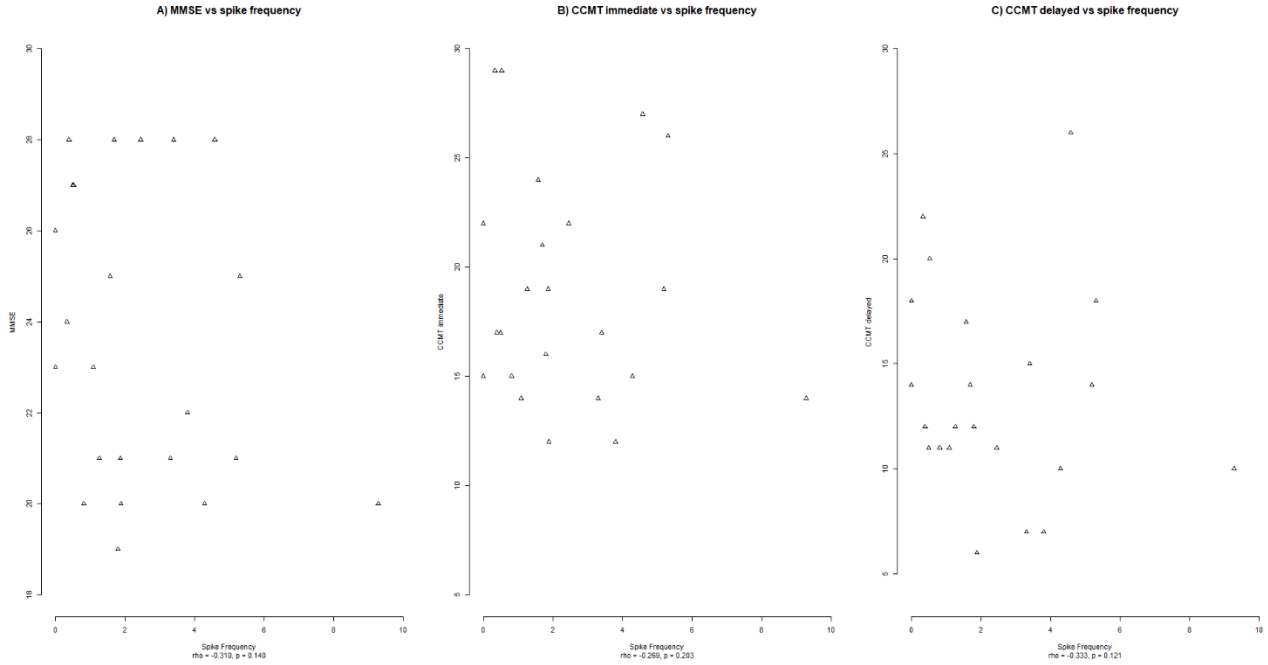
In HC, one participant had focal temporal slowing.



Supplementary figure 1: Spaghetti plot showing the number of spikes/24 hours of recording. Solid black line shows the spike/slow wave frequency per 24 hours for visit 1-3, which increased between visit 1 and 2.



Supplementary figure 2: Spaghetti plot showing the MMSE.



Supplementary figure 3: Spearman's correlations between spikes or sharp waves/24 hours of artifact-free data for all recordings and A) MMSE (p = 0.140), B) immediate recall (p = 0.203), and C) delayed recall (p=0.121) from the CCMT. One patient did not want to complete the CCMT, which explains that only 23 patients had a delayed recall score.

Comparison of cognitive scores between patients with and without epileptiform discharges

Wilcoxon rank sum test was used to compare cognitive test scores between patients with and without epileptiform discharges at baseline.

Supplementary table 1: Cognitive scores between patients with AD and epileptiform discharges compared to patients without.

	AD with ED	AD without ED	p-value
Number of participants, n	18	6	
MMSE, mean (SD)	23.1 (3.61)	24.8 (1.94)	0.2
CCMT, immediate recall, mean (SD)	17.7 (5.5)	20.5 (6.16)	0.4
CCMT, delayed recall, mean (SD)	12.2 (5.29)	15.8 (4.22)	0.09
CCMT, recognition, mean (SD)	38.1 (4.78)	38.3 (4.76)	0.8

ED: Epileptiform discharges, MMSE: Mini-Mental State Examination, CCMT: Category Cued Memory Test

A comparison of the signal between ear-EEG and scalp-EEG

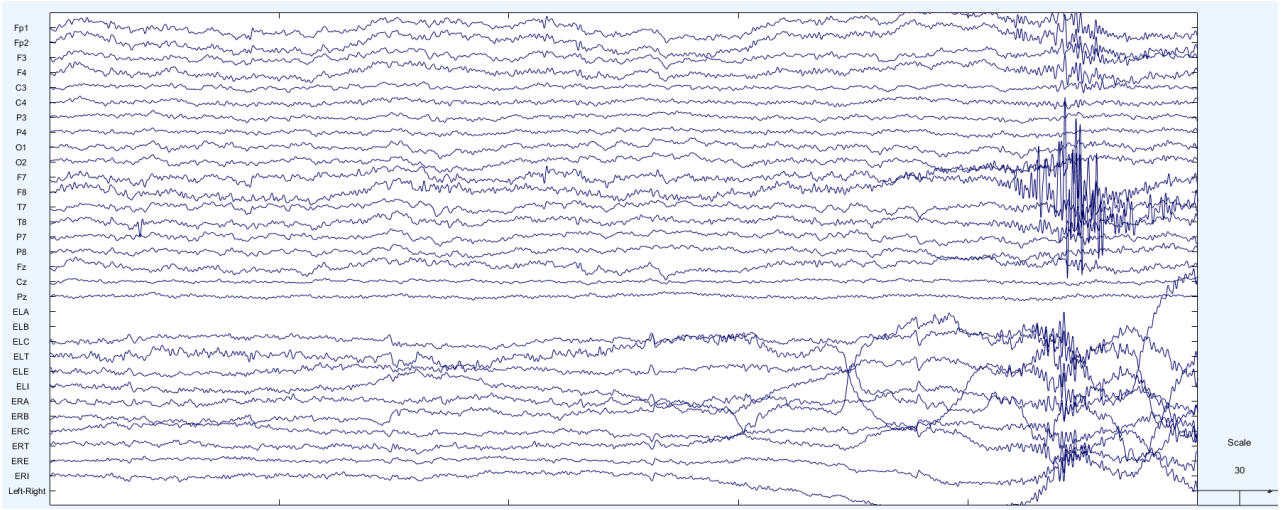
During the co-registered recordings of ear-EEG and scalp-EEG, we did not use the same equipment/amplifier, which led to the signals not being aligned. As a result, we used both the manual marking in the EEG, clear artifacts in both the ear-EEG and scalp EEG as well as the function *finddelay* in MATLAB (between temporal electrode T7 and the ear-EEG electrodes on the left side to align the signals) to support finding the alignment delay.

In the current examples, both signals are bandpass filtered form 0.5-70Hz and the ear-EEG signal is resampled to the lowest sampling rate of the two recordings (ear-EEG is recorded at 2000 Hz during the co-registered recordings). However, due to the lack of a reference channel for the ear-EEG, it was referenced to the average while the scalp-EEG had the same reference as recorded.

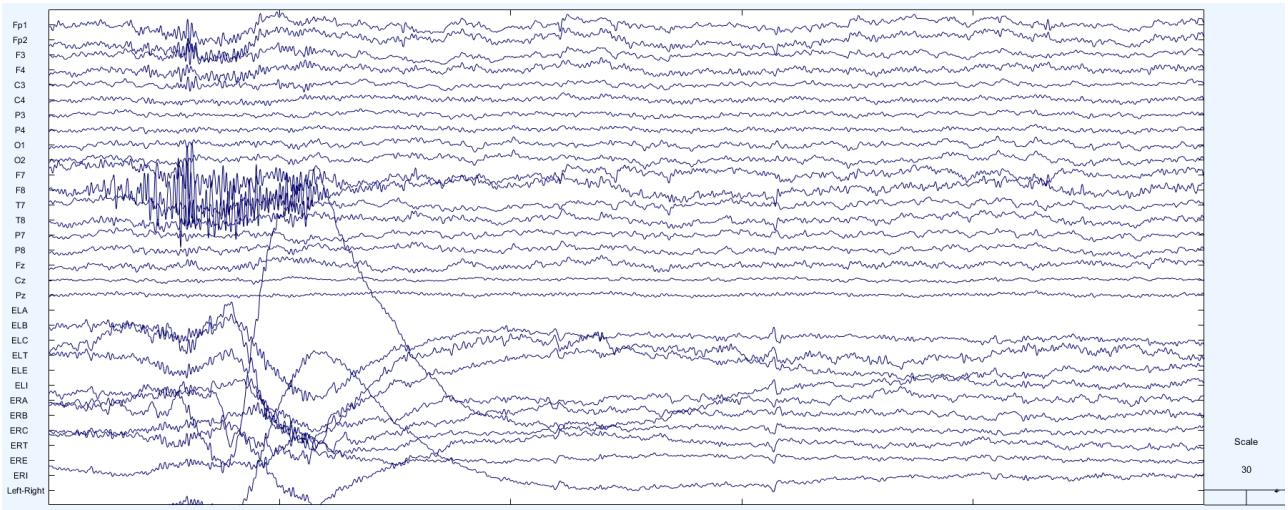
Examples of comparison between the signals can be seen below using a combination of manual and automatic alignment as stated above.

Patient #1: A similar signal can be found between the temporal electrodes on scalp-EEG (T7 and T8) and the ear-EEG signal including the muscle artifact. The periodic spikes in the scalp and ear-EEG are pulse artifacts (occurring regularly every second).

Example #1

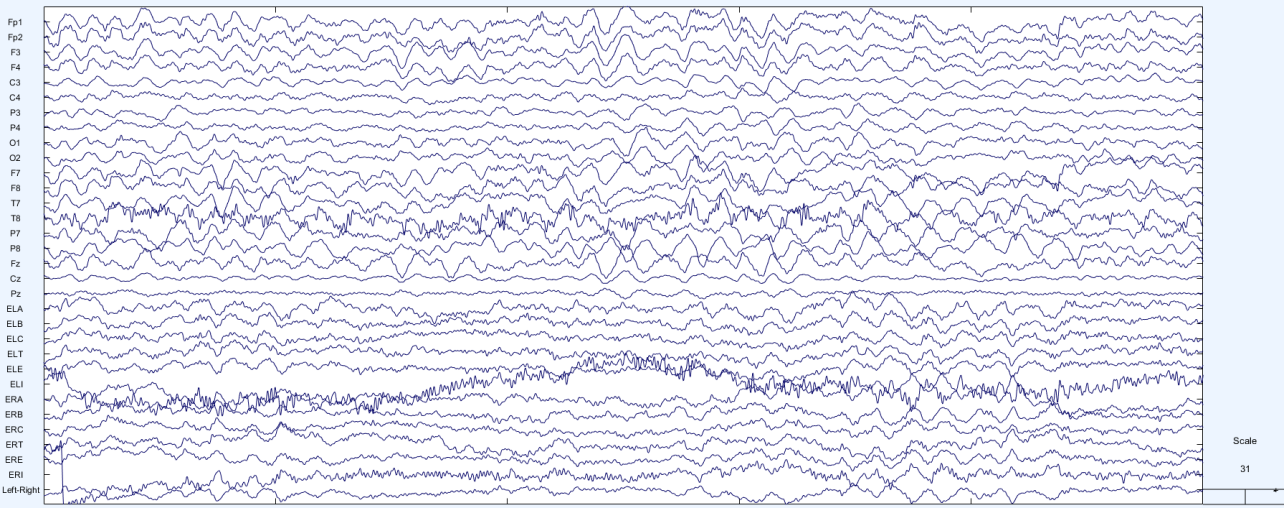


Example #2

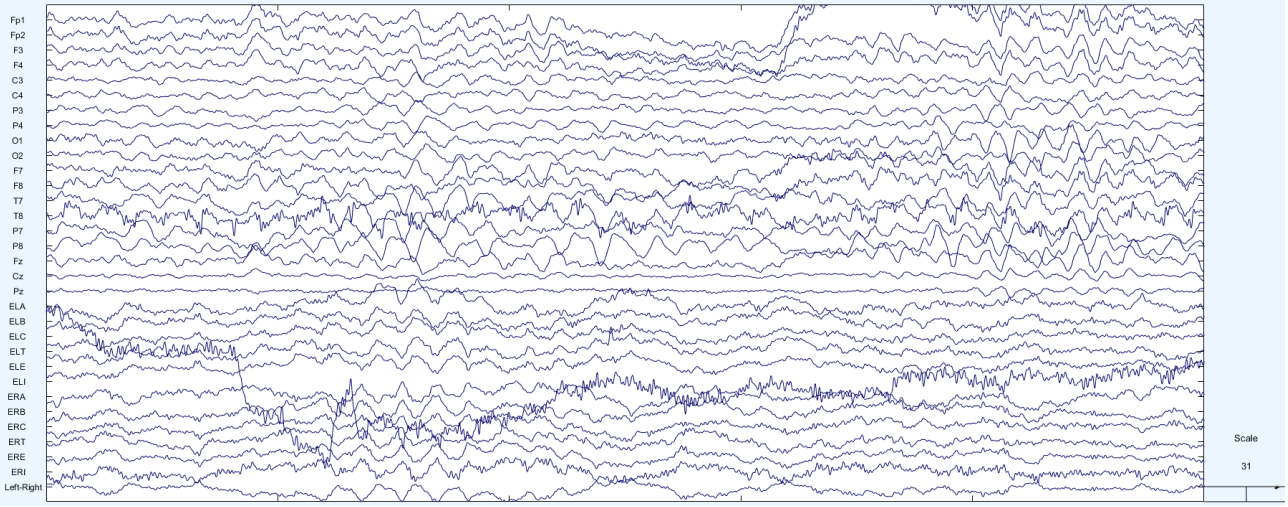


Patient #2: Examples of 7-8 Hz activity in the temporal lobes (as identified on the scalp-EEG) can be found in the ear-EEG. Although this rhythm is present for the majority of window for the frontal, parietal, and occipital electrodes, it is only present in the ear-EEG when the temporal electrodes (T7 and T8) have dominant 7-8 Hz activity.

Example #1

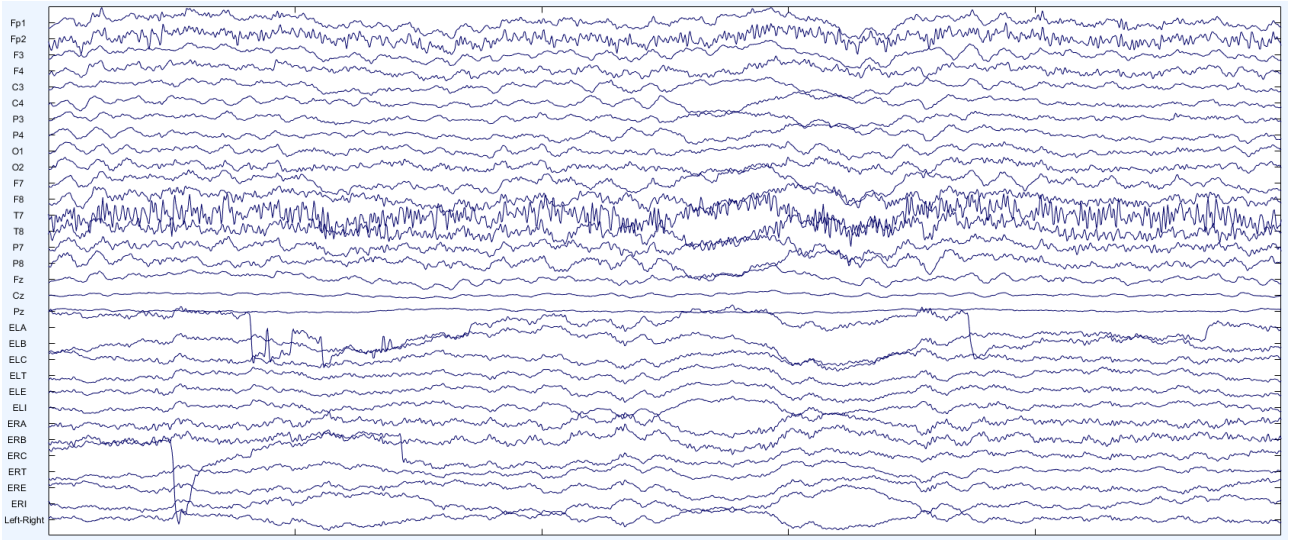


Example #2

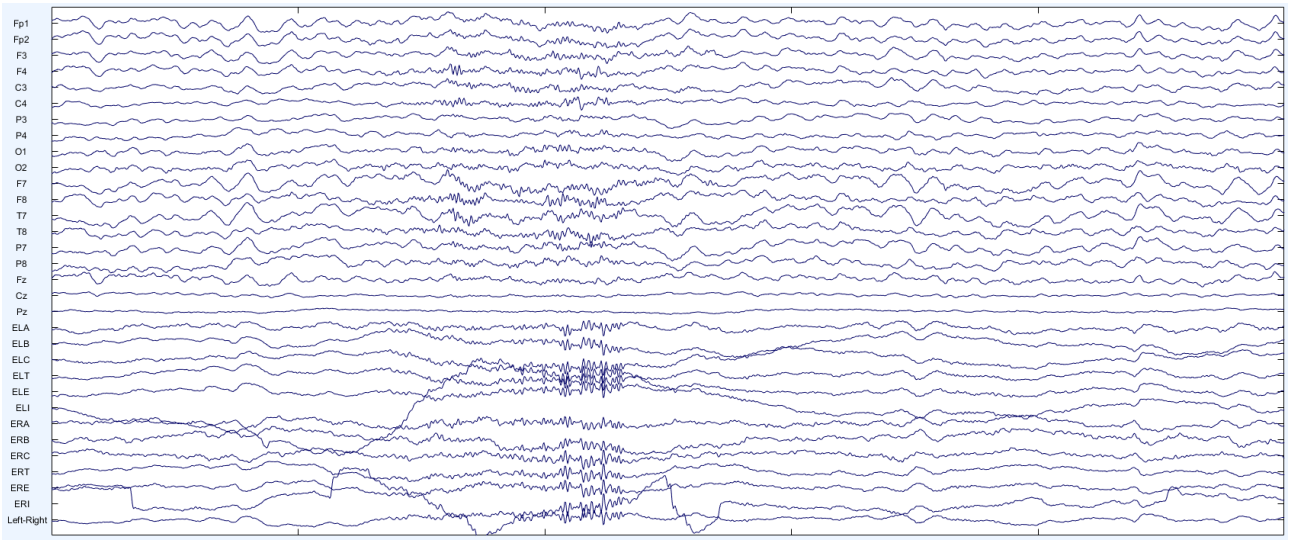


Patient #3: Example of artifact and EEG slowing.

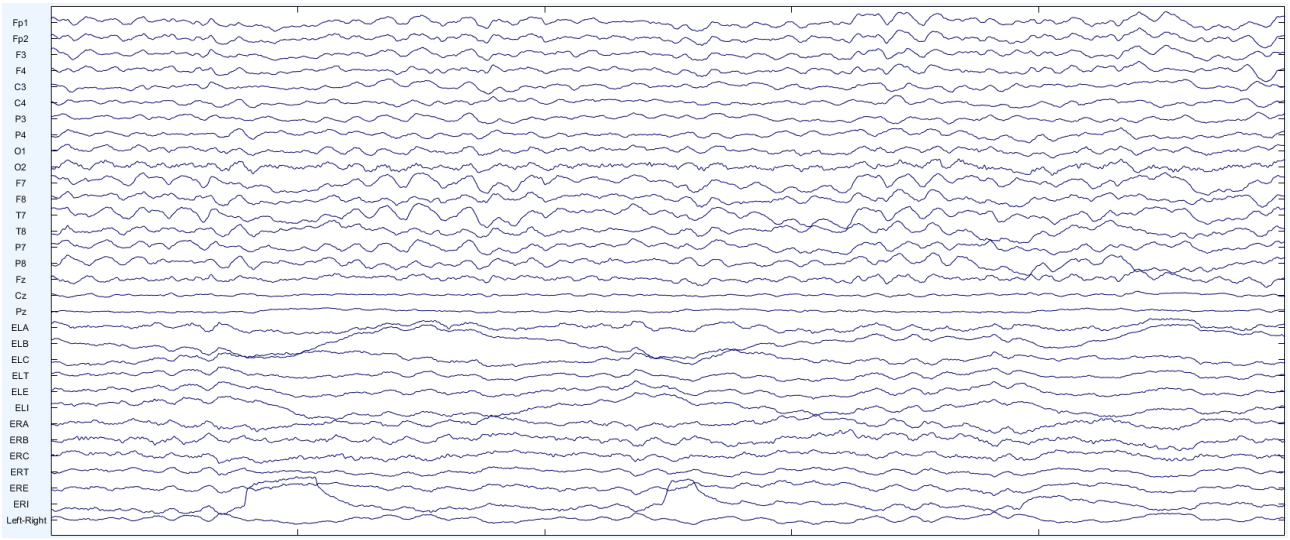
Example #1



Example #2

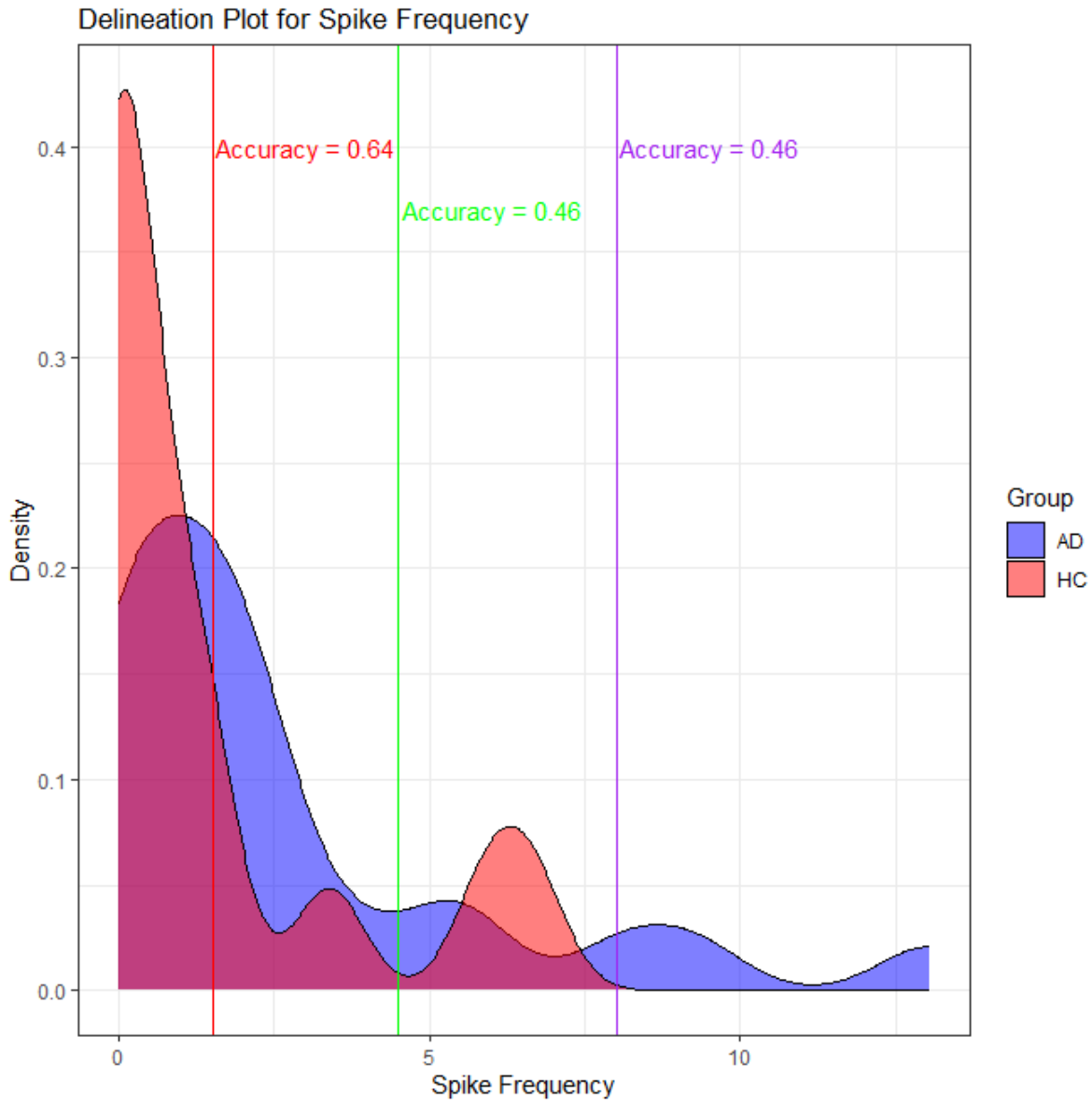


Example #3


Fp1
Fp2
F3
F4
C3
C4
P3
P4
O1
O2
F7
F8
T7
T8
P7
P8
Fz
Cz
Pz
ELA
ELB
ELC
ELT
ELE
ELI
ERA
ERB
ERC
ERT
ERE
ERI
Left-Right

Delineation plot between AD and HC with different threshold and accuracy

A delineation plot showing an analysis of the classification accuracy between AD and HC based on the spike frequency using three different thresholds (1.5, 4.5, and 8). As can be seen from the plot, the spike frequency can not be used to separate AD and HC. This indicates that hyperexcitability is not present in all patients with AD but possibly only in a subset of the group.



Supplementary figure 4: A delineation plot of the density of participants and spike frequency. The classification accuracy was calculated for three different spike frequency thresholds (1.5, 4.5, 8).

R code for creating the delineation plot

```
library(ggplot2)

# Threshold
thresholds <- c(1.5, 4.5, 8)
data$Frequency <- data$`Spike Frequency`
health_freq <- data[data$Group == "HC", "Frequency"]
patient_freq <- data[data$Group == "AD", "Frequency"]
accuracy <- sapply(thresholds, function(t) {
  TP <- sum(patient_freq >= t)
  FP <- sum(health_freq >= t)
  TN <- sum(health_freq < t)
  FN <- sum(patient_freq < t)
  (TP + TN) / (TP + FP + TN + FN)
})

# Create delineation plot
ggplot(data, aes(x = `Spike Frequency`, fill = Group)) +
  geom_density(alpha = 0.5) +
  scale_fill_manual(values = c("blue", "red")) +
  labs(x = "Spike Frequency", y = "Density", fill = "Group") +
  ggtitle("Delineation Plot for Spike Frequency") +
  theme_bw()+
  geom_vline(xintercept = thresholds, linetype = "solid", color = c("red", "green", "purple"))+
  annotate("text", x = thresholds+1.5, y = c(0.4, 0.37, 0.4),
           label = paste0("Accuracy = ", round(accuracy, 2)), color = c("red", "green", "purple"))
```

Comparison of the HC with and without epileptiform discharges

Supplementary table 2: Participant characteristics between HC and epileptiform discharges compared to HC without.

	HC with ED	HC without ED	p-value
Number of participants, n	7	8	
Age, mean (SD)	73.0 (5.57)	66.5 (8.75)	0.132
Males/females	5/2	3/5	0.315
Education, mean (SD)	14.57 (2.3)	15.75 (1.75)	0.303
MMSE, mean (SD)	29.0 (1.0)	29.5 (0.76)	0.339
CCMT, immediate recall, mean (SD)	37.86 (4.67)	38.38 (5.58)	1
CCMT, delayed recall, mean (SD)	36.0 (5.1)	38.25 (6.27)	0.448
CCMT, recognition, mean (SD)	47.71 (0.49)	47.37 (0.74)	0.385

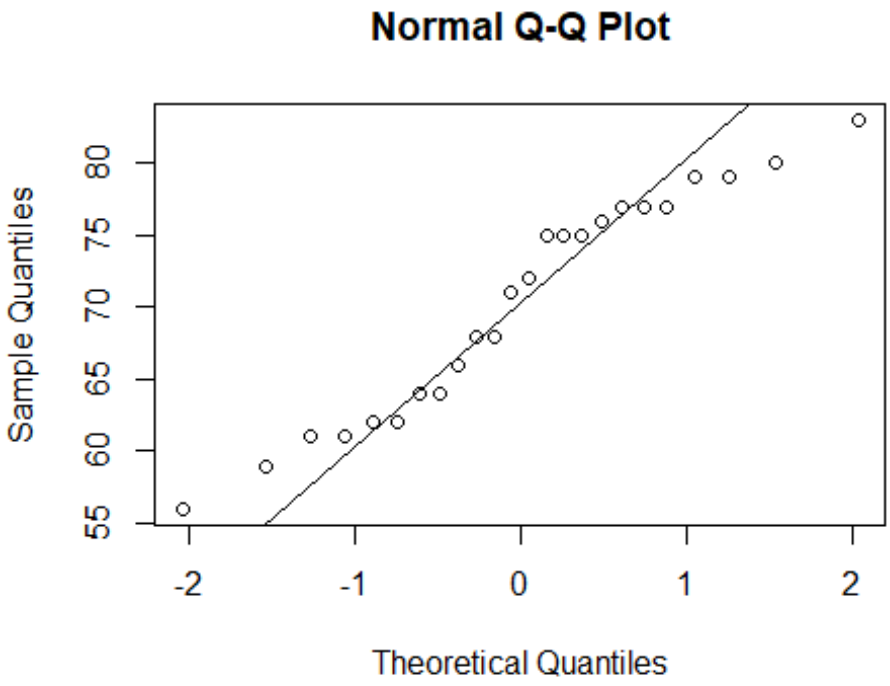
ED: Epileptiform discharges, MMSE: Mini-Mental State Examination, CCMT: Category Cued Memory Test

Statistics as part of: Subclinical epileptiform discharges in Alzheimer's disease using long-term outpatient EEG monitoring

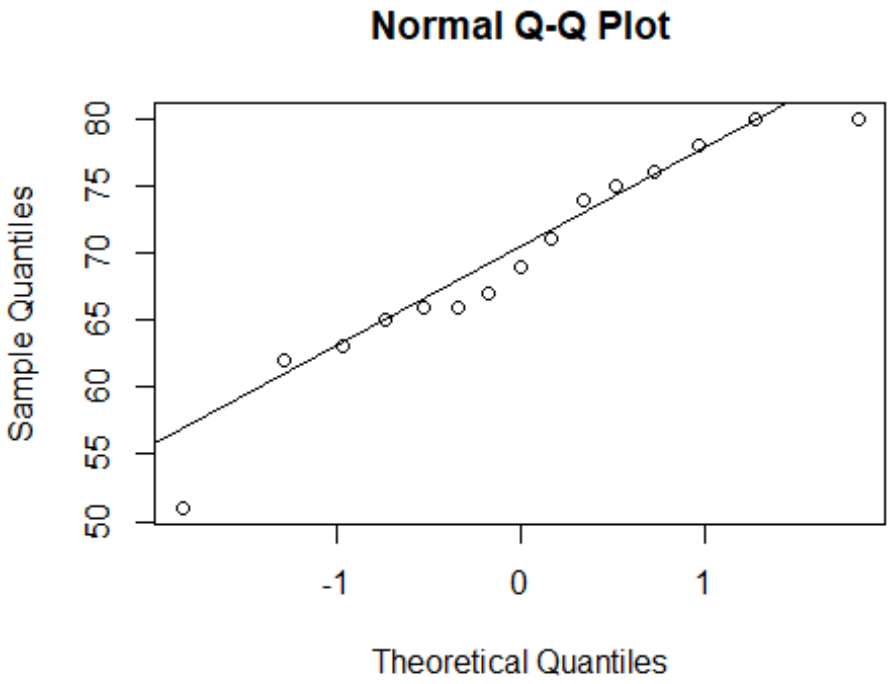
See author list

Analysis: Demographics

```
# Age
qqnorm(AD$age_inclusion)
qqline(AD$age_inclusion)
```

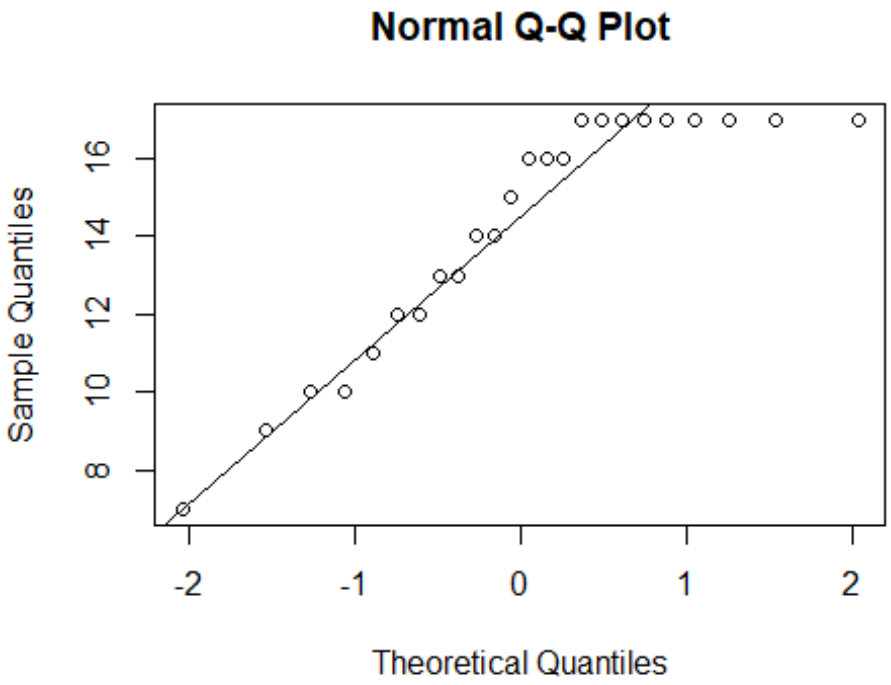



```
qqnorm(HC$age_inclusion)
qqline(HC$age_inclusion)
```

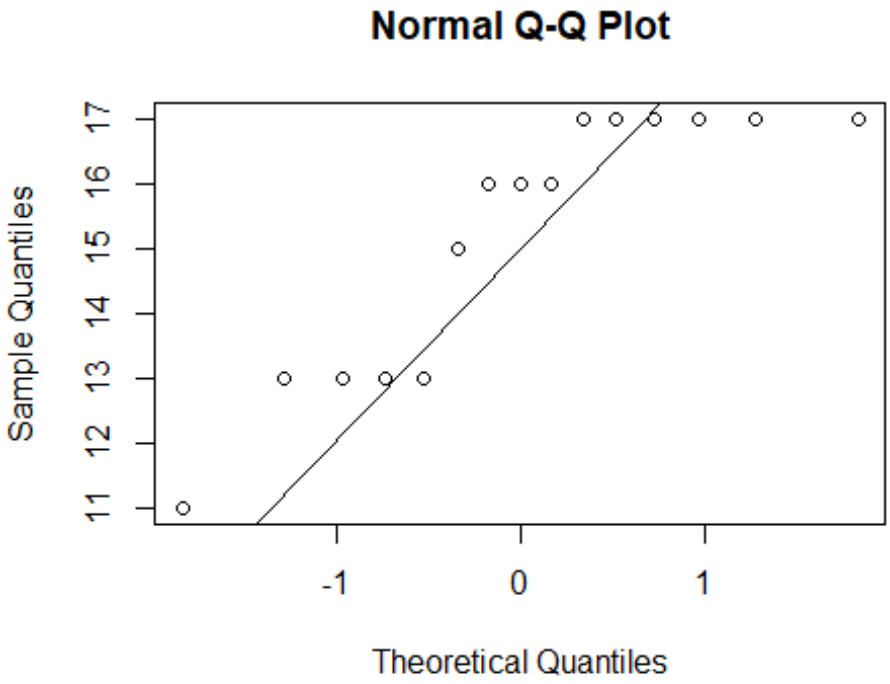



```
var.test(AD$age_inclusion, HC$age_inclusion)

##
##  F test to compare two variances
##
## data:  AD$age_inclusion and HC$age_inclusion
## F = 0.96518, num df = 23, denom df = 14, p-value = 0.9107
## alternative hypothesis: true ratio of variances is not equal to 1
## 95 percent confidence interval:
##  0.3446019 2.4096864
## sample estimates:
## ratio of variances
##          0.9651846

res <- t.test(AD$age_inclusion, HC$age_inclusion, var.equal = TRUE)
res

##
##  Two Sample t-test
##
## data:  AD$age_inclusion and HC$age_inclusion
## t = 0.29385, df = 37, p-value = 0.7705
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -4.470709  5.987376
## sample estimates:
## mean of x mean of y
##  70.29167  69.53333
```

```r
# Education
qqnorm(AD$education) # Ceiling effect
qqline(AD$education)
```

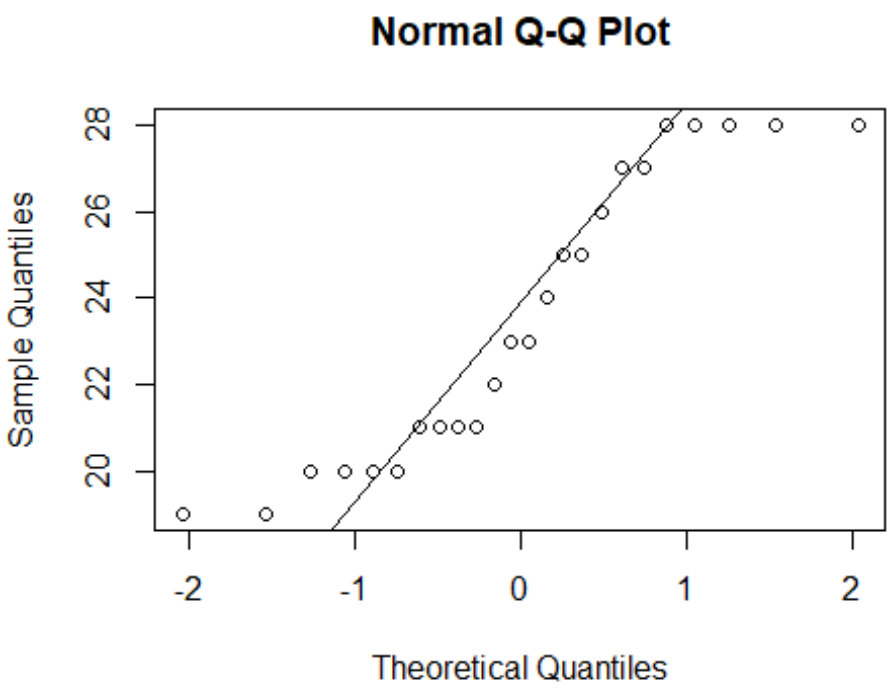



```r
qqnorm(HC$education) # Ceiling effect
qqline(HC$education)
```

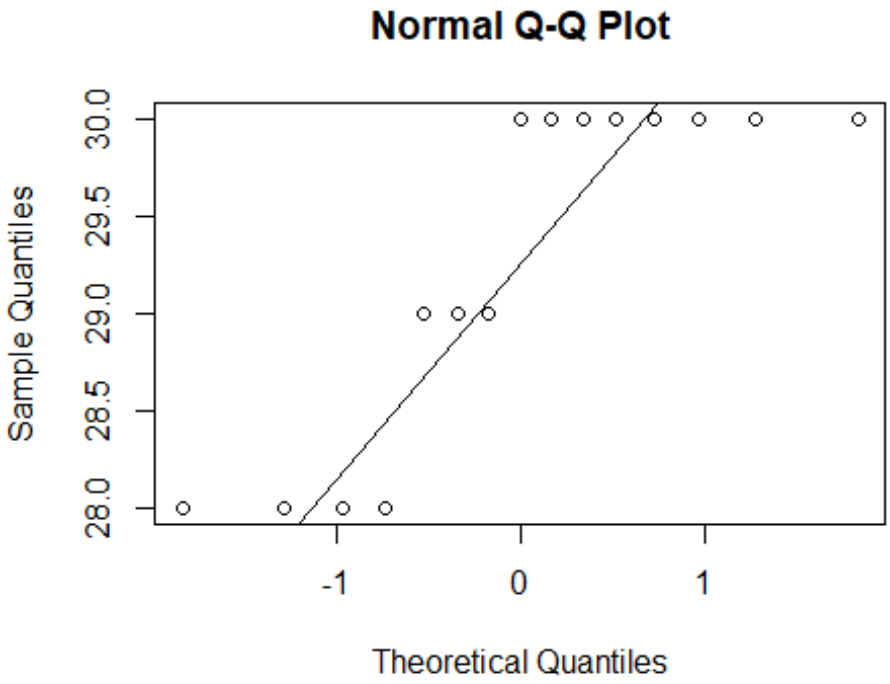



```
var.test(AD$education, HC$education)

##
##  F test to compare two variances
##
## data:  AD$education and HC$education
## F = 2.2718, num df = 23, denom df = 14, p-value = 0.115
## alternative hypothesis: true ratio of variances is not equal to 1
## 95 percent confidence interval:
##  0.8110881 5.6716703
## sample estimates:
## ratio of variances
##           2.271752

res <- t.test(AD$education, HC$education, var.equal = TRUE)
res

##
##  Two Sample t-test
##
## data:  AD$education and HC$education
## t = -1.1024, df = 37, p-value = 0.2774
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -2.8142910  0.8309577
## sample estimates:
## mean of x mean of y
##  14.20833  15.20000
```

```r
# MMSE
qqnorm(AD$mmse_1)
qqline(AD$mmse_1)
```

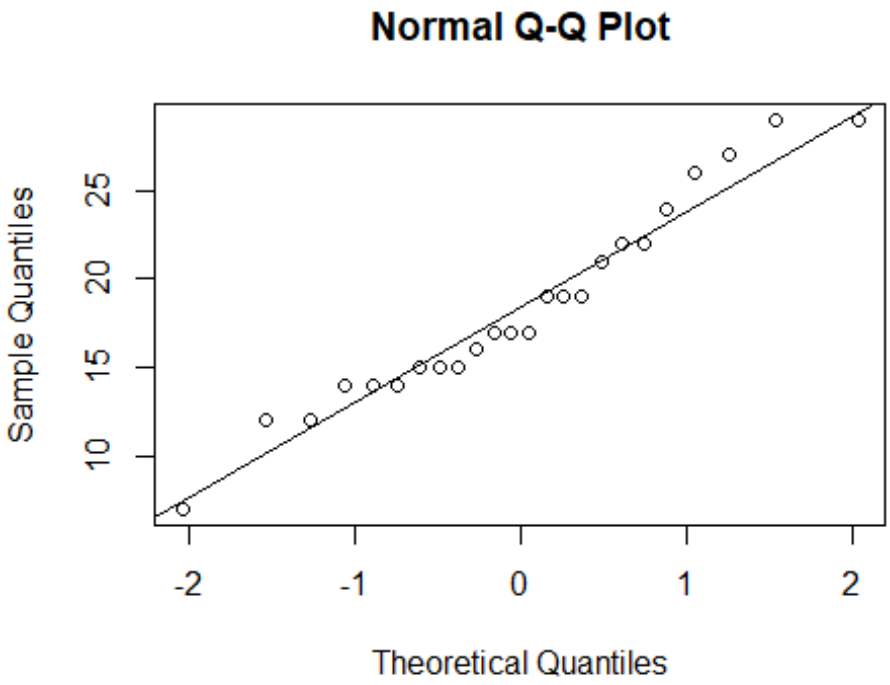



```r
qqnorm(HC$mmse_1) # Ceiling effect
qqline(HC$mmse_1)
```

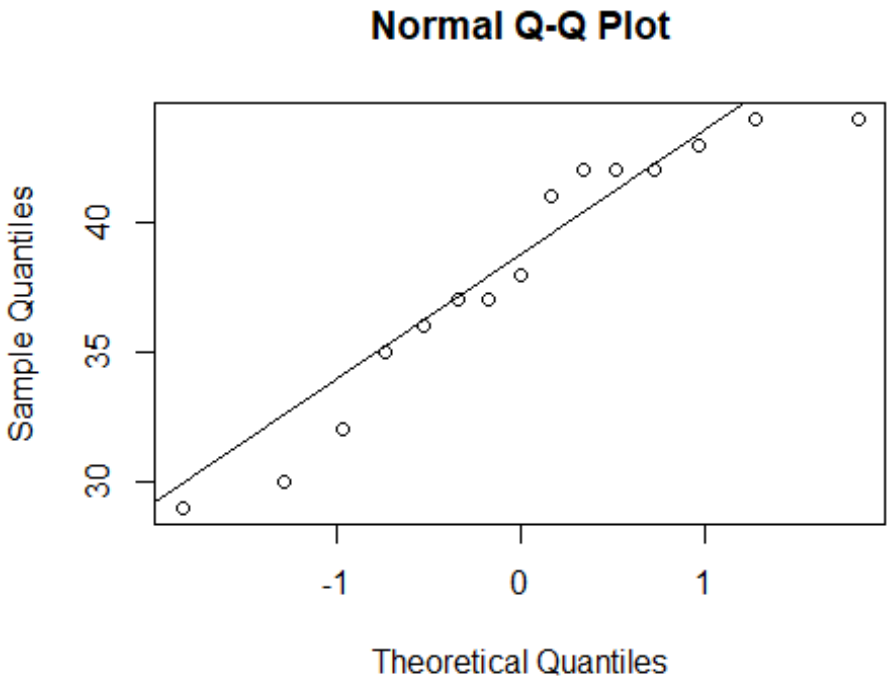



```
var.test(AD$mmse_1, HC$mmse_1)

##
##  F test to compare two variances
##
## data:  AD$mmse_1 and HC$mmse_1
## F = 14.141, num df = 23, denom df = 14, p-value = 6.932e-06
## alternative hypothesis: true ratio of variances is not equal to 1
## 95 percent confidence interval:
##   5.048804 35.304615
## sample estimates:
## ratio of variances
##           14.14104

res <- t.test(AD$mmse_1, HC$mmse_1, var.equal = FALSE)
res

##
##  Welch Two Sample t-test
##
## data:  AD$mmse_1 and HC$mmse_1
## t = -8.0575, df = 27.912, p-value = 9.179e-09
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -7.232894 -4.300440
## sample estimates:
## mean of x mean of y
##  23.50000  29.26667
```

```r
# CCMT Immediate
qqnorm(AD$ccmt_umiddelbar)
qqline(AD$ccmt_umiddelbar)
```

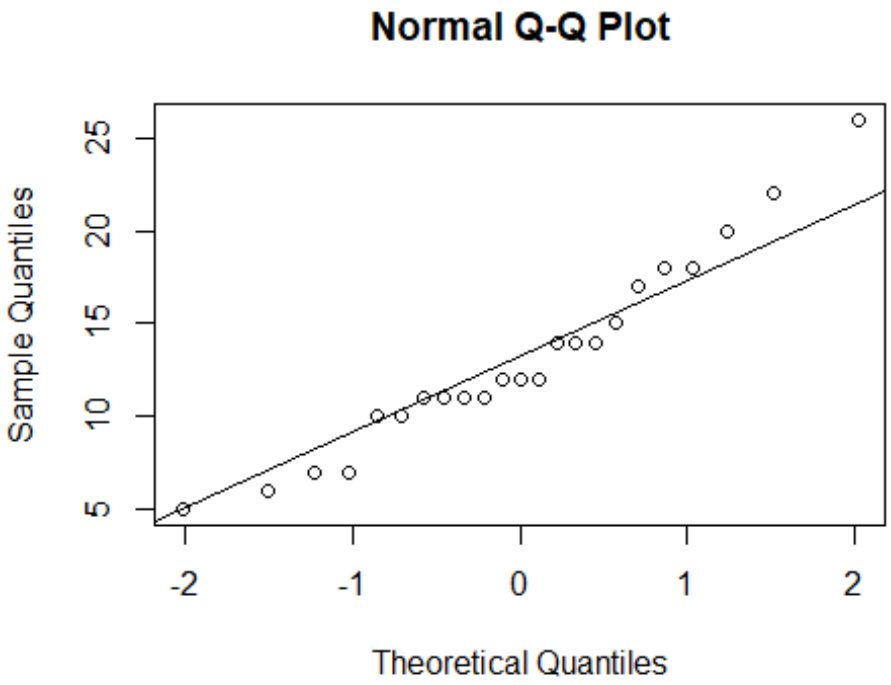



```r
qqnorm(HC$ccmt_umiddelbar)
qqline(HC$ccmt_umiddelbar)
```

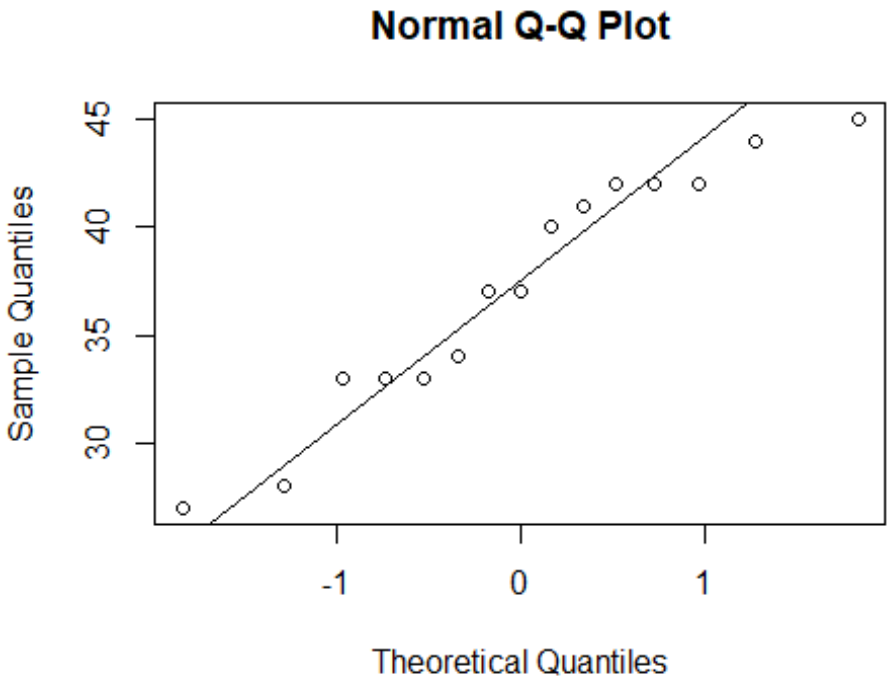



```
var.test(AD$ccmt_umiddelbar, HC$ccmt_umiddelbar)

##
##  F test to compare two variances
##
## data:  AD$ccmt_umiddelbar and HC$ccmt_umiddelbar
## F = 1.2842, num df = 23, denom df = 14, p-value = 0.6386
## alternative hypothesis: true ratio of variances is not equal to 1
## 95 percent confidence interval:
##  0.4584887 3.2060596
## sample estimates:
## ratio of variances
##           1.284167

res <- t.test(AD$ccmt_umiddelbar, HC$ccmt_umiddelbar, var.equal = TRUE)
res

##
##  Two Sample t-test
##
## data:  AD$ccmt_umiddelbar and HC$ccmt_umiddelbar
## t = -11.049, df = 37, p-value = 2.824e-13
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -23.33234 -16.10100
## sample estimates:
## mean of x mean of y
##  18.41667  38.13333
```

```r
# CCMT Delayed
qqnorm(AD$ccmt_forsinket)
qqline(AD$ccmt_forsinket)
```

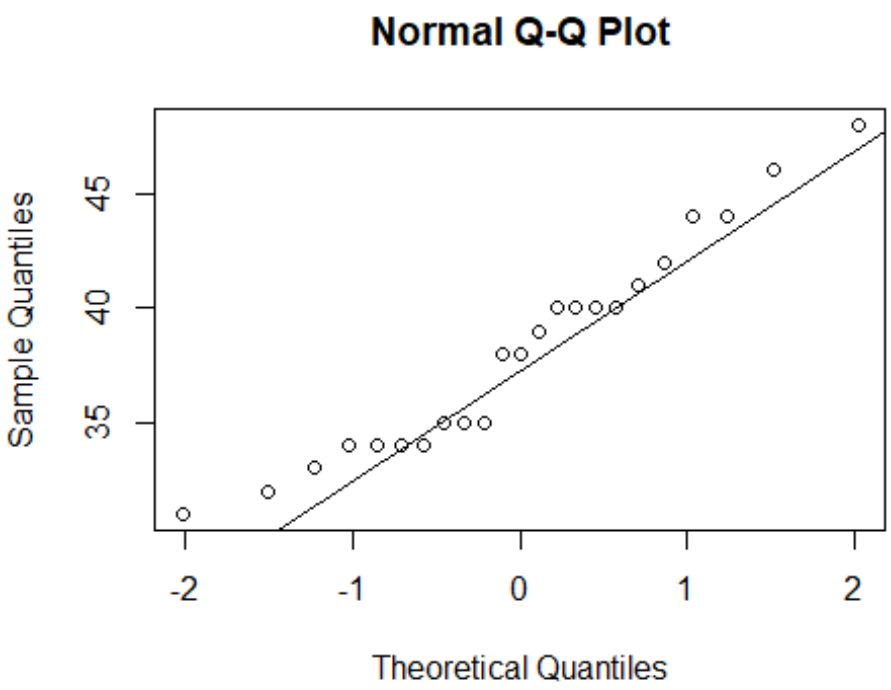



```r
qqnorm(HC$ccmt_forsinket)
qqline(HC$ccmt_forsinket)
```

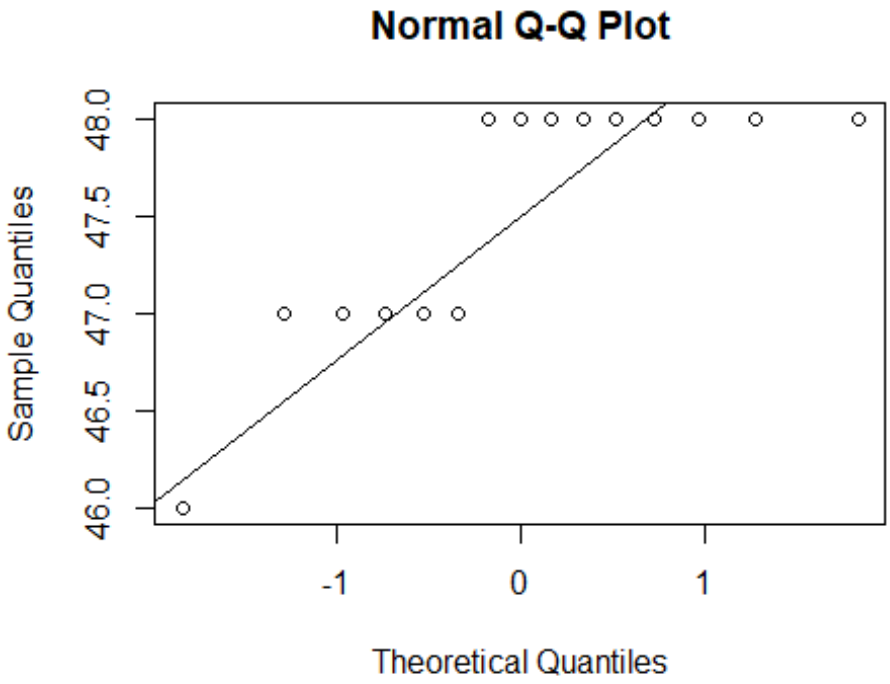



```
var.test(AD$ccmt_forsinket, HC$ccmt_forsinket)

##
##  F test to compare two variances
##
## data:  AD$ccmt_forsinket and HC$ccmt_forsinket
## F = 0.83827, num df = 22, denom df = 14, p-value = 0.6907
## alternative hypothesis: true ratio of variances is not equal to 1
## 95 percent confidence interval:
##  0.2978994 2.1195531
## sample estimates:
## ratio of variances
##          0.8382711

res <- t.test(AD$ccmt_forsinket, HC$ccmt_forsinket, var.equal = TRUE)
res

##
##  Two Sample t-test
##
## data:  AD$ccmt_forsinket and HC$ccmt_forsinket
## t = -13.445, df = 36, p-value = 1.329e-15
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -27.65025 -20.40192
## sample estimates:
## mean of x mean of y
##  13.17391  37.20000
```

```r
# CCMT Recognition
qqnorm(AD$ccmt_genkendelse)
qqline(AD$ccmt_genkendelse)
```




```r
qqnorm(HC$ccmt_genkendelse)
qqline(HC$ccmt_genkendelse) # Ceiling effect
```




```
var.test(AD$ccmt_genkendelse, HC$ccmt_genkendelse)

##
##  F test to compare two variances
##
## data:  AD$ccmt_genkendelse and HC$ccmt_genkendelse
## F = 53.123, num df = 22, denom df = 14, p-value = 1.142e-09
## alternative hypothesis: true ratio of variances is not equal to 1
## 95 percent confidence interval:
##   18.87834 134.31934
## sample estimates:
## ratio of variances
##           53.12253

res <- t.test(AD$ccmt_genkendelse, HC$ccmt_genkendelse, var.equal = FALSE)
res

##
##  Welch Two Sample t-test
##
## data:  AD$ccmt_genkendelse and HC$ccmt_genkendelse
## t = -9.5316, df = 23.258, p-value = 1.684e-09
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -11.442364  -7.363434
## sample estimates:
## mean of x mean of y
##  38.13043  47.53333
```

```r
df_gender <- data.frame(HC=c(7,8), patients=c(10,14))
chisq.test(df_gender, correct = F)

##
##  Pearson's Chi-squared test
##
## data:  df_gender
## X-squared = 0.09385, df = 1, p-value = 0.7593
```

Analysis: Spikes at baseline

```r
df <- data.frame(HC=c(7,8), patients=c(18,6))
chisq.test(df, correct = F)

##
##  Pearson's Chi-squared test
##
## data:  df
## X-squared = 3.2203, df = 1, p-value = 0.07273

library("epitools")

Spikes <- c(HC = 18, AD = 72) # Number of spikes
Days_data <- c(HC = 17.30044787, AD = 23.74376036) # Number of days
rateratio(Spikes,Days_data)

## $data
##       Spikes Days_data
## HC        18  17.30045
## AD        72  23.74376
## Total     90  41.04421
##
## $measure
##                          NA
## rate ratio with 95% C.I. estimate    lower    upper
##                       HC 1.000000       NA       NA
##                       AD 2.894544 1.766036 5.009254
##
## $p.value
##          Outcome
## Predictor   midp.exact         wald
##        HC           NA           NA
##        AD 1.014154e-05 2.085106e-05
##
## attr(,"method")
## [1] "Median unbiased estimate & mid-p exact CI"

# Distributions of epileptiform discharges (day and night) in HC vs AD
df_day <- data.frame(HC=c(11,5), patients=c(25,46))
chisq.test(df_day, correct = F)

##
##  Pearson's Chi-squared test
```

```
##
## data:  df_day
## X-squared = 6.0551, df = 1, p-value = 0.01387
```

Analysis: Conventional EEG

```
df_slowing <- data.frame(HC=c(1,14), patients=c(7,17))
test_slowing <- fisher.test(df_slowing)
test_slowing

##
##  Fisher's Exact Test for Count Data
##
## data:  df_slowing
## p-value = 0.1214
## alternative hypothesis: true odds ratio is not equal to 1
## 95 percent confidence interval:
##  0.003602359 1.684582661
## sample estimates:
## odds ratio
##  0.1801667
```

Analysis: Correlations

```
model_MMSE <- cor.test(Cor_SpikeCCMT$Spikerate, Cor_SpikeCCMT$MMSE, method = "s
pearman",exact=FALSE)
model_MMSE

##
##  Spearman's rank correlation rho
##
## data:  Cor_SpikeCCMT$Spikerate and Cor_SpikeCCMT$MMSE
## S = 3076.1, p-value = 0.1068
## alternative hypothesis: true rho is not equal to 0
## sample estimates:
##       rho
## -0.3374464

model_CCMT <- cor.test(Cor_SpikeCCMT$Spikerate, Cor_SpikeCCMT$CCMT_imme, method
= "spearman",exact=FALSE)
model_CCMT

##
##  Spearman's rank correlation rho
##
## data:  Cor_SpikeCCMT$Spikerate and Cor_SpikeCCMT$CCMT_imme
## S = 2961.4, p-value = 0.173
## alternative hypothesis: true rho is not equal to 0
## sample estimates:
##       rho
## -0.2875568
```

```r
model_CCMT <- cor.test(Cor_SpikeCCMT$Spikerate, Cor_SpikeCCMT$CCMT_delay, metho
d = "spearman",exact=FALSE)
model_CCMT

##
##  Spearman's rank correlation rho
##
## data:  Cor_SpikeCCMT$Spikerate and Cor_SpikeCCMT$CCMT_delay
## S = 2759.5, p-value = 0.0883
## alternative hypothesis: true rho is not equal to 0
## sample estimates:
##        rho
## -0.3633925
```

Analysis: Compare cognitive test scores depending on epileptiform discharges

```r
# MMSE
wilcox.test(AD_Y$mmse_1, AD_N$mmse_1, alternative = "two.sided",exact=FALSE)

##
##  Wilcoxon rank sum test with continuity correction
##
## data:  AD_Y$mmse_1 and AD_N$mmse_1
## W = 35.5, p-value = 0.2256
## alternative hypothesis: true location shift is not equal to 0

# ccmt_umiddelbar
wilcox.test(AD_Y$ccmt_umiddelbar, AD_N$ccmt_umiddelbar, alternative = "two.side
d",exact=FALSE)

##
##  Wilcoxon rank sum test with continuity correction
##
## data:  AD_Y$ccmt_umiddelbar and AD_N$ccmt_umiddelbar
## W = 41, p-value = 0.4027
## alternative hypothesis: true location shift is not equal to 0

# ccmt_forsinket
wilcox.test(AD_Y$ccmt_forsinket, AD_N$ccmt_forsinket, alternative = "two.sided"
,exact=FALSE)

##
##  Wilcoxon rank sum test with continuity correction
##
## data:  AD_Y$ccmt_forsinket and AD_N$ccmt_forsinket
## W = 26.5, p-value = 0.0912
## alternative hypothesis: true location shift is not equal to 0

# ccmt_genkendelse
wilcox.test(AD_Y$ccmt_genkendelse, AD_N$ccmt_genkendelse, alternative = "two.si
ded",exact=FALSE)

##
##  Wilcoxon rank sum test with continuity correction
```

```
##
## data:  AD_Y$ccmt_genkendelse and AD_N$ccmt_genkendelse
## W = 46.5, p-value = 0.778
## alternative hypothesis: true location shift is not equal to 0
```

Analysis: Compare over visits

```
library(lme4)

library(lmerTest)

library(LMMstar)



Model1 = glmer(Spikes ~ Visit + offset(log(Time)) + (1 | ID), family = poisson,
nAGQ=0, data = Over_time_Long, na.action = na.exclude) # Added nAGQ=0 due to pr
oblem converging - most likely due to low sample size
summary(Model1)
```

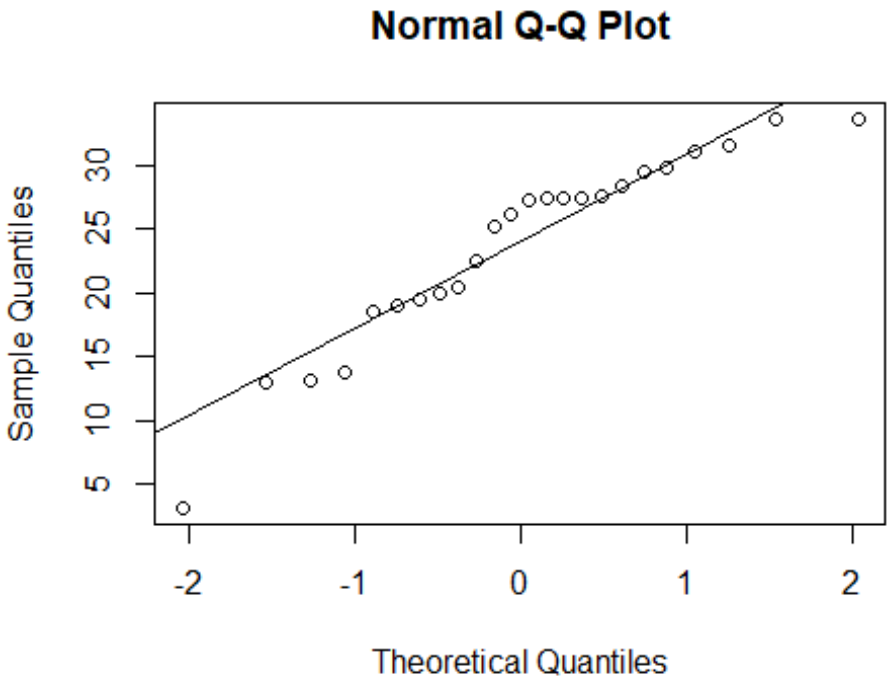
```
## Generalized linear mixed model fit by maximum likelihood (Adaptive
##   Gauss-Hermite Quadrature, nAGQ = 0) [glmerMod]
##  Family: poisson  ( log )
## Formula: Spikes ~ Visit + offset(log(Time)) + (1 | ID)
##    Data: Over_time_Long
##
##      AIC      BIC   logLik deviance df.resid
##    199.2    206.0    -95.6    191.2       37
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -2.2969 -1.0185 -0.6259  0.2931  4.3857
##
## Random effects:
##  Groups Name        Variance Std.Dev.
##  ID     (Intercept) 0.4954   0.7039
## Number of obs: 41, groups:  ID, 15
##
## Fixed effects:
##             Estimate Std. Error z value Pr(>|z|)
## (Intercept)  -6.8144     0.2638 -25.831   <2e-16 ***
## Visit2        0.2918     0.2494   1.170    0.242
## Visit3        0.2453     0.2598   0.944    0.345
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##        (Intr) Visit2
## Visit2 -0.477
## Visit3 -0.446  0.528
```

```
e.lmmUN = lmm(MMSE ~ Visit, repetition = ~Visit|ID, data = Over_time_Long)
summary(e.lmmUN)
```

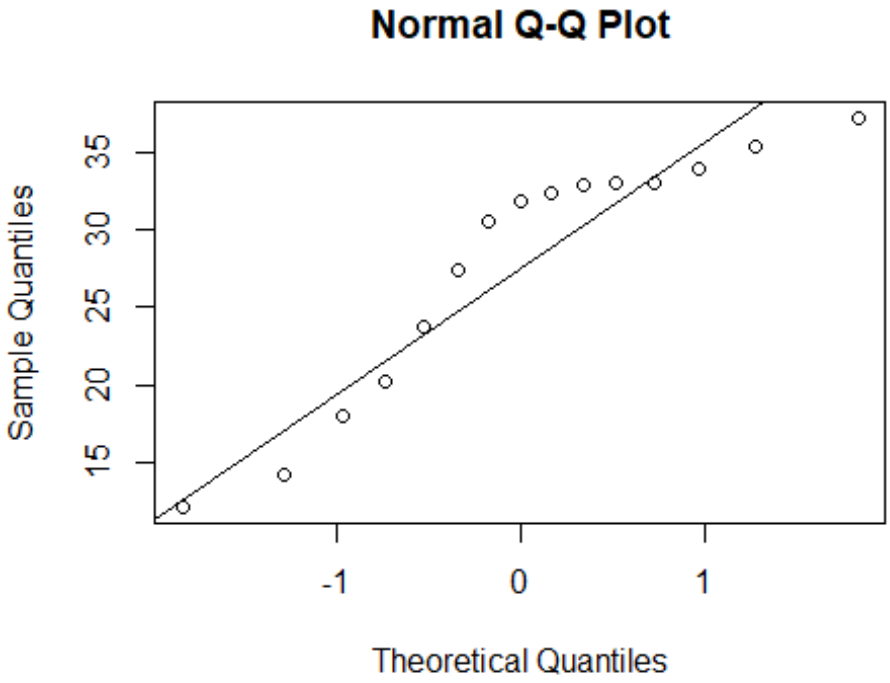
```
##            Linear Mixed Model
##
## Dataset: Over_time_Long
##
##   - 15 clusters
##   - 41 observations were analyzed, 4 were excluded because of missing values
##   - between 2 and 3 observations per cluster
##
## Summary of the outcome and covariates:
##
##     $ MMSE : num   ...
##     $ Visit: Factor w/ 3 levels "1","2","3": ...
##     reference level: Visit=1
##
## Estimation procedure
##
##   - Restricted Maximum Likelihood (REML)
##   - log-likelihood :-85.24987
##   - parameters: mean = 3, variance = 3, correlation = 3
##
## Residual variance-covariance: unstructured
##
##   - correlation structure: ~Visit - 1
##           1     2     3
##     1 1.000 0.939 0.857
##     2 0.939 1.000 0.817
##     3 0.857 0.817 1.000
##
##   - variance structure: ~Visit
##             standard.deviation    ratio
##     sigma.1           3.369328 1.000000
##     sigma.2           3.622679 1.075193
##     sigma.3           4.072123 1.208586
##
## Fixed effects: MMSE ~ Visit
##
##             estimate    se     df  lower  upper p.value
## (Intercept)   23.733  0.87 14.001 21.867 25.599  <0.001 ***
## Visit2          -0.6 0.321 13.997 -1.288  0.088  0.0824   .
## Visit3        -1.076 0.734  4.525 -3.025  0.872  0.2085
##
## Uncertainty was quantified using model-based standard errors (column se).
## Degrees of freedom were computed using a Satterthwaite approximation (column
df).
## The columns lower and upper indicate a 95% confidence interval for each coef
ficient.
```

Analysis: Pre-processed data

```
qqnorm(AD_Preprocess$CleanData)
qqline(AD_Preprocess$CleanData)
```




```
qqnorm(HC_Preprocess$CleanData)
qqline(HC_Preprocess$CleanData)
```




```
var.test(AD_Preprocess$CleanData, HC_Preprocess$CleanData)
```

```
##
##  F test to compare two variances
##
## data:  AD_Preprocess$CleanData and HC_Preprocess$CleanData
## F = 0.89391, num df = 23, denom df = 14, p-value = 0.7865
## alternative hypothesis: true ratio of variances is not equal to 1
## 95 percent confidence interval:
##  0.3191552 2.2317468
## sample estimates:
## ratio of variances
##           0.893912
```

```
res <- t.test(AD_Preprocess$CleanData, HC_Preprocess$CleanData, var.equal = TRU
E)
res
```

```
##
##  Two Sample t-test
##
## data:  AD_Preprocess$CleanData and HC_Preprocess$CleanData
## t = -1.54, df = 37, p-value = 0.1321
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -9.116770  1.242858
## sample estimates:
## mean of x mean of y
##  23.74376  27.68072
```

Analysis: Adverse events

```
df_adverseevent <- data.frame(HC=c(9,6), patients=c(19,5))
fisher.test(df_adverseevent)
```

```
##
##  Fisher's Exact Test for Count Data
##
## data:  df_adverseevent
## p-value = 0.2773
## alternative hypothesis: true odds ratio is not equal to 1
## 95 percent confidence interval:
##  0.0743921 2.0730070
## sample estimates:
## odds ratio
##  0.4048773
```

Manuscript III

Subclinical epileptiform activity in dementia with Lewy bodies

Musaeus CS, Kjær TW, Andersen BB, Høgh P, Kidmose P, Fabricius M, Hribljan MC, Hemmsen MC, Rank ML, Waldemar G, Frederiksen KS. Subclinical epileptiform activity in dementia with Lewy bodies. Mov Disord. 2023 Jul 11. doi: 10.1002/mds.29531. Online ahead of print.

RESEARCH ARTICLE

Subclinical Epileptiform Activity in Dementia with Lewy Bodies

Christian Sandøe Musaeus, MD,[1*] Troels Wesenberg Kjær, MD, PhD,[1] Melita Cacic Hribljan, MD, PhD,[2] Birgitte Bo Andersen, MD, DMSc,[1] Peter Høgh, MD, PhD,[3,4] Preben Kidmose, MSc, PhD,[5] Martin Fabricius, MD, DMSc,[2,4] Martin Christian Hemmsen, PhD,[6] Mike Lind Rank, MSc, PhD,[6] Gunhild Waldemar, MD, DMSc,[1,4] and Kristian Steen Frederiksen, MD, PhD[1]

[1]*Danish Dementia Research Centre (DDRC), Department of Neurology, Copenhagen University Hospital—Rigshospitalet, Copenhagen, Denmark*
[2]*Department of Clinical Neurophysiology, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark*
[3]*Regional Dementia Research Centre, Department of Neurology, Zealand University Hospital, Roskilde, Denmark*
[4]*Department of Clinical Medicine, University of Copenhagen, Copenhagen, Denmark*
[5]*Department of Electrical and Computer Engineering, Aarhus University, Aarhus, Denmark*
[6]*T&W Engineering, Lillerød, Denmark*

ABSTRACT: Background: Patients with dementia with Lewy bodies (DLB) have a higher probability of seizures than in normal aging and in other types of neurodegenerative disorders. Depositions of α-synuclein, a pathological hallmark of DLB, can induce network excitability, which can escalate into seizure activity. Indicator of seizures are epileptiform discharges as observed using electroencephalography (EEG). However, no studies have so far investigated the occurrence of interictal epileptiform discharges (IED) in patients with DLB.

Objectives: To investigate if IED as measured with ear-EEG occurs with a higher frequency in patients with DLB compared to healthy controls (HC).

Methods: In this longitudinal observational exploratory study, 10 patients with DLB and 15 HC were included in the analysis. Patients with DLB underwent up to three ear-EEG recordings, each lasting up to 2 days, over a period of 6 months.

Results: At baseline, IED were detected in 80% of patients with DLB and in 46.7% of HC. The spike frequency (spikes or sharp waves/24 hours) was significantly higher in patients with DLB as compared to HC with a risk ratio of 2.52 (CI, 1.42–4.61; *P*-value = 0.001). Most IED occurred at night.

Conclusions: Long-term outpatient ear-EEG monitoring detects IED in most patients with DLB with an increased spike frequency compared to HC. This study extends the spectrum of neurodegenerative disorders in which epileptiform discharges occurs at an elevated frequency. It is possible that epileptiform discharges are, therefore, a consequence of neurodegeneration. © 2023 The Authors. *Movement Disorders* published by Wiley Periodicals LLC on behalf of International Parkinson and Movement Disorder Society.

Key Words: EEG; long-term EEG monitoring; Lewy body dementia; interictal epileptiform discharges; fluctuations

*__Correspondence to:__ Dr. Christian Sandøe Musaeus, Danish Dementia Research Centre, Copenhagen University Hospital–Rigshospitalet, Inge Lehmanns Vej 8, 2100 Copenhagen, Denmark; E-mail: christian.sandoee.musaeus@regionh.dk

Relevant conflicts of interest/financial disclosures: C.S.M. received an unrestricted grant from T&W Engineering. Two authors (M.L.R. and M.C.H.) who are employees of T&W Engineering contributed to the interpretation and writing of the report. T.W.K. consults for T&W engineering.

Funding agencies: The study was funded by the Alzheimer Research Foundation (grant number: 181003), Toyota Foundation (KJ/BG-9171 F), Axel Juul Muusfeldts Foundation (2016–527), Ellen Mørchs foundation (J.nr. 32491419), Rigshospitalet Research Foundation and T&W Engineering. None of the funding parties had a role in the collection, analysis, and interpretation of data or in the writing of the manuscript or whether to publish the results of the study.

Received: 21 March 2023; **Revised:** 26 May 2023; **Accepted:** 20 June 2023

Published online in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/mds.29531

Introduction

Patients with Lewy body dementia (DLB) have a higher risk of developing seizures than the general population.[1] Case reports have shown that patients with DLB may present with epilepsy[2,3] or patients with epilepsy showing symptoms mimicking DLB.[4] Further, since the early features of DLB may bear resemblance to nonconvulsive seizures, as for example, fluctuating cognition, a common feature in patient with DLB,[5] seizures may go undetected as has been shown in patients with Alzheimer's disease (AD).[1]

As a possible biomarker for developing epileptic seizures, interictal epileptiform discharges (IED) such as spikes or sharp waves can be observed using electroencephalography (EEG). Recently, studies have found that patients with AD have a higher rate of IED when measured with long-term EEG[6-8] and a study found improved cognition after administration of levetiracetam in patients with AD who had IED.[9] However, no studies have so far investigated the prevalence of IED using long-term EEG in patients with DLB. In mouse models of α-synucleinopathy, one study found a link between network hyperexcitability as detected by IED, occurring during non-rapid eye movement (REM) sleep.[10] Although atrophy of the temporal lobes is generally not as pronounced in DLB as in AD,[11] one study has suggested that a higher density of Lewy bodies is present in the temporal lobes.[12] Furthermore, in Parkinson's disease visual hallucinations may be related to IED originating from the temporal lobes.[13]

Because of the possible clinical relevance of detecting IED, there is a need for accessible and dependable EEG monitoring in an outpatient setting. One method is ear-EEG[14] where the EEG signals are recorded from electrodes placed within a customized earpiece inserted into each ear and is associated with little interference of everyday life.[14-16] Recently, ear-EEG has shown to be feasible for long-term outpatient ear-EEG monitoring in patients with AD[17] and has been shown to be able to detect IED in patients with temporal lobe seizures.[18] Because of the symptoms being associated with temporal lobe structures, it is plausible that ear-EEG can capture IED in patients with DLB.

In the current study exploratory study, we investigated if IED as measured with ear-EEG occurs with a higher frequency in patients with DLB compared to healthy controls.

Methods

Participants

Patients with mild to moderate DLB were recruited from the Memory Clinic at Copenhagen University Hospital-Rigshospitalet and the Memory Clinic at Zealand University Hospital, Roskilde. All patients met the DLB Consortium criteria for DLB.[19] Inclusion criteria were (1) a Mini-Mental State Examination (MMSE)[20] score of 16 to 28; (2) age between 50 and 90 years; (3) native Danish speaker; (4) at least 7 years of education; (5) hearing and vision sufficient for neuropsychological examination; (6) no alcohol or drug abuse within the last 2 years; (7) no contraindications for magnetic resonance imaging (MRI); (8) an MRI or computed tomography (CT); (9) the general health conditions of the patient allowed participation in the study (as judged by the principal investigator); and (10) living with a caregiver who was able to assist the patient with the home recordings.

The following exclusion criteria were applied: (1) epilepsy before the diagnosis of DLB; (2) focal pathology (except DLB related atrophy) in the hippocampus (ie, hippocampal sclerosis); (3) living with a relative with serious illness or impaired activities of daily living; (4) living in a nursing home; (5) psychiatric (except mild depression) or neurological conditions that affects the brain except DLB; (6) currently treated with antiepileptic medication, tricyclic antidepressants, or antipsychotics; (7) daily or almost daily administration of medication with known anticholinergic or adrenergic effect, which may affect cognitive abilities or EEG; (8) large cerebral infarctions or more than four lacunar infarctions on MRI; (9) suffering from facial tics/facial hyperkinetic disorders; or (10) daily use of hearing aids.

The healthy controls (HC) were all participants in other studies and had expressed interest in participating in new studies. The inclusion criteria were (1) normal cognition (as judged by the principal investigator); (2) the general health allowed participation in the study; and (3) did not suffer from any psychiatric (except mild depressive symptoms or mild depression) or neurological conditions that affect the brain as well as criteria 2 to 7 as applied in patients with DLB. The following exclusion criteria were applied: (1) diagnosed with epilepsy; and (2) focal pathology in the brain (except mild hippocampal atrophy) as well as exclusion criteria 5 to 10 as applied in patients with DLB, but without DLB. The data from the HC has also been published in a previous study.[21]

The study was approved by the Capital Region Ethics Committee (H-17035751), the Danish Medicines Agency (2017112288), and the Data Protection Agency (P-2021-866). All participants gave their written informed consent before participating in the study. The study is registered at clinicaltrials.gov (NCT04436341).

Study Design

In this longitudinal study, a total of eight visits were planned over a 6-month period for patients with DLB and a total of four visits for the HC (see Figure 1).

In patients with DLB, the 3 2-day ear-EEG recordings were planned 3 months apart, that is, at the beginning of the study (baseline), at 3 months, and at 6 months follow-up. At visit 1 (baseline), informed consent was obtained followed by assessment of medical history, a physical and neurological examination, the MMSE and an imprint of the ears using Otoform A Soft X (Dreve, Germany), a soft ear impression silicone. Subsequently, the patient underwent the following: visit 2, MRI scan to evaluate exclusion criteria related to possible pathology or infarctions; visit 3 standard EEG recording together with ear-EEG, Functional Assessment Questionnaire Instrumental Activities of Daily Living (FAQ IADL)[22] (to assess everyday function), neuropsychiatric inventory (NPI)[23] (to assess behavioral and psychological symptoms), and the Category Cued Memory Test (CCMT)[24] to assess memory function. Further, the patient and the caregiver were briefly instructed about seizures and introduced to a seizure diary to take home to note any symptoms of seizures (such abdominal aura or automatisms, jerks, rapid changes of awareness, and in cognition). Information and training on how to use the ear-EEG at home were conducted. This included a log-sheet to take notes on when the participant wore the ear-EEG equipment. After ~48 hours (visit 4) the patient and/or the caregiver returned with the equipment and the patient filled a questionnaire. The external ear was examined for any minor lesions when the patient was present to return the equipment (visit 4, 6, and 8) and participants were queried regarding any possible adverse events. Visit 5 and 6, and visits 7 and 8 were similar to visits 3 and 4 except that MMSE was performed at visit 5 and 7. Based on the experiences gathered in the feasibility study,[17] breaks were permitted throughout the recording periods where the participants were allowed to take out the ear-EEG. Emphasis was put on recordings during the night, so participants were encouraged to wear the ear-EEG especially during the night. HC only had a single recording session with ear-EEG. The program for the HC was similar to the program for DLB patients except for the fact that the FAQ IADL and NPI were not administered.

A short questionnaire about the use of the ear-EEG was administered to DLB patients at visit 4, 6, and 8. See Supplementary Data S1.

Ear-EEG Recording

The ear-EEG earpieces were custom-made in silicone and mounted with six dry-electrodes[14] (T&W Engineering, Denmark). The earpiece was placed inside the ear canal and in the concha[25] in both ears with the labeling of the ear-EEG electrodes being previously described.[26] The ground electrode was placed ~2 cm under the midline of the clavicle on either one side. The ear-EEG recordings were performed using the TMSi Mobita EEG amplifier (TMSi systems, the Netherlands), which was worn in either a small bag around the neck or in a belt bag and connected to the electrodes. The sampling rate was 1000 Hz. The EEG data were loaded into MATLAB (The MathWorks, Natick, MA, v2020b) and the EEGLAB[27] data structure was used throughout the analysis. See feasibility study for a complete description of the preprocessing.[17]

The amount of time when data from at least one electrode in each ear were present was calculated (afterward called preprocessed data). The amount of available data for each individual electrode and the amount of time preprocessed data on each ear were present during the night (10 PM–8 AM) and day (8 AM–10 PM) was examined.

Standard EEG Recordings

We performed 30-minutes 19-channel EEG recording at visit 4, 6, and 8. The protocol involved 10 minutes of resting state followed by 20 minutes of sleeping, see Supplementary Data S1 for full detail of the recording and assessment.

Ear-EEG Review and Annotation

Ear-EEG recordings were visually reviewed and annotated by C.S.M. using the EEGLAB toolbox (v13.6.5b) implemented in MATLAB (2020b). Specifics for montage can be seen in the Supplementary Data S1.

All annotations of epileptiform discharges were based on visual assessment. A sharp asymmetric negative potential of 20 to 200 millisecond duration was considered an epileptiform discharge (spike/sharp wave) according to the International Federation of Clinical Neurophysiology criteria for defining IED.[28] The criteria compromised of (1) di- or tri-phasic with a pointed peak with (2) a different wave duration than the background activity; (3) asymmetry of the waveform; (4) background activity was disrupted by the presence of the epileptiform discharge and in some cases was (5) followed by a slow after-wave. Because of the nature of ear-EEG, we could not investigate the spatial distribution of the spikes/sharp waves (criteria 6). Practically, this led to most epileptiform discharges fulfilling at least four out of the six criteria. All annotations underwent review by a board-certified clinical neurophysiologist (T.W.K.), who made the final ruling. Both C.S.M. and T.W.K. were blinded to the diagnosis when reviewing the EEG results.

For the ear-EEG analysis, we also included six of the HC to ensure blinding during the analysis. However, as these six HC had already been analyzed, the results from the reanalysis were discarded. The subsequent statistical analysis was, therefore, carried out on the original data as has previously been reported on.[21] The number of epileptiform discharges between the first

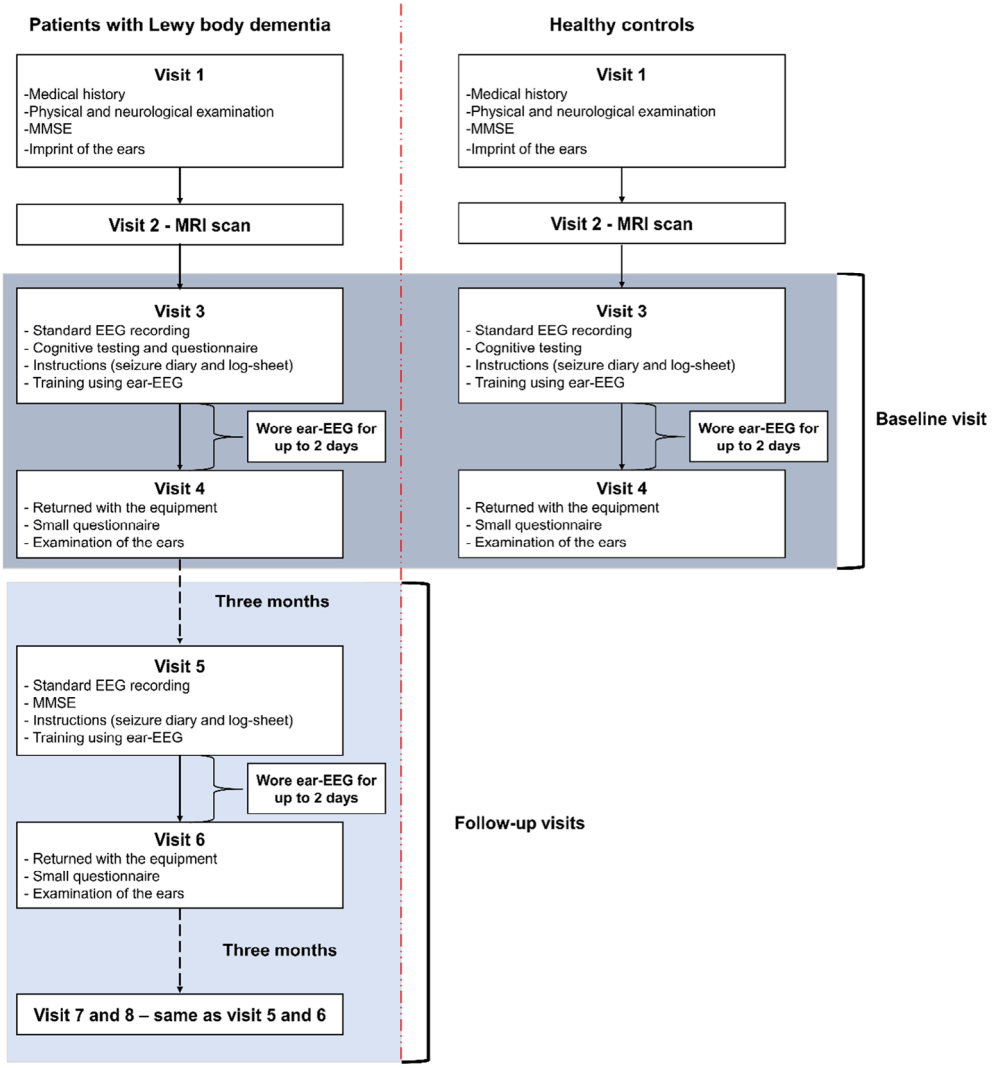


FIG. 1. Study design. Both participants with dementia with Lewy bodies (DLB) and healthy controls underwent the first session of ear-electroencephalography (EEG) recording (baseline) with the participants with DLB also undergoing up to two follow-up visits. The figure is based on the study figure in a previous publication.[21] [Color figure can be viewed at wileyonlinelibrary.com]

analysis (n = 8) and the reanalysis (n = 9) was almost similar.

Statistics

All statistics were performed in RStudio (v1.2.1335). When comparing age, education, cognitive scores, and the length of ear-EEG recordings between DLB (at baseline) and HC, we performed two-sample *t* tests. The distribution of the data as well as the variance was checked. χ^2 test or Fisher's exact test (if one input variable being ≤5) was performed for comparing sex, the number of participants with EEG slowing, the number of participants with spike/sharp waves at baseline, and the number of spikes/night and spikes/day between DLB and HC.

When comparing the number of spikes or sharp waves/24 hours (spike frequency) between HC, and DLB at baseline, we calculated the rate ratio using the function rateratio from the epitools toolbox.

Spearman's correlation between the spike frequency across all recordings in DLB and cognitive tests was performed.

Changes in spikes or sharp waves between visits were analyzed using a generalized linear mixed-effect model including visit number (categorical variable) and log-transformed the amount of pre-processed EEG (in minutes) as the offset variable with a person specific random intercept in a Poisson regression model using the lme4 package.

Changes in MMSE over visits were analyzed using a linear mixed model including visit number (categorical) as fixed effect because a decrease was expected over time. An unstructured covariance pattern was used to account for repeated measurements on the same subjects with the LMMStar package.

A comparison of participant characteristics of the HC with and without epileptiform discharges was performed using two-sample Wilcoxon test (see Supplementary Data S1).

Results

Demographics

Ten patients with DLB and 15 HC were eligible for inclusion and gave informed consent. For the follow-up visits, eight patients with DLB participated in two ear-EEG recordings and seven in all three visits (see Supplementary Data S1 for the reasons for dropping out of the study). See Table 1 for demographics and cognitive scores. One patient with DLB was prescribed quetiapine after the first ear-EEG recording because of visual hallucinations.

Prevalence of Epileptiform Discharges with Ear-EEG at Baseline

See Figure 2A for examples of IED. IED were detected in 80% (8/10) of patients with DLB and in 46.7% (7/15) of HC at baseline (*P*-value = 0.210), see Figure 2B. At baseline, the spike frequency was significantly higher in patients with DLB (range, 0–5.97; mean, 2.63 spikes/24 hours) as compared to HC (range, 0–6.66; mean, 1.04 spikes/24 hours) with a risk ratio of 2.52 (CI, 1.42–4.61, *P*-value = 0.001). See Figure 2C for an overview of the distribution of spikes. In patients with DLB, 59.3% of spikes occurred between 8 PM and 8 AM (see Fig. 2D), whereas this was true for 31.3% of HC (see Fig. 2E), but this was not significant between the groups (*P*-value = 0.076). One patient with DLB did not note time when wearing the ear-EEG at baseline. A comparison of the signal between ear-EEG and scalp-EEG can be seen in Supplementary Data S1.

TABLE 1 *Baseline demographics and clinical scales*

	Healthy controls	Lewy body dementia	*P*-value
Number of participants	15	10	
Age, years, mean (SD)	69.5 (7.93)	69.9 (8.49)	0.9
Males/females	8/7	10/0	0.020
Education, mean (SD)	15.2 (2.04)	14.3 (2.91)	0.4
Cholinesterase inhibitor, n (%)		9 (90%)	
Levodopa, n (%)		3 (30%)	
SSRI/SNRI, n (%)	1 (7%)	2 (20%)	
MMSE, mean (SD)	29.3 (0.88)	26.7 (1.83)	0.001
CCMT, immediate recall	38.1 (5)	26.8 (7.64)	<0.001
CCMT, delayed recall	37.2 (5.67)	24.3 (9.43)	<0.001
CCMT, recognition	47.5 (0.64)	44.5 (4.48)	0.061
NPI		7.67 (7.19)	
FAQ IADL		12.67 (5.79)	

Abbreviations: SD, standard deviation; SSRI/SNRI, selective serotonin reuptake inhibitor/serotonin and noradrenaline reuptake inhibitor; MMSE, Mini-Mental State Examination; CCMT, Category Cued Memory Test; NPI, Neuropsychiatric Inventory; FAQ IADL, Functional Assessment Questionnaire Instrumental Activities of Daily Living.

Variability in Epileptiform Discharges across Recordings

A considerable variance between the number of spikes across recordings was seen by significant difference in spike frequency between the baseline and the follow-up visits (visit 1–2: estimate, 0.33; *P*-value = 0.029; visit 1–3: estimate, 2.45; *P*-value = 0.002), see Supplementary Figure S1. At visit 3, one patient had 22 IED, which was considerably higher than the rest. If this observation was removed, no significant differences were found between visits 1 to 3. Combining all the recordings, we found that IED were found in 90% of DLB patients. When the spike frequency for all recordings was combined in patients with DLB, we found a spike frequency of 2.75 spikes/24 hours (70 IED, 25.45 days of preprocessed data). No significant differences were found for MMSE across visits (visit1–2: estimate, 0.88; *P*-value = 0.809; visit1–3: estimate, 1.34; *P*-value = 0.588) (Supplementary Figure S2 and Table 2).

Association between Memory Function and Epileptiform Discharges

A significant correlation was found between the spike frequency in patients with DLB and the baseline MMSE ($\rho = -0.80$, *P*-value = 0.006). No significant correlations were found for immediate recall and delayed recall (Supplementary Figure S3).

A comparison of the participant characteristics of the HC with and without epileptiform discharges can be found in the Supplementary Data S1.

Conventional EEG and Patient Diaries

Regarding conventional EEG at both baseline and follow-up, we found that 80% (8/10) of patients with DLB had slowing in the EEG as compared to 6.7% (1/15) of HC (*P*-value = <0.001). Specifically, we found that half of the DLB patients (5/10) had disorganization or slowing of the background activity and 60% had

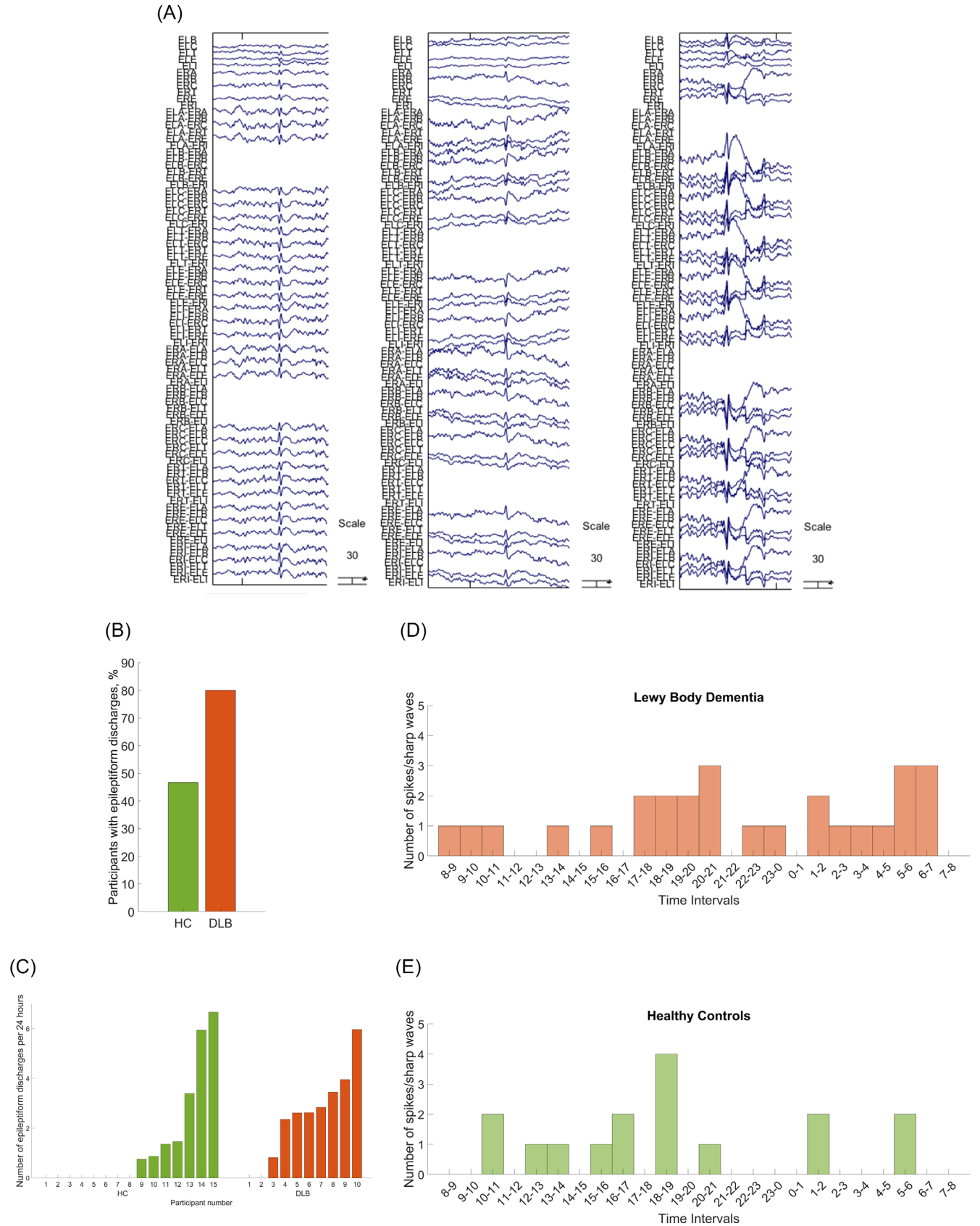


FIG. 2. Characterization of spikes/sharp waves in participants with dementia with Lewy bodies (DLB) compared to healthy controls (HC) at baseline. (**A**) Shows examples of spikes (<70 millisecond) in patients with DLB. (**B**) The proportion of patients with DLB and HC with epileptiform discharges. (**C**) Spike frequency (spikes/sharp waves per 24 hours) in each patient with DLB or HC with epileptiform discharges at baseline. Distribution of epileptiform discharges by time intervals for (**D**) for patients with DLB and (**E**) for HC. [Color figure can be viewed at wileyonlinelibrary.com]

focal slowing in one or both temporal lobes in one or more recordings. One patient with DLB had a low-voltage EEG throughout three recordings. No IED were found during standard EEG recordings that were usually performed in early afternoon when a higher probability for short sleep was expected. A comparison between scalp-EEG and ear-EEG can be found in the Supplementary Data S1. The findings from the patient diaries can also be found in the Supplementary Data S1.

Data Quality

At baseline, the mean number of recorded hours were 38.1 hours (standard deviation [SD], 10.5 hours) in patients with DLB as compared to 40.9 hours (SD, 9.5) in HC. The amount of preprocessed data was almost equal between patients with DLB with 28.2 hours (SD, 10.1 hours; range, 10.2–45.7 hours) as compared to HC with 27.7 hours (SD, 8.0 hours; range, 12.1–37.2 hours), P-value = 0.9, t-value = 0.2, df = 23. During the nighttime, 81.8% of the data in patients with DLB and 78.7% of data in HC remained after preprocessing as compared to the daytime with 66.1% of data in patients with AD and 63.9% of the data in HC.

Adverse Events at Baseline

A total of 70% of patients with DLB had adverse events related to the ear-EEG as compared to 60% for HC. In patients with DLB, 60% reported some difficulty sleeping during the baseline recording. There were no reports that earplugs were dislodged by accident during routine activities. None of the adverse events related to ear-EEG were classified as serious adverse events.

Discussion

This is the first study to provide data on the prevalence of IED in patients with DLB using long-term EEG monitoring. At baseline, 80% of patients showed IED with a significantly increased risk ratio of 2.5 for spike frequency as compared to HC. Most IED occurred at night in patients with DLB. Considerable variability was observed between visits in patients with DLB. A significant negative correlation ($\rho = -0.80$) between MMSE and spike frequency was found in DLB. The data quality of the ear-EEG was of the same magnitude in DLB and HC, and participants experienced only mild adverse events related to the ear-EEG.

This is the first study to investigate the prevalence of IED in patients with DLB. Multiple studies have found that a larger percentage of patients with AD without epilepsy have IED (22%–75%) on 24-hour EEG recordings as compared to HC.[6-8,21] In our study, there was no significant differences in the proportions of patients with DLB who had IED at baseline (80%) as compared to HC (46.7%), which we suspect might be because of the low sample size. That the majority of DLB patients had IED in our study was in contrast to no IED being found using short-term EEG recordings in the included DLB patients and that short-term conventional EEG recordings in a large mixed memory clinic cohort only found that 3% of participants had IED.[29] This is in line with studies showing an association between recording length and number of IED.[21,30] As the probability of detecting IED increases with the total observation time, long-term EEG recordings are important when investigating the use of antiepileptic medication in patients with DLB as to be able to measure a possible decrease in the occurrence of IED after antiseizure medication.

The spike frequency was significantly higher in patients with DLB as compared to HC at baseline,

TABLE 2 *Epileptiform discharges across ear-EEG recordings*

	1st ear-EEG recording	2nd ear-EEG recording	*P*-value (1st vs. 2nd)	3nd ear-EEG recording	*P*-value (1st vs. 3rd)
No. of patients	10	8		7	
No. with ED, %	80	38		71	
Spikes/24 h	2.63	0.69	0.029	5.32	0.002
Spikes, n	31	5		34	
Clean data, mean hours (SD)	28.24 (10.06)	21.88 (9.79)		21.92 (8.07)	
MMSE, mean (SD)	26.7 (1.83)	27 (3.02)	0.809	27.43 (3.05)	0.588
Time since last recording, mean (SD)		115.13 (44.71)		96.71 (37.01)	

Abbreviations: EEG, electroencephalography; ED, epileptiform discharges; h, hour; SD, standard deviation; MMSE, Mini-Mental State Examination.

which has also been found in patients with AD.[6-8,21] One explanation for the increased spike frequency could be depositions of α-synuclein, which has been shown to induce network excitability in transgenic mouse models[31] that can escalate into seizure activity.[32] In addition, one study found an association between the densities of Lewy bodies in the temporal lobes and visual hallucinations,[12] and visual hallucinations have been linked to IED in the temporal lobes in patients with Parkinson's disease.[13] However, because of a high frequency of amyloid deposition in DLB,[33,34] it is also possible that AD related pathology leads to IED in DLB patients (ie, that amyloid is the common denominator leading to IED) or that the neurodegenerative process per se leads to IED. However, studies in transgenic mouse models have found that α-synuclein aggregation alone is sufficient to cause network hyperexcitability.[10,31,32] It is also possible that more IED can be detected if measurements were made over the parietal or occipital cortex because of the location of neurodegeneration in patients with DLB.[11]

A tendency toward more IED occurring at night was seen in patients with DLB (Fig. 2). In DLB, multiple studies have shown that REM sleep behavior disorder is common[35] and studies using polysomnography has shown sleep fragmentation and shortened REM time.[36] Because REM sleep has been shown to possibly inhibit IED[37,38] and this process becomes altered in patients with DLB, it is likely to worsen or possibly lead to IED during the neurodegenerative processes. This is in line with a study using mouse models of α-synucleinopathy, which found increasing IED during non-REM sleep.[10] Because of the small sample size, there is a need for a larger study to confirm the findings.

Considerable variability between recordings was observed. It is possible that the variability is associated with cognitive fluctuations. A qualitative study suggested that fluctuation observed in DLB has a transient quality, which reflects an interruption of awareness.[39] Several theories have been proposed to explain the mechanism of fluctuations in patients with DLB.[40] One theory being atrophy of the anterior cingulate cortex and bilateral insular regions observed in patients with prodromal DLB, because these regions harbor neurons implicated in the integrity of attentional circuits.[41] Because of the location of the insular regions, it is plausible that the ear-EEG can capture IED originating from these areas. However, more studies are needed to understand if there is a link between fluctuations and IED in DLB.

Our study has several limitations. First, the number of participants was low, which limits the generalizability of the study, and more studies are needed to understand the prevalence of spikes in patients with DLB. However, findings are in line with studies in AD, which have also shown increased epileptiform activity. We found a relatively high spike frequency in HC. This was surprising and may be because of the long recordings or undetected neurodegenerative pathology. Furthermore, no significant differences were found for the participant characteristics for HC with and without epileptiform discharges. Because of the low spatial resolution of ear-EEG, it is possible that false positive epileptiform discharges were annotated leading to an overestimation both in HC and DLB. Future studies should carry out studies on the validity of epileptiform discharges in ear-EEG as compared to scalp-EEG to directly compare epileptiform discharges as recorded by ear-EEG and scalp-EEG. Furthermore, we did not have multiple recordings in HC, which makes it difficult to understand if variability between recordings is seen in participants without a neurodegenerative disease. Here, we did not have any clinical scales for measuring fluctuations and could not directly investigate the link with IED. Another important aspect is the lack of characterization of sleep stages. However, automatic sleep staging tools for ear-EEG has been developed,[42] but validation in older cognitively healthy controls and/or patients with neurodegenerative diseases is needed. Furthermore, because of the low spatial resolution of the ear-EEG, it is possible that IED were missed because IED occur at areas associated with neurodegeneration in DLB. However, we were able to conduct long-term outpatient EEG recordings in patients with DLB, which showed significantly higher spike frequency as compared to HC.

Conclusions

This is the first study to show that IED can be detected in patients with DLB using long-term outpatient ear-EEG monitoring. In patients with DLB, a significantly increased spike frequency was observed that mostly occurred at night. This supports previous findings in mouse models of α-synucleinopathy, which showed increased IED during sleep. Because IED have been shown to occur in both patients with AD and now DLB, it is likely that IED are an unspecific marker of neurodegeneration. However, studies are needed to understand if IED are more prevalent in other areas in DLB as compared to AD. We hypothesize that the variability between recordings could be associated with cognitive fluctuations as seen in DLB. Because recent clinical trials have shown promising effect of anti-seizure medication in patients with AD and IED, this study shows the relevance of investigating the effect of anti-seizure medication in patients with DLB and IED.

Acknowledgments: We thank specialist nurses Susanne Kristiansen and Oda Jakobsen for their assistance in recruiting participants and helping during study visits.

Data Availability Statement

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

References

1. 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;60:211–223.
2. Sun L, Cao J, Chu FN, Wang Z, Lv Y. Dementia with Lewy bodies versus nonconvulsive status epilepticus in the diagnosis of a patient with cognitive dysfunction, complex visual hallucinations and periodic abnormal waves in EEG: a case report. BMC Neurol 2014; 14:112.
3. Tun MZ, Soo WK, Wu K, Kane R. Dementia with Lewy bodies presenting as probable epileptic seizure. BMJ Case Rep 2017;2017: bcr2017221454.
4. Park IS, Yoo SW, Lee K-S, Kim J-S. Epileptic seizure presenting as dementia with Lewy bodies. Gen Hosp Psychiatry 2014;36:230.e3–230.e5.
5. Matar E, Shine JM, Halliday GM, Lewis SJG. Cognitive fluctuations in Lewy body dementia: towards a pathophysiological framework. Brain 2020;143:31–46.
6. Vossel KA, Ranasinghe KG, Beagle AJ, et al. Incidence and impact of subclinical epileptiform activity in Alzheimer's disease. Ann Neurol 2016;80:858–870.
7. Lam AD, Sarkis RA, Pellerin KR, et al. Association of epileptiform abnormalities and seizures in Alzheimer disease. Neurology 2020; 95:e2259–e2270.
8. Horvath AA, Papp A, Zsuffa J, et al. Subclinical epileptiform activity accelerates the progression of Alzheimer's disease: a long-term EEG study. Clin Neurophysiol Off J Int Fed Clin Neurophysiol 2021;132: 1982–1989.
9. Vossel K, Ranasinghe KG, Beagle AJ, et al. Effect of Levetiracetam on cognition in patients with Alzheimer disease with and without Epileptiform activity: a randomized clinical trial. JAMA Neurol 2021;78:1345–1354.
10. 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 α-Synuclein. Front Neurol 2020;11:563.
11. Mak E, Su L, Williams GB, O'Brien JT. Neuroimaging characteristics of dementia with Lewy bodies. Alzheimers Res Ther 2014;6:18.
12. Harding AJ, Broe GA, Halliday GM. Visual hallucinations in Lewy body disease relate to Lewy bodies in the temporal lobe. Brain 2002; 125:391–403.
13. Fry A, Singh D, Manganas L, Gordon ML, Christodoulou C, Leung H-C, Schwartz GJ. Parkinson's disease with visual hallucinations is associated with Epileptiform activity on EEG. Front Neurol 2022;12:788632.
14. Kappel SL, Rank ML, Toft HO, Andersen M, Kidmose P. Dry-contact electrode ear-EEG. IEEE Trans Biomed Eng 2019;66: 150–158.
15. Looney D, Kidmose P, Park C, Ungstrup M, Rank M, Rosenkranz K, Mandic D. The In-the-ear recording concept: user-centered and wearable brain monitoring. IEEE Pulse 2012;3:32–42.
16. Looney D, Park C, Kidmose P, Rank ML, Ungstrup M, Rosenkranz K, Mandic DP. An in-the-ear platform for recording electroencephalogram; 2011. In *2011 Annual International Conference of the IEEE Engineering in Medicine and Biology Society* IEEE.
17. Musaeus CS, Waldemar G, Andersen BB, et al. Long-term EEG monitoring in patients with Alzheimer's disease using ear-EEG: a feasibility study. J Alzheimers Dis 2022;90(4):1713-1723. doi: 10.3233/JAD-220491
18. Zibrandtsen IC, Kidmose P, Christensen CB, Kjaer TW. Ear-EEG detects ictal and interictal abnormalities in focal and generalized epilepsy-a comparison with scalp EEG monitoring. Clin Neurophysiol 2017;128:2454–2461.
19. McKeith IG, Dickson DW, Lowe J, et al. Diagnosis and management of dementia with Lewy bodies. Neurology 2005;65:1863–1872.
20. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975;12:189–198.
21. Musaeus CS, Frederiksen KS, Andersen BB, et al. Detection of subclinical epileptiform discharges in Alzheimer's disease using long-term out-patient EEG monitoring. Neurobiol Dis 2023;183:106149. doi:10.1016/j.nbd.2023.106149
22. Pfeffer RI, Kurosaki TT, Harrah CH, Chance JM, Filos S. Measurement of functional activities in older adults in the community. J Gerontol 1982;37:323–329.
23. Cummings JL, Mega M, Gray K, Rosenberg-Thompson S, Carusi DA, Gornbein J. The neuropsychiatric inventory: comprehensive assessment of psychopathology in dementia. Neurology 1994; 44:2308.
24. Vogel A, Stokholm J, Andreasen R, et al. Psychometric properties and reference data for Danish versions of free and cued selective reminding test. Category Cued Memory Test and Logical Memory 2018;59:496–502.
25. Mikkelsen KB, Kappel SL, Mandic DP, Kidmose P. EEG recorded from the ear: characterizing the ear-EEG method. Front Neurosci 2015;9:438.
26. Kidmose P, Looney D, Mandic DP. Auditory evoked responses from Ear-EEG recordings; 2012. In *2012 Annual International Conference of the IEEE engineering in Medicine and Biology Society* IEEE.
27. Delorme A, Makeig S. EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. J Neurosci Methods 2004;134:9–21.
28. Kural MA, Duez L, Sejer Hansen V, et al. Criteria for defining interictal epileptiform discharges in EEG. Neurology 2020;94:e2139–e2147.
29. Liedorp M, Stam CJ, van der Flier WM, Pijnenburg YAL, Scheltens P. Prevalence and clinical significance of Epileptiform EEG discharges in a large memory clinic cohort. Dement Geriatr Cogn Disord 2010;29:432–437.
30. Horváth A, Szűcs A, Barcs G, Kamondi A. Sleep EEG detects Epileptiform activity in Alzheimer's disease with high sensitivity. J Alzheimers Dis 2017;56:1175–1183.
31. Tweedy C, Kindred N, Curry J, et al. Hippocampal network hyperexcitability in young transgenic mice expressing human mutant alpha-synuclein. Neurobiol Dis 2021;149:105226.
32. Morris M, Sanchez PE, Verret L, et al. Network dysfunction in α-synuclein transgenic mice and human Lewy body dementia. Ann Clin Transl Neurol 2015;2:1012–1028.
33. Nelson PT, Jicha GA, Kryscio RJ, et al. Low sensitivity in clinical diagnoses of dementia with Lewy bodies. J Neurol 2010;257: 359–366.
34. Kim WS, Kågedal K, Halliday GM. Alpha-synuclein biology in Lewy body diseases. Alzheimers Res Ther 2014;6:73.
35. Chan P-C, Lee H-H, Hong C-T, Hu C-J, Wu D. REM sleep behavior disorder (RBD) in dementia with Lewy bodies (DLB). Behav Neurol 2018;2018:9421098.
36. Bugalho P, Salavisa M, Marto JP, Borbinha C, Alves L. Polysomnographic data in dementia with Lewy bodies: correlation with clinical symptoms and comparison with other α-synucleinopathies. Sleep Med 2019;55:62–68.
37. Frauscher B, von Ellenrieder N, Dubeau F, Gotman J. EEG desynchronization during phasic REM sleep suppresses interictal epileptic activity in humans. Epilepsia 2016;57:879–888.
38. Campana C, Zubler F, Gibbs S, et al. Suppression of interictal spikes during phasic rapid eye movement sleep: a quantitative stereo-electroencephalography study. J Sleep Res 2017;26:606–613.
39. Bradshaw J, Saling M, Hopwood M, Anderson V, Brodtmann A. Fluctuating cognition in dementia with Lewy bodies and Alzheimer's disease is qualitatively distinct. J Neurol Neurosurg Psychiatry 2004;75:382–387.

40. O'Dowd S, Schumacher J, Burn DJ, Bonanni L, Onofrj M, Thomas A, Taylor J-P. Fluctuating cognition in the Lewy body dementias. Brain 2019;142:3338–3350.

41. Cauda F, Torta DME, Sacco K, et al. Functional anatomy of cortical areas characterized by Von Economo neurons. Brain Struct Funct 2013;218:1–20.

42. Mikkelsen KB, Tabar YR, Kappel SL, et al. Accurate whole-night sleep monitoring with dry-contact ear-EEG. Sci Rep 2019;9:16824.

Supporting Data

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

SGML and CITI Use Only
DO NOT PRINT

Author Roles

(1) Research project: A. Conception, B. Organization, C. Execution; (2) Statistical Analysis: A. Design, B. Execution, C. Review and Critique; (3) Manuscript: A. Writing of the First Draft, B. Review and Critique.

Christian Sandøe Musaeus: 1A, 1B, 1C, 2A, 2B, 2C, 3A, 3B
Troels Wesenberg Kjær: 1A, 1B, 1C, 2A, 2B, 3B
Melita Cacic Hribljan: 1A, 1B, 1C, 3B
Birgitte Bo Andersen: 1A, 1B, 1C, 3B
Peter Høgh: 1A, 1B, 1C, 3B
Preben Kidmose: 1A, 1B, 1C, 2A, 3B
Martin Fabricius: 1A, 1B, 1C, 3B
Martin Christian Hemmsen: 1A, 1B, 1C, 3B
Mike Lind Rank: 1A, 1B, 1C, 3B
Gunhild Waldemar: 1A, 1B, 1C, 2A, 3B
Kristian Steen Frederiksen: 1A, 1B, 1C, 2A, 3B

Supplementary materials to: "Subclinical epileptiform activity in patients with Lewy body dementia"

Reason for dropping out of the study:

- Discomfort with ear-EEG (n = 1)
- Too difficult for the participant to complete study (n = 1)
- Diagnosed with subdural hematoma during clinical visit (n = 1)

The questionnaire included the following questions:

a) how did it feel to wear the ear-EEG

b) did you have any problems sleeping with the equipment

c) did the ear-EEG ever fall out of the ear

d) do you have any suggestions for improvement.

Question a) and b) was used to evaluate if the participant had experienced any adverse events together with the examination of the ears.

Specification of conventional EEG recording and assessment: This consisted of 10 minutes of alternating eyes open, and eyes closed (30 seconds each) followed by 20 minutes of sleep. If the participants got drowsy, the length of the eyes open/closed segments were adjusted. The EEGs were recorded using Nicolet One EEG (Nervus) recording software 5.82 (Natus) with a standard 44-channel headbox. Each subject was fitted with a cap using silver-silver-chloride-coated electrodes. The EEGs was recorded from 19 electrodes positioned according to the International 10-20 system. For impedance, the aim was to reach below 10 kOhm for all electrodes during the recordings.

The EEGs were assessed by an experienced clinical neurophysiologist (MCH) who was blinded to the diagnosis. The results from the EEGs were either classified as 1) Normal EEG, 2) Slowing with subclassification into focal slowing as defined by intermittent or persistent low frequency theta or delta activity and/or diffuse slowing as defined by a background activity below 7 Hz or disorganized background defined as a lack of waveforms in the background forming a sinusoidal, approximately monofrequent rhythm [1] and 3) abnormal EEG with IED.

Reference:

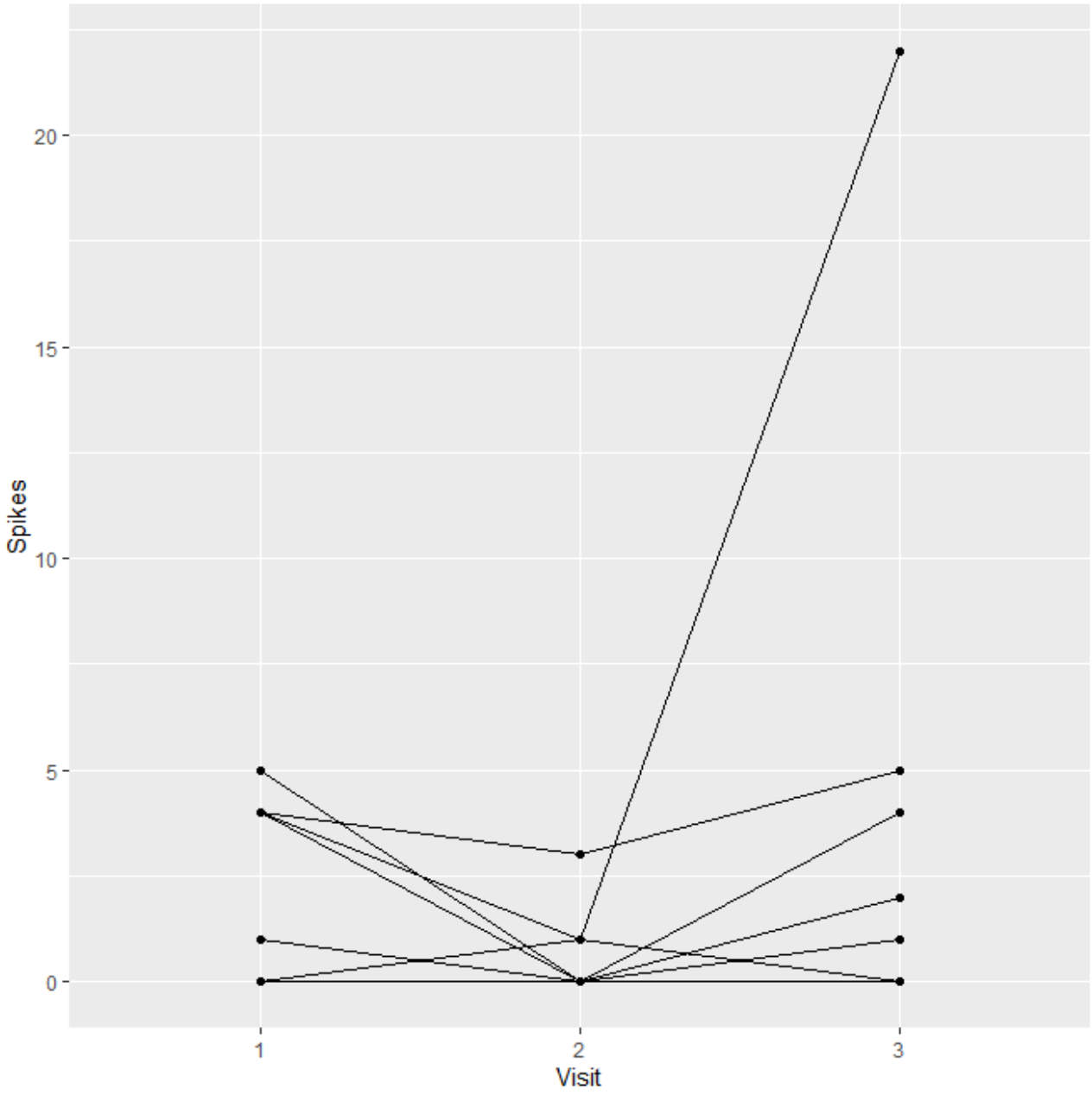
[1] Kane N, Acharya J, Benickzy S, Caboclo L, Finnigan S, Kaplan PW, Shibasaki H, Pressler R, van Putten MJAM (2017) A revised glossary of terms most commonly used by clinical electroencephalographers and updated proposal for the report format of the EEG findings. Revision 2017. *Clin Neurophysiol Pract* **2**, 170–185.

Montage for reviewing ear-EEG

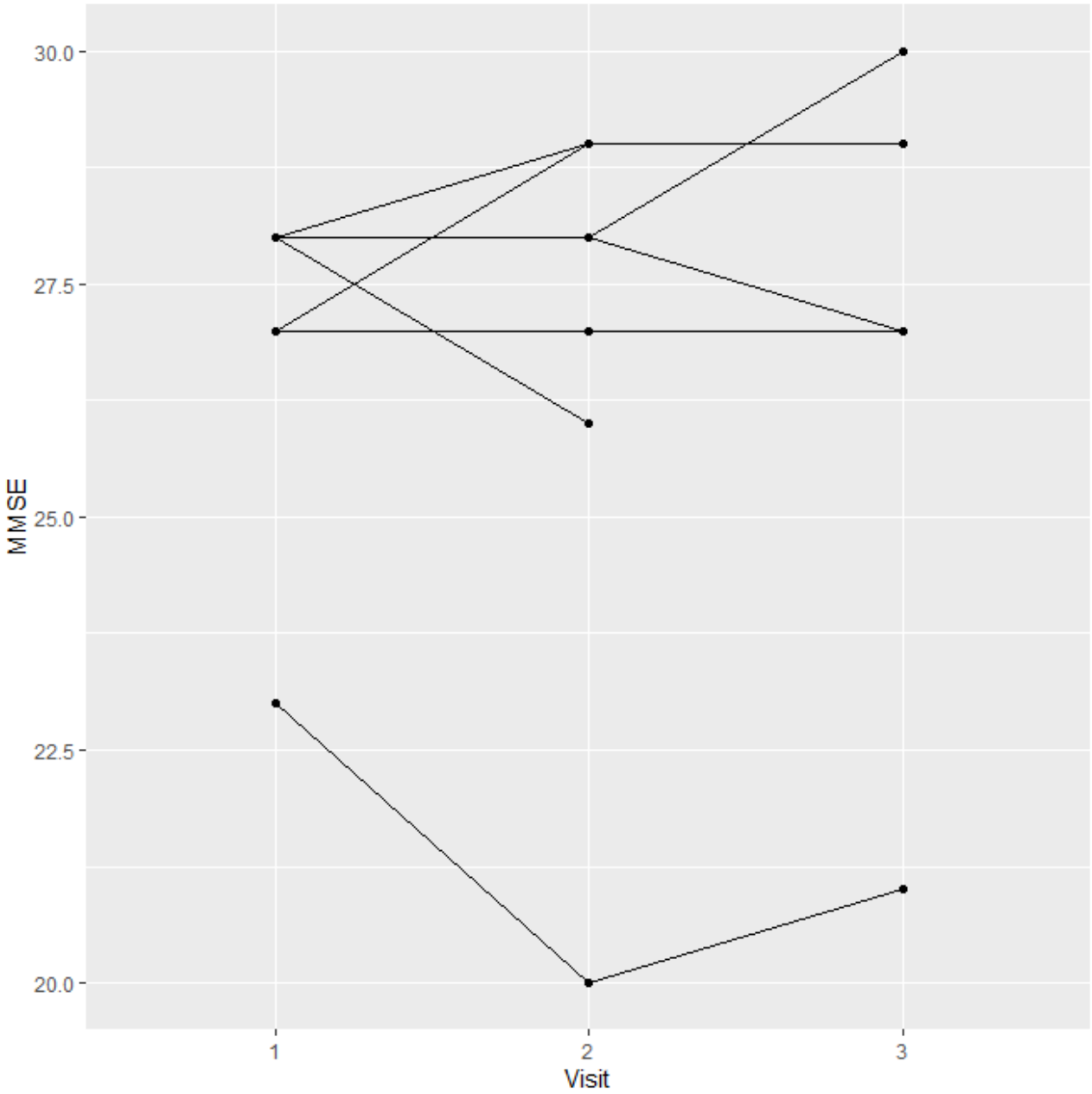
The average referenced montage was used and 10 second epochs were inspected at a time with ±30 µV amplitude range. In this montage, the first 12 channels were low-pass filtered at 70 Hz, high-pass filtered at 1 Hz, and notch filtered at 50 Hz. The next 12 channels were after pre-processing. The subsequent 36 channels were the comparisons between electrodes with the active electrodes being on the left (e.g., ELA-ERA, ELA-ERB [2]) and the last 36 channels were created using the opposite laterality. The segments disregarded by the pre-processing pipeline and segments only containing recordings from one ear were not inspected.

Reference:

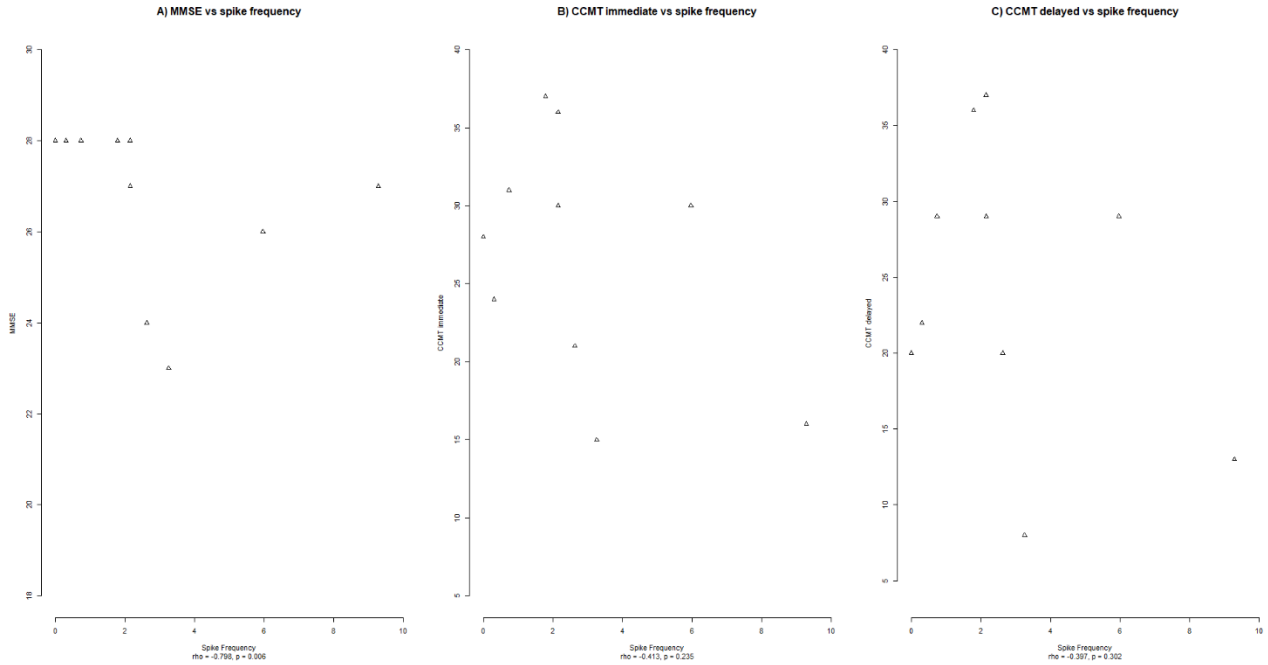
[2] Kidmose P, Looney D, Mandic DP (2012) Auditory evoked responses from Ear-EEG recordings. In *2012 Annual International Conference of the IEEE engineering in Medicine and Biology Society* IEEE.



Supplementary figure 1: Spaghetti plot showing the number of spikes/24 hours of recording.



Supplementary figure 2: Spaghetti plot showing the MMSE.



Supplementary figure 3: Spearman's correlations between spike frequency and MMSE, immediate recall, and delayed recall from the CCMT.

Results from patient diaries

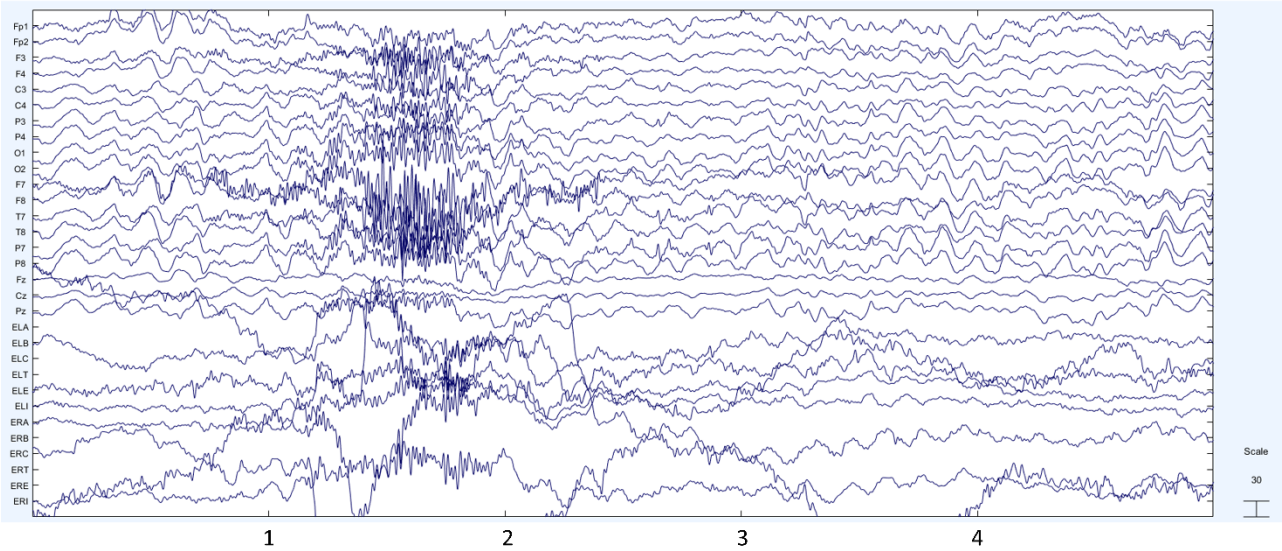
As for the patient diaries, we received the following reports regarding three separate patients: 1) a caregiver reported minor jerks of the patients' body during sleep lasting only a few seconds, 2) a patient reported visual hallucinations at night and 3) the caregiver reported three instances of suspected symptoms: a) a major jerk of the body in the morning, b) a caregiver reported that the patient reported seeing family members who were not present and that the caregiver interpreted as hallucinations and c) the patient reported an unfamiliar taste in the mouth.

A comparison of the signal between ear-EEG and scalp-EEG

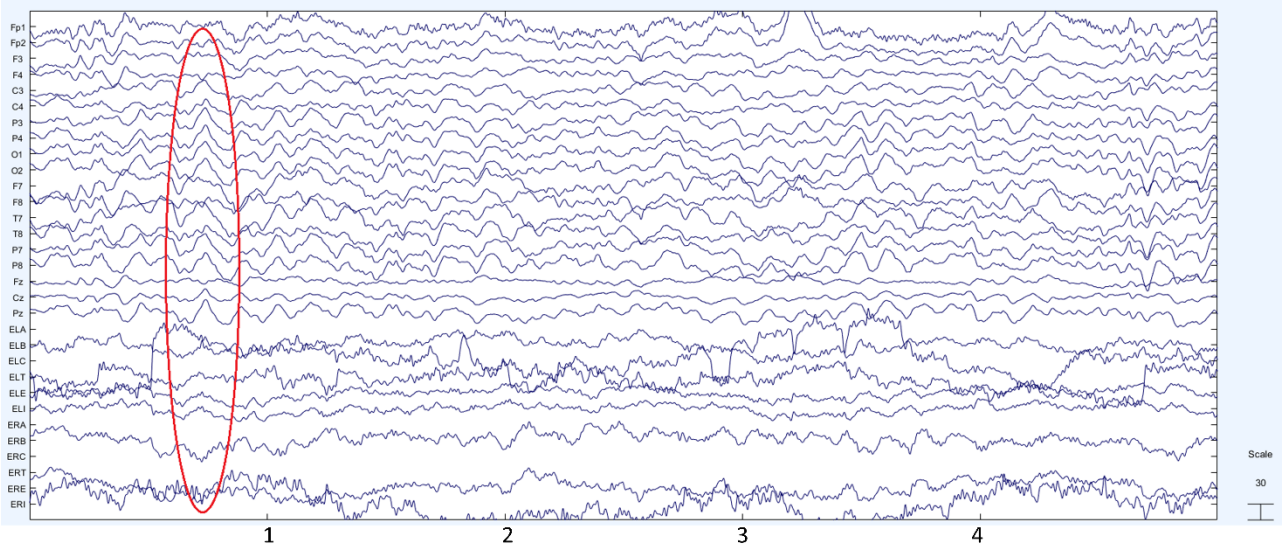
During the co-registered recordings of ear-EEG and scalp-EEG, we did not use the same equipment/amplifier, which led to the signals not being synchronized. As a result, we used both the manual marking in the scalp EEG, clear artifacts in both the ear-EEG and scalp EEG as well as different functions in MATLAB to support finding the alignment delay. Therefore, we can not with absolute certainty confirm that the segments are completely aligned.

In the current examples, both signals are bandpass filtered form 0.5-70Hz and the ear-EEG signal is resampled to the lower sampling rate of the scalp EEG. Examples of comparison between the signals can be seen below using a combination of manual and automatic alignment as stated above. The examples show artifacts, 7-8 Hz activity and slowing.

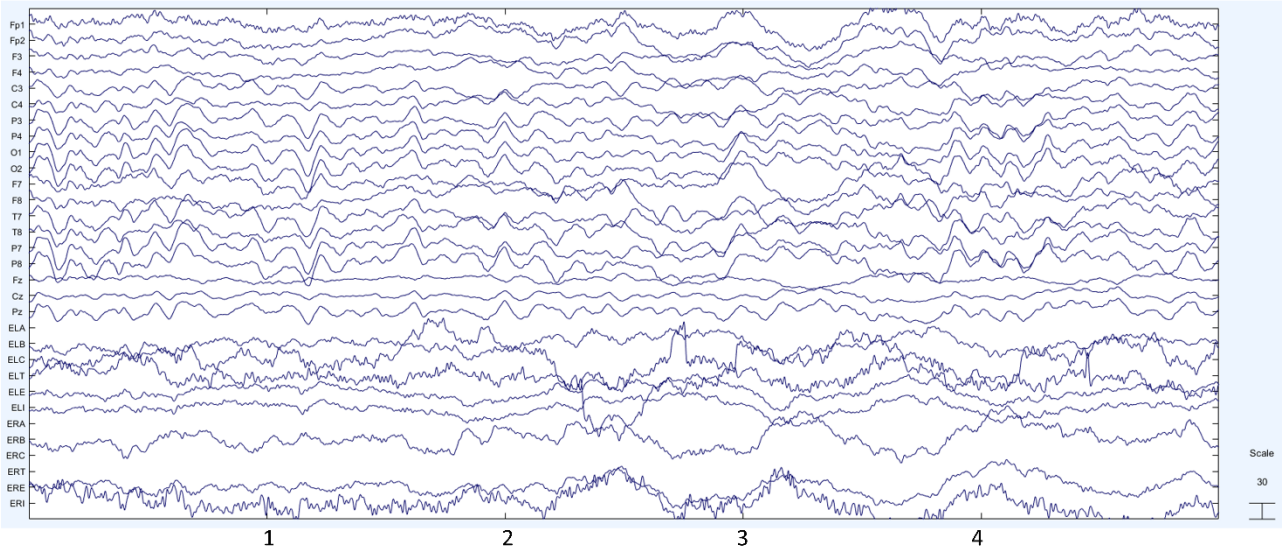
Example #1: Artifact seen in both scalp and ear-EEG



Example #2: 7-8 Hz activity seen in both scalp and ear-EEG (window is 5 seconds)



Example #3: Theta/delta activity seen in both the scalp EEG as well as the ear-EEG



Comparison of the HC with and without epileptiform discharges

Supplementary table 1: Participant characteristics between HC and epileptiform discharges compared to HC without.

	HC with ED	HC without ED	p-value
Number of participants, n	7	8	
Age, mean (SD)	73.0 (5.57)	66.5 (8.75)	0.132
Males/females	5/2	3/5	0.315
Education, mean (SD)	14.57 (2.3)	15.75 (1.75)	0.303
MMSE, mean (SD)	29.0 (1.0)	29.5 (0.76)	0.339
CCMT, immediate recall, mean (SD)	37.86 (4.67)	38.38 (5.58)	1
CCMT, delayed recall, mean (SD)	36.0 (5.1)	38.25 (6.27)	0.448
CCMT, recognition, mean (SD)	47.71 (0.49)	47.37 (0.74)	0.385

ED: Epileptiform discharges, MMSE: Mini-Mental State Examination, CCMT: Category Cued Memory Test

Manuscript IV

Subclinical epileptiform discharges in Alzheimer's disease are associated with increased hippocampal blood flow

Musaeus CS, Kjær TW, Lindberg U, Vestergaard MB, Larsson HBW, Press DZ, Andersen BB, Høgh P, Kidmose P, Hemmsen MC, Rank ML, Hasselbalch SG, Waldemar G, Frederiksen KS. Subclinical epileptiform discharges in Alzheimer's disease are associated with increased hippocampal blood flow (submitted).

Subclinical epileptiform discharges in Alzheimer's disease are associated with increased hippocampal blood flow

Christian Sandøe Musaeus[a*], Troels Wesenberg Kjær[a], Ulrich Lindberg[c], Mark B. Vestergaard[c], Henrik Bo Wiberg Larsson[c], Daniel Zvi Press[f], Birgitte Bo Andersen[a], Peter Høgh[b,d], Preben Kidmose[e], Martin Christian Hemmsen[g], Mike Lind Rank[g], Steen Gregers Hasselbalch[a,d], Gunhild Waldemar[a,d], Kristian Steen Frederiksen[a,d]

[a] Danish Dementia Research Centre (DDRC), Department of Neurology, Copenhagen University Hospital – Rigshospitalet, Inge Lehmanns vej 8, 2100 Copenhagen, Denmark

[b] Regional Dementia Research Centre, Department of Neurology, Zealand University Hospital, Vestermarksvej 11, 4000 Roskilde, Denmark

[c] Functional Imaging Unit, Department of Clinical Physiology and Nuclear Medicine, University of Copenhagen, Valdemar Hansens Vej 13, 2600 Glostrup, Denmark

[d] Department of Clinical Medicine, University of Copenhagen, Blegdamsvej 3B, 2200 Copenhagen, Denmark

[e] Department of Electrical and Computer Engineering, Aarhus University, Finlandsgade 22, 8200 Aarhus N, Denmark

[f] Berenson-Allen Center for Non-invasive Brain Stimulation, Beth Israel Deaconess Medical Center, Harvard Medical School, 330 Brookline Ave, Boston, MA 02215, USA

[g] T&W Engineering, Borupvang 2, 3450 Lillerød, Denmark

Running title: Association between rCBF and epileptiform activity

***** Corresponding author:

Christian Sandøe Musaeus

Danish Dementia Research Centre

Copenhagen University Hospital – Rigshospitalet

Inge Lehmanns Vej 8

2100 Copenhagen

Denmark

E-mail address: Christian.sandoee.musaeus@regionh.dk

Abstract

Background: In epilepsy, the ictal phase leads to cerebral hyperperfusion while hypoperfusion is present in the interictal phases. Patients with Alzheimer's disease (AD) have an increased prevalence of epileptiform discharges and a study using intracranial electrodes have shown that these are very frequent in the hippocampus. However, it is not known whether there is an association between hippocampal hyperexcitability and regional cerebral blood flow (rCBF). The objective of the study was to investigate the association between rCBF in hippocampus and epileptiform discharges as measured with ear-EEG in patients with Alzheimer's disease. Our hypothesis was that increased spike frequency may be associated with increased rCBF in hippocampus

Methods: A total of 24 patients with AD, and 15 HC were included in the analysis. Using linear regression, we investigated the association between rCBF as measured with arterial spin-labelling MRI (ASL-MRI) in the hippocampus and the number of spikes/sharp waves per 24 hours as assessed by ear-EEG.

Results: A significant linear association between spike frequency and normalized rCBF in the hippocampus was found for patients with AD (estimate: 0.109, t-value = 4.03, p-value <0.001). Changes in areas that typically show group differences (temporal-parietal cortex) were found in patients with AD, compared to HC.

Conclusions: Increased spike frequency was accompanied by a hemodynamic response of increased blood flow in the hippocampus in patients with AD. This phenomenon has also been shown in patients with epilepsy and supports the hypothesis of hyperexcitability in patients with AD. Hippocampal rCBF may serve as a marker of hyperexcitability in patients with AD.

Trial registration: The study is registered at clinicaltrials.gov (NCT04436341).

Keywords: Alzheimer's disease; hyperperfusion; epileptiform discharges; spike frequency; EEG

Background

Patients with Alzheimer's disease (AD) and dementia with Lewy bodies (DLB) are at an increased risk of developing epileptic seizures (1–7). A common indicator of seizures are epileptiform discharges identified with electroencephalography (EEG). Recently, studies using long-term EEG monitoring have found an increased frequency of epileptiform discharges in patients with AD as compared to healthy controls (HC) (8–10), and those with discharges showed accelerated disease progression (8,10).

In patients with temporal lobe epilepsy, ictal activity leads to increased regional cerebral blood flow (11–13), while interictal periods are associated with a decreased rCBF (14–16). Although studies in AD have found epileptiform discharges (8–10), which are often associated with epilepsy, this may also be due to underlying hyperexcitability. As hyperexcitability can be defined as an increased probability of neuronal activation by a certain stimulus (17), it may be less transient compared to ictal activity and may underly the increased number of epileptiform discharges. In general, most studies that measured the rCBF changes in patients with AD have shown a decreased rCBF in the regions affected by neurodegeneration, which includes the hippocampus (18–21). However, a few studies found increased rCBF in the temporal lobes including the hippocampus (22,23). It is unclear whether the conflicting results can be attributed to a biological difference as opposed to methodological differences but if so, it may be due to varying levels of hyperexcitability. Although the epileptiform discharges seen in AD only involve spikes/sharp waves and not ictal activity (8–10) the latter beingis characterized by a sustained and rhythmic pattern (24), it is possible that a similar hemodynamic response may underly epileptiform discharges. This is supported by a study showing hippocampal hyperexcitability (25) but this association has not been investigated.

Ear-EEG is a new method for long-term EEG recording in the out-patient setting. Ear-EEG is a method where EEG signals are recorded from electrodes placed within a customized earpiece inserted into each ear allowing the individual to engage in their daily routine. (26–28). The signals recorded using ear-EEG has been shown to correspond to the temporal electrodes on scalp EEG (26) and a study in patients with temporal lobe epilepsy has shown that epileptiform discharges can be detected with ear-EEG (29). Recently, the ear-EEG has been shown to be safe and feasible in patients with AD (30) and a recent study using the same cohort as presented here have found a significantly increased spike frequency in AD (31). Arterial spin labeling MRI (ASL-MRI) is a non-invasive imaging technique that allows for quantitative measurements of rCBF (19,20,32–36). By combining these techniques, it is possible to understand the relationship between epileptiform discharges and rCBF in the hippocampus.

The aim of this study was to investigate the association between rCBF in hippocampus and the frequency of epileptiform discharges in patients with AD as measured with ear-EEG. The reason for hippocampus being investigated was due to previous finding of hippocampal hyperexcitability in patients with AD (25). Our hypothesis was that increased spike frequency as a measure of hyperexcitability was associated with increased rCBF in hippocampus as seen in patients with epilepsy.

Methods

Participants

In the current study, patients with mild to moderate AD, and healthy controls (HC) were recruited from the Memory Clinic at Copenhagen University Hospital – Rigshospitalet and the Memory Clinic at Zealand University Hospital, Roskilde. Patients with AD met the NIA-AA criteria for probable AD with amnestic presentation (37) with the diagnosis being determined based on a consensus conference. The consensus conference included information from structural imaging and, in most instances, [^{18}F]FDG positron emission tomography (PET). Some patients underwent lumbar puncture (n_{AD}=18) with evaluation of amyloid-β42, phosphorylated tau, and total tau while some underwent a [^{11}C] Pittsburgh compound-B-PET scan (n_{AD}=2) or both (n_{AD}=3) as part of the clinical work-up.

In patients with AD, the inclusion criteria included 1) a mini-mental state examination (MMSE) (38) score of 16-28, 2) age between 50-90 years, 3) native Danish speaker, 4) at least 7 years of education, 5) hearing and vision sufficient for neuropsychological examination, 6) no alcohol or drug abuse within the last two years, 7) no contraindications for MRI, 8) an MRI or CT scan that supported the diagnosis of AD, 9) the general health conditions of the patient allowed participation in the study (as judged by the principal investigator), and 10) living with a caregiver who was able to assist the patient with the home EEG recordings.

The following exclusion criteria were applied: 1) epilepsy prior to the diagnosis of AD, 2) focal pathology (except AD related atrophy) in the hippocampus, i.e. hippocampal sclerosis, 3) living with a relative with serious illness or impaired activities of daily living since the participant may need help to participate in the study, 4) living in a nursing home, 5) psychiatric (except mild depression) or neurological conditions that affects the brain except AD, 6) currently treated with anti-epileptic

medication, tricyclic antidepressants or antipsychotics, 7) daily or almost daily administration of medication with known anticholinergic or adrenergic effect, which may affect cognitive abilities or EEG, 8) large cerebral infarctions or more than four lacunar infarctions on MRI, 9) suffering from facial tics/facial hyperkinetic disorders or 10) daily use of hearing aids.

The HC were recruited from a pool of participants in other studies who had expressed interest in participating in new studies. The inclusion criteria were 1) normal cognition (as judged by the principal investigator), 2) a general health compatible with participation in the study as well as criteria 2-7 as applied in patients with AD. The following exclusion criteria were applied: 1) diagnosed with epilepsy, 2) focal pathology in the brain (except mild hippocampal atrophy) as well as exclusion criteria 5-10 as applied in patients with AD but without AD.

The study was approved by the Capital Region Ethics Committee (H-17035751), and by the Danish Medicines Agency (2017112288), and registered at the Data Protection Agency (P-2021-866). All participants gave written and oral informed consent before participating in the study. The study is registered at clinicaltrials.gov (NCT04436341).

Study design

In this cross-sectional study, a total of four visits were planned for patients with AD, and HC, see Figure 1.

At visit 1, informed consent was obtained followed by assessment of medical history, a physical and neurological examination, the Mini-Mental State Examination (MMSE) (for assessment of global cognitive function) and an imprint of the ears using Otoform A Soft X (Dreve, Germany), a soft ear

impression silicone. Subsequently, the patient underwent the following: visit 2) MRI scan (either before or after the ear-EEG recording), visit 3) standard EEG recording together with ear-EEG, Functional Assessment Questionnaire IADL (FAQ IADL) (39) (to assess everyday function), and the neuropsychiatric inventory (NPI) (40) (to assess behavioral and psychological symptoms).

Ear-EEG recording and review

The participants underwent up to two days of out-patient ear-EEG recording. A full description of the ear-EEG equipment and pre-processing can be found in the supplementary material.

A sharp asymmetric negative potential of 20-200 ms duration was considered an epileptiform discharge (spike/sharp wave) if it was clearly distinct from ongoing background activity and unlikely to be artifactual (41). Due to the nature of ear-EEG, we could not investigate the spatial distribution of the spikes/sharp wave. All annotations performed by CSM underwent review by a board-certified clinical neurophysiologist (TWK), who made the final ruling. The term epileptiform discharges cover both spikes and sharp waves and assumes an underlying irritative process as seen in epilepsy, even if this cannot be stated with absolute certainty. Both CSM and TWK were blinded to the diagnosis when reviewing the EEGs.

The spike frequency was calculated by dividing the number of spikes by the amount of time (in days) when data from at least one electrode in each ear was being recorded.

The results from the ear-EEG recordings from the patients with AD and HC have been presented elsewhere (31).

MRI acquisition

All scans were recorded on a 3T Achieva dStream (Philips, Best, The Netherlands) with a 32-channel head receive coil.

Structural image acquisition and analysis

A sagittal 3D T1-weighted magnetization prepared rapid acquisition gradient echo (T1-MPRAGE) was recorded with the following acquisition parameters: repetition time (TR) 6.9 ms, echo time (TE) 2.82 ms, flip angle 9, matrix size 256x255, 155 slices , voxel size 1.1×1.1×1.1 mm^3, The T1-weighted images were segmented using Freesurfer (version 7.2.0, https://surfer.nmr.mgh.harvard.edu/) and the volumes for each hippocampus and the inferior lateral ventricles were obtained. Both were normalized to the intracranial volume (ICV). The estimated total intracranial volume (eTIV) generated by FreeSurfer was used as an estimate for ICV in this study as has previously been shown (42).

Phase contrast mapping

The mean global cerebral blood flow (CBF) was obtained using velocity sensitive phase contrast mapping (PCM) MRI (43,44). Blood velocity contrast maps were acquired by a turbo field echo sequence. Measurements were acquired from an imaging plane perpendicular to the carotid arteries and one perpendicular to the basilar artery.

The blood flow in both internal carotids and the basilar artery was calculated by multiplying the mean blood velocity by the cross-sectional area from regions of interest defining each vessel. The global mean CBF was calculated by normalizing the total blood flow from each artery to the total brain

weight, which was estimated from the segmentation of the structural MRI images with an assumed brain density of 1.05 g/mL. Calculations were performed using a custom-built script in Python (https://github.com/MarkVestergaard/PCMCalculator).

Arterial spin labeling MRI analysis

A pseudo continuous ASL (PCASL) sequence with Look-Locker Echo Planar Imaging was chosen. The labeling plane was placed across the neck 9 cm beneath the center of the imaging slab and the labeling duration was 1650 ms. The acquisition parameters were: 13 slices, TE 10.8 ms, voxel size 3.44x3.44x6.6 mm^3, FoV 220x220x85 mm^3, TR was 300 ms, Look-Locker Flip-Angle 40º, slice acquisition duration 22 ms, SENSE factor 2.3, The post-labeling delays were set at [100, 400, 700, 1000, 1300, 1600, 1900 ms]. Each ASL pair (label and control) took 8 seconds. In the current study, the region of interest was the hippocampus, which resulted in the acquisition plane being placed parallel to the inferior lateral ventricles. After each ASL scan, a single equilibrium magnetization scan (M0) was acquired with the same parameters as the previously described ASL images except for a 10,000 ms TR.

ASL images were quantified using BASIL in FSL (FMRIB software library, version 6.0.5.1, www.fmrib.ox.ac.uk) with quantification (45) and fitting of the macrovascular compartment (46). The initial prior of bolus arrival time was adjusted to account for the delayed arrival as seen in our sample, which resulted in a selected arrival time prior of 1.6 s. Lastly, the ASL data were registered to the T1wscan. The ASL quantification was not corrected for differences in hematocrit values.

To quantify rCBF in the hippocampus, we first extracted all the values within the hippocampus ROI segmented with FreeSurfer and then removed the voxels with zero values as these were assumed to be contaminated by CSF. Afterwards, any values more than two standard deviations above the mean were removed since they were assumed most likely to represent arteries. Finally, the median value of CBF was extracted for each hippocampus and normalized to the global mean CBF as measured with PCM and the mean of the two values were computed. Due to the both the structural and functional connectivity between the hippocampus and the precuneus (47), we wanted to investigate if a similar association between spike frequency and rCBF in precuneus was present. Here, the same approach for computing the normalized rCBF in the precuneus was used. No other regions were investigated.

Statistics

The statistical analyses were performed in RStudio (v1.2.1335). When comparing age, education, MMSE, and the time difference between the ear-EEG recording and MRI scan, we performed t-test between AD and HC. Chi-squared tests was performed for testing for sexual distribution.

When comparing the hippocampus volume, global cerebral blood flow, and rCBF in the hippocampus or precuneus, we performed a t-test between AD and HC. Here, the distribution of the data as well as variance between groups were investigated before performing t-tests.

When comparing the number of spikes or sharp waves/24 hours (spike frequency) between HC, and AD, we calculated the rate ratio using the function *rateratio* from the *epitools* toolbox.

Simple linear regression was used to test if spike frequency significantly predicted the normalized mean rCBF in the hippocampus in patients with AD. In the exploratory analysis, we tested if the spike

frequency was associated with the normalized rCBF in the precuneus and whether a similar association between rCBF and spike frequency could be seen in HC.

The R code and output from the subsequent analyses can be found in the supplementary material.

When conducting the voxel-to-voxel analysis to compare normalized rCBF between HC and AD, we performed two-sample unpaired t-tests in randomize from FSL with cluster correction.

Results

Patient characteristics

A total of 25 patients with AD, and 15 HC were included. One patient with AD had less than one hour of ear-EEG data after pre-processing and was not included in the linear regression analyses. The average time from MRI scan to ear-EEG recording was 19.18 days and was not significantly different between groups (p-value = 0.332). Both left and right hippocampus were significantly smaller in patients with AD as compared to HC (p-value<0.05). See table 1 for baseline demographics.

Spike frequency measured with ear-EEG

The spike frequency was significantly higher in patients with AD (range: 0–13.04 (mean: 3.03 spikes/24 hours) as compared to HC (range: 0–6.66 (mean: 1.04 spikes/24 hours) with a risk ratio of 2.9 (CI: 1.77-5.01, p-value = <0.001).

Global and regional CBF

Global cerebral blood flow was not significantly (p-value = 0.872, t-value = 0.16, df =38) different between AD (mean (SD): 43.23 ml/100g/min (12.28)) and HC (mean (SD): 42.60 ml/100g/min (11.01)).

No significant differences were found for the normalized rCBF value in the hippocampus between the AD, and HC for left hippocampus (p-value = 0.367, t-value = -0.91, df = 38), right hippocampus (p-value = 0.092, t-value = -1.73, df = 38), or mean hippocampus (p-value = 0.118, t-value = -1.60, df = 38). See Figure 2. A significant difference was found between AD and HC for the normalized rCBF in the precuneus (p-value = 0.010, t-value = -2.73, df = 38) with a higher normalized rCBF in the HC, see Supplementary Figure 1.

In an exploratory manner, we conducted a voxel-to-voxel comparison and found significant bilateral decreased rCBF in the temporal and parietal lobe in patients with AD as compared to HC, see Figure 3.

Association between ASL and spike frequency

A significant positive linear association (estimate: 0.109, t-value = 4.03, p-value <0.001) between spike frequency and normalized rCBF in the hippocampus was found for AD, see Figure 4. This effect was also present when the outlier was kept in the model (p-value = 0.028). See Supplementary Figure 2 and page 4-7 in the supplementary material for detection of outliers. No significant association between rCBF and spike frequency was found for the HC (estimate: 0.021, t-value = 0.491, p-value = 0.632), see Supplementary figure 5.

As part of the exploratory analysis, we also found a positive association between normalized rCBF in precuneus and spike frequency in patients with AD (estimate: 0.069, p-value = 0.037), see Supplementary Figure 6. This may be explained by the positive association between normalized rCBF in precuneus and hippocampus (estimate: 0.504, p-value = 0.013), see Supplementary Figure 7.

Discussion

In the present study, we investigated the association between rCBF in the hippocampus and epileptiform discharges (in the form of spike/wave activity) in patients with AD. As hypothesized, a significant linear association between spike frequency and the normalized rCBF in the hippocampus was found in patients with AD. In an exploratory analysis a significant positive association between spike frequency and the normalized rCBF in precuneus was found in patients with AD. Spike frequency was significantly higher in patients with AD compared to HC. No difference in the mean hippocampal rCBF was found between AD and HC. The exploratory analysis revealed a significantly lower normalized rCBF in the parietal lobes including precuneus in AD as compared to HC.

The findings of an association between spike frequency and normalized rCBF in the hippocampus suggests that these two biological phenomena may be coupled in AD. Similarly, in patients with epilepsy, rCBF increases at the epileptic focus during the ictal phase (11–13). Even though we only measured spikes/sharp waves and not ictal activity, the results indicate a common hemodynamic response to epileptiform discharges in both conditions. Due to this resemblance with epilepsy, it is likely that the increasing rCBF in hippocampus with more epileptiform discharges is linked to an increased metabolic demand (48), which may again be linked to accelerated neurodegeneration although this remains speculative. The epileptiform discharges measured in patients with AD in this study may represent a hyperexcitable state of neurons in the hippocampus that does not directly resemble the pathophysiological mechanisms in epilepsy. Using a more sensitive measuring technique, which enables recordings of much smaller amplitudes of spikes, it was found that these discharges are much more common in the hippocampus as compared to what is detected using scalp EEG and possible indicating a hyperexcitable state (25). Such a hyperexcitable state may also represent a less transient state than seen in epileptic seizures. This would also explain the positive

association despite the difference in time between ASL acquisition and ear-EEG recording. Furthermore, the association between hippocampal rCBF and spike frequency in HC was not significant, which suggests that this coupling is specific to AD, but more studies are needed. To test the effect of anti-seizure medication on this hyperexcitability, a recent study investigated the changes in rCBF in patients with AD after administration of levetiracetam using ASL-MRI but without long-term EEG monitoring and found an increased hippocampal rCBF (49), which is in contrast to the current findings. Based on the current findings of an association between spike frequency and hippocampal rCBF, we would expect a decrease in rCBF after administration of levetiracetam due to a decrease in the hippocampal excitability. Due to the limited studies in this field, further investigations of the rCBF response to anti-seizure medications while performing long-term EEG monitoring are needed to better understand the relationship between epileptiform discharges and rCBF in AD. Specifically, it remains to be determined how chronic administration of anti-seizure medication in patients with AD will alter hippocampal perfusion and if increased perfusion due to epileptiform discharges will be decreased (i.e., normalization) after administration of anti-seizure medication in patients with AD.

In the exploratory analysis, we found significantly decreased normalized rCBF in the precuneus in patients with AD as compared to HC. In the early phases of AD, the neuropathological abnormalities are present in the precuneus (50). Using resting-state functional MRI, the precuneus is considered a main hub of the default mode network and is both structurally and functionally connected to the hippocampus (47) and lower default mode network connectivity has been linked to faster cortical thinning in older adults with amyloid depositions (51). Due to the interconnectivity between the precuneus and the hippocampus, we investigated the role of the precuneus in epileptiform discharges and found a positive association between spike frequency and rCBF in the precuneus in patients with AD. Although previous studies have shown that most epileptiform discharges in patients with AD

occur primarily in the temporal lobe, it is likely that the strong functional and structural connectivity between the hippocampus and the precuneus (47,52) fully explains this positive association. However, more studies are needed to understand the role of the intrinsic network between hippocampus and precuneus in relation to epileptiform discharges.

The study has several limitations. Due to the focus on the hippocampus, we did not acquire ASL images from the whole brain, which limits further exploration into the possible association between epileptiform discharges and other brain regions. In addition, the MRI acquisition was not performed on the same day as the beginning of the ear-EEG recording, which may lead to the association being weaker due to variability in the measured epileptiform discharges. Furthermore, due to the low spatial resolution of the ear-EEG, it will be of importance to systematically compare epileptiform discharges between ear-EEG and scalp EEG, which was not possible in the current study since only 30 minutes of scalp EEG was recorded. Although scalp EEG is the gold standard for detection of epileptiform discharges, a study has shown that scalp EEG is unable to detect a large proportion of the epileptiform discharges (25), which may be an advantage of ear-EEG but more studies are needed. Although ear-EEG has been able to detect epileptiform discharges in patients with epilepsy (29), no studies have so far directly compared ear-EEG to scalp EEG in detection of epileptiform discharges. Therefore, it is possible that accuracy is lower with ear-EEG than conventional scalp EEG. Overall, we were able to demonstrate in a relatively small sample that an association between spike frequency and normalized rCBF is present in AD, which underlines the neuropathological link between epileptiform discharges and hippocampal rCBF.

Conclusions

As hypothesized, increasing spike frequency was associated with increasing rCBF in the hippocampus in patients with AD while this could not be shown in HC. The hemodynamic coupling between epileptiform discharges and blood flow has previously been found in patients with epilepsy and may be a shared mechanism across pathologies. Our findings indicate that hyperperfusion may be an accompanying phenomenon to hyperexcitability in patients with AD, which is in support of the theory of a hyperexcitable hippocampus. However, more studies investigating the range of rCBF and epileptiform discharges in AD are needed.

Declarations

Ethics approval and consent to participate

The study was approved by the Capital Region Ethics Committee (H-17035751), and by the Danish Medicines Agency (2017112288). All participants gave written and oral informed consent before participating in the study.

Consent for publication

Not applicable

Availability of data and materials

The datasets generated and/or analyzed during the current study are not publicly available due Danish data protection regulations but are available from the corresponding author on reasonable request.

Funding

The study was funded by the Alzheimer Research Foundation (grant number: 181003), Toyota Foundation (KJ/BG-9171 F), Axel Juul Muusfeldts Foundation (2016-527), Ellen Mørchs foundation (J.nr. 32491419), Rigshospitalet Research Foundation and T&W Engineering. None of the funding parties had a role in the collection, analysis, and interpretation of data or in the writing of the

manuscript or whether to publish the results of the study. CSM received funding from the Lundbeck Foundation (R358-2020-2344)

Competing interests

CSM received an unrestricted grant from T&W engineering. Two authors (MLR, and MCH) who are employees of T&W Engineering contributed to the interpretation and writing of the report. TWK consults for T&W engineering.

Authors' contributions

CSM: Conceptualization, Data Curation, Formal Analysis, Funding Acquisition, Investigation, Methodology, Project Administration, Software, Visualization, Writing – Original Draft Preparation

TWK: Methodology, Formal analysis, Resources, Writing - Review & Editing, Visualization, Supervision, Project administration

UL: Conceptualization, Data Curation, Formal Analysis, Resources, Methodology, Software, Visualization, Writing – Review & Editing

MBV: Conceptualization, Formal Analysis, Resources, Methodology, Software, Writing – Review & Editing

HBWL: Conceptualization, Resources, Methodology, Writing – Review & Editing

DZP: Conceptualization, Methodology, Writing – Review & Editing

BBA: Conceptualization, Investigation, Resources, Writing – Review & Editing

PH: Conceptualization, Investigation, Resources, Writing – Review & Editing

PK: Conceptualization, Methodology, Software, Visualization, Writing – Review & Editing

MCH: Conceptualization, Methodology, Resources, Writing – Review & Editing

MLR: Conceptualization, Methodology, Resources, Writing – Review & Editing

SGH: Conceptualization, Writing – Review & Editing

GW: Conceptualization, Methodology, Resources, Investigation, Writing - Review & Editing, Supervision, Project administration, Funding acquisition

KSF: Conceptualization, Methodology, Investigation, Writing - Review & Editing, Supervision, Project administration

Acknowledgements

We would like to thank specialist nurses Susanne Kristiansen and Oda Jakobsen for their assistance in recruiting participants and helping during study visits.

References

1. 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(1):211–23.

2. Scarmeas N, Honig LS, Choi H, Cantero J, Brandt J, Blacker D, et al. Seizures in Alzheimer Disease. Arch Neurol [Internet]. 2009 Aug;66(8). Available from: https://doi.org/10.1001%2Farchneurol.2009.130

3. 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 [Internet]. 2006;47(5):867–72. Available from: https://doi.org/10.1111%2Fj.1528-1167.2006.00554.x

4. Lozsadi DA, Larner AJ. Prevalence and Causes of Seizures at the Time of Diagnosis of Probable Alzheimer's Disease. Dement Geriatr Cogn Disord [Internet]. 2006;22(2):121–4. Available from: https://doi.org/10.1159%2F000093664

5. Bernardi S, Scaldaferri N, Vanacore N, Trebbastoni A, Francia A, D'Amico A, et al. Seizures in Alzheimer's disease: a retrospective study of a cohort of outpatients. Epileptic Disord [Internet]. 2010 Mar;12(1):16–21. Available from: https://doi.org/10.1684%2Fepd.2010.0290

6. Rao SC, Dove G, Cascino GD, Petersen RC. Recurrent seizures in patients with dementia: Frequency, seizure types, and treatment outcome. Epilepsy Behav [Internet]. 2009 Jan;14(1):118–20. Available from: https://doi.org/10.1016%2Fj.yebeh.2008.08.012

7. Risse SC, Lampe TH, Bird TD, Nochlin D, Sumi SM, Keenan T, et al. Myoclonus, Seizures,

and Paratonia in Alzheimer Disease. Alzheimer Dis Assoc Disord [Internet]. 1990;4(4):217–25. Available from: https://doi.org/10.1097%2F00002093-199040400-00003

8. 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 Off J Int Fed Clin Neurophysiol. 2021 Aug;132(8):1982–9.

9. 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 Oct;95(16):e2259–70.

10. 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 [Internet]. 2016;80(6):858–70. Available from: http://dx.doi.org/10.1002/ana.24794

11. Duncan R. Epilepsy, cerebral blood flow, and cerebral metabolic rate. Cerebrovasc Brain Metab Rev. 1992;4(2):105–21.

12. Dupont P, Zaknun JJ, Maes A, Tepmongkol S, Vasquez S, Bal CS, et al. Dynamic perfusion patterns in temporal lobe epilepsy. Eur J Nucl Med Mol Imaging. 2009 May;36(5):823–30.

13. Nguyen D, Kapina V, Seeck M, Viallon M, Fedespiel A, Lovblad KO. Ictal hyperperfusion demonstrated by arterial spin-labeling MRI in status epilepticus. Vol. 37, Journal of neuroradiology = Journal de neuroradiologie. France; 2010. p. 250–1.

14. Pendse N, Wissmeyer M, Altrichter S, Vargas M, Delavelle J, Viallon M, et al. Interictal arterial spin-labeling MRI perfusion in intractable epilepsy. J Neuroradiol = J Neuroradiol. 2010 Mar;37(1):60–3.

15. Lim Y-M, Cho Y-W, Shamim S, Solomon J, Birn R, Luh WM, et al. Usefulness of pulsed

arterial spin labeling MR imaging in mesial temporal lobe epilepsy. Epilepsy Res. 2008 Dec;82(2–3):183–9.

16. Haller S, Zaharchuk G, Thomas DL, Lovblad K-O, Barkhof F, Golay X. Arterial Spin Labeling Perfusion of the Brain: Emerging Clinical Applications. Radiology [Internet]. 2016 Oct 18;281(2):337–56. Available from: https://doi.org/10.1148/radiol.2016150789

17. Targa Dias Anastacio H, Matosin N, Ooi L. Neuronal hyperexcitability in Alzheimer's disease: what are the drivers behind this aberrant phenotype? Transl Psychiatry [Internet]. 2022;12(1):257. Available from: https://doi.org/10.1038/s41398-022-02024-7

18. Roquet D, Sourty M, Botzung A, Armspach J-P, Blanc F. Brain perfusion in dementia with Lewy bodies and Alzheimer's disease: an arterial spin labeling MRI study on prodromal and mild dementia stages. Alzheimers Res Ther [Internet]. 2016;8(1). Available from: http://dx.doi.org/10.1186/s13195-016-0196-8

19. Johnson NA, Jahng G-H, Weiner MW, Miller BL, Chui HC, Jagust WJ, et al. Pattern of Cerebral Hypoperfusion in Alzheimer Disease and Mild Cognitive Impairment Measured with Arterial Spin-labeling MR Imaging: Initial Experience. Radiology [Internet]. 2005;234(3):851–9. Available from: http://dx.doi.org/10.1148/radiol.2343040197

20. Yoshiura T, Hiwatashi A, Yamashita K, Ohyagi Y, Monji A, Takayama Y, et al. Simultaneous Measurement of Arterial Transit Time, Arterial Blood Volume, and Cerebral Blood Flow Using Arterial Spin-Labeling in Patients with Alzheimer Disease. Am J Neuroradiol [Internet]. 2009;30(7):1388–93. Available from: http://dx.doi.org/10.3174/ajnr.a1562

21. Wolk DA, Detre JA. Arterial spin labeling MRI: an emerging biomarker for Alzheimer's

disease and other neurodegenerative conditions. Curr Opin Neurol. 2012 Aug;25(4):421–8.

22. Alsop DC, Casement M, de Bazelaire C, Fong T, Press DZ. Hippocampal hyperperfusion in Alzheimer's disease. Neuroimage [Internet]. 2008;42(4):1267–74. Available from: http://dx.doi.org/10.1016/j.neuroimage.2008.06.006

23. Fleisher AS, Podraza KM, Bangen KJ, Taylor C, Sherzai A, Sidhar K, et al. Cerebral perfusion and oxygenation differences in Alzheimer's disease risk. Neurobiol Aging [Internet]. 2009;30(11):1737–48. Available from: http://dx.doi.org/10.1016/j.neurobiolaging.2008.01.012

24. Meritam Larsen P, Wüstenhagen S, Terney D, Gardella E, Aurlien H, Beniczky S. Duration of epileptic seizure types: A data-driven approach. Epilepsia. 2023 Feb;64(2):469–78.

25. 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 Jun;23(6):678–80.

26. Kappel SL, Rank ML, Toft HO, Andersen M, Kidmose P. Dry-Contact Electrode Ear-EEG. IEEE Trans Biomed Eng [Internet]. 2019 Jan;66(1):150–8. Available from: https://doi.org/10.1109%2Ftbme.2018.2835778

27. Looney D, Kidmose P, Park C, Ungstrup M, Rank M, Rosenkranz K, et al. The In-the-Ear Recording Concept: User-Centered and Wearable Brain Monitoring. IEEE Pulse [Internet]. 2012 Nov;3(6):32–42. Available from: https://doi.org/10.1109%2Fmpul.2012.2216717

28. Looney D, Park C, Kidmose P, Rank ML, Ungstrup M, Rosenkranz K, et al. An in-the-ear platform for recording electroencephalogram. In: 2011 Annual International Conference of the IEEE Engineering in Medicine and Biology Society [Internet]. IEEE; 2011. Available

from: https://doi.org/10.1109%2Fiembs.2011.6091733

29. Zibrandtsen IC, Kidmose P, Christensen CB, Kjaer TW. Ear-EEG detects ictal and interictal abnormalities in focal and generalized epilepsy - A comparison with scalp EEG monitoring. Clin Neurophysiol [Internet]. 2017 Dec;128(12):2454–61. Available from: https://doi.org/10.1016%2Fj.clinph.2017.09.115

30. Musaeus CS, Waldemar G, Andersen BB, Høgh P, Kidmose P, Hemmsen MC, et al. Long-Term EEG Monitoring in Patients with Alzheimer's Disease Using Ear-EEG: A Feasibility Study. J Alzheimer's Dis. 2022;In Press.

31. 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 out-patient EEG monitoring. Accept Neurobiol Dis. 2023;

32. Alsop DC, Detre JA, Grossman M. Assessment of cerebral blood flow in Alzheimer's disease by spin-labeled magnetic resonance imaging. Ann Neurol [Internet]. 2000;47(1):93–100. Available from: http://dx.doi.org/10.1002/1531-8249(200001)47:1%3C93::aid-ana15%3E3.0.co;2-8

33. Asllani I, Habeck C, Scarmeas N, Borogovac A, Brown TR, Stern Y. Multivariate and Univariate Analysis of Continuous Arterial Spin Labeling Perfusion MRI in Alzheimer's Disease. J Cereb Blood Flow Metab [Internet]. 2007;28(4):725–36. Available from: http://dx.doi.org/10.1038/sj.jcbfm.9600570

34. Dai W, Lopez OL, Carmichael OT, Becker JT, Kuller LH, Gach HM. Mild Cognitive Impairment and Alzheimer Disease: Patterns of Altered Cerebral Blood Flow at MR Imaging. Radiology [Internet]. 2009;250(3):856–66. Available from: http://dx.doi.org/10.1148/radiol.2503080751

35. Binnewijzend MAA, Kuijer JPA, Benedictus MR, van der Flier WM, Wink AM, Wattjes MP, et al. Cerebral Blood Flow Measured with 3D Pseudocontinuous Arterial Spin-labeling MR Imaging in Alzheimer Disease and Mild Cognitive Impairment: A Marker for Disease Severity. Radiology [Internet]. 2013;267(1):221–30. Available from: http://dx.doi.org/10.1148/radiol.12120928

36. Ding B, Ling HW, Huang J, Zhang H, Wang T, Yan FH, et al. Pattern of cerebral hyperperfusion in Alzheimer's disease and amnestic mild cognitive impairment using voxel-based analysis of 3D arterial spin-labeling imaging: initial experience. Clin Interv Aging [Internet]. 2014;493. Available from: http://dx.doi.org/10.2147/cia.s58879

37. McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack CR, 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 [Internet]. 2011 Apr;7(3):263–9. Available from: https://doi.org/10.1016%2Fj.jalz.2011.03.005

38. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res. 1975/11/01. 1975;12(3):189–98.

39. Pfeffer RI, Kurosaki TT, Harrah CH, Chance JM, Filos S. Measurement of Functional Activities in Older Adults in the Community. J Gerontol [Internet]. 1982;37(3):323–9. Available from: https://doi.org/10.1093%2Fgeronj%2F37.3.323

40. Cummings JL, Mega M, Gray K, Rosenberg-Thompson S, Carusi DA, Gornbein J. The Neuropsychiatric Inventory: Comprehensive assessment of psychopathology in dementia. Neurology [Internet]. 1994 Dec;44(12):2308. Available from:

https://doi.org/10.1212%2Fwnl.44.12.2308

41. Kural MA, Duez L, Sejer Hansen V, Larsson PG, Rampp S, Schulz R, et al. Criteria for defining interictal epileptiform discharges in EEG. Neurology [Internet]. 2020 May 19;94(20):e2139 LP-e2147. Available from: http://n.neurology.org/content/94/20/e2139.abstract

42. Voevodskaya O, Simmons A, Nordenskjöld R, Kullberg J, Ahlström H, Lind L, et al. The effects of intracranial volume adjustment approaches on multiple regional MRI volumes in healthy aging and Alzheimer's disease [Internet]. Vol. 6, Frontiers in Aging Neuroscience . 2014. Available from: https://www.frontiersin.org/articles/10.3389/fnagi.2014.00264

43. Bakker CJ, Hartkamp MJ, Mali WP. Measuring blood flow by nontriggered 2D phase-contrast MR angiography. Magn Reson Imaging. 1996;14(6):609–14.

44. Vestergaard MB, Lindberg U, Aachmann-Andersen NJ, Lisbjerg K, Christensen SJ, Rasmussen P, et al. Comparison of global cerebral blood flow measured by phase-contrast mapping MRI with (15) O-H(2) O positron emission tomography. J Magn Reson Imaging. 2017 Mar;45(3):692–9.

45. Chappell MA, Groves AR, Whitcher B, Woolrich MW. Variational Bayesian Inference for a Nonlinear Forward Model. Trans Sig Proc [Internet]. 2009 Jan;57(1):223–236. Available from: https://doi.org/10.1109/TSP.2008.2005752

46. Chappell MA, MacIntosh BJ, Donahue MJ, Günther M, Jezzard P, Woolrich MW. Separation of macrovascular signal in multi-inversion time arterial spin labelling MRI. Magn Reson Med. 2010 May;63(5):1357–65.

47. Cunningham SI, Tomasi D, Volkow ND. Structural and functional connectivity of the

precuneus and thalamus to the default mode network. Hum Brain Mapp. 2017 Feb;38(2):938–56.

48. Alavi A, Yakir S, Newberg AB. Positron emission tomography in seizure disorders. Ann N Y Acad Sci. 2011 Jun;1228:E1–12.

49. Press DZ, Musaeus CS, Zhao L, Breton JM, Shafi MM, Dai W, et al. Levetiracetam Increases Hippocampal Blood Flow in Alzheimer's Disease as Measured by Arterial Spin Labelling MRI. J Alzheimers Dis. 2023;93(3):939–48.

50. Yokoi T, Watanabe H, Yamaguchi H, Bagarinao E, Masuda M, Imai K, et al. Involvement of the Precuneus/Posterior Cingulate Cortex Is Significant for the Development of Alzheimer's Disease: A PET (THK5351, PiB) and Resting fMRI Study [Internet]. Vol. 10, Frontiers in Aging Neuroscience . 2018. Available from: https://www.frontiersin.org/articles/10.3389/fnagi.2018.00304

51. Hampton OL, Buckley RF, Manning LK, Scott MR, Properzi MJ, Peña-Gómez C, et al. Resting-state functional connectivity and amyloid burden influence longitudinal cortical thinning in the default mode network in preclinical Alzheimer's disease. NeuroImage Clin [Internet]. 2020;28:102407. Available from: https://www.sciencedirect.com/science/article/pii/S2213158220302448

52. Wang L, Laviolette P, O'Keefe K, Putcha D, Bakkour A, Van Dijk KRA, et al. Intrinsic connectivity between the hippocampus and posteromedial cortex predicts memory performance in cognitively intact older individuals. Neuroimage. 2010 Jun;51(2):910–7.

Table 1: Baseline demographics.

	Healthy controls	Alzheimer's disease	p-value
Number of participants	15	25	
Age, mean (SD)	69.5 (7.93)	70.3 (7.79)	0.723
Males/females	8/7	15/10	0.680
Education, mean (SD)	15.2 (2.04)	14.3 (3.08)	0.219
Cholinesterase inhibitor, n (%)	0	24 (96%)	
SSRI/SNRI, n (%)	1 (7%)	4 (16%)	
MMSE, mean (SD)	29.3 (0.88)	23.4 (3.29)	<0.001
NPI		4.83 (4.06)	
FAQ IADL		14.28 (5.78)	
Time in days between MRI and ear-EEG, mean (SD)*	21.4 (12.46)	17.8 (10.26)	0.332
Left hippocampus volume, % of ICV, mean (SD)	0.26 (0.03)	0.21 (0.03)	<0.001
Right hippocampus volume, % of ICV, mean (SD)	0.26 (0.3)	0.21 (0.03)	<0.001

SSRI/SNRI: Selective Serotonin Reuptake Inhibitor/ Serotonin and Noradrenaline Reuptake Inhibitor, MMSE: Mini-Mental State Examination, ADL: Activities of Daily Living, NPI: Neuropsychiatric Inventory, Time in days refers to the number of days between first day of ear-EEG recording and MRI scan in patients who were included in the regression analysis, SD: Standard deviation, ICV: intracranial volume, * Only for the 24 patients with AD with sufficient ear-EEG data

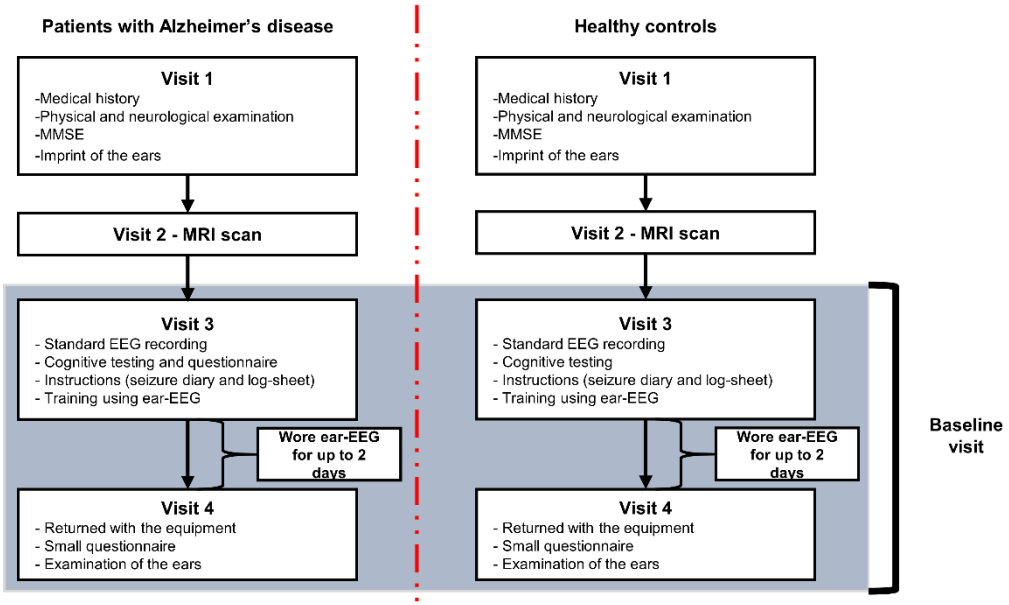


Figure 1: Study design. The patients with AD underwent the same procedures as HC except that no questionnaires were administered for the HC in visit 3

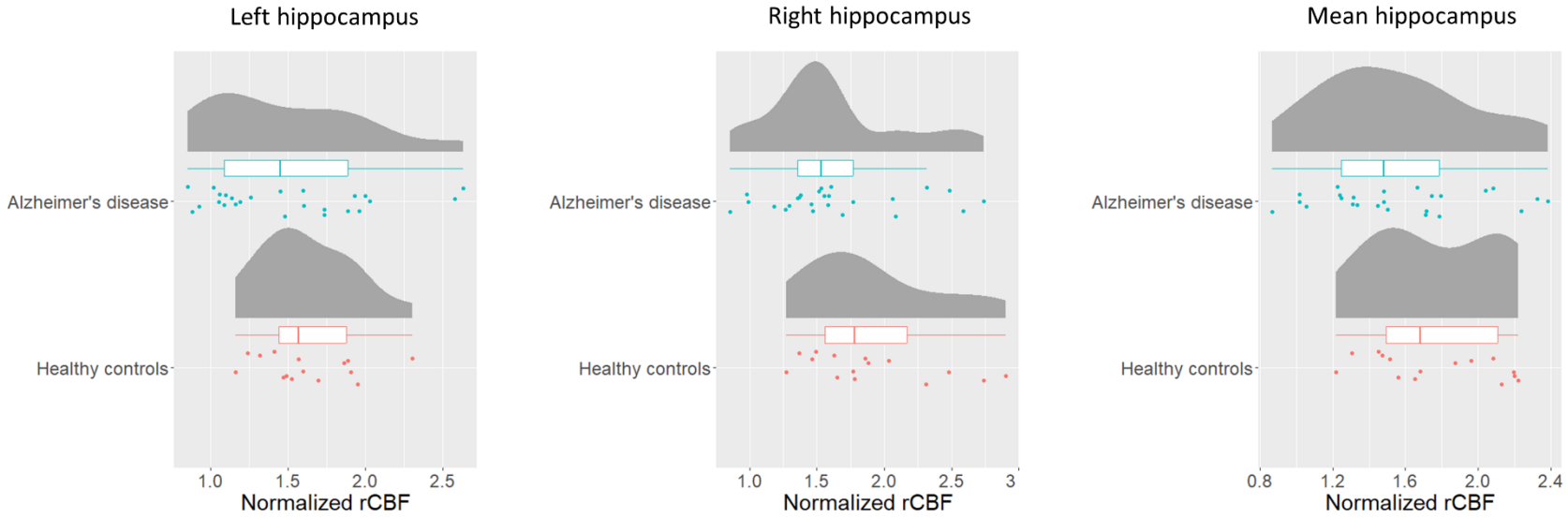


Figure 2: Raincloud plots showing mean normalized rCBF. No significant differences were found between AD, and HC for left hippocampus, right hippocampus, or mean hippocampus.

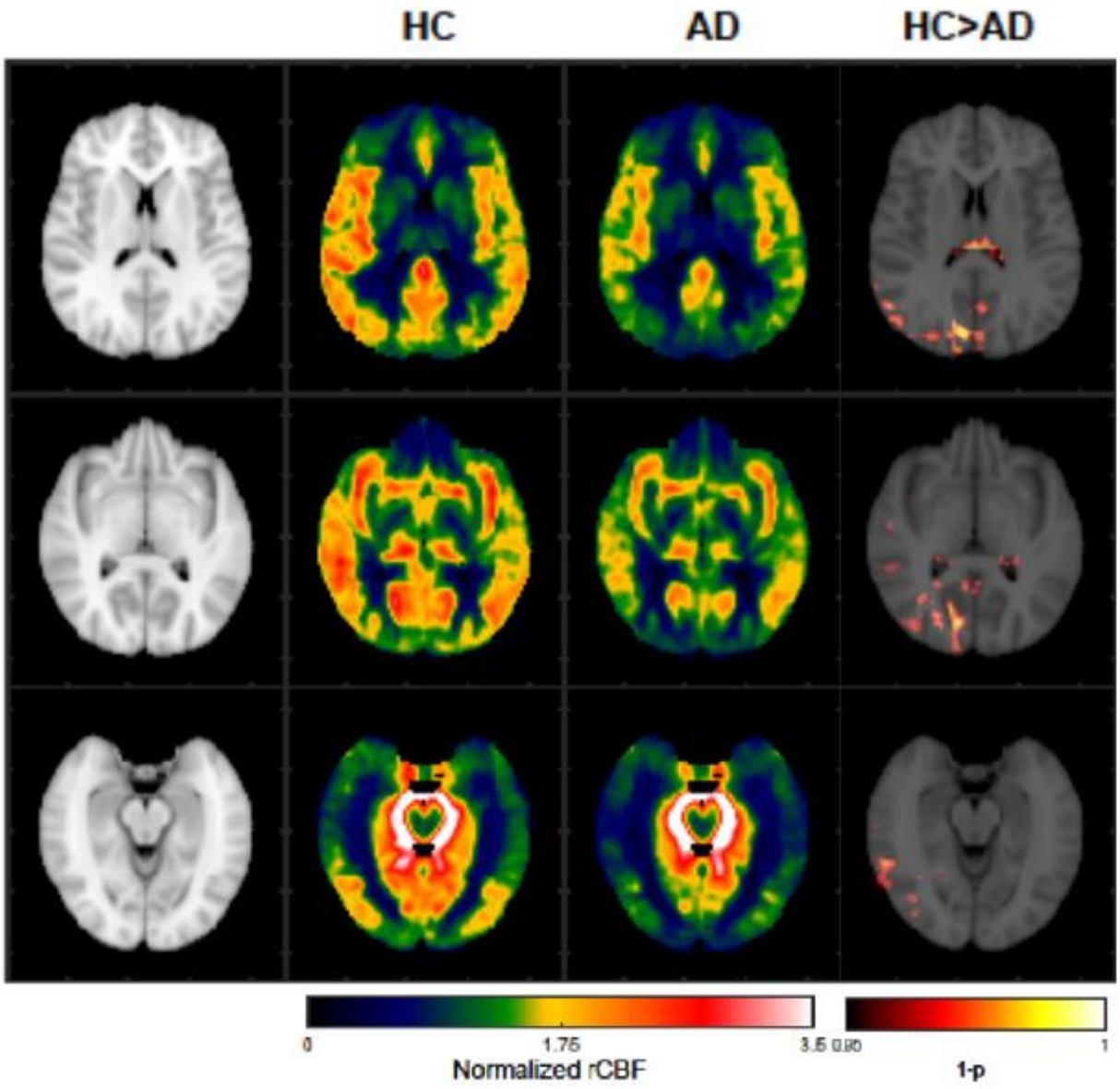


Figure 3: Exploratory analysis of rCBF. A significantly decreased normalized rCBF in the temporal and parietal lobes (including precuneus) in AD as compared to HC.

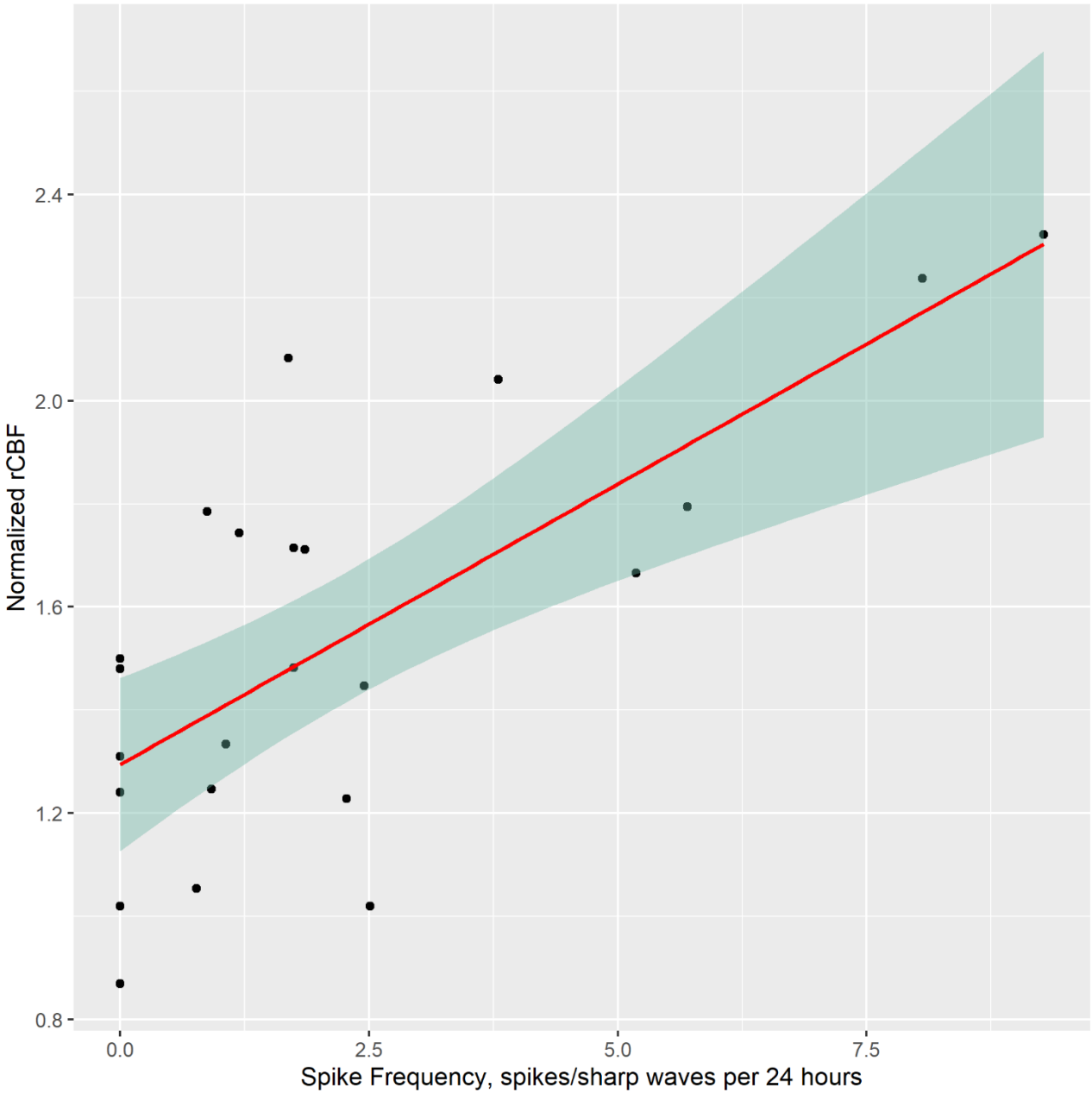
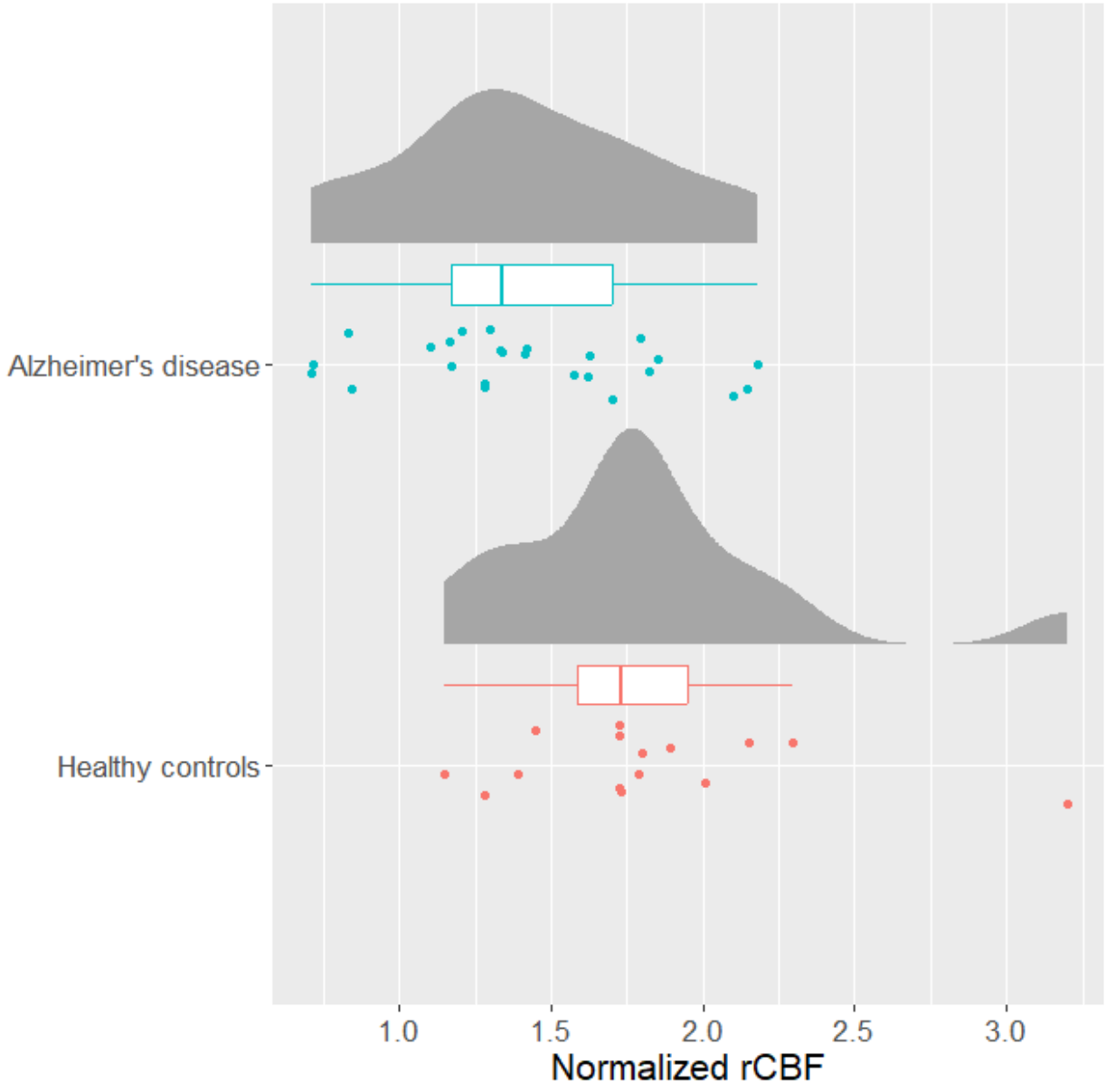
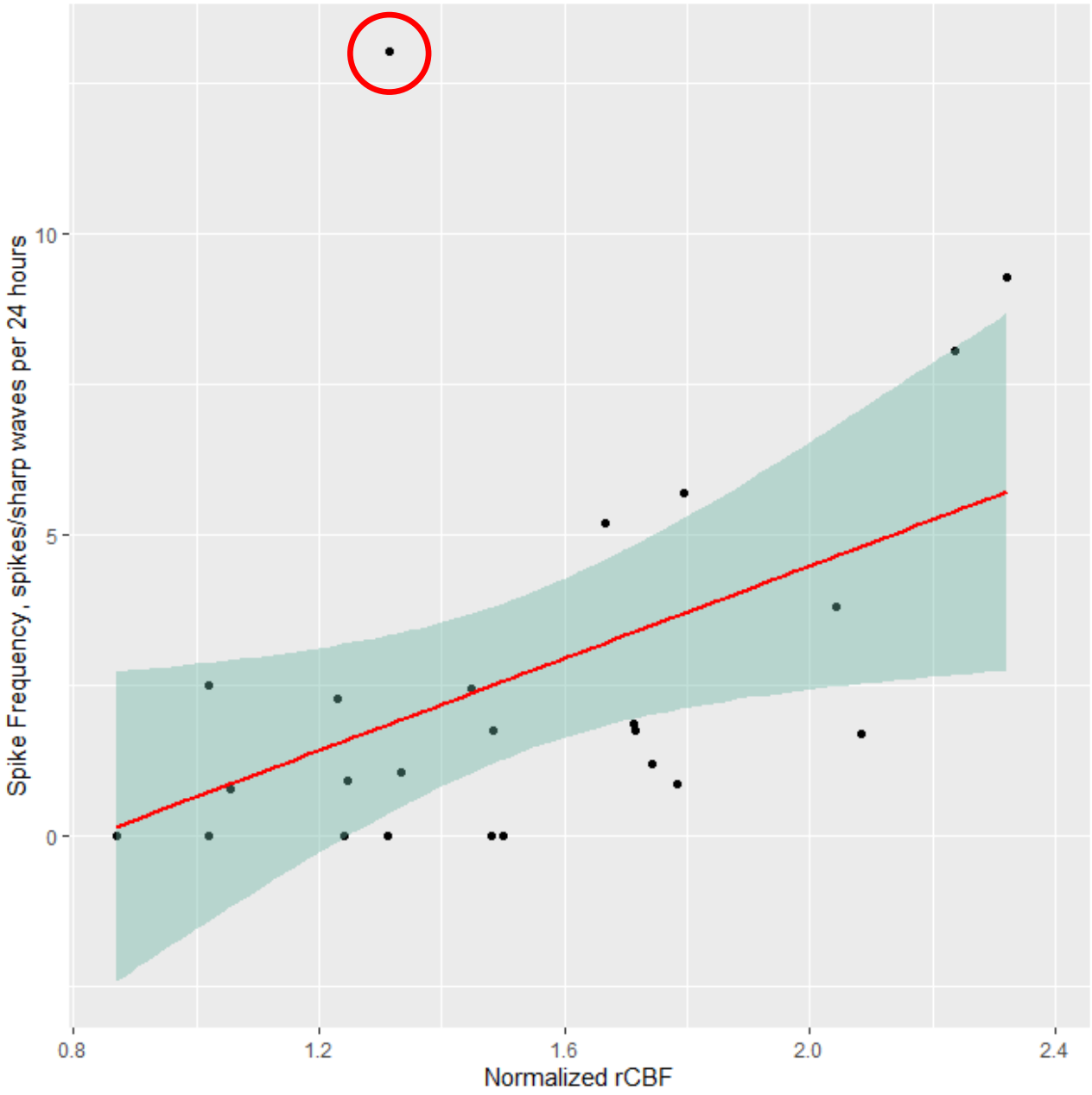


Figure 4: Association between normalized rCBF in hippocampus and spike frequency as measured with ear-EEG. Simple linear regression was applied to test if spike frequency was associated with the normalized mean rCBF in the hippocampus in patients with AD ($p<0.001$)

Supplementary materials to: "Subclinical epileptiform discharges in Alzheimer's disease are associated with increased hippocampal blood flow"



Supplementary figure 1: Normalized rCBF in the precuneus for HC and AD.



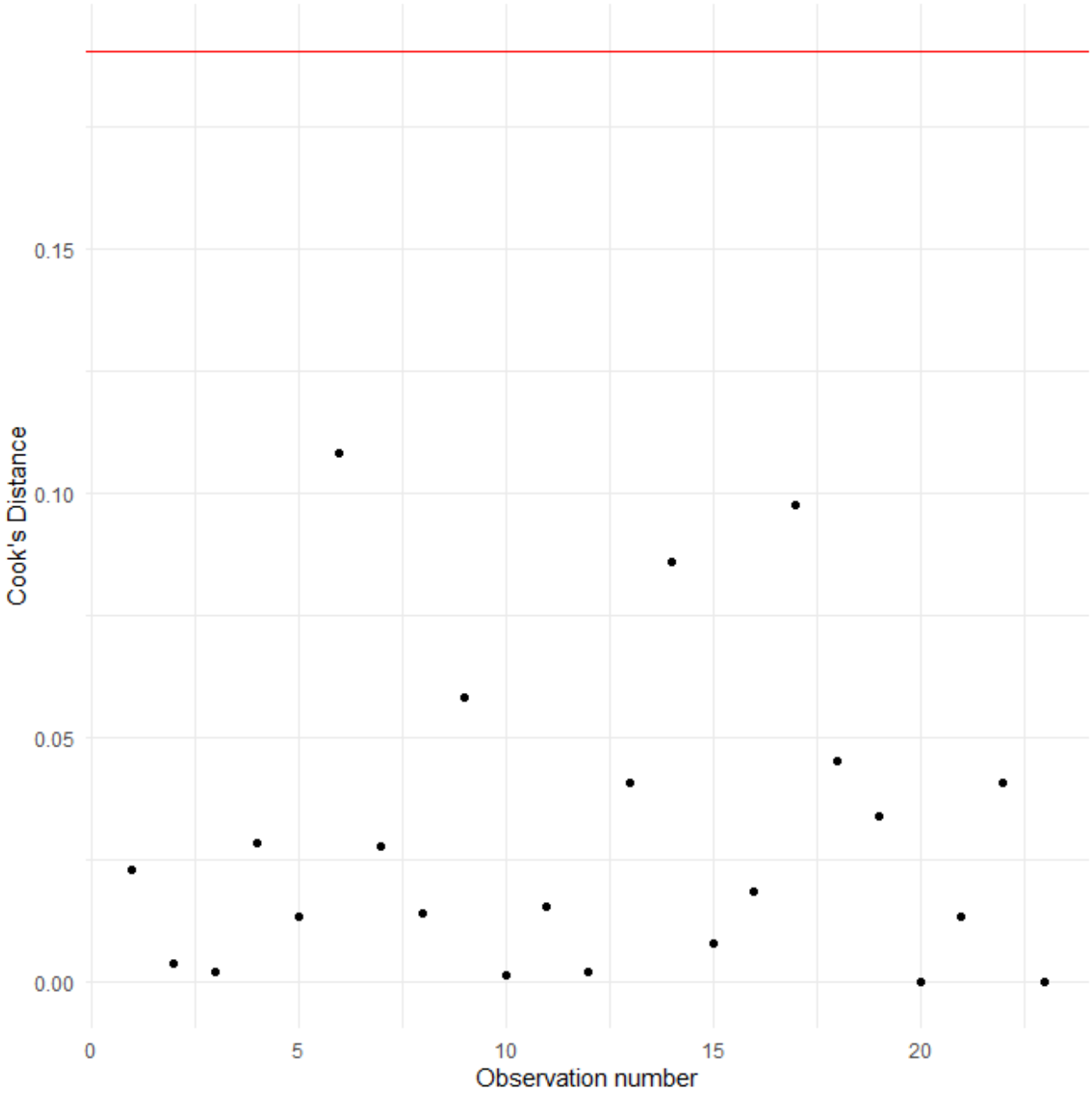
Supplementary figure 2: Plot showing the number of spikes/24 hours of recording and the normalized rCBF in the hippocampus in patients with AD. The outlier (marked with red circle) was removed from the subsequent analyses.

Checking of the linear regression

A full description of the statistical analysis can found in the following pages including a QQ-plot of the residuals. Here, we wanted to fully understand whether Figure 3 was affected by outliers after removing the most pronounced outlier (Supplementary figure 2). We therefore performed Cook's distance as follows in R:

```
library(ggplot2)
cooksd <- cooks.distance(model1_AD)
cooksd_df <- data.frame(observation = seq_along(cooksd), cooksd = cooksd)
n <- nrow(cooksd_df)
k <- length(coefficients(model1_AD)) - 1
threshold <- 4 / (n - k - 1)
ggplot(cooksd_df, aes(x = observation, y = cooksd)) +
  geom_point() +
  geom_hline(yintercept = threshold, color = "red", linetype = "solid") +
  labs(x = "Observation number", y = "Cook's Distance") +
  theme_minimal()
```

Here, we found that none of the remaining data points were substantially higher or exceeded the threshold set at 4/(n - k – 1) where n is the sample size and k is the number of independent variables (see Supplementary figure 3).

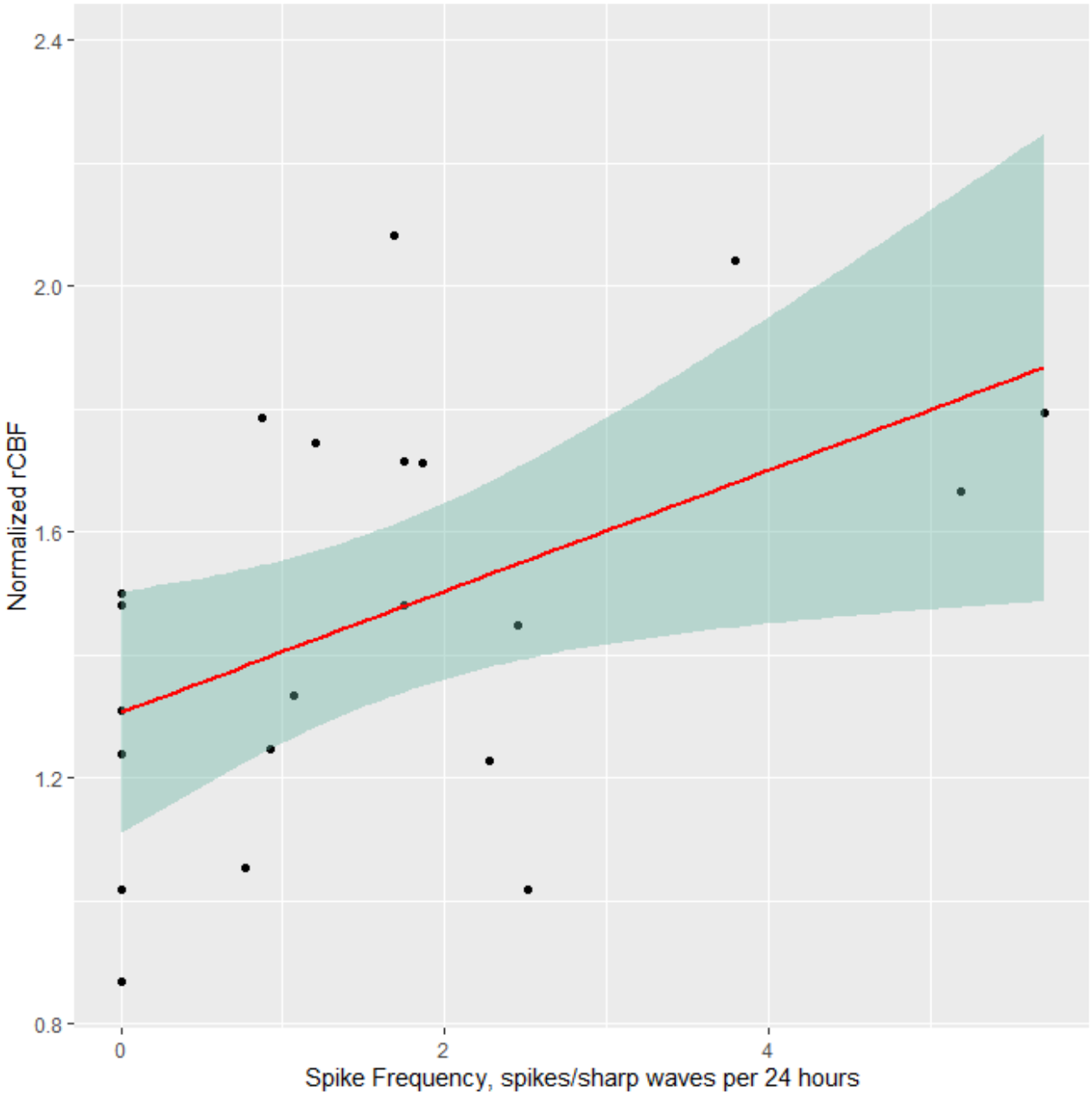


Supplementary figure 3: Figure showing the Cook's distance for each observation and the calculated threshold (red line at 0.19). None of the observations were considered outliers.

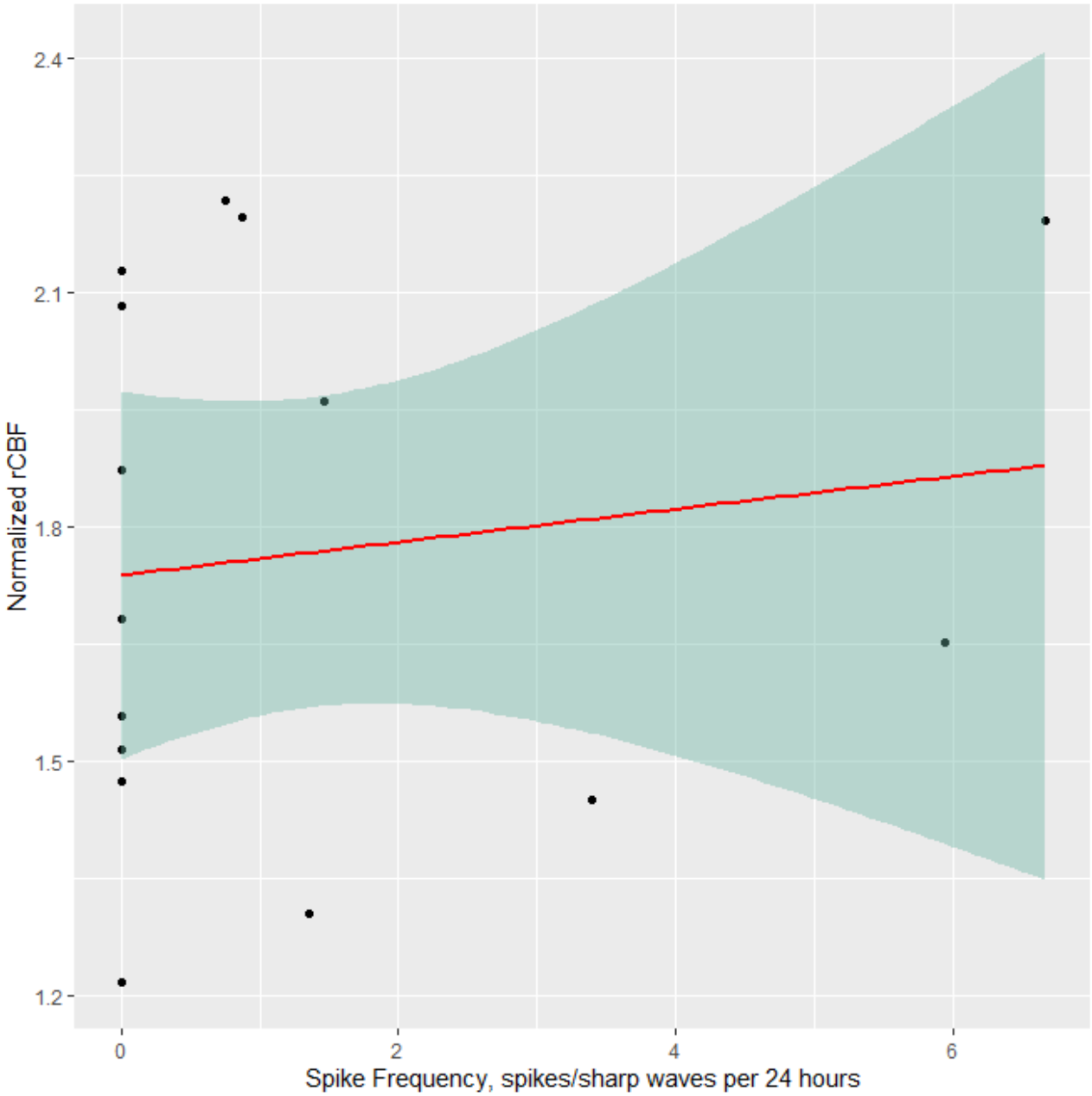
However, to understand if the linear regression was affected by the two individuals with high spike frequency, we conducted an additional analysis excluding these individuals as follows:

```
AD <- AD[-5,]
AD <- AD[-3,]

model1_AD <- lm (AD$Mean_hip_norm~AD$SpikeFrequency)
summary(model1_AD)
plot(model1_AD)

ggplot(AD, aes(x=SpikeFrequency, y=Mean_hip_norm)) +
  geom_point() +
  geom_smooth(method=lm , color="red", fill="#69b3a2", se=TRUE) +
  xlab("Spike Frequency, spikes/sharp waves per 24 hours") + ylab("Normalized rCBF")
ggsave("figure4_high_res.tiff", device='tiff', dpi=300)
```

Here, we found that the linear regression was still significant (see Supplementary figure 4) with a slightly lower estimate (estimate: 0.09833, p-value: 0.0281.



Supplementary figure 4: Plot showing the number of spikes/24 hours of recording and the normalized rCBF in the hippocampus in patients with AD. The outlier (as marked in Supplementary figure 3) as well as the two observations with high spike frequency were removed from the analyses.



Supplementary figure 5: Plot showing the number of spikes/24 hours of recording and the normalized rCBF in the hippocampus in patients with HC. No significant was found (estimate: 0.021, t-value = 0.491, p-value = 0.632).

Ear-EEG equipment and pre-processing

The ear-EEG earpieces were custom-made in silicone and mounted with six dry-electrodes [1] (T&W engineering, Denmark). The ear-piece was placed inside the ear canal and in the concha [2] in both ears with the labeling of the ear-EEG electrodes being previously described [3]. The ground electrode was placed approximately 2 cm under the midline of the clavicle on the left or right side. The ear-EEG recordings were performed using the TMSi Mobita EEG amplifier (TMSi systems), which was worn in either a small bag around the neck or in a belt bag and connected to the electrodes. The sampling rate was 1000 Hz. The EEG data were loaded into MATLAB (MathWorks, v2020b) and the EEGLAB [4] data structure was used throughout the analysis. See feasibility study for a complete description of the pre-processing [5].

Ear-EEG recordings were visually reviewed and annotated by CSM using the EEGLAB toolbox (v13.6.5b) implemented in MATLAB. The average referenced montage was used and 10 second epochs were inspected at a time with ±30 μV amplitude range. In this montage, the first 12 channels were low-pass filtered at 70 Hz, high-pass filtered at 1 Hz, and notch filtered at 50 Hz. The next 12 channels were after pre-processing, which included removal of segments with excessive artifacts. The subsequent 36 channels were the comparisons between electrodes with the active electrodes being on the left (e.g., ELA-ERA, ELA-ERB [3]) and the last 36 channels were created using the opposite laterality. The segments disregarded by the pre-processing pipeline and segments only containing recordings from one ear were not inspected.

References

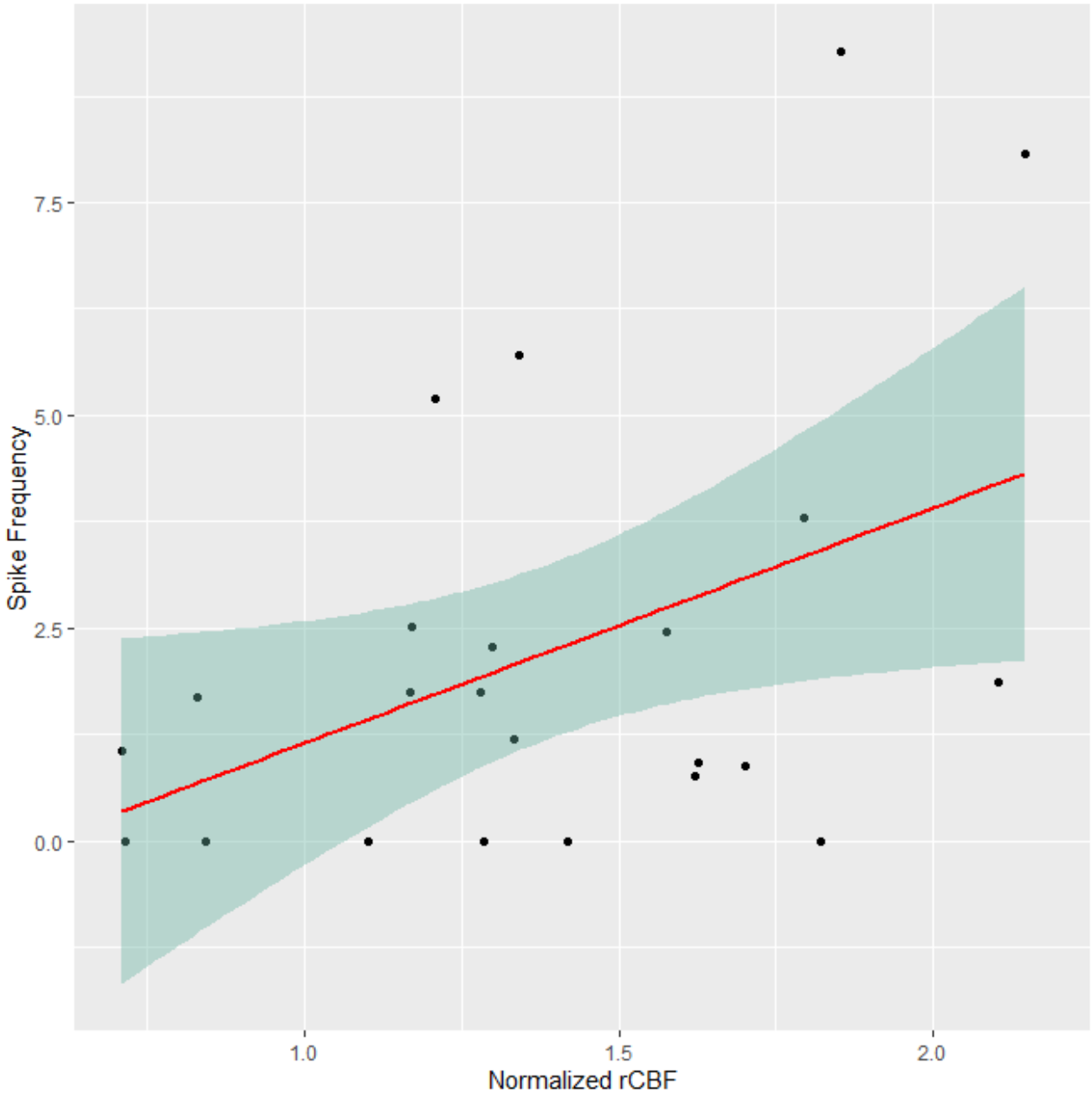
[1] Kappel SL, Rank ML, Toft HO, Andersen M, Kidmose P (2019) Dry-Contact Electrode Ear-EEG. *IEEE Trans Biomed Eng* **66**, 150–158.

[2] Mikkelsen KB, Kappel SL, Mandic DP, Kidmose P (2015) EEG Recorded from the Ear: Characterizing the Ear-EEG Method. *Front Neurosci* **9**,.

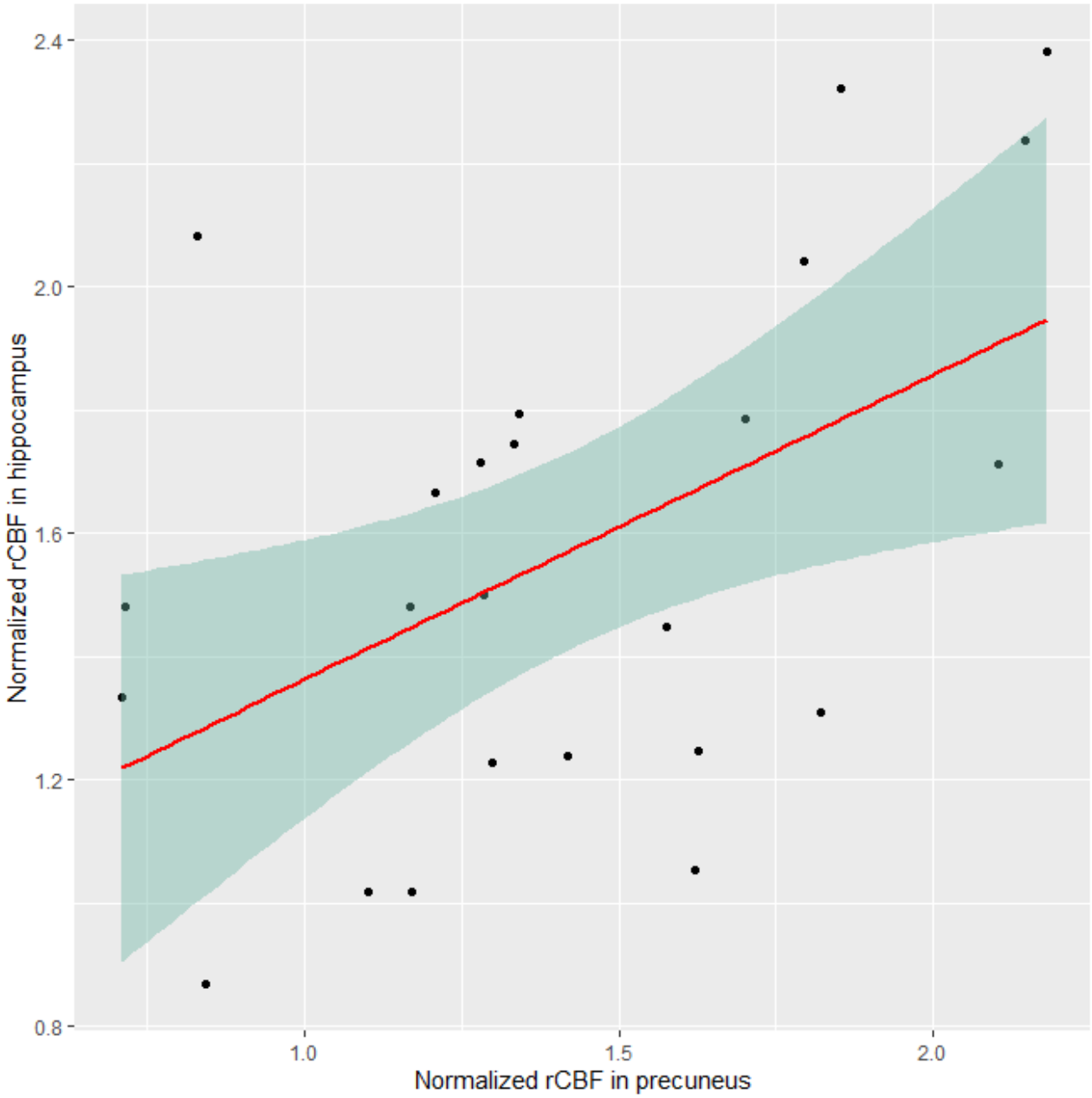
[3] Kidmose P, Looney D, Mandic DP (2012) Auditory evoked responses from Ear-EEG recordings. In *2012 Annual International Conference of the IEEE engineering in Medicine and Biology Society* IEEE.

[4] Delorme A, Makeig S (2004) EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. *J Neurosci Methods* **134**, 9–21.

[5] Musaeus CS, Waldemar G, Andersen BB, Høgh P, Kidmose P, Hemmsen MC, Rank ML, Kjær TW, Frederiksen KS (2022) Long-Term EEG Monitoring in Patients with Alzheimer's Disease Using Ear-EEG: A Feasibility Study. *J Alzheimer's Dis* **In Press**,.



Supplementary figure 6: Figure showing the linear association between spike frequency and normalized rCBF in precuneus in patients with AD



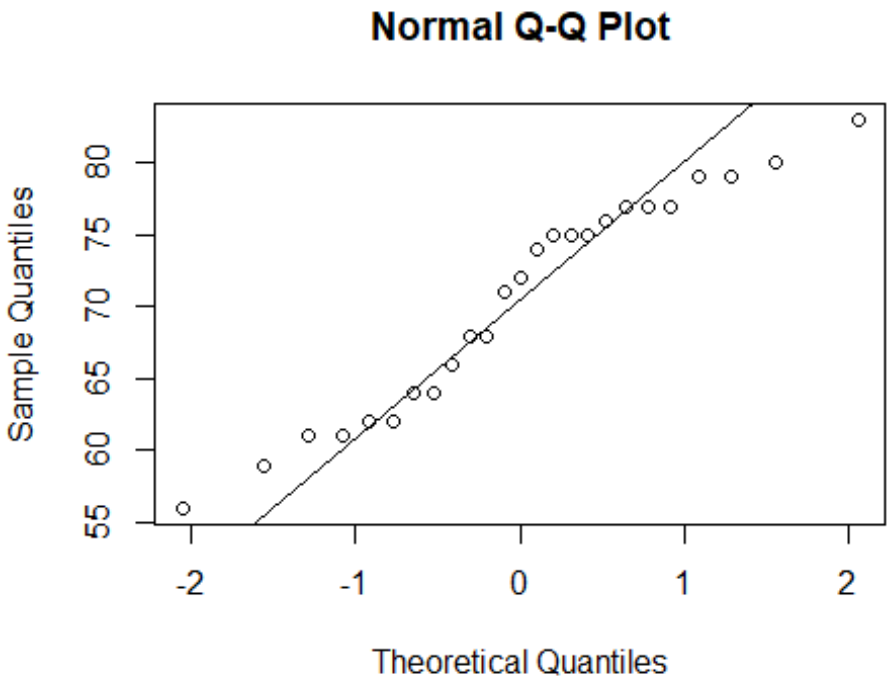
Supplementary figure 7: The association between normalized rCBF in precuneus and normalized rCBF in the hippocampus in patients with AD

Subclinical epileptiform discharges in Alzheimer's disease are associated with increased hippocampal blood flow

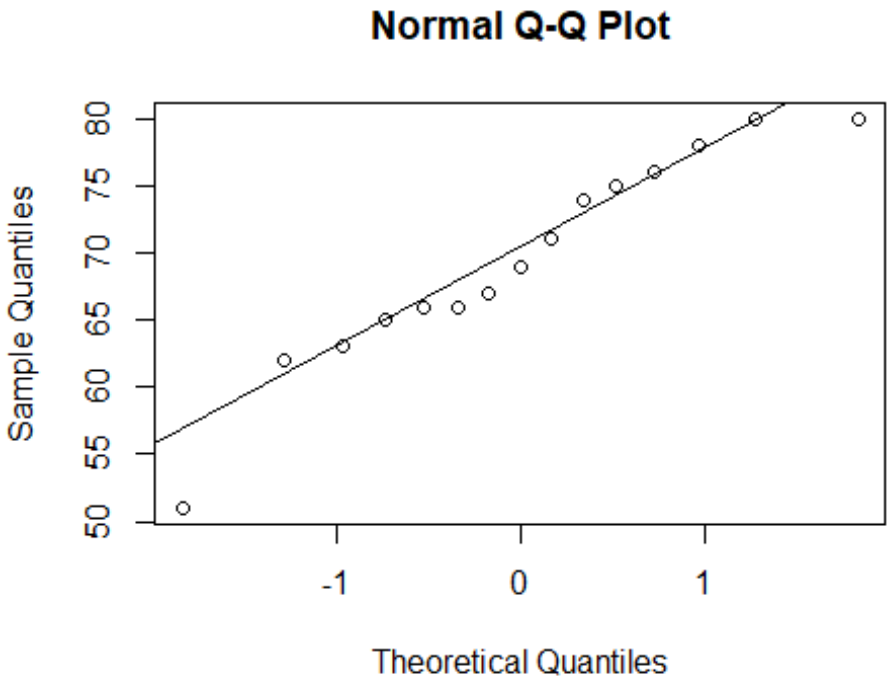
Musaeus CS et al.

15/3/2023

```r
## Demographics
Demogra$Diagnosis <- as.factor(Demogra$Diagnosis)

#Subset
AD <- subset(Demogra, Diagnosis == "Alzheimer's disease")
HC <- subset(Demogra, Diagnosis == "Healthy Control")

# Age
qqnorm(AD$Age)
qqline(AD$Age)
```




```r
qqnorm(HC$Age)
qqline(HC$Age)
```



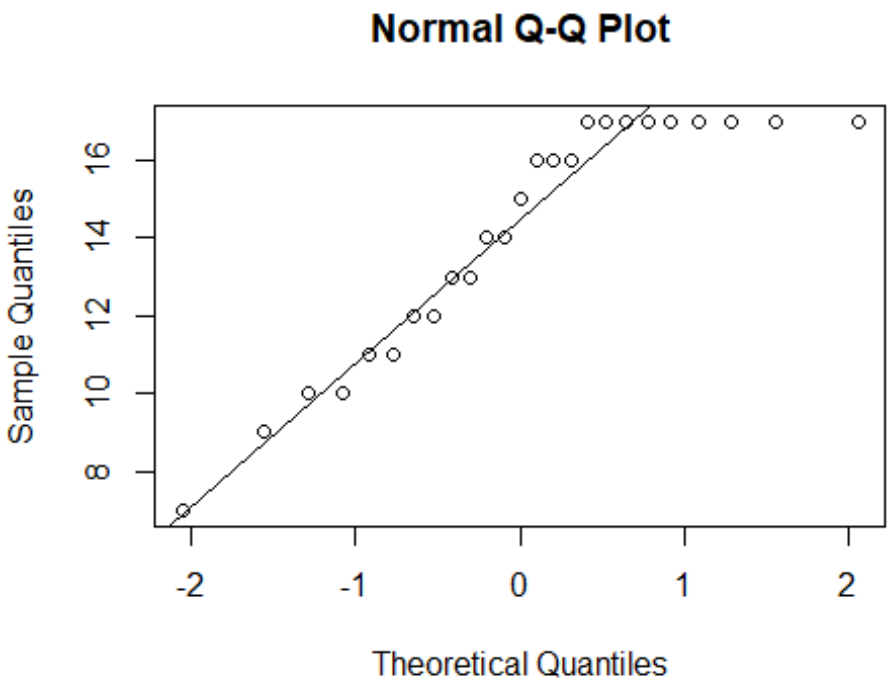

```r
var.test(AD$Age, HC$Age)
```

```
##
##  F test to compare two variances
##
## data:  AD$Age and HC$Age
## F = 0.93372, num df = 24, denom df = 14, p-value = 0.8535
## alternative hypothesis: true ratio of variances is not equal to 1
## 95 percent confidence interval:
##  0.3348101 2.3041107
## sample estimates:
## ratio of variances
##          0.9337223
```

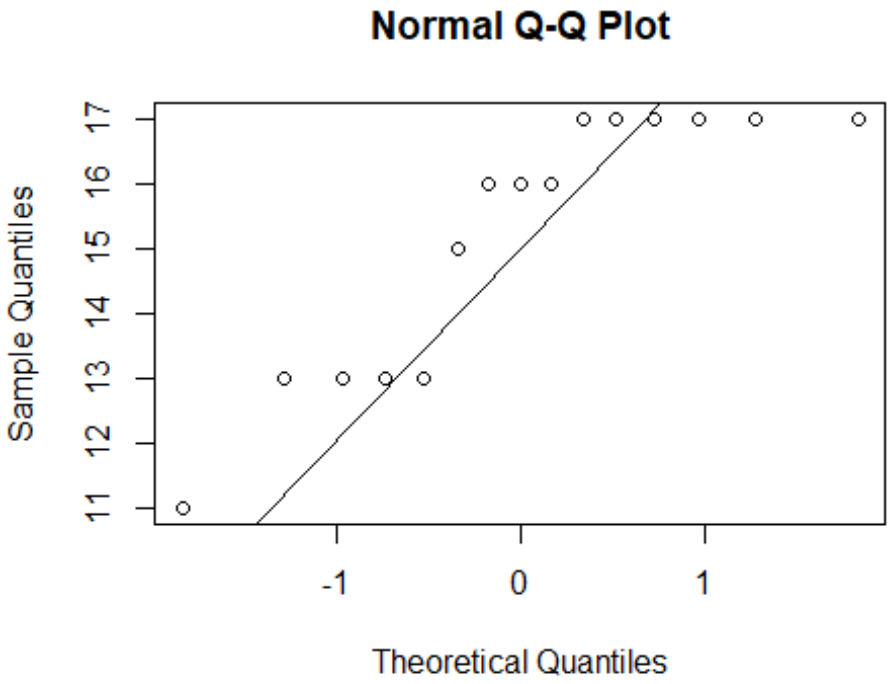
```r
t.test(AD$Age, HC$Age, var.equal = TRUE)
```

```
##
##  Two Sample t-test
##
## data:  AD$Age and HC$Age
## t = 0.35777, df = 38, p-value = 0.7225
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -4.223548  6.036881
## sample estimates:
## mean of x mean of y
##  70.44000  69.53333
```

```r
# Gender
Gender_diagnosis <- data.frame(G1=c(as.numeric(8),as.numeric(7)),
                               G2=c(as.numeric(15),as.numeric(10)))


chisq.test(Gender_diagnosis, correct = F)

##
##  Pearson's Chi-squared test
##
## data:  Gender_diagnosis
## X-squared = 0.1705, df = 1, p-value = 0.6797

# Education

qqnorm(AD$Education)
qqline(AD$Education)
```

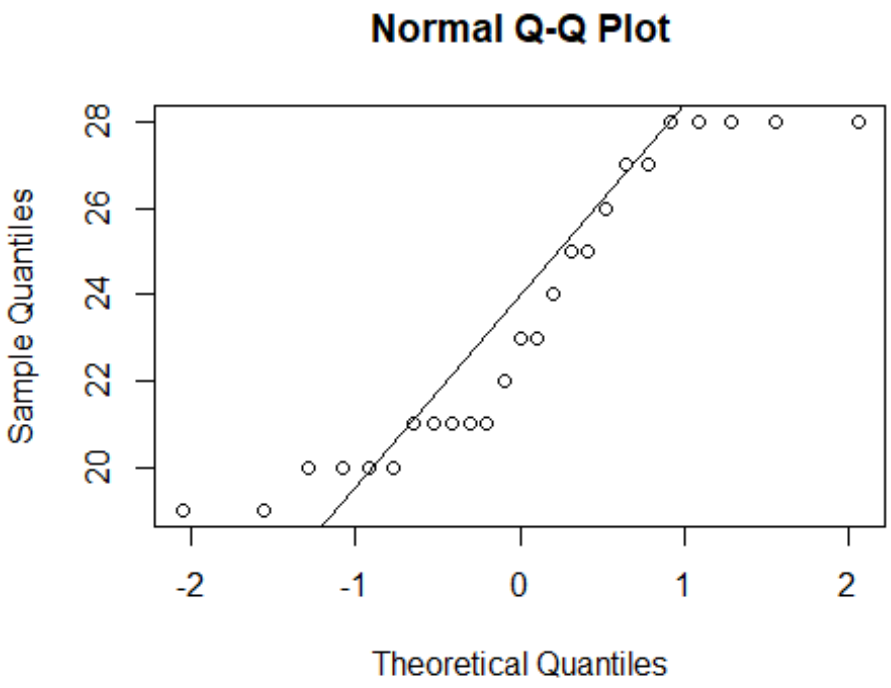



```r
qqnorm(HC$Education)
qqline(HC$Education)
```

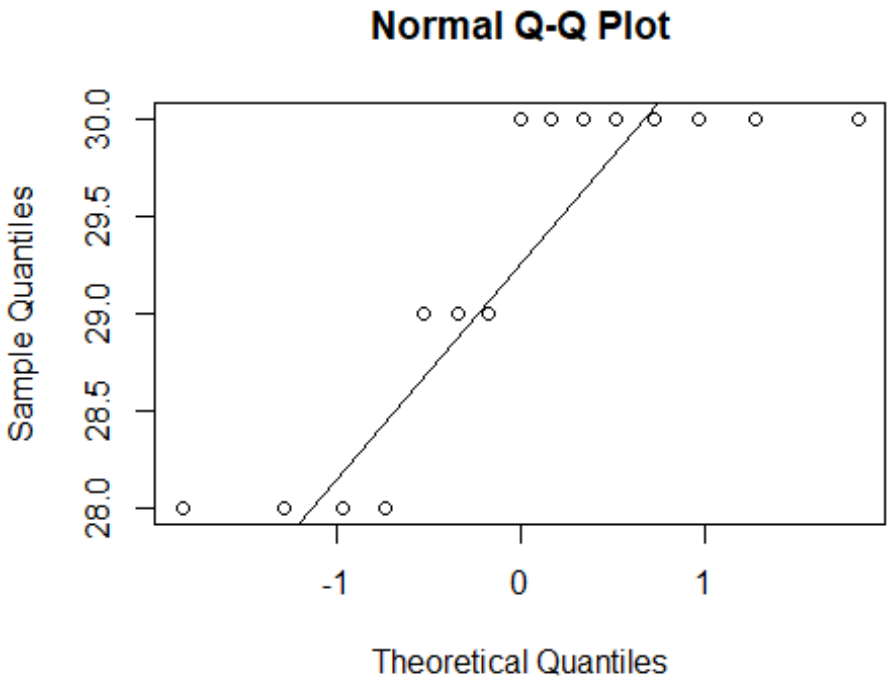



```
var.test(AD$Education, HC$Education)

##
##  F test to compare two variances
##
## data:  AD$Education and HC$Education
## F = 2.2758, num df = 24, denom df = 14, p-value = 0.1127
## alternative hypothesis: true ratio of variances is not equal to 1
## 95 percent confidence interval:
##  0.8160463 5.6159018
## sample estimates:
## ratio of variances
##           2.275799

t.test(AD$Education, HC$Education, var.equal = TRUE)

##
##  Two Sample t-test
##
## data:  AD$Education and HC$Education
## t = -1.2495, df = 38, p-value = 0.2191
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -2.9346079  0.6946079
## sample estimates:
## mean of x mean of y
##     14.08     15.20
```

```r
# MMSE

qqnorm(AD$MMSE_1)
qqline(AD$MMSE_1)
```

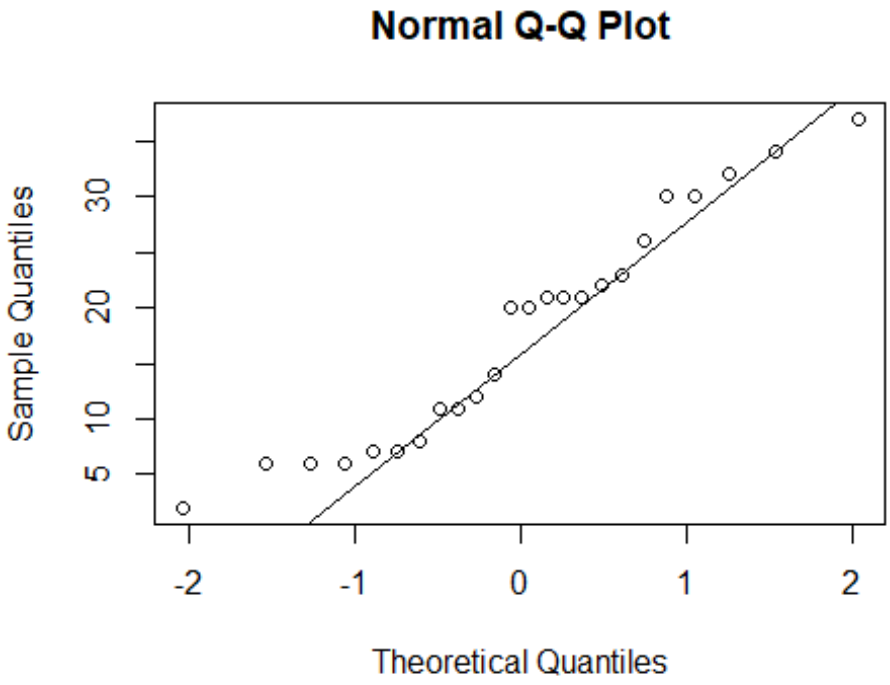



```r
qqnorm(HC$MMSE_1)
qqline(HC$MMSE_1)
```

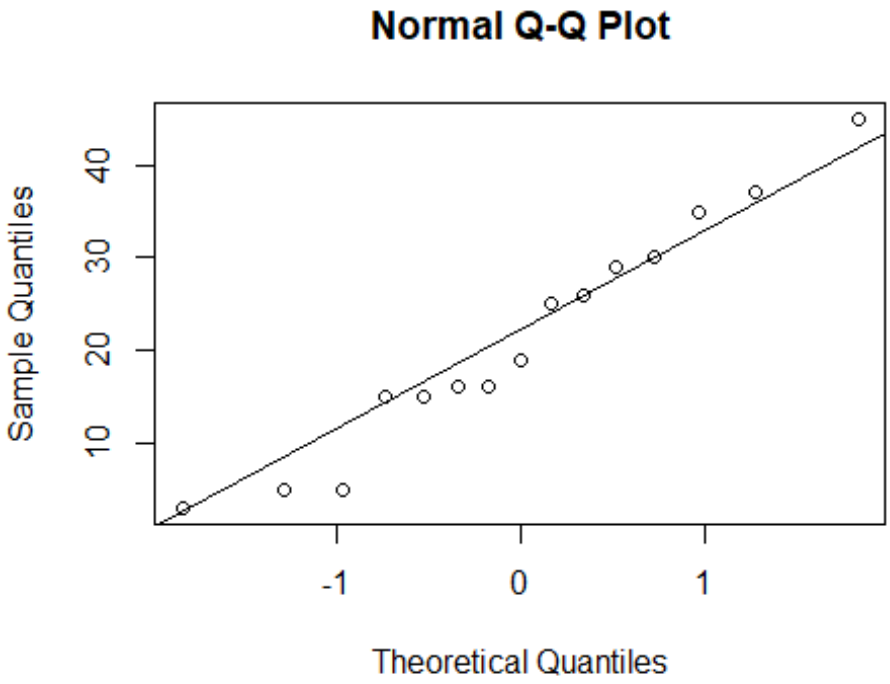



```
var.test(AD$MMSE_1, HC$MMSE_1)

##
##  F test to compare two variances
##
## data:  AD$MMSE_1 and HC$MMSE_1
## F = 13.872, num df = 24, denom df = 14, p-value = 7.478e-06
## alternative hypothesis: true ratio of variances is not equal to 1
## 95 percent confidence interval:
##   4.974145 34.231280
## sample estimates:
## ratio of variances
##           13.87195

t.test(AD$MMSE_1, HC$MMSE_1, var.equal = FALSE)

##
##  Welch Two Sample t-test
##
## data:  AD$MMSE_1 and HC$MMSE_1
## t = -8.4206, df = 29.386, p-value = 2.515e-09
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -7.290774 -4.442560
## sample estimates:
## mean of x mean of y
##  23.40000  29.26667
```

```r
tid_dif <- read_excel("Tid_dif.xlsx") #
tid_dif$Diagnosis <- as.factor(tid_dif$Diagnosis)

tid_dif <- tid_dif[-17,] # Remove patient without ear-EEG

AD_tiddif <- subset(tid_dif, Diagnosis == "2")
HC_tiddif <- subset(tid_dif, Diagnosis == "1")


qqnorm(AD_tiddif$Tid_dif)
qqline(AD_tiddif$Tid_dif)
```

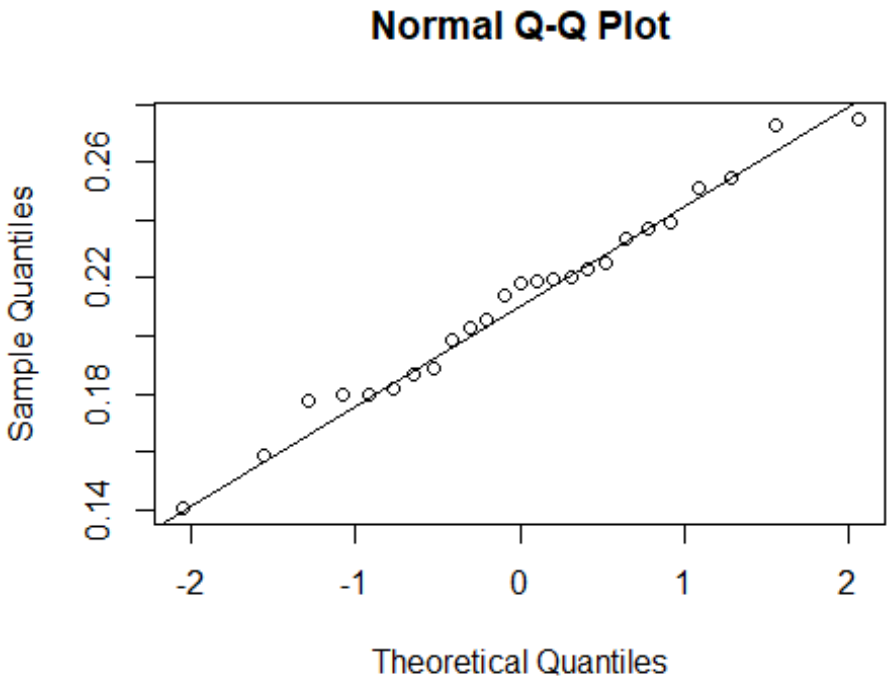



```r
qqnorm(HC_tiddif$Tid_dif)
qqline(HC_tiddif$Tid_dif)
```

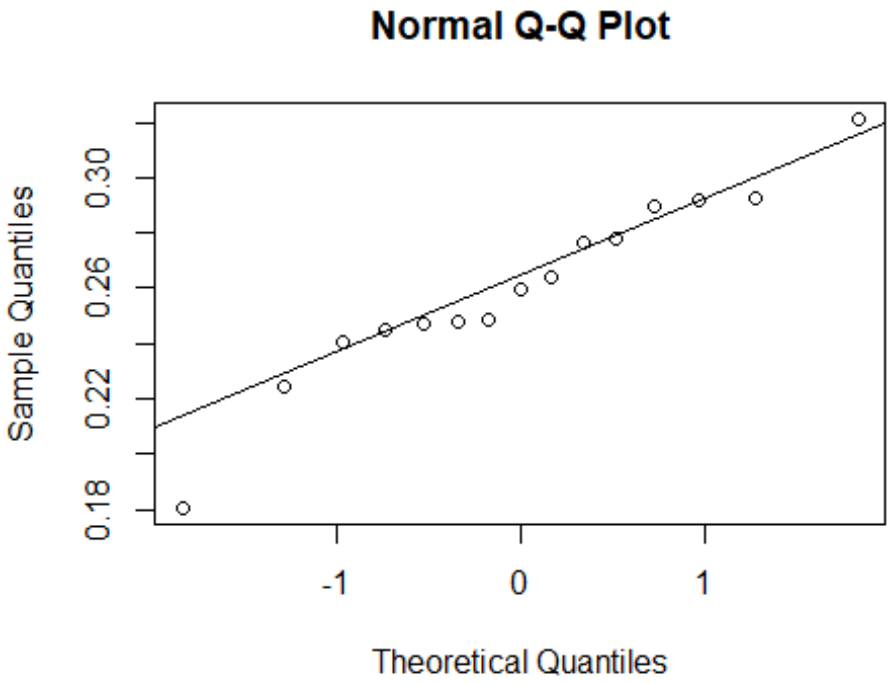



```
var.test(AD_tiddif$Tid_dif, HC_tiddif$Tid_dif)

##
##  F test to compare two variances
##
## data:  AD_tiddif$Tid_dif and HC_tiddif$Tid_dif
## F = 0.67769, num df = 23, denom df = 14, p-value = 0.3954
## alternative hypothesis: true ratio of variances is not equal to 1
## 95 percent confidence interval:
##  0.2419556 1.6919153
## sample estimates:
## ratio of variances
##          0.6776859

t.test(AD_tiddif$Tid_dif, HC_tiddif$Tid_dif, var.equal = TRUE)

##
##  Two Sample t-test
##
## data:  AD_tiddif$Tid_dif and HC_tiddif$Tid_dif
## t = -0.9839, df = 37, p-value = 0.3316
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -11.039132   3.822466
## sample estimates:
## mean of x mean of y
##  17.79167  21.40000
```

```r
AD_tiddif_both <- subset(tid_dif, Diagnosis == "2" | Diagnosis == "1")
mean(AD_tiddif_both$Tid_dif)

## [1] 19.17949

#
#
## Structural ##
#
#


Hippo$Diagnose <- as.factor(Hippo$Diagnose)
Hippo$Gender <- as.factor(Hippo$Gender)

Hippo$Left_hippo_c <- Hippo$Left_hippo/Hippo$Total_vol*100
Hippo$Right_hippo_c <- Hippo$Right_hippo/Hippo$Total_vol*100

AD_hippo <- subset(Hippo, Diagnose == "2")
HC_hippo <- subset(Hippo, Diagnose == "1")

# Left
qqnorm(AD_hippo$Left_hippo_c)
qqline(AD_hippo$Left_hippo_c)
```

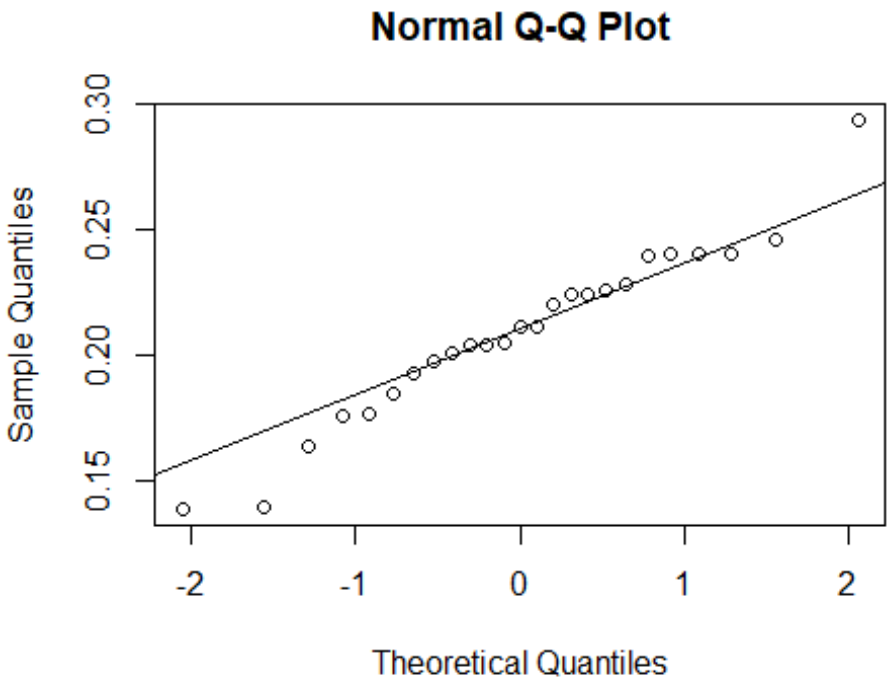



```r
qqnorm(HC_hippo$Left_hippo_c)
qqline(HC_hippo$Left_hippo_c)
```

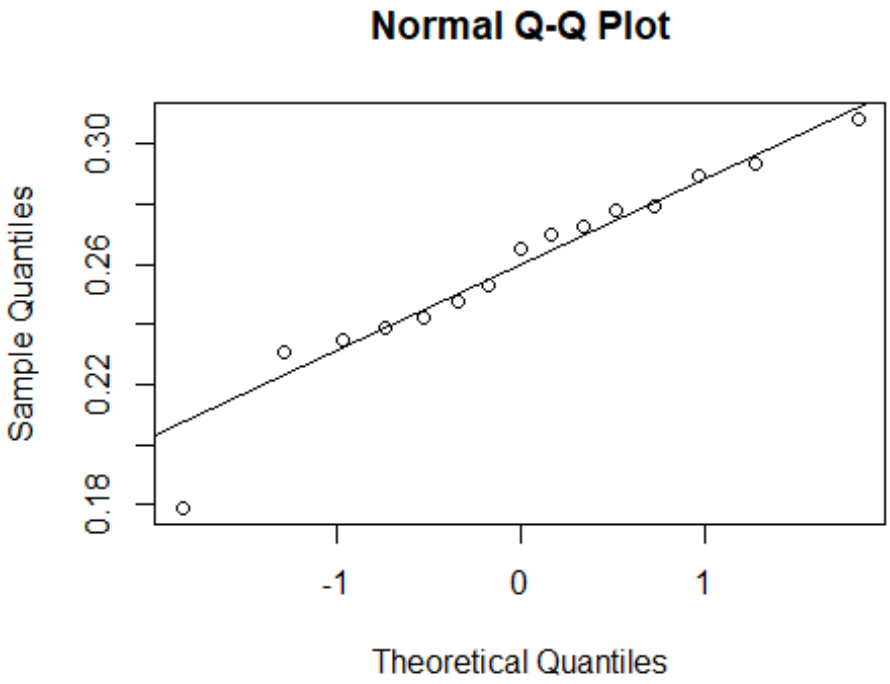



```
var.test(AD_hippo$Left_hippo_c, HC_hippo$Left_hippo_c)

##
##  F test to compare two variances
##
## data:  AD_hippo$Left_hippo_c and HC_hippo$Left_hippo_c
## F = 0.98454, num df = 24, denom df = 14, p-value = 0.9407
## alternative hypothesis: true ratio of variances is not equal to 1
## 95 percent confidence interval:
##  0.3530307 2.4295019
## sample estimates:
## ratio of variances
##          0.9845361

t.test(AD_hippo$Left_hippo_c, HC_hippo$Left_hippo_c, var.equal = TRUE)

##
##  Two Sample t-test
##
## data:  AD_hippo$Left_hippo_c and HC_hippo$Left_hippo_c
## t = -4.3958, df = 38, p-value = 8.584e-05
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -0.07045393 -0.02602313
## sample estimates:
## mean of x mean of y
## 0.2122014 0.2604400
```

```r
#Right
qqnorm(AD_hippo$Right_hippo_c)
qqline(AD_hippo$Right_hippo_c)
```

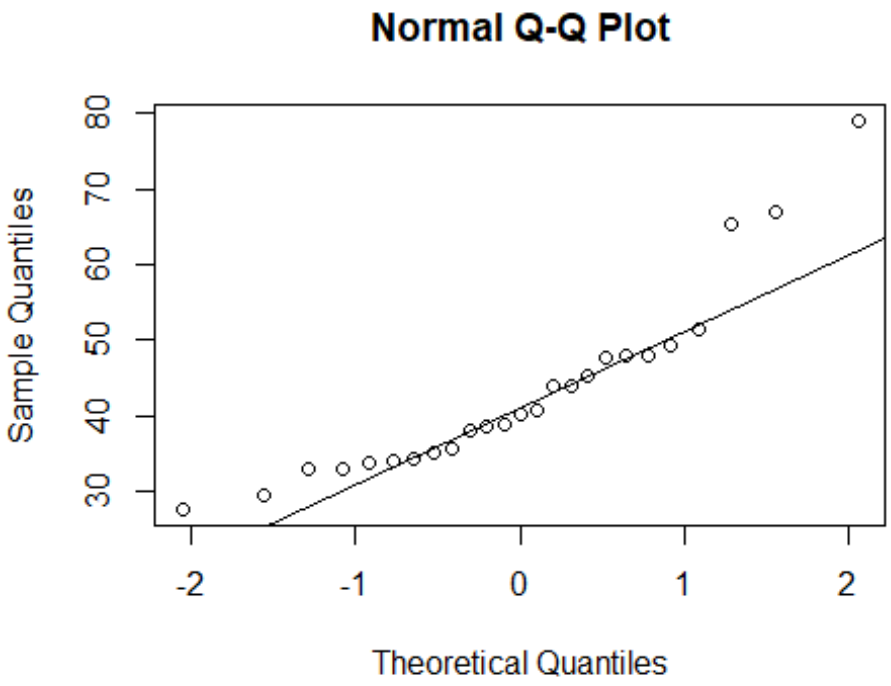



```r
qqnorm(HC_hippo$Right_hippo_c)
qqline(HC_hippo$Right_hippo_c)
```

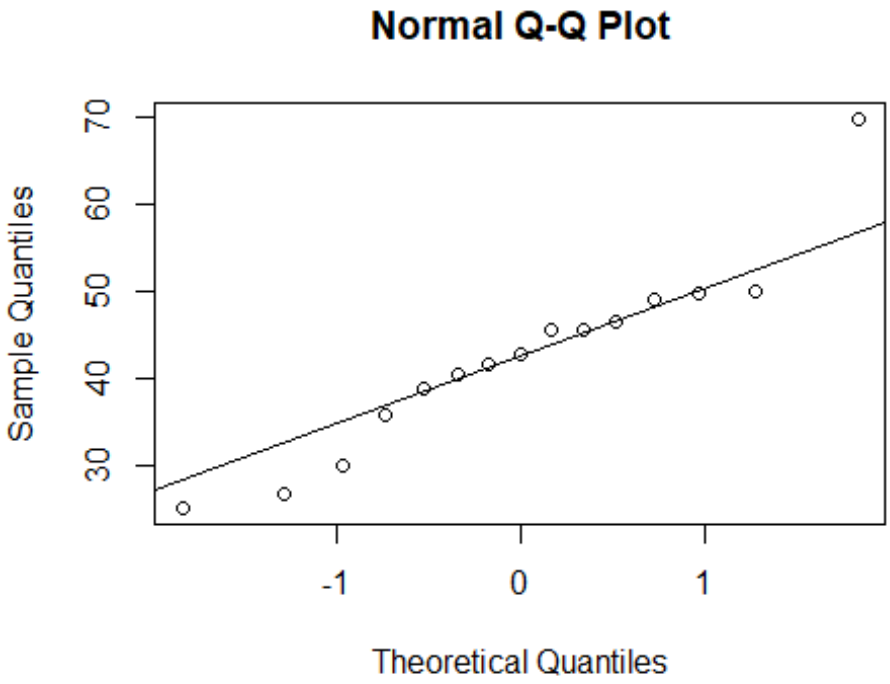



```
var.test(AD_hippo$Right_hippo_c, HC_hippo$Right_hippo_c)

##
##  F test to compare two variances
##
## data:  AD_hippo$Right_hippo_c and HC_hippo$Right_hippo_c
## F = 1.1758, num df = 24, denom df = 14, p-value = 0.7708
## alternative hypothesis: true ratio of variances is not equal to 1
## 95 percent confidence interval:
##  0.4215992 2.9013790
## sample estimates:
## ratio of variances
##           1.175761

t.test(AD_hippo$Right_hippo_c, HC_hippo$Right_hippo_c, var.equal = TRUE)

##
##  Two Sample t-test
##
## data:  AD_hippo$Right_hippo_c and HC_hippo$Right_hippo_c
## t = -4.5053, df = 38, p-value = 6.142e-05
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -0.07177459 -0.02727045
## sample estimates:
## mean of x mean of y
## 0.2092538 0.2587763
```

```r
#
#
# Flow
#
#


Flow <- read_excel("Flow.xlsx") #
Flow$Diagnosis <- as.factor(Flow$Diagnosis)
Flow$Gender <- as.factor(Flow$Gender)

AD_Flow <- subset(Flow, Diagnosis == "2")
HC_Flow <- subset(Flow, Diagnosis == "1")

# Flow
qqnorm(AD_Flow$Flow)
qqline(AD_Flow$Flow) # Two outliers
```

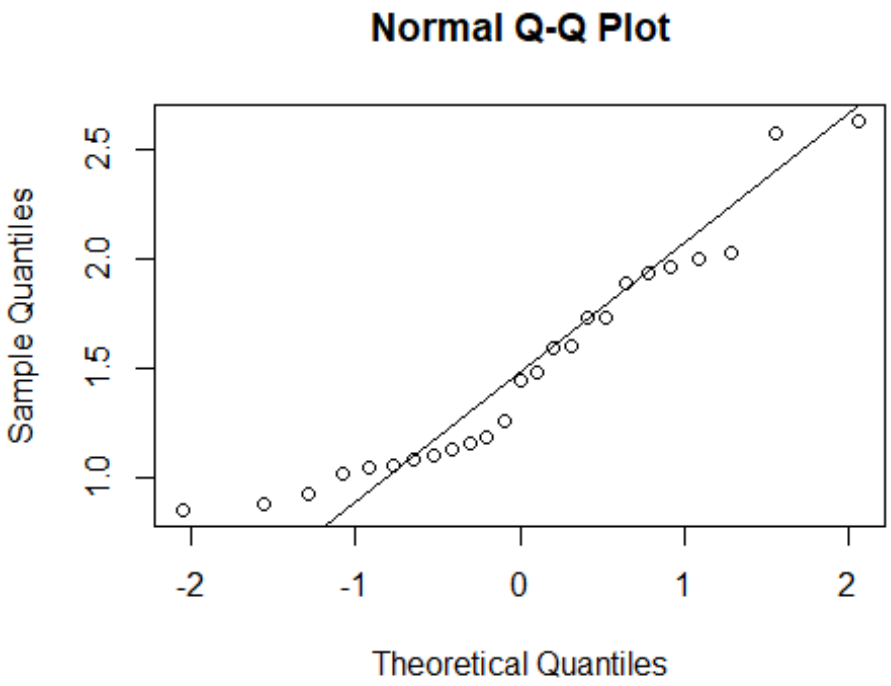



```r
qqnorm(HC_Flow$Flow)
qqline(HC_Flow$Flow)
```

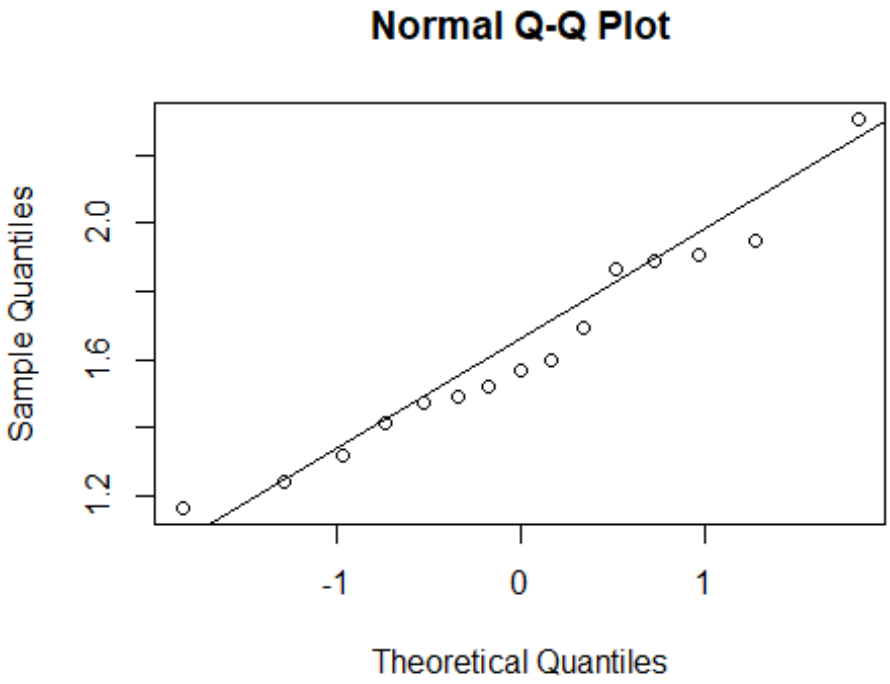



```
var.test(AD_Flow$Flow, HC_Flow$Flow)

##
##  F test to compare two variances
##
## data:  AD_Flow$Flow and HC_Flow$Flow
## F = 1.2441, num df = 24, denom df = 14, p-value = 0.6845
## alternative hypothesis: true ratio of variances is not equal to 1
## 95 percent confidence interval:
##  0.4460962 3.0699637
## sample estimates:
## ratio of variances
##           1.244078

t.test(AD_Flow$Flow, HC_Flow$Flow, var.equal = TRUE)

##
##  Two Sample t-test
##
## data:  AD_Flow$Flow and HC_Flow$Flow
## t = 0.16231, df = 38, p-value = 0.8719
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -7.190683  8.444229
## sample estimates:
## mean of x mean of y
##  43.22720  42.60043
```

```r
#
#
# ASL
#
#

ASL$Diagnosis <- as.factor(ASL$Diagnosis)

ASL$Mean_hippo_c <- (Hippo$Left_hippo+Hippo$Right_hippo)/Hippo$Total_vol*100

ASL$Left_hip_norm <- ASL$Left_hip/ASL$Flow
ASL$Right_hip_norm <- ASL$Right_hip/ASL$Flow
ASL$Mean_hip_norm <- (ASL$Mean_hip)/ASL$Flow

AD_ASL <- subset(ASL, Diagnosis =="2")
HC_ASL <- subset(ASL, Diagnosis =="1")

# Left hippocampus
qqnorm(AD_ASL$Left_hip_norm)
qqline(AD_ASL$Left_hip_norm)
```

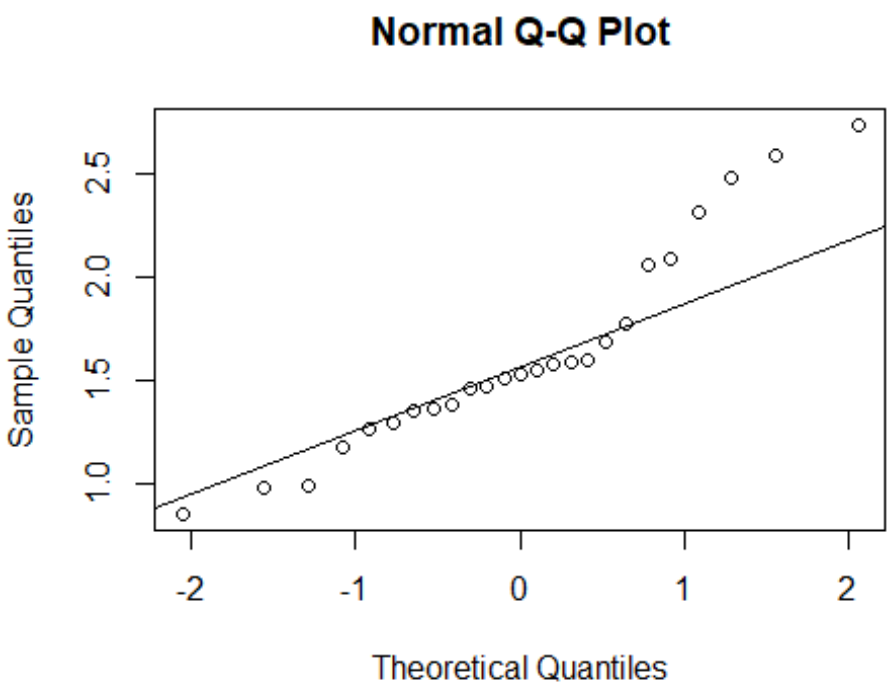



```r
qqnorm(HC_ASL$Left_hip_norm)
qqline(HC_ASL$Left_hip_norm)
```

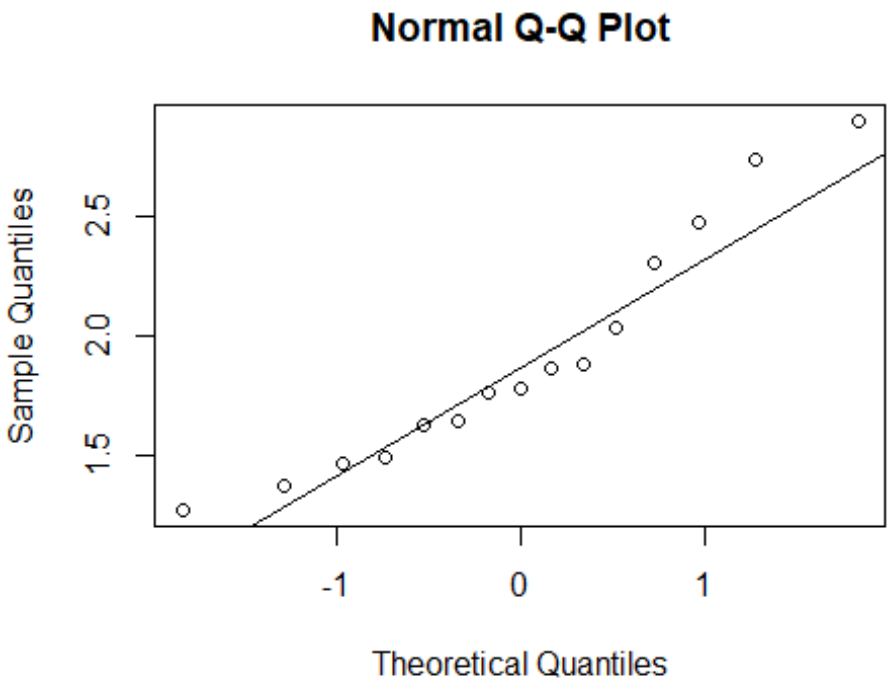



```
var.test(AD_ASL$Left_hip_norm, HC_ASL$Left_hip_norm)

##
##  F test to compare two variances
##
## data:  AD_ASL$Left_hip_norm and HC_ASL$Left_hip_norm
## F = 2.6925, num df = 24, denom df = 14, p-value = 0.05793
## alternative hypothesis: true ratio of variances is not equal to 1
## 95 percent confidence interval:
##  0.9654724 6.6442287
## sample estimates:
## ratio of variances
##            2.69252

t.test(AD_ASL$Left_hip_norm, HC_ASL$Left_hip_norm, var.equal = TRUE)

##
##  Two Sample t-test
##
## data:  AD_ASL$Left_hip_norm and HC_ASL$Left_hip_norm
## t = -0.91265, df = 38, p-value = 0.3672
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -0.4258929  0.1612105
## sample estimates:
## mean of x mean of y
##  1.494728  1.627070
```

```r
# Right Hippocampus
qqnorm(AD_ASL$Right_hip_norm)
qqline(AD_ASL$Right_hip_norm)
```

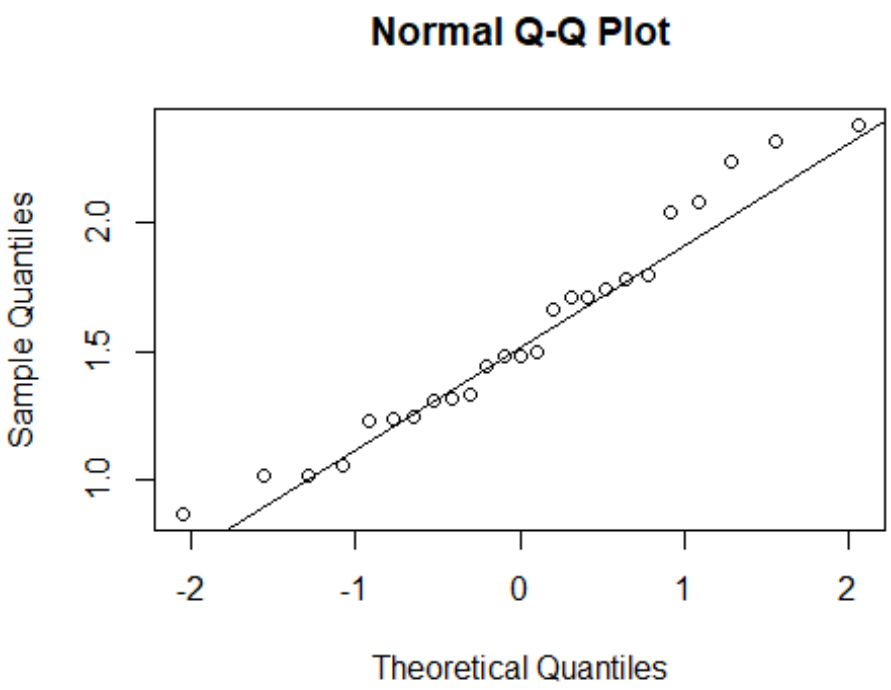



```r
qqnorm(HC_ASL$Right_hip_norm)
qqline(HC_ASL$Right_hip_norm)
```



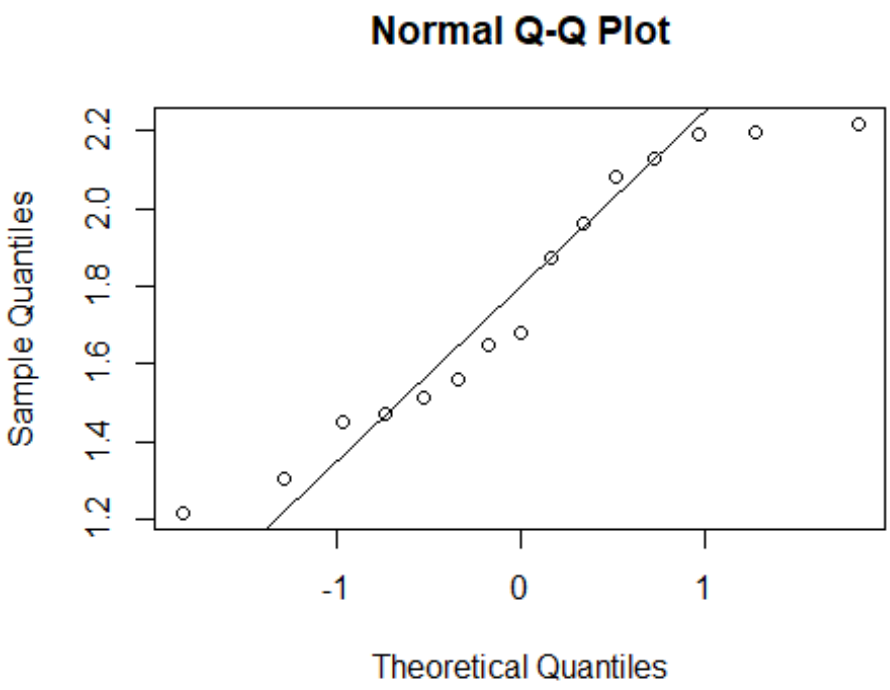

```
var.test(AD_ASL$Right_hip_norm, HC_ASL$Right_hip_norm)

##
##  F test to compare two variances
##
## data:  AD_ASL$Right_hip_norm and HC_ASL$Right_hip_norm
## F = 1.0064, num df = 24, denom df = 14, p-value = 0.977
## alternative hypothesis: true ratio of variances is not equal to 1
## 95 percent confidence interval:
##  0.3608717 2.4834620
## sample estimates:
## ratio of variances
##           1.006403

t.test(AD_ASL$Right_hip_norm, HC_ASL$Right_hip_norm, var.equal = TRUE)

##
##  Two Sample t-test
##
## data:  AD_ASL$Right_hip_norm and HC_ASL$Right_hip_norm
## t = -1.7302, df = 38, p-value = 0.09172
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -0.60918590  0.04774172
## sample estimates:
## mean of x mean of y
##  1.627635  1.908357
```

```r
# Mean hippocampus
qqnorm(AD_ASL$Mean_hip_norm)
qqline(AD_ASL$Mean_hip_norm)
```



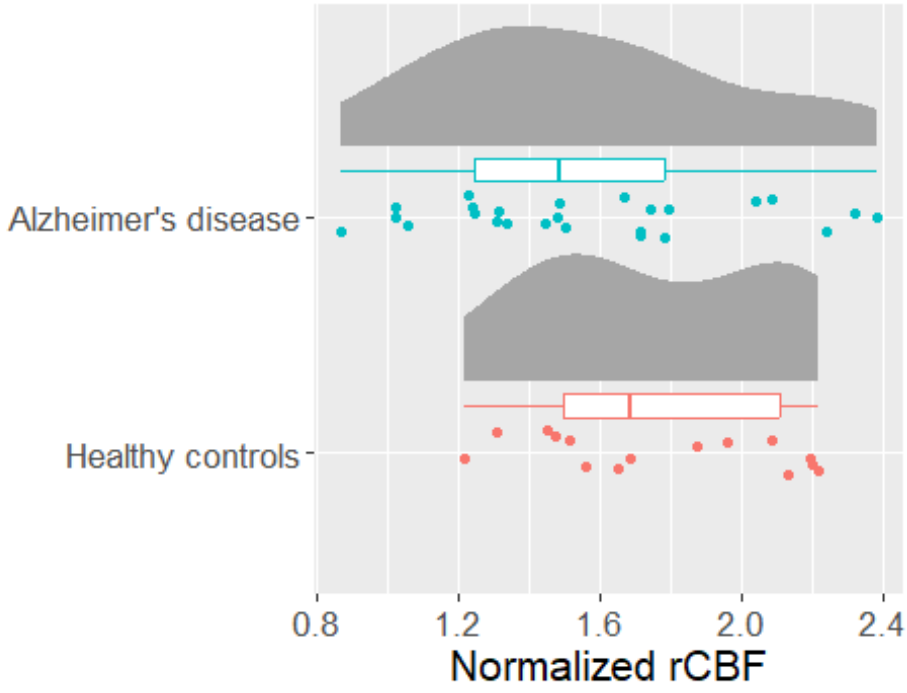

```r
qqnorm(HC_ASL$Mean_hip_norm)
qqline(HC_ASL$Mean_hip_norm)

var.test(AD_ASL$Mean_hip_norm, HC_ASL$Mean_hip_norm)

##
##  F test to compare two variances
##
## data:  AD_ASL$Mean_hip_norm and HC_ASL$Mean_hip_norm
## F = 1.4764, num df = 24, denom df = 14, p-value = 0.453
## alternative hypothesis: true ratio of variances is not equal to 1
## 95 percent confidence interval:
##  0.5294086 3.6433067
## sample estimates:
## ratio of variances
##           1.476421

t.test(AD_ASL$Mean_hip_norm, HC_ASL$Mean_hip_norm, var.equal = TRUE)

##
##  Two Sample t-test
##
## data:  AD_ASL$Mean_hip_norm and HC_ASL$Mean_hip_norm
## t = -1.6014, df = 38, p-value = 0.1176
## alternative hypothesis: true difference in means is not equal to 0
```

```
## 95 percent confidence interval:
##  -0.46762018  0.05455832
## sample estimates:
## mean of x mean of y
##  1.561178  1.767709

library(tidyverse)

## -- Attaching packages --------------------------------------- tidyverse 1.3.
2 --
## v ggplot2 3.4.0     v purrr   0.3.4
## v tibble  3.1.7     v dplyr   1.0.9
## v tidyr   1.2.0     v stringr 1.4.0
## v readr   2.1.2     v forcats 0.5.2
## -- Conflicts ------------------------------------------ tidyverse_conflicts(
) --
## x dplyr::filter() masks stats::filter()
## x dplyr::lag()    masks stats::lag()
```




```
library(ggdist)
library(ggplot2)

ASL_plot <- subset(ASL, Diagnosis == "1" | Diagnosis == "2")

ggplot(ASL_plot, aes(x = Mean_hip_norm, y = Diagnosis, color = Diagnosis)) +
  stat_halfeye(
    point_color = NA, .width = 0, height = 0.6,
    position = position_nudge(y = 0.3)
  ) +
```

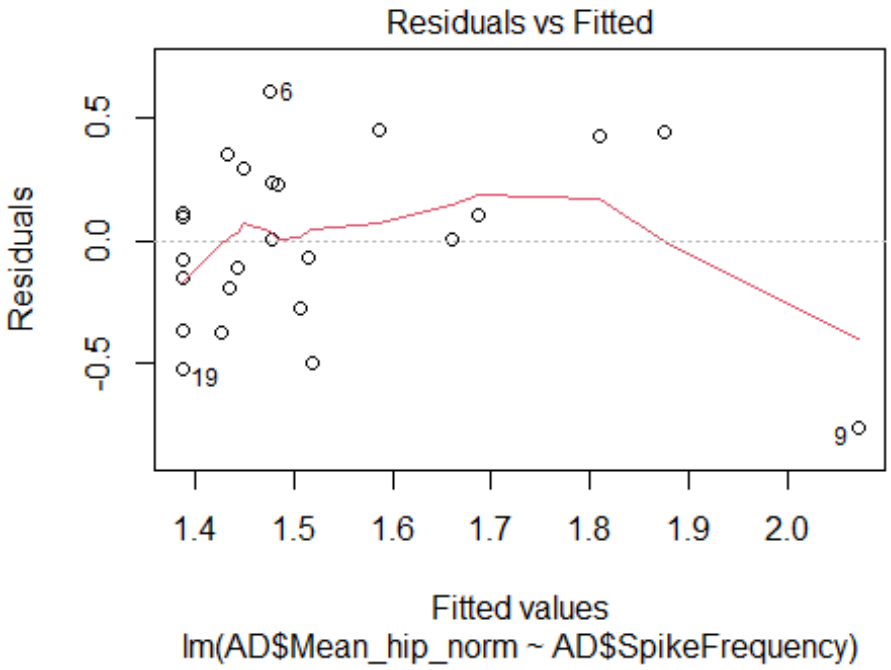
```
  geom_boxplot(
    position = position_nudge(y = 0.2),
    width = 0.1, outlier.shape = NA
  ) +
  geom_point(position = position_jitter(width = 0, height = 0.1, seed = 1)) +
  ggtitle("") + xlab("Normalized rCBF") + ylab("") +scale_y_discrete(breaks=c("
1","2"),labels=c("Healthy controls", "Alzheimer's disease")) + theme(legend.pos
ition = "none") +
  theme(text = element_text(size = 16))
```

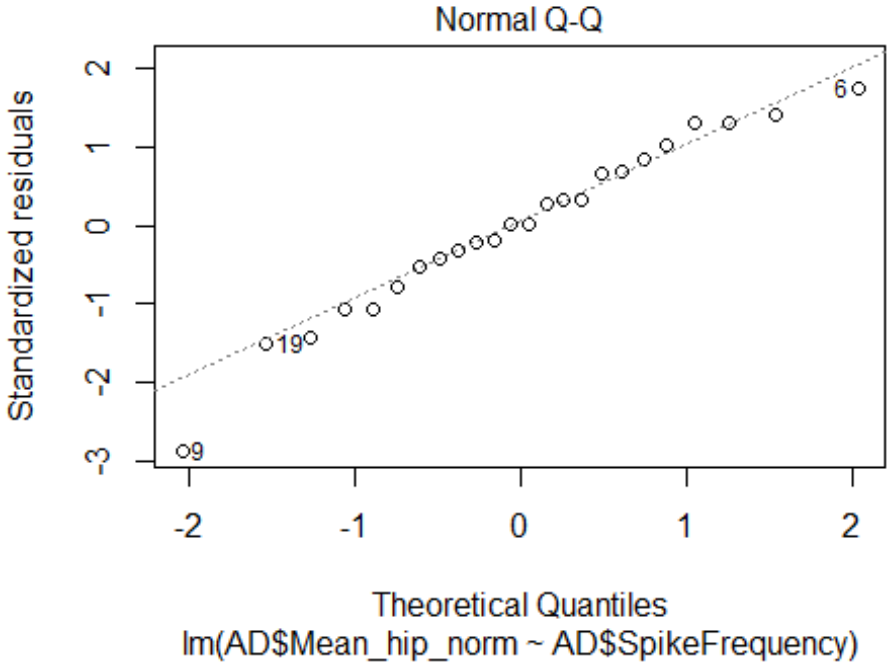


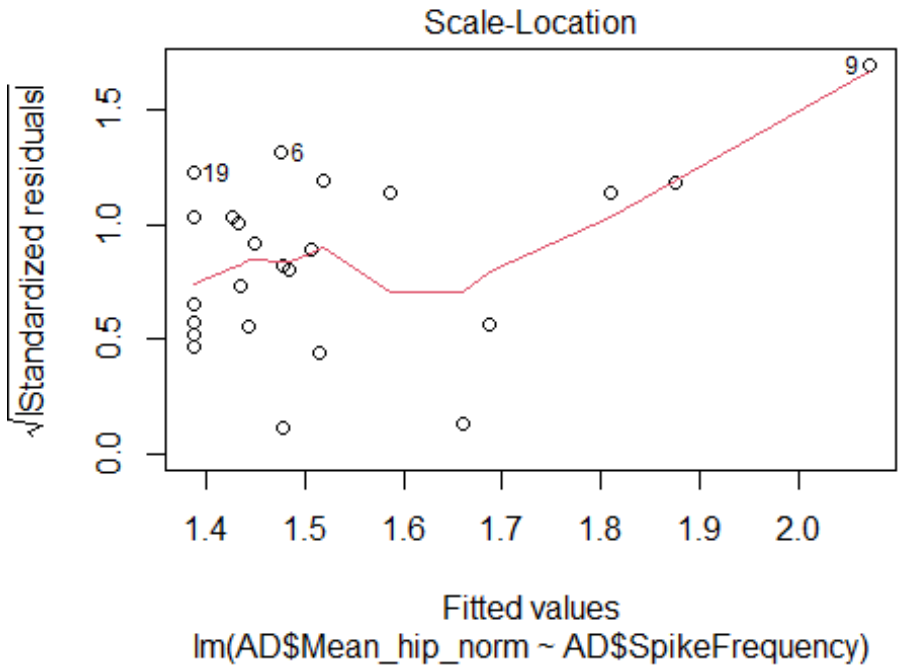

```
#
#
# Plot Linear regression
#
#

AD <- subset(ASL, Diagnosis == "2")

model1_AD <- lm (AD$Mean_hip_norm~AD$SpikeFrequency)
summary(model1_AD)

##
## Call:
## lm(formula = AD$Mean_hip_norm ~ AD$SpikeFrequency)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -0.7578 -0.2100  0.0053  0.2512  0.6079
##
```

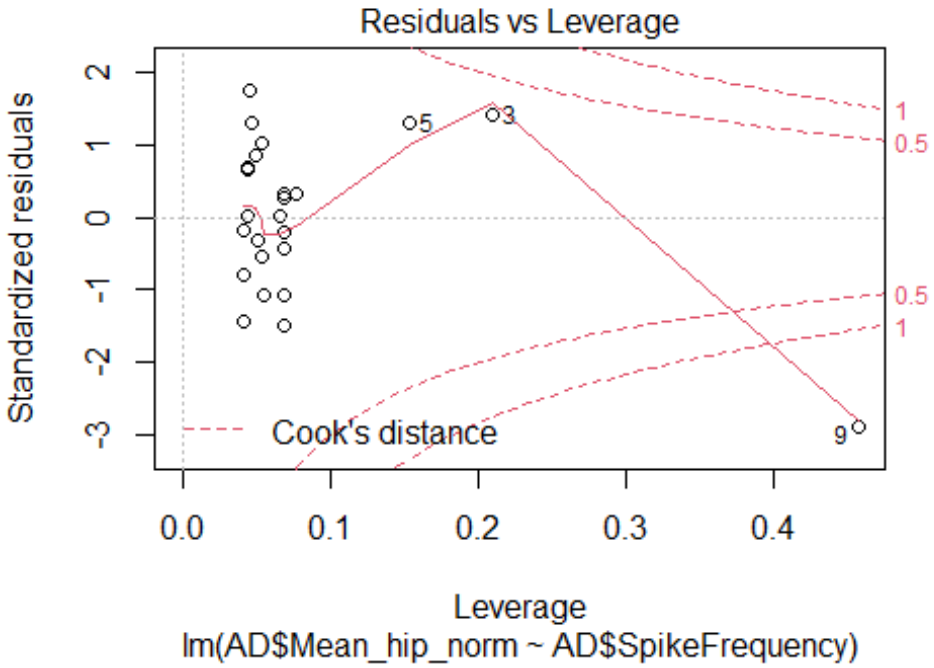
```
## Coefficients:
##                   Estimate Std. Error t value Pr(>|t|)
## (Intercept)        1.38625    0.09449  14.672 7.66e-13 ***
## AD$SpikeFrequency  0.05263    0.02233   2.356   0.0278 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.3587 on 22 degrees of freedom
##   (1 observation deleted due to missingness)
## Multiple R-squared:  0.2015, Adjusted R-squared:  0.1652
## F-statistic: 5.552 on 1 and 22 DF,  p-value: 0.02778
```

```
plot(model1_AD)
```


Residuals vs Fitted
Residuals
0.5
0.0
-0.5
6
19
9
1.4
1.5
1.6
1.7
1.8
1.9
2.0
Fitted values
lm(AD$Mean_hip_norm ~ AD$SpikeFrequency)


Normal Q-Q
Standardized residuals
2
1
0
-1
-2
-3
6
19
9
-2
-1
0
1
2
Theoretical Quantiles
lm(AD$Mean_hip_norm ~ AD$SpikeFrequency)

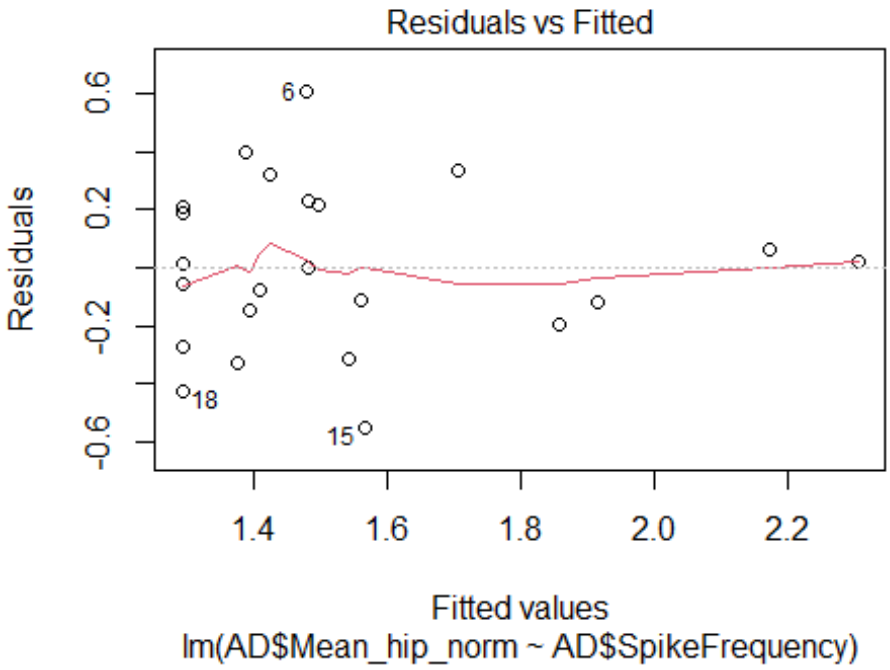


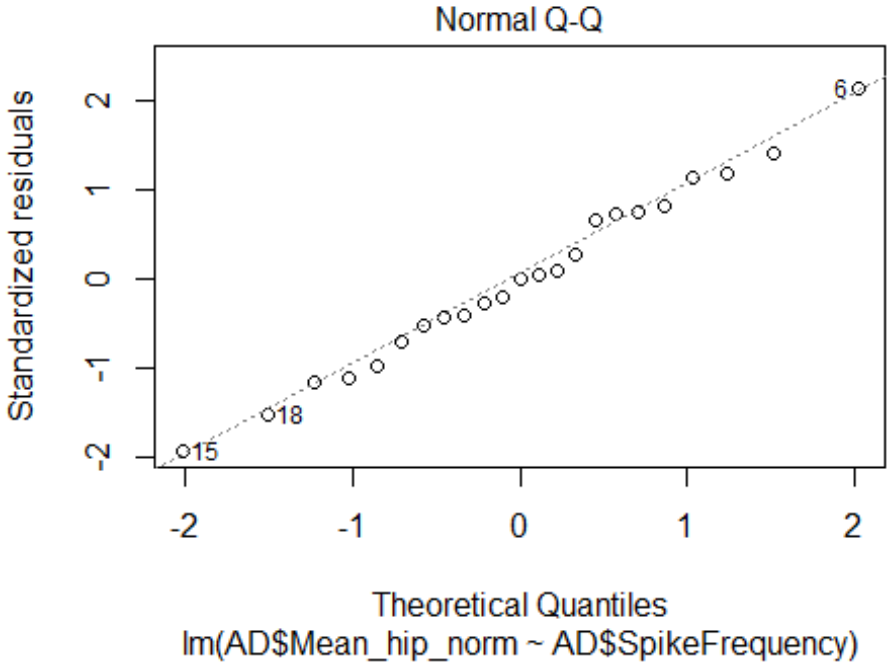


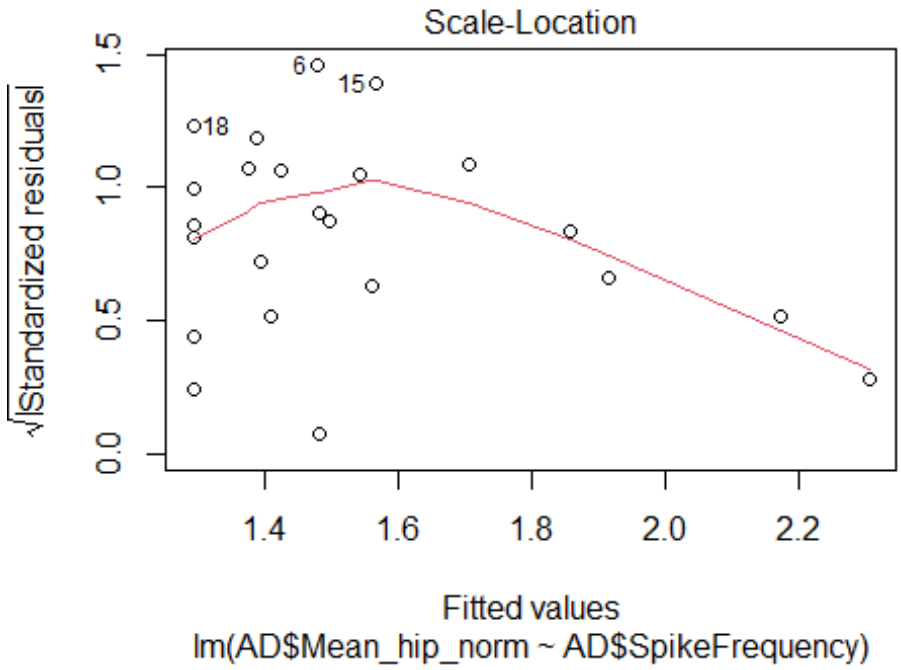

```
AD <- AD[-9,]

model1_AD <- lm (AD$Mean_hip_norm~AD$SpikeFrequency)
summary(model1_AD)
```

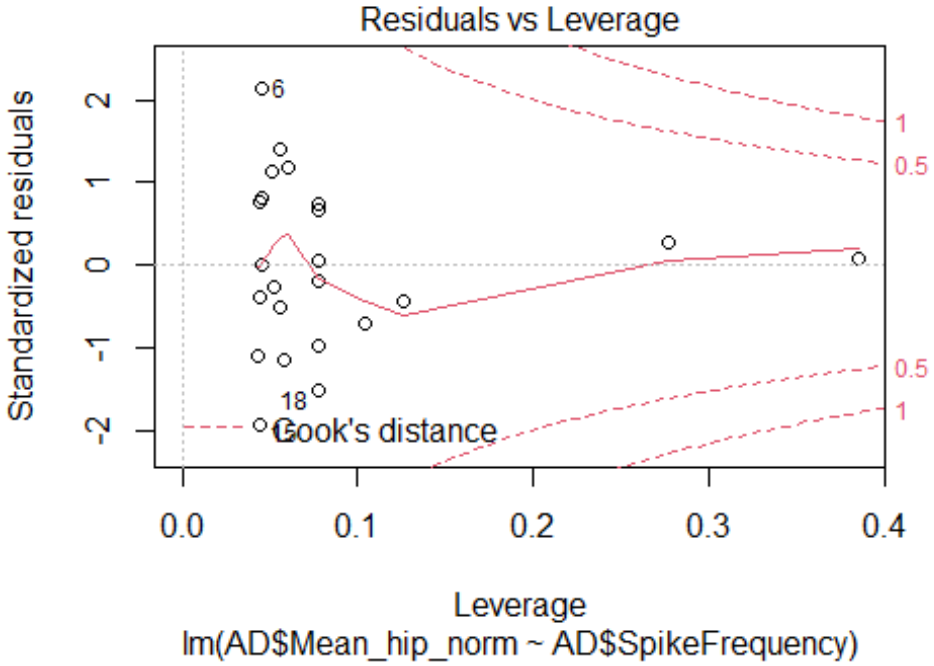
```
##
## Call:
## lm(formula = AD$Mean_hip_norm ~ AD$SpikeFrequency)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.54794 -0.17011 -0.00172  0.21061  0.60487
##
## Coefficients:
##                   Estimate Std. Error t value Pr(>|t|)
## (Intercept)        1.29437    0.08078  16.024 2.99e-13 ***
## AD$SpikeFrequency  0.10877    0.02404   4.524 0.000186 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.2904 on 21 degrees of freedom
##   (1 observation deleted due to missingness)
## Multiple R-squared:  0.4936, Adjusted R-squared:  0.4695
## F-statistic: 20.47 on 1 and 21 DF,  p-value: 0.0001857
```

```
plot(model1_AD)
```


Residuals vs Fitted
Residuals
0.6
0.2
-0.2
-0.6
6
18
15
1.4
1.6
1.8
2.0
2.2
Fitted values
lm(AD$Mean_hip_norm ~ AD$SpikeFrequency)

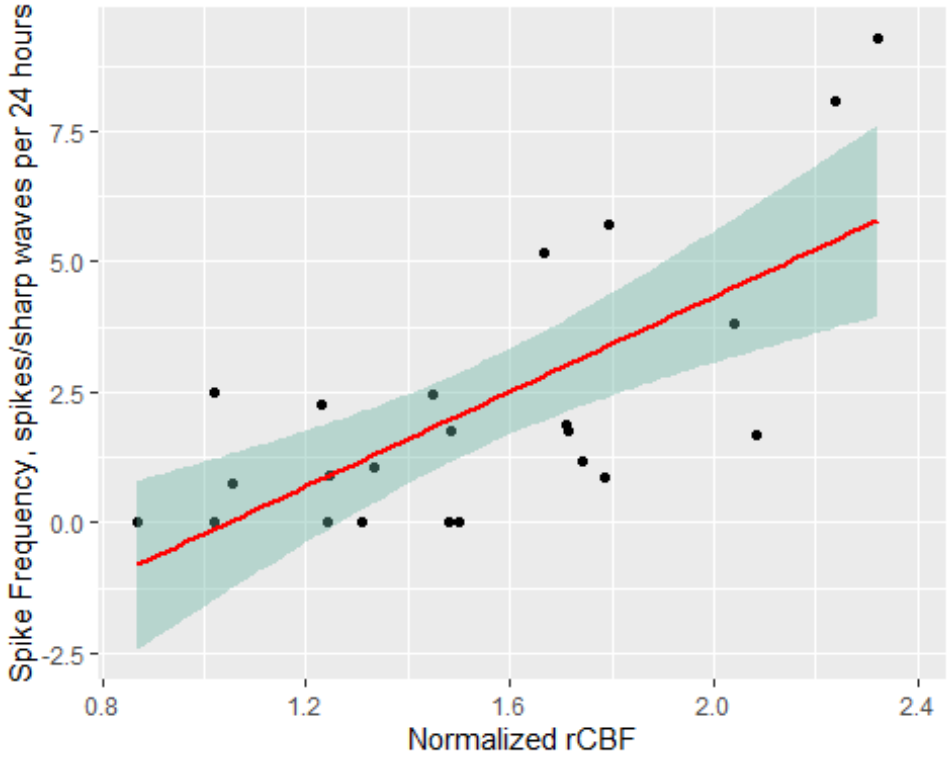

Normal Q-Q
Standardized residuals
2
1
0
-1
-2
6
18
15
-2
-1
0
1
2
Theoretical Quantiles
lm(AD$Mean_hip_norm ~ AD$SpikeFrequency)



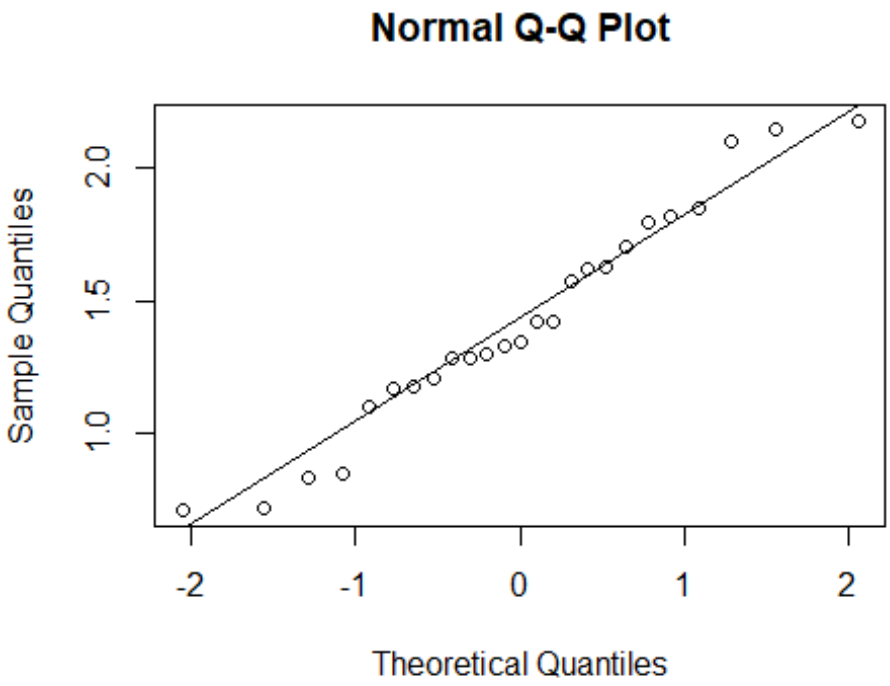



```
ggplot(AD, aes(x=Mean_hip_norm, y=SpikeFrequency)) +
  geom_point() +
  geom_smooth(method=lm , color="red", fill="#69b3a2", se=TRUE) +
  xlab("Normalized rCBF") + ylab("Spike Frequency, spikes/sharp waves per 24 ho
urs")
```

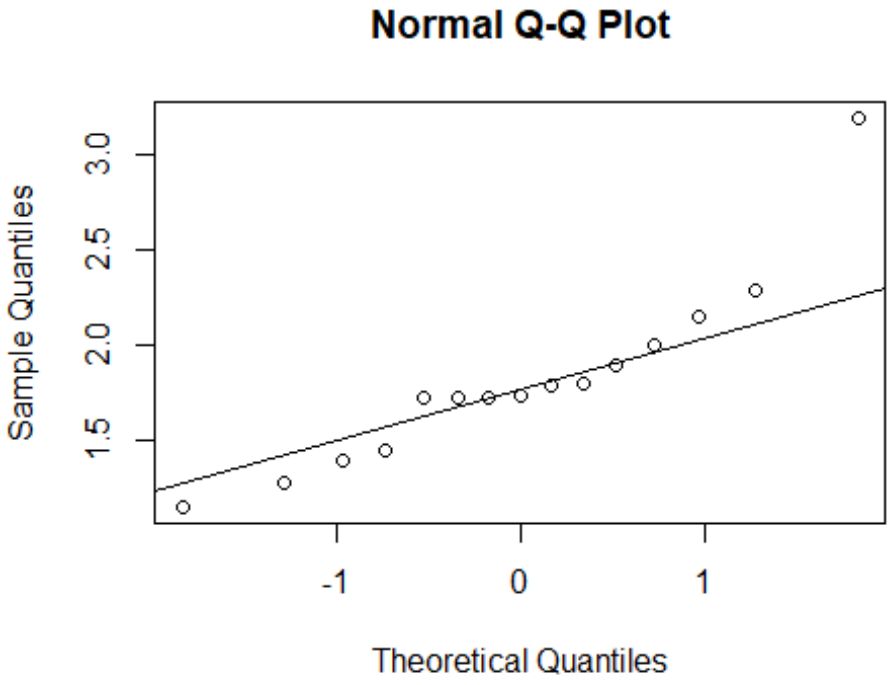
```
## `geom_smooth()` using formula = 'y ~ x'

## Warning: Removed 1 rows containing non-finite values (`stat_smooth()`).

## Warning: Removed 1 rows containing missing values (`geom_point()`).
```




```
# Precuneus


Precuneus <- read.delim("MyFile_precuneus.txt", sep = ",", dec = ".")

Precuneus$Diagnosis <- Flow$Diagnosis

Precuneus$Diagnosis <- as.factor(Precuneus$Diagnosis)

Precuneus$Precuneus_norm <- Precuneus$Precuneus/Precuneus$Flow
Precuneus$gender <- as.factor(Precuneus$gender)

AD_precuneus <- subset(Precuneus, Diagnosis == "2")
HC_precuneus <- subset(Precuneus, Diagnosis == "1")

# Precuneus
qqnorm(AD_precuneus$Precuneus_norm)
qqline(AD_precuneus$Precuneus_norm)
```




```
qqnorm(HC_precuneus$Precuneus_norm)
qqline(HC_precuneus$Precuneus_norm)
```



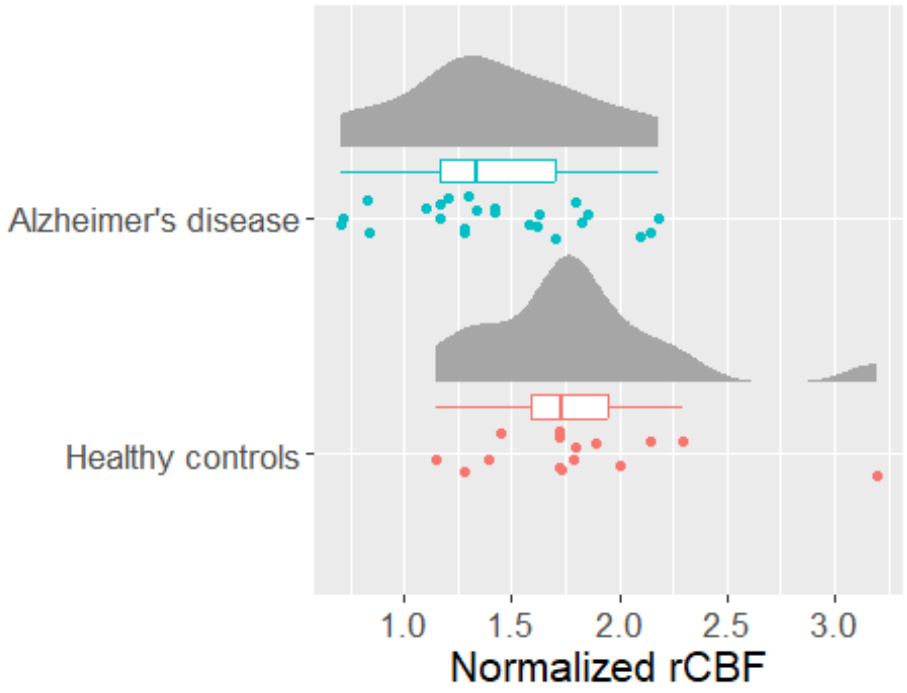

```
var.test(AD_precuneus$Precuneus_norm, HC_precuneus$Precuneus_norm)
```

```
##
##  F test to compare two variances
##
## data:  AD_precuneus$Precuneus_norm and HC_precuneus$Precuneus_norm
## F = 0.73881, num df = 24, denom df = 14, p-value = 0.4983
## alternative hypothesis: true ratio of variances is not equal to 1
## 95 percent confidence interval:
##  0.2649192 1.8231320
## sample estimates:
## ratio of variances
##          0.7388096
```

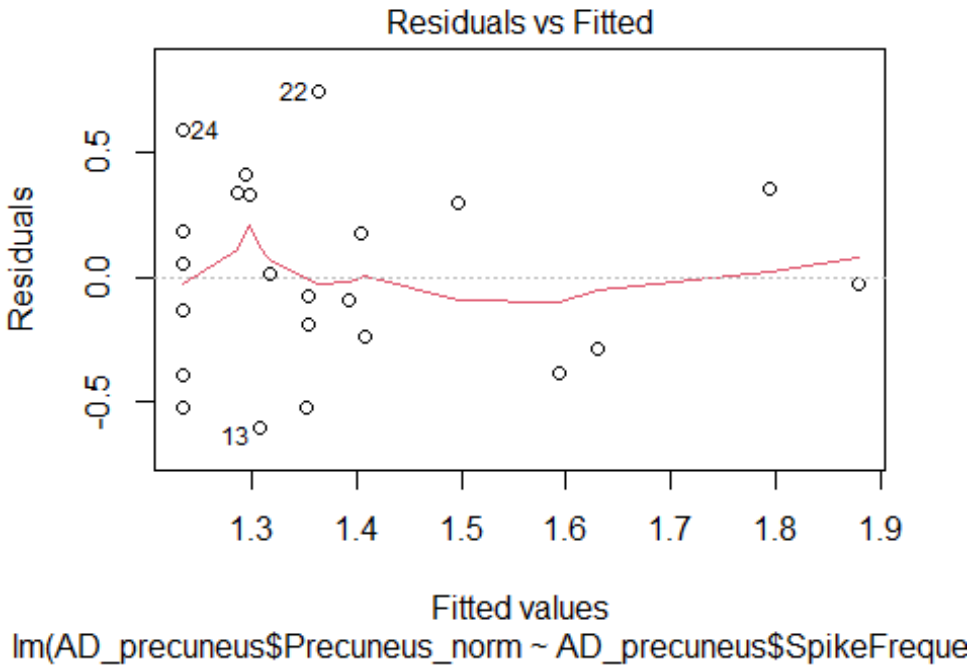
```
t.test(AD_precuneus$Precuneus_norm, HC_precuneus$Precuneus_norm, var.equal = TR
UE)
```

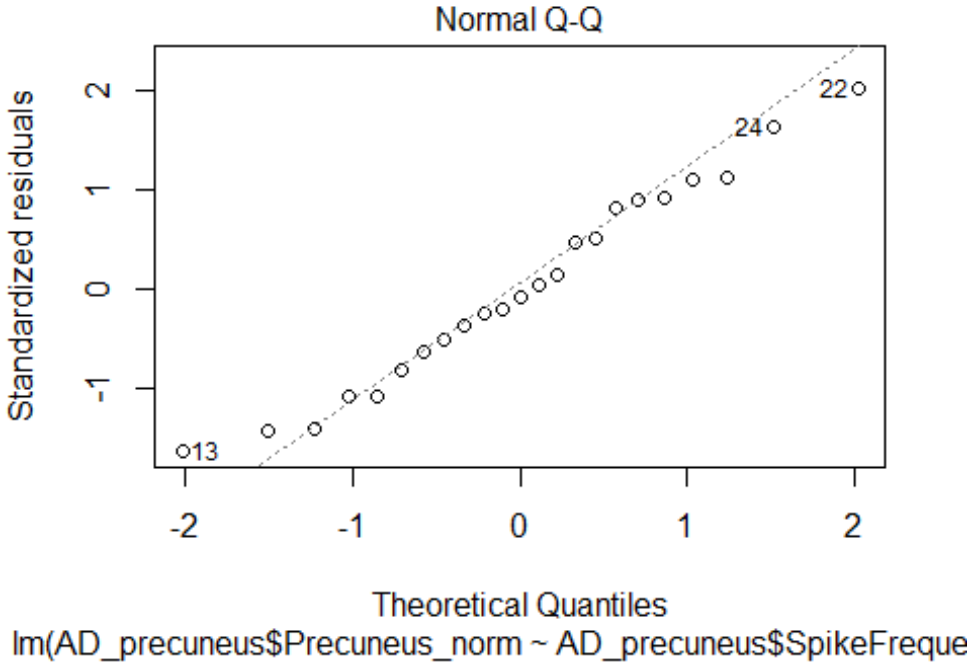
```
##
##  Two Sample t-test
##
## data:  AD_precuneus$Precuneus_norm and HC_precuneus$Precuneus_norm
## t = -2.7293, df = 38, p-value = 0.009563
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -0.6956283 -0.1031489
## sample estimates:
## mean of x mean of y
##  1.421242  1.820631
```

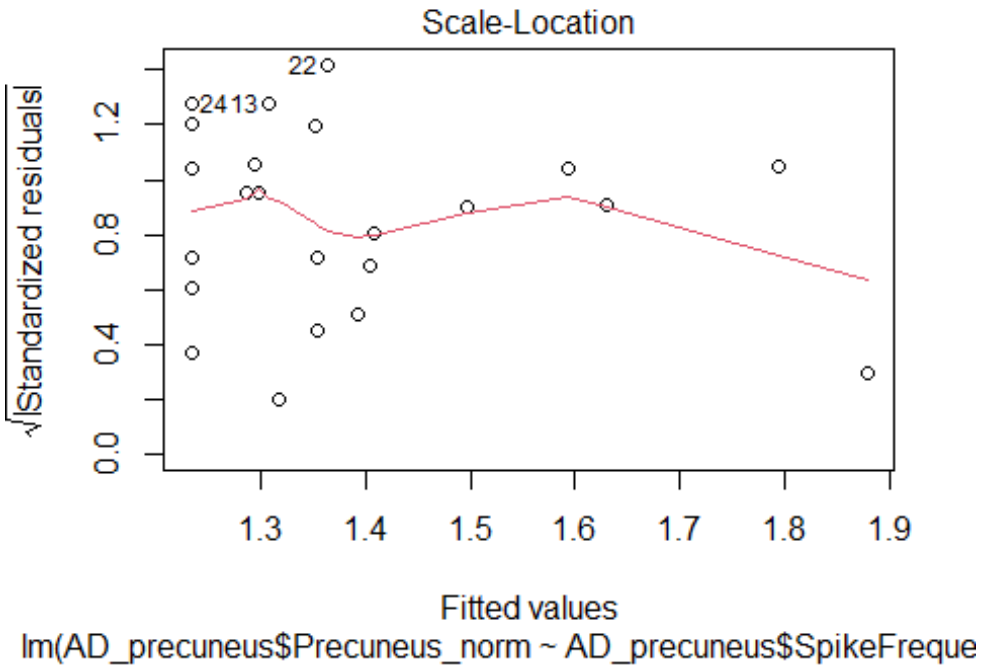
```
ASL_plot_precuneus <- subset(Precuneus, Diagnosis == "1" | Diagnosis == "2")

ggplot(ASL_plot_precuneus, aes(x = Precuneus_norm, y = Diagnosis, color = Diagn
osis)) +
  stat_halfeye(
    point_color = NA, .width = 0, height = 0.6,
    position = position_nudge(y = 0.3)
  ) +
  geom_boxplot(
    position = position_nudge(y = 0.2),
    width = 0.1, outlier.shape = NA
  ) +
  geom_point(position = position_jitter(width = 0, height = 0.1, seed = 1)) +
  ggtitle("") + xlab("Normalized rCBF") + ylab("") +scale_y_discrete(breaks=c("
1","2"),labels=c("Healthy controls", "Alzheimer's disease")) + theme(legend.pos
ition = "none") +
  theme(text = element_text(size = 16))
```

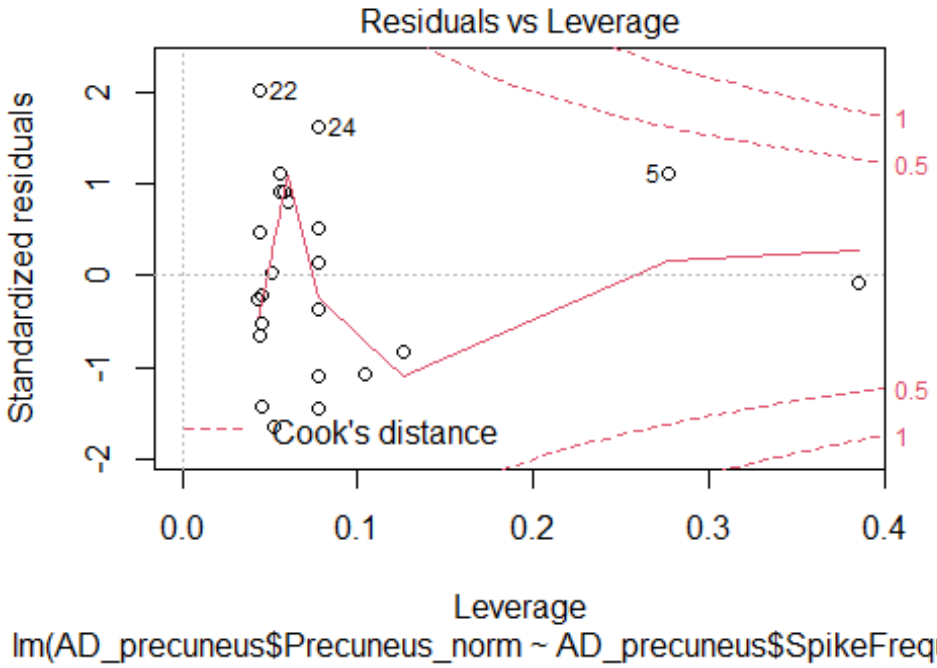



```
# Association SF and norm rCBF in precuneus
AD_precuneus <- AD_precuneus[-9,]
library(ggplot2)
model1 <- lm (AD_precuneus$Precuneus_norm~AD_precuneus$SpikeFrequency)
summary(model1)

##
## Call:
## lm(formula = AD_precuneus$Precuneus_norm ~ AD_precuneus$SpikeFrequency)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.59895 -0.26321 -0.02564  0.31213  0.73855
##
## Coefficients:
##                              Estimate Std. Error t value Pr(>|t|)
## (Intercept)                   1.23417    0.10443  11.818 9.65e-11 ***
## AD_precuneus$SpikeFrequency  0.06940    0.03108   2.233   0.0366 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.3755 on 21 degrees of freedom
##   (1 observation deleted due to missingness)
## Multiple R-squared:  0.1919, Adjusted R-squared:  0.1534
## F-statistic: 4.986 on 1 and 21 DF,  p-value: 0.03657

plot(model1)
```


Residuals vs Fitted
Residuals
0.5
0.0
-0.5
22
24
13
1.3
1.4
1.5
1.6
1.7
1.8
1.9
Fitted values
lm(AD_precuneus$Precuneus_norm ~ AD_precuneus$SpikeFreque

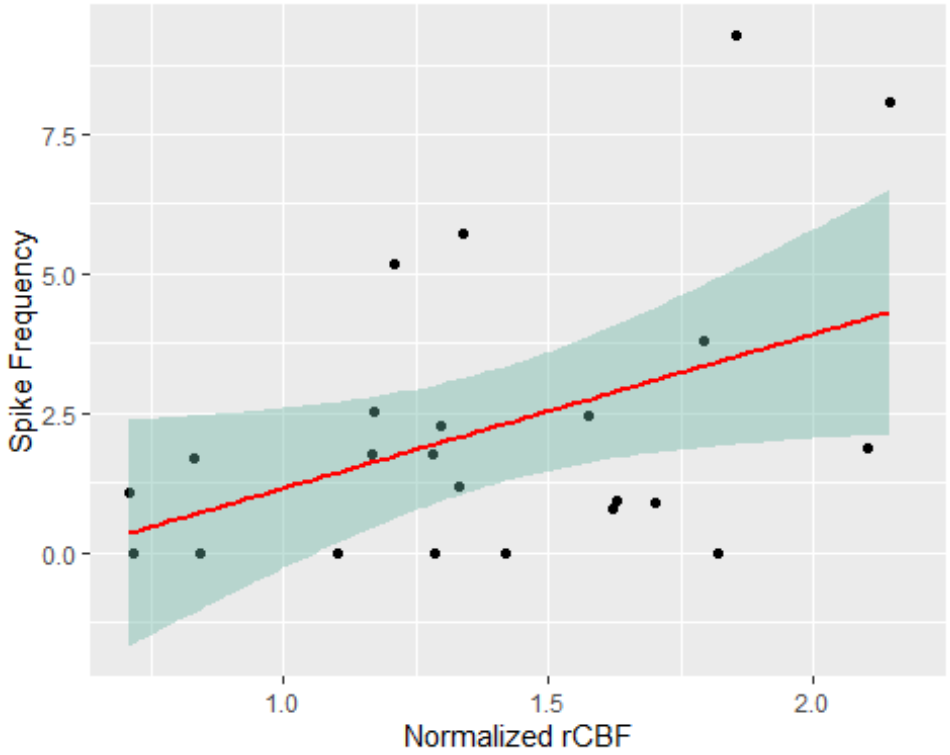

Normal Q-Q
Standardized residuals
2
1
0
-1
22
24
13
-2
-1
0
1
2
Theoretical Quantiles
lm(AD_precuneus$Precuneus_norm ~ AD_precuneus$SpikeFreque






```
ggplot(AD_precuneus, aes(x=Precuneus_norm, y=SpikeFrequency)) +
  geom_point() +
  geom_smooth(method=lm , color="red", fill="#69b3a2", se=TRUE) +
  xlab("Normalized rCBF") + ylab("Spike Frequency")

## `geom_smooth()` using formula = 'y ~ x'
```

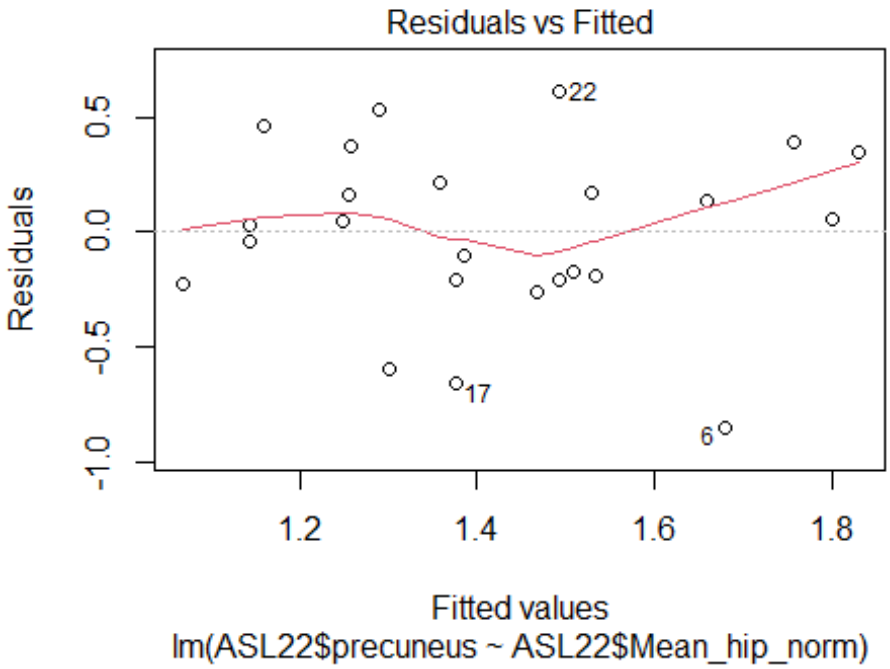
```
## Warning: Removed 1 rows containing non-finite values (`stat_smooth()`).
## Removed 1 rows containing missing values (`geom_point()`).
```

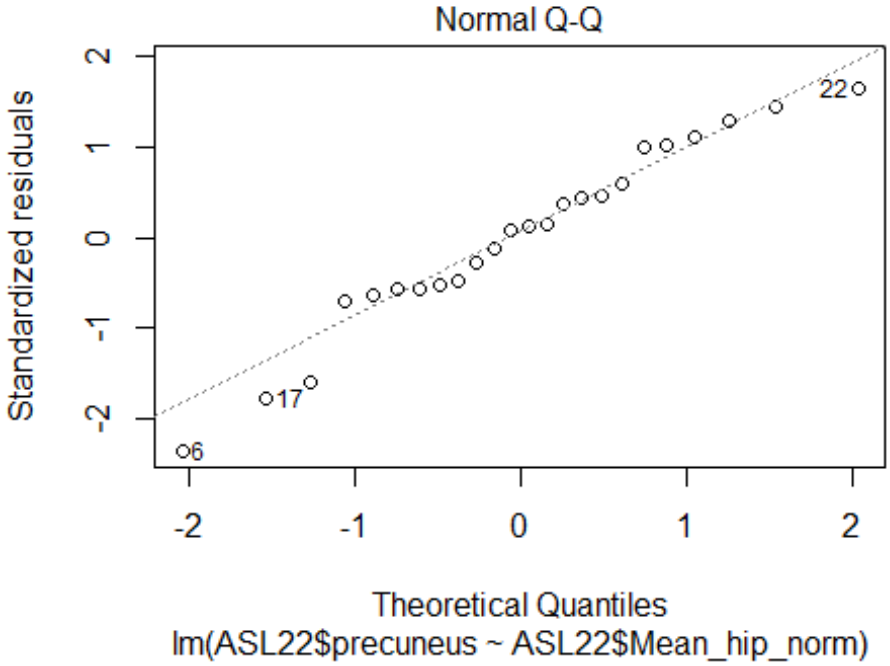


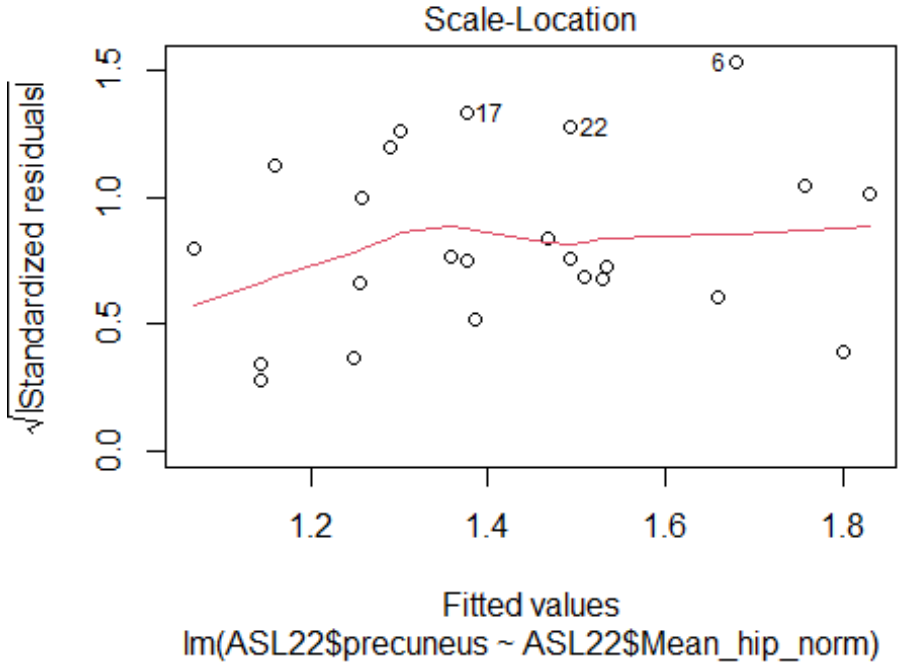

```
confint(model1)

##                                    2.5 %    97.5 %
## (Intercept)                 1.016983580 1.4513470
## AD_precuneus$SpikeFrequency 0.004764957 0.1340434

# Association Precuneus and Hippocampus in AD
ASL2 = subset(ASL, Diagnosis == "2" )
ASL22 <- ASL2[-9,]
ASL22$precuneus <- AD_precuneus$Precuneus_norm

model1 <- lm (ASL22$precuneus~ASL22$Mean_hip_norm)
summary(model1)

##
## Call:
## lm(formula = ASL22$precuneus ~ ASL22$Mean_hip_norm)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.84977 -0.21041  0.03865  0.25064  0.60950
##
## Coefficients:
##                      Estimate Std. Error t value Pr(>|t|)
## (Intercept)            0.6291     0.3031   2.075   0.0498 *
## ASL22$Mean_hip_norm    0.5042     0.1864   2.704   0.0130 *
```

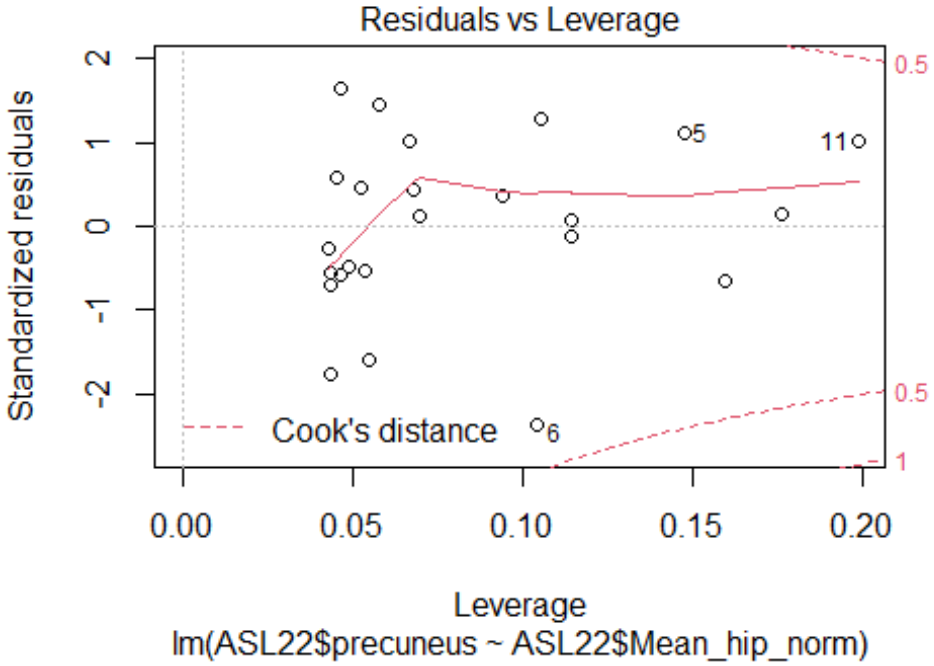
```
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.3813 on 22 degrees of freedom
## Multiple R-squared:  0.2495, Adjusted R-squared:  0.2154
## F-statistic: 7.313 on 1 and 22 DF,  p-value: 0.01295
```

```
plot(model1)
```


Residuals vs Fitted
Residuals
0.5
0.0
-0.5
-1.0
22
17
6
1.2
1.4
1.6
1.8
Fitted values
lm(ASL22$precuneus ~ ASL22$Mean_hip_norm)

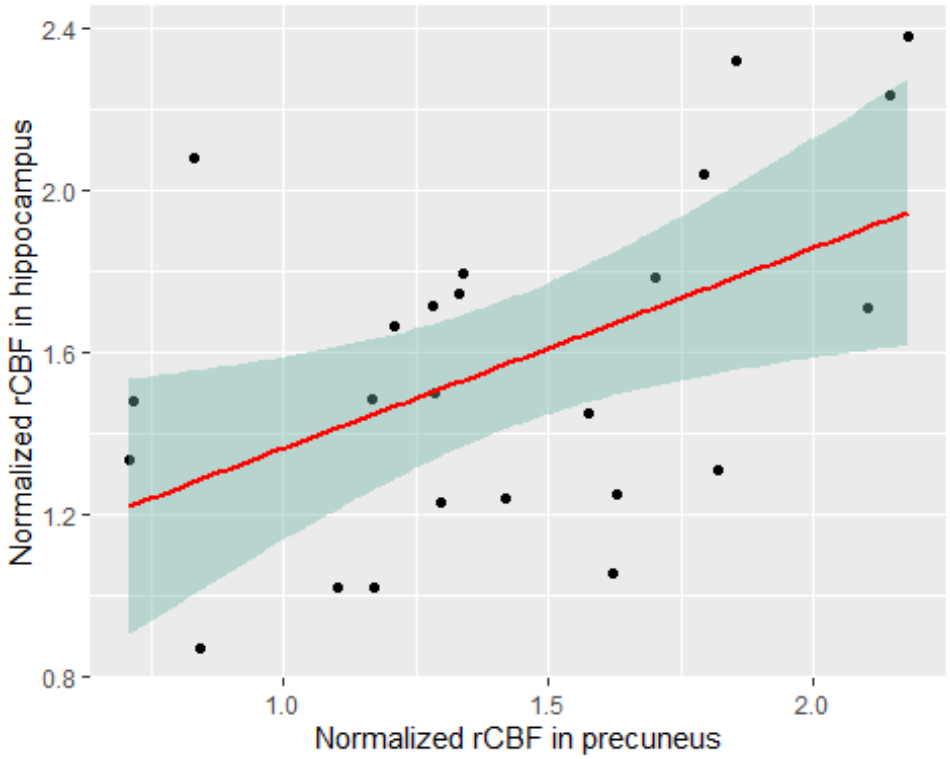

Normal Q-Q
Standardized residuals
2
1
0
-1
-2
22
17
6
-2
-1
0
1
2
Theoretical Quantiles
lm(ASL22$precuneus ~ ASL22$Mean_hip_norm)






```r
library(ggplot2)
ggplot(ASL22, aes(x=precuneus, y=Mean_hip_norm)) +
  geom_point() +
  geom_smooth(method=lm , color="red", fill="#69b3a2", se=TRUE) +
  xlab("Normalized rCBF in precuneus") + ylab("Normalized rCBF in hippocampus")
```

```
## `geom_smooth()` using formula = 'y ~ x'
```



UNIVERSITY OF COPENHAGEN

FACULTY OF HEALTH AND MEDICAL SCIENCES