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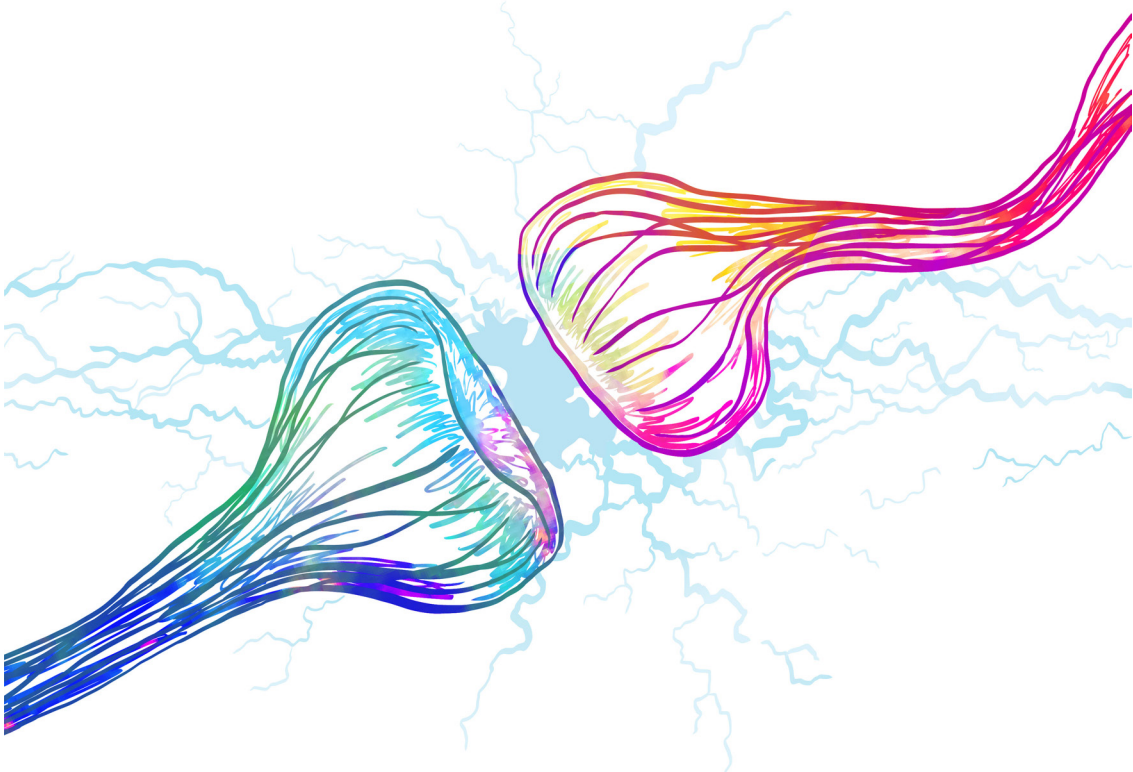
Multimodal phenotyping of synaptic damage in Alzheimer's disease: Translational perspective with focus on quantitative EEG



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MULTIMODAL PHENOTYPING OF SYNAPTIC DAMAGE IN ALZHEIMER'S DISEASE: TRANSLATIONAL PERSPECTIVE WITH FOCUS ON QUANTITATIVE EEG

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THESIS FOR DOCTORAL DEGREE (Ph.D.)

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'Nothing in life is to be feared, it is only to be understood.
Now is the time to understand more, so that we may fear less.'

Marie Skłodowska Curie

ABSTRACT

Alzheimer's disease (AD) is a progressive neurodegenerative disorder and the most common form of dementia. Accumulation of AD-associated pathology in the brain may begin a decade or more before the appearance of the first symptoms of the disease. The pathological-clinical "continuum of AD" therefore encompasses time between the initial neuropathological changes and symptoms of advanced disease. Besides cognitively healthy individuals at risk, it includes subjects with subjective cognitive decline (SCD), mild cognitive impairment (MCI) and eventually dementia when the severity of cognitive impairment affects patients' ability to carry out everyday activities. Timely detection of the disease would therefore recognize patients that are at risk for future cognitive deterioration and provide time window for the prevention and novel therapeutical interventions. Accumulating evidence suggests that degeneration and dysfunction of brain neuronal connections, *i.e.* synapses, is one of the earliest and best proxies of cognitive deficits in patients along AD continuum. Human electroencephalography (EEG) is a non-invasive and widely available diagnostic method that records real-time large-scale synaptic activity. The commonly used method in research settings is quantitative EEG (qEEG) analysis that provides objective information on EEG recorded at the level of the scalp. Quantitative EEG analysis unravels complex EEG signal and adds relevant information on its spectral components (frequency domain), temporal dynamics (time domain) and topographic estimates (space domain) of brain cortical activity. The general aim of the present thesis was to characterize different aspects of synaptic degeneration in AD, with the focus on qEEG and its relationship to both conventional and novel synaptic markers. In study I, global qEEG measures of power and synchronization were found to correlate with conventional cerebrospinal fluid (CSF) biomarkers of Aβ and tau pathology in patients diagnosed with SCD, MCI and AD, linking the markers of AD pathology to the generalized EEG slowing and reduced brain connectivity in fast frequency bands. In study II, qEEG analysis in the time domain (EEG microstates) revealed alterations in the organization and dynamics of large-scale brain networks in memory clinic patients compared to healthy elderly controls. In study III, topographical qEEG analysis of brain functional connectivity was associated with region-specific cortical glucose hypometabolism ([^{18}F]Fluorodeoxyglucose positron-emission tomography) in MCI and AD patients. Study IV provided evidence that qEEG measures of global power and synchronization correlate with CSF levels of synaptic marker neurogranin, both modalities being in combination independent predictors of progression to AD dementia in MCI patients. Study V and associated preliminary study introduced in the thesis assessed the translational potential of CSF neurogranin and qEEG as well as their direct relationship to AD neuropathology in *App* knock-in mouse models of AD. In study V, changes in CSF neurogranin levels and their relationship to conventional CSF markers in *App* knock-in mice corresponded to the pattern observed in clinical AD cohorts. These findings highlighted the potential use of mouse CSF biomarkers as well as *App* knock-in mouse models for translational investigation of synaptic dysfunction due to AD. In general, the results of the thesis invite for further clinical validation of multimodal synaptic markers in the context of early AD diagnosis, prognosis, and treatment monitoring in individual patients.

LIST OF SCIENTIFIC PAPERS INCLUDED IN THE THESIS

I. **Smailovic U**, Koenig T, Kåreholt I, Andersson T, Kramberger MG, Winblad B, Jelic V. Quantitative EEG power and synchronization correlate with Alzheimer's disease CSF biomarkers. *Neurobiology of Aging.* 2018;63:88-95.

II. **Smailovic U**, Koenig T, Laukka EJ, Kalpouzos G, Andersson T, Winblad B, Jelic V. EEG time signature in Alzheimer´s disease: Functional brain networks falling apart. *Neuroimage Clinical*. 2019;24:102046.

III. **Smailovic U**, Koenig T, Savitcheva I, Chiotis K, Nordberg A, Blennow K, Winblad B, Jelic V. Regional disconnection in Alzheimer dementia and amyloid-positive mild cognitive impairment: Association between EEG functional connectivity and brain glucose metabolism. *Brain Connectivity.* 2020; doi: 10.1089/brain.2020.0785. *Epub ahead of print.*

IV. **Smailovic U**, Kåreholt I, Koenig T, Ashton NJ, Winblad B, Höglund K, Nilsson P, Zetterberg H, Blennow K, Jelic V. Synaptic molecular and neurophysiological markers are independent predictors of progression in Alzheimer's disease. *Manuscript under review*.

V. **Smailovic U**, Delac L, Liman V, Kvartsberg H, Winblad B, Jelic V, Höglund K, Zetterberg H, Blennow K, Nilsson P. Translational potential of Alzheimer's disease CSF biomarkers from man to mouse – CSF neurogranin correlates with tau and amyloid-β pathology in *App* knock-in mouse models. *Manuscript*.

LIST OF PUBLICATIONS NOT INCLUDED IN THE THESIS

I. Koenig T, **Smailovic U**, Jelic V. Past, present and future EEG in the clinical workup of dementias. *Psychiatry Research: Neuroimaging*. 2020;306:111182.

II. Höglund K, Schussler N, Kvartsberg H, **Smailovic U**, Brinkmalm G, Liman V, Becker B, Zetterberg H, Cedazo-Minguez A, Janelidze S, Lefevre IA, Eyquem S, Hansson O, Blennow K. Cerebrospinal fluid neurogranin in an inducible mouse model of neurodegeneration: A translatable marker of synaptic degeneration. *Neurobiology of Disease*. 2020;134:104645.

III. **Smailovic U**, Jelic V. Neurophysiological markers of Alzheimer's disease: Quantitative EEG approach. *Neurology and Therapy*. 2019;8(Suppl 2):37-55.

IV. Knezovic A, Loncar A, Homolak J, **Smailovic U**, Osmanovic Barilar J, Ganoci L, Bozina N, Riederer P, Salkovic-Petrisic M. Rat brain glucose transporter-2, insulin receptor and glial expression are acute targets of intracerebroventricular streptozotocin: risk factors for sporadic Alzheimer's disease? *Journal of neural transmission (Vienna)*. 2017;124(6):695-708.

V. Salkovic-Petrisic M, Knezovic A, Osmanovic-Barilar J, **Smailovic U**, Trkulja V, Riederer P, Amit T, Mandel S, Youdim MB. Multi-target iron-chelators improve memory loss in a rat model of sporadic Alzheimer's disease. *Life Sciences*. 2015;136:108-119.

CONTENTS

LIST OF ABBREVIATIONS

Aβ	Amyloid β
APOE	Apolipoprotein E
APP	Amyloid β precursor protein
AD	Alzheimer's disease
CDR	Clinical dementia rating scale
CSF	Cerebrospinal fluid
CT	Computed tomography
DLB	Dementia with Lewy bodies
fAD	Familial Alzheimer's disease
[^{18}F]FDG-PET	[^{18}F]Fluorodeoxyglucose positron-emission tomography
fMRI	Functional magnetic resonance imaging
FTD	Frontotemporal dementia
IWG	International working group
LORETA	Low resolution electromagnetic tomography
LTP	Long term potentiation
MAPT	Microtubule-associated protein tau
MCI	Mild cognitive impairment
MMSE	Mini-Mental State Examination
MRI	Magnetic resonance imaging
NFT	Neurofibrillary tangle
PSEN	Presenilin
p-tau	Phosphorylated tau
qEEG	Quantitative electroencephalography
REM	Rapid eye movement
ROI	Region of interest
t-tau	Total tau
SCD	Subjective cognitive decline
SUV	Standardized uptake value

1 INTRODUCTION

1.1 FROM NORMAL BRAIN AGING TO NEURODEGENERATION

The global population aged 65 years or over has doubled in the last 30 years. The number of elderly people is expected to further increase and reach over 1.5 billion by 2050 (1). A positive trend in worldwide population aging is one of the great achievements of humankind. Still, our brain is not resistant to aging, since it is characterized by functional and structural changes such as age-related cognitive decline, grey and white matter atrophy, impairment of functional connectivity and neurotransmission (2). These alterations are often subtle, non-progressive, and do not result in functional impairment (2). However, advanced age and increased complexity of cognitive functions developed during the evolution of the human brain may have accentuated vulnerabilities of our species, including susceptibility to multifactorial neurodegenerative disorders (3).

1.1.1 Continuum of Alzheimer's disease (AD)

Dementia is a neurocognitive disorder associated with old age. In the world aging population, Alzheimer's disease (AD) is the most common form of dementia characterized by a progressive cognitive decline that ultimately affects the ability to carry out everyday activities (4, 5). Quantitative and qualitative differences between normal aging and AD dissociate AD as an entity with disease-related changes in the brain structure and function (2). These AD-associated brain changes may begin a decade or more before first clinically evident symptoms appear. Consequently, the "continuum of AD" refers to the time between the initial brain changes and symptoms of advanced disease (6). Besides cognitively healthy individuals at risk, the continuum of AD includes subjects that experience a self-perceived decline in cognition with a performance on cognitive testing within the range of a normal variation, which is referred to as subjective cognitive decline (SCD) (7). A proportion of SCD individuals may represent the incipient stage of AD since it has been shown that they have an increased probability of AD biomarker positivity and future cognitive decline (7-11).

Further along the continuum, individuals have mild but objectively measurable deficits in one or more cognitive domains that still do not affect the ability to carry out everyday activities, referred to as mild cognitive impairment (MCI) (6, 12). MCI has been identified as an at-risk condition, with an annual transition rate to dementia of 8% to 15% (13). The variability in the reported transition rates stems presumably from different clinical and/or research diagnostic criteria used across studies.

SCD and MCI subjects comprise a substantial percentage of memory clinic patients (Figure 1). However, in the absence of supportive biomarkers, they represent rather heterogeneous conditions that differ in neuropathology, clinical presentation, and long-term prognosis, with a substantial number of patients remaining cognitively stable over time (14). Furthermore, the trajectory of decline in subjects with the same biological risk may be different due to individual variability in functional capacity determined by the brain and cognitive reserve (15). Since the disease's biological processes can be diagnosed in the early clinical stages without considerable functional impairment, the most recent clinical diagnostic criteria, DSM-5, are proposing

grading of neurocognitive impairment from minor to major and exclusion of dementia as a diagnostic term (16).

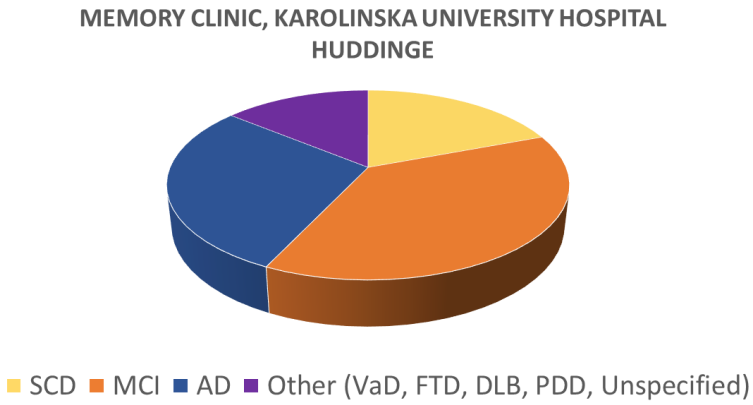

Figure 1. Distribution of diagnoses at Memory Clinic, Karolinska University Hospital Huddinge, Sweden. The colored portions represent the percentage (%) of diagnoses of the total number of 270 patients during January – October 2020. AD = Alzheimer's disease; DLB = dementia with Lewy bodies; FTD = frontotemporal dementia; MCI = mild cognitive impairment; PDD = Parkinson's disease dementia; SCD = subjective cognitive decline; VaD = vascular dementia.

1.1.2 Neuropathology in AD

German psychiatrist and neuropathologist Alois Alzheimer was the first to identify and describe typical neuropathological changes of the disease that bears his name. Distinctive plaques and neurofibrillary tangles remain the neuropathological hallmarks of AD to date (17). The main component of the extracellular plaques is amyloid β protein (Aβ) produced from a larger amyloid β precursor protein (APP) by the consecutive cleavage by β- and γ-secretase (18). According to the amyloid cascade hypothesis, an imbalance between production and clearance of highly self-aggregating Aβ42 is an early and most likely initiating factor of AD (19, 20). The majority of AD cases are sporadic (sAD) with a minority of patients suffering from familial AD (fAD) caused by mutations within the APP or the γ-secretase component presenilin (PSEN) gene that alter APP processing and result in elevated levels or more aggregation-prone forms of Aβ42 (21-23). The main known genetic risk factor for sAD is the apolipoprotein E (APOE) ε4 genotype (24). According to the comprehensive meta-analysis, homozygous APOE ε4 carriers have 2 – 33 fold increased risk of sAD which is thought to be mediated by the effect of the ε4 allele on Aβ deposition and clearance, maintenance of neuronal and synaptic function, and neuroinflammation (25, 26). Duplication of the wild-type APP gene, due to the 21 trisomy in Down's syndrome, leads to increased Aβ deposition and typical Alzheimer neuropathology, further supporting the amyloid hypothesis (27). The distribution of Aβ deposition during the course of AD follows a certain sequence, involving the neocortex, followed by the hippocampus and entorhinal cortex, diencephalic nuclei and striatum, cholinergic nuclei of the basal forebrain, brainstem and finally

cerebellum (28, 29). Another hallmark of AD neuropathology is the intraneuronal accumulation of abnormally phosphorylated tau protein that results in the formation of intracellular neurofibrillary tangles (NFT) (30). In AD, referred tau pathology starts with the "transentorhinal" and "limbic stages" and ends with a widespread "isocortical stage" (29). Regional distribution of tau pathology additionally correlates well with the neurodegeneration as well as the severity of cognitive impairment (31-33). Since most of the neuropathological studies included patients with advanced, end-stage of AD, the development and implementation of early and reliable disease-specific biomarkers will further elucidate the clinicopathological trajectory of AD continuum.

1.1.3 Synaptic dysfunction in AD

Decreased brain synaptic density appears to be a consistent finding and the closest anatomical correlate of cognitive impairment in AD (34-36). Several studies have reported substantial loss of synapses in the hippocampus and neocortex early during the disease course, present already in the MCI stage (34-39). Besides, loss of synapses appears to be more substantial than neuronal loss in the same brain regions (37). These discoveries suggested that AD might be primarily a disease of synaptic failure (40, 41). In addition, it raised interest in downstream mechanisms of AD pathological processes that have selective toxic effects on synapses (40, 42).

Recent findings point towards soluble Aβ42 oligomers as the most toxic amyloid species in the brain (19, 43, 44). It has been shown that Aβ oligomers can dose-dependently impair long term potentiation (LTP), decrease dendritic spine density, and deplete number of brain synapses (43-46). Moreover, cognitively healthy individuals with an abundance of amyloid plaques in the brain may have low Aβ oligomer levels (low oligomer-to plaque ratios), which might represent a missing link in understanding the (lack of) relationship between amyloid burden in the brain and cognition (19, 47). It has been proposed that Aβ42 oligomers have the potential to drive AD-like tau pathology since they can induce tau hyperphosphorylation and neuritic degeneration (48). Moreover, tau pathology can induce synaptic alterations independent of Aβ accumulation as shown in the mouse models of tauopathy (49-51). Activation of microglia and astrocytes that accompanies buildup of Aβ and tau pathology in the brain is an additional contributor to the synaptic degeneration and loss in AD (52-54). These recent findings raise compelling evidence that the key pathophysiological events leading to synaptic dysfunction may have a central role in AD (Figure 2).

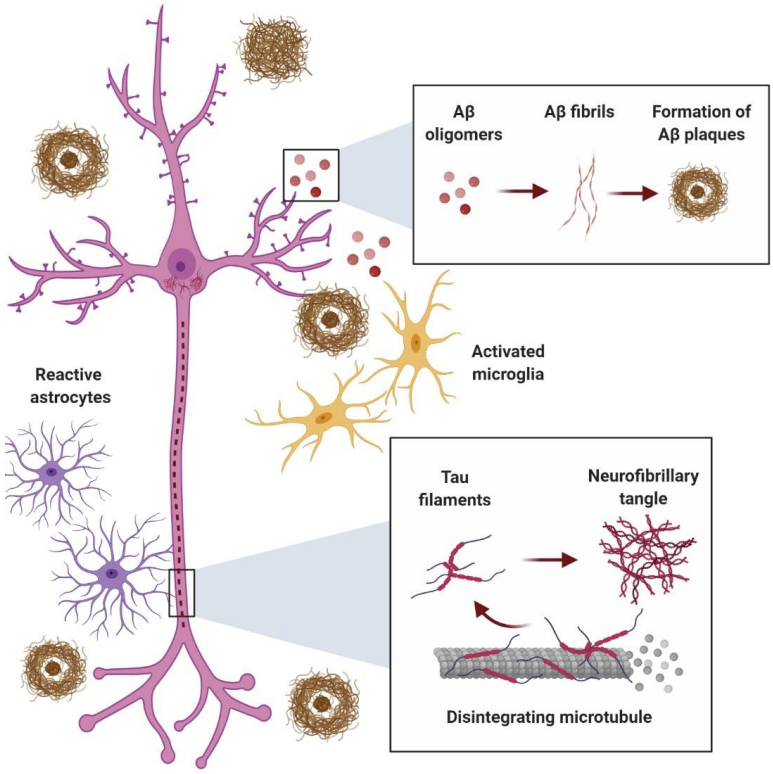

Figure 2. Illustration of the key pathophysiological events leading to synaptic dysfunction in AD. Aβ oligomers exhibit synaptotoxic effects, form Aβ fibrils and extracellular amyloid plaques. Pathological forms of tau form intracellular neurofibrillary tangles and have an additional impact on synapses. Activation of microglia and astrocytes that accompanies Aβ and tau pathology is another contributor to the synaptic degeneration and loss in AD.

1.1.4 Clinical and research diagnostic criteria

Clinical diagnostic criteria define AD as a progressive disorder of middle or late life with an insidious onset and progressive deterioration of memory and other cognitive domains, in the absence of any other systemic or brain disease as a possible underlying cause of referred memory and/or other cognitive deficits (5, 55). Revised clinical criteria of National Institute on Aging-Alzheimer's Association workgroups recognized AD dementia as a part of the continuum and referred to it as a fundamentally clinical diagnosis but emphasized the use of AD pathophysiological biomarkers in clinical trials and research settings (56). However, McKhann's clinical diagnostic criteria underline post-mortem histopathological proved AD as a definite diagnosis of the disease (55-57). On the other hand, in 2007 International Working Group (IWG) published the research diagnostic criteria of AD that rely on the specific clinical phenotype (episodic memory impairment) and presence of supportive biomarkers that provide *in vivo* evidence of the disease. The aim was to incorporate biomarkers of AD, such as structural magnetic resonance imaging (MRI), molecular imaging with positron-emission tomography (PET) and conventional cerebrospinal fluid (CSF) AD biomarkers in order to recognize and detect the earliest stages of the disease (58). The IWG criteria were first refined in 2010,

proposing a distinction between the clinical disease and disease pathology which led to defining the preclinical stage of the disease as “asymptomatic at risk for AD” (involving biomarker positive but asymptomatic individuals) and “presymptomatic AD” (referring to autosomal dominant mutation carriers) (59). The IWG-2 further refined the research criteria in 2014 based on weighting of the biomarkers and defined typical AD as an entity with episodic memory impairment with the presence of *in vivo* supportive biomarkers of amyloid and tau pathology. Additionally, IWG-2 criteria outlined structural MRI and [^{18}F]Fluorodeoxyglucose positron-emission tomography ([^{18}F]FDG-PET) as valuable markers of disease progression (60). A substantial number of ongoing studies have focused on the identification of novel AD biomarker candidates. Diagnostic modalities that prove to capture the earliest stages of the disease will presumably lead to further refinements of research and clinical diagnostic criteria.

1.2 ALZHEIMER’S DISEASE BIOMARKERS

The ongoing investigations of AD pathophysiological processes provide insights into new potential therapeutic strategies. Concurrently, they have urged the identification of reliable and translational biomarkers that reflect core elements of disease pathophysiology. A biomarker is an objective measure of a normal biological or pathogenic process used to guide clinical diagnosis, assess disease risk and prognosis, aid drug selection and monitor therapeutic interventions (61). Many studies have shown that biomarker abnormalities consistent with the central AD pathophysiological processes are detectable before the appearance of significant clinical symptomatology and are predictive of cognitive decline (Figure 3) (6, 61, 62).

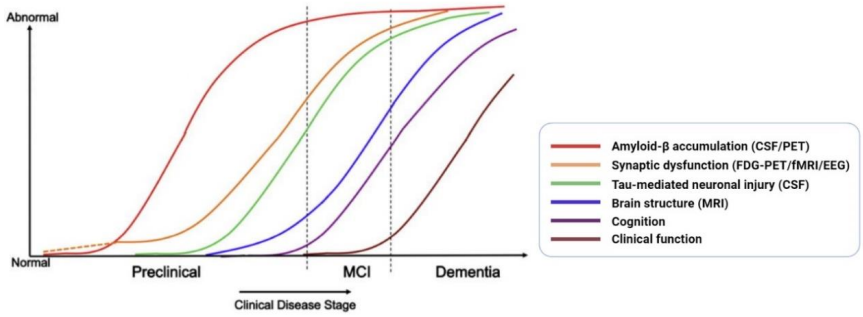

Figure 3. Proposed model of dynamic biomarker changes along Alzheimer's disease continuum. Adapted with permission from Sperling RA, Aisen PS, Beckett LA, et al. Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement. 2011;7:280-292. © 2011 Published by Elsevier B.V.

1.2.1 Conventional CSF biomarkers

Cerebrospinal fluid (CSF) is a clear and proteinaceous fluid contained in the brain ventricular system and subarachnoidal space. It is continuously produced, reaching an average rate of around 500 mL per day in adult humans (63). CSF has a potential to reflect biochemical changes in the brain due to direct contact with its extracellular space (61). In addition to its flow through the brain ventricular system and into the subarachnoid space, CSF passes through

interstitial space of brain parenchyma, mixes with the extracellular fluid and carries away brain waste products (64). CSF, which can be directly obtained by a lumbar puncture, is therefore an easily accessible source of AD-associated biomarkers.

Core CSF biomarkers of AD seem to reflect the underlying molecular pathology of the disease. CSF AD profile includes reduced concentrations of Aβ42 and increased total tau (t-tau) and phosphorylated tau (p-tau) protein levels (61, 65) (Figure 4). The general explanation for the reduction in CSF Aβ42 levels found in AD patients is that Aβ42 preferentially deposits in the brain tissue which leads to decreased diffusion of Aβ42 into the CSF (66). Reduced CSF Aβ42 levels in patients along AD continuum were found to correlate well with cortical plaque load in the brain tissue (67-69) and *in vivo* brain amyloid load as detected by [^{11}C]Pittsburgh Compound-B PET ([^{11}C]PIB-PET) imaging (70-72). CSF Aβ42 positivity (*i.e.* reduction) in patients with cognitive impairment is indicative of underlying AD pathology (6, 61) and is predictive of increased rate of brain atrophy even in healthy elderly subjects (73).

P-tau levels in the CSF presumably reflect phosphorylation state of tau and the development of neurofibrillary tangles in the AD brain (61, 74). CSF t-tau is considered a less specific AD marker compared to CSF p-tau as it is thought to mirror the intensity of neuronal and axonal damage in the AD brain (61) and is also increased in different acute states such as stroke and brain trauma (75, 76). Recently, the role of tau secretion in the brain and Aβ pathology-induced alterations in tau phosphorylation and release have been emphasized in several preclinical and clinical studies (77-80). Elevated CSF tau levels have been associated with faster cognitive decline (81, 82), progression from MCI to AD (83, 84) and cortical and hippocampal atrophy (85-87).

Considerable progress has been recently made in regard to the blood-based biomarkers of core AD pathology. Even though additional efforts are needed to overcome challenges in their development and widespread clinical use, blood-based biomarkers may offer straightforward, non-invasive, and cost-effective detection of AD pathology (88).

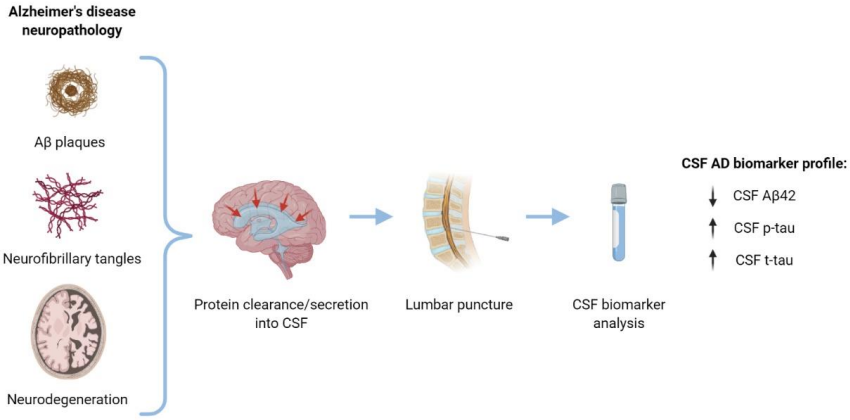

Figure 4. Overview of cerebrospinal fluid (CSF) biomarkers in Alzheimer's disease (AD). AD biomarkers in the CSF are considered to reflect core elements of disease pathology including amyloid accumulation, tau phosphorylation and formation of neurofibrillary tangles and neuronal damage and degeneration. AD biomarker profile includes decreased CSF Aβ42 and increased CSF p- and t-tau protein levels.

1.2.2 Neuroimaging

Structural imaging

Structural brain changes, such as atrophy and variations in brain tissue characteristics, can be assessed by widely available computed tomography (CT) or magnetic resonance imaging (MRI). Brain atrophy is a macroscopic manifestation of degenerative brain changes, such as loss of dendrites and neurons in AD (89). First changes are usually observed in the entorhinal cortex, amygdala and hippocampus in MCI and early AD patients (90-92), followed by atrophy in temporal neocortex and neocortical association areas (93, 94). The AD-associated pattern of structural MRI changes is closely related to cognitive decline (95-97) and is considered as a marker of disease progression (60, 98). More recently, the biological heterogeneity of AD has been substantiated by structural neuroimaging studies that revealed distinct AD subtypes of brain atrophy such as typical AD, limbic-predominant, hippocampal sparing and minimal atrophy AD (99, 100).

Functional imaging

An abnormal local neuronal (synaptic) activity can be indirectly detected by [^{18}F]FDG-PET, an imaging method that measures regional cerebral glucose metabolism (101). Sperling et al. proposed a biomarker model where decreased [^{18}F]FDG uptake, particularly in temporoparietal regions, is a marker of synaptic dysfunction in patients on the AD continuum (Figure 3) (6) that has already been implemented in the clinical use (102). Reduced [^{18}F]FDG uptake within these regions was shown to emerge before clinically evident cognitive impairment (103-105) and to be associated with clinical progression in MCI and AD patients (106-108). In memory clinic settings, [^{18}F]FDG PET adds to the differential diagnosis of dementia (60, 109). A recent study, however, questioned the general notion of [^{18}F]FDG-PET as an exclusive marker of neuronal activity since it has been shown that [^{18}F]FDG PET signal is determined by the glucose uptake in astrocytes (110).

Molecular imaging

In vivo imaging and quantification of brain Aβ burden can be assessed by PET imaging with amyloid tracers such as [^{11}C]-Pittsburgh compound-B, [^{18}F]flutemetamol, [^{18}F]florbetapir, [^{18}F]florbetaben (111-115). Amyloid PET positivity in MCI patients is associated with an increased likelihood of cognitive decline and a progression to clinical diagnosis of AD dementia (112). Interestingly, approximately 30% of cognitively healthy elderly show amyloid positivity (116, 117), which places them in the preclinical stage of AD pathophysiological continuum according to the research criteria (6, 60). Recent advances in the development of tau PET ligands have provided an opportunity for *in vivo* imaging of tau pathology in the brain. Increased retention of tau tracers was found in AD patients compared to healthy elderly controls, and accumulating evidence suggests relationship between tau PET findings, neurodegeneration and cognition (118-121). Future studies will elucidate the role of *in vivo* imaging of tau deposition in the pathophysiology of AD and other non-AD tauopathies.

1.3 NOVEL CANDIDATE MARKERS – FOCUS ON SYNAPSES

Biomarkers that have a potential to detect alterations in synaptic function during the earliest stages of AD, before a significant neuronal loss has occurred, may be important for successful prevention and timely therapeutic intervention. There are several available imaging methods that can provide *in vivo* information on brain synaptic function. Resting-state and task-based functional MRI (fMRI) and [^{18}F]FDG-PET have been shown to provide valuable anatomical information on synaptic and network abnormalities in AD, even though both of these modalities pick up hemodynamic and metabolic signals and hence detect indirect neuronal functioning (6, 122-124). A more direct *in vivo* imaging of synapses has been recently developed by employing synaptic vesicle glycoprotein 2A (SV2A) PET (125-127). This method may provide a unique opportunity to image brain synaptic density in living patients although several challenges, such as the development of optimal PET tracer and strength and consistency in the relationship between decreased SV2A expression and synaptic loss in AD, are yet to overcome (128). The closest non-invasive measures of synaptic (dys)function to date may be obtained by electroencephalography (EEG) and magnetoencephalography (MEG) since these complementary methods directly measure electric and magnetic fields, respectively, generated by brain synaptic activity (129).

1.4 QUANTITATIVE ELECTROENCEPHALOGRAPHY (QEEG)

Hans Berger, a German psychiatrist, was the first to record human EEG (130). His extensive research yielded the first detailed descriptions of many EEG features in healthy subjects as well as in patients with neurological disorders. Hans Berger considered EEG a "window into the brain" which was later substantiated by the widespread use of EEG in different research settings as well as routine clinical practice (129, 130).

Human EEG is a non-invasive diagnostic method that captures direct and real-time functioning of brain synapses. EEG, recorded at the scalp, mainly reflects summated excitatory and inhibitory postsynaptic potentials of a large number of neurons in the cerebral cortex (131). Synchronous polarization and laminar arrangement of cortical pyramidal neurons, that are spatially aligned and perpendicular to the cortical surface, make them ideal generators of dipolar current flow that can be recorded by scalp EEG (Figure 5) (129, 131).

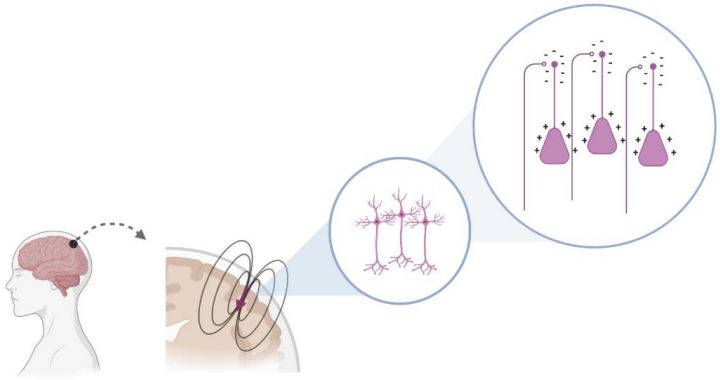

Figure 5. Illustration of human EEG recording at the level of the scalp. EEG mainly reflects summated excitatory and inhibitory postsynaptic potentials of a large number of spatially aligned and synchronously active neurons in the cerebral cortex.

Resting-state EEG recordings are subjected to visual analysis in routine clinical practice. It includes a description of EEG waveforms in the terms of frequency, amplitude, morphology, polarity, etc., and is important for assessing the age- and state- appropriate EEG patterns as well as distinctive neurophysiological phenomena (131, 132). However, the reliability of visual EEG assessment depends on the skills and experience of electroencephalographers as well as the unambiguity of changes of interest (131). In addition, some of the EEG characteristics cannot be observed by sole visual inspection. Quantitative EEG (qEEG) allows objective and reproducible analysis of EEG signals and is defined as "*the mathematical processing of digitally recorded EEG in order to highlight specific waveform components, transform the EEG into a format or domain that elucidates relevant information, or associate numerical results with the EEG data for subsequent review or comparison.*" (133). Therefore, qEEG unfolds complex EEG signal in time, frequency and space domain and provides objective information on frequency (or spectral) components, temporal dynamics, and topographic estimates of cortical activity (Figure 6) (129).

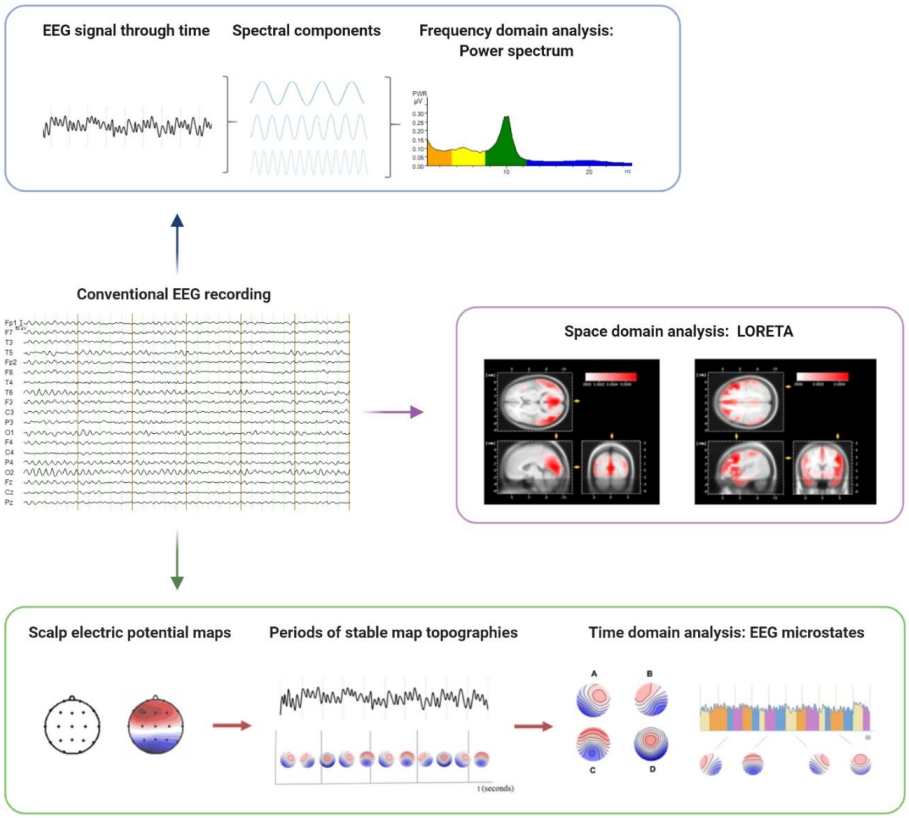

Figure 6. Quantitative EEG analysis in frequency (FFT spectral analysis; blue square), time (EEG microstate analysis; green square) and space domains (LORETA = low resolution brain electromagnetic tomography analysis; purple square). Adapted with permission from Smailovic U, Jelic V. Neurophysiological Markers of Alzheimer's Disease: Quantitative EEG Approach. Neurol Ther. 2019;8(Suppl 2):37-55. © 2019 The Authors.

1.4.1 Frequency domain analysis

EEG is primarily characterized by its oscillatory nature (131). Hans Berger, the discoverer of EEG, was also the first to describe its spectral components (134-136). Fast Fourier transform (FFT) spectral analysis is a method that transforms raw and continuous EEG data into the frequency domain and therefore allows for the quantification of the amplitude and power of EEG oscillations, averaged through time, across pre-defined frequency bands (Figure 6) (129). The definition of frequency bands stemmed initially from visual assessment of physiologically and pathologically relevant phenomena in EEG signal but was later substantiated by mathematical approaches such as factor analysis (137, 138). EEG frequency spectrum is usually characterized by four major categories of interest: delta (0 – 4 Hz), theta (4 – 7 Hz), alpha (8 – 12 Hz) and beta (>13 Hz) frequency bands. Normal resting-state EEG of an awake adult is predominantly characterized by frequencies in alpha range, also named "posterior dominant rhythm". Alpha rhythm is attenuated with eyes opening and other mental alerting activities (131, 132). In addition to the thalamus and thalamo-cortical connections as presumed "pacemakers" of cortical rhythmic activity, the horizontal intracortical connections are thought to be important for the generation and spread of alpha rhythms (131, 139, 140). Theta activity is typically seen in drowsiness and sleep with only a small amount present in normal awake adult recordings. Mammalian hippocampus and entorhinal cortex were found to be the main sources of theta activity (131). In addition, cholinergic deafferentation of the cortex results in increased slow wave (delta and theta) activity, which highlighted central cholinergic system as the modulator of EEG oscillations (131, 141). Frequencies in delta range are related to sleep and anesthesia and were found to originate from both thalamus and cortex since their appearance survives thalamectomy, as revealed by experimental studies (131). Beta rhythms are a normal EEG finding with dominance over the frontocentral regions, associated with wakefulness and rapid eye movement (REM) sleep (131). Even though a lot has been learned about EEG current sources since the first human recording made by Hans Berger, the exact generators and mechanism of cortical rhythmic activity picked up by EEG remains to be fully elucidated.

Generalized EEG slowing, characterized by increase in slow frequency (delta and theta) and decrease in fast frequency (alpha and beta) amplitude and power, is a consistent finding in patients along the clinical AD continuum (Figure 7) (142-151). These changes seem to develop with a certain temporal pattern including early alterations in power in theta and beta bands, followed by a decrease in alpha and an increase in delta power (142-144, 152). Additionally, referred qEEG alterations were shown to correlate with the degree of cognitive impairment (144, 147, 150, 153), performance in specific cognitive domains including memory, attention and verbal deficits (151, 154) and to be predictive of loss of ability to carry out activities of daily living (155). Several studies have provided evidence that generalized EEG slowing is of intermediate magnitude in SCD and MCI patients compared to AD and healthy controls (144, 151, 153, 156). Moreover, an increase in theta and a decrease in alpha power were shown to be predictive of future cognitive decline in MCI (153, 156) and amyloid positive SCD subjects (157).

Global field power (GFP) has been introduced as a simplified qEEG measure of generalized EEG amplitude. It summarizes multichannel recordings to a single measure that corresponds to the root mean of the spectral amplitudes across all channels (129, 148). Increase in GFP theta

and delta together with decrease in GFP alpha were reported in AD patients compared to the healthy controls (148, 158) while decrease in GFP alpha and beta was described in AD compared to MCI patients (148). Moreover, decrease in GFP alpha was predictive of progression to AD in the MCI patients during 2 years follow-up (148).

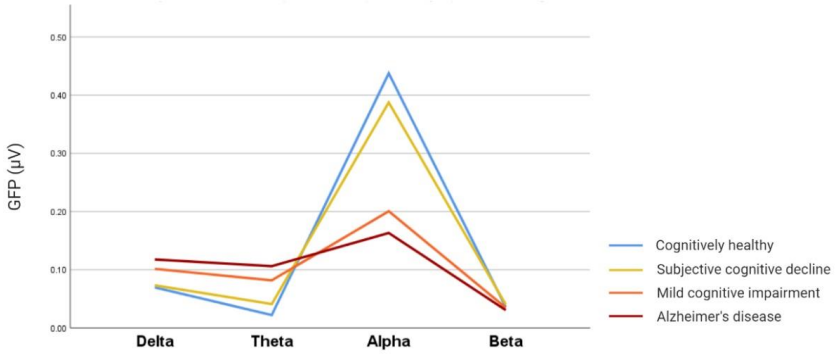

Figure 7. Comparative EEG power spectrum in a cognitively healthy subject, patient with a clinical diagnosis of subjective cognitive decline, mild cognitive impairment, and Alzheimer's disease. The global field power (GFP) is increased in a gradient-like manner in delta and theta bands and decreased in alpha and beta bands with the more severe stage of cognitive impairment.

AD is characterized by disturbances in brain structural and functional connectivity as a result of widespread neuronal and synaptic loss and dysfunction (159, 160). Growing evidence from neuropathological, neuroimaging and electrophysiological studies further support the hypothesis of AD as a "global disconnection syndrome" (161). EEG is a convenient technique for the investigation of brain functional connectivity since it provides direct information on the synchronous firing of a large number of cortical neurons with a uniquely high temporal resolution. EEG coherence is a measure of the stability of phase differences, or rather the common variance of spectral activity detected at two electrodes (129, 162). The most prominent decrease in EEG coherence was reported in alpha band which was also found to correlate with neurophysiological performance in AD patients (145, 146, 149, 163, 164). EEG coherence in the sensor space, however, poses several limitations. It is computed and mapped between channel pairs and its interpretation is influenced by volume conduction effect, *i.e.*, coupling of activities at two electrode sites may represent a single process that affects both electrodes at the same time (129).

Global field synchronization (GFS) has been introduced as a qEEG measure of relative phase synchrony of oscillating neuronal networks over all electrodes at a given frequency (165). It is computed using a principal component analysis (PCA) of the electrode positions in the sine-cosine diagram (sine and cosine coefficients are the results of FFT of the EEG signal and are entered for each electrode into the diagram). GFS corresponds to the normalized difference between the two resulting eigenvalues of PCA and is therefore the measure of the cloud spread of entry points, *i.e.*, electrode entries. It indicates the degree (percentage of EEG activity) that oscillates with a common phase over all electrodes at the investigated frequency (129, 165). Reduced GFS in alpha and beta and increased in delta frequency band has been reported in

patients with SCD, MCI and AD compared to the cognitively healthy controls (166). These GFS changes were additionally found to correlate with Mini-Mental State Examination (MMSE), Montreal Cognitive Assessment (MoCA) score and Clinical Dementia Rating scale (CDR) in AD (167, 168). Several other measures of EEG synchronization, including synchronization likelihood (SL) and phase lag index (PLI), were reported to decrease in alpha and beta bands in AD patients and to correlate with the disease severity (169-172). Overall, qEEG measures in the sensor space seem to consistently show decreased EEG synchronization in fast frequencies in AD.

1.4.2 Time domain analysis

"Is it possible to demonstrate the influence of intellectual work upon the human electroencephalogram, insofar as it has been reported here? Of course, one should not at first entertain too high hopes with regard to this, because mental work, as I explained elsewhere, adds only a small increment to the cortical work which is going on continuously and not only in the waking state."

Hans Berger, 1929 (130, 173)

EEG signal averaging, which has been conventionally performed in EEG analyses, extracts valuable information on EEG components from both resting-state and event-related EEG recordings. However, resting brain state is characterized by continuous and spontaneous mental activity that vigilantly changes in a sub-second time scale. This ongoing intrinsic brain activity demands considerably high energy consumption and is thought to be organized in distinctive functional networks (174, 175). EEG analysis in the time domain therefore provides a valuable tool for investigating the dynamics of large-scale brain networks with a millisecond time resolution (129, 175). Temporal organization of neuronal networks can be analyzed using topographies of electric fields, *i.e.*, representation of voltages recorded at all electrodes on a single scalp map at a given moment in time. Previous studies using resting-state EEG recordings have shown that these topographies of electric fields remain stable for short periods before abruptly transitioning into the new topographic arrangement (Figure 6) (176). It was assumed that they may represent basic elements of brain information processing and were referred to as "atoms of thoughts" (177).

Referred periods of the stable scalp electric potential topographies were named functional microstates. There are several computational approaches in microstate analysis, with commonly used modified k-means spatial cluster analysis that groups together similar topographies (at GFP peaks) and eventually determines the best-fitting maps that optimally explain the variance in the EEG data. Previous studies have shown that the mean duration of microstates is 60 to 150 ms (178, 179) which also coincides with the resolution of human information processing (180). Moreover, they have demonstrated that microstate topographies are remarkably consistent across EEGs of different subjects, only 4 distinct topographies (named map A, B, C and D) appear to be optimal to explain most of the human resting-state EEGs (Figure 8 and 9) (178, 179). Following these findings, numerous studies investigated differences in microstates topographies and parameters in association with age, medication, and different psychiatric and neurological conditions (179, 181, 182). Reduced microstates duration and anteriorization of the centers of gravity of the microstate topography were initially reported in AD patients compared to healthy controls (183-185). Conversely, two studies

employing more advanced methodological approaches, such as k-means spatial cluster analysis, reported no differences in microstate parameters nor topographies in AD patients compared to healthy controls, however, they involved a modest number of study participants (186, 187). A recent multi-centric study that involved over two hundred cognitively impaired patients reported increased duration, occurrence, and coverage of microstate map A in AD and MCI patients compared to healthy controls (188). Distinct organization and reproducibility of EEG microstate maps suggest them as a potential tool for detecting changes in the organization and activation of large-scale brain networks.

Study	N. Elect.	N. Subj.	Filter (Hz)	GEV (%)	A	B	C	D
König 1999	19	18	1-30	NR				
König 2002	19	496	2-20	79				
Lehmann 2005	16-21	27	2-20	84				
Britz 2010	64	9	1-40	66				
Kindler 2011	74	9	2-20	79				
Schlegel 2012	33	19	2-20	NR				
Brodbeck 2012	30	32	1-40	NR				
Andreaou 2013	64	22	2-20	NR				
Nishida 2013	19	8	2-20	NR				
Tomescu 2014	204	28	1-40	80				
Tomescu 2015	64	27	1-40	84				
Khanna 2014	32	10	1-50	70				
Diaz 2016	32	20	2-20	71				
Pascual-Marqui 2014	109	61	2-20	NR				
Pipinis 2016	64	94	NR	NR				
Milz 2016	64	70	2-20	77				
Katayama 2007	19	12	2-20	NR				
Corradini 2014	19	26	NR	58				
Gschwind 2016	204	49	1-40	NR				
Grieder 2016	19	24	2-20	74				
Drissi 2016	64	16	1-40	NR				
Seitzman 2017	61	24	2-20	68				
Santarnecchi 2017	20	74	1-30	NR				

Figure 8. Four representative EEG microstates named as maps (classes) A, B, C and D are highly consistent across independent studies. With permission from Michel CM, Koenig T. EEG microstates as a tool for studying the temporal dynamics of whole-brain neuronal networks: A review. Neuroimage. 2018;180(Pt B):577-593. © 2017 The Authors. Published by Elsevier Inc.

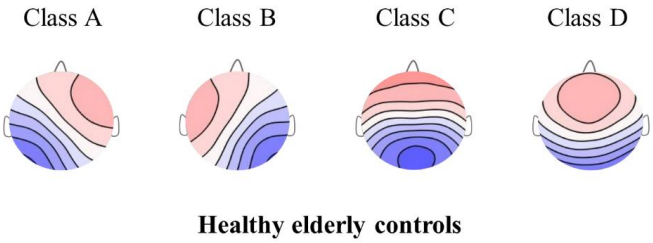

Figure 9. Four representative EEG microstates named as maps (classes) A, B (asymmetric), C and D (symmetric) in 308 healthy elderly controls (age range: 60 – 93 years). Adapted with permission from Smailovic U, Koenig T, Laukka EJ, et al. EEG time signature in Alzheimer´s disease: Functional brain networks falling apart. Neuroimage Clin. 2019;24:102046. © 2019 The Authors. Published by Elsevier Inc.

1.4.3 Space domain analysis

Direct interpretation of scalp electric field topographies in the context of activation of specific brain regions requires additional EEG source localization analysis. These methods involve EEG inverse solutions that estimate 3-dimensional (3D) distribution of neuronal activity that could generate EEG signal recorded at the level of the scalp. Inverse calculations do not provide a unique solution, however, they are constrained to the cortical grey matter and synchronous activation of large clusters of cortical neurons (129). One of the EEG source localization techniques is a low-resolution electromagnetic tomography (LORETA) which estimates and displays the 3D cortical sources of electric brain activity (Figure 6) (189). The performance of LORETA as an EEG imaging method has been previously validated through studies that combined LORETA and structural and functional MRI (190, 191) as well as [^{18}F]FDG-PET imaging (Figure 10) (192).

Several studies have employed LORETA to locate cortical sources of altered EEG activity in AD. These studies reported a reduction in alpha activity in parietal and occipital lobes as well as a widespread increase in delta and theta activity in AD patients compared to healthy controls (150, 193-196). Similar changes in resting-state EEG sources were also described in subjects with subjective cognitive complaints and MCI patients (197) and were reported to be sensitive to the disease progression (196). EEG measures of functional connectivity have also been employed in the source space where decreased lagged connectivity in the alpha band in the temporal and parietal cortex (195) as well as more widespread increase in lagged connectivity in theta and delta band were reported in AD patients (195, 198).

1.4.4 qEEG in relation to AD biomarkers

Disturbances in qEEG measures in frequency, time and space domain have been repeatedly reported in patients along the AD continuum, however, their relationship with AD neuropathology and other molecular, structural, and functional (synaptic) markers have not been extensively investigated. A previous study has reported a negative correlation between CSF Aβ42 levels and current source density in the right temporal lobe in the theta band and between t-tau levels and lagged phase synchronization between the left frontal eye field and

the right auditory area in alpha band in AD patients (199). Conversely, another small-scale study reported a negative correlation between EEG slowing and tau levels in patients with AD (158). The correlation between t-tau/Aβ42 ratio and EEG theta power was further revealed in cognitively healthy elderly subjects (200). A study that included a larger cohort consisting of over 300 healthy elderly with subjective memory complaints reported a lack of associations between global amyloid load, as assessed by amyloid PET, and PLI measure (201). The association between hippocampal and cortical gray matter volume and sources of resting-state EEG, as assessed by LORETA, was the most consistently reported in alpha frequency band in MCI and AD patients (202-205).

The coupling between glucose metabolism and synaptic activity in the brain was further explored with [^{18}F]FDG-PET and EEG studies. Association between regional glucose metabolism and localization of intracerebral EEG generators across distinct frequency bands was reported in both healthy controls and in patients with cognitive impairment (Figure 10) (192, 206, 207). Although both modalities are considered to reflect brain synaptic activity, [^{18}F]FDG-PET assesses an indirect metabolic signal and involves the use of radioactive tracer.

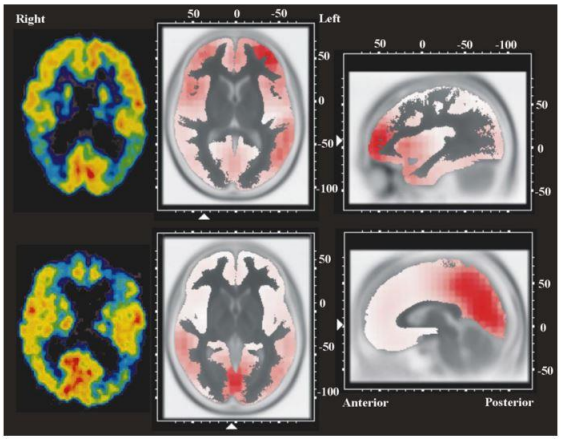

Figure 10. The correspondence between brain glucose metabolism determined by [^{18}F]FDG-PET and brain electric activity as estimated by EEG LORETA. The images show transaxial PET (left) and transaxial and sagittal EEG LORETA images (middle and right) of two subjects (top and bottom row). The more intense red color indicates higher metabolism/alpha band activity. With permission from Dierks T, Jelic V, Pascual-Marqui RD, et al. Spatial pattern of cerebral glucose metabolism (PET) correlates with localization of intracerebral EEG-generators in Alzheimer's disease. Clin Neurophysiol. 2000;111(10):1817-1824. © 2000 Elsevier Science Ireland Ltd.

Several studies investigated the relationship between APOE ε4 genotype and AD-associated alterations in EEG measures. Increased slow EEG activity and reduced activity and coherence in alpha band were reported in APOE ε4 carriers with AD (146, 195, 208, 209). However, the referred relationship between APOE genotype and oscillatory brain activity was brought into question following a report from a larger-scale study that showed a more pronounced EEG slowing in AD patients that were APOE ε4 non-carriers (210).

EEG offers several methodological advantages; direct access to neuronal signaling, high time resolution, noninvasiveness, portability, low cost, and wide availability (129). However, the relationship between qEEG measures and biological processes associated with AD, especially in the early stages of the disease, requires further large-scale investigation before establishing the potential role of qEEG in the diagnostic workup of AD.

1.5 CSF SYNAPTIC BIOMARKERS

Synaptic proteins emerged as candidate markers for early AD due to their close relationship with the cognitive status and disease progression (34, 35, 211, 212). Several pre- and postsynaptic proteins were shown to be altered in the brains of AD patients, with a recent focus on neuron-specific protein neurogranin (213). Neurogranin is a post-synaptic protein mainly expressed by excitatory neurons of the cortex and hippocampus (214, 215). It has a crucial role in synaptic plasticity and LTP by regulating calmodulin availability in response to the increased intracellular calcium levels (216, 217). A reduction in neurogranin levels in the hippocampus and cortex was found in AD patients compared to cognitively healthy controls (211, 213). Referred findings motivated the investigation of neurogranin as a potential fluid-based marker of synaptic pathology in AD. Several studies have shown that CSF neurogranin concentration is increased in MCI and AD patients, predictive of cognitive deterioration and metabolic and structural AD-related biomarker changes (218, 219, 220). Increased leakage and/or secretion of neurogranin into the brain interstitial fluid and CSF as a result of AD-related neuropathology was proposed as a potential mechanism behind its alterations in the CSF (221), however, the exact relationship between changes in neurogranin levels in the CSF and brain tissue remains to be elucidated.

In addition to neurogranin, several other CSF synaptic proteins are under investigation as potential novel markers of AD-related synaptic degeneration, including growth-associated protein-43 (GAP-43), synaptic vesicle protein 2A (SV2A), synaptosomal-associated protein 25 (SNAP-25), and synaptotagmin (128, 222-225).

1.6 TRANSLATIONAL POTENTIAL OF SYNAPTIC MARKERS

The urgency to understand the pathophysiological processes of AD, develop novel and reliable biomarkers as well as to provide a platform for testing potential novel therapeutic agents has given a strong initiative to model the disease in animals (226, 227). Animal models of AD have proven to be of significant value for a thorough investigation of the isolated key elements of the disease (226). However, preclinical findings on pathophysiological mechanisms and neuropathological changes in AD often require brain tissue investigations and hence do not have direct translational value. In parallel use of biomarkers in preclinical and clinical research studies opens a possibility for a more straightforward translation of basic and clinical findings in the AD field. It includes discovery of novel disease markers and their relationship with neuropathological changes, selecting potential therapeutics and estimating preclinical and clinical drug efficacy (Figure 11). Early and reliable synaptic markers that closely reflect clinical symptoms and underlying pathology together with animal models that exhibit AD-related synaptic degeneration may therefore represent an important step in bridging the translational gap between preclinical and clinical research fields. In this context, animal models of isolated pathological pathways of AD pose a certain advantage since they disentangle the

interplay of known (and yet unknown) causes of the disease. It complements investigations of novel synaptic biomarkers in the large and heterogeneous cohorts of patients along the AD continuum.

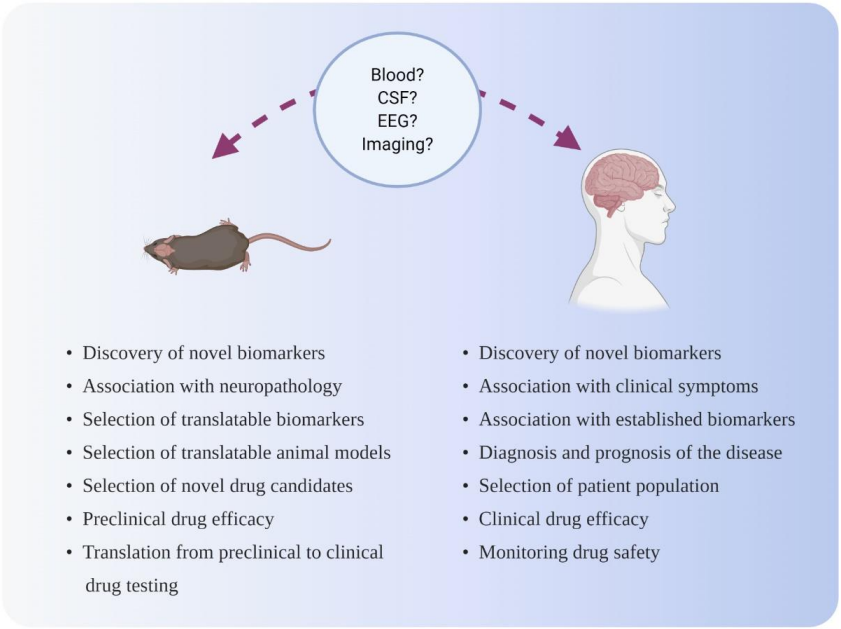

Figure 11. The importance of in parallel use of biomarkers in preclinical and clinical AD research highlighted by the different (and common) investigations that can be performed in preclinical animal and clinical studies.

1.7 ANIMAL MODELS OF ALZHEIMER'S DISEASE

1.7.1 Overview of animal models

Animal models of AD span from invertebrates such as roundworm (*Caenorhabditis elegans*) and fruit fly (*Drosophila melanogaster*) across vertebrates such as zebrafish and rodents, all the way to the nonhuman primates (226, 228). The size and reproduction characteristics of the rodents, in particular mouse (*Mus musculus*), made them one of the most widely used models in research (229). Meanwhile, the discovery of mutations in the *APP* and *PSEN* gene in familial AD provided crucial knowledge for generating animal models of the disease (226). The first transgenic mouse model of AD, named PDAPP, overexpressed human *APP* with the Indiana mutation which led to the 10-fold elevation of APP and development of amyloid plaques at 6 to 9 months of age (230). Following the development of the referred model, various APP transgenic models with different fAD *APP* mutations and promotor combinations were developed. Some of the common models are Tg2576 and APP23 mice that overexpress human *APP* with the Swedish mutation that increases production of both Aβ40 and 42 (226, 231-233). Since then, several mutant presenilin models have been crossed with APP mice in order to produce double transgenic mice models, such as APP/PS1, that exhibit early and accelerated

amyloid deposition (234, 235). 5XFAD transgenic mouse model was further produced using five fAD mutations (three *APP* and two *PSEN* 1 mutations) and has shown to develop rapid and severe amyloid pathology and neuronal loss (236). Even though these models are valuable for the investigation of progressive amyloid pathology and its downstream effects, most of them do not develop typical signs of tau pathology, *i.e.*, NFTs, and/or (severe) neurodegeneration. Consequently, animals that model tau pathology, by expressing mutant human microtubule-associated protein tau (MAPT), were developed. These mice exhibit extensive neurodegeneration, NFTs and cognitive impairment (54, 237). In order to recapitulate two pathological hallmarks of AD, triple Aβ-tau transgenic mice models were further introduced (238). However, multiple gene mutations pose an increased risk of artificial phenotypes and more importantly, mutant *MAPT* gene is found in frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17) and not in AD (239). Several non-transgenic mouse models, including senescence-accelerated, seeding, neurotransmitter, and toxin-induced, were additionally developed in an attempt to model aspects of sAD pathology and cognitive impairment (226, 240). However, mouse models of AD often fail to mimic sufficiently well an interplay of different pathophysiological pathways found in human AD and/or they utilize multiple mutations found in familial forms of the disease. Nevertheless, they provide a valuable platform for in-depth investigation of main neuropathological components of the disease, temporal and spatial spread of pathology as well as their downstream effects.

1.7.2 *App* knock-in mouse models of AD

Transgenic mouse models of amyloid pathology in AD have dominated the field of preclinical animal research, however, they exhibit certain limitations in terms of APP overexpression-related artifacts. Overproduction of APP fragments in these models, in addition to Aβ, induces artificial phenotypes that are hard to distinguish from the ones that develop due to sole Aβ accumulation (239). Additionally, different transgene constructs and promoters, lack of clear negative controls, use of certain mutations that are not present in AD, and high mortality exemplify further disadvantages of transgenic models (239). *App* knock-in mice have been introduced as models of AD that overproduce Aβ without the overexpression of APP and therefore bypass some of the shortcomings of the previous models (241). *App* knock-in mouse models harbor Swedish and Beyreuther/Iberian mutations (App^{NL-F}) while the App^{NL-G-F} mice additionally possess the Arctic mutation (241). The Swedish mutation facilitates β-secretase cleavage of APP and therefore increases the total amount of Aβ40 and Aβ42 (233, 242) while the Beyreuther/Iberian mutation affects γ-cleavage which results in increased Aβ42/Aβ40 ratio (243). The Arctic mutation additionally enhances the aggregation of Aβ peptides by promoting Aβ protofibrils formation (244). App^{NL-F} and App^{NL-G-F} mice develop amyloid plaques and memory impairment in an age-dependent manner (Figure 12) (241). App^{NL-F} mice exhibit initial Aβ deposition at 6 months and memory impairment at 18 months of age (241), even though a more recent study reported only modest cognitive deficits in this model (245). App^{NL-G-F} mice exhibit aggressive and early Aβ pathology with initial cortical and subcortical deposition at 2 and 4 months, respectively, while memory impairment was observed by 6 months of age (241, 246). Both of these models present with neuroinflammation characterized by accumulation of microglia and activated astrocytes around Aβ plaques (241). Even though *App* knock-in mice do not model the most common, sporadic form of AD nor exhibit neuronal loss or NFTs, they were shown to accumulate phosphorylated tau in dystrophic neuronal processes around Aβ

plaques (247). Besides the differences in the level and rate of induced pathologies (Figure 12), these two models present with several differences. *App*$^{NL-F}$ mice lack subcortical Aβ deposition and, importantly, Aβ sequence in *App*$^{NL-F}$ mice corresponds to the wild-type sequence since the arctic mutation in *App*$^{NL-G-F}$ affects twenty-second amino acid of Aβ (244). Additionally, modest cognitive deficits reported in *App*$^{NL-F}$ mice suggest them as a model of "preclinical AD". On the other hand, *App*$^{NL-G-F}$ mice may be useful models for investigating aggressive Aβ pathology and its downstream pathophysiological effects, more pronounced neuroinflammation and behavioral deficits.

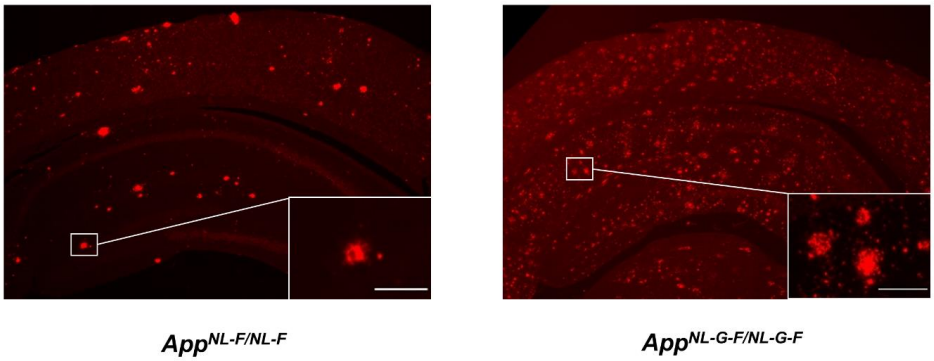

Figure 12. Aβ plaques in the cortex and hippocampus of 12 months old App^{NL-F} *and App*$^{NL-G-F}$ *mice. Brain sections stained for Aβ using mouse monoclonal OMAB antibody (Agrisera, AS10 932). Lower right corners show Aβ plaques with higher magnification. Scale bars = 100 µm. Adapted from Smailovic U, Delac L, Liman V, et al. Translational potential of Alzheimer's disease CSF biomarkers from man to mouse – CSF neurogranin correlates with tau and amyloid-β pathology in App knock-in mouse models. Manuscript in preparation.*

1.7.3 Synaptic pathology in *App* knock-in mouse models

The initial publication that introduced *App* knock-in mice also described synaptic alterations in these animal models of AD. The changes included reduction of pre- and post-synaptic proteins, synaptophysin and postsynaptic density protein 95 (PSD-95), respectively, in the proximity of Aβ plaques (241). Additionally, loss of mushroom postsynaptic spines, as a result of extracellular Aβ42 accumulation, was demonstrated in the hippocampal neurons of *App*$^{NL-F}$ mice (248). More recent studies reported impairment of synaptic function, including deficits in LTP in 3-4 months old *App*$^{NL-G-F}$ mice (249) as well as aberrant synaptic hyperactivity in the entorhinal cortex of *App*$^{NL-F}$ mice present before the formation of amyloid plaques (250). Overall, *App*$^{NL-F}$ and *App*$^{NL-G-F}$ mice display pathology at the synaptic level, however, its influence on synaptic function and brain oscillatory activity in general remains to be investigated in more detail. Moreover, biomarker characterizations, such as the investigation of fluid biomarkers of synaptic degeneration and AD-associated pathology, may further facilitate the translational potential of research findings in *App* knock-in mouse models of AD.

2 SYDAD – PROJECT IN CONTEXT

This project was part of the European Training Network (ETN) "Synaptic Dysfunction in Alzheimer Disease" (SyDAD) sponsored by Horizon 2020 Marie Sklodowska Curie Actions. SyDAD Training Network included 15 Early Stage Researchers (PhDs) in an international collaborative program with interdisciplinary training, translational approach stemming from both preclinical and clinical research conducted within academia as well as pharmaceutical companies, and mutual exchange programs between participating organizations. The main aim of the SyDAD Training Network was to elucidate pathophysiological pathways underlying synaptic dysfunction in AD and to identify potential pharmaceutical targets and novel biomarkers of the disease. Most of the PhD projects within the Training Network included preclinical investigations of different mechanisms behind synaptic pathology in AD, including the role of APP processing and Aβ, propagation of tau pathology, mitochondrial dysfunction, and cholesterol homeostasis. The aim of the present project was to bridge preclinical and clinical studies and investigate potential novel synaptic biomarkers in both animal models of AD and clinical cohorts of patients with different stages of cognitive impairment. The experimental part of the project, involving *App* knock-in mouse models, was partly performed as an exchange program (secondment) and extensive collaboration with University of Gothenburg (CSF analyses) and Janssen Pharmaceutica R&D[1], Beerse, Belgium (EEG recordings and analysis).

[1] Pharmaceutical Companies of Johnson & Johnson

3 AIMS

The present thesis aimed to provide new insights on markers of synaptic dysfunction and their relationship to Alzheimer's disease pathology in both patients and animal models. The primary focus was on qEEG as a potential modality for detecting early functional (synaptic) deficits in AD. The specific aims for each study were as follows:

In **Paper I**, to investigate the association between qEEG measures of global EEG power and synchronization and conventional CSF biomarkers of AD in a cohort of patients diagnosed with SCD, MCI and AD.

In **Paper II**, to examine differences in EEG microstate topographies and parameters between healthy elderly and memory clinic patients and their relationship to conventional CSF markers of AD in patients diagnosed with SCD, MCI and AD.

In **Paper III**, to investigate the correspondence between brain functional connectivity measured by means of topographical EEG analysis and regional glucose metabolism registered by [^{18}F]FDG-PET in patients with MCI and AD and with biomarker-verified AD molecular pathology.

In **Paper IV**, to investigate the association of CSF synaptic marker neurogranin with qEEG measures as well as their potential to predict clinical progression to AD in MCI patients.

In **Paper V**, to assess the translational potential of CSF neurogranin and its relationship to AD pathology (Aβ and tau) in *App* knock-in mouse models.

In **preliminary experimental study** presented in the method section of the thesis, to investigate changes in EEG power spectrum in relation to the accumulating Aβ pathology in *App* knock-in mouse models of AD.

4 METHODOLOGY

4.1 ETHICAL CONSIDERATIONS

All studies were performed in accordance with the ethical standards of the national and institutional research committees and Declaration of Helsinki. Study I and II were approved by regional ethics committees in Stockholm and Uppsala, Sweden. The use of SNAC-K data was additionally authorized by written approval from the responsible person and data manager for SNAC-K population study. Study III and IV were approved by regional ethics committees in Stockholm and Lund, Sweden. Study V was approved by Linköping's animal experiment ethical committee, Sweden. Preliminary data presented in the thesis was conducted in collaboration with Janssen Research & Development, Belgium, while all animal experiments were performed in strict accordance with the guidelines of the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC) and with the European Council Directive of 24 November 1986 (86/609/EEC) and European Ethics Committee directive (2010/63/EU) for the protection of laboratory animals. In line with Belgian governmental directives, all protocols were approved by the Animal Care and Use Committee of Janssen Pharmaceutica NV.

4.2 PARTICIPANTS

A general overview of the study participants (Study I – IV) involved in the present thesis is presented in Table 1.

4.2.1 Study I and II

The patient population in study I and II included patients recruited at the Memory Clinic, Karolinska University Hospital-Huddinge, Sweden. The study cohort consisted of 637 patients clinically diagnosed with SCD (n = 210), MCI (n = 230) and AD (n = 197) according to the Jessen et al. 2014, Winblad et al. 2004, and ICD-10 criteria (5, 7, 12). All patients underwent standard assessment consisting of general clinical and neurological examination, neuropsychological testing, brain imaging with MRI or CT, screening blood tests, CSF sampling and conventional biomarker analysis and resting-state EEG recording.

Study II additionally included healthy elderly controls that were part of the SNAC-K population-based study and that resided in Kungsholmen district, Stockholm. SNAC-K study recruited healthy elderly individuals above 60 years of age that have been followed-up over time. The study participants underwent comprehensive baseline assessment including neuropsychological test battery and general clinical and neurological examination, MRI imaging and resting-state EEG recordings. A total number of 308 out of 484 participants with EEG recordings were included in the study II. The exclusion criteria were:

- MMSE score lower than 27 points
- decline in MMSE > 2 points during any of the follow-ups (6 years)
- dementia diagnosis during any of the follow-ups (6 years)
- presence of any major neurological or psychiatric disorder.

More information about SNAC-K cohort can be found on the SNAC-K population study webpage: https://www.snac-k.se/.

4.2.2 Study III

Study III included a subsample of patients recruited at Karolinska University Hospital-Huddinge, Sweden that were clinically diagnosed with MCI (n = 41) and AD (n = 26) and that underwent [^{18}F]FDG-PET imaging and EEG recordings in addition to the standard clinical assessment. The study was conducted in two parts, the first part involved the whole cohort of MCI and AD patients and the second part involved a subsample of patients that were CSF amyloid positive. For the second part, only patients that had available CSF sample from the biobank were selected (24 MCI and 18 AD) and stratified into amyloid positive according to the CSF Aβ42/40 ratio status, with the cut-off for amyloid positivity CSF Aβ42/40 ratio < 0.89. In comparison to CSF Aβ42, Aβ42/40 ratio was shown to compensate for inter-individual differences in the total Aβ production and to have superior diagnostic performance (251, 252). The second part of the analysis therefore included 14 amyloid positive MCI (or "MCI due to AD") and 18 amyloid positive AD patients.

4.2.3 Study IV

Study IV included 99 patients recruited at Karolinska University Hospital-Huddinge, Sweden that were clinically diagnosed with MCI. All patients underwent standard assessment as described above for studies I and II. A subsample of MCI patients has been followed-up over time (n = 72) and stratified into stable MCI (n = 41) and progressive MCI (n = 31) according to the clinical progression to AD dementia during 2-years follow-up. Stable and progressive MCI patients were further stratified into CSF amyloid positive (12/41 of patients in stable and 19/31 of patients in progressive MCI group) according to the cut-off for CSF Aβ42 positivity < 550 ng/L (Laboratory at Karolinska University Hospital Huddinge). Clinically available CSF Aβ42 value was used for the stratification since insufficient number of patients had available frozen CSF from the biobank for the complementary CSF Aβ42/40 analysis.

Table 1. Overview of the study design, participants, qEEG methods and main aims of the clinical studies presented in the thesis.

	Study I	**Study II**	**Study III**	**Study IV**
Study design	Cross-sectional	Cross-sectional	Cross-sectional	Prospective
Subjects	SCD (n = 210) MCI (n = 230) AD (n = 197)	HC (n = 308) SCD (n = 210) MCI (n = 230) AD (n = 197)	MCI (n = 41) AD (n = 26) - stratified according to CSF amyloid status	Stable MCI (n = 41) Progressive MCI (n = 31) (progression to AD during 2 years follow-up)
qEEG analysis	Frequency domain	Time domain	Space domain	Frequency domain
qEEG measures	GFP GFS	Microstate topography Microstate parameters	LORETA – instantaneous and lagged linear connectivity	GFP GFS
Main aims	- Correlation with conventional CSF markers: Aβ42, p-tau, t-tau	- Differences in EEG microstates between HC, SCD, MCI and AD - Correlation with conventional CSF markers: Aβ42, p-tau, t-tau	- Correlation with [^{18}F]FDG-PET in vulnerable brain regions	- Correlation with CSF neurogranin - Predictive potential of CSF neurogranin and qEEG

AD = Alzheimer's disease; CSF = cerebrospinal fluid; GFP = global field power; GFS = global field synchronization; HC = healthy controls; LORETA = low resolution electromagnetic tomography; MCI = mild cognitive impairment; SCD = subjective cognitive decline.

4.3 ANIMAL MODELS

4.3.1 Study V

Experimental preclinical studies in the present thesis were conducted on two *App* knock-in mouse models of AD that harbor double (Swedish and Beyreuther/Iberian mutations; App^{NL-F} mice) and triple mutations (additional Arctic mutation, App^{NL-G-F} mice). In general, these mouse models overproduce Aβ and exhibit enhanced Aβ protofibril formation. Study V investigated AD biomarkers in the CSF of 12 months old App^{NL-F}, App^{NL-G-F}, and wild type controls ($App^{wt/wt}$). It was conducted on a total of 25 animals, including heterozygous and homozygous *App* knock-in mice: 8 $App^{wt/wt}$, 5 $App^{wt/NL-F}$, 5 $App^{NL-F/NL-F,}$ 2 $App^{wt/NL-G-F}$ and 5 $App^{NL-G-F/NL-G-F}$ mice. Both male and female mice were used.

4.3.2 Preliminary study

Preliminary/unpublished study presented in the thesis introduces longitudinal *in vivo* EEG recordings on *App* knock-in mice and age-matched wild type controls. The experiments involved both male and female homozygous App^{NL-F} (n = 29), App^{NL-G-F} (n = 20) and age-matched App^{wt} mice (n = 40). All animals underwent surgical electrode implantation at 10 weeks of age with baseline EEG recording at 12 weeks of age and four subsequent longitudinal recordings performed at three (for App^{NL-G-F}) or six/twelve weeks (for App^{NL-F}) interval. For App^{NL-F} it included recordings at 12, 18, 24, 36 and 48 weeks, while for App^{NL-G-F} at 12, 15, 18, 21 and 24 weeks of age (Figure 13). All mice were single housed in a soundproof holding room with continuously controlled temperature, humidity, and 12h light/dark cycle regime (lights on 07:00 – 19:00 hours, light intensity: ~ 100 lux) and tap water and food (SAFE diet A05) available *ad libitum*.

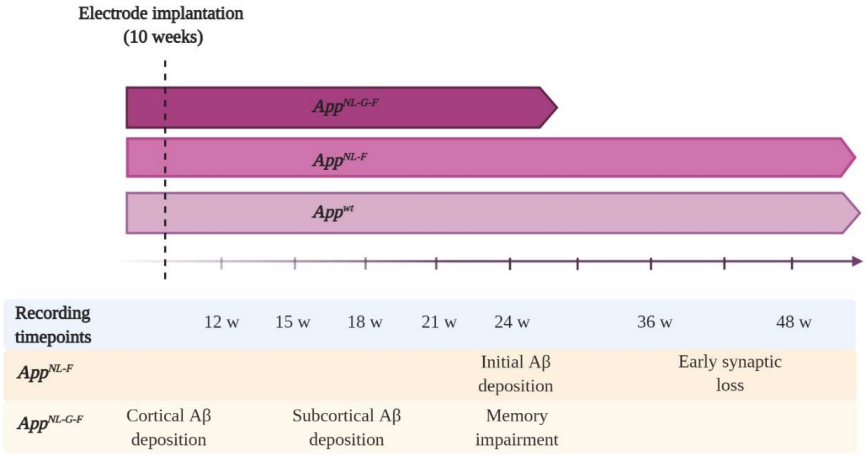

Figure 13. Experimental design of the study involving in-vivo EEG recordings in two App knock-in mouse models of AD. The study included App^{NL-F}, App^{NL-G-F}, and age-matched App^{wt} mice that underwent surgical electrode implantation and longitudinal EEG recordings at five different time points with respect to the pathology progression. Model characteristics that typically develop in an age-dependent manner in App^{NL-F} and App^{NL-G-F} mice are outlined in the yellow sections underneath the time axis.

4.4 METHODS – CLINICAL STUDIES

4.4.1 qEEG analysis

EEG recording setup and preprocessing

All EEGs from the patient population were recorded on the Nervus System at the Department of Clinical Neurophysiology, Karolinska University Hospital-Huddinge. The electrode placement followed the standard 10/20 international system, including 19 scalp, two earlobe and two electrooculogram (EOG) electrodes, which made the recording procedure comparable to the standard setups used at the international clinical and research sites. In addition, this number and electrode placement was shown to be adequate to record and analyze characteristics of brain electric activity (132). All EEGs were recorded during awake resting-state which poses certain advantages when it comes to the neurophysiological studies, such as its common use in the clinical setting, straightforward implementation in both research and clinical investigations, minimal requirements for the patient engagement and less variation compared to the task- and stimuli- EEG paradigms. Patients' vigilance states were closely monitored during the whole recordings by medical technicians. Electrode impedances were below 5 kΩ and EEGs were sampled at a rate of 256 Hz with band-pass filters between 0.5 Hz and 70 Hz. All EEG recordings were exported in average reference montage.

Resting-state EEG recordings of the healthy elderly from SNAC-K database were recorded on the Schwarzer EEG Natus Incorporated System by using Easy Caps. The electrode placement and recording setup corresponded to the procedure performed in the patient population, as outlined above.

All EEG recordings (patient population and healthy elderly) were preprocessed in Brain Vision Analyzer, version 2.0 software (Gilching, Germany) with the following procedure:

- semiautomatic independent component analysis (ICA) algorithm for the removal of ocular, blink and electrocardiographic artifacts
- visual inspection and manual rejection of the remaining artifacts, episodes of drowsiness and other non-resting-state vigilance states

Frequency domain analysis

QEEG analysis in the frequency domain (Figure 6) was conducted in study I and IV. This type of investigation was selected for several reasons. First, it is the most common form of qEEG analysis that provides measures of power and synchronization of EEG oscillations across different frequency bands that bear physiological significance. Second, measures of EEG power and synchronization were shown to capture changes in brain oscillatory activity in cognitively impaired patients (253). Third, these measures have straightforward computation in user-friendly software which suggests them as candidates for broad research and clinical implementation. In particular, measures of global field power (GFP) and synchronization (GFS) were employed in study I and IV. GFP and GFS have been previously investigated in well-characterized memory clinic cohorts (148, 166), study with longitudinal design (148) and in several correlative studies involving CSF markers and measures of symptoms severity (158, 167, 168). Both GFP and GFS recapitulate multichannel EEG data in a single measure of global

EEG power and synchronization which makes them appealing candidates for investigations of their relationship with other biological markers. Even though conventional FFT power spectra analysis provides more detailed results (one parameter per derivation), the advantage of global EEG measures is that they consider all electrodes equally and avoid problems of multiple testing and a priori selection of derivations for further correlative analyses. Both GFP and GFS were analyzed in four conventional frequency bands: delta (1 – 3.5 Hz), theta (4 – 7.5 Hz), alpha (8 – 11.5 Hz) and beta (12 – 19.5 Hz).

Time domain analysis

Quantitative EEG analysis in the time domain (Figure 6) was conducted in study II. EEG microstate analysis can be used to investigate the temporal organization of large-scale brain networks with a high time resolution. Microstate maps represent voltages recorded at all electrode sites on a single scalp map in one moment in time. Four distinct microstate maps have been shown to be sufficient to explain around 60-80% of the total variance of the human EEG data (Figure 9) (179). In addition, previous studies have shown that these four maps are highly reproducible across different clinical cohorts, including patients with cognitive impairment (179, 186, 187). These characteristics made them appealing for studying dysfunction of resting-state networks in patients along AD continuum. Microstate topographies last for a certain period (60 – 150 ms) and do not overlap in time. Therefore, 19 channel EEG recording can be visualized as a time-series of microstate maps that alternate through time.

Steps of microstate analysis conducted in study II are outlined in Figure 14. Microstate maps can be evaluated in terms of the change in their topographies and parameters. Microstate parameters refer to the duration, occurrence (time per second) and contribution (expressed as percentage of the time that each map occupies). Microstate maps were selected at GFP peaks throughout the whole preprocessed EEG recording and subjected to the modified k-means spatial cluster algorithm since microstate maps tend to change their topographies during low GFP values (Figure 14). The number of clusters was set to the four most representative maps to be comparable to the previous studies and between patients and healthy subjects in study II. Cluster analysis yielded four most representative maps per subject (both healthy and patients) that were further averaged across all healthy elderly controls. These four grand mean microstate maps of healthy control group were classified as A, B, C and D based on their spatial similarity to the normative microstate maps as published previously (178). Next, individual microstate maps from both patient and healthy groups were assigned as maps A, B, C or D based on the spatial similarity to the controls' grand mean maps (Figure 14). This sorting procedure was selected for several reasons. First, in order to compare the topographies of individual microstate maps between patients and healthy elderly controls, one would need to assign the maps to one of the microstate classes based on their spatial similarity. Second, this procedure was selected after visual inspection of the quality of several different sorting approaches to the individual microstate classification. These included sorting and assigning maps based on the grand mean maps of both healthy elderly and patient groups, separate sorting of patient and healthy groups based on their respective grand mean maps and sorting and assigning of the both patient and healthy groups based on the normative microstate maps from Koenig et al., 2002 (178). Visual inspection revealed that sorting based on the grand mean maps from the healthy elderly controls yielded the most consistent results, especially in the patient groups. This may be due to the use of the maps from the healthy elderly subjects for the correct classification of the patients' maps

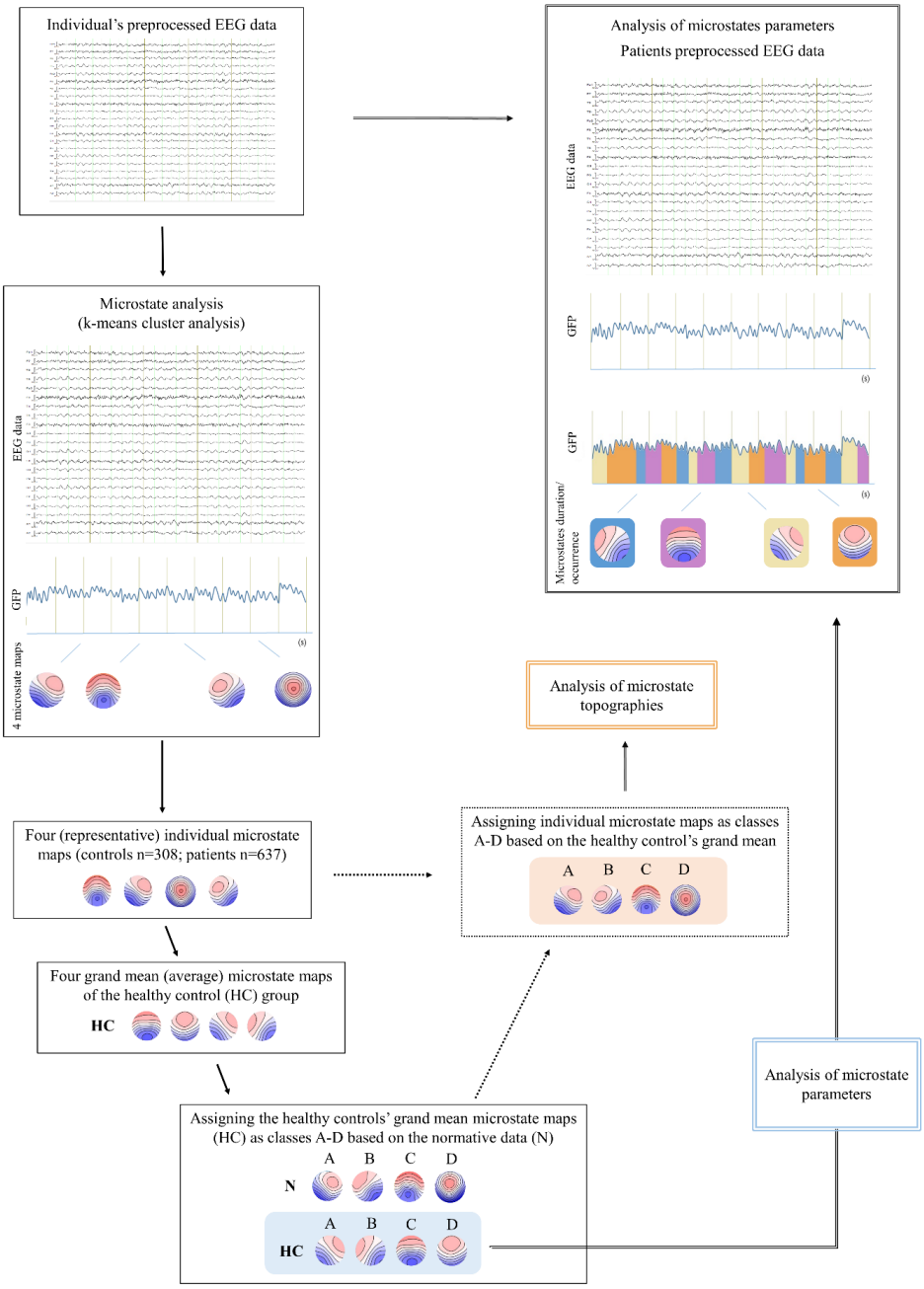

Figure 14. Steps of EEG microstate analysis: computation, sorting and assigning procedure in study II. With permission from Smailovic U, Koenig T, Laukka EJ, et al. EEG time signature in Alzheimer´s disease: Functional brain networks falling apart. Neuroimage Clin. 2019;24:102046. © 2019 The Authors. Published by Elsevier Inc.

compared to the use of normative data that included a substantial number of younger subjects. Finally, the healthy elderly microstate maps presented in study II stem from a cohort that has been extensively evaluated and characterized and could therefore serve as normative data for future investigations of the microstate alterations in elderly and cognitively impaired patients.

The hypothesis of study II was that microstate maps of healthy elderly and patients with cognitive impairment differ in their topographies, implying that map A of the patient with MCI or AD diagnosis could substantially differ from the topography of map A of the healthy elderly subject. For this reason, microstate parameters were analyzed by back-fitting healthy controls' grand mean maps to the original EEG data of the patient groups. This approach yielded microstate parameters (duration, occurrence, contribution) of healthy elderly maps in the EEG data of patients with SCD, MCI and AD (Figure 14).

Space domain analysis

Space domain analysis in study III was performed using a specific solution to the EEG inverse (or source imaging) problem named LORETA (low resolution electromagnetic tomography). This particular type of analysis was selected for correlative [^{18}F]FDG-PET – qEEG study since LORETA can provide topographical estimates of cortical electric activity based on the scalp EEG recordings (254). In study III, current sources estimated by LORETA were averaged across voxels in the five regions of interest (ROIs) bilaterally: frontal, parietal, temporal, occipital and limbic lobes. The selection of ROIs was matched to the corresponding PET ROIs.

Correlation of LORETA estimates of current source densities has been previously related to cortical brain glucose metabolism in patients with AD (192, 207). Study III further investigated the relationship between cortical glucose metabolism and topographical EEG measures of brain functional connectivity in MCI and AD patients with and without biomarker evidence of AD pathology. EEG analysis of brain functional connectivity included measures of instantaneous and lagged linear connectivity. Previous EEG studies have mostly employed measures of lagged functional connectivity in the inverse solutions due to the volume conduction effect, *i.e.* instantaneous spread of the electric fields through the biological tissue that confounds EEG measures of instantaneous connectivity. However, instantaneous synchronization of neuronal activity has physiological significance and may complement the investigation of functional disconnection due to AD. Both instantaneous and lagged linear connectivity measures were computed for each main brain lobe and all the remaining ROIs (brain lobes) yielding the measures of average connectivity between the respective main lobe and remaining cortex. The analysis focused on temporoparietal lobes for the correlative [^{18}F]FDG-PET study. LORETA measures of functional connectivity were computed for each conventional frequency band.

4.4.2 CSF biomarkers analysis

CSF was obtained by lumbar puncture according to the standard procedure and as part of the routine clinical assessment. All samples were analyzed at Clinical Neurochemistry Laboratory, Mölndal, Sweden. The local cut-offs for conventional CSF biomarkers were as follows: Aβ42 > 550 ng/L, t-tau < 400 ng/L and p-tau < 80 ng/mL (study I, II and IV) and CSF Aβ42/40 ratio > 0.89 (used for stratification of patients into CSF amyloid positive and negative in study III). In study IV, neurogranin was additionally analyzed on frozen CSF samples, kept at -80 °C and obtained from the biobank, using an in-house-developed ELISA protocol (213).

4.4.3 [^{18}F]FDG-PET analysis

Patients in study III underwent metabolic [^{18}F]FDG-PET imaging at the Medical Radiation Physics and Nuclear Medicine, Section for Nuclear Medicine, Karolinska University Hospital, Stockholm, Sweden. [^{18}F]FDG-PET imaging was performed on a Siemens Biograph mCT scanner and preprocessed using Syngo.via program. Following the automatic template-based spatial normalization, mean [^{18}F]FDG standardized uptake values ([^{18}F]FDG-SUV) were calculated for bilateral five main brain lobes: frontal, parietal, temporal, occipital and limbic lobes as published in automated anatomical labeling atlas by Tzourio-Mazoyer et al. (255). In order to reduce interindividual variability, absolute [^{18}F]FDG-SUV were normalized to the [^{18}F]FDG-SUV in the cerebellum yielding [^{18}F]FDG-SUV ratios (SUVR). Cerebellum was selected as a reference region since the sensitivity of cerebellar normalization in detecting AD-related metabolic abnormalities has been previously validated in [^{18}F]FDG-PET studies (256). The [^{18}F]FDG-SUVR parietal and temporal lobes were selected for further correlative analysis with EEG LORETA measures due to their pronounced vulnerability and early involvement in AD.

4.4.4 Statistical analysis

Different statistical methods were used in the present thesis based on the study aim, hypothesis, and type of data available for analysis. Description of statistical analyses is reported in detail in respective papers.

Multiple linear regression analysis

Multiple linear regression models were employed in studies I and IV to investigate the relationship between CSF biomarkers and qEEG measures of global power and synchronization. In study I, conventional CSF biomarkers of AD (Aβ42, t-tau and p-tau) were used as independent and GFP and GFS in four conventional frequency bands as dependent continuous variables, while accounting for age, sex and MMSE in the whole group of memory clinic patients diagnosed with SCD, MCI and AD. More specifically, the analysis was controlled for MMSE to exclude group differences in qEEG measures that were present between patients due to the different severity of cognitive impairment. MMSE was selected as a more objective controlling variable compared to the group diagnosis. The same regression analyses were additionally performed in the separate diagnostic groups while controlling for age and sex. In study IV, multiple linear regression analysis was employed to investigate the relationship between CSF neurogranin (independent variable) and qEEG measures GFP and GFS (dependent variable) in MCI patients. The first model was controlled for age and sex while the second model was additionally controlled for CSF t-tau/Aβ42 ratio since both of these CSF measures correlate with CSF neurogranin and qEEG measures, as reported in the literature. The regression analyses were additionally performed in separate groups: stable, progressive, amyloid negative and positive MCI groups.

Randomization statistics

Multichannel scalp field EEG data can be analyzed using randomization statistics since they do not require many assumptions or data manipulations while providing a robust and physiologically meaningful interpretation of the data analysis. Basic principles of

randomization statistics and their implementation for EEG analysis in Ragu software have been provided in detail in Michel, 2009 (129), and Koenig et al., 2011 (257). In brief, randomization statistics test whether differences in EEG scalp field data occur by chance, *i.e.* due to random variance across the groups, by random shuffling the scalp field data across the subjects and groups, and then recomputing and comparing the differences (effect sizes) that stem from the real data against the data obtained by randomization (and then repeating this procedure multiple times). In study II, differences in microstate topographies between healthy controls and patient groups were assessed by non-parametric randomization topographic analysis of variance (TANOVA). TANOVA compares map differences between conditions. Topographic analysis of covariance (TANCOVA) was used to investigate the association between CSF biomarkers and changes in topography of different microstate maps since the predictors were continuous variables (CSF Aβ42, p- and t-tau). Age and sex were regressed out of the microstate data prior to the analyses. Both TANOVA and TANCOVA were conducted with 5000 randomization runs and a level of significance $p < 0.05$ (257).

Correlations

In study III, the associations between brain glucose metabolism and EEG LORETA measures of instantaneous and lagged linear connectivity in temporal and parietal lobes were investigated using Spearman's rank correlation tests due to the non-normal distribution of the data. The analysis was exploratory, so the findings were presented with both corrected and uncorrected p-values. Correction for multiple comparisons was performed using Benjamini-Hochberg correction with 5% false discovery rate (FDR) since it adjusts p-values according to their ranking and minimizes false negatives reports.

The association between conventional CSF biomarkers and EEG microstate parameters (study II) and between CSF neurogranin and conventional markers (study IV) were also investigated by means of Spearman's rank correlation coefficients.

Logistic regression analysis

In study IV, the potential of CSF neurogranin and qEEG measures to predict clinical progression to AD dementia in patients diagnosed with MCI was investigated by logistic regression analysis, adjusted for age, sex and MMSE. Classification accuracy of correctly categorized progressive and stable MCI patients was used to explore the predictive potential of CSF neurogranin and qEEG measures. The dependence of significant predictors (CSF neurogranin and qEEG measures) on patients' amyloid status was additionally tested with interaction models.

4.5 METHODS – PRECLINICAL STUDIES

4.5.1 CSF biomarkers analysis

CSF sampling

In study V, CSF samples were collected from cisterna magna by penetrating dura mater with a glass capillary while the mice were anesthetized. Collection under the dissection microscope allowed for the inspection of any blood contamination that may affect the results (Figure 15). All mice underwent the same procedure and the amount of collected CSF sample ranged from 5 to 20 µl per mouse.

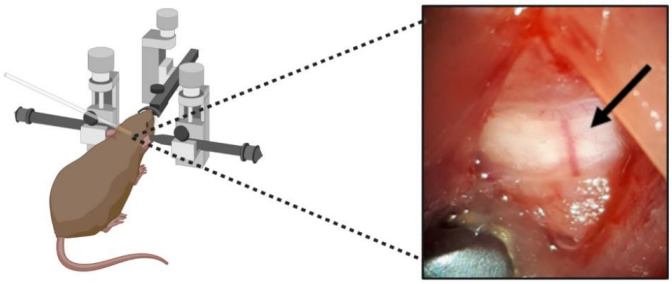

Figure 15. Mouse CSF was collected by surgical procedure performed under the dissection microscope. The visualization of the cisterna magna and a large blood vessel that was avoided during CSF sampling is presented on the right.

CSF analysis

All mouse CSF samples were analyzed at Neurochemistry Laboratory, Mölndal, Sweden. Due to the limited amount of available CSF samples, a new ultra-sensitive assay named single molecule array (Simoa), which allows for the detection of proteins at very low levels, was used for the analyses. Simoa assay for mouse neurogranin analysis was recently developed, validated on both mouse and human CSF, and described in detail in Höglund et al., 2020 (258). Mouse CSF Aβ42 and tau were analyzed using Simoa HD-1 commercially available human and mouse kits, respectively.

4.5.2 Brain tissue analysis

Mouse brain tissue was divided into right and left hemispheres and dissected into hippocampus and cortex (right hemisphere) and dehydrated and embedded in paraffin (left hemisphere). The same Simoa assays, as used for the CSF analyses, were used to investigate neurogranin and tau levels in the mouse cortical and hippocampal lysates. The protein concentrations were normalized with the total protein concentrations analyzed in the respective brain lysates. For the purpose of study V, sections of paraffin-embedded left hemisphere were used for establishing immunohistochemical (IHC) staining protocol for the in-house developed anti-Ng antibody (Ng2) that was used in the Simoa assay. IHC staining was then used to investigate difference in neurogranin expression in *App* knock-in mouse models compared to age-matched

wild-type controls. Double neurogranin – Aβ IHC staining was further performed to investigate relationship between neurogranin expression and amyloid pathology in *App* knock-in mice.

4.5.3 EEG recordings and analysis

All mice underwent surgical implantation of surface screw electrodes at 10 weeks of age. Before surgery, animals were subcutaneously injected with 0.025 mg/kg analgesic Piritramide (Dipidolor). The whole surgical procedure was performed under deep isoflurane anesthesia (5% induction and 2% maintenance) while a heating pad was used to control animal body temperature. The animal was placed in the stereotactic frame instrument (Robot Stereotaxic, Neurostar, Germany) with the isoflurane anesthesia delivered via an inhalation mask. At the surgery site, an additional local spray analgesic was applied (Xylocaine, 10%). The head was shaved, and a sagittal incision was made to expose the skull. Holes were drilled for the placement of three depth needle recording electrodes (right hemisphere) and three surface (epidural) screw recording electrodes (left hemisphere). Three epidural electrodes were stereotaxically implanted in the left hemisphere at the following anterior-posterior (AP), medial-lateral (ML), dorsal-ventral (DV) coordinates relative to the bregma: Frontal left (FL) at AP +1.94 mm, ML -1.5 mm, Parietal left (PL) at AP -1.7 mm, ML -1.5 mm, Occipital left (OL) at AP -2.8 mm, ML -3.2 mm. Depth electrodes were inserted into the right hemisphere as follows: frontal cortex (Frontal right AP +1.94 mm, ML +1.5 mm, DV -1.2 mm), dorsal CA1 region of hippocampus (dCA1 right AP -1.7 mm, ML +1.5 mm, DV -1.7 mm) and ventral CA1 region of hippocampus (vCA1 right AP -3.16 mm, ML +3.0 mm, DV -3.6 mm). Reference electrode was placed 0.5 mm posterior and to the right of bregma (AP -0.5 mm, ML +0.5 mm) and ground electrode was placed in the midline above cerebellum (Figure 16; all coordinates according to the atlas by Paxinos and Franklin, 2012 (259)). Electrodes were connected to a pin (Future Electronics: 0672-2-15-15-30-27-10-0) with a small insert (track pins; DataFlex: TRP-1558-0000) and then inserted into a 10-hole connector, which was carefully fixed to the skull using dental cement. The wound was sutured, and mice were closely monitored during two-week recovery.

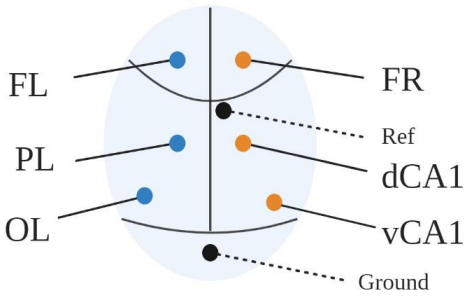

Figure 16. Surgical placement of the three surface (epidural) screw electrodes in the left hemisphere above frontal (FL), parietal (PL) and occipital (OL) left cortex, and three depth electrodes in the frontal cortex (FR), dorsal CA1 (dCA1) and ventral CA1 (vCA1) region of the hippocampus in the right hemisphere. The position of Reference (Ref) and Ground electrodes are shown in black.

After surgery, the animals received 0.3 ml analgesic subcutaneously (Carprofen, Rimadyl, 50 mg/ml, Pfizer Ltd, UK diluted 1:10) and local analgesic on the wounds (Lidocaine spray Xylocaine, 1% solution, Astra Pharmaceuticals Ltd, UK). Animals were next individually placed in their home cage and kept in a heating box set at 26 °C ± 2 °C to avoid hypothermia with the temperature steadily decreasing over days until it reached room temperature. After two weeks recovery, the animals were habituated to the recording setup for 24 hours prior to the recording of the spontaneous EEG and locomotor activity for another 20 hours (10 h of light and dark phases). Recordings were taken in the animal's home cages placed in the environmentally controlled, sound-attenuated Faraday cages. Locomotor activity of the animals was measured by two passive infrared (PIR) detectors located above every recording cage. Motion levels were analyzed from both PIR detectors. A notch finite impulse response (FIR) filter (50 Hz) was used to filter out possible noise caused by the main power line interference. EEGs were recorded at a sampling rate of 512 Hz with BioSemi ActiveTwo system (BioSemi, Amsterdam, Netherlands) and digitized with a resolution of 24 bits. All animals were single caged and allowed to move freely during recordings. The recording setup followed the same procedure at all recording timepoints.

The vigilance states of the animals during each recording were assessed as active wakefulness (AW), quiet wakefulness (QW), non-rapid eye movement sleep (NREM) or rapid-eye movement sleep (REM) on the basis of EEG recording and locomotor activity of the animal according to the pre-defined criteria (Table 2 conform e.g., Ahnaou et al. 2009 (260)) and analyzed using a machine-learning Scoretool on 4 s artifact-free epochs. Examples of mice EEG recordings during different vigilance states of the animal are given in Figure 17. For the purpose of developing a machine-learning algorithm for the scoring of the vigilance states of the longitudinal 20 hours recordings, 20% of all available, $App^{NL\text{-}F}$, $App^{NL\text{-}G\text{-}F}$ and App^{wt} recordings at different time points were randomly selected for visual inspection and manual scoring of animals' vigilance states according to the criteria outlined above. Following this procedure, automated machine-learning scoring tool was uniformly applied on all available EEG recordings. Next, difference in the duration spent in different vigilance states was calculated during both the dark and light phases of the total recording time (20 hours).

EEG power spectra will be calculated in consecutive artifact-free 4 s epochs of QW state of the animals during the first 5 hours of dark period of EEG recordings. QW state and the first half of dark period were selected due to the closest relationship to the resting-state EEG and timing of clinical recordings in humans (mice are nocturnal animals and spend more time in sleep-states during the light phase). To further facilitate the translational approach of the present study, recordings from the three surface (epidural) electrodes will be used for the subsequent analysis. EEG power spectra will be computed using FFT analysis and calculated as relative power (expressed as a percentage of power spectra in every Hz relative to total power).

Table 2. EEG and locomotor characteristics of different vigilance states of the mouse.

Vigilance state	EEG and locomotor characteristics
AW	Low EEG amplitude, high and variable locomotor activity
QW	Low EEG amplitude, low or no locomotor activity
NREM	High amplitude slow-wave EEG activity, no locomotor activity
REM	Regular theta oscillations, no locomotor activity

AW = active wake state, QW = quiet wake state, NREM = non-rapid eye movement sleep, REM = rapid-eye movement sleep.

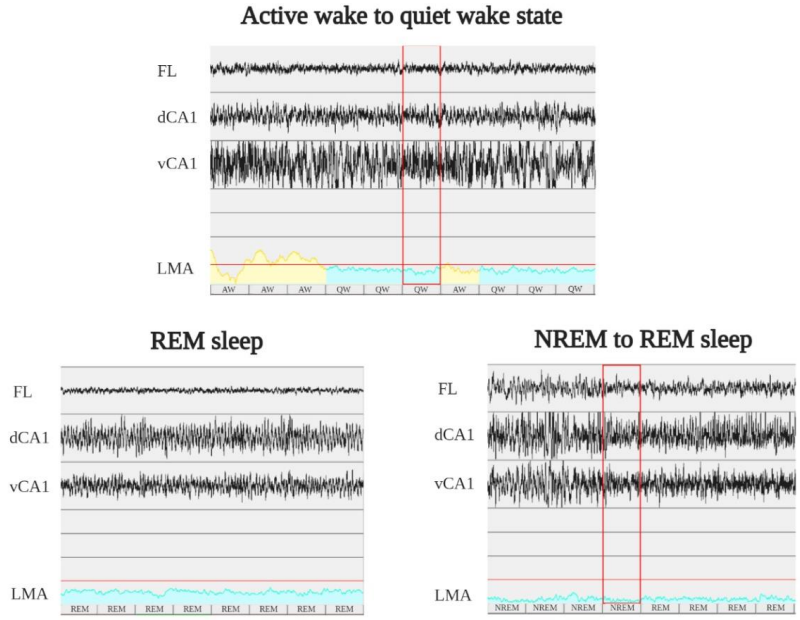

Figure 17. Example of different vigilance states and transitions between vigilance states of the mouse based on EEG, depth electrode recordings and locomotor activity (LMA). The difference in AW versus QW state is mainly based on LMA activity. Recordings presented from the following electrodes: FL = surface frontal left electrode; dCA1 = depth electrode in dorsal right CA1 region; vCA1 = depth electrode in ventral right CA1 region. Vigilance states defined as AW = active wake; QW = quiet wake; NREM = non-rapid eye movement sleep; REM = rapid-eye movement sleep.

4.5.4 Statistical analysis

The data in study V was analyzed using non-parametric tests (Kruskal-Wallis test and Spearman rank correlation tests) due to the small sample sizes that prevented a more thorough investigation of the data distribution. Even though the experiments were conducted in line with the common practice in neuroscience and the 3Rs (replace, reduce, refine), small sample sizes should introduce caution when interpreting results of the respective statistical analyses.

5 MAIN FINDINGS AND THESIS SUMMARY

The following section describes the main findings of the studies included in this thesis. More detailed results together with reflections and discussion are reported in the respective publications/manuscripts.

5.1 PAPER I – ASSOCIATION BETWEEN QEEG AND CSF BIOMARKERS OF AD

The main aim of study I was to investigate the association between qEEG measures of global power (GFP) and synchronization (GFS) and conventional CSF biomarkers of AD. The relationship between CSF markers and qEEG measures was investigated in the whole cohort of SCD, MCI and AD patients and subsequently in separate diagnostic groups. In the whole memory clinic cohort, CSF Aβ42 levels were significantly negatively associated with GFP in delta and theta frequency bands. CSF p- and t-tau levels were significantly negatively associated with GFP in alpha and beta frequency bands. Results indicated that lower CSF Aβ42 levels are associated with increased EEG power in slow frequencies while higher CSF p- and t-tau levels correlate with decreased power in fast frequencies (Table 3).

Multiple linear regression analysis revealed that CSF Aβ42, p- and t-tau levels significantly correlate with GFS in alpha and beta frequency bands. Specifically, lower CSF Aβ42 and higher p- and t-tau levels were associated with decreased GFS in fast frequencies (Table 3).

Analysis of separate diagnostic groups revealed that significant associations of CSF AD biomarkers with GFP and GFS are dominantly present in MCI group, with a significant association with GFS beta present already in SCD patients.

Table 3. Association between CSF biomarkers of AD, GFP and GFS in memory clinic patients.

	CSF Aβ42	CSF p-tau	CSF t-tau
GFP delta	-0.304**	-0.107	-0.048
GFP theta	-0.514***	-0.076	0.011
GFP alpha	0.170	-0.409***	-0.222**
GFP beta	0.002	-0.278***	-0.175***
GFS delta	0.002	0.002	0.001
GFS theta	0.001	-0.001	-0.001
GFS alpha	0.024***	-0.015***	-0.010***
GFS beta	0.013***	-0.005	-0.004*

CSF = cerebrospinal fluid; GFP = global field power; GFS = global field synchronization. Data presented as β coefficients. Controlled for age, sex and MMSE. *p < 0.05; **p < 0.01; ***p < 0.001. Adapted with permission from Smailovic U, Koenig T, Kåreholt I, et al. Quantitative EEG power and synchronization correlate with Alzheimer's disease CSF biomarkers. Neurobiol Aging. 2018;63:88-95. © 2017 The Authors. Published by Elsevier Inc.

5.2 PAPER II – EEG MICROSTATES IN HEALTHY ELDERLY AND MEMORY CLINIC PATIENTS

The aim of study II was to investigate differences in EEG microstate topographies in healthy elderly, SCD, MCI and AD patients. The second aim was to investigate changes in microstate parameters in patients with different stages of cognitive impairment. The third aim was to investigate the association of microstate topographies and parameters with conventional CSF biomarkers of AD.

The first finding was that the four representative grand mean maps from the healthy control group were compatible with the four normative maps available from the literature as well as to the maps from the previous studies employing microstate analysis (Figure 18) (178, 179).

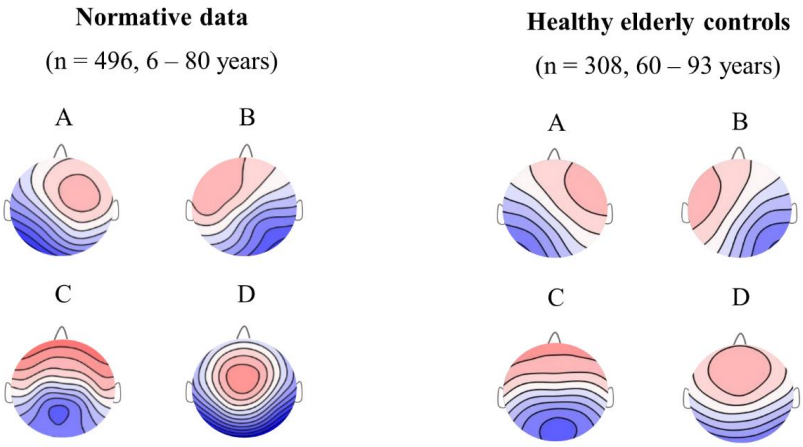

Figure 18. Four representative microstate maps from the healthy population published as normative data (age 6 – 80 years, on the left) and from healthy elderly controls from study II (60 – 93 years, on the right). Adapted with permission from Smailovic U, Koenig T, Laukka EJ, et al. EEG time signature in Alzheimer´s disease: Functional brain networks falling apart. Neuroimage Clin. 2019;24:102046. © 2019 The Authors. Published by Elsevier Inc.

Study II further reported significant differences in microstate topographies between healthy elderly, SCD, MCI and AD patients for microstate classes A, C and D. Differences in microstate topographies between healthy elderly and memory clinic patients precluded direct comparison of microstate parameters between referred groups. Differences in microstate parameters (duration, occurrence, and contribution) were therefore investigated by back-fitting healthy elderly maps (serving as healthy controls) onto the patients' EEG data (Figure 14). This analysis revealed a statistically significant increase in the duration of maps A and B (asymmetrical maps) and a significant decrease in the occurrence of maps C and D (symmetrical maps) with the more severe stage of cognitive impairment (comparing SCD, MCI and AD patients). Microstate contribution parameter resembles both duration and occurrence since it indicates the dominance of each map (expressed as the percentage) through the whole EEG recording. Mean contribution of maps A and B was significantly increased, while contribution of maps C and D significantly decreased with the more severe stage of cognitive impairment (Figure 19).

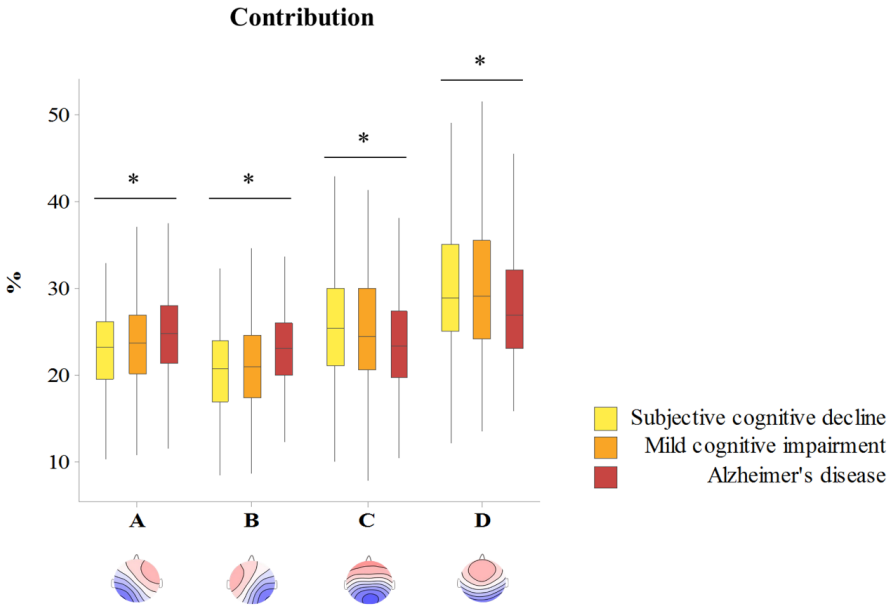

*Figure 19. Contribution of healthy elderly microstate maps in the EEG of patients diagnosed with subjective cognitive decline, mild cognitive impairment, and Alzheimer's disease. Microstate contribution parameter indicates the dominance of each map (expressed as the percentage) through to whole EEG recording and therefore summarizes parameters such as microstate duration and occurrence. *p < 0.05. Adapted with permission from Smailovic U, Koenig T, Laukka EJ, et al. EEG time signature in Alzheimer´s disease: Functional brain networks falling apart. Neuroimage Clin. 2019;24:102046. © 2019 The Authors. Published by Elsevier Inc.*

Study II additionally reported a significant association between MMSE score and topography of microstate map A, CSF Aβ42 levels and the topography of microstate map C and CSF p-tau levels and topography of microstate map B when analyzed in the whole patient group. The direction of significant associations of microstate parameters with MMSE and conventional CSF biomarkers of AD followed the pattern of differences observed between SCD, MCI and AD patients (Supplementary data of study II).

5.3 PAPER III – QEEG IMAGING IN RELATION TO [^{18}F]FDG-PET

Study III investigated the relationship between glucose hypometabolism in temporoparietal lobes, as evidenced by [^{18}F]FDG-PET, and EEG LORETA functional connectivity measures within the same vulnerable brain regions in MCI and AD patients. The analysis was conducted in the whole cohort of clinically diagnosed MCI and AD patients and in the subgroup of CSF amyloid positive MCI and AD patients. The main finding was that decreased glucose metabolism in temporal and parietal lobes significantly correlated with decreased EEG instantaneous linear connectivity in alpha and beta frequency bands within the same vulnerable brain regions. Simultaneously, decreased [^{18}F]FDG uptake in temporoparietal lobes significantly correlated with increased EEG lagged linear connectivity in slow frequencies, *i.e.*

delta and theta frequency bands, within the same vulnerable brain regions in MCI and AD patients. The pattern of referred [^{18}F]FDG-PET and EEG connectivity correlations was consistent in the amyloid positive MCI and AD patients, especially when employing measures of EEG instantaneous connectivity (Figure 20).

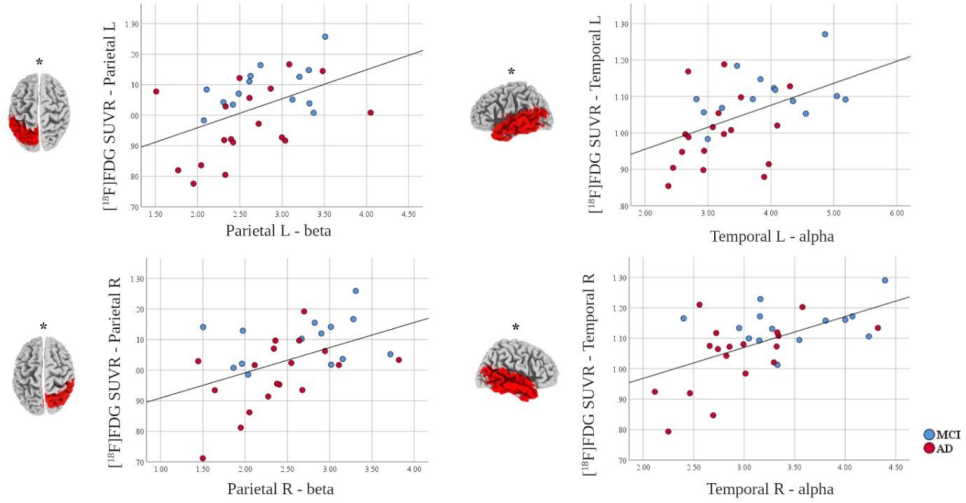

*Figure 20. Significant positive correlations between brain [^{18}F]FDG SUVR and LORETA instantaneous linear connectivity in alpha and beta frequency bands in the corresponding brain regions of amyloid positive MCI and AD patients (based on CSF Ab42/40 ratio). *p < 0.05. The regression line was fitted for visualization.*

5.4 PAPER IV – CSF NEUROGRANIN AND QEEG IN STABLE AND PROGRESSIVE MCI

The main aim of study IV was to investigate the association between measures of global EEG power and synchronization and molecular synaptic marker neurogranin in the CSF of MCI patients. The second aim was to investigate their potential to predict clinical deterioration to AD dementia in MCI patients that have been followed-up for two years.

Baseline neurogranin levels were significantly increased in the CSF of progressive compared to stable MCI patients (Figure 21). Quantitative EEG measure of global power, GFP, indicated significantly higher power in slow frequencies (delta and theta) in the progressive compared to stable MCI patients.

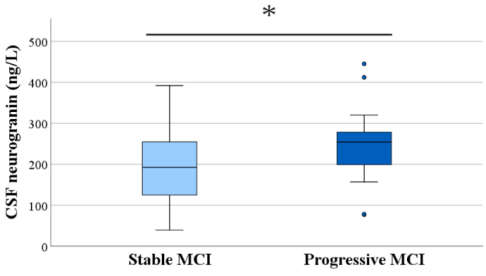

*Figure 21. Baseline differences in CSF neurogranin levels in stable and progressive MCI patients. *p < 0.05.*

Study IV further reported a significant negative association between CSF neurogranin levels and GFP and GFS in theta frequency band in the progressive MCI group, after controlling for age, sex, and CSF t-tau/Aβ42 ratio.

The main finding of IV study was that CSF neurogranin, GFP theta and GFS beta were all significant and independent predictors of progression to AD dementia in MCI patients. GFP delta was close to the statistical significance ($p = 0.059$) and was further tested in the models that combined several predictors. GFP delta and theta were not assessed in the same model due to their strong correlation ($r > 0.7$). As a single predictor, GFP theta exhibited higher classification accuracy (74.6%) compared to CSF neurogranin (70.0%) or GFS beta alone (67.6%). A model that combined GFP delta or theta with GFS beta revealed both measures as significant predictors, however, the classification accuracy remained the same as for GFP theta alone (74.6%). A combination of CSF neurogranin, GFP theta and GFS beta reached the highest classification accuracy (81.4%) in differentiating progressive from stable MCI patients (Table 4).

Table 4. Classification accuracy of baseline CSF neurogranin and qEEG measures in differentiating progressive from stable MCI patients.

Significant predictor(s)	CSF Ng	GFP delta	GFP theta	GFS beta	Classification accuracy
CSF Ng	*				70.0%
GFP delta		(p = 0.059)			69.0%
GFP theta			*		74.6%
GFS beta				*	67.6%
GFP delta + GFS beta		*		*	74.6%
GFP theta + GFS beta			*	*	74.6%
CSF Ng + GFP delta	*	n.s.			74.3%
CSF Ng + GFP theta	*		*		74.3%
CSF Ng + GFS beta	*			n.s.	74.3%
CSF Ng + GFP delta + GFS beta	*	*		*	78.6%
CSF Ng + GFP theta + GFS beta	*		*	*	81.4%

Logistic regression with CSF neurogranin, GFP and GFS as predictors and progression to AD dementia in MCI patients during two years follow-up as an outcome. CSF = cerebrospinal fluid; GFP = global field power; GFS = global field synchronization; Ng = neurogranin. N.s. = non-significant; *$p < 0.05$. Adapted from Smailovic U, Kåreholt I, Koenig T, et al. Synaptic molecular and neurophysiological markers are independent predictors of progression in Alzheimer's disease. Manuscript under review.

The dependence of CSF neurogranin and qEEG measures on patients' baseline CSF Aβ42 status (stratified according to the clinical cut-off for amyloid positivity < 550 ng/L) was additionally tested with interaction models, which revealed that these measures were significant predictors of progression to AD dementia independent of baseline CSF amyloid status in MCI patients.

5.5 PAPER V – CSF NEUROGRANIN, AB42 AND TAU IN *APP* KNOCK-IN MOUSE MODELS

Neurogranin has emerged as a potential CSF marker of synaptic pathology in AD. Study V assessed the translational potential of CSF neurogranin and its relationship to AD-related pathology (Aβ and tau) in the CSF and brain tissue of *App* knock-in mouse models. The experiments were performed on 12-months old *App* knock-in and wild-type control mice. Study V employed novel ultra-sensitive single molecule assay (Simoa) that allowed for several separate protein analyses from the limited amount of mouse CSF.

The main finding was that neurogranin levels are increased in the CSF of *App* knock-in mouse models compared to age-matched wild-type controls (Figure 22). Additionally, study V revealed that $App^{NL\text{-}G\text{-}F}$ mice have increased tau levels in the CSF (Figure 22). The Simoa analyses of brain tissue showed that tau levels are decreased in the soluble fraction of cortex of $App^{NL\text{-}G\text{-}F}$ mice compared to wild-type controls. Simultaneously, increased CSF neurogranin levels correlated with decreased neurogranin levels in the soluble fraction of cortex when analyzed in *App* knock-in mice and age-matched wild-type controls.

Study V further reported the relationship between AD CSF biomarkers in *App* knock-in mice that closely resembled the associations reported in clinical AD cohorts. These included a significant positive correlation of CSF neurogranin and CSF tau and a negative correlation of CSF neurogranin and CSF Aβ42 levels. Additionally, neurogranin and tau levels positively correlated in the soluble fractions of both cortex and hippocampus.

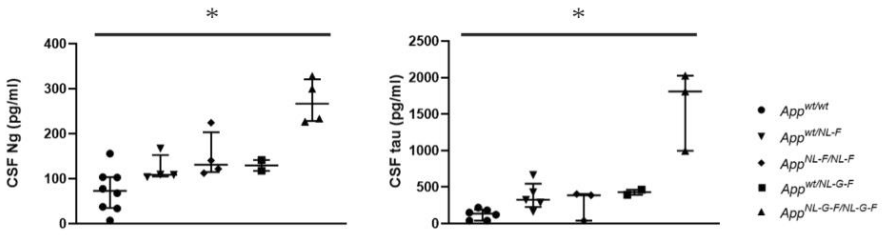

Figure 22. CSF neurogranin and tau levels in the App knock-in mice models and age-matched wild-type controls. Data are presented as median and interquartile range. The $App^{wt/NL\text{-}G\text{-}F}$ *is presented in the figures for graphical comparisons but was not included in the statistical analyses. * p < 0.05. Adapted from Smailovic U, Delac L, Liman V, et al. Translational potential of Alzheimer's disease CSF biomarkers from man to mouse – CSF neurogranin correlates with tau and amyloid-β pathology in App knock-in mouse models. Manuscript in preparation.*

An IHC staining protocol was established with the in-house developed anti-Ng antibody (corresponding to the antibody used in the Simoa assay) to investigate the expression of neurogranin in *App* knock-in mouse brain tissue (Figure 23). The first finding was the decrease in neurogranin immunostaining in the CA1 region of the hippocampus in the $App^{NL\text{-}F/NL\text{-}F}$ and especially in $App^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ mice compared to $App^{wt/wt}$ controls. The second finding followed double neurogranin – Aβ immunostaining and revealed loss of neurogranin immunoreactivity in the close vicinity of Aβ plaques in the hippocampus of *App* knock-in mice (Figure 23).

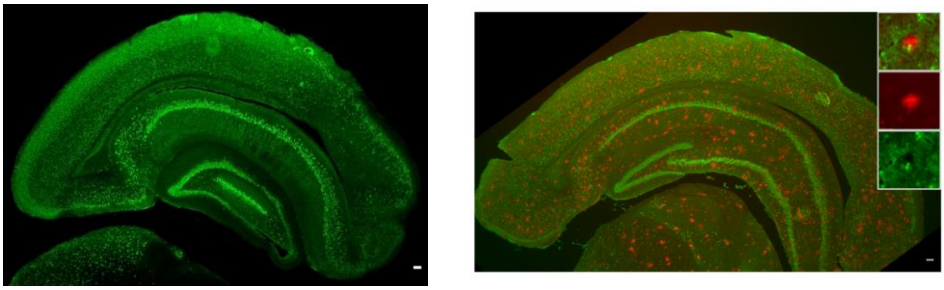

Figure 23. Fluorescent immunostaining for neurogranin in adult wild type mouse cortex and hippocampus using in-house developed anti-Ng Ng2 antibody (on the left). Double neurogranin – Aβ immunostaining and decreased neurogranin immunoreactivity around Aβ plaques in the App$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ mice shown in higher magnification (on the right). Scale bars = 100 µm. Adapted from Smailovic U, Delac L, Liman V, et al. Translational potential of Alzheimer's disease CSF biomarkers from man to mouse – CSF neurogranin correlates with tau and amyloid-β pathology in App knock-in mouse models. Manuscript in preparation.

5.6 THESIS SUMMARY

The general aim of the thesis was to contribute to the characterization of synaptic degeneration in Alzheimer's disease. The main focus was on qEEG as a potential early, non-invasive, and readily available add-on to the assessment of synaptic dysfunction due to AD. The main conclusions can be summarized in the following points:

- General EEG slowing and reduced global EEG synchronization correlated with decreased CSF Aβ42 and increased CSF p- and t-tau levels in memory clinic patients diagnosed with SCD, MCI and AD.

- Topographies of EEG microstates deviated in SCD, MCI and AD patients compared to healthy elderly controls and were associated with changes in MMSE and conventional CSF biomarkers of AD. In memory clinic patients, EEG microstate parameters were altered in a gradient-like manner with the more severe stage of cognitive impairment.

- EEG sLORETA analysis of brain functional connectivity detected AD-related regional dysfunction as evidenced by correlation and topographical overlap with [^{18}F]FDG-PET findings of cortical glucose hypometabolism in MCI and AD patients. These findings were corroborated in a subsample of patients with the evidence of amyloid pathology.

- qEEG measures of global power and synchronization and CSF synaptic marker neurogranin were independent predictors of progression to AD dementia in MCI patients. A combination of qEEG measures and CSF neurogranin increased the classification accuracy for differentiating progressive versus stable MCI patients.

- *App* knock-in mouse models of AD exhibited increased neurogranin and tau and decreased Aβ42 levels in the CSF. Neurogranin levels interrelated in the CSF and brain tissue and were associated with Aβ pathology. Relationships between CSF biomarker alterations were similar to the patterns found in clinical AD cohorts. In parallel use of mouse CSF biomarkers and *App* knock-in models have translational value for investigating synaptic degeneration due to AD.

6 DISCUSSION

6.1 ALZHEIMER'S DISEASE CONTINUUM – IMPORTANT CONSIDERATIONS

Most of the studies presented in the thesis included patients on a whole spectrum of cognitive impairment, even though a significant proportion of SCD and MCI patients will not progress to AD or other types of dementia. However, this type of "all-inclusive" analysis was performed for several reasons. First, there may not be strict transition points in pathological biomarker nor cognitive status since it was postulated that AD develops on a pathological-clinical continuum (6). Therefore, including patients on the whole continuum may be crucial when it comes to the investigation of a relationship between different molecular and functional markers. Second, cut-offs for conventional biomarker changes, such as for CSF Aβ42 positivity, are often set for clinical diagnosis of AD dementia and possibly require more lenient thresholds in intermediate stages such as SCD and MCI. Otherwise, a certain proportion of subjects may be neglected as representing a non-AD trajectory even though the biomarker values were showing abnormal trends towards or close to cut-off values for AD dementia (261, 262). Third, the results can be further substantiated by a sub-analyses in separate, biomarker defined, diagnostic groups as it was performed in the present thesis. It is also important to note that while molecular and imaging biomarkers are undeniably important for both research and clinical investigations, their use is still restricted to the large medical centers. Studies with well-phenotyped naturalistic cohorts are therefore crucial for the validation of novel biomarker candidates. At the same time, the use of clinically defined diagnostic groups warrants caution due to the enrollment of etiologically heterogeneous patient groups. Therefore, it is important to consider regular clinical follow-ups as well as biomarker dynamics in SCD and MCI patients in future large-scale qEEG studies.

6.2 DEMENTIA ASSESSMENT – WHERE DOES EEG STAND?

The European Federation of Neurological Societies (EFNS) guidelines suggest that EEG may aid differential diagnosis of AD (263). EFNS guidelines highlight the use of EEG in the case of suspected Creutzfeldt-Jakob Disease, toxic metabolic disorders, and transient epileptic amnesia (263). Its contribution to the differential diagnosis of dementia was further supported by the recent consensus criteria from Dementia with Lewy Bodies (DLB) Consortium which include EEG findings of dominant posterior slow-wave EEG activity as a supportive biomarker for DLB (264). Guidelines from National Institute for Health and Care Excellence (NICE), UK, explicitly state that EEG should not be used to diagnose AD (265). Lack of clinical recommendations for the use of EEG in the assessment and diagnosis of AD stem from the insufficient evidence of its pathological and clinical validity as well as lack of standards in reporting research findings. Furthermore, the nature of EEG signal made it appealing for different algorithmic approaches and an enthusiastic introduction of numerous qEEG measures. While the complexity and variability of EEG signal call for a comprehensive analytical approach, research has often been method-driven and outside the context of underlying disease pathologies, leaving the question where does the EEG stand in the dementia assessment largely unresolved. The present thesis attempted to shed light on these issues, such as the need for different EEG domain analyses, the relation of qEEG markers to AD pathology and the prognostic potential of qEEG measures in the context of AD, as discussed throughout the following sections.

6.3 IS THERE A NEED FOR DIFFERENT QEEG DOMAIN ANALYSES?

Most EEG studies so far have reported generalized EEG slowing in patients along AD continuum, resulting from the EEG power spectral analysis, which was also confirmed in the present thesis. Even though power spectra analysis captures disturbances across different frequency bands that bear physiological significance, it may not fully portray the extent of brain functional impairment. Additionally, frequency domain analysis does not utilize everything that qEEG, as a method, has to offer. The present thesis employed qEEG analyses in frequency (study I, III and IV), time (study II) and space domain (study III). Study I and IV employed more conventional analyses and measures of global EEG power and synchronization that have been previously investigated in comparable studies and large memory clinic cohorts (148, 158, 166). In the present thesis, these qEEG measures were further evaluated in relation to the conventional CSF biomarkers of AD (study I), synaptic CSF marker neurogranin and their potential to predict clinical deterioration in MCI patients (study IV). Study II involved EEG microstate analysis in the time domain that may capture the (dis)organization of large-scale resting-state brain networks in AD. In contrast to other methods, the discovery of microstates has opened a possibility to detect and study disturbances in spontaneous mental processes with a uniquely high time resolution (176, 179). Additionally, study II involved EEG recordings from a large group of healthy elderly controls that may serve as normative data for future EEG and microstate analyses in brain aging studies. Study III further investigated the association between cortical glucose hypometabolism, as evidenced by [^{18}F]FDG-PET, and EEG functional connectivity measures in MCI and AD patients. In order to detect the topographical sources of brain functional disturbances, an EEG imaging (LORETA) analysis was employed. LORETA has been previously validated against other imaging modalities and employed in studies involving cognitively impaired patients (190-192, 195, 266). Altogether, the choice of qEEG methods was fitted to the study design, involving different diagnostic modalities, and research questions of interest. Recent studies have additionally emphasized the advantage of combining multiple EEG markers when it comes to the diagnostic classifiers of AD and prediction of clinical progression of the disease (267-269). Altogether, and taking into account the inter-individual variability in EEG signal, integrating different qEEG methods may be necessary for a comprehensive assessment of different aspects of functional (synaptic) deficits due to AD.

6.4 TOWARDS VALIDATION OF QEEG WITH MOLECULAR AND NEUROIMAGING MARKERS

The diagnosis of dementia has several challenges, among which are the different etiologies underlying clinically evident cognitive deficits. Therefore, it is important to relate novel diagnostic approaches to the available AD biomarkers and disease-associated neuropathology. In addition, biological substrates of the qEEG alterations observed in AD patients have not been extensively investigated in large and well-defined clinical cohorts so far. The present thesis investigated the association of conventional CSF biomarkers of AD (study I and II), [^{18}F]FDG-PET (study III) and novel CSF synaptic marker neurogranin (study IV) with qEEG in cognitively impaired subjects and patients with AD. The main finding of study I was the distinctive pattern of associations between qEEG power abnormalities and CSF markers, *i.e.* the association of decreased CSF Aβ42 levels with increased EEG delta and theta power and the association of increased CSF p- and t-tau levels with decreased EEG alpha and beta power

in SCD, MCI and AD patients. While the study presented some encouraging findings when it comes to the use of qEEG in patients along the AD continuum, it should be noted that EEG, as a method, cannot be offered as a substitute for molecular diagnostic tests for specific brain pathologies. However, the study provided insight into potential pathological changes associated with observed alterations in EEG power spectra that have been long related to AD. Furthermore, these findings may contribute to the selection of qEEG features that may help differentiate AD from non-AD pathologies as causes of cognitive impairment. Study III further investigated the association of cortical glucose metabolism with qEEG measures of brain functional connectivity indicating that EEG imaging methods, such as LORETA, have the potential to detect functional disconnection of AD vulnerable brain regions. Despite moderate correlations and the need for further validation in larger cohorts, these findings have several implications. First, alterations of qEEG measures of brain functional connectivity observed in AD were associated with specific brain regional dysfunction as evidenced by typical [^{18}F]FDG-PET pattern of decreased glucose metabolism in temporoparietal lobes in MCI and AD patients. Second, these findings further support the potential role of qEEG as a marker for synaptic alterations in AD since [^{18}F]FDG uptake was shown to be closely related to synaptic density and function (110, 123, 270). Third, the utility of [^{18}F]FDG-PET to differentiate AD from other types of dementia and the relationship between cortical glucose metabolism and cognitive reserve in preclinical AD (271-273) hints that qEEG may find a similar clinical application. In study IV, qEEG measures of power and synchronization in theta frequency band were negatively associated with CSF neurogranin levels in the MCI group progressing to AD dementia. Interestingly, the association between increased CSF neurogranin levels and decreased GFP in theta band was significant only after controlling for CSF t-tau and Aβ42 levels. This should be further investigated in order to disentangle the effects of neuronal injury and possible neuronal compensatory mechanisms in MCI patients. Lastly, the direct relationship of qEEG abnormalities with AD pathology may be explored in preclinical models of the disease. In that context, emerging studies on fluid, imaging and neurophysiological markers in animal models offer a possibility to assess biomarker inter-relations as well as their direct link to the core AD neuropathology.

6.5 TRANSLATIONAL PERSPECTIVE – SYNAPTIC DYSFUNCTION IN AD MOUSE MODEL

Translational biomarkers of AD may facilitate the selection of relevant preclinical models, investigation of their direct correlates with neuropathology and therefore different disease mechanisms, and eventually selection of novel drug candidates. Study V reported an increase in CSF neurogranin, an emerging marker of AD synaptic pathology according to the clinical studies, in *App* knock-in mouse models of AD. Neurogranin levels in the brain tissue interrelated with its CSF levels and were affected by the presence of Aβ plaques. These findings implicate that neurogranin alterations in the CSF and brain tissue are downstream to the accumulating amyloid pathology. Additionally, it puts forward neurogranin as a potential translational marker of synaptic pathology due to AD. While mouse CSF experiments require a surgical procedure that provides a limited amount of the sample for further analyses, the use of novel ultra-sensitive assays for protein quantification opens a possibility for simultaneous investigation of several relevant biomarkers from the small quantity of CSF

specimens. This suggests in parallel use of fluid biomarkers as a translational bridge between preclinical and clinical AD research.

Interestingly, study V further reported an increase in tau levels in the CSF of *App* knock-in mice, a model of β-amyloid pathology in AD. This finding is in line with some previous studies on transgenic mice models of amyloid-β pathology, such as APP/PS1 and APP23, that reported increase in CSF tau levels compared to age-matched wild-type controls (274). None of these models of amyloid-β pathology develop considerable tau hyperphosphorylation, NFTs or neuronal loss. The inconsistencies between tau pathology in the brain and CSF tau levels were previously reported in the clinical studies that included CSF investigations of autopsy-confirmed AD cases (275) as well as in patients with tauopathies (79). More recently, tau release into the extracellular space was shown to be directly associated with the level of brain synaptic activity (276, 277). Even though amyloid-β was shown to alter synaptic activity in AD (278), further studies are warranted to investigate the exact mechanisms and pathways behind amyloid-induced tau pathology in AD (279).

The aim of the preliminary study introduced in this thesis is to evaluate changes in synaptic function, assessed by EEG recordings, in *App* knock-in mouse models. The study will evaluate longitudinal changes in EEG power spectrum in relation to the accumulating Aβ pathology. The recordings from epidural electrodes as well as analyses of quiet wake vigilance state facilitate the translational perspective of the project. Interestingly, a recent study reported decreased power in lower frequencies (delta and theta) and increased power in higher frequencies (alpha and beta) in a previous generation of transgenic mouse models of amyloid-β pathology (280). These findings seem to be in direct contrast to the reports from qEEG analyses in patients with AD, even though the study did not assess longitudinal EEG changes that could detect the emergence of different phenotypes as transgenic mice age. The longitudinal qEEG analysis in *App* knock-in mice may therefore elucidate the relationship between amyloid accumulation and neurophysiological findings in AD, in the absence of substantial tau pathology and neuronal loss.

Results showing CSF biomarker changes in *App* knock-in mice models encourage their use in the translational studies of synaptic degeneration and core pathophysiological mechanisms of AD, including potential drug candidate testing. Nonetheless, the limitations of these preclinical models should be recognized, such as the lack of some hallmark aspects of AD pathology and the introduction of a combination of mutations that are found in the different familial and not sporadic, the most common form of the disease.

6.6 PREDICTIVE VALUE OF SYNAPTIC MARKERS

An integrated approach to dementia diagnosis calls for the use of interdisciplinary tools that would provide a comprehensive assessment of structural and functional deficits due to AD. In this context, potential of the diagnostic tests to identify subjects with high risk for rapid deterioration to AD dementia is of main importance. Study IV investigated the relationship between molecular and functional synaptic markers, *i.e.*, CSF neurogranin and qEEG measures and whether they have any additive value in predicting progression to AD dementia in MCI patients that have been followed-up for two years. The results showing that CSF neurogranin and qEEG measures of global field power and synchronization are all significant predictors of progression to AD dementia implicate that these markers provide complementary information

about synaptic degeneration in AD. Extensive phenotyping of synaptic damage due to AD may be warranted for reliable identification of subjects and patients that are at risk to decline to AD dementia and have a faster progression of the disease. This was further corroborated by showing that a combination of CSF neurogranin and qEEG measures increased the classification accuracy of cases identified as progressive versus stable MCI, compared to the use of individual markers. Another interesting finding was that CSF neurogranin and qEEG measures were predictors of progression to AD dementia irrespective of the patients' baseline amyloid status. These results are seemingly in contrast to the previous reports showing that CSF neurogranin and t-tau are primarily associated with clinical deterioration in the presence of Aβ pathology (220). However, the referred study conducted separate analyses in CSF Aβ42 positive and negative subjects, which was precluded by the smaller sample size in study IV. Another explanation may be related to the inclusion of MCI patients only (study IV), where these associations may not be as pronounced compared to the analyses conducted in patients covering the full spectrum of the disease, as well as the use of conservative clinical cut-offs for CSF Aβ42 positivity. Altogether, complementarity and additive value of different synaptic markers in predicting AD dementia call for multimodal phenotyping of synaptic damage due to AD. These results require further validation in larger cohorts including healthy subjects and patients with clinically- and biomarker-verified diagnoses, thorough neurophysiological profiling at the baseline, and longer follow-ups.

7 FUTURE PERSPECTIVES

The research criteria for AD diagnosis has emphasized the importance of biomarker inclusion into the diagnostic process in order to ascertain the underlying pathophysiology and cover the full continuum of the disease (60). Recent advancements in molecular imaging and fluid biomarkers provide a unique opportunity to *in vivo* assess pathological changes associated with AD. However, there is a certain concern regarding their widespread clinical use. In addition, a considerable number of healthy elderly show amyloid positivity (281) and stable cognitive status over a long period of time despite abnormal core markers of the disease pathophysiology (282). Disclosing such clinical findings to the patients and healthy elderly raises many ethical considerations (283, 284). In this regard, an accurate assessment of the risk of disease progression and estimated time to transition to the next clinical stage are of great priority for patients, their families, and clinicians. Quantitative EEG is a non-invasive, low-cost, and widely accessible technique that could contribute to the multimodal assessment of the individual brain functional status and risk for impending cognitive deterioration. EEG shows relatively high inter-individual variability (285, 294), and lacks sensitivity and specificity of molecular markers (286), however, it provides a "window" into the complex brain functioning that is influenced by many genetic, pathologic and environmental factors (287). Recent identification of numerous low-risk genes that affect different AD pathways and contribute to the late onset AD (288) supports the utility of "umbrella" diagnostic and prognostic classifiers. However, several additional research and clinical considerations should be addressed before establishing the potential role of qEEG in a clinical work-up of dementia.

In addition to the standardized recording setup and preprocessing approaches, there is a need for the use of *harmonized* qEEG measures across studies at different research centers. So far, numerous qEEG approaches and measures of brain functional connectivity, the topography of cortical activity and microstate algorithms and sorting procedures have been utilized in AD research field, most of which have not replicated methodological approaches used in complementary studies.

In this context, initiatives for freely *available and multi-center EEG datasets* would aid methodological harmonization of EEG research studies. It would expedite collection, utilization and validation of the data and open a possibility for the coordinated use of data from large normative cohorts and other contrast diagnostic groups.

A significant proportion of cognitively impaired patients are on a different clinicopathologic spectrum of dementia with underlying mixed and other types of neurodegenerative processes (289-292). Several studies aimed to investigate resting-state EEG as a potential tool in the *differential diagnosis* of dementia. To date, EEG is included as a supportive diagnostic biomarker for DLB (264). Quantitative EEG abnormalities in time, frequency and space domain that were reported in patients along the AD continuum in the present thesis, however, await further validation in the studies involving contrast groups such as patients diagnosed with frontotemporal dementia (FTD), DLB and mixed dementia with both neurodegenerative and cerebrovascular pathology. Another important role of biomarkers in the context of biological heterogeneity of the disease is the detection of entities that comprise the clinical AD continuum but have considerably different clinical outcomes, such as primary age-related tauopathy (292, 293). The genetic basis of variation in individual EEG was supported by studies that showed

high concordance in EEG signal between monozygotic twins (294, 295). It would thus be of interest to additionally assess the utility of qEEG in patients with fAD, including pre-symptomatic mutation carriers.

Investigation of EEG phenotypes in animal models of AD will further disentangle the association between neurophysiological abnormalities and molecular pathology underlying the disease. It will additionally aid the selection of animal models with relevant neurophysiological phenotypes for AD translational studies. Overall, in parallel use of qEEG and fluid synaptic biomarkers in future preclinical and clinical studies on synaptic dysfunction in AD would expedite the *translation* of preclinical findings to clinical use.

Relatively large inter-individual variability in EEG signal and reported abnormalities across several qEEG domains in AD suggests the use of a *combination of EEG features* for the improved diagnostic and prognostic accuracy of AD. In addition, applying more advanced statistical models may improve the selection and classification performance of qEEG markers. So far, encouraging results were obtained by using automated tools and integrating multiple EEG markers for the diagnostic classification of AD (267-269). Future studies may elucidate the optimal combination of the EEG features, such as measures of power spectra, instantaneous and lagged connectivity, microstate topographies and parameters that may provide comprehensive information on synaptic dysfunction due to AD. Additionally, the utility of EEG in dementia assessment may go *beyond resting-state* EEG. Disturbances in sleep architecture were shown to be indicative of future cognitive decline and dementia in MCI patients (296), highlighting the potential use of sleep EEG recordings and analysis.

The validity of qEEG as a synaptic marker in AD will be further assessed in studies combining several novel CSF pre- and postsynaptic markers as well as synaptic SV2A PET imaging. However, the purpose of multimodal studies goes beyond interrelating novel and established markers of AD. *Multimodal approaches* that combined EEG with other imaging and molecular markers were shown to significantly improve the differential diagnosis of AD (297-300) and may be therefore not just advantageous, but necessary in the diagnostic work-up of dementia. These notions require further investigations, however, in these circumstances, qEEG could be applied as a diagnostic add-on at multiple time points starting from the early stages of the disease. In addition, qEEG may reflect the functional deficits in patients with different levels of cognitive reserve and possibly aid the differentiation of AD from other causes of cognitive impairment.

Clinically silent epileptiform activity, as a manifestation of brain network hyperexcitability, was reported in early pre-dementia stages of the disease (301), suggesting interventions with available treatment options that were not initially approved for AD. The potential of anticonvulsant levetiracetam, a modulator of SV2A, to improve cognitive deficits in MCI and AD patients is currently being tested in clinical trials (302, 303). These recent clinical drug trials incorporate EEG as a part of the baseline assessment battery as well as the secondary outcome measure. In addition to the currently established AD biomarkers, EEG may therefore aid the selection of patients that may benefit from the novel *pharmacological interventions* as well as in monitoring drug efficacy in both drug trials and clinical settings.

Overall, the findings of the present thesis indicated plausible associations between qEEG measures and molecular and functional markers of AD. It addressed the importance of comprehensive analyses across several EEG domains and harmonized use of qEEG markers in the context of AD research. The results further suggested complementary value of molecular synaptic and qEEG markers in predicting cognitive deterioration to AD dementia in memory clinic patients that are at risk for cognitive decline. Ultimately, the results of the thesis invite for the research and clinical validation of multimodal synaptic markers for early AD diagnosis, accurate prognosis, and treatment monitoring in individual patients.

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