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Article

Keywords: Alzheimer's disease, functional MRI, brain pulsations, brain physiology

Posted Date: September 10th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-9725762/v1>

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Additional Declarations: Competing interest reported. J. Krüger has served on the advisory board of Novartis, Nutricia, Eisai, Lilly and Roche and received honoraria from lectures from Bioarctic and Lilly and received support for congress participation from Merck. Vesa Kiviniemi has received a lecture honorarium from Insightec. Other authors have no competing interests to declare.

Amplitude of physiological brain pulsations in Alzheimer's disease

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Abstract

Physiological pulsations maintaining brain neurofluidic homeostasis are altered early in the course of Alzheimer's disease (AD). In this study, we analyzed amplitude changes in physiological brain pulsations of 43 AD patients diagnostically validated by cerebrospinal fluid (CSF) biomarkers and 72 controls using ultrafast functional magnetic resonance imaging. We thereby tested the hypothesis that pulsation changes would correlate with the commonly used AD CSF biomarkers. The very low frequency (< 0.1 Hz) vasomotor waves, respiratory and cardiac frequencies and cardiorespiratory modulation all displayed significantly higher amplitudes in overlapping brain structures of AD patients compared to the controls. The increased amplitude was most pronounced in gray matter (GM) (49 %), but also substantial in white matter (39 %) with lesser increases in CSF spaces (12 %). The amplitude increases in AD correlated spatially with GM atrophy ($r = 0.47$), relative hypometabolism measured with [^{18}F]fluorodeoxyglucose positron emission tomography ([^{18}F]FDG-PET) (0.26), and with early amyloid- β deposition measured with [^{18}F]florbetapir PET (0.17). There was no linear correlation to CSF biomarkers. In conclusion, the amplitudes of all physiological brain pulsations increase in AD, possibly in association with failed solute efflux.

Key words: Alzheimer's disease, functional MRI, brain pulsations, brain physiology

INTRODUCTION

Alzheimer's disease (AD) is the most common form of dementia, and by far the most common neurodegenerative disease¹. Elucidation of the pathogenesis of AD is of paramount importance for early diagnosis, timely intervention and possible remediation of disease pathways. The National Institute on Aging and Alzheimer's Association has steered AD diagnostics towards a biomarker-based definition², and AD diagnosis currently requires evidence of both amyloid β ($A\beta$) and tau pathologies, assessed with lumbar puncture or molecular imaging by positron emission tomography (PET), in conjunction with manifestation of a typical clinical AD phenotype. Comorbidity is common, and a considerable proportion of biomarker positive individuals never develop AD. Compensatory mechanisms promoting brain resilience may delay onset of clinical disease, while failure of compensatory mechanisms results in cognitive decline³.

Failure of perivascular solute transport may precede significant amyloid plaque formation. Soluble forms of $A\beta_{40}$ seem to be more neurotoxic than previously thought, contributing to the formation of amyloid plaques in early AD, and actually impairing CSF influx more than $A\beta_{42}$ does, thus producing a feed-forward deficit in cortical solute clearance⁴. Aging further exacerbates this AD-related deficit, through a reduction in $A\beta$ transport mediated by endothelial low-density lipoprotein receptor-related protein 1 and loss of aquaporin-4 polarization in astrocytic endfeet, together impairing $A\beta$ removal to blood^{5,6}. Furthermore, changes in the amplitude and kinetics of the hyperemic response, which are regulated by nitric oxide (NO) and Piezo1 receptor activities, affect cerebrovascular pulsatility and blood flow, with effects on CSF outflow and memory retrieval^{7,8}. Consistent with this, our recent research suggests that combined NO and Piezo1 stimulation can increase brain effluxes of soluble $A\beta_{40}$ and $A\beta_{42}$ ⁹.

It is increasingly appreciated that AD is generally accompanied by comorbidities such as neurovascular dysfunction, which has been shown to contribute to AD pathology possibly before or

in conjunction with A β deposition^{10,11,12,13}. Recent solute transport investigations showed that diminished convection of soluble metabolites from the brain parenchyma into the CSF and thence out of the brain via pulsatile flow along paravascular CSF spaces potentially precedes neurodegeneration^{14,15}.

Physiological brain pulsations induced by < 0.1 Hz vasomotion, respiration and cardiovascular pulse waves are possible drivers of brain solute flow through the cortical perivascular solute pathways¹⁶. Vasomotion driven by noradrenaline signaling promotes paravascular clearance of solutes^{17,18}. The importance of sleep for brain health, waste removal and memory consolidation is well known; during sleep vasomotor pulsation promotes clearance of water through the brain parenchyma¹⁹. Interestingly, in narcolepsy type 1, a sleep disorder characterized by sudden daytime sleep attacks, is associated with powerful vasomotor waves mediated by the low orexin levels²⁰. Respiratory waves are the predominant pulsation in normal human brain function²¹, and respiratory pulsation abnormalities have been observed in epilepsy²². Cardiac arterial pulsation is the main driver of paravascular flow in mice^{23,24}.

Increased variability in the resting-state functional magnetic resonance imaging (fMRI) blood oxygenation level dependent (BOLD) signal sampled around 1 Hz has been investigated as a non-invasive biomarker in AD patients^{25,26}. Higher frequency fMRI acquisitions showed increased brain pulsatility in patients with mild cognitive impairment (MCI) six months before onset of dementia²⁷ and ultrafast (10 Hz) fMRI studies of cardiovascular impulse propagation in the AD brain have revealed increased proximal, but decreased intraparenchymal propagation speed together with reversed direction of pulsations in hippocampal memory encoding and parietal cortical areas²⁸.

In this study, we investigated how all known physiological human brain pulsations are altered in AD. Using ultrafast fMRI scanning with magnetic resonance encephalography (MREG), we quantified the amplitudes of vasomotor (< 0.1 Hz VLF), respiratory (Resp 0.3 Hz), cerebrovascular (Card 1 Hz) and cardiorespiratory amplitude modulations (CREM) of cerebrovascular pulsations individually and

compared AD patients with age and sex matched controls (Table 1). We also explored for spatial correlation between the detected pulsation amplitude changes in AD with gray matter (GM) atrophy, relative dysmetabolism (assessed with [^{18}F]fluorodeoxyglucose ([^{18}F]FDG)-PET), and amyloid deposition maps (assessed with [^{18}F]florbetapir-PET) thus testing the joint hypothesis that cortical atrophy, dysmetabolism, and cerebral amyloid depositions may not account fully for the detected abnormalities in AD brain pulsations.

[Table 1]

RESULTS

Significant signal amplitude increases in AD

The group mean amplitude maps of AD patients and controls are presented in Figure 1 in the three planes of Montreal Neurological Institute (MNI) space, where hot colors indicate high pulsation amplitudes. Comparison of the signal amplitude maps between AD patients and controls revealed significantly higher ($p < 0.05$, false discovery rate (FDR) corrected) amplitude in all physiological brain pulsations in the AD group compared to controls.

[Figure 1]

The spatial extent of significant amplitude increases was greatest for the slower pulsations, i.e. the VLF and respiratory bands, with more circumscribed increases for the CREM modulation and cardiovascular frequency bands. MREG signal mean amplitude increases in AD predominated in left temporal, frontal and occipital cortices, bilaterally in the caudate and putamen, and in the midline hypothalamus as depicted in Figure 2.

[Figure 2]

Signal amplitude was elevated in widespread brain areas of the AD patients, encompassing both gray and white matter (WM) and revealing considerable overlapping abnormality in all brain pulsations

regardless of relatively short disease duration (mean disease duration 3.1 ± 3.3 years) and presence of mild cognitive impairment (MCI) (mean Mini-Mental State Examination (MMSE) 24.1 ± 4.5) (Figure 2, Table 1). Half of the volume showing pulsation abnormality was localized to GM, with CREM appearing to best depict GM changes in the AD group (Figure 1-2, Table 2). However, almost 40 % of the pulsation abnormality was found in deep WM, implying notable subcortical and WM contribution to disease pathology. The remaining volume (<15 %) with increased pulsation amplitude was mainly present in the CSF spaces. A dissection of the results into separate frequency bands of VLF, respiratory, CREM and cardiac activity (Figure 2) indicated broadest results in the lower frequency bands, i.e. VLF and respiration, and declining volumes with increasing frequency.

The cumulative map of increased pulsation amplitude in AD comprising all four frequency bands (Figure 2, bottom row) highlights involvement of the occipital visual cortex, temporal cortex, fusiform cortex, frontal pole and middle frontal gyrus with left hemisphere predominance, as well as the periventricular WM, however sparing the ventricles. VLF increases predominated in occipital and frontal regions while CREM-related amplitude increases were mostly in the temporal lobes. The regions of increased pulsations did not completely respect anatomical outlines, suggesting contribution of other global physiological factors.

[Table 2]

After establishing the extent and location of brain pulsation abnormalities in CSF biomarker-validated AD patients (Figure 3a), we set out to investigate the physiological elements underlying the AD pulsation properties by comparing the mean map of amplitude fluctuation (AF) to the regions of GM atrophy (Figure 3b), areas of relative hypo- and hyperactive glucose metabolism ($[^{18}\text{F}]\text{FDG}$ -PET) in comparison to healthy controls (Figure 3c) and finally to areas of early $\text{A}\beta$ accumulation ($[^{18}\text{F}]\text{florbetapir}$ -PET) in AD (Figure 3d). GM atrophy patterns aligned with findings of relative hypometabolism in the FDG-PET Alzheimer's Disease Related Pattern (ADRP) map (4c) in temporoparietal cortices, posterior cingulate, the precuneus and the thalamus, whereas the $[^{18}\text{F}]\text{FDG}$ -

PET images also showed compensatory increased relative metabolic activity in the cerebellum. [¹⁸F]Florbetapir-A β PET showed accumulation mainly in midline structures in the posterior cingulate and frontobasal regions of the AD patients.

[Figure 3]

There was a strong spatial overlap between the anatomical locations of GM atrophy, relative hypometabolism and amyloid accumulation in AD. Most of the volume with increased AF including all frequency bands was explicable by respiratory and VLF pulsation abnormalities in AD (Table 3) and the CREM and cardiac band results also mostly aligned with the same regions. The known markers of neurodegeneration, namely GM atrophy and decreased relative glucose metabolism, correlated best with the AF all-bands map, with Pearson correlation coefficients of 0.47 for atrophy and 0.26 for relative hypometabolism. There were considerably weaker relationships between increased AF and early A β deposition ($r = 0.17$) and increased AF and relative hypermetabolism ($r = 0.06$).

[Table 3]

We compared our MREG results to an ADRP map presented by Perovnik et al²⁹ validated by comparison with large AD cohorts and with CSF biomarker-verified AD patients to ensure disease specificity. Relative hypometabolism ([¹⁸F]FDG-PET) had a spatial correlation coefficient of 0.26 with the present finding of amplitude increase, and a slightly higher correlation ($r = 0.36$) with GM atrophy. The respiration frequency and cardiorespiratory modulation were best linked to reduced glucose metabolism ($r = 0.27$ and 0.28 , respectively). [¹⁸F]FDG-PET relative hypermetabolism in the ADRP was in least accord with the increased amplitude map, demonstrating a separation between cerebellar metabolic changes³⁰ and pulsation amplitude increases in the present early AD patients (Table 3). However, we did observe a small cerebellar area of increased pulsation amplitude in the VLF frequency band.

CSF biomarkers vs. brain pulsation amplitude

CSF biomarkers (A β 42, tau and hyperphosphorylated tau), measured via lumbar puncture, in the local AD group (Table 1) showed no significant correlation with any of the MREG amplitude results. Neither analysis of CSF biomarkers and the whole brain mean AF per subject nor a voxelwise analysis within the AD group yielded significant correlations.

DISCUSSION

Our objective in this study was to establish a comprehensive quantitation in AD patients of the altered amplitudes of the physiological brain pulsations driving brain solute homeostasis using critically sampled imaging with MREG fMRI. We found increased amplitudes of *all* recognized physiological brain pulsations in early AD patients, including the novel respiratory-driven amplitude modulation of cardiovascular pulsation (CREM). Pulsation increases occurred predominantly in the GM, but also extended to the WM (involving 39 % of voxels), with lesser involvement of CSF (12 % of result voxels). Regions of increased AF overlapped with quantified GM atrophy of these subjects with a correlation coefficient 0.47, and 0.26 for relative hypometabolism ([^{18} F]FDG-PET), versus only 0.17 for A β deposition ([^{18} F]florbetapir-PET). The CSF biomarkers A β 42, tau and hyperphosphorylated tau, did not show significant linear correlations with any of the AF amplitudes in voxel-wise analysis, nor with the AF whole brain mean.

Increased physiological pulsations in AD – compensation or a sign of pathology?

Critically sampled MREG has previously detected increased physiological brain pulsation power or amplitude in narcolepsy²⁰, epilepsy²², and primary central nervous system lymphoma³¹. In patients with the latter condition, in which the lymphoma cells invade the brain parenchyma especially in the

perivascular and periventricular areas, we have detected increased amplitude in all brain pulsations, suggesting a global alteration of the tissue. In epilepsy patients, respiratory pulsation was increased²², whereas in narcolepsy we saw increased VLF and decreased cardiac pulsations, with respiratory pulsations mostly unaffected²⁰. Thus, prior research indicates signatures of altered pulsation power across a range of brain disorders.

Neurodegenerative diseases such as AD manifest impairments of vascular autoregulation, resulting in increased cerebrovascular resistance^{32,33}, lower brain tissue oxygenation³⁴, lower cerebral blood flow velocity and increased oscillations in blood pressure³². AD is often accompanied by autopsy findings of at least mild cerebral amyloid angiopathy (CAA), which involves loss of vascular smooth muscle cells, vessel wall thickening and endothelial dysfunction, all in association with augmented pulsation and cerebral hypoperfusion^{35,36,37,38}. Thus, the extreme MREG amplitudes discovered in this study could be a reflection of abnormally high, uncontrolled pulsatility. The intact blood brain barrier (BBB) in healthy brain functions to control transport of water, ions, glucose and other biomolecules, thus maintaining the composition of the interstitial fluid adequate for synaptic activity³⁹. CAA and blood vessel damage contribute to disruption of BBB integrity in AD³⁹. We suppose that high amplitude fluctuations capture an early phase of malfunctioning cerebrovascular autoregulation, which imposes mechanical strain on blood vessels leading to BBB breakdown. Concomitantly, protein accumulation within the BBB obstructs fluid flow and waste clearance, conceivably seen as increased pulsation amplitude.

Prior research in AD patients revealed increased speed of cardiovascular impulses in upper cortical and periventricular WM²⁸ suggestive of increased CSF and blood flow. The same brain areas showed reduced diastolic impulse complexity, with the cardiovascular impulses becoming more sinusoidal and stacking of diastolic harmonic impulses more adjacent to the impulse peak⁴⁰. Furthermore, the overall physiological signal amplitude variance had increased six months prior to onset of cognitive

decline in ADNI data²⁷. These findings strongly suggest that cerebrovascular pulsatility is indeed affected in early AD.

In sleep studies of physiological pulsations, sleep pressure caused by sleep deprivation augmented particularly the VLF pulsation⁴¹ and non-rapid eye movement sleep increased all physiological brain pulsations⁴². As physiological pulsations drive brain solute flow, present findings of increased pulsation amplitude in AD could reflect a compensatory mechanism for impaired solute efflux, aiming to increase the convection of proteins accumulating in the CSF also in the awake state. Further investigations are needed to examine whether the increased amplitudes are a compensatory response or the result of a pathological process.

Relation of physiological pulsations to other imaging biomarkers.

AD pathology initiates characteristic changes in brain energy metabolism, A β deposition, and structural changes including GM atrophy. A previous study has linked amyloid positivity in brain to widespread reductions in the amplitude of low frequency fluctuations (ALFF) in preclinical AD⁴³. However, we now report that all AF bands were increased, and furthermore the MREG_{AF} increases extended spatially beyond the zones of [¹⁸F]florbetapir A β -PET findings. GM loss can only partially account for the MREG AF increases seen in early AD⁴⁴.

Even though GM atrophy did not spatially match with the AF increases in the present AD group, it may still contribute to the increase of pulsation amplitudes due to the relative increase of CSF to brain volumes. The increased free CSF in AD brain may enable stronger pulsations at the voxel level, even though these two factors can be spatially remote. Furthermore, there could be relative amplitude increases in perivascular CSF spaces due to stagnation of interstitial fluid and CSF flow, which might also affect neurofluidic solute convection within the brain tissue.

Studies with classical slow sampling rate fMRI BOLD have produced inconsistent results in AD, with reports of both increased and decreased ALFF in AD^{44,45,46,47}. A recent meta-analysis of 26 whole brain ALFF studies of AD/MCI summarized the afflicted brain regions as having decreased ALFF in the bilateral posterior cingulate gyrus, precuneus and the right angular gyrus and increased ALFF in the bilateral parahippocampal gyrus⁴⁵. Furthermore, decreased ALFF has been found in the medial and inferior parietal lobe, several temporal regions⁴⁴, the medial prefrontal cortex⁴⁵ and the lentiform nucleus⁴⁶, as well as in the direction of increased ALFF in the superior parietal, inferior temporal⁴⁴, superior frontal regions⁴⁶ and the hippocampi⁴⁴.

Before our present AF analysis, the fastest previous repetition time (TR) used was 720 ms in a single T2* weighted imaging study, which displayed reduced ALFF of around 13 % in most of the frontal association cortex, the bilateral sensorimotor, supplementary motor, insular, posterior cingulate, precuneus, and lateral parietal cortices and in thalamus and striatum of amyloid-positive MCI patients⁴³. Present results encompass the same anatomical structures, but with much broader global involvement, showing increased AF in AD compared to controls in all bands. The ambiguity in earlier research about the direction of amplitude change was presumably due to aliasing effects and the earlier focus on specific regions of interest (ROI) rather than whole brain analysis. Also, the prior focus on GM pathology and regressing out WM and CSF signals could account for incongruent results, as our study aimed to explore the behavior of physiological pulsations, without removing information about CSF and WM. However, some previous findings demonstrate amplitude increase in MCI, which possibly reverts to decreased ALFF with advancement of AD^{46,47}. In our patient sample, the average disease duration from first symptoms to scanning was $3.1 \pm (3.3)$ years, and as a group represents early AD (mean MMSE 24.1), which speaks to the concept of global amplitude increase as an early compensatory or disease mechanism. Furthermore, we have recently shown that vasomechanically stimulated increase of brain pulsation augments the efflux of harmful proteins such as soluble A β ⁹, which could be the purpose of the amplitude increase.

Technical and analytical perspectives of AF scanning.

To our knowledge, this is the first study mapping AF in AD patients using ultrafast MREG sequencing, separating all physiological pulsation bands without aliasing. Slow, non-critical sampling of the BOLD signal induces aliasing of the cardiorespiratory signals over the VLF range⁴⁸ and with increasing TR, the repeatability of the VLF pulse detection becomes clearly reduced⁴⁸. Furthermore, the faster physiological factors like cardiorespiratory amplitude modulations alter the BOLD signal and can mask VLF vasomotor activity in resting state BOLD signals^{49,50}.

We have earlier found aliasing to be strongest in slow sampling rates with TRs around 1000-2000 ms. Previous ALFF studies of AD patients employed TRs of 750-5000 ms, most frequently a TR of 2000 ms, which is highly prone to aliasing. Ultra-fast critically sampled MREG utilized in the current study can separate cardiovascular ~ 1 Hz and respiratory ~ 0.3 Hz pulsations from VLF, thereby avoiding aliasing and generating more accurate results. As earlier works have not separated cardiovascular, respiratory and cardiorespiratory modulation frequencies from the data, they are not directly comparable with present findings, even in the VLF range (< 0.1 Hz).

We have recently shown MREG to be sensitive to flow-related signal changes^{19,20,28}. Our method intrinsically combines information originating from cerebral fluid flow, very low frequency vasomotion, neuronal activity, respiration enhanced waste clearance, arterial pulsations, and the newly described CREM²⁰, which seems to best highlight GM pathology.

Strengths and limitations. We endeavored to identify the origin of the alterations in physiological brain pulsations reported earlier in AD^{26,27,51}. In addition to its markedly increased precision in detecting physiological pulsations, the fast MREG sampling enabled us to apply individual subject-specific cardiorespiratory pulsation frequencies with a tight 0.1 Hz window instead of a group range for each pulsation; this makes our analysis markedly more accurate for the purposes of this study, as

harmonic, heterodyne, and thermal noise frequencies are removed^{21,40}. In fact, the specific analysis of the heterodyne peaks reflecting the CREM amplitude modulation of the cardiovascular pulsation proved to be most specific for AD-related changes in GM areas (Table 2).

Within the vasomotor VLF range, the present amplitude-based analysis is likely more sensitive than our earlier coefficient of variation (CV) -based approach²⁷. After bandpass filtering, the CV effectively reduces to a standard-deviation measure of the filtered time series, whereas the current method quantifies the amount of oscillatory amplitude concentrated within the spectral band. Thus, this approach may more accurately detect weaker differences in vasomotor low-frequency pulsations. In addition, the larger sample size and more stringent preprocessing improved sensitivity further and enhanced statistical power. Motion was controlled rigorously by excluding high-motion subjects, regressing residual head-motion effects, and removing motion-related spikes by despiking. These improvements enabled the current finding of coinciding regions of increased amplitude in all brain pulsations driving neurofluids.

We note the limitation that our [¹⁸F]FDG-PET and [¹⁸F]florbetapir A β -PET scans were derived from databases, thus limiting their comparison with our AF and GM atrophy results. In the future, recently developed plasma AD biomarkers should be analyzed with respect to the MREG_{AF} findings.

Conclusions. The amplitudes of all physiological brain pulsations were increased in AD. The changes overlapped only partially with regions of atrophy and relative hypometabolism ([¹⁸F]FDG-PET), and minimally with regions of relative hypermetabolism and A β deposition. The AD CSF biomarkers did not correlate with the physiological pulsation amplitudes.

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Subject group	Number (females)	Age, years (SD)	MMSE score (SD)	AD duration years (SD)	CSF A β 42 mean (SD)*	CSF tau mean (SD)*	CSF p-tau mean (SD)*
Control	72 (48) 67 % female	59.8 (5.4)	28.5 (1.5)	n.a.	n.a.	n.a.	n.a.
AD	43 (24) 56 % female	60.2 (4.6)	24.1 (4.5)	3.1 (3.3)	581 (156) **	400 (123) **	38 (16) **

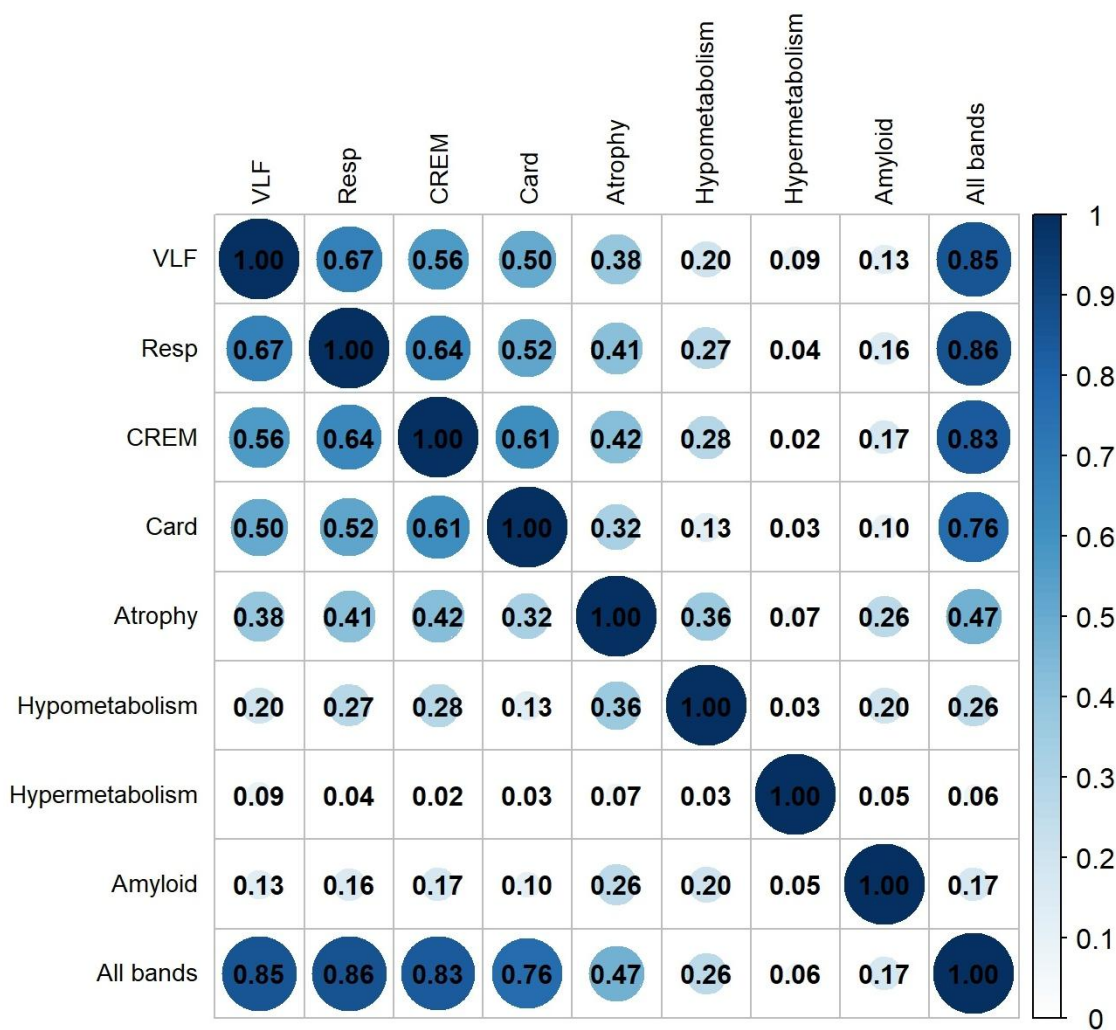
* unit for CSF values is pg/ml

** CSF biomarker values are missing for 5 subjects

Table 1. Characteristics of control and Alzheimer’s disease (AD) groups with cerebrospinal fluid (CSF) laboratory findings for AD patients. *Regarding the AD CSF biomarker profiles: all AD patients had pathological CSF profiles with abnormal A β 42 and tau/p-tau; however 6 AD patients exhibited abnormal levels of only A β 42 or only p-tau/tau instead of both according to the following cut-off values used: A β 42 < 715 pg/ml, tau > 275 pg/ml and p-tau > 26 pg/ml; borderline values: A β 42 < 845 pg/ml, tau > 230 pg/ml and p-tau > 21 pg/ml. We considered borderline results pathological. The group mean CSF values indicate all three biomarkers to be markedly pathological.*

Frequency band	Gray matter	White matter	Other (mainly CSF)
Cardiovascular	51.7 %	37.7 %	10.6 %
CREM	60.5 %	33.6 %	5.9 %
Respiratory	49.6 %	43 %	7.4 %
VLF	48.7 %	38.5 %	12.8 %
All bands combined	49 %	38.7 %	12.3 %

Table 2. Distribution of increased amplitude in Alzheimer’s disease (AD). *The distribution of increased amplitude results across gray matter (GM), white matter (WM) and other regions (mainly cerebrospinal fluid spaces) by frequency band (read left to right). Each row shows the percentages for each frequency band of the increased magnetic resonance encephalography amplitude fluctuation ($MREG_{AF}$) located in GM, WM, and other structures i.e. CSF. GM and WM areas were defined as regions of interest (ROIs) in Montreal Neurological Institute (MNI152) space and percentages were calculated as the proportion of voxels with increased amplitude within each ROI over the total number of increased amplitude voxels per frequency band.*



Tþble 3. Correlogram of spatial correlation coefficients. *The correlogram indicates the relationships between each of the areas of significantly increased amplitude fluctuation (AF) by frequency band: very low frequency (VLF), respiratory (Resp), cardiorespiratory modulation (CREM), and cardiac (Card) and locations of gray matter atrophy (Atrophy), relatively reduced glucose metabolism (Hypometabolism) and relatively increased glucose metabolism (Hypermetabolism) measured with [¹⁸F]fluorodeoxyglucose positron emission tomography (PET) amyloid accumulation measured with [¹⁸F]florbetapir PET (Amyloid) and the combined result area of the AF analysis (All bands). The spatial cross-correlations are reported as correlation coefficients that indicate the extent of similarity. The color and size of the circles indicate the strength of correlation coefficient, also indicated by the color scale on the right side of the figure.*

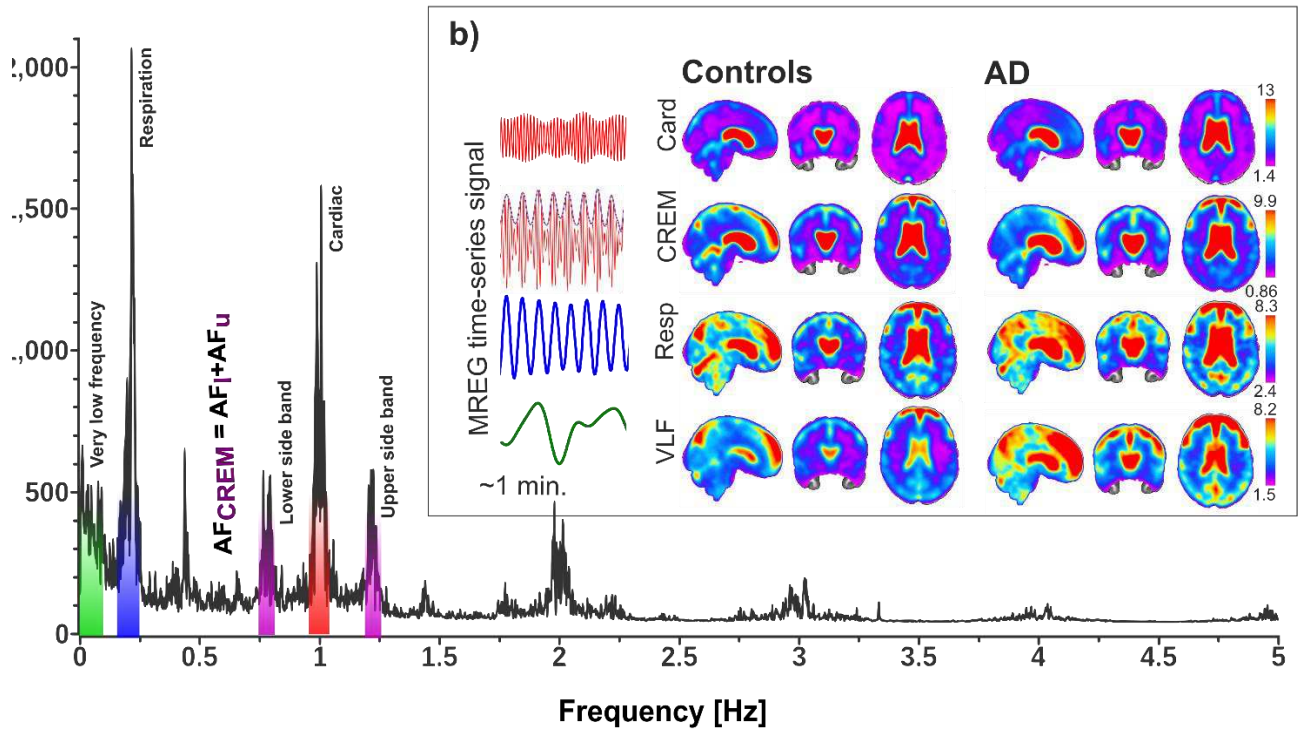


Figure 1. Amplitude spectrum, signal examples and group mean amplitude maps. a) *Healthy control data illustrating the frequency distribution of the whole brain amplitude spectrum, which is color-coded into bands of very low frequency (0.01–0.1 Hz, green), respiration (individual respiratory peak ± 0.05 Hz, blue), cardiac (individual cardiac peak ± 0.05 Hz, red) and cardiorespiratory amplitude modulation (CREM, violet). The CREM band was calculated as the sum of the lower and upper heterodyne side bands on both sides of the cardiac peak.* b) *Representative MREG time signals (~1 minute of data) with respective color-coding for (top to bottom) cardiovascular (Card), cardiorespiratory modulation (CREM), respiratory (Resp) and very low frequency (VLF) bands of a healthy control, shown beside the group mean amplitude maps for controls (n=72) and AD (n=43) patients of the aforementioned physiological brain pulsations. The color bar on the right indicates amplitude values in arbitrary units.*

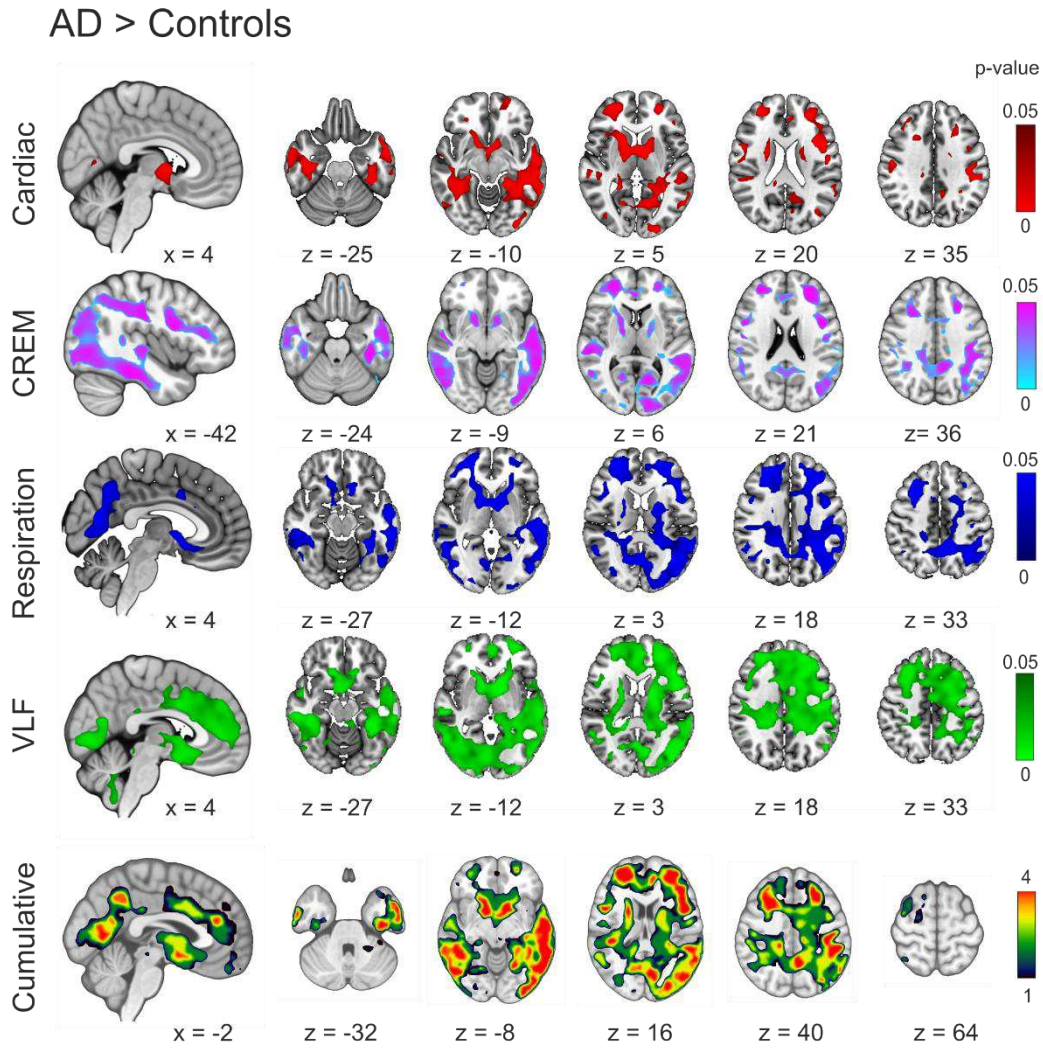


Figure 2. Increased pulsation amplitudes in Alzheimer's disease. Mean maps of statistically significant amplitude increase in Alzheimer's disease (AD) patients ($n = 43$) compared with age-matched controls ($n = 72$) in Montreal Neurological Institute (MNI) space. The MNI coordinates for each plane are indicated below the images, with color bars on the right representing p -values for cardiac, cardiorespiratory modulation (CREM), respiratory and very low frequency (VLF) bands (top to bottom). For all bands the AD patients showed extensive regions of significantly increased amplitudes, as compared to healthy controls (FDR corrected, $p < 0.05$).

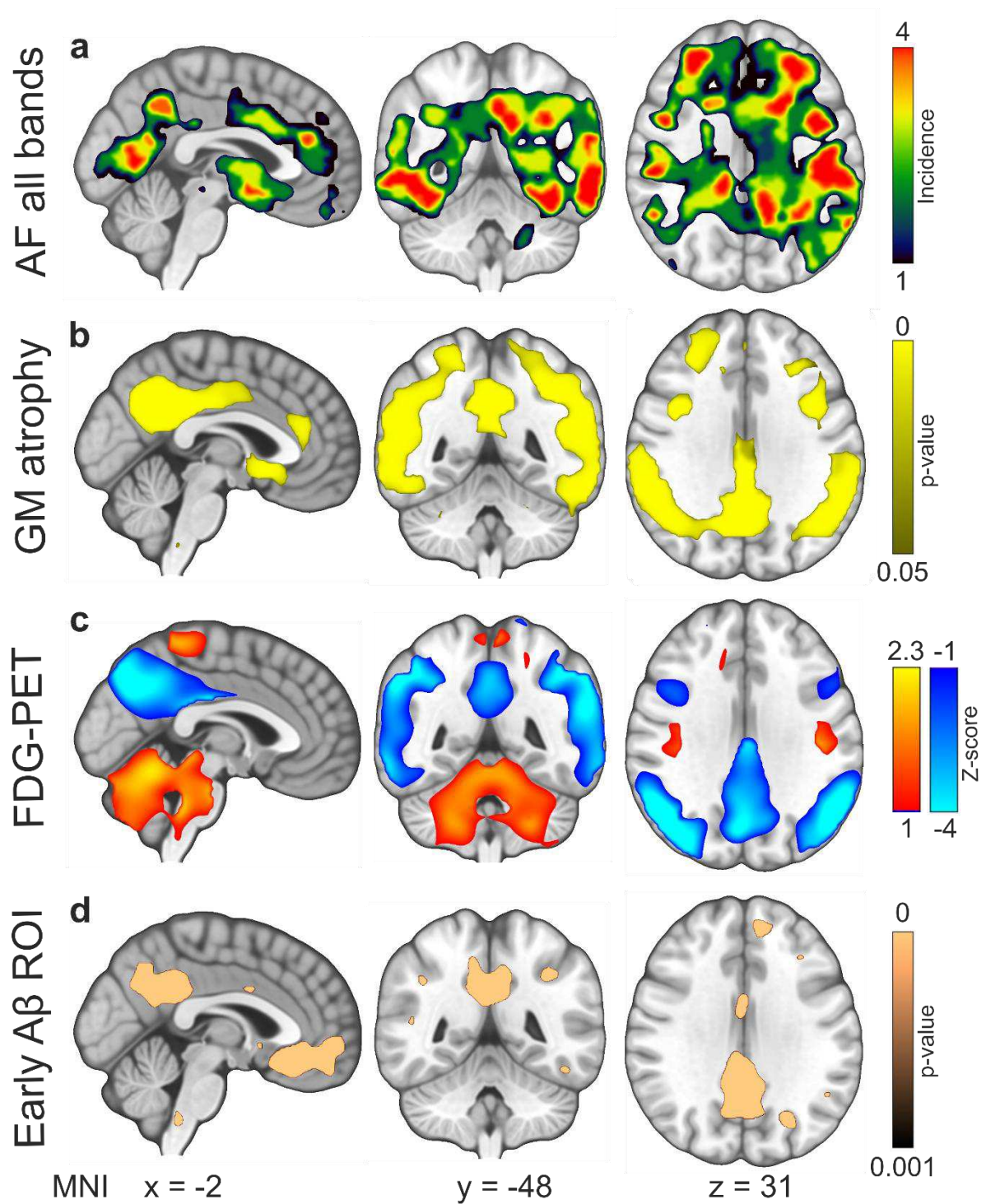


Figure 3. Regions of significant amplitude increase, cortical atrophy, altered relative glucose metabolism, and amyloid β accumulation. Comparison of the magnetic resonance encephalography amplitude fluctuation (MREG_{AF}) result maps to the location of gray matter atrophy

in the same subjects, altered relative glucose metabolism measured with [^{18}F]fluorodeoxyglucose positron emission tomography ([^{18}F]FDG-PET) and known regions of early β -amyloid ($A\beta$) accumulation measured with [^{18}F]florbetapir-PET. a) Cumulative incidence map of significantly increased pulsation amplitude within all four bands (very low frequency, respiration, cardiorespiratory modulation and cardiac frequencies together,) with the color legend indicating the number of overlapping bands of the MREG_{AF} analysis results showing increased amplitude (AF all bands) in Alzheimer's Disease (AD). A single band result is shown in black, two overlapping bands in green, three overlapping bands in yellow and the red color illustrates the hotspots of increased amplitude in AD patients seen in all four examined frequency bands (FDR, $p < 0.05$). b) Gray matter (GM) voxel-based morphometry atrophy maps (GM atrophy) for the same AD patients compared to local age-matched controls. The color bar indicates p-value. c) Altered relative glucose metabolism in AD patients as measured with [^{18}F]FDG-PET by Perovnik et al (2022). Cold blue indicates regions of relative hypometabolism and hot yellow-orange relative hypermetabolism in AD patients compared with healthy controls. The color bar indicates the z-value threshold. d) Map of early β -amyloid accumulation (early $A\beta$ ROI) based on [^{18}F]florbetapir- $A\beta$ PET data from the ADNI dataset (Palmqvist et al 2017). Color bar indicates p-value. Note that the GM atrophy, relative hypometabolism and $A\beta$ -positivity tend to overlap in the same cortical regions such as the posterior cingulate, while the physiological pulsation abnormality tends to surround the previous changes extending more deeply into the white matter as well.

Materials and methods

Participants

This study adheres to the Declaration of Helsinki and was conducted after obtaining institutional approval by the Ethical Committee of Pohde Wellbeing Services County of North Ostrobothnia (formerly known as the Northern Ostrobothnia Hospital District of Oulu University Hospital) (permits 53/2012, 274/2020, 181/2023, Fimea 2023/005604). Written informed consent was obtained from all subjects.

Seventy-six healthy subjects and fifty-seven AD patients were enrolled in the study. Of the original 57 AD patients, we excluded 8 due to excessive head motion, 3 due to data corruption, and 3 due to incidental intracranial tumors. Of the 76 healthy controls, we excluded 3 due to excessive motion and 1 due to data corruption. This left 43 AD patients (age 60.2 ± 4.6 years) and 72 healthy subjects (age 59.8 ± 5.4 years) for this study.

All AD group patients met the current NINCDS-ADRDA (National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association) diagnosis criteria for probable AD¹. All patients were examined and diagnosed by experienced senior neurologists specialized in dementia and memory disorders at the memory outpatient clinic of the Department of Neurology, Neurocenter in Oulu University Hospital. The patients underwent routine clinical and neurological examinations, laboratory screening tests, comprehensive neuropsychological examination, and structural and functional MRI of the brain. For confirmation of their AD diagnosis, cerebrospinal fluid (CSF) samples were collected for the analysis of the biomarkers A β 42, tau, and phosphorylated tau. CSF samples were collected except in cases of contraindication for lumbar puncture or when the patient did not give consent for lumbar puncture. Age-matched control subjects were interviewed and screened for cognitive impairment with the

MMSE examination. Descriptive quantities of control and AD subjects are presented in Table 1.

Data acquisition and preprocessing

Subjects reclined inside the MRI scanner and were asked to lie still and keep their eyes open with gaze fixated on a cross projected on a monitor, while thinking of nothing in particular (eyes open, resting state). Ear plugs were provided to reduce scanner noise. Cushions were placed beside the ears to restrict head movement and to further block scanner noise.

Subjects were scanned using a Siemens MAGNETOM 3T SKYRA with a 32-channel head coil respectively. Additional cardiorespiratory data were collected with an MRI-compatible multimodal neuroimaging Hepta-Scan concept². MREG is a 3D single shot stack of spirals (SOS) sequence that under-samples k-space to reach a sampling rate of 10 Hz, thus allowing critical imaging of physiological pulsations³. The SOS gathers k-space in 80 ms intervals with spiral in/out to minimize the air-sinus off-resonance artifact³. MREG scanning parameters were TR = 100 ms, TE = 36 ms, flip angle = 25°, 3D matrix = 64³, FOV = 192 mm with voxel size of 3 × 3 × 3 mm³ for a 5-minute scan, yielding approximately 3000 brain volumes. Spin saturation effects were minimized by deleting 20 s from the beginning of each scan. Magnetization spoiling gradient between scans was set to 0.1 to avoid stimulated echo drifts, while still maximizing sensitivity to physiological brain pulsations. For anatomical T1W 3D MPRAGE images the parameters were TR = 1900 ms, TE = 2.49 ms, TI = 900 ms, flip angle = 9°, FOV = 240 mm, voxels size = 0.9 × 0.9 × 0.9 mm³. Cardiorespiratory frequencies were verified both with an MRI compatible anesthesia monitor (GE Datex-Ohmeda Aestive 5) and from scanner physiological data recordings.

MREG data were reconstructed using L2-Tikhonov regularization with lambda 0.1, where the latter regularization parameter was determined by the L-curve method with a MATLAB recon-tool from the sequence developers^{4,5,6}. Image reconstruction included a dynamic off-resonance in the k-space

(DORK) method, which corrected for scanner warming and respiration-induced dynamic B0-field changes before preprocessing^{7,8}.

The data were preprocessed with the standard FSL (Functional Magnetic Resonance Imaging of the Brain's Software Library) pipeline⁹, using high-pass filtration with a cut-off frequency of 0.008 Hz (125 s). Motion correction was performed using FSL MCFLIRT, and FSL BET was used for brain extraction. Data was spatially smoothed with a 5 mm FWHM Gaussian kernel using *fslmaths*. The anatomical 3D MPRAGE images were used to register MREG data into MNI152 standard space using FSL FLIRT^{9,10}. The AFNI *3dDespike* function was used to remove motion produced spikes from the remaining data. To minimize unwanted movement-related effect, subjects with a mean absolute movement > 0.3 mm or having any timepoints with average movement > 1.5 mm were excluded from the study. Subsequently, nuisance head motion was removed from the MREG data using multiple linear regression, performed using FSL *fsl_glm* function to remove the six head realignment parameters (3 translation and 3 rotation) detected by MCFLIRT. As this study focuses on the physiological BOLD signals, we aimed to retain physiological pulsations as much as possible in the data. Therefore, CSF, WM and global signals were not regressed out of the datasets but instead thoroughly analyzed.

ALFF analysis

For every subject, the time courses of each voxel from whole brain MREG data were transformed using AFNI *3dPeriodogram* to the frequency domain via fast Fourier transformation, which yielded the voxel-wise power spectrum. A global power spectrum was then combined from all brain voxels to investigate detectable physiological pulsation bands affecting the global BOLD signal. From the global power spectrum, we obtained frequency peaks of respiratory and cardiac pulsations. These MREG signal pulsation frequencies were verified with the SpO2 and respiratory belt data from the

simultaneous physiological monitoring, and it has been shown that the MREG pulsation frequencies accurately match these measurements^{11,12,13}.

We used a modified ALFF method to study amplitude of fluctuation (AF) of very low frequency (AF_{VLF}), respiratory (AF_{resp}), and cardiovascular (AF_{card}) pulsations^{14,15}. The frequency band for AF_{VLF} was 0.01–0.1 Hz, while the bands for AF_{resp} and AF_{card} were 0.1 Hz wide, centered around the subject-specific individual band FFT peaks (i.e., center peak $\pm$ 0.05 Hz). To define the respiratory-induced modulation of cardiovascular pulsation (CREM), we quantified the 0.1 Hz wide heterodyne peaks with equal respiratory frequency distance away from the principal cardiac peak on both sides, as described before¹⁵.

For every subject, the time courses of each voxel from the whole brain time series MREG data were transformed using AFNI *3dPeriodogram* to the frequency domain via a fast Fourier transformation, which yielded the voxel-wise power spectrum. The square root of the power spectral density was calculated to yield an amplitude spectrum (Figure 1a), and amplitudes calculated over individual physiological pulsation frequency bands of interest were summed to obtain a corresponding AF map. The AF value in a given voxel represents the total voxelwise amplitude of the chosen frequency band, which reflects the local features of brain oscillatory activities^{14,16}. Group mean AD and control group images represent the respective mean voxelwise AF over the whole brain (Figure 1b).

[Figure 1]

Statistical analysis

Voxelwise comparisons between AD patients and controls for AF maps for different frequency bands were performed by a two-sample nonparametric threshold-free permutation test (10 000 permutations) implemented in *vlisa_twosample* from LIPSIA¹⁷. A multiple linear regression model was applied to evaluate the differences in AF metrics between AD patients and control subjects. In

this model, age and sex were included as nuisance variables to account for their potential confounding effects. We used a whole brain mask including WM, GM and CSF. The voxel-based statistical tests were corrected for FDR at a significance level of $p < 0.05$. All data are reported as mean $\pm$ standard deviation (SD) unless otherwise stated. For visualization, the resulting p-value maps of significant comparisons ($p < 0.05$) were overlayed into 1mm MNI152 standard space using the MRICroGL MNI152 standard brain and FSLEyes spline interpolation.

Gray matter voxel-based morphometry analysis.

The structural data were inspected visually by an experienced neuroradiologist (VKi). First, the structural images from the 43 AD individuals and 72 age-matched controls were analyzed using FSL-VBM, an optimized voxel-based morphometry analysis (VBM) technique conducted using FSL tools^{18,19,20,21}. In this procedure, the T1W 3D MPRAGE 0.9 mm structural images were brain-extracted and GM segmented before being registered to the 2 mm MNI 152 standard space using non-linear registration. The images were averaged and flipped along the x-axis to generate a study-specific GM template that was symmetrical from left to right. Next, all native GM images were non-linearly registered to this study-specific template and modulated to correct any local expansion (or contraction) that had occurred because of the non-linear stage of the spatial transformation. After that, an isotropic Gaussian kernel with a sigma of 2 mm was used to smooth the modulated GM images. Finally, voxel-wise GLM was used with permutation-based non-parametric testing to adjust for multiple comparisons across space. We then used the population-based GM probabilistic map to deduce the overall GM for each patient and control. Statistical comparison between AD and control groups was performed with *vlisa_twosample* from LIPSIA and was corrected for FDR at a significance level of $p < 0.05$. Age and sex were included as nuisance variables to account for their potential confounding effects.

Anatomical regions of dysmetabolism and amyloid β accumulation

In order to analyze the spatial overlap between the physiological pulsation changes, dysmetabolic findings and amyloid β ($A\beta$) accumulation occurring in AD, we used PET imaging results from two international datasets for the comparison with our data. Thus > 300 subjects from three different cohorts were utilized in this study^{22,23}.

The fluorodeoxyglucose-positron emission tomography (FDG-PET) images of dysmetabolism and [^{18}F]florbetapir PET images of early $A\beta$ accumulation were acquired from other research groups^{22,23}. The corresponding maps (Figures 3c-d) were validated using large cohorts, derived from different study groups than those in the present AF and atrophy analyses. The globally normalized [^{18}F]FDG-PET images show regions of relative hypo- and hypermetabolism (Figure 3c), designated as the Alzheimer's Disease Related Pattern (ADRP). This was identified in a small group of CSF-validated AD patients ($n = 20$) by Perovnik et al (2022) and validated by comparison with a second internal cohort as well as with ADNI database subjects ($n = 60$). The ADRP differentiated AD patients from healthy controls with high specificity (90–100%) and sensitivity (80–91%), even when adjusted for age and is correlated with cognitive impairment. The pattern could also differentiate AD from the behavioral variant of frontotemporal dementia. The ADRP shows relative hypometabolism in the parietal lobe and posterior cingulate and relative hypermetabolism in the cerebellum.

The [^{18}F]florbetapir $A\beta$ map (Figure 3d) shows areas of early $A\beta_{42}$ accumulation determined by Palmqvist et al (2017) using longitudinal ADNI data. Early $A\beta$ accumulators were defined as being CSF $A\beta$ positive, amyloid-PET negative individuals; declining CSF $A\beta_{42}$ levels have been shown to mark the onset of $A\beta$ accumulation^{24,25,26,27,28}. The [^{18}F]florbetapir accumulation rate of these early accumulators was compared with non-accumulators, defined as subjects with normal cognition and negative CSF and PET biomarker profiles. The brain regions showing early $A\beta$ accumulation were the bilateral posterior cingulate cortex, precuneus, and medial orbitofrontal cortex.

The local results of MREG AF analysis and GM atrophy were binarized, aligned into identical MNI space with the acquired [^{18}F]FDG ADRP and [^{18}F]florbetapir A β accumulation maps, and the various AD-related changes were binarized and compared using the FSL function *fsldcc* for spatial correlation (Table 3). Additionally, as all of the local AD patients had abnormal CSF profiles as established via lumbar puncture, a linear correlation analysis at voxel level was performed to seek possible correlation between MREG AF data and the CSF biomarkers A β 42, tau and p-tau.

CSF biomarker collection

Lumbar punctures were performed and CSF collected from all AD patients, except for (n=5) who had a contraindication for lumbar puncture or otherwise refrained from the procedure. The biomarkers A β 42, tau and p-tau were quantified by the University of Eastern Finland (UEF) Clinical Research Center in Kuopio. The CSF samples collected in 2016-2019 were analyzed with the Fujirebio Innostest ELISA method and the CSF samples collected in 2020-2021 with a Roche 8000 analyzer and the Elecsys method. Because of the change of method and resulting altered cut-off values, all CSF measurements were scaled to correspond to the current cut-off values measured with the newer method using conversion equations used routinely by the UEF laboratory.

DATA AVAILABILITY

The data from individuals in this study cannot be shared because of privacy issues for clinical data. Group data are available for research upon reasonable request from the corresponding author after ethical approval for the specific study.

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ACKNOWLEDGEMENTS

This study was funded by Research Council of Finland: TERVA grants 1-2, 275342, 338599, 314497, 335720 (VKi), 360508 (Jka); The EU Joint Programme – Neurodegenerative Disease Research 2022-120 (VKi); Jane & Aatos Erkko Foundation: 2017-2021, 210043 (VKi.); VTR grants from Oulu University Hospital (VKi, Vko, JKr, Jka); The Finnish Medical Foundation (VKi, Jka, MJ); Finnish Brain Foundation (VKi, JT, LR, HM); Maire Taponen Foundation (LR); Orion Research Foundation (HH, MJ, JT, LR); Emil Aaltonen Foundation (HH, MJ, LR); Tauno Tönning Foundation (LR); Juhani Aho Foundation for Medical Research (TV, LR); Päivikki and Sakari Sohlberg Foundation (JKr, LR); Paulo Foundation (HH, MJ, LR); Uulo Arhio Memorial Foundation (LR); Instrumentarium Science Foundation (MJ, JT, TV); Uniogs/MRC Oulu DP-grant (HH); Pohjois-Suomen Terveystieteiden tutkimuskeskus (Vko, Jka, HH). We thank CSC – IT Center for Science Ltd., Finland for providing high-performance computational resources used in this study. We further thank research nurse Annastiina Kivipää and Jussi Kantola for computational administration. We thank Adj. Prof. Paul Cumming of Queensland University of Technology, Brisbane for comments on the manuscript.

AUTHOR CONTRIBUTIONS

VP, VKi designed the study; VP, LR, HM, AE, NH, TV, JT, HH, MJ, Jka, Vko, JKr, VKi performed research; VP, HM, NH, TV, JT, HH, MJ, Jka, Vko, JKr collected the data; Jussi Kantola performed MREG image reconstruction; NH, LR performed pre-processing; VP, LR analyzed data; VP, VKi wrote original draft; VP, LR, AE, NH, TV, JT, MJ, Jka, JKr, Vko, VKi contributed to writing, review & editing.

COMPETING INTEREST DECLARATION

J. Krüger has served on the advisory board of Novartis, Nutricia, Eisai, Lilly and Roche and received honoraria from lectures from Bioarctic and Lilly and received support for congress participation from Merck. Vesa Kiviniemi has received a lecture honorarium from Insightec. Other authors have no competing interests to declare.