Early stages of tau pathology and its associations with functional connectivity, atrophy and memory

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Abstract

In Alzheimer’s disease, postmortem studies have shown that the first cortical site where neurofibrillary tangles appear is the transentorhinal region, a subregion within the medial temporal lobe that largely overlaps with area 35, and the entorhinal cortex. Here we used tau-PET imaging to investigate the sequence of tau pathology progression within the human medial temporal lobe and across regions in the posterior-medial system. Our objective was to study how medial temporal tau is related to functional connectivity, regional atrophy, and memory performance. We included 215 β-amyloid negative cognitively unimpaired, 81 β-amyloid positive cognitively unimpaired and 87 β-amyloid positive individuals with mild cognitive impairment, who each underwent [18]F-RO948 tau and [18]F-flutemetamol amyloid PET imaging, structural T1-MRI and memory assessments as part of the Swedish BioFINDER-2 study. First, event-based modelling revealed that the entorhinal cortex and area 35 show the earliest signs of tau accumulation followed by the anterior and posterior hippocampus, area 36 and the parahippocampal cortex. In later stages, tau accumulation became abnormal in neocortical temporal and finally parietal brain regions. Second, in cognitively unimpaired individuals, increased tau load was related to local atrophy in the entorhinal cortex, area 35 and the anterior hippocampus and tau load in several anterior medial temporal lobe subregions was associated with distant atrophy of the posterior hippocampus. Tau load, but not atrophy, in these regions was associated with lower memory performance. Further, tau-related reductions in functional connectivity in critical networks between the medial temporal lobe and regions in the posterior-medial system were associated with this early memory impairment. Finally, in patients with mild cognitive impairment, the association of tau load in the hippocampus with memory performance was partially mediated by posterior hippocampal atrophy. In summary, our findings highlight the progression of tau pathology across medial temporal lobe subregions and its disease-stage specific association with memory performance. While tau pathology might affect memory performance in cognitively unimpaired individuals via reduced functional connectivity in critical medial temporal lobe-cortical networks, memory impairment in mild cognitively impaired patients is associated with posterior hippocampal atrophy.
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**Introduction**

Tau pathology is closely associated with cognition and particularly with memory performance in Alzheimer’s disease (AD). Studies on the functional architecture of memory have identified two major cortical memory networks that are highly connected with the hippocampus and differentially involved in episodic memory – the anterior-temporal (AT) and the posterior-medial (PM) system. While early β-amyloid deposition occurs in regions of the PM system such as the posterior cingulate cortex and the precuneus, neurofibrillary tangle accumulation occurs early in medial temporal lobe (MTL) subregions of the AT system.

Specifically, neurofibrillary tangles have been described to appear first in the transentorhinal region, a subregion within the MTL that largely overlaps with area 35, as well as in the entorhinal cortex. Later, neurofibrillary tangles can be found in hippocampal subfield cornu ammonis (CA) before they extend to adjacent regions such as the subiculum and then brain regions outside the MTL.

While tau begins to accumulate in MTL regions in the AT system, it can be increasingly seen in regions in the PM system with disease progression. Furthermore, tau pathology is tightly linked to atrophy and future neurodegeneration, and both are associated with cognitive impairment which likely affects cognitive functions associated with the AT system before those associated with the PM system.

In order to understand to what extent functional memory networks are affected during the course of AD, it is important to know the exact order of tau pathology progression throughout subregions in the MTL and the cortical PM system. However, this remains to date unknown as neuropathological studies have not studied tau pathology across all these subregions in the same individuals. It is also unclear, whether tau pathology leads to cognitive impairment primarily via grey matter atrophy, or whether there are changes in other modalities such as functional connectivity that may precede gross atrophy especially in early stages of AD.

With the recent advent of tau-PET, it has become possible to investigate the accumulation of tau pathology in vivo, and second-generation tau-PET tracers now allow the investigation of hippocampal tau-PET binding due to reduced off-target binding in the choroid plexus. Here, we use [18]F-RO948 tau-PET imaging in combination with event-based modelling to characterize the sequence of tau-PET binding in MTL subregions, including MTL regions of the AT system, and the cortical PM system in cognitively unimpaired (CU) individuals and patients with mild cognitive impairment (MCI). We utilize whole brain analyses as well as MTL subregional segmentation to investigate how tau-PET binding is associated with functional connectivity, memory function and atrophy.
Materials and methods

Participants

We analyzed data from 383 non-demented individuals from the Swedish BioFINDER-2 study (NCT03174938) which was approved by the ethical review board in Lund, Sweden. Participants were enrolled between 2017-2020 and gave their written informed consent to participate. Participants were stratified in CU and MCI patients (see supplementary methods for details on inclusion criteria and missing data). Aβ-status (positive/negative) was defined using CSF Aβ42/Aβ40 (cut-off 0.63).

Twenty-five individuals were excluded during quality assessment of automated MTL subregional segmentations (9 CU Aβ-, 6 CU Aβ+ and 10 MCI Aβ+). As [18]F-RO948 tau PET shows off-target binding in the skull and the meninges in some individuals that could interfere with tau-PET signal in MTL subregions, we calculated a ratio of tau-PET signal in the skull/meninges compared to signal in grey matter to identify individuals with high amounts of off-target binding (>1.5 SD, n=37). Subsequently, each case was visually assessed and excluded in case of confirmed bleeding of off-target binding signal from the meninges in MTL subregions (n=17).

Imaging acquisition

MRI

T1-weighted images were acquired on a Siemens Prisma scanner (Siemens Medical Solutions, Erlangen, Germany) with a 64-channel head coil using an MPRAGE sequence (in-plane resolution=1×1mm², slice thickness=1mm, TR=1900ms, TE=2.54ms, flip-angle=9°). Spontaneous BOLD oscillations were acquired with a gradient-echo planar sequence (eyes closed, in-plane resolution=3×3mm², slice thickness=3.6mm, TR=1020ms, TE=30ms, flip-angle=63°, 462 dynamic scans, 7.85min).

Tau and Aβ-PET

All study participants underwent PET scans on a digital GE Discovery MI scanner (General Electric Medical Systems). Approval for PET imaging was obtained from the Swedish Medical Products Agency. Participants were injected with 365±20 MBq of [18F]RO948, and LIST mode emission data was acquired for each scan 70–90 min ([18F]RO948) post injection. Aβ-PET imaging was performed on the same platform 90-110 min after the injection of ~185 MBq [18F]Flutemetamol.

Imaging analysis

ROI segmentation and estimates of volume and thickness

Individual volume and median thickness of MTL subregions including anterior (3416mm³, SD=291) and posterior hippocampus (3206mm³, SD=470), perirhinal cortex (divided in area 35 (1202mm³, SD=204) and area 36 (3754mm³, SD=604)), entorhinal (1094mm³, SD=171) and parahippocampal cortex (1952mm³, SD=582) were defined on T1-weighted images (1x1x1mm³).
resolution) using ASHS-T1 (Xie et al., 2019) (find details in the supplementary material) and a multi-template thickness analysis pipeline. All subregional masks were visually assessed. Regions-of-interest for non MTL regions such as inferior temporal (20337mm³, SD=2880), middle temporal (20512mm³, SD=2842), retrosplenial (isthmus cingulate, 5078mm³, SD=746), inferior parietal (24518mm³, SD=3575), posterior cingulate cortex (6029mm³, SD=869) and precuneus (19121mm³, SD=2426), were derived from T1-weighted scans using FreeSurfer v6.0 (https://surfer.nmr.mgh.harvard.edu/).

**SUVr measures**

[^F]RO948: Standardized uptake value ratio (SUVr) images were calculated for each subregion of interest using an inferior cerebellar reference region. Partial volume correction (PVC) was performed using the geometric transfer matrix method extended to voxel-level using region-based voxel-wise correction. To reduce the influence of off-target binding, choroid plexus tau-PET signal was regressed from hippocampal measures.

[^F]Flutemetamol: A cortical composite SUVR was calculated using the whole cerebellum as reference region.

**Event-based modelling**

An event-based modelling (EBM) framework was used to derive a sequence of brain regions that become sequentially abnormal based on their individual tau-PET SUVr. The biomarkers in our analysis constitute 11 transition ‘events’ (tau-PET SUVr in area 35 and 36, entorhinal and parahippocampal cortex, anterior and posterior hippocampus, inferior and middle temporal cortex, inferior parietal and retrosplenial cortex and precuneus), where each event corresponds to a biomarker becoming abnormal with high probability. The most likely ordering of events and the respective uncertainty was estimated using the EBM, where each biomarker is either treated as ‘normal’, i.e. non-pathological, or ‘abnormal’. Individual regional tau-PET SUVr from regions-of-interest were z-standardized and probabilistic cut-offs were derived using Gaussian mixture modelling as previously described. Specifically, for each region-of-interest, we fit a two-component mixture model to the SUVr vector and used repeated 5-fold cross-validation to determine the probability a given value falls within the right-most (i.e. abnormal) Gaussian distribution. This resulted in a probability value between 0-1 that an individual regional SUVr was abnormal. We repeated this process to find the probability each SUVr was normal, and these two subject x region probability matrices were used as input to the EBM algorithm. Code for the probability estimation can be found at: https://github.com/illdopejake/data_driven_pathology/blob/master/esm/ESM_utils.py. EBM was run using the MatLab implementation of EBM-SuStaIn with k=1 (see https://github.com/ucl-pond). In this implementation, an ‘event’ represents the switch from a normal to an abnormal state. The EBM calculates the event sequence that maximizes the data likelihood, which in turn represents the most likely
ordering of the events. In addition to defining the most likely event sequence, the EBM evaluates the relative likelihood of the position of each event along the sequences using Markov chain Monte Carlo resampling. This provides information about the uncertainty of the event ordering. In addition, the EBM provides an individual stage (0-11, corresponding to the number of biomarkers) for each participant, indicating which stage of the sequence of abnormal tau events that participant has reached. More details on the EBM and the calculation of uncertainties can be found in Young et al., Brain, 2014). For visualization, tau-PET SUVr from individual regions-of-interest was regressed against tau-PET stages derived from the EBM using monotone non-linear splines where the Akaike information criterion was used to select the number of knots. Uncertainties represent 95% confidence intervals from the model estimated variance-covariance matrix.

Voxel-wise tau-PET analyses

Non-smoothed and partial volume corrected tau-PET SUVr images were transformed to template space using the non-linear warp obtained by normalizing the T1-weighted image to the MNI152 template (Avants et al., 2014). SUVr images from individuals were binned into four different groups across EBM stages with increasing disease severity with the aim to result in groups of roughly comparable size (stage 0 (n=281), stage 1-3 (n=35), stage 4-8 (n=29), stage 9-11 (n=38) (see supplementary table 6 for sample characteristics). For voxel-wise group comparisons of SUVr images, we used the Statistical Parametric Mapping software (SPM, Version 12; Wellcome Trust Centre for Neuroimaging, London, UK). Two-sample t-tests were used to contrast tau-PET SUVr images from groups of individuals in higher stages against those in stage 0 with the aim to visualize tau-PET signal binding for the different stages. Voxel-wise results were corrected using family-wise error correction at a threshold of p<0.05 and a cluster size of 50 mm$^3$.

Voxel-based morphometry

T1-weighted images were preprocessed using SPM12. All images were segmented into grey matter, white matter, and cerebrospinal fluid. Then, the diffeomorphic non-linear image registration tool (DARTEL) was used to create a study-specific template based on the grey matter and white matter tissues of the whole sample. Once the template was created, the grey matter tissues were warped into MNI space using the individual flow fields resulting from the DARTEL registration, and voxel values were modulated for volumetric changes introduced by the normalization. Finally, the images were smoothed with an isotropic Gaussian kernel with 4mm full width at half maximum. In order to account for differences in head size in the statistical analyses in addition to age and sex, we calculated the total intracranial volumes of each subject as the sum of the grey matter, white matter and cerebrospinal fluid volumes. All results were adjusted for multiple comparisons using a family wise error rate correction set at p < 0.05 and a cluster size of 50 mm$^3$. 

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**rs-fMRI preprocessing**

Rs-fMRI data preprocessing was performed with a pipeline composed of FSL, AFNI and ANTS. Anatomical processing involved skull stripping, segmentation of CSF, white and grey matter, and normalization to MNI152-space. After bulk motion and slice timing correction, nuisance regression compensated WM/CSF signal, physiological noise, motion parameters and scanner drift. Distortion correction was performed by unwarping the EPI average to a high resolution (1x1x1mm) T2 scan. Finally, the functional data was band-pass filtered (0.01-0.1 Hz) and transformed to MNI space. Frames causing outliers in total frame-to-frame signal variation (75 percentile plus 1.5 times interquartile range) were censored. Subjects with a mean/maximum frame-wise displacement exceeding 0.7/3.0mm were excluded.

The ROIs in the template space atlas employed in the connectivity analysis consisted of the BrainNetTome atlas, in which the MTL subcortical regions were replaced with 50% thresholded probability fields generated by averaging all individual MTL subregional segmentations warped to template space. Regions-of-interest in the AT/PM system were selected based on regions that showed significant functional connectivity with the MTL in Aβ-cognitively unimpaired older adults as identified in a previous publication and subdivided in individual BrainNetTome regions. In the functional connectivity analysis, network components involving connections between MTL subregions and AT/PM subsystems (described in Berron et al., 2020) that correlated with regional tau-PET SUVr were extracted and corrected for age, sex and a cortical composite measure of Aβ-PET. In a second stage of this calculation, subcomponents of the tau-correlating network component associated with memory performance were identified (see supplementary methods for details).

**Cognitive measure**

As a measure representing episodic memory, we used the delayed 10-word-list-recall test from ADAS-cog, measured on a scale from 0-10, with 0 being least impaired. The learning trial of the 10 words was repeated three times. After a distraction task (Boston Naming Test – 15 items short version), the participant was asked to freely recall the 10 words (“delayed recall”). Delayed recall was scored as number of errors (i.e., 10 minus correct recalled words), so that a higher score equaled worse memory performance.

**Statistical analysis**

Multiple regression analyses were carried out between measures of tau- and Aβ-PET, MTL subregional atrophy and memory in Rv3.3.2 (www.r-project.org). All models were adjusted for sex, age and Aβ-PET in addition to years-of-education (for models including cognitive measures) and intracranial volume (for models including volumes). Results were corrected for multiple comparisons using False-Discovery Rate correction (p<0.05) where appropriate. Mediation models were calculated using the lavaan package using a bootstrap method for the mediation effect (Rosseel et al., 2012). Robust
regression models estimated using iteratively re-weighted least squares (ILRS) were calculated using the MASS package (rlm function).

Data availability
Anonymized data will be shared by request from a qualified academic investigator for the sole purpose of replicating procedures and results presented in the article and as long as data transfer is in agreement with EU legislation on the general data protection regulation and decisions by the Ethical Review Board of Sweden and Region Skåne, which should be regulated in a material transfer agreement.
Results

Participant sample

The sample consisted of 217 β-amyloid negative (CU Aβ-, 57% female, mean age 67) and 79 β-amyloid positive cognitively unimpaired older adults (CU Aβ+, 51% female, mean age 71) as well as 87 β-amyloid positive patients with mild cognitive impairment (MCI, 56% female, mean age 72, see table 1).

Accumulation of AD pathology in MTL subregions and regions in the posterior-medial system

We used an EBM approach in order to derive a sequence of brain regions becoming abnormal with respect to accumulation of AD pathology. Every region becoming abnormal represents an individual event in the EBM. We were interested in the sequence of individual brain regions becoming abnormal with respect to tau accumulation in MTL subregions and across regions of the PM system. We found that tau-PET signal in entorhinal cortex and area 35 showed abnormality first and were followed by anterior and posterior hippocampus, area 36, parahippocampal, middle-temporal, inferior-temporal, inferior-parietal and retrosplenial cortex, and finally the precuneus (see Fig.1A). Individuals with positive amyloid status and cognitive impairment were assigned higher tau-EBM stages (see Fig.1B). Notably, CU individuals were almost exclusively positioned in stages 0-6 where tau abnormality was limited to MTL subregions. Many amyloid-positive MCI patients nonetheless presented with low tau-EBM stages. Supplementary tables 7/8 compare sample characteristics and comorbidities to amyloid-positive MCI patients at high tau-EBM stages.

In order to visualize tau accumulation across tau-EBM stages, we regressed subregional tau-PET SUVr against tau-EBM stages for each individual and region-of-interest using non-linear splines (see Fig.1C). Entorhinal cortex and area 35 showed the steepest increase before they reach a plateau around stage 8 and 10 respectively. Anterior and posterior hippocampus followed with less steep increases reaching a plateau around stage 6 and never reached similar levels compared to extrahippocampal regions. Area 36 and the parahippocampal cortex began to increase in stage 2-3, followed by middle-temporal, inferior-temporal and inferior parietal cortex, which started to increase in stage 4-5. Finally, retrosplenial cortex and precuneus began to increase in stage 7.

Stages 5-8 were less populated than other stages which could theoretically have an influence on the observed dynamics in tau-PET increase across stages. Thus, we ran two additional analyses with less events which yielded almost identical results (see supplementary figure 1).

Spatial distribution of tau accumulation in early disease stages

With the aim to visualize the spatial distribution of tau accumulation in different tau-EBM stages, we followed a voxel-wise approach and grouped individuals in different stage groups (see stages in Fig.1B). Individuals in EBM stages 1-3 were mostly characterized by tau-PET binding in area 35 and the entorhinal cortex (Fig.2A, left column). In addition, there were two bilateral clusters in the subiculum...
and cornu ammonis (CA) 1 which are in line with early depictions of Braak stage I-II in the neuropathological literature 8 and correspond roughly to the prosubiculum 10,39,40. Notably, we also observed significant tau-PET binding in the amygdala which can be appreciated in the sagittal view in the most left bottom panel in Figure 2A. Individuals in tau-EBM stages 4-8 show additional tau-PET binding in the posterior hippocampus (mostly subiculum/CA1 or prosubiculum), more widespread accumulation in the anterior hippocampus and extended neocortical temporal accumulation (e.g area 36 and parahippocampal cortex) (Fig.2A, middle column). Thus, tau pathology seems to accumulate predominantly in anterior MTL regions in the beginning before it progresses more posteriorly and to lateral temporal regions, nicely in line with our region-of-interest based results displayed in Figure 1D. Finally, tau-EBM stages 9-11 were characterized by even stronger tau accumulation in lateral temporal brain regions as well as middle parietal and orbitofrontal brain regions (see Fig.2A, right column). Voxel-wise results are available as videos going through all posterior-anterior brain slices in the supplementary material. Peak cluster coordinates can be found in supplementary tables 1-3.

MTL subregional tau is related to local and distant subregional atrophy in cognitively unimpaired individuals

Next, we were interested in whether tau accumulation in MTL subregions was associated with subregional atrophy. Analyses were carried out separately in CU individuals and MCI patients to investigate disease stage specific relationships. Based on our previous finding that CU individuals were almost entirely restricted to stages where tau deposition was limited to MTL subregions (mostly in tau-EBM stages <7), we focused on relationships between tau deposition and atrophy of MTL subregions. Using linear regression models, we found two types of relationships. Local relationships where tau in a given MTL subregion was associated with atrophy of the very same region (Fig.3A, diagonal highlighted in blue) were observed for the entorhinal cortex, area 35 and the anterior and posterior hippocampus (see Fig.3) – subregions that were the earliest in the sequence identified by the EBM (see Fig.1D). We also found distant effects where tau in one region was associated with atrophy in a distant region. Here, tau in all MTL subregions except parahippocampal cortex was related to posterior hippocampal atrophy. In MCI patients, we found a local effect of tau on atrophy in the posterior hippocampus (see Fig.3B, pFDR<.005). All models were corrected for continuous cortical Aβ-PET levels (see supplementary material for uncorrected analyses). Aβ-PET levels were not associated with cortical thickness or volume of most MTL subregions (even when not accounting for tau-PET) in CU individuals (all pFDR values>0.5) and MCI patients (all pFDR>0.14), except for posterior hippocampal volume (MCI: β=-251, SE=88.2, pFDR=0.03; trend in CU individuals: β=-103.7, SE=41.8, pFDR=0.08).

Finally, we used voxel-based morphometry to analyze volumetric differences between all three stage groups derived from the EBM on a whole-brain level by contrasting them against individuals in stage 0. We found no differences in the earliest stages 1-3, but clear differences in stage 4-8 and 9-11.
While atrophy in stage 4-8 was limited to the MTL (see Fig.2B, middle column and Table S4), there was more widespread atrophy in stage 9-11 including more neocortical temporal regions as well as medial and lateral parietal and frontal brain regions (see Fig.2B, right column and Table S5). These results largely mirrored our stage-specific voxel-wise tau-PET binding patterns (see Fig.2A). Importantly, while we did not find differences in the earliest group in stages 1-3 at a corrected statistical threshold \( p_{FWE} < 0.05 \), we found a convincing pattern at an uncorrected threshold \( p < 0.001 \). With evident tau binding in anterior MTL regions, atrophy was limited to area 35 (transentorhinal region), the lateral portion of the entorhinal cortex and the posterior hippocampus (see Fig.2B, left column). Although at an uncorrected threshold, these results mirror our region-of-interest-based finding arguing for the presence of early posterior hippocampal atrophy in absence of measurable local tau accumulation.

**Medial temporal lobe atrophy is related to memory performance in MCI patients, but not CU individuals**

Next, we were interested in how MTL subregional tau and atrophy are related to memory performance in CU individuals. Following multiple comparisons correction, we found that tau-PET SUVr in entorhinal cortex, area 35 and anterior hippocampus was associated with delayed word recall performance (Entorhinal: \( \beta = 0.73, SE = 0.27, p_{FDR} = 0.017 \); area 35: \( \beta = 0.9, SE = 0.34, p_{FDR} = 0.017 \); anterior hippocampus: \( \beta = 2.16, SE = 0.7, p_{FDR} = 0.014 \), see Fig.4A. All relationships also survived multiple comparison correction after applying robust regression models). However, there were no significant relationships between memory performance and volume/thickness of any MTL subregions (all \( p \) values >0.6, see Fig.4B). While cortical A\( \beta \)-PET SUVr was associated with memory performance (\( \beta = 0.98, SE = 0.39, p = 0.012 \)), a mediation analysis showed that tau-PET binding in area 35, entorhinal cortex and anterior hippocampus each significantly and fully mediated the effect of A\( \beta \)-PET on memory, leaving no additional significant direct effect (area 35: \( \beta = 0.112, [0.029-0.195], p = 0.008 \); Entorhinal: \( \beta = 0.125, [0.036-0.215], p = 0.006 \); Anterior hippocampus: \( \beta = 0.098, [0.025-0.170], p = 0.008 \)).

In order to compare early effects of tau accumulation on MTL atrophy and memory functioning with later disease stages, we conducted the same analysis in the A\( \beta \)+ MCI patients. While tau accumulation in the anterior and posterior hippocampus was related to memory (anterior hippocampus: \( \beta = 2.47, SE = 0.75, p_{FDR} = 0.004 \); posterior hippocampus: \( \beta = 3.7, SE = 0.96, p_{FDR} = 0.001 \), see Fig.4C), we observed statistical trends in area 35 and the entorhinal cortex \( p_{FDR} = 0.051 \) and \( p_{FDR} = 0.077 \) respectively). Furthermore, posterior hippocampal atrophy was significantly related to memory performance \( \beta = -0.003, SE = 0.00096, p_{FDR} = 0.009 \), see Fig.4D). Mediation analyses showed that posterior hippocampal atrophy mediated the effect of anterior hippocampal tau on memory performance \( \beta = 0.105, [0.002-0.207], p = 0.049 \), see Fig.4E and F) and showed a trend for a mediation of the effect of posterior hippocampal tau on memory performance \( \beta = 0.09, [-0.005-0.184], p = 0.063 \). In both mediation

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analyses, there remained a significant direct effect of tau on memory performance. Again, Aβ-PET SUVr was associated with memory performance ($\beta=0.4$, $SE=0.1$, $p=0.0001$) and a mediation analysis confirmed that anterior ($\beta=0.145$, $[0.039-0.252]$, $p=0.007$) and posterior hippocampal tau ($\beta=0.105$, $[0.013-0.197]$, $p=0.025$) significantly mediated the effect of Aβ-PET on memory to a large extent, but there remained a direct effect of Aβ-PET on memory.

**Tau accumulation disrupts MTL-cortical functional connectivity**

Our findings point towards a role of area 35, entorhinal and anterior hippocampal tau pathology in memory impairment for CU older adults while hippocampal tau pathology and posterior hippocampal atrophy were associated with memory impairment in MCI patients. We have recently reported that changes in functional connectivity between the MTL (especially entorhinal cortex and area 35) and regions in the AT and PM system seemingly preceded significant atrophy in CU individuals. Given that measures of atrophy were not associated with memory performance in CU individuals in the present study, we were interested in how functional connectivity between the MTL and cortical memory systems changes with increasing area 35 and entorhinal tau-PET signal. We found decreased functional connectivity between area 35 and 36, entorhinal cortex and the anterior hippocampus on the one hand, and regions in the AT system (mostly temporal pole, medial prefrontal and orbitofrontal cortex) on the other hand (see Fig.5A and B). While this represents functional connectivity networks that were significantly reduced with increasing tau-PET signal in area 35 and entorhinal cortex, reduced connectivity across both components was not significantly associated with memory performance (area 35 tau SUVr: $\beta=-0.019$, $SE=0.016$, $p=0.223$; entorhinal tau SUVr: $\beta=-0.03$, $SE=0.04$, $p=0.467$). In order to identify the subset of links in the network component that are both reduced with increasing tau pathology and relevant to memory performance, we identified 3 subcomponents (see Fig.5C) correlating with memory performance. These subcomponents consisted of reduced functional connectivity between (i) area 35 and the orbitofrontal/medial prefrontal cortex, (ii) the posterior hippocampus, the parahippocampal cortex and the angular gyrus as well as between the (iii) entorhinal cortex, anterior hippocampus and the posterior cingulate cortex (including the retrosplenial cortex) (see Fig.5C).


Discussion

Using a second-generation tau-PET tracer, detailed subdivision of MTL subregions and event-based modelling, we identified the sequence of tau progression across the MTL and the PM system in AD. The entorhinal cortex and area 35 (i.e. transentorhinal region) were affected very early by tau pathology and showed the steepest increase in tau-PET signal in early disease stages. Other MTL subregions such as the hippocampus, area 36 and parahippocampal cortex followed, before middle and inferior temporal and inferior parietal regions, retrosplenial cortex and precuneus became abnormal. In CU individuals, tau accumulation was mostly limited to MTL subregions. Atrophy mirrored the spatial distribution pattern of tau-PET binding with the notable exception of early posterior hippocampal atrophy. While tau-PET binding was tightly associated with MTL subregional atrophy, only tau in area 35, entorhinal cortex and the anterior hippocampus was related to memory performance. Importantly, no measure of grey matter volume or thickness was associated with memory performance in CU individuals. In contrast, tau-PET binding in the hippocampus, as well as posterior hippocampal atrophy, were both strongly associated with memory in MCI patients. Mediation analyses revealed that atrophy mediated the effect of tau pathology on memory. Finally, functional connectivity analyses revealed that transentorhinal tau pathology in CU individuals resulted primarily in reduced MTL-AT functional connectivity. Meanwhile, tau-related reductions in functional connectivity in critical MTL-PM networks were associated with early subtle memory impairment.

Early locus of tau-PET binding in the MTL using a second-generation tracer resembles neuropathological findings

The neuropathological literature suggests the earliest cortical neurofibrillary tau tangles accumulate in an area in the anterior MTL that covers the transentorhinal region and part of the entorhinal cortex. Following the first stage of Braak’s staging scheme, tau tangles increase in numbers in area 35 and the entorhinal cortex and can be detected in CA1 in Braak stage II, however, in a region adjacent to the subiculum which roughly matches a region referred to as the prosubiculum. While neurofibrillary tangle counts increase in the mentioned subregions in stage III/IV, they can also be found in lower numbers in other hippocampal subfields and more lateral temporal regions such as area 36. Finally, in Braak stages V/VI, tau pathology can be found in neocortical association cortices.

Using tau-PET imaging with a second-generation tracer that allows more accurate measurement of hippocampal tau-PET binding, our results are in line with Braak and Braak’s findings of the earliest tau accumulation in the entorhinal cortex, area 35 and the anterior hippocampus. Our voxel-wise results show an almost exact replication of their original findings where tau-PET binding could be observed in a region spanning area 35 (i.e. transentorhinal region), entorhinal cortex and an area in the anterior hippocampus consistent with boundaries of CA1 with the subiculum, roughly matching prosubiculum. This is also in line with a recent autopsy imaging study combining ex vivo MRI and serial histopathology which identified an almost identical pattern as well as a study showing that Braak stage was associated
with neuron loss in the subicular end of CA1. We also found consistent tau-PET signal in the amygdala early on congruent with earlier studies. In later stages, tau binding was detected in other temporal cortical regions such as area 36, again consistent with Braak and Braak’s findings. While tau-PET binding could be observed in early stages in the anterior MTL, binding in later stages was seen in the subiculum/CA1 of the posterior hippocampus and in the parahippocampal cortex. These findings suggest that specific regions in the posterior MTL are affected in later disease stages. Hippocampal tau-PET signal never reached the levels of neocortical regions. One underlying reason might be that tau pathology is particularly accumulating in subiculum and CA1 while other subregions such as CA2-3 and the dentate gyrus seem rather spared in non-demented individuals (see movies in supplementary material). Given that we report the mean signal of all voxels in the anterior/posterior hippocampus, the mean tau-PET signal is likely lower on average compared to cortical regions.

Taken together, we found the earliest signs of abnormal tau-PET signal in regions of the AT system while progressively more regions of the PM system became abnormal in later tau-EBM stages. As it is likely that the nature of cognitive and particularly memory impairment will change depending on the spatial distribution of tau pathology in functional memory systems, these findings can inform research on disease stage-specific cognitive markers.

Medial temporal tau is locally and remotely associated with grey matter atrophy

Tau binding has recently been shown to be tightly linked to atrophy. In line with these earlier findings, our voxel-based morphometry results across different tau-EBM stages showed a very similar atrophy pattern compared to voxel-wise tau-PET binding. In addition, our region-of-interest analysis revealed that tau pathology was associated with local thickness and volume within regions such as the entorhinal cortex, area 35 and the hippocampus. This is congruent with earlier studies that reported cross-sectional and longitudinal atrophy in MTL subregions associated with MTL tau-PET binding. However, while earlier studies collapsed across Aβ+ participants including CU, MCI and AD patients, we show in a larger sample that anterior MTL tau-PET binding is associated with atrophy already in CU individuals. In addition, while earlier studies used an MTL-tau-composite, we used tau-PET signal from individual regions-of-interest, and the reduced off-target binding of [18F]RO948 tau-PET allowed us to analyze the signal from the hippocampus. With this approach, we could separate local and distant relationships between tau accumulation and atrophy.

In addition to local relationships, we found a robust effect where tau in distant MTL subregions was strongly associated with lower volume of the posterior hippocampus suggesting remote mechanisms of atrophy in addition to local effects. This finding was additionally confirmed by our voxel-based morphometry analysis. The relationship between primarily posterior hippocampal atrophy and tau pathology has recently been reported in several studies. De Flores and colleagues reported that tau pathology, measured as a mean score of tau tangles in entorhinal cortex, subiculum and CA1, was associated with ante mortem posterior hippocampal volume and Xie and colleagues reported the
earliest longitudinal decline in preclinical AD in area 35 and the posterior hippocampus. Furthermore, a study from our group showed additional evidence for lower posterior hippocampal volume of the subiculum with increased levels of CSF p-Tau. In sum, while earlier studies have shown consistent local relationships between tau and MTL atrophy, our findings extend these by revealing early tau-related atrophy of the posterior hippocampus in vivo that likely results from distant rather than local mechanisms. While we can only speculate on the underlying mechanisms, it is interesting that anterior-lateral regions in the entorhinal cortex neighboring the collateral sulcus and area 35, receive mostly projections from the posterior hippocampus (CA1/Subiculum). The same region shows the earliest tau-related atrophy, suggesting that posterior hippocampal atrophy could be driven by a loss of connections between the anterior-lateral entorhinal cortex and the posterior hippocampus.

_Tau and functional connectivity, but not atrophy, are associated with memory performance in cognitively unimpaired individuals_

Earlier work has suggested a tight link between tau, neurodegeneration and cognitive performance. Here we extend that literature with a fine-grained anatomical analysis. We show that entorhinal, area 35 and anterior hippocampal tau-PET binding were related to memory performance in CU individuals, while only hippocampal tau-PET binding was associated with memory in MCI patients. Critically, while memory was not associated with atrophy in any MTL region in CU, posterior hippocampal atrophy mediated the effect of hippocampal tau on memory in MCI patients. Taken together, this suggests that while tau-related memory impairment might be less dependent on atrophy in early disease stages, it seems to depend on posterior hippocampal atrophy at the MCI stage. Recent work using lesion mapping further strengthens the role of the posterior hippocampus in memory impairment showing that lesions causing amnesia were all functionally connected to an area spanning the posterior hippocampus and parts of the retrosplenial cortex. In light of these findings, our results suggest that memory impairment in AD becomes fully evident once the posterior hippocampus, as the core region of an episodic memory circuit, is affected by atrophy. Whether there is a qualitative difference between subtle tau-related memory impairment in CU individuals and tau- and atrophy-related impairment in MCI patients, needs to be addressed in future studies using several different memory measures.

We have recently reported changes in functional connectivity between the MTL and regions in the AT/PM system in different stages of AD. While there was primarily reduced MTL-AT connectivity in early disease stages (CU Aβ+ vs. CU Aβ-), predominant reductions in MTL-PM functional connectivity characterized individuals in later disease stages (Aβ+ MCI patients vs. CU Aβ-). In addition, reduced MTL-PM functional connectivity was more consistently associated with memory impairment. In line with these earlier results, the findings of our present study show that increases in tau-PET signal in the transentorhinal and entorhinal region in CU individuals were mainly associated with reduced MTL-AT functional connectivity. This is in agreement with a recent study that reported a hippocampus-AT system disconnection, paired with increased regional hippocampal homogeneity, to
be associated with increasing MTL tau-PET signal. While these recent results as well our analyses point towards a predominant tau-related MTL-AT disconnection, results from our subcomponent analysis suggest that functional connectivity within critical MTL-PM networks connecting the entorhinal cortex, hippocampus, parahippocampal cortex, angular gyrus and the posterior cingulate/retrosplenial cortex is particularly associated with tau-related verbal memory impairment. These resulting subcomponents are congruent with recent reports highlighting functional connectivity between the MTL and parietal nodes of the default mode network to be particularly relevant for episodic memory. While we could identify subnetworks that are particularly vulnerable to early tau accumulation and were associated with memory impairment, our data driven analysis approach does not allow to test to what degree these changes in functional connectivity can explain tau-related memory impairment. Future studies focusing on the interactions of specific functional connectivity networks, tau pathology and memory function in independent cohorts of cognitively unimpaired individuals are needed.

Limitations

Firstly, our identified order of biomarker abnormality in tau-PET SUVr across different subregions is based on cross-sectional measurements instead of longitudinal data. Secondly, the implementation of the EBM used here assumes one common trajectory for participants. While this might be true, there could still be individuals with non-typical spread of tau. Thirdly, while our overall sample consists of 383 individuals, groups of individual stages were not large enough to carry out individual stage-specific analyses, requiring us to collapse across stages. Thirdly, while we followed a unique approach using individually derived fine-grained MTL subregions, the resulting regions-of-interest are in part quite small and challenge the resolution offered by tau-PET. However, even the area 35 region-of-interest in our study has a volume of 1202±204 mm$^3$ (mean±SD); resulting in ~10 voxels in the tau-PET resolution). Fourthly, while the tau-PET tracer in our study shows considerably reduced off-target binding in the choroid plexus, tau tracers with even lower choroid plexus binding may be even better suited for assessing MTL subregions. Finally, we used ADAS-delayed-10-word-recall as a measure of memory performance which limits our results to a specific memory measure. Given the specificity of tau-related effects on atrophy and functional connectivity in different disease stages, future studies with several different and more specific memory measures are needed to further understand the nature of early tau-related memory impairment.

Conclusion

Taken together, our findings provide an anatomically detailed insight into tau progression across fine-grained MTL subregions and memory-relevant cortical regions in non-demented individuals. While tau pathology might affect memory performance in cognitively unimpaired individuals via reduced
functional connectivity in critical MTL-cortical networks, memory impairment in MCI seems to be associated with posterior hippocampal atrophy.
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Competing interests

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Figure legends

**Figure 1: Sequence of biomarker abnormality in non-demented older adults.** (A) Positional variance plot for the event-based model including Tau-PET regions of interest in the medial temporal lobe and posterior-medial system showing the distribution of event sequences. The positional variance diagram shows the uncertainty in the maximum likelihood event ordering estimated by taking MCMC (Markov chain Monte Carlo) samples using the EBM and each entry represents the proportion of MCMC samples in which events appear at a particular position in the sequence (x-axis). The proportion ranges from 0 in white to 1 in black. (B) Distributions of study participants in diagnostic groups across tau-EBM stages. (C) Non-linear splines visualizing differences in tau-PET SUVr across tau-EBM stages for individual regions of interest. Mean tau-PET SUVr from individuals in stage 0 was subtracted from tau PET SUVr in individual ROIs. Abbreviations: EBM, event-based model; CU, cognitively unimpaired; MCI, mild cognitive impairment; Aβ, β-amyloid; ERC, entorhinal cortex; IPC, inferior parietal cortex; MTC, middle temporal cortex; ITC, inferior temporal cortex; A35, area 35; RSC, retrosplenial cortex; PRE, precuneus; A36, area 36; PHC, parahippocampal cortex.

**Figure 2: Voxel-wise tau-PET binding and voxel-based morphometry across tau-EBM stages.** (A) Voxel-wise group differences in Tau-PET SUVr images resulting from two-sample t-tests between a group corresponding to tau-EBM stage (stage 0 (n=281) and groups corresponding to 1-3 (n=35), stage 4-8 (n=29) and stage 9-11 (n=38) respectively. (B) Group differences derived from voxel-based morphometry between identical groups. Voxel-wise results in all analyses were corrected using family-wise error correction at a threshold of p < 0.05 and a cluster size of 50 mm³. The voxel-based morphometry group comparison between individuals in stage 1-3 and individuals in stage 0 (B, left column) did not yield significant results using that statistical threshold and is thus reported at an uncorrected threshold of p < 0.001.

**Figure 3: Relationship between MTL subregional tau SUVr and atrophy.** (A) represents relationships in cognitively unimpaired individuals and (B) in patients with mild cognitive impairment. Correlation matrices show the relationship between subregional measures of tau SUVr (rows) and atrophy measures (columns). Relationships lying on the diagonal (highlighted in blue) indicate local relationships between a given subregions tau SUVr and local thickness or volume. Relationships off the diagonal indicate distant effects, where tau SUVr in one region
was associated with atrophy in another region. Dark green represents multiple regression models that are significant at $p_{FDR} < 0.005$, while light green indicate significance at $p_{FDR} < 0.05$. All regression models were corrected for age, sex and continuous $A\beta$-PET SUVr in the cortical composite region. Regression models including volumetric measures (anterior and posterior hippocampus) were additionally corrected for intracranial volume. Abbreviations: A35, area 35; ERC, entorhinal cortex; antHC, anterior hippocampus; postHC, posterior hippocampus; A36, area 36; PHC, parahippocampal cortex.

**Figure 4:** Relationships between MTL tau SUVr, memory performance and atrophy in cognitively unimpaired individuals and patients with mild cognitive impairment. Relationships between MTL tau SUVr and memory in CU (A) and MCI (C) as well as between MTL atrophy and memory in CU (B) and MCI (D). A mediation analysis revealed that posterior hippocampal volume partially mediates the relationship between anterior hippocampal tau SUVr and memory. (E) The direct effect (c) of anterior hippocampal tau SUVr on delayed memory performance. (F) The mediated effect of posterior hippocampal volume is designated $c-c'$. The remaining effect of anterior hippocampal tau SUVr on delayed memory performance after adjusting for posterior hippocampal volume is designated $c'$. The direct effect of anterior hippocampal tau-PET SUVr on posterior hippocampal volume is a, the effect of posterior hippocampal volume on delayed memory performance is b. All regression models were corrected for age, sex, years of education and continuous $A\beta$-PET SUVr in a cortical composite region. Regression models including volumetric measures (anterior and posterior hippocampus) were additionally corrected for intracranial volume. Note that ADAS delayed recall performance is reported in number of errors. Abbreviations: A35, area 35; ERC, entorhinal cortex.

**Figure 5:** Changes in functional connectivity between the MTL and regions in the anterior-temporal and posterior-medial system in cognitively unimpaired individuals. (A) Network component of reduced functional connectivity with increasing tau-PET signal in area 35 and (B) the entorhinal cortex. (C) Subcomponents of A that are significantly associated to delayed recall memory performance. Line width and colour in the connectograms are proportional to the number of links between regions of interest as indicated in the corresponding scale. aHI = anterior hippocampus; pHI = posterior hippocampus; ERC, entorhinal cortex; Br35, area 35; Br36, area 36; PHC, parahippocampal cortex; mPFC = medial prefrontal cortex; OFC =
orbitofrontal cortex; TP = temporal pole; SFG = superior frontal gyrus; ANG = angular gyrus; PCC = posterior cingulate cortex; PREC = precuneus.
Figure 1: Sequence of biomarker abnormality in non-demented older adults. (A) Positional variance plot for the event-based model including Tau-PET regions of interest in the medial temporal lobe and posterior-medial system showing the distribution of event sequences. The positional variance diagram shows the uncertainty in the maximum likelihood event ordering estimated by taking MCMC (Markov chain Monte Carlo) samples using the EBM and each entry represents the proportion of MCMC samples in which events appear at a particular position in the sequence (x-axis). The proportion ranges from 0 in white to 1 in black. (B) Distributions of study participants in diagnostic groups across tau-EBM stages. (C) Non-linear splines visualizing differences in tau-PET SUVr across tau-EBM stages for individual regions of interest. Mean tau-PET SUVr from individuals in stage 0 was subtracted from tau PET SUVr in individual ROIs. Abbreviations: EBM, event-based model; CU, cognitively unimpaired; MCI, mild cognitive impairment; Aβ, β-amyloid; ERC, entorhinal cortex; IPC, inferior parietal cortex; MTC, middle temporal cortex; ITC, inferior temporal cortex; A35, area 35; RSC, retrosplenial cortex; PRE, precuneus; A36, area 36; PHC, parahippocampal cortex.
Figure 2: Voxel-wise tau-PET binding and voxel-based morphometry across tau-EBM stages. (A) Voxel-wise group differences in Tau-PET SUVr images resulting from two-sample t-tests between a group corresponding to tau-EBM stage (stage 0 (n=281) and groups corresponding to 1-3 (n=35), stage 4-8 (n=29) and stage 9-11 (n=38) respectively. (B) Group differences derived from voxel-based morphometry between identical groups. Voxel-wise results in all analyses were corrected using family-wise error correction at a threshold of $p < 0.05$ and a cluster size of 50 mm$^3$. The voxel-based morphometry group comparison between individuals in stage 1-3 and individuals in stage 0 (B, left column) did not yield significant results using that statistical threshold and is thus reported at an uncorrected threshold of $p < 0.001$. 
Figure 3: Relationship between MTL subregional tau SUVr and atrophy. (A) represents relationships in cognitively unimpaired individuals and (B) in patients with mild cognitive impairment. Correlation matrices show the relationship between subregional measures of tau SUVr (rows) and atrophy measures (columns). Relationships lying on the diagonal (highlighted in blue) indicate local relationships between a given subregions tau SUVr and local thickness or volume. Relationships off the diagonal indicate distant effects, where tau SUVr in one region was associated with atrophy in another region. Dark green represents multiple regression models that are significant at $p_{FDR} < 0.005$, while light green indicate significance at $p_{FDR} < 0.05$. All regression models were corrected for age, sex and continuous Aβ-PET SUVr in the cortical composite region. Regression models including volumetric measures (anterior and posterior hippocampus) were additionally corrected for intracranial volume. Abbreviations: A35, area 35; ERC, entorhinal cortex; antHC, anterior hippocampus; postHC, posterior hippocampus; A36, area 36; PHC, parahippocampal cortex.
Figure 4: Relationships between MTL tau SUVr, memory performance and atrophy in cognitively unimpaired individuals and patients with mild cognitive impairment. Relationships between MTL tau SUVr and memory in CU (A) and MCI (C) as well as between MTL atrophy and memory in CU (B) and MCI (D). A mediation analysis revealed that posterior hippocampal volume partially mediates the relationship between anterior hippocampal tau SUVr and memory. (E) The direct effect (c) of anterior hippocampal tau SUVr on delayed memory performance. (F) The mediated effect of posterior hippocampal volume is designated c-c'. The remaining effect of anterior hippocampal tau SUVr on delayed memory performance after adjusting for posterior hippocampal volume is designated c'. The direct effect of anterior hippocampal tau-PET SUVr on posterior hippocampal volume is a, the effect of posterior hippocampal volume on delayed memory performance is b. All regression models were corrected for age, sex, years of education and continuous Aβ-PET SUVr in a cortical composite region. Regression models including volumetric measures (anterior and posterior hippocampus) were additionally corrected for intracranial volume. Note that ADAS delayed recall performance is reported in number of errors. Abbreviations: A35, area 35; ERC, entorhinal cortex.
Figure 5: Changes in functional connectivity between the MTL and regions in the anterior-temporal and posterior-medial system in cognitively unimpaired individuals. (A) Network component of reduced functional connectivity with increasing tau-PET signal in area 35 and (B) the entorhinal cortex. (C) Subcomponents of A that are significantly associated to delayed recall memory performance. Line width and colour in the connectograms are proportional to the number of links between regions of interest as indicated in the corresponding scale. aHI = anterior hippocampus; pHI = posterior hippocampus; ERC, entorhinal cortex; Br35, area 35; Br36, area 36; PHC, parahippocampal cortex; mPFC = medial prefrontal cortex; OFC = orbitofrontal cortex; TP = temporal pole; SFG = superior frontal gyrus; ANG = angular gyrus; PCC = posterior cingulate cortex; PREC = precuneus.
Table 1 displays mean values (standard deviations) unless otherwise stated. Aβ positivity was defined based on CSF Aβ42/40 levels. N = number of participants; Aβ = β-amyloid; CU = cognitively unimpaired; MCI = mild cognitive impairment; MMSE = Mini-Mental State Examination.

|                  | CU Aβ- (N=217) | CU Aβ+ (N=79) | MCI Aβ+ (N=87) | Total (N=383) |
|------------------|----------------|---------------|----------------|---------------|
| Age (years)      | 66.9 (10.1)    | 71.2 (8.54)   | 72.4 (6.89)    | 69.0 (9.48)   |
| Education (years)| 12.6 (3.47)    | 12.7 (3.75)   | 13.3 (4.82)    | 12.8 (3.87)   |
| Sex (female)     | 57.1 %         | 50.6 %        | 56.3 %         | 55.6 %        |
| MMSE a           | 29 (1.23)      | 28.9 (1.24)   | 26.7 (1.91)    | 28.4 (1.69)   |
| Delayed-Word-Recall (errors) b | 2.34 (1.64) | 3.18 (1.51) | 7.05 (2.31) | 3.58 (2.62) |

Aβ positivity was defined based on CSF Aβ42/40 levels. MMSE is presented in points. ADAS Delayed Word Recall is presented in number of errors.