Structural tract alterations predict downstream tau accumulation in amyloid-positive older individuals

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Animal models of Alzheimer’s disease have suggested that tau pathology propagation, facilitated by amyloid pathology, may occur along connected pathways. To investigate these ideas in humans, we combined amyloid scans with longitudinal data on white matter connectivity, hippocampal volume, tau positron emission tomography and memory performance in 256 cognitively healthy older individuals. Lower baseline hippocampal volume was associated with increased mean diffusivity of the connecting hippocampal cingulum bundle (HCB). HCB diffusivity predicted tau accumulation in the downstream-connected posterior cingulate cortex in amyloid-positive but not in amyloid-negative individuals. Furthermore, HCB diffusivity predicted memory decline in amyloid-positive individuals with high posterior cingulate cortex tau binding. Our results provide in vivo evidence that higher amyloid pathology strengthens the association between HCB diffusivity and tau accumulation in the downstream posterior cingulate cortex and facilitates memory decline. This confirms amyloid’s crucial role in potentiating neural vulnerability and memory decline marking the onset of preclinical Alzheimer’s disease.

Amyloid-beta plaques and tau neurofibrillary tangles, the hallmark lesions of Alzheimer’s disease (AD), progress in predictable topographical patterns. Amyloid plaques accumulate first in neocortical areas and then in subcortical regions as the disease progresses. Tau neurofibrillary tangles are detected first in the entorhinal cortex, and after affecting other medial temporal regions, they spread to other limbic structures and finally to the neocortex. Cognitive functions, especially memory, gradually decline as pathology slowly progresses in load and extent, marking the insidious onset of AD.

Animal studies have provided evidence that the topographical patterns of tau pathology reflect spreading of tau via synaptic connectivity. The highest concentration of normally occurring tau protein is found in axons, the connections between cells. As axons do not have the required processes to render tau pathology in a fibrillar state, pathological tau remains in the axon for a long period of time, allowing these soluble aggregates of the misfolded protein to propagate via axons and cell-to-cell synaptic transmission. It remains unclear whether in human disease tau propagation occurs mainly via direct cellular connectivity or by a combination of connectivity and proximity, showing that tau pathology in hippocampal neurons spreads to connected regions and affects the white matter tracts connecting the hippocampus with the distal region. As the perforant pathway cannot be captured with the current spatial resolution of DTI, we focused on a fiber tract strongly connected to the hippocampus to explore associations between hippocampal neurodegeneration and tract diffusivity. Since the novel tau-tracer FTP was added to the study around year 3–4, we considered variations in hippocampal volume associations for memory in a healthy older population. We combined the recently developed positron-emission tomography (PET) tracer flortaucipir (FTP), which binds specifically to tau pathology, with established diffusion tensor imaging (DTI) methods, which measure fiber microstructural properties, and amyloid PET imaging. These methods allowed us to model the untested hypothesis of propagation via connectivity or proximity in vivo in humans from an imaging perspective, assuming parallels to the biological mechanisms examined in animal studies, albeit on different resolution scales. To that end, we evaluated hypothesized associations (Fig. 1) using longitudinal imaging and memory data from the Harvard Aging Brain Study (HABS), an ongoing study of cognitively healthy individuals (n = 256) followed for up to 7 years.

Rationale for investigating limbic pathways in our tau propagation model. Our design is motivated in part by the approach of a recent animal study investigating tau propagation via connectivity or proximity, showing that tau pathology in hippocampal neurons spreads to connected regions and affects the white matter tracts connecting the hippocampus with the distal region. Figure 1 shows the hypothesized associations investigated consecutively in this study. Histological data suggests that early propagation of tau pathology occurs from the entorhinal cortex to the hippocampus, via the perforant pathway. As the perforant pathway cannot be captured with the current spatial resolution of DTI, we focused on a fiber tract strongly connected to the hippocampus to explore associations between hippocampal neurodegeneration and tract diffusivity. Since the novel tau-tracer FTP was added to the study around year 3–4, we considered variations in hippocampal volume...
at baseline as a proxy for aging-associated hippocampal tau-related neurodegeneration. The tract that can be measured most reliably and has the densest connections in the hippocampus is the hippocampal cingulum bundle (HCB). We predicted that hippocampal volume at baseline would predict abnormal HCB diffusivity over time (Fig. 1). Diffusivity of the HCB would in turn be associated with tau accumulation in the downstream-connected posterior cingulate cortex (PCC) and both processes would be associated with memory decline over a 6-year period. Recent cross-sectional work from our group suggested that the interaction between amyloid and tau occurs in posterior hippocampal and PCC regions, supporting the selection of these regions. We additionally included the uncinate fasciculus (UF) as a control tract, since this tract innervates the medial temporal lobe but not the hippocampus. Furthermore, the UF innervates the prefrontal lobe, a region with limited tau binding in cognitively healthy older individuals. For tau accumulation, we included inferior temporal (IT) tau load as a control measure for spread via proximity versus connectivity. The tau accumulation, we included inferior temporal (IT) tau load as a control measure for spread via proximity versus connectivity. The inferior temporal lobe is spatially proximate to the hippocampus and does not primarily have connections with the HCB. Finally, using this approach, we also investigated the potential moderating role of amyloid in tau propagation and memory decline. Previous postmortem studies have shown that tau pathology in the hippocampus is higher in amyloid-positive individuals compared to amyloid-negative individuals and that tau pathology outside of the medial temporal lobe in the context of amyloid pathology is associated with increased cognitive decline. We therefore hypothesized that neocortical amyloid deposition is associated with increased hippocampal volume loss and potentiates the effect of abnormal tract diffusivity on increased tau-accumulation in the PCC (Fig. 1).

Results
Participants (n = 256) from the HABS underwent serial imaging and annual neuropsychological assessments over 7 years (Supplementary Fig. 1). At baseline, the median age of the participants was 73.5 years (interquartile range (IQR): 68.5–78.25 years), their median educational level was 16 years (IQR: 13–18 years) and their median Mini-Mental State Examination (MMSE) screening score was 29 points (IQR: 28–30). One hundred forty-five participants (60.16%) were female. At baseline, all participants were cognitively healthy as determined by the MMSE, had a Clinical Dementia Rating Scale of 0, and normal age- and education-adjusted scores on the Logical Memory delayed-recall test. All participants underwent a comprehensive medical and neurological evaluation to exclude major psychiatric or neurological disorders (see Methods).

Amyloid deposition is associated with increased hippocampal volume loss. We first examined whether smaller hippocampal volume at baseline and volume loss over time (or atrophy) was associated with neocortical amyloid deposition at baseline. Hippocampal volume, adjusted for intracranial volume, was determined by FreeSurfer. Amyloid deposition was examined with PET using the Pittsburgh compound-B (PiB) tracer expressed as the distribution volume ratio, with cerebellar gray as reference tissue. Amyloid status was assessed using a cutoff of 1.20 (amyloid-positive, n = 61 subjects; amyloid-negative, n = 183; missing cases, n = 12) determined by a previous Gaussian mixture modeling approach for the total sample, for a large cortical region-of-interest aggregate that included frontal, lateral, temporal and retrosplenial cortices.

Amyloid-positive individuals were significantly older and, as expected, were more likely to be APOE-e4 carriers compared to amyloid-negative individuals. No differences were found for sex, education, MMSE or memory scores. Even though amyloid-positive individuals showed lower adjusted hippocampal volumes at baseline (Table 1), after correcting for age, sex and education the regression analyses showed no relationship between hippocampal volume and amyloid status at baseline (left hippocampus: β = –85.58, t120 = –1.35, P = 0.18; 95% confidence interval (CI): –210.88, 39.72; right hippocampus: β = –86.85, t139 = –1.54, P = 0.13; 95% CI: –197.97, 24.27). Longitudinal analyses with linear mixed-effect models showed a steeper decline in hippocampal volume in amyloid-positive compared to amyloid-negative individuals over a 6-year follow-up period (right hippocampus: β = –25.25, t206 = –2.49, P = 0.014; 95% CI: –45.26, –5.24; left hippocampus: β = –17.86, t253 = –2.01, P = 0.046; 95% CI: –35.36, –0.35; 453 observations, n = 244). These findings corroborate previous findings from our group and are consistent with disease models suggesting that neurodegeneration occurs downstream of amyloid pathology. We should note, however, that tissue volume measures can reflect multiple underlying pathologies contributing to neurodegeneration, of which neurofibrillary tangles are the most characteristic of AD. Entorhinal tau and hippocampal volume measured at the nearest time point correlated significantly (left: r = –0.43, right: r = –0.36; P < 0.001), suggesting that hippocampal volume may be a reasonable proxy for early tau pathology.

Lower hippocampal volume is associated with white matter abnormalities of HCB and UF at baseline. We examined whether there was a relationship between hippocampal volume at baseline with tract diffusivity at baseline. Tract diffusivity was expressed in four metrics: fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AxD) and radial diffusivity (RD), using DTI. Using robust regression analyses with age, education and sex as covariates, we observed associations between right and left hippocampal volume and right and left FA, MD and RD of the HCB (n = 256) respectively (Supplementary Table 1 and Supplementary Fig. 2). Associations between hippocampal volume and diffusivity of the UF (n = 253) were only found for the right hemisphere. These results confirm an association between local volumetric measures and local structural tract properties. Because Braak neurofibrillary tangles at stage I–II (involvement of entorhinal cortices and hippocampal formation) were present in more than 80% of individuals aged 60 years and older, these associations are likely a reflection of age-related processes, including tau deposition. However, for determining the propagation of tau pathology potentially indicative of memory decline as part of preclinical AD, it will be important to look at tau accumulation in regions associated with later neurofibrillary tangle stages and, hence, the presence of tau lesions in limbic regions.

Fig. 1 | The hypothesized model motivating our design and analyses. The relationships that were specifically analyzed in this article are within the blue box. The green boxes hypothesize that hippocampal volume loss results from neuronal damage, partially due to early tau pathology in the medial temporal lobe (MTL). As tau PET was acquired later in the study and thus this information was not available at baseline, we could not examine the green boxes. The relationship between hippocampal volume and diffusivity of the HCB was analyzed using both baseline measures of hippocampal volume and change in hippocampal volume over time.
Hippocampal volume predicts change in diffusion over time of HCB, but not in UF. In accordance with our model (Fig. 1), we next assessed the hypothesis that hippocampal volume would predict changes in HCB diffusivity over time, but not changes in UF diffusivity. To that end, we used a linear mixed-effects model to predict annual change in tract diffusivity, with baseline hippocampal volume as predictor. We also investigated whether diffusion changes and hippocampal volume changes occur simultaneously. Age, education and sex were included as covariates. We report the statistics of the MD component, as this metric is most commonly associated with aging and the earliest episodic memory deficits⁶. Statistics for these and the other metrics can be found in Supplementary Figs. 3 and 4 and Supplementary Table 2.

Hippocampal volume at baseline predicted a change in HCB diffusivity in such a way that lower hippocampal volume at baseline was associated with more abnormal white matter diffusivity over time (higher MD, AxD, RD). These associations were only significant for the right side (for MD: left: $\beta = -0.0006, t_{128} = -0.82, P = 0.417$; right: $\beta = -0.002, t_{128} = -2.26, P = 0.025$; 386 observations; Fig. 2 and Supplementary Fig. 3). Hippocampal volume did not predict changes in UF diffusivity over time (for MD: left: $\beta = 0.001, t_{128} = 0.42, P = 0.675$; right: $\beta = 0.0005, t_{128} = 1.07, P = 0.283$; 379 observations; Fig. 2 and Supplementary Table 2). Similar relationships were observed when investigating the association between change in hippocampal volume with change in HCB or UF diffusivity (Supplementary Fig. 4).

Right hippocampal volume change predicted a change in right HCB MD, but no associations were found for the left hemisphere (for MD: left: $\beta = -0.029, t_{128} = -1.06, P = 0.29$; right: $\beta = -0.037, t_{128} = -2.19, P = 0.03$; 386 observations). No associations were found between change in hippocampal volume and change in UF diffusivity (for MD: left: $\beta = 0.041, t_{128} = -0.48, P = 0.63$; right: $\beta = 0.004, t_{128} = 0.50, P = 0.62$; 379 observations; Supplementary Table 2).

To establish directionality, we also modeled reverse associations, investigating whether baseline or change in white matter diffusivity of these tracts predicted hippocampal volumes changes over time. Neither baseline nor change in HCB or UF diffusivity (left and right) predicted changes in hippocampal volume over time (Supplementary Table 3).

These results support the hypothesis that hippocampal volume specifically is associated with white matter abnormalities of the HCB and not another nearby tract. Notably, we only found associations for the right hemisphere, even though right hippocampal volume was larger than the left (mean difference = 106.11, paired t test $t = -6.75, P < 0.001$). Furthermore, we were able to show directionality in these associations.

Hippocampal cingulum diffusivity selectively predicts accumulation of tau pathology in the connected PCC in amyloid-positive individuals. Our previous analyses suggested that hippocampal neurodegenerative processes are associated with structural abnormalities of nearby tracts. However, the question remains whether neurodegenerative-associated connectivity loss predicts increased accumulation in a region at the anatomic terminus of the HCB. To that end, the linear mixed-effects models included annual tau accumulation in the PCC as the outcome measure and white matter diffusivity at baseline as the predictor. Tau accumulation was measured beginning, on average, 3.01 years (±0.96) after baseline MRI measurement ($n = 141$), with an average follow-up ($n = 71$) duration of 2.24 ± 0.49 years. In all models, tau binding was expressed as annual increase (from baseline MRI) in regional binding (with cerebellar gray as reference and corrected for partial volume effects)⁷. All models were corrected for age, education and sex. The baseline FTP-subsample ($n = 141$) did not differ from the original baseline sample ($n = 256$) with respect to age (Welch’s t test, $t_{279.68} = -0.38, P = 0.70$), education (Welch’s t test, $t_{280.64} = 0.92, P = 0.36$), sex (chi-squared test, $\chi^2 = 0.009, P = 0.95$), MMSE scores (Welch’s t test, $t_{172.61} = 1.23, P = 0.22$) or PiB-PET levels (Welch’s t test, $t_{167.06} = -0.26, P = 0.79$). PCC tau binding at baseline was not different between individuals with only baseline data versus those with follow-up data (left: Welch’s t test, $t_{124.76} = -0.45, P = 0.65$; right: Welch’s t test, $t_{104.9} = -1.25, P = 0.22$).

Diffusion values (MD, AxD, RD) at baseline of the HCB predicted annual changes in PCC tau for the right hemisphere (MD: $\beta = 0.002, t_{98.5} = 2.55, P = 0.012, n = 212$ observations), not the left (MD: $\beta = 0.001, t_{98.5} = -0.93, P = 0.356$; Fig. 3 and Supplementary Table 4). These effects were not changed when we included baseline values.

**Table 1 | Baseline characteristics of amyloid-negative and amyloid-positive individuals**

|                | Amyloid-negative ($n = 183$) | Amyloid-positive ($n = 61$) | Group difference |
|----------------|-------------------------------|-----------------------------|------------------|
|                | Mean and s.d. (or % for dichotomous variables) | Mean and s.d. (or % for dichotomous variables) | t or $\chi^2$ value | P (unadjusted) |
| Age (years)    | 73.48 (6.23)                 | 75.62 (6.16)                | -2.34            | 0.021*        |
| Education (years) | 15.62 (3.20)                 | 16.34 (2.81)                | -1.68            | 0.097         |
| Sex (female, n %) | 110 (60.11%)                 | 37 (60.66%)                | 0.01             | 0.940         |
| APOE* (n, %)    | 33 (21.29%)                  | 29 (53.70%)                 | 20.64            | 5.541 $\times 10^{-6}$*** |
| MMSE (score)    | 29.04 (1.08)                 | 28.85 (1.08)                | 1.17             | 0.247         |
| Memory factor score | -0.037 (0.75)             | -0.035 (0.73)                 | -0.02            | 0.987         |
| PiB-PET levels (DVR) | 1.085 (0.05)               | 1.421 (0.15)                | -17.72           | 2.2 $\times 10^{-14}$*** |
| Left hippocampal volume (mm$^3$) | 3,680.64 (451.37)           | 3,533.08 (484.21)          | 2.10             | 0.039*        |
| Right hippocampal volume (mm$^3$) | 3,791.47 (404.59)           | 3,639.10 (468.65)          | 2.27             | 0.025*        |
| Left HcB, FA    | 0.354 (0.03)                 | 0.353 (0.03)                | 0.28             | 0.784         |
| Right HcB, FA   | 0.348 (0.03)                 | 0.346 (0.04)                | 0.46             | 0.648         |
| Left HCB, MD$^2$ | 0.879 (0.10)                 | 0.887 (0.10)                | -0.53            | 0.598         |
| Right HCB, MD$^2$ | 0.890 (0.11)                 | 0.911 (0.13)                | -1.14            | 0.256         |
| Left PCC tau (SUVR)$^a$ | 1.192 (0.16)               | 1.301 (0.20)                | -2.91            | 0.005**       |
| Right PCC tau (SUVR)$^a$ | 1.242 (0.16)               | 1.309 (0.19)                | -1.93            | 0.059         |

Mean and s.d. (or % for dichotomous variables) are provided. Two-sided Welch’s t tests were used to calculate group differences for the continuous variables, and $\chi^2$ tests were used for comparing proportions across both groups. Hippocampal volumes were adjusted for intracranial volume. DVR, distribution volume ratio; SUVR, standardized uptake value ratio. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; Tau binding levels are from the first tau PET measurement (103 amyloid-negative; 36 amyloid-positive individuals). Missing data for APOE ε4 status for amyloid-negative ($n = 28$) and amyloid-positive ($n = 7$) individuals. MD measures were multiplied by 1000. Axial and radial diffusivity values correlated with the MD measurements at $r = 0.96$ and $r = 0.99$, respectively.
hippocampal volume in the model (right: for MD: $\beta = 0.002$, $t_{\alpha} = 2.47$, $P = 0.016$; left: for MD: $\beta = -0.001$, $t_{\alpha} = -0.96$, $P = 0.341$).

Even though hippocampal volume at baseline by itself did not predict PCC tau accumulation over time (right side: $\beta = 0.0001$, $t_{\alpha} = -0.83$, $P = 0.409$; left: $\beta = -0.00001$, $t_{\alpha} = -0.23$, $P = 0.821$), these results provide evidence that HCB diffusivity has a more proximal relationship to PCC tau accumulation over time than does hippocampal volume. Baseline diffusion values of the UF were not associated with accumulation of tau in the PCC (right: for MD: $\beta = 0.001$, $t_{\alpha} = 0.61$, $P = 0.544$; left: for MD: $\beta = 0.001$, $t_{\alpha} = 1.33$, $P = 0.186$; Fig. 3 and Supplementary Table 4).

These analyses suggest that tau pathology associated with reduced hippocampal volume affects specific tracts and that more abnormal diffusivity in those tracts is associated with increased tau accumulation in a downstream-connected region. To show that this tau accumulation is specific to the PCC, we ran the same models but with tau binding in the IT cortex as the outcome. As shown in Fig. 3, HCB diffusivity values at baseline did not predict tau accumulation in the IT cortex (right: for MD: $\beta = 0.0004$, $t_{\alpha} = 0.65$, $P = 0.517$; left: for MD: $\beta = -0.0004$, $t_{\alpha} = -0.45$, $P = 0.657$; Supplementary Table 5). As tau PET data was acquired later in the study, we were not able to investigate the opposite direction, i.e., whether accumulation of PCC tau also has detrimental effects on HCB diffusivity.

We next investigated the role of neocortical amyloid deposition in the propagation of tau via white matter connectivity. Amyloid status was assessed using the cutoff 1.20 ($n = 2$ missing for participants who underwent FTP-PET). Baseline amyloid status had no direct effect on tau accumulation in the PCC (left: $\beta = 0.01$, $t_{\alpha} = 0.58$, $P = 0.57$; right: $\beta = -0.028$, $t_{\alpha} = 1.74$, $P = 0.086$; 210 observations). We then investigated a three-way interaction, baseline amyloid $\times$ baseline HCB diffusivity $\times$ time, in predicting PCC tau accumulation over time. The three-way interaction (210 observations) was significant for right-sided associations (FA, MD and RD) and not for left-sided associations (right: for MD: $\beta = 0.004$, $t_{\alpha} = 2.58$, $P = 0.012$; left: for MD: $\beta = 0.001$, $t_{\alpha} = 0.29$, $P = 0.775$; Supplementary Table 6 and Supplementary Fig. 5). The associations between baseline right HCB diffusivity and tau accumulation in the right PCC were found in amyloid-positive individuals (amyloid-positive ($n = 36$), for MD: $\beta = 0.005$, $t_{\alpha} = 3.35$, $P = 0.003$; amyloid-negative ($n = 103$), for MD: $\beta = 0.001$, $t_{\alpha} = 1.36$, $P = 0.179$; Fig. 3).

Together, these results suggest that the mechanism underlying tau propagation from the hippocampus to the PCC is related to reduced diffusivity of a connected white matter fiber bundle, the HCB, and not via a proximate connection. The strength of the association between HCB diffusivity and PCC tau is stronger under higher levels of amyloid pathology.

**Memory-tract diffusivity associations are driven by tau pathology.** Previous studies have shown that tau pathology outside the medial temporal lobe is closely linked with changes in cognition. Baseline FTP-PET data were classified into two groups: participants with low ($n = 93$) or high ($n = 48$) PCC tau binding. The cutoff, a standardized uptake value ratio of 1.28, was determined with a Gaussian mixture modeling approach (Supplementary Fig. 6). Running these models with PCC tau binding as a continuous measure led to similar results. Memory performance scores were based on factor scores from the total HABS cohort. We performed linear mixed-effects models with baseline HCB diffusivity as the predictor and annual changes in memory performance as the outcome. In the second step, we examined the interaction between HCB diffusivity by PCC tau status in predicting memory changes, hypothesizing that memory decline associated with increased HCB diffusivity would be stronger in participants with higher tau levels. As in the previous analyses, tau in the IT cortex was also used as a control region for PCC tau (for the Gaussian mixture model defining the cutoff for IT tau (standardized uptake value ratio = 1.73, partial volume-corrected; see Supplementary Fig. 6). In all models, age, education, and sex were included as covariates. To investigate cognitive domain specificity, control analyses were performed with an executive composite score based on factor scores from the total cohort.

Left and right HCB diffusivity (lower FA, higher MD and RD) at baseline predicted memory decline (right: for MD: $\beta = -0.002$, $t_{\alpha} = -2.70$, $P = 0.007$; left: for MD: $\beta = -0.003$, $t_{\alpha} = -2.56$, $P = 0.011$; $n = 1,167$ observations), not executive performance decline (right: for MD: $\beta = -0.0004$, $t_{\alpha} = -0.55$, $P = 0.58$; left: for MD: $\beta = 0.0008$, $t_{\alpha} = 0.80$, $P = 0.42$; $n = 1,157$ observations). The three-way interaction, HCB diffusivity $\times$ PCC tau status $\times$ time, on memory performance was significant for the right HCB (MD, AxD and RD) and not the left HCB (right: MD: $\beta = -0.006$, $t_{\alpha} = -2.43$, $P = 0.015$; left: MD: $\beta = 0.002$, $t_{\alpha} = 0.73$, $P = 0.466$; 714 observations, $n = 141$.
Fig. 3 | Associations between tract diffusivity and tau accumulation in the PCC or IT. The orange and pink boxes depict part of the model under investigation, as depicted in Fig. 1 (tract diffusivity predicting annual change in tau binding). Images below the boxes depict the anatomical locations of the HCB (red), PCC (blue), UF (yellow), IT (pink) and the investigated links (arrows) in the columns below each brain drawing. The line plots (top, left hemisphere; bottom, right hemisphere) show that higher levels of MD in the right HCB predicted increased PCC tau accumulation (black box; n = 141 unique participants). The middle column shows the associations between the control tract, UF and PCC. No significant associations were found for left or right, consistent with our hypothesis (n = 139 unique participants). The right column shows the associations between the HCB and the control tau region (IT cortex). No significant associations were found for left or right (n = 141 unique participants). Bottom: a more detailed view of the black boxed graph, showing the link between amyloid pathology, tract diffusivity and annual change in PCC tau accumulation (model depicted in the yellow, orange and pink boxes). The line plots show that the association between right HCB diffusivity and right PCC tau change is only found in amyloid-positive individuals (right, n = 36 unique participants) and not amyloid-negative individuals (left, n = 103 unique participants). In all line plots, time was defined as the time since the baseline MRI measurement and MD of the tracts is depicted as mean ± 1 s.d., but analyses were done continuously using linear mixed-effects models. Shaded areas around the fit lines show the 95% CI. All P values are two-sided and unadjusted for multiple comparisons. *P < 0.05; **P < 0.01.
show regional specificity, we ran another three-way interaction, observations: MD:

\[ \beta = 0.006, t_{101} = -3.20, P = 0.001 \]

low PCC tau (n = 93, 466 observations): MD:

\[ \beta = 0.001, t_{100} = -0.93, P = 0.352 \]

This interaction showed that participants with high PCC tau were driving these effects (amyloid-positive individuals: \( n = 18 \) unique participants) or amyloid-positive (\( n = 18 \) right). In all line plots, HCB MD is depicted as mean ± 1 s.d., but analyses were done continuously using linear mixed-effects models. Shaded areas around the fit lines show 95% CI. All P values are two-sided and unadjusted for multiple comparisons. *P < 0.05; **P < 0.01; ***P < 0.001.

A defining feature of AD is the progressive accumulation of amyloid and tau pathology. Current disease models suggest a temporal trajectory in which amyloid pathology initiates a chain of events including the increases in amount and extent of tau pathology. The question of how tau pathology propagates from one region to another is crucial for understanding the pathophysiological mechanisms of AD, but is also important for developing novel therapeutics. Potential mechanisms of tau propagation that have been suggested in histological and animal studies include connectivity, proximity, diffusion along axons and neural activity.

In the present work, we provide in vivo evidence that amyloid pathology—a key determinant in the onset of AD—accompanies the stronger association between structural alterations of the HCB and accumulation of tau pathology in the downstream PCC. By combining several established and innovative in vivo neuroimaging methods, we showed that accumulation of tau pathology in a downstream-connected region is specifically associated with properties of connections of that region and not with other proximate connections, such as the UF. While we recognize that current neuroimaging techniques do not have the resolution to understand the molecular mechanisms of tau propagation, these results are consistent with several animal studies and current disease models, and they also establish a link to memory decline.

Propagation of tau has been investigated predominantly in animal studies. Several studies have shown a stereotypic increase in tau pathology in a time-dependent manner, both locally and distal to regions with synaptic connections, involving axonal degeneration. Tau propagation has been observed from tau-infused rodent hippocampus to distal regions such as olfactory and retrosplenial cortices. These observations are consistent with our findings of downstream-increased PCC tau (including in the retrosplenial cortex) in relation to abnormalities of the connecting HCB, which in turn was associated with lower hippocampal volume at baseline. The fact that hippocampal volume was not directly associated with PCC tau accumulation suggests that changes in tract diffusivity may be an observation related to the underlying mechanisms mediating the spreading of tau. The fact that these associations showed regional selectivity and were not related to diffusivity of another tract or tau in an adjacent region supports the idea that tau pathology propagates via connectivity. Since the majority of these findings were found in MD, AxD and RD metrics, hippocampal tau pathology seems to induce disruption of both axons and myelin. Variations in the AxD and RD components need to be interpreted.
cautiously, as these inferences are largely based on animal studies. Even though we corrected for partial volume effects, it is possible that hippocampal atrophy may have influenced our results. Future studies should therefore consider acquiring multishell sequences, such as NODDI, to increase the specificity of these findings.

We did not find evidence for the reverse association (baseline HCB diffusivity predicting hippocampal volume changes over time), suggesting a biological ordering that fits with the spatiotemporal topography of tau pathology, in which the hippocampus is affected prior to a tract leaving the medial temporal lobe. We note that neurodegeneration is a slow process, and tau and neurodegeneration may be occurring in parallel, with different magnitudes. Tau propagation may be necessary but not sufficient for volume loss. Most of the animal studies also found no evidence for tau-induced neurodegeneration, but the animals were on average not very aged. As for retrograde associations, immunohistochemistry studies have shown evidence for both anterograde cell-to-cell propagation and combined anterograde and retrograde spread of tau to and from the hippocampus and mammillary nuclei, and inconsistencies between our results and these studies may be related to differences in spatial resolution inherent to the methodology.

To our knowledge, this is the first study suggesting that changes in tract diffusivity are likely related to the biological mechanisms underlying the association between hippocampal volume and PCC tau accumulation over time. The PCC is an important neuronal hub that displays extensive amyloid deposition in animal studies and human neuroimaging studies and plays a crucial role in spatial learning and memory. Animal studies have also suggested propagation from the entorhinal cortex to medial frontal regions or olfactory cortex, and thus this phenomenon is most likely not limited to the hippocampus-PCC connection. Since we investigated cognitively healthy older individuals, the variability in frontal tau binding was limited. Longer follow-ups or inclusion of early AD patients will allow extension of this model to other brain networks.

Abnormalities of the HCB have been associated with increased amyloid deposition. We found that amyloid is associated with an increased relationship between PCC tau and the HCB, suggesting that amyloid is a crucial part of the chain of events promoting tau spread and specifically linked to tau-related memory decline. While the role of amyloid as a driving force for tau propagation to distal regions has been shown in vitro and in vivo models, the exact molecular mechanism underlying tau propagation and its facilitation by amyloid are still poorly understood. Hyperphosphorylation is one potential mechanism, as dephosphorylation in animal models has been shown to reduce tau propagation. Whether tau pathology in turn can exacerbate amyloid plaques is still under debate. We did not test this in our study, as variability in PiB-PET measures over time is low and requires longer follow-up.

These data do not preclude the possibility that other factors, or a combination of factors, induce tau propagation. Animal studies have also provided evidence for functional spread, in which regions that fire synchronously and have higher metabolism are more vulnerable. Khan et al. reported that tau pathology in the medial temporal lobe of APP/tau mice led to secondary functional changes in medial parietal regions. Notably, neuronal networks with a high load of neurofibrillary tangles can remain functionally intact, suggesting that more downstream elements at the axonal or synaptic level may show tau-related defects.

We note that all associations we observed were lateralized to the right. Some degree of asymmetry of tau is common in AD pathology. A recent study showed bilateral tau pathology with a stronger involvement of the right hemisphere when comparing cognitively healthy with cognitively impaired individuals. This lateralization may be related to disease stage, so that in cognitively healthy older individuals, tau accumulation in the left hemisphere has not yet crossed the detection threshold. However, it has been shown that asymmetries in amyloid and tau pathology occur and remain stable during disease progression. Alternatively, these right-sided associations may also be specific to the cognitive domain under investigation. Recent work indicates that amyloid-memory (verbal and visuospatial) associations are slightly more right lateralized, and work from our group showed stronger right-side relationships between tau pathology and hypometabolic topoparietal patterns in amyloid-positive individuals. It is also possible that the right-sided effects reflect a selection bias. Left-sided pathology may induce more pronounced cognitive deficits, such as language problems, which would exclude such individuals from our study at baseline. Whether this lateralization is also seen in patients or is associated with other cognitive domains needs to be further examined.

The recent development of FTP-PET tracers allows us to measure and visualize tau pathology in vivo, but as they were recently introduced in the HABS cohort, we did not have this information available at baseline. Therefore, we started our cascade of events with hippocampal volume as a proxy of tau pathology. Hippocampal volume changes can also reflect amyloid deposition or various non-AD-related pathological processes, including Lewy bodies, vascular lesions, hippocampal sclerosis, TDP-43 inclusions and argyrophilic grain disease. Nonetheless, hippocampal atrophy is a common accompaniment to AD-related pathology, especially neurofibrillary degeneration.

In summary, our findings suggest that amyloid contributes to increased spreading of tau via the HCB, confirming that amyloid is a crucial part of the chain of events leading to increased tau pathology and contributing to tau-related memory decline. Possible upstream molecular mechanisms enabling the spread of tau pathology or pathways by which amyloid may potentiate this spread have not been tested with our data and remain open questions. Nonetheless, these findings provide empirical foundations for future work on disease models and suggest that the extent of tau pathology spreading may be an interesting outcome measure for clinical trials focused on removal of amyloid plaques in the earliest stages, as amyloid potentiates tau propagation.

Methods

Methods, including statements of data availability and any associated accession codes and references, are available at https://doi.org/10.1038/s41593-018-0070-z.

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Author contributions

H.I.L.J designed the study, analyzed the diffusion and behavioral data, performed statistical analyses and wrote the manuscript. T.H. performed the factor analyses and aided in data interpretation and manuscript preparation. A.P.S. analyzed the PET and structural data and aided in manuscript preparation, J.S. aided in the connectivity data analysis and manuscript preparation. R.E.P. aided in data analysis and manuscript preparation, K.A., K.V.P. and D.M.R. aided in study screening procedures, neuropsychological assessments and manuscript preparation. R.A.S. provided the participants and data analytic tools and aided in study design and manuscript preparation. K.A.J. designed the study, aided in data analyses and interpretation and wrote the manuscript.

Competing interests

A.S. has been a paid consultant for Janssen Pharmaceuticals and Biogen. K.P. has served as a paid consultant for Biogen. D. Rentz has done consulting for Eli Lilly and served on the Scientific Advisory Board for Neurotrack. K.J. has served as paid consultant for Bayer, GE Healthcare, Janssen Alzheimer’s Immunotherapy, Siemens Medical Solutions, Genzyme, Novartis, Biogen, Roche, ISIS Pharma, AZTherapy, GEHC, Lundberg and Abbvie; and he is a site co-investigator for Lilly/AviD, Janssen Immunotherapy and Pfizer. R.S. has served as a paid consultant for Abbvie, Biogen, Bracket, Genentech, Lundbeck, Roche and Sanofi; has served as co-investigator for Avid, Eli Lilly and Janssen Alzheimer Immunotherapy clinical trials; and has spoken at symposia sponsored by Eli Lilly, Biogen and Janssen. R.S. receives research support from Janssen Pharmaceuticals and Eli Lilly and Co.; these relationships are not related to the content in the manuscript.

Additional information

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Methods
Participants. Healthy older individuals from the Harvard Aging Brain Study who underwent 3T MRI including diffusion tensor imaging (DTI) at baseline were included in the present study ($n = 256$, mean age $74.07$ ± $6.21$). The Harvard Aging Brain Study is a longitudinal study on aging and AD. Participants undergo annual neuropsychological testing and multiple imaging sessions. Participants were included if they had a global score of 0 on the Clinical Dementia Rating scale (CDR), a Mini-Mental State Examination score $\geq 25$, and performed within education-adjusted norms on the Logical Memory delayed-recall test ($> 10$ for elementary school completion and $> 6$ for 8–15 years of education). All participants underwent at least one comprehensive medical and neurological evaluation and had no major psychiatric or neurological disorders. Presence of clinical depression (Geriatric Depression Scale below $11/20$) or other psychiatric illnesses, history of alcoholism, drug abuse, head trauma, or a family history of autosomal dominant Alzheimer’s disease were exclusion criteria. All behavioral and neuroimaging experiments were performed during daytime.

Study protocols were approved by the Partners Human Research Committee at Massachusetts General Hospital, and all participants provided written informed consent. We complied with all ethical regulations.

Structural MRI. All imaging was performed at the Massachusetts General Hospital, Athinoula A. Martinos Center for Biomedical Imaging on a 3 T imaging system (TIM Trio; Siemens) with a 12-channel phased-array head coil. Head motion was controlled with a foam pillow and extendable padded head clamps.

The protocol included a structural T1-weighted volumetric magnetization-prepared rapid acquisition-gradients-echo (MPRAGE) sequence (repetition time (TR) = $2,300$ ms, echo time (TE) = $2.93$ ms, and inversion time = $900$ ms, flip angle = $9°$, and $1 	imes 1 	imes 1.2$ mm resolution). Diffusion-weighted imaging was acquired with a single-shot echoplanar imaging sequence (TR = $8,000$ ms, TE = $84$ ms, flip angle = $90°$, field of view = $256 	imes 256 	imes 128$, voxel size = $2$ mm isotropic, $30$ isotropically distributed diffusion-sensitizing gradients with a $b$-value of $700$ s/mm$^2$ and $5$ nondiffusion weighted images ($b = 0$ s/mm$^2$)). Participants were scanned at baseline ($n = 256$) and a second time after an average of $2.82$ years ($n = 134$, median follow-up $2.62$ years ($IQR = 2.51–2.85$)). The diffusion MRI data were processed with ExploreDTI version 4.8.6. Data preprocessing included correction of subject motion, eddy current distortion correction, incorporating the B-matrix rotation to preserve the diffusion gradient orientation information correctly, echoplanar image susceptibility correction based on each individual’s baseline skull-stripped anatomical image, and tensor estimation using the robust nonlinear least-squares Restore algorithm. Tracks of interest were the hippocampal cingulum bundle (HCB), and the uncinate fasciculus (UF) was chosen as control tract. In three individuals, the UF could not be estimated accurately due to signal dropout, and these individuals were discarded for analyses including this tract. The bootstrapped ($n = 500$) intraclasc correlation coefficient (ICC), adapted for longitudinal data, demonstrated adequate measurement reliability over time. For the HCB, the ICC was $0.63$, $0.66$, $0.65$ and $0.62$ for the MD, FA, AxD and RD components, respectively. For the UF, the ICC was $0.81$, $0.56$, $0.74$ and $0.84$ for the MD, FA, AxD and RD components, respectively.

T1-weighted images were processed in FreeSurfer (FS) version 5.1 using the software package’s default, automated reconstruction protocol, as described previously. Fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AxD) and radial diffusivity (RD) values were extracted from the tracts of interests after thresholding $FA > 0.20$ to exclude partial volume effects. Additionally, tract size was included in our statistical models. Partial volume effects vary with the size of the tract, and simulations have shown that this approach can account partially for this contamination. The tract of interest was the hippocampal cingulum bundle (HCB), and the uncinate fasciculus (UF) was chosen as control tract. In three individuals, the UF could not be estimated accurately due to signal dropout, and these individuals were discarded for analyses including this tract. The bootstrapped ($n = 500$) intraclasc correlation coefficient (ICC), adapted for longitudinal data, demonstrated adequate measurement reliability over time. For the HCB, the ICC was $0.63$, $0.66$, $0.65$ and $0.62$ for the MD, FA, AxD and RD components, respectively. For the UF, the ICC was $0.81$, $0.56$, $0.74$ and $0.84$ for the MD, FA, AxD and RD components, respectively.

Hippocampal volume was adjusted for the estimated intracranial volume (eTIV) using the following equation:

$$\text{Adjusted hippocampal volume} = \text{raw hippocampal volume} - b \times \text{eTIV} - \text{Mean eTIV}$$

where $b$ indicates the regression coefficient when hippocampal volume is regressed against eTIV.

PIB-positive emission tomography. Pittsburgh compound B–positive emission tomography (PiB-PET) was performed at the Massachusetts General Hospital PET facility. Carbon $11$–PiB was synthesized using a previously published protocol, and PiB-PET was performed at baseline using a PET system (ECAT EXACT HR+; Siemens). $\delta$-PiB PET was acquired with an $8.5–15$ mCi bolus injection followed immediately by a $60$-min dynamic acquisition in $69$ frames ($12$ frames $\times 15$, $57$ frames $\times 60$). $\delta$-PiB PET data were expressed as the distribution volume ratio (DVR) with cerebellar gray as reference tissue, using the Logan graphical method applied to data from $40$ to $60$ min after injection. DVR was further assessed using a large cortical ROI aggregate that included frontal, lateral, temporal and retrosplenial cortices (FLR) as described previously. Amyloid status was ascertained by a previously determined cutoff value based on Gaussian mixture modeling approach (cutoff value = $1.20^\text{a}$). Based on this cutoff, $183$ individuals were classified as amyloid-negative and $61$ as amyloid-positive at baseline (for $12$ cases the no baseline PiB-PET measurement). The global PiB-DVR was $1.109$ ($IQR = 1.063–1.202$); the median baseline PiB imaging delay from the first neuropsychological assessment was $0.35$ years ($IQR = 0.24–0.49$ years) and the median delay from the first MRI scan was $0.003$ years ($IQR = 0.20$ to $0.12$ years).

Flortaucipir (FTP)-PET. Flortaucipir $18$-FTP was prepared at MGH with a radiochemical yield of $14 \pm 3$% and specific activity of $216 \pm 60$ GBq/mol at the end of synthesis (60 min), and validated for human use. PET images were acquired on a Siemens/CTI (Knoxville, TN) ECAT HR+ scanner (3D mode; $63$ image planes; $15.2$-cm axial field of view; $5.6$-mm transaxial resolution and $2.4$-mm axial resolution). FTP was acquired from $89–100$ min after injection and a $9–10$ mCi bolus injection in four $5$-min frames. PET data were reconstructed and attenuation corrected, and each frame was evaluated to verify adequate count statistics and the absence of head motion. To evaluate the anatomy of cortical FTP binding, each individual PET dataset was rigidly co-registered to the subject’s MPRAGE data using SPM8 (Welcome Department of Cognitive Neurology, Function Imaging Unit, London). FreeSurfer’s cerebral gray ROI as reference. IT and entorhinal tau have so far been investigated more closely than PCC in older individuals. Zero-order Pearson’s product-moment correlation coefficients show similar behavior across these measures ($r = 0.58, P < 0.001$ for PCC and IT and $r = 0.45$ and $P < 0.001$ for PCC and entorhinal tau). The ICC values showed adequate reliability over time (IT tau ICC = $0.86$ and PCC tau ICC = $0.77$).

As the FTP was only recently developed, FTP-PET was introduced later in the HABS study, on average $3.33 \pm 0.77$ years after the first neuropsychological assessment, $3.01 \pm 0.96$ years after the first MRI scan and $2.99 \pm 0.83$ years after the PiB-PET scan. As of the writing of this manuscript, $141$ individuals had received a first FTP-PET scan and $71$ individuals had been followed up (after on average $2.24$ years from their first tau PET scan). The median follow-up time for tau-PET was $2.16$ years ($IQR = 1.95–2.53$).

Cognitive performance. A memory and an executive function composite score were created based on a factor analysis from the entire HABS cohort ($n = 284$). The memory composite (with factor loadings between parentheses) included the $z$-score transformations of the delayed recall scores of the 6-Trial Selective Reminding Test ($r = 0.739$), free recall of the Free and Cued Selective Reminding Test (0.605) and delayed recall of the Logical Memory Test ($r = 0.534$). Memory was evaluated annually in HABS and therefore we included $1,167$ evaluations ($n = 256$ at baseline, $n = 246$ at year $1$, $n = 233$ at year $2$, $n = 194$ at year $3$, $n = 157$ at year $4$ and $n = 81$ at year $5$). The executive function composite included the $z$-score transformations of the Trail Making Test form B – $A^\text{c}$ (0.666), the Letter Number Sequencing Test ($r = 0.533$) and the phonemic fluency FAS test ($r = 0.622$). Executive function was also evaluated annually and, at the time of the analyses, $1,157$ observations were included (10 participants did not yet had scores for follow-up up to year 5). The median follow-up duration was $4.08$ years (IQR = $1.96–5.19$ years). Experimenters collecting MRI or behavioral data were blind to amyloid status or level of tau binding, and experimenters collecting PET data were blind to the behavioral data and MRI results.

Statistical analyses. Statistical analyses were performed using statistical software (R version 3.3.0: http://www.r-project.org/). All analyses were done during January 2016 through May 2017. No statistical methods were used to predetermine sample sizes, but our sample sizes are similar to those reported in previous publications. Group characteristics were assessed with the chi-square test. Differences between amyloid-positive and -negative individuals were tested with the Wilcoxon’s two-sample $t$ test to account for unequal variances or with the $\chi^2$ test. Baseline associations between adjusted hippocampal volume and amyloid status or white matter tract diffusivity (diffusion metrics) were investigated using linear robust regression methods with the Huber-M estimator. Robust regression is a more conservative test that is less sensitive to outliers and atypical methods, as the resulting models are stable against outliers, which were present in the baseline absolute diffusion data.
Longitudinal analyses were performed with a stepwise hypotheses-driven linear mixed-effects (LME) modeling approach using the maximum likelihood estimation, containing a fixed effect for the predictor of interest, a random intercept for each subject and random slope for time (number of years between baseline and follow-up). For all LME models, we compared the Akaike Information Criteria between models with either a random intercept and random slope or a random intercept alone, using the log-likelihood ratio test, and chose the most parsimonious model. To control for longitudinal changes in voxel selection of tracts, we included analysis weights in the LME models examining diffusion values and weighing the error variance inversely by the tract size. In all LME models, age, sex and education and their interaction with time were included as covariates if $P < 0.10$ (using the Wald statistic).

To further examine the hypothesized relationships (Fig. 1), we performed several LME models. We provide the formula for the most complex model (with three-way interactions):

$$
\text{Outcome}_i = \beta_0 + \beta_1 \text{Age}_i + \beta_2 \text{Education}_i + \beta_3 \text{Sex}_i + \beta_4 \text{PreditorA}_i \\
+ (\beta_5 \text{PreditorB}_i \times \beta_6 \text{Time}_i + \beta_7 \text{Age}_i \times \text{Time}_i) \\
+ (\beta_8 \text{Education}_i \times \text{Time}_i + \beta_9 \text{Sex}_i \times \text{Time}_i) \\
+ (\beta_{10} \text{PreditorA}_i \times \text{Time}_i + \beta_{11} \text{PreditorB}_i \times \text{Time}_i) \\
+ (\beta_{12} \text{PreditorA}_i \times \text{PreditorB}_i \times \text{Time}_i) + \epsilon_i,
$$

where $\text{Var}(\epsilon_i) = \sigma^2/\text{tract-size}_i$, $\text{Var}(\beta_7) = \tau^2$, $\text{Var}(\beta_8) = \tau^2$, and $\text{Cov}(\beta_7, \beta_8) = p \times \tau_1 \times \tau_2$. Outcome is the outcome variable measured over time; Age, Education, Sex, indicate age, education or sex at baseline testing session (i); Predictor A/B-variables of interest depending on the investigated model; Time, is the time at testing session, relative to baseline testing session; $\epsilon_i$ is the random intercept for each subject; $\tau_1$, $\tau_2$ is the random slope for each subject (j); $\tau_1$, $\tau_2$ is the variance of the residuals of the random intercept; and $\epsilon_i$ is the variance of the residuals of predicting the random slope.

We first investigated differences in annual change in hippocampal volume between amyloid-positive and amyloid-negative individuals. The second models estimated mean annual change in each DTI metric in each tract predicted by baseline hippocampal volume and its interaction with time. To explore directionality, we also estimated mean annual change in adjusted hippocampal volume predicted by diffusivity of the tracts of interest.

In the next step, we estimated mean annual change in PCC tau predicted by tract diffusivity over time. The time variable reflects annual change in PCC tau since baseline MRI measurements (to check the robustness of our models, we also ran these models with the first tau measurement as baseline and also within a complete-case design ($n = 71$); these LMEs provided similar results). The same model was performed with a control tau region, the IT cortex, to establish regional specificity of the findings.

In the fourth step, we examined whether the change in tau predicted by HCB diffusivity was different for amyloid-positive versus amyloid-negative individuals by adding a three-way interaction in the model (HCB diffusivity at baseline × amyloid status × time).

In the final step, we investigated associations with annual changes in memory performance. First, we investigated whether HCB diffusivity predicted memory performance over time. Associations with executive functioning were investigated as control cognitive measure. Next, we added the interaction with PCC tau performance. First, we investigated whether HCB diffusivity predicted memory performance. In the fourth step, we examined whether the change in tau predicted by HCB diffusivity interacted to influence hippocampal volume cross-sectional and longitudinal associations: four comparisons were performed for the tract of interest and four for the control tract per side and per diffusion metric; for the association between tau and diffusion, total of three comparisons were performed for the tract of interest and control region per side and per diffusion metric; for the cognitive analyses, a total of three comparisons were performed per cognitive measure, per side and per diffusion metric.

Life Sciences Reporting Summary. Further information on experimental design is available in the Life Sciences Reporting Summary.

Data availability. Relevant data that support the findings of this study are available from the authors upon reasonable request. Baseline data from the Harvard Aging Brain Study are also publicly available online at: http://nmr.mgh.harvard.edu/lab/harvardagingbrain/data26. Follow-up data from the Harvard Aging Brain Study is expected be released in 2018.

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Experimental design

1. Sample size
   Describe how sample size was determined.
   There is no justification of sample size. This is a longitudinal cohort (panel) study and considering the novelty of the tau PET tracer and the multi-modal design, this could not be predicted before hand. However, taking into account previous work from our group, we were convinced that N=256 at baseline (and up to 7 years follow-up measurements) would be adequate to detect changes in tract integrity and associations with neurodegeneration and cognition. Previous studies: - Sepulcre et al., 2016 investigated cross-sectional relationships between whole-brain tau and amyloid PET and grey matter volume (FDR corrected, N=88) - Mormino et al. 2014: showed that longitudinal changes in cognition are related to amyloid-status and the amount of neurodegeneration (N=166, 3 years follow-up).

2. Data exclusions
   Describe any data exclusions.
   We excluded three data points for DTI measures of the uncinate fasciculus due to poor data (signal loss in the region of interest). This has been described on page 29. Missing data on amyloid status was also reported on page 7. Missing data on genotype for APOE is provided in Table 1. Missing data for the longitudinal data is reported in the online methods section.

3. Replication
   Describe whether the experimental findings were reliably reproduced.
   No formal replication was done. However, the absolute diffusivities (AxD, RaD and MD) correlated highly, indicating that they measure a similar underlying process. Results for the these diffusivities were similar across the various analyses.

4. Randomization
   Describe how samples/organisms/participants were allocated into experimental groups.
   N/A. No randomization was used. This was a cohort study.

5. Blinding
   Describe whether the investigators were blinded to group allocation during data collection and/or analysis.
   N/A. However, experimenters collecting MRI or behavioral data were blind to amyloid status or level of tau binding and experimenters collecting PET data were blind to the behavioral data and MRI results.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.
6. Statistical parameters
For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

| n/a | Confirmed |
|-----|-----------|
| ☑   | The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.) |
| ☑   | A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| ☑   | A statement indicating how many times each experiment was replicated |
| ☑   | The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section) |
| ☑   | A description of any assumptions or corrections, such as an adjustment for multiple comparisons |
| ☑   | The test results (e.g. P values) given as exact values whenever possible and with confidence intervals noted |
| ☑   | A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range) |
| ☑   | Clearly defined error bars |

See the web collection on statistics for biologists for further resources and guidance.

Software
Policy information about availability of computer code
7. Software
Describe the software used to analyze the data in this study.

Batch scripts were written in Matlab for the PET and MRI analyses (making use of FreeSurfer, SPM, ExploreDTI), this is reported on page 28 for DTI, page 29 last paragraph for MRI analyses, and page 30-31 for PET data. Data analyses was done in R as described on page 33.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). Nature Methods guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents
Policy information about availability of materials
8. Materials availability
Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

No unique materials were used

9. Antibodies
Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

No antibodies were used

10. Eukaryotic cell lines
a. State the source of each eukaryotic cell line used.
No eukaryotic cell lines were used

b. Describe the method of cell line authentication used.
No eukaryotic cell lines were used

c. Report whether the cell lines were tested for mycoplasma contamination.
No eukaryotic cell lines were used

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.
No eukaryotic cell lines were used
Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

N/A, no animals were used in this study.

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

Healthy older participants (n=256) were recruited within the Harvard Aging Brain Study and underwent serial imaging and annual neuropsychological assessments up to seven years. At baseline, the median age of the participants was 73.5 years (interquartile range [IQR], 68.5 – 78.25 years), their median educational level was 16 years (IQR, 13 – 18 years) and their median Mini-Mental State Examination (MMSE) screening score was 29 points (IQR, 28 – 30). One hundred forty-five participants (60.16%) were female. Sixty-one individuals were amyloid positive and 183 amyloid negative (12 missing amyloid status values). Sixty-two individuals carried at least one APOE E4 allele, while 159 individuals were APOE E4 negative (35 individuals did not have genotyping). This information is also provided on page 6 and Table 1 provides an overview of the differences between amyloid positive and amyloid negative individuals.
MRI Studies Reporting Summary

Experimental design

1. Describe the experimental design. No task, structural MRI data

2. Specify the number of blocks, trials or experimental units per session and/or subject, and specify the length of each trial or block (if trials are blocked) and interval between trials. NA

3. Describe how behavioral performance was measured. No functional task was included in this study. Cognition measures were measured off-line. We used scores from neuropsychological tests of memory and executive function. See page 32 and 33

Acquisition

4. Imaging

   a. Specify the type(s) of imaging. Structural MRI: volumetrics and diffusion tensor imaging

   b. Specify the field strength (in Tesla). 3 Tesla (page 28))

   c. Provide the essential sequence imaging parameters. T1-weighted volumetric magnetization–prepared rapid-acquisition gradient-echo (MPRAGE) sequence (repetition time (TR) = 2300 ms, echo time (TE) = 2.95 ms, and inversion time = 900 ms, flip angle = 9°; and 1.05×1.05×1.2mm resolution). These parameters are also provided on page 28

   d. For diffusion MRI, provide full details of imaging parameters. TR = 8040 ms, TE = 84 ms, flip angle = 90°, field of view = 256 x 256 x 128, voxel size= 2mm isotropic, 30 isotropically distributed diffusion-sensitizing gradients with a b-value of 700 s/mm² and 5 non-diffusion weighted images (b = 0 s/mm²). These parameters are also provided on page 28

5. State area of acquisition. Whole-brain acquisition using a 12-channel phased-array head coil (page 28)

Preprocessing

6. Describe the software used for preprocessing. For T1 data: FreeSurfer version 5.1 was used for volumetrics (page 28-29)
For diffusion data: ExploreDTI version 4.8.6 was used to analyze the data and elastix (provided within ExploreDTI) was used to perform diffeomorphic registrations of atlas to subject space (page 29).
7. Normalization  
   a. If data were normalized/standardized, describe the approach(es).
   
   T1 data was intensity normalized using FreeSurfer’s `mri_normalize` script (page 29).  
   DTI data was corrected for echo-planar imaging susceptibility by applying an affine registration to the skull-stripped T1 image of FreeSurfer (this step includes B-matrix rotation to preserve correct diffusion gradient rotation) See page 28

   b. Describe the template used for normalization/transformation.
   
   All data was kept in subject-specific space, no templates were used. Note that for FreeSurfer, volumes are defined in several steps during which a high-dimensional non-linear registration is done to the MNI305 template to increase the accuracy of the segmentation and to facilitate the segmentation and tissue-probability assessment for region labeling. Volumes of the hippocampus are ultimately extracted from a subject-specific subcortical volumetric atlas (aseg) in 1mm isotropic subject-space. For the diffusion analyses, we used the JHU Mori atlas template for tract labeling, but tracts of interests were warped on each subject’s data to not affect the gradient orientation. These tracts of interests were warped using diffeomorphic registration methods (see page 28-29).

8. Describe your procedure for artifact and structured noise removal.

   The standard preprocessing pipeline of FreeSurfer incorporates motion correction, intensity correction and skull-stripping (page 29-30).  
   Diffusion data was corrected for eddy-current distortions, subject motion and echo-planar imaging susceptibility. Additionally, to correct for partial volume effects, images were thresholded at a FA-value of 0.20 (during the statistical analyses, additional correction was done by correcting for tract size). Motion correction, eddy current correction and handling of outliers was done with the REKINDLE robust non-linear least-squares estimation method. The correction for echo-planar imaging susceptibility was performed by applying an affine registration to the skull-stripped T1 image (including B-matrix rotation to preserve correct diffusion gradient rotation) (see page 28-29).

9. Define your software and/or method and criteria for volume censoring, and state the extent of such censoring.

   NA, no functional MRI data was included

### Statistical modeling & inference

10. Define your model type and settings.

   All statistical analyses were done in R, not in the imaging software packages. Subject-specific values were extracted from predefined ROIs and group analyses were performed in R. We have performed regression analyses (robust regressions and mixed effects models for longitudinal data). See page 33-36

11. Specify the precise effect tested.

   Associations between amyloid status and hippocampal volume were determined cross-sectionally and longitudinally. Associations between hippocampal volume and tract integrity were assessed cross-sectionally and longitudinally. No ANOVA or factorial designs were used, but robust regressions and mixed effects models for longitudinal analyses were performed. See page 33-36
12. Analysis  
   a. Specify whether analysis is whole brain or ROI-based.  
      ROI-based in which a limited set of ROIs (1 for structural data (hippocampus) and 2 for diffusion data (hippocampal cingulum bundle and uncinate fasciculus)) were chosen based on the hypotheses and model (figure 1, page 6; the rationale behind this model and these ROIs is provided on page 4-5)  
   b. If ROI-based, describe how anatomical locations were determined.  
      All ROIs were chosen based on the model in figure 1.  
      FreeSurfer: a subject-independent probabilistic atlas (hand labeled atlas by neuroanatomists on a training set) provides subject-specific measured values. Subject-specific segmentations of the hippocampus are based on these probabilities and on the white and pial surfaces. Hippocampal volumes, corrected for intracranial volume, were used in the analyses. Page 30  
      For diffusion data: masks of the tracts were based on the Johns Hopkins University Mori atlas. Tracts in this atlas were based on fiber orientations and hand segmentations on diffusion images. The atlas and tracts were registered to each subject using diffeomorphic methods. Page 29  

13. State the statistic type for inference. (See Eklund et al. 2016.)  
   NA, structural data with analyses limited to specific ROIs  

14. Describe the type of correction and how it is obtained for multiple comparisons.  
   No statistical adjustments were done as the analyses were planned in the hypothesized model (this is indicated on page 36). We controlled these analyses by including control regions for the tract analyses (uncinate fasciculus), the tau analyses (inferior temporal cortex was control region) and the cognitive domain (executive function as control domain) as explained on page 4-5. Furthermore, the diffusion analyses were complementary as shown in the consistency in all the diffusion metrics outcomes (the chance for a type I error decreases when tests are positively correlated; the absolute diffusivity metrics are highly positively correlated, see correlations below Table 1 (r=0.96-0.99)). The results of the absolute diffusivities point in all analyses in the same direction.  

15. Connectivity  
   a. For functional and/or effective connectivity, report the measures of dependence used and the model details.  
      NA  
   b. For graph analysis, report the dependent variable and functional connectivity measure.  
      NA  

16. For multivariate modeling and predictive analysis, specify independent variables, features extraction and dimension reduction, model, training and evaluation metrics.  
   NA