

Published in final edited form as:

*Ann Neurol.* 2011 October ; 70(4): 657–661. doi:10.1002/ana.22509.

## Amyloid- $\beta$ associated volume loss occurs only in the presence of phospho-tau

Rahul S. Desikan, MD, PhD<sup>1</sup>, Linda K. McEvoy, PhD<sup>1</sup>, Wes Thompson, PhD<sup>3</sup>, Dominic Holland, PhD<sup>2</sup>, J. Cooper Roddey, PhD<sup>2</sup>, Kaj Blennow, MD, PhD<sup>4</sup>, Paul S. Aisen, MD<sup>2</sup>, James B. Brewer, MD, PhD<sup>1,2</sup>, Bradley T. Hyman, MD, PhD<sup>5</sup>, Anders M. Dale, PhD<sup>1,2</sup>, and the Alzheimer's Disease Neuroimaging Initiative\*

<sup>1</sup>Department of Radiology, University of California, San Diego, La Jolla, CA, USA

<sup>2</sup>Department of Neuroscience, University of California, San Diego, La Jolla, CA, USA

<sup>3</sup>Department of Psychiatry, University of California, San Diego, La Jolla, CA, USA

<sup>4</sup>Clinical Neurochemistry Laboratory, The Sahlgrenska Academy at Göteborg University, Mölndal, 40530, Gotheburg, Sweden

<sup>5</sup>Department of Neurology, Massachusetts General Hospital, Boston, MA, USA

### Abstract

The relationship between neurodegeneration and the two hallmark proteins of Alzheimer's disease, amyloid- $\beta$  ( $A\beta$ ) and tau, is still unclear. Here, we examined 286 non-demented participants (107 cognitively normal older adults and 179 memory impaired individuals) who underwent longitudinal MR imaging and lumbar puncture. Using mixed effects models, we investigated the relationship between longitudinal entorhinal cortex atrophy, CSF p-tau<sub>181p</sub> and CSF  $A\beta_{1-42}$ . We found a significant relationship between elevated entorhinal cortex atrophy and decreased CSF  $A\beta_{1-42}$  only with elevated CSF p-tau<sub>181p</sub>. Our findings indicate that  $A\beta$ -associated volume loss occurs only in the presence of phospho-tau in humans at risk for dementia.

### INTRODUCTION

A fundamental tenet of the amyloid hypothesis is that amyloid- $\beta$  ( $A\beta$ ) deposition triggers the neurodegenerative process underlying Alzheimer's disease (AD) <sup>1</sup>. The presence of  $A\beta$  initiates downstream loss of dendritic spines and synapses <sup>2</sup> and functional disruption of neuronal networks <sup>3</sup>. However in humans, amyloid plaques are present in cognitively normal older adults <sup>4</sup>, correlate poorly with memory decline <sup>5</sup> and immunotherapy-induced plaque removal may not prevent progressive neurodegeneration <sup>6</sup> suggesting that other entities may be required for AD-related degeneration. Recent studies using mouse models show that tau reduction can block  $A\beta$ -induced cognitive impairment <sup>7</sup> and tau potentiates  $A\beta$ -associated

Correspondence should be addressed to: Dr. Anders M. Dale Department of Radiology University of California, San Diego 8950 Villa La Jolla Drive, Suite C101 La Jolla, CA, USA 92037-0841 amdale@ucsd.edu Phone: (858)-822-6671 Fax: (858)-534-1078.

\*Data used in the preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database ([www.loni.ucla.edu/ADNI](http://www.loni.ucla.edu/ADNI)). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in analysis or writing of this report. ADNI investigators include (complete listing available at [http://adni.loni.ucla.edu/wp-content/uploads/how\\_to\\_apply/ADNI\\_Authorship\\_List.pdf](http://adni.loni.ucla.edu/wp-content/uploads/how_to_apply/ADNI_Authorship_List.pdf))

#### Disclosure Statement

Dr. Anders M. Dale is a founder and holds equity in CorTechs Labs, Inc, and also serves on the Scientific Advisory Board. The terms of this arrangement have been reviewed and approved by the University of California, San Diego in accordance with its conflict of interest policies.

Dr. Linda K. McEvoy's spouse is CEO of CorTechs Labs, Inc.

synaptic toxicity<sup>8</sup>. Though these experimental findings suggest a link between tau and A $\beta$ , it is unknown whether tau (specifically, phospho-tau or p-tau) interacts with A $\beta$  to effect dendritic and synaptic degeneration in humans at risk for AD.

In humans, structural MRI and CSF biomarkers allow for the indirect assessment of the cellular changes underlying AD *in vivo*. Structural MRI provides measures of cerebral atrophy, which reflects loss of dendrites and synapses<sup>9</sup>. Low CSF levels of A $\beta$  strongly correlate with high binding of the amyloid PET ligand Pittsburgh Compound B (PiB)<sup>10</sup> and with increased intracranial plaques<sup>11</sup>. High concentrations of CSF p-tau correlate with AD-specific neurofibrillary pathology<sup>12</sup> and elevated total tau correlates with neuronal and axonal damage<sup>13</sup>. Here, we investigated whether an interaction between increased CSF p-tau<sub>181p</sub> and decreased CSF A $\beta$ <sub>1-42</sub> is associated with increased cerebral atrophy over time in non-demented older individuals at risk for developing AD.

## SUBJECTS AND METHODS

Selection of participants and analysis methods for MRI and CSF biomarkers are briefly summarized here, with details provided in Supplemental Information.

We evaluated healthy older controls (HC, n = 107) and individuals diagnosed with amnesic mild cognitive impairment (MCI, n = 179) from the Alzheimer's Disease Neuroimaging Initiative. Using recently proposed CSF cutoffs<sup>14</sup>, we classified all participants based on high (>23 pg/ml, "positive") and low (<23 pg/ml, "negative") ptau<sub>181p</sub> levels and based on low (<192 pg/ml, "positive") and high (>192 pg/ml, "negative") A $\beta$ <sub>1-42</sub> levels (Table 1). We restricted participants to those with CSF data and quality-assured baseline and follow-up MRI scans (6 months to 3.5 years, mean of 2.14 years, standard deviation 0.75 years) available as of January 2011.

We examined 1,177 T<sub>1</sub>-weighted MRI scans. We performed quantitative surface-based analysis of all MRI scans using an automated region-of-interest labeling technique and focused on the entorhinal cortex, a medial temporal lobe region that is selectively affected in the earliest stages of AD<sup>5</sup>. We additionally examined other neuroanatomic regions affected in later stages of the disease process (see Supplemental Methods and Results). Using a recently developed method from our laboratory<sup>15</sup>, we assessed longitudinal sub-regional change in gray matter volume (atrophy) on serial MRI scans.

We asked whether a statistical interaction between CSF p-tau<sub>181p</sub> and CSF A $\beta$ <sub>1-42</sub> status is associated with entorhinal cortex atrophy over time (Figure 1). We examined the main and interactive effects of CSF p-tau<sub>181p</sub> and CSF A $\beta$ <sub>1-42</sub> on entorhinal cortex atrophy while controlling for age, sex, group status (MCI vs. HC), and disease severity (CDR-Sum of Boxes), using the following mixed effects model:  $\Delta v = \beta_0 + \beta_1 \text{CSF\_p-tau}_{181p\_status} \times \Delta t + \beta_2 \text{CSF\_A}\beta_{1-42\_status} \times \Delta t + \beta_3 [\text{CSF\_ptau}_{181p\_status} \times \text{CSF\_A}\beta_{1-42\_status} \times \Delta t] + \text{covariates} \times + \epsilon$ . Here,  $\Delta v$  = entorhinal cortex atrophy (millimeters<sup>3</sup>) and  $\Delta t$  = change in time from baseline MRI scan (years). We also investigated whether an interaction between increased CSF ptau<sub>181p</sub> and decreased CSF A $\beta$ <sub>1-42</sub> is associated with longitudinal clinical decline as assessed with the Alzheimer's Disease Assessment Scale-cognitive subscale (ADAS-cog) (see Supplemental Results).

## RESULTS

Across all participants, the interaction between CSF p-tau<sub>181p</sub> and CSF A $\beta$ <sub>1-42</sub> status on entorhinal cortex atrophy was significant ( $\beta_3 = -0.01$ , standard error (SE) = 0.004,  $p = 0.002$ ) indicating elevated atrophy over time in individuals with positive CSF p-tau<sub>181p</sub> and positive CSF A $\beta$ <sub>1-42</sub> status (Figure 2a). With this interaction term in the model, neither the main

effect of CSF p-tau<sub>181p</sub> nor CSF A $\beta$ <sub>1-42</sub> was significantly different from zero. Follow-up analyses demonstrated that CSF A $\beta$ <sub>1-42</sub> status was associated with elevated entorhinal cortex atrophy only among CSF p-tau<sub>181p</sub> positive individuals ( $\beta$ -coefficient = -0.01, SE = 0.003,  $p$  = 0.0002) (Figure 2b). There was no association between CSF A $\beta$ <sub>1-42</sub> status and entorhinal cortex atrophy among CSF p-tau<sub>181p</sub> negative individuals ( $\beta$ -coefficient = -0.0007, SE = 0.002,  $p$  = 0.75). Similar results were obtained when CSF p-tau<sub>181p</sub> and CSF A $\beta$ <sub>1-42</sub> were treated as continuous rather than categorical variables (see Supplemental Results).

Subgroup analyses within the MCI cohort demonstrated that CSF A $\beta$ <sub>1-42</sub> status was associated with elevated entorhinal cortex atrophy only among CSF p-tau<sub>181p</sub> positive individuals ( $\beta$ -coefficient = -0.02, SE = 0.0066,  $p$  = 0.003). There was no association between CSF A $\beta$ <sub>1-42</sub> status and entorhinal cortex atrophy among CSF p-tau<sub>181p</sub> negative MCI individuals ( $\beta$ -coefficient = -0.005, SE = 0.004,  $p$  = 0.30) (Table 1a).

Consistent with the MCI results, subgroup analyses within the HC cohort demonstrated that CSF A $\beta$ <sub>1-42</sub> status was associated with entorhinal cortex atrophy only in CSF p-tau<sub>181p</sub> positive individuals ( $\beta$ -coefficient = -0.009, SE = 0.003,  $p$  = 0.007). There was no association between CSF A $\beta$ <sub>1-42</sub> status and entorhinal cortex atrophy among CSF p-tau<sub>181p</sub> negative HC individuals ( $\beta$ -coefficient = 0.002, SE = 0.002,  $p$  = 0.32) (Table 1b).

Supplementary analyses demonstrated a similar pattern of results in regions affected later in the disease process, with CSF A $\beta$ <sub>1-42</sub> status associated with increased atrophy only among CSF p-tau<sub>181p</sub> positive individuals. Similarly, CSF A $\beta$ <sub>1-42</sub> status was associated with a worsening ADAS-cog score over time only among CSF p-tau<sub>181p</sub> positive individuals (See Supplemental Results).

We also examined the possibility that a nonspecific form of tau, total tau (t-tau), which correlates strongly with p-tau (Spearman's  $\rho$  = 0.82,  $p$  < 0.001), interacts with A $\beta$  to influence AD-associated entorhinal cortex atrophy. We classified all participants based on high ("positive") and low ("negative") t-tau levels using a CSF cutoff value of 93 pg/ml<sup>14</sup>. Using the same mixed effects framework described above, we found that neither the main effect of CSF t-tau status nor its interaction with CSF A $\beta$ <sub>1-42</sub> significantly differed from zero. Treating t-tau and A $\beta$ <sub>1-42</sub> as continuous variables did not change these results.

## DISCUSSION

Here, we show for the first time that in non-demented humans at risk for AD, A $\beta$ -associated entorhinal cortex degeneration occurs only in the presence of p-tau. In the absence of p-tau, the effect of A $\beta$  on entorhinal cortex volume loss is not significantly different from zero. Our results also indicate that A $\beta$  is associated with accelerated clinical decline only in the presence of p-tau.

Though several cross-sectional studies have found a significant relationship between A $\beta$  deposition and cortical thickness<sup>18, 19</sup>, the role of p-tau in modulating this relationship has not been examined. Our longitudinal data indicate that in non-demented individuals A $\beta$  deposition by itself is not associated with neurodegeneration; the presence of p-tau likely represents a critical link between A $\beta$  deposition and accelerated volume loss in the entorhinal cortex and other AD-vulnerable regions.

These findings point to p-tau as an important marker of AD-associated neurodegeneration. Though CSF p-tau and t-tau are highly correlated, p-tau may represent a more specific marker of the disease process. Elevations in CSF t-tau are seen in a number of neurologic disorders characterized by neuronal and axonal damage whereas increased CSF p-tau correlates with cognitive decline in MCI, increased neurofibrillary pathology in AD, and can

distinguish AD from other neurodegenerative diseases.<sup>20</sup> Our results are consistent with these prior observations and indicate that the interaction between p-tau and A $\beta$  likely reflects underlying AD pathobiology.

Recent work using neuronal cultures and mouse models suggests that a synergistic interaction between tau and A $\beta$  effects neurodegeneration. Specifically, A $\beta$  triggers tau hyperphosphorylation<sup>16</sup> leading to tau dissociation from microtubules and increased tau accumulation in the somatodendritic compartment<sup>8</sup>. Accumulated tau interacts with tyrosine protein kinases, such as FYN, leading to sensitization of NMDA receptors and rendering neurons susceptible to A $\beta$ -associated toxicity.<sup>17</sup> Our human findings are consistent with this experimentally-derived pathobiologic cascade.

Limitations of our study include its observational nature, which precludes conclusions regarding causation and the use of CSF biomarkers, which provide an indirect assessment of amyloid and neurofibrillary pathology. Finally, we primarily focused on CSF biomarkers of the two pathologic hallmarks of AD. Additional markers, such as the e4 allele of apolipoprotein E (APOE e4) or other p-tau isoforms, may also interact with A $\beta$  to predict entorhinal cortex volume loss.

From a clinical perspective, this work illustrates the importance and feasibility of testing hypotheses generated from experimental models in humans. Although substantial progress has been made in understanding the cellular and molecular mechanisms of neurodegeneration, problems arise in translating these findings into the clinical setting. Our results indicate that an analytic framework that integrates multiple biomarkers to test experimental hypotheses in living individuals may help bridge the translational divide between basic research and clinical medicine. By elucidating various aspects of AD biology, the combination of longitudinal MRI and CSF biomarkers may additionally be useful for detecting and quantifying the biochemical effects of therapeutic interventions.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

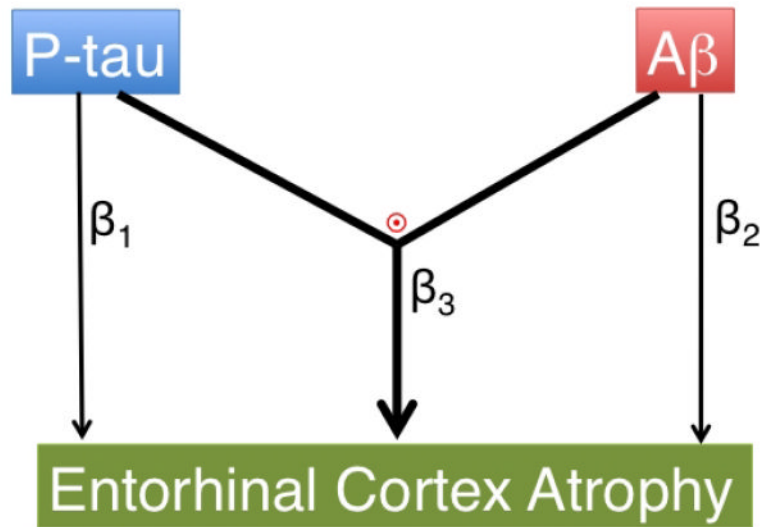
## Acknowledgments

The authors would like to thank Roberto Malinow, Edward Koo, and Anne Fagan for helpful discussions. This research was supported by grants from the National Institutes of Health (R01AG031224; K01AG029218; K02 NS067427; T32 EB005970). Data collection and sharing for this project was funded by the Alzheimer's Disease Neuroimaging Initiative (ADNI) (National Institutes of Health Grant U01 AG024904). ADNI is funded by the National Institute on Aging, the National Institute of Biomedical Imaging and Bioengineering, and through generous contributions from the following: Abbott, AstraZeneca AB, Bayer Schering Pharma AG, Bristol-Myers Squibb, Eisai Global Clinical Development, Elan Corporation, Genentech, GE Healthcare, GlaxoSmithKline, Innogenetics, Johnson and Johnson, Eli Lilly and Co., Medpace, Inc., Merck and Co., Inc., Novartis AG, Pfizer Inc, F. Hoffman-La Roche, Schering-Plough, Synarc, Inc., and Wyeth, as well as non-profit partners the Alzheimer's Association and Alzheimer's Drug Discovery Foundation, with participation from the U.S. Food and Drug Administration. Private sector contributions to ADNI are facilitated by the Foundation for the National Institutes of Health ([www.fnih.org](http://www.fnih.org)). The grantee organization is the Northern California Institute for Research and Education, and the study is coordinated by the Alzheimer's Disease Cooperative Study at the University of California, San Diego. ADNI data are disseminated by the Laboratory for Neuro Imaging at the University of California, Los Angeles. This research was also supported by NIH grants P30 AG010129, K01 AG030514, and the Dana Foundation.

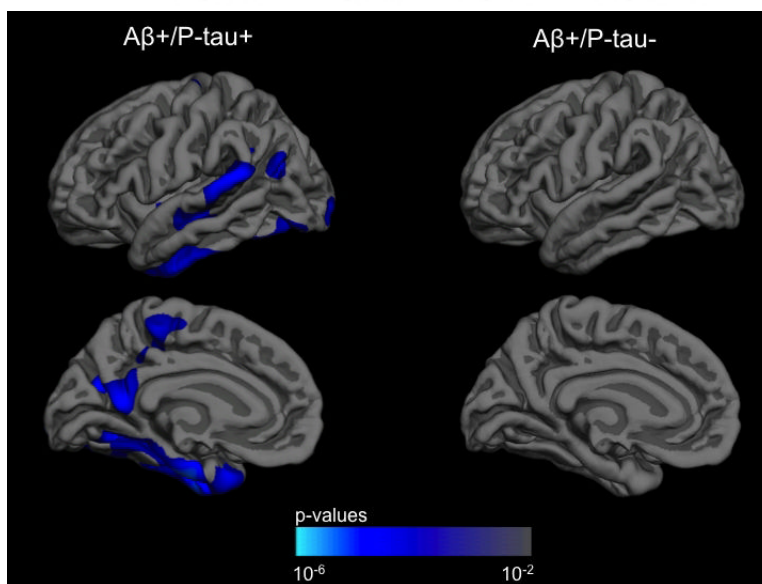
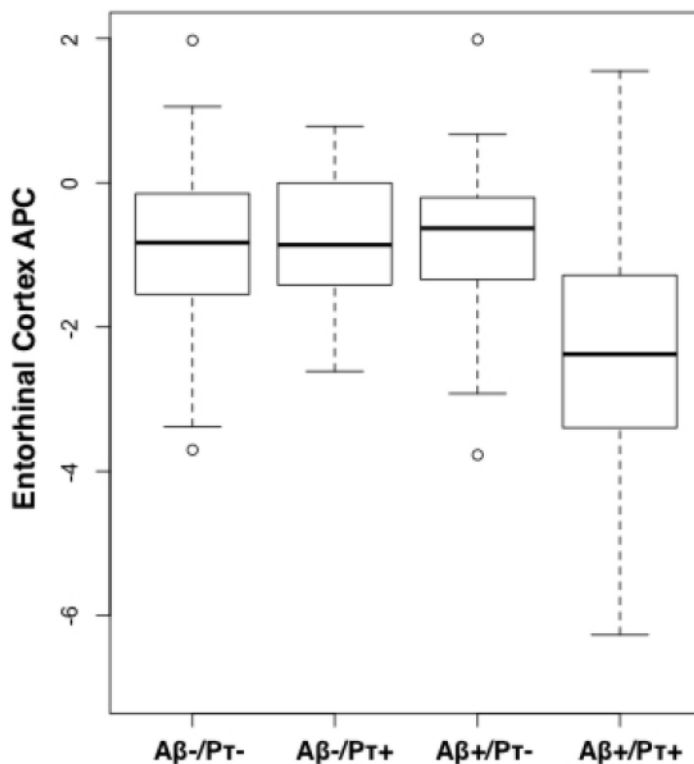
## References

1. Hardy J, Selkoe DJ. *Science*. 2002; 297:353–6. [PubMed: 12130773]
2. Spiros-Jones TL, et al. *Am J Pathol*. 2007; 171:1304–11.
3. Kuchibhotla KV, et al. *Neuron*. 2008; 59:214–225. [PubMed: 18667150]

4. Crystal H, et al. *Neurology*. 1988; 38:1682–7. [PubMed: 3185902]
5. Arriagada PV, et al. *Neurology*. 1992; 42:631–9. [PubMed: 1549228]
6. Holmes C, et al. *Lancet*. 2008; 372:216–23. [PubMed: 18640458]
7. Roberson ED, et al. *Science*. 2007; 316:750–4. [PubMed: 17478722]
8. Ittner LM, et al. *Cell*. 2010; 142:387–97. [PubMed: 20655099]
9. Freeman SH, et al. *J Neuropathol Exp Neurol*. 2008; 67:1205–12. [PubMed: 19018241]
10. Fagan AM, et al. *Ann Neurol*. 2006; 59:512–519. [PubMed: 16372280]
11. Strozyk D, et al. *Neurology*. 2007; 60:652–6. [PubMed: 12601108]
12. Buerger K, et al. *Brain*. 2006; 129:3035–41. [PubMed: 17012293]
13. Hesse C, et al. *J Alzheimer Disease*. 2000; 2:199–206.
14. Shaw LM, et al. *Ann Neurol*. 2009; 65:403–13. [PubMed: 19296504]
15. Holland D, Dale AM. *Med Image Anal*. Feb 23.2011
16. Jin M, et al. *Proc Natl Acad Sci USA*. 2011; 108:5819–24. [PubMed: 21421841]
17. Ittner LM, Gotz J. *Nat Rev Neurosci*. 2011; 12:65–72. [PubMed: 21193853]
18. Mormino EC, et al. *Brain*. 2009; 132:1310–23. [PubMed: 19042931]
19. Becker JA, et al. *Ann Neurol*. Nov 17.2010
20. Hampel H, et al. *Exp Gerontol*. 2010; 45:30–40. [PubMed: 19853650]



**Figure 1.** Diagrammatic representations of the primary hypotheses evaluated in the current study: ( $\beta_1$ ) main effect of p-tau on entorhinal cortex atrophy, ( $\beta_2$ ) main effect of A $\beta$  on entorhinal cortex atrophy, and ( $\beta_3$  and  $\odot$ ) an interactive effect between p-tau and A $\beta$  is associated with entorhinal cortex atrophy.



**Figure 2.** (a) Box and whisker plots for all participants illustrating entorhinal cortex atrophy rate, measured as annualized percent change (APC), based on CSF  $A\beta_{1-42}$  ( $A\beta$ ) and CSF p-tau<sub>181p</sub> ( $P\tau$ ) status for all participants. For each plot, thick black lines show the median value. Regions above and below the black line show the upper and lower quartiles, respectively. The dashed lines extend to the minimum and maximum values with outliers shown as open circles. As illustrated, the  $A\beta +/P\tau +$  individuals demonstrated the largest cortical atrophy rate (i.e. more negative percent change). (b) ANCOVA results for all participants displayed across the lateral and medial hemispheres of the cerebral cortex. The

cortex-wide ANCOVA co-varied for age, gender, group status (MCI vs. HC) and CDR-SB. The strongest association between CSF  $A\beta_{1-42}$  status and cortical atrophy rate, measured as annualized percent change, was observed in the entorhinal/medial temporal cortex and present only among p-tau positive individuals. Among p-tau negative individuals, CSF  $A\beta_{1-42}$  status was not associated with rate of cortical atrophy. For illustration, only the left hemisphere is visualized.



**Table 1a**

Demographic, clinical, and imaging data for all Mild Cognitive Impairment (MCI) individuals in this study. MMSE = Mini-mental status exam, CDR-SB = Clinical Dementia Rating-Sum of Boxes score, APC = Annualized Percent Change, SE = Standard error of the mean, CI = Confidence Interval

	<b>A<math>\beta</math> -/P-tau - (n = 36)</b>	<b>A<math>\beta</math> -/P-tau + (n = 9)</b>	<b>A<math>\beta</math> +/P-tau - (n = 15)</b>	<b>A<math>\beta</math> +/P-tau + (n = 119)</b>
Age, Mean (SE)	74.2 (1.4)	80.4 (1.9)	76.9 (1.5)	74.1 (0.6)
Female, %	24	29	31	38
Education Years, Mean (SE)	15.9 (0.5)	16.1 (1.1)	16.5 (0.7)	15.6 (0.3)
MMSE, Mean (SE)	27.3 (0.3)	25.8 (0.6)	27.0 (0.5)	26.8 (0.2)
CDR-SB, Mean (SE)	1.3 (0.1)	1.3 (0.2)	1.8 (0.3)	1.6 (0.1)
Years Between MRI Scans, Mean (SE)	2.0 (0.1)	1.8 (0.1)	2.1 (0.2)	2.1 (0.1)
Entorhinal Cortex APC, Mean (95% CI)	-1.4 (-1.0, -1.8)	-1.1 (0.1, -2.3)	-1.1 (-0.3, -1.9)	-2.6 (-2.4, -2.8)

**Table 1b**

Demographic, clinical, and imaging data for all healthy older controls (HC) in this study. MMSE = Mini-mental status exam, APC = Annualized Percent Change, SE = Standard error of the mean, CI = Confidence Interval

	<b>A<math>\beta</math> -/P-tau - (n = 46)</b>	<b>A<math>\beta</math> -/P-tau + (n = 19)</b>	<b>A<math>\beta</math> +/P-tau - (n = 20)</b>	<b>A<math>\beta</math> +/P-tau + (n = 21)</b>
Age, Mean (SE)	74.3 (0.6)	78.0 (1.4)	74.9 (1.1)	78.2 (1.0)
Female, %	24	29	31	38
Education Years, Mean (SE)	15.5 (0.4)	15.5 (0.4)	14.8 (0.8)	16.7 (0.6)
MMSE, Mean (SE)	29.1 (0.1)	28.8 (0.3)	29.1 (0.2)	29.3 (0.2)
Years Between MRI Scans, Mean (SE)	2.3 (0.1)	2.3 (0.2)	2.3 (0.1)	2.4 (0.2)
Entorhinal Cortex APC, Mean (95% CI)	-0.6 (-0.4, -0.8)	-0.6 (-0.2, -1.0)	-0.6 (-0.2, -1.0)	-1.2 (-0.8, -1.6)