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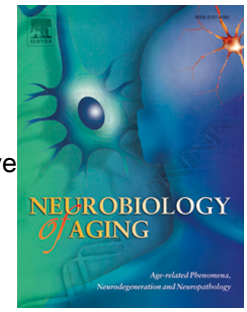

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Braak staging, plaque pathology and APOE status in elderly persons without cognitive impairment

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Abstract

Clinico-pathological studies reveal that some elderly people with no cognitive impairment have high burdens of neurofibrillary tangles (NFTs) a pathology associated with Alzheimer's disease. We examined a total of 123 elderly participants without dementia and free of other neurological disorders or pathologies who at autopsy were classified as Braak NFT stages of I-V. We found that females were significantly more likely to have a high Braak score. Significant associations were found between high Braak scores and entorhinal cortex amyloid load, combined hippocampal and entorhinal cortex amyloid loads with perceptual speed in the low Braak group after adjusting for age, gender and APOE ε4 status. Elderly with preserved cognitive function show a wide range of Braak scores and plaque pathology similar to that seen in prodromal and frank AD at death. These data suggest that some older people with extensive NFT and plaque pathology demonstrate brain resilience or reserve leading to preserved cognitive function.

Key words: Aging, amyloid, Alzheimer's, dementia, neurofibrillary tangles, neuropathology

Highlights:

1. Braak stage failed to correlate with cognition in non-demented elders.
2. Elderly with preserved cognition show a range of Braak scores.
3. Elderly with extensive NFT and plaque pathology demonstrate brain resilience, reserve or plasticity

Introduction

Elderly people without dementia accumulate Alzheimer's disease (AD) neuropathology (Bennett et al., 2002; Guillozet et al., 2003; Markesbery, 2010; Morris & Price, 2001; Morris et al., 2009; Tomlinson et al., 1970), but the association with premortem cognitive function remains relatively unexplored. Braak and Braak (1991) proposed a neuropathological staging to differentiate initial, intermediate and advanced AD based upon the spread of neurofibrillary tangles (NFTs) within the medial temporal lobe (MTL) memory circuit: Braak Stage 0 corresponds to absence of NFTs, Stages I–II to entorhinal-perirhinal cortex NFTs, Stages III–IV to NFTs additionally in hippocampus and Stages V–VI to NFTs distributed in wider neocortical areas. Although previous studies evaluated the association between the Consortium to Establish a Registry in Alzheimer's Disease (CERAD) (Mirra et al., 1991) and NIA Reagan AD (The National Institute on Aging, 1997) pathological criteria and clinical findings in older people without cognitive impairment (Bennet et al., 2002; Guillozet et al., 2003; Morris & Price, 2001; Morris et al., 2009), there is limited detailed information relating Braak staging and neurocognitive measures (Bennett et al., 2002; Erten-Jones et al., 2009; Gold et al., 2000; Guillozet et al 2003; Markesbery, 2010; Nelson et al., 2009) in this population.

We have been conducting clinical pathobiological studies on the onset of dementia in the elderly (Mufson et al., 1999; Mufson et al., 2014) using tissue obtained from the Rush Religious Orders Study (RROS), a longitudinal clinicopathologic investigation of aging and AD (Bennett et al., 2005; Mufson et al., 1999; Mufson et al, 2012a; Mufson et al, 2014). Subjects with a premortem clinical diagnosis of no cognitive impairment (NCI) were classified postmortem with a wide range of Braak scores (I-V) (Gilmor et al, 1999; Mufson et al, 1999; Mufson et al, 2012b; Perez

et al, 2012). To our knowledge there is not a detailed evaluation between NFTs based on Braak scoring, cognitive status and plaque load in non-demented subjects. Therefore, we performed a cross sectional analysis of our NCI individuals grouped by Braak stage and compared these findings with change in various cognitive domains and plaque load values.

Methods

Braak staging and cognitive status were examined in a 123 older deceased and autopsied subjects with no cognitive impairment and no coexisting clinical or neurological condition judged to be contributing to cognitive impairment at the last evaluation (Bennett et al., 2005; Mufson et al., 1999) who agreed to annual clinical evaluations and signed an informed consent and an Anatomic Gift Act donating their brains at time of death (see Table 1). Cases have been used in a large number of clinical pathological investigations supported by our ongoing NIA program project grant (PPG) entitled the "Neurobiology of Mild Cognitive Impairment in the Elderly" (AG14449) over the last almost twenty years. Cases were chosen from all RROS brains that came to autopsy, which has a rolling admission (see Bennett et al., 2006). Subjects were selected using s stringent set of exclusion criteria; no large strokes, cerebral vascular disease, Lewy body disease, Parkinson's disease, frontal temporal dementia, mixed pathologies or any condition that contributed to dementia). The pathological methods employed to determine these conditions have been reported previously (Bennett et al., 2004; Bennett et al., 2005; Bennett et al., 2006). In addition, those taking anticholinesterases or medication for depression were also excluded from our studies. The average interval from last evaluation to brain autopsy was 0.73 ± 0.79 years. The Human Investigation Committee of Rush University Medical Center approved the study.

Clinical Evaluation

Participants underwent annual uniform, structured, clinical evaluation and self-report medical history obtained by a team led by a neurologist and annual cognitive function was determined by a trained neuropsychological test technician (Bennett et al., 2002; Bennett et al, 2005; Mufson et al., 1999; Schneider et al., 2009a). All medications used by the participants within the previous two weeks of the examination were reviewed and classified. After review of all clinical data and examination of the participant, a board certified neurologist or geriatrician with expertise in the evaluation of elderly persons with dementia made a final diagnosis. Diagnostic classification of no cognitive impairment was performed as previously described (Bennett et al., 2006; Schneider et al., 2009b). After death, a neurologist reviewed the medical history, medication use, neurologic examination and results of cognitive performance testing as well as the neuropsychologist's opinion of cognitive impairment and dementia to render a final clinical diagnosis blinded to pathology data. Each participant was evaluated in his/her home, emphasizing findings deemed clinically relevant.

Cognitive Domain Composite Scores

Composite scores are based on the results of 17 individual cognitive tests divided into five domains of cognition as reported previously (Bennett et al., 2002; Bennett et al., 2005; Bennett et al., 2006; Wilson et al., 2011). Mini-Mental State Examination (MMSE) was used to describe the cohort but not used in the composite scores. Briefly, episodic memory was evaluated with tests including immediate and delayed recall of story A from Logical Memory and of the East Boston Story, and Word List Memory, Recall, and Recognition from the Consortium to Establish a Registry for AD (CERAD). Semantic memory was assessed with three tests including a 15-item

version of the Boston Naming Test, Verbal Fluency, which involves naming examples of semantic categories (i.e., animals, vegetables) in 1-min trials; and a reading test that involves reading single words aloud and a 10-item reading test. Scores on the 3 tests are converted to a standard scale and averaged to get the composite score. Working memory was assessed using Digit Span Forward and Backward and Digit Ordering. Two tests of perceptual speed included Symbol Digit Modalities Test, and Number Comparison. Finally, two tests of visuospatial ability included a 15-item version of Judgment of Line Orientation and a 9-item version of Standard Progressive Matrices (Wilson et al., 2011). For each test, raw scores were converted into z-scores based on the mean and standard deviation of the sample. The z-scores from the individual tests were averaged to create individual domain composite scores. The Global Composite Score (GCS) is an average of 17 individual domain z-scores.

Tissue Preparation and Neuropathological Diagnosis

Brains accruement and processing was described previously (Mufson et al., 1999; Perez et al., 2015; Schneider et al., 2009a) (see Table). Briefly, one hemisphere of the brain was cut into 1 cm thick coronal slabs using a brain slice apparatus and immersion fixed in 4% paraformaldehyde for at least 72 hours). Tissue blocks including the midfrontal cortex, middle or superior temporal cortex, entorhinal cortex, hippocampus, inferior parietal cortex were paraffin embedded and cut at 6 µm. Examination for cerebral infarctions was conducted on these fixed slabs (Bennett et al., 2006). Bielschowsky stain was used to visualize neuritic plaques (NPs), diffuse plaques (DPS) and NFTs (Schneider et al., 2009b). Paired helical filament tau (AT8; 1:800, Covance) immunohistochemistry (Bennett et al., 2004) was also used to label NFTs and for quantitation (see below). Additional blocks were used to collect data on amyloid load from hippocampus and entorhinal cortex. Sections were stained for amyloid load using amyloid-β antibodies (6F/3D,

1:50; DAKO, CA and 4G8, 1:9000, Covance, WI) (Bennett et al., 2004; Markesbery, 2010). Neuropathological diagnoses were made according to CERAD (Mirra et al., 1991) and Braak score (Braak & Braak, 1991) as recommended by the National Institute on Aging (NIA)-Reagan (National Institute on Aging, 1997) criteria (Bennett et al., 2006; Mufson et al., 1999; Schneider et al., 2007).

Pathologic Quantitation

A board-certified neuropathologist or trained technician blinded to all clinical data counted total number of NPs, DPs, and NFTs in one square mm area (100x magnification) per cortical region (see Bennett et al., 2005a, Bennett et al., 2005b). Quantitation of amyloid load was performed using a scheme to capture images of amyloid beta stained sections employing a custom algorithm as previously described (Mitchell et al., 2000). In brief, following camera and illumination calibration, 24-bit color images obtained at each sampling site were converted to 8-bit gray scale images. Calculation of percent area occupied by immunopositive amyloid-β pixels was carried out using the public domain Object-Image 1.62p15 (Norbert Vischer;*http://simon.bio.uva.nl/object-image.html*). The analysis algorithm segmented labeled images and background compartments using 1 of 2 histogram-dependent automatic thresholding methods (Iterative Self-Organizing Data Analysis) and triangulation. The percent areas for each section were averaged and the number used for analyses. Since the distribution of plaques and tangles was not normally distributed, standardized plaque and tangle count from each area were converted to standard scores by dividing the SD of mean raw counts per marker and region from the entire deceased cohort. Scaled scores for NPs and DPs, and NFTs for each region was averaged across the four brain regions examined to develop a summary AD pathology score for

each subject. Cronbach's coefficient alpha, a measure of internal consistency, was 0.90 for the 12 postmortem indices, supporting the formation of the global measure of AD pathology.

Since the cohort of RROS NCI subjects we examined did not contain Braak stage VI cases, we operationally divided the present cohort in three different ways: First, the cohort was divided as low 0 - II to high=III – V Braak stages, where the intermediate stage III was included in the high Braak group. Alternatively, we used the more conventional grouping of low 0-II, medium=III-IV and high=V-VI (Braak and Braak, 1991). Finally, we compared low (I-II) and high (IV-V) with the intermediate stage III cases. The first low/high grouping was our primary approach, the additional cohort splitting allowed for a determination of whether stage III subjects would affect the interaction with cognitive test scores.

Statistical Analysis

Shapiro-Wilk test determined normality of cognitive domains/test data, demographics, amyloid load and plaque count variables. APOE ε4 status and gender differences among the cognitive domains were analyzed using a two-sample t-test. Chi-square test determined differences in gender frequency and APOE ε4 genotype by Braak group. Since post-mortem interval and MMSE significantly violated the assumption of normality and demonstrated a significantly skewed distribution, we used the non-parametric Mann-Whitney test. Linear regression models used to test Braak group differences on the cognitive domains. Age at death, gender, education, and time between death and last assessment were included in the models to account for their effects. Cohen's *d* assessed the magnitude of the group differences. Level of agreement between NIA-Reagan and CERAD criteria was determined using the weighted kappa test. Logistic

regression analyses were performed for hippocampal, entorhinal cortex and total (hippocampus+entorhinal) amyloid loads as predictor variables for Braak group membership, with the Braak 0 to II group as the reference. Linear regression analyses, adjusted for age at death, gender, education, and time between death and last assessment, were used to assess the associations between amyloid loads with the cognitive domains. The same logistic and linear models were used to assess associations with hippocampal and entorhinal plaque counts. False discovery rate was used to adjust for multiple comparisons. Statistical analyses were performed using Systat 13 (Systat; San Jose, CA). Significance level was set at 0.05 (two sided). A false discovery rate (FDR) significance level of 0.003, equivalent to corrected type-I error of 0.05, was used to adjust for multiple comparisons for the amyloid load and Braak stage analyses among the different cognitive domains.

Results

The cohort was comprised of 63 males and 60 females, who did not have cognitive impairment proximate to death with an average age at death of 83.90±6.12 years and a mean of 18.29±3.59 years of education (Table 1). The MMSE was 29 and 28 for the low and high Braak groups, respectively and the average age from last examination to autopsy was 0.73 months and average postmortem interval was 5.8 hours (Table 1). Neuropathological diagnosis revealed that, out the 123 cases, 45 (35%) were classified with low and 78 (63%) as high Braak scores with a median stage of III and a range of 0 to V (Table 1). Twenty-one individuals were APOE ε4 carriers and 101 were non-ε4 carriers (APOE was not available for one individual). Among the non-carriers, 16 exhibited an APOE ε2/3 and 85 were ε3/3. Of the 21 ε4 carriers, only one was APOE ε4/4 homozygous and none were APOE ε2/4. Chi-square analysis indicated a significant difference in

frequency of males and females exhibiting low and high Braak stages (χ^2 = 8.87, df = 1, p = 0.003). A logistic regression analysis revealed that females were significantly more likely to have a high Braak score (OR = 3.18, 95% Confidence Interval: 1.47, 6.92, p = 0.003). There was no significant association between APOE ε4 genotype and Braak stage group (χ^2 = 0.62, df = 1, p = 0.43).

Of the cases meeting NIA-Reagan criteria, 56% (95% Confidence Interval: 47%, 65%) were categorized with a low likelihood of AD and 40% (95% Confidence Interval: 31%, 49%) with an intermediate likelihood of AD. Low likelihood Braak scores ranged from I – IV and from II to V in the intermediate likelihood category. CERAD criteria revealed 41% as no AD (95% Confidence Interval: 32%, 50%), 13% as possible (95% Confidence Interval: 8%, 20%), 36% as probable (95% Confidence Interval: 28%, 45%) and 11% as definite AD: (95% Confidence Interval: 6%, 18%) (Table 2). The level of agreement between the NIA-Reagan and CERAD criteria was moderate (κ = 0.49, 95% Confidence Interval: 0.43, 0.56).

Low and High Braak Stage and Cognition in Non-demented Subjects

Although the low Braak group showed higher performance on most cognitive domains examined, only semantic memory showed significantly higher values in the unadjusted (p = 0.01, Fig. 1A) but not in the adjusted analysis (p = 0.14; Table 2, Fig. 1A). Statistical analysis of the factors underlying the non-significant adjusted p-value revealed that both gender and education showed a significant association with semantic memory scores (p = 0.05 and p < 0.001, respectively). Linear regression determined that only the interaction between education and

Braak group was statistically significant (p = 0.02). The unadjusted and adjusted group analyses of cognitive composite z-scores are shown in Table 3.

APOE, Gender and Cognition in Non-demented Subjects

None of the cognitive domains showed significant differences between ε4 carriers and non-carriers (Table 3 and Fig. 1B), however, males performed significantly better than females on the semantic memory (p = 0.006) and the visuospatial (p = 0.04) domains (Table 4 and Fig. 1C). Effect size for the semantic memory difference was moderate (d = 0.51) while the visuospatial effect size was small (d = 0.39).

Hippocampal and Entorhinal Amyloid Load Associations

No significant amyloid load differences were found between low and high Braak groups for either hippocampus (p = 0.71) or entorhinal cortex (p = 0.68). Hippocampal amyloid plaque load was not significantly associated with Braak stage in either the crude (OR = 1.57, 95% Confidence Interval: 0.88, 2.80, p = 0.12) or adjusted analyses (OR = 1.24, 95% Confidence Interval: 0.66, 2.36, p = 0.50). By contrast, entorhinal cortex amyloid plaque load was significantly associated with higher Braak score in the unadjusted (OR = 2.03, 95% Confidence Interval: 1.23, 3.33, p = 0.005) and adjusted (OR = 1.94, 95% Confidence Interval: 1.06, 3.58, p = 0.03) analysis. APOE ε4 status demonstrated non-significant associations with hippocampal (p = 0.06) and entorhinal cortex (p = 0.29) amyloid load. Unadjusted analysis of the total amyloid load (hippocampus+entorhinal) found higher amyloid load was significantly associated with the high Braak group (OR = 1.42, 95% Confidence Interval: 1.07, 1.90, p = 0.02), but not after adjustment (OR = 1.32, 95% Confidence Interval: 0.95, 1.85, p = 0.10). Analysis of total

amyloid load also showed non-significant results for the other individual variables and their interaction.

None of the adjusted analyses showed a significant association between hippocampal or entorhinal amyloid load and cognitive composite scores for the entire sample (Table 4). However, a subgroup analyses found that entorhinal cortex alone and hippocampal plus entorhinal cortex amyloid loads were significantly associated with perceptual speed in the low Braak group (Table 5).

Hippocampal and Entorhinal Cortex Plaque Count Associations

Analysis of hippocampal CA1 and entorhinal cortex neuritic (NPs) and diffuse (DPs) plaque counts were not significantly associated with Braak groups ($p > 0.05$). Combined hippocampal and entorhinal cortex NPs showed a significant association with high Braak scores (OR = 1.09; 95% Confidence Interval: 1.02, 1.15; $p = 0.007$), however, DP counts were not associated with Braak group (OR = 1.02; 95% Confidence Interval: 0.99, 1.06; $p = 0.23$). Analyses of the associations between plaque counts and the cognitive domains found that hippocampal NPs counts were significantly negatively associated with GCS ($r = -0.33$, $p = 0.001$) and episodic memory ($r = -0.32$, $p = 0.002$) after adjusting for multiple comparisons.

Low, medium and high vs low (I-II) and high (IV-V) Braak stages

The more conventional Braak staging paradigm comparing stages I-II, III-IV and V-VI revealed similar findings to that reported described above. Comparing Braak stages I-II and IV-V with III (an intermediate stage) revealed that none of the cognitive domains examined showed significant

differences between groups. However, hippocampal and entorhinal and combined NP counts for these regions were significantly greater in the Braak IV-V compared to other groups (hippocampal NP: Braak IV-V vs Braak III, $p = 0.01$ and Braak IV-V vs Braak I-II, $p < 0.001$; hippocampal+entorhinal NP: Braak IV-V vs Braak III, $p = 0.01$ and Braak IV-V vs Braak I-II, $p < 0.001$. Braak III cases were not significantly different from the I-II individuals.

Discussion

We evaluated the association of low and high Braak scores upon cognitive domains and plaque pathology in a large cohort of elderly persons without cognitive impairment. The majority of these subjects were Braak stage III and none were Braak stage VI, which is highly associated with AD-dementia (Braak & Braak, 1991). Females were significantly more likely to have higher Braak scores (III-V). The low Braak group showed higher performance on most cognitive domains examined. However, only semantic memory was significantly higher in the unadjusted but not when adjusted for age, gender and years of education suggesting that this relationship was not directly related to Braak score. Although it is possible that there was either a problem with word retrieval or word knowledge that would impair performance, it remains to be determined how common pure word retrieval or pure word knowledge problems are in this group. These data suggest that neurofibrillary pathology within the entorhinal-perirhinal cortex (stages I–II) and hippocampus (stages III–IV) and neocortex (stage V) are not a necessary precondition for cognitive decline in the elderly. Perhaps the addition of stage VI cases would have resulted in correlations with cognitive test results.

Although hippocampal amyloid plaque load was not associated with Braak stage, entorhinal cortex amyloid load correlated significantly with high Braak scores. Entorhinal cortex alone and

combined hippocampal and entorhinal cortex amyloid loads were also significantly associated with perceptual speed only in the low Braak group. Group analysis found no significant effects for either hippocampal or entorhinal cortex amyloid load or APOE ε4 status. Our results suggest that cognitively non-impaired elderly exhibit a wide range of Braak scores and plaque pathology similar to prodromal AD (Mufson et al., 2012a; Perez et al., 2015; Price et al., 2009; Schneider et al., 2009a;) and AD (Bennett et al., 2002; Mufson, et al., 1999) without concomitant dementia.

Numerous studies have investigated the neuropathology of probable AD and mild cognitive impairment (MCI) in various clinical cohorts but very few have concentrated on the relationship of Braak staging to clinical dysfunction in people with no cognitive impairment (Gold et al., 2000; Price et al., 2009). An investigation of a small number of cognitively non-impaired people from the RROS cohort revealed similar percentages of low (40%) and high (60%) Braak. However, this study revealed a minor association between episodic memory and NIA-Reagan criteria (Bennett et al., 2006), which combines plaque and tangle pathology including Braak scores. The combination of pathologies may drive the minor association found in this report. Interestingly, females, which display significantly greater NFT pathology, performed worse than males on semantic memory and visuospatial domain tests in the adjusted analysis, suggesting that NFTs pathology influences decline associated with these cognitive domains in females. In addition, we found significant correlations between hippocampal CA1 NPs, GCS and episodic memory suggesting that this pathology plays a critical role in cognitive decline. Erten-Jones and coworkers (2009) reported that individuals with low and high Braak scores with moderate to frequent CERAD criteria did not manifest overt cognitive impairment, but only the high Braak cases had larger hippocampal and total brain volume suggesting that brain volume plays a role in

whether cognitive decline occurs in the face of AD neuropathology (Erten-Jones et al., 2009), which may become more pronounced in the oldest old (Ewbank et al., 2009; Haroutunian et al., 2008). Although we failed to find an association between APOE ε4 carrier status, with any of the pathological or clinical variable examined here, other genes such as those related to the regulation of apoptosis (Su et al., 2003) may lead to neurodegenerative resistance and cognitive stability.

Our results expand and support earlier studies suggesting that some form of brain resilience/reserve allows some older individuals to withstand significant amounts of AD pathology without concomitant dementia (Bennett et al., 2005; Dickson et al., 1992; Iacono et al., 2009; Mufson et al., 1999; Negash et al., 2013; Nelson et al., 2011; Price & Morris, 1999; Price et al., 2009). Although the mechanism(s) underlying these constructs are unclear, it is posited that the MTL memory circuit is neuroplastic during the onset of dementia (Mufson et al., 2015). Previous studies using RROS tissue noted similarities in entorhinal cortex amyloid load between non-cognitively impaired controls, MCI and AD cases (Mufson et al., 1999). Other RROS studies examining the hippocampus show a preservation of synapse number (Scheff et al., 2007) and protein levels (Counts et al., 2012) as well as neurotrophic factor receptor levels (Mufson et al., 2012b) in non-demented controls with a wide range of Braak stages. Findings derived from other clinical pathological cohorts report preserved numbers and larger neuronal sizes in the hippocampus in “asymptomatic AD” compared with normal, MCI and clinical AD cases (Iacono et al., 2009; Riudavets et al., 2007) indicating an early reaction to AD pathology. This compensatory structural remodeling and biochemical stability within the hippocampus in the face of extensive NFT pathology illustrates the neuroplastic capacity of the MTL to

counteract mounting pathology and perhaps maintain cognition. The early MTL reorganization may represent a viable window for potential therapeutic strategies aimed at restoring or maintaining function in the elderly. However, we did note that combined hippocampal and entorhinal cortex NP but not DP counts were associated with Braak scores, but only hippocampal NPs correlated with GCS and episodic memory suggesting combined pathologies play a more significant role in dementia onset that single pathologies.

Finally, it is important to consider our study limitations. Subjects were from a community based group of highly educated retired clergy who had excellent health care and nutrition and were used in multiple clinical pathological (Mufson et al., 2012a) and epidemiological investigations (Bennett et al., 2002). Individuals who volunteer may introduce bias by decreasing pathology but this is partially mitigated by high in RROS follow-up and autopsy rates (Schneider et al., 2009a). None of the cases evaluated were Braak stage VI, which is highly associated with AD-dementia (Braak & Braak, 1991). Although subtle changes in neuropsychological testing may not detect cognitive changes in non-demented people (Smith et al., 2007), our cognitive tests are standard in the field (Bennett et al., 2005). Strengths include uniform premortem clinical and postmortem pathological evaluation and that final pathologic classification was performed without knowledge of clinical evaluation. These study characteristics lend strength to our findings that older people can withstand the onslaught of AD pathology without degraded cognition.

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Disclosure statement

None of the authors have any disclosures.

References

Bennett DA, Wilson RS, Schneider JA, Evans DA, Beckett LA, Aggarwal NT, et al. Natural history of mild cognitive impairment in older persons. Neurology 2002; 59:198-205.

Bennett DA, Schneider JA, Wilson RS, Bienias JL, Arnold SE. Neurofibrillary tangles mediate the association of amyloid load with clinical Alzheimer disease and level of cognitive function. Arch Neurol 2004; 61:378-84.

Bennett DA, Schneider JA, Bienias JL, Evans DA, Wilson RS. Mild cognitive impairment is related to Alzheimer disease pathology and cerebral infarctions. Neurology. 2005a 8; 64:834-41.

Bennett DA, Schneider JA, Buchman AS, Mendes de Leon C, Bienias JL, Wilson RS. The Rush Memory and Aging Project: Study design and baseline characteristics of the study cohort. Neuroepidemiology 2005b; 25:163-75.

Bennett DA, Schneider JA, Arvanitakis Z, Kelly JF, Aggarwal NT, Shah RC, et al. Neuropathology of older persons without cognitive impairment from two community-based studies. Neurology 2006; 66:1837-44.

Braak H, Braak E. Neuropathological stageing of Alzheimer-related changes. Acta Neuropathol 1991; 82:239-59.

Counts SE, He B, Nadeem M, Wuu J, Scheff SW, Mufson EJ. Hippocampal drebrin loss in mild cognitive impairment. Neurodegenerative Dis 2012; 10:216-19.

Dickson DW, Crystal HA, Mattiace LA, Masur DM, Blau AD, Davies P, et al. Identification of normal and pathological aging in prospectively studied nondemented elderly humans. Neurobiol Aging 1992; l13:179-89.

Erten-Jones D, Woltjer RL, Dodge H, Nixon R, Vorobik R, Calvert JF, et al. Factors associated with resistance to dementia despite high Alzheimer disease pathology. Neurology 2009; 72:354-60.

Ewbank DC, Arnold SE. Cool with plaques and tangles. N Engl J Med 2009; 360:2357-59.

Gilmor ML, Erickson JD, Varoqui H, Hersh LB, Bennett DA, Cochran EJ, et al. Preservation of nucleus basalis neurons containing choline acetyltransferase and the vesicular acetylcholine

transporter in the elderly with mild cognitive impairment and early Alzheimer's disease. J Comp Neurol 1999; 411:693-704.

Gold G, Bouras C, Kövari E, Canuto A, Glaría AB, Malky A, et al. Clinical validity of Braak neuropathological staging in the oldest-old. Acta Neuropathol 2000; 99:579-82.

Guillozet AL, Weintraub S, Mash DC, Mesulam MM. Neurofibrillary tangles, amyloid, and memory in aging and mild cognitive impairment. Arch Neurol 2003; 60:729-36.

Haroutunian V, Schnaider-Beeri M, Schmeidler J, Wysocki M, Purohit DP, Perl DP, et al. Role of the neuropathology of Alzheimer disease in dementia in the oldest-old. Arch Neurol 2008; 65:1211-17.

Iacono D, Markesbery WR, Gross M, Pletnikova O, Rudow G, Zandi P, et al. The Nun study: clinically silent AD, neuronal hypertrophy, and linguistic skills in early life. Neurology 2009; 73:665-73.

Markesbery WR. Neuropathologic alterations in mild cognitive impairment: a review. J Alzheimers Dis 2010; 19:221-28.

Mitchell TW, Nissanov J, Han LY, Mufson EJ, Schneider JA, Cochran EJ, Bennett DA, Lee VM, Trojanowski JQ, Arnold SE. Novel method to quantify neuropil threads in brains from elders with or without cognitive impairment. J Histochem Cytochem 2000; 48:1627-38.

Mirra SS, Heyman A, McKeel D, Sumi SM, Crain BJ, Brownlee LM, et al. The consortium to establish a registry for Alzheimer’s disease (CERAD). part II. Standardization of the neuropathologic assessment of Alzheimer’s disease. Neurology 1991; 41:479-86.

Morris JC, Price JL. Pathologic correlates of nondemented aging, mild cognitive impairment, and early-stage Alzheimer's disease. J Mol Neurosci 2001; 17:101-18.

Mufson EJ, Chen EY, Cochran EJ, Beckett LA, Bennett DA, Kordower JH. Entorhinal cortex beta-amyloid load in individuals with mild cognitive impairment. Exp Neurol 1999; 158:469-90.

Mufson EJ, Binder L, Counts SE, De Kosky ST, de Toledo-Morrell L, Ginsberg SD, et al. Mild cognitive impairment: pathology and mechanisms. Acta Neuropathol 2012a; 123:13-30.

Mufson EJ, He B, Nadeem M, Perez SE, Counts SE, Leurgans S, et al. Hippocampal proNGF signaling pathways and β-amyloid levels in mild cognitive impairment and Alzheimer disease. J Neuropathol Exp Neurol 2012b; 71:1018-29.

Mufson EJ, Ward S, Binder L. Prefibrillar tau oligomers in mild cognitive impairment and Alzheimer's disease. Neurodegener Dis 2014; 13:151-53.

Mufson EJ, Mahady L, Waters D, Counts SE, Perez SE, DeKosky ST, et al. Hippocampal plasticity during the progression of Alzheimer’s disease. Neuroscience, Available online 12 March 2015, ISSN 0306-4522, http://dx.doi.org/10.1016/j.neuroscience.2015.03.006.

Negash S, Wilson RS, Leurgans SE, Wolk DA, Schneider JA, Buchman AS, et al. Resilient brain aging: Characterization of discordance between Alzheimer's disease pathology and cognition. *Curr Alzheimer Res* 2013; 10:844-51.

Nelson PT, Braak H, Markesbery WR. Neuropathology and cognitive impairment in Alzheimer disease: A complex but coherent relationship. J Neuropathol Exp Neurol 2009; 68:1-14.

Nelson PT, Head E, Schmitt FA, Davis PR, Neltner JH, Jicha GA, et al. Alzheimer's disease is not "brain aging": neuropathological, genetic, and epidemiological human studies. Acta neuropathol 2011; 121:571-87.

Perez SE, Getova DP, He B, Counts SE, Geula C, Desire L, et al. Rac1b Increases with Progressive Tau Pathology within Cholinergic Nucleus Basalis Neurons in Alzheimer's Disease. The Am J Path 2012; 180:526-40.

Perez SE, He B, Nadeem M, Wuu J, Scheff SW, Abrahamson EE, et al. Resilience of precuneus neurotrophic signaling pathways despite amyloid pathology in prodromal Alzheimer's disease. Biol Psychiatry 2015; 77:693-703.

Price JL, Morris JC. Tangles and plaques in nondemented aging and "preclinical" Alzheimer's disease. Ann Neurol 1999; 45:358-68.

Price JL, McKeel DW Jr, Buckles VD, et al. Neuropathology of nondemented aging: Presumptive evidence for preclinical Alzheimer disease. Neurobiol Aging 2009; 30:1026-36.

Riudavets MA, lacono D, Resnick SM, O'Brien R, Zonderman AB, Martin LJ, et al. Resistance to Alzheimer's pathology is associated with nuclear hypertrophy in neurons. Neurobiol Aging 2007; 28:1484-92.

Schneider JA, Boyle PA, Arvanitakis Z, Beinias JL, Bennett DA. Subcortical infarcts, Alzheimer's disease pathology, and memory function in older persons. Ann Neurol 2007; 62:59-66.

Schneider JA, Aggarwal NT, Barnes L, Boyle P, Bennett DA. The neuropathology of older persons with and without dementia from community versus clinic cohorts. J Alz Dis 2009a; 18:691-701.

Schneider JA, Arvanitakis Z, Leurgans SE, Bennett DA. The neuropathology of probable Alzheimer disease and mild cognitive impairment. Ann Neurol 2009b; 66:200-08.

Su JH, Anderson AJ, Cribbs DH, Tu C, Tong L, Kesslack P, et al. Fas and Fas Ligand are associated with neuritic degeneration in the AD brain and participate in β-amyloid-induced neuronal death. Neurobiol Dis 2003; 12:182-93.

Scheff SW, Price DA, Schmitt FA, DeKosky ST, Mufson EJ. Synaptic alterations in CA1 in mild Alzheimer disease and mild cognitive impairment. Neurology 2007; 68:1501-08.

Smith GE, Pankratz VS, Negash S, Machulda MM, Petersen RC, Boeve BF, et al. A plateau in pre-Alzheimer memory decline: evidence for compensatory mechanisms? Neurology 2007; 69:133-39.

The National Institute on Aging, and Reagan Institute Working Group on Diagnostic Criteria for the Neuropathological Assessment of Alzheimer's Disease: Consensus recommendations for the postmortem diagnosis of Alzheimer's disease. Neurobiol Aging 1997; 18:S1-S2.

Tomlinson BE, Blessed G, Roth M. Observations on the brains of demented old people. J Neurol Sci 1970; 11: 205-42.

Wilson RS, Leurgans SA, Boyle PA, Bennett DA. Cognitive decline in prodromal Alzheimer's disease and mild cognitive impairment. Arch Neurol 2011; 68:351-56.

Table 1. Demographic and clinical characteristics for Low and High Braak Groups.

	Low Braak Stage (0 - II)	High Braak Stage (III - V)	Total	p-value	Cohen's *d*
N	45	78	123	na	na
Male/Female	31/14	32/46	63/60	0.003	na
APOEε (2/3, 3/3, 2/4, 3/4, 4/4)	13, 50, 14, 1	3, 35, 6, 0	16, 85, 20, 1	0.27	na
Time Between Last Assessment and Death, y	0.72 (0.69)	0.72 (0.94)	0.73 (0.79)	0.98	0.00
Age at Death, y	80.23 (6.36)	86.01 (4.88)	83.90 (6.12)	<0.001	1.02
PMI, h	5.50 [2.17, 33.50]	5.83 [1, 66]	5.58 [1, 66]	0.33	na
Education, y	18.71 (3.79)	18.05 (3.47)	18.29 (3.59)	0.32	0.19
MMSE	29.00 [25.00, 30.00]	28.00 [25.00, 30.00]	29.00 [25.00, 30.00]	0.39	na

Mean (SD); PMI and MMSE – Median [Range]

Table 2. Distribution of Braak Stage by NIA-Reagan and CERAD Criteria.

Braak Stage*	NIA-Reagan			
	Not AD	Low Likelihood	Intermediate Likelihood	High Likelihood
0	3	0	0	0
I	0	20	0	0
II	0	19	3	0
III	0	20	15	0
IV	0	10	28	0
V	0	0	3	2
Totals	3	69	49	2

Braak Stage	CERAD			
	No AD	Possible AD	Probable AD	Definite AD
0	3	0	0	0
I	14	5	1	0
II	11	3	5	3
III	14	6	13	2
IV	8	2	22	6
V	0	0	3	2
Totals	50	16	44	13

*No individuals were Braak stage VI.

Table 3. Cognitive Domains Characteristics for Low and High Braak Groups.

Cognitive Domain	Low Braak Stage (0 - II)	High Braak Stage (III - V)	Total	Unadjusted p-value	*Adjusted p-value	Cohen's *d*
Global Composite	0.65 (0.34)	0.55 (0.34)	0.59 (0.34)	0.10	0.29	0.32
Episodic Memory	0.92 (0.35)	0.82 (0.44)	0.86 (0.41)	0.16	0.41	0.25
Semantic Memory	0.59 (0.42)	0.38 (0.47)	0.46 (0.46)	0.01	0.14	0.49
Working Memory	0.52 (0.58)	0.47 (0.48)	0.49 (0.52)	0.62	0.66	0.10
Visuospatial Ability	0.51 (0.57)	0.53 (0.63)	0.52 (0.61)	0.09	0.74	0.32
Perceptual Speed	0.72 (0.58)	0.53 (0.60)	0.60 (0.60)	0.83	0.69	0.04

Mean (SD); *adjusted for age at death, education, gender, and time between last assessment and death

Table 4. Group Analyses of Cognitive Domain Scores for APOE ε4 Carrier Status and Gender

	APOE ε4 Carriers	APOE ε4 Non-Carriers	p-value	Cohen's *d*
N	21	101	na	na
Global Composite	0.51 (0.26)	0.60 (0.36)	0.28	0.29
Episodic Memory	0.78 (0.40)	0.87 (0.41)	0.32	0.22
Semantic Memory	0.33 (0.43)	0.48 (0.47)	0.16	0.33
Working Memory	0.52 (0.43)	0.48 (0.53)	0.73	0.08
Visuospatial Ability	0.40 (0.57)	0.55 (0.62)	0.33	0.15
Perceptual Speed	0.53 (0.60)	0.62 (0.60)	0.54	0.25
	Males	Females	p-value	Cohen's *d*
N	63	60	na	na
Global Composite	0.62 (0.38)	0.55 (0.29)	0.27	0.21
Episodic Memory	0.87 (0.40)	0.85 (0.42)	0.79	0.05
Semantic Memory	0.57 (0.44)	0.34 (0.46)	0.006	0.51
Working Memory	0.56 (0.54)	0.42 (0.49)	0.13	0.27
Visuospatial Ability	0.61 (0.67)	0.43 (0.52)	0.04	0.39
Perceptual Speed	0.49 (0.63)	0.72 (0.55)	0.11	0.30

Mean (standard deviation); *adjusted for age at death, education, gender, and time between last assessment and death

Table 5. Associations between Hippocampal, Entorhinal Cortex, and Amyloid Load and Cognitive Domains.

Amyloid Load	Cognitive Domain	Low Braak Adjusted p-value	High Braak Adjusted p-value	Low and High Adjusted p-value
Hippocampus	Global Composite	0.69	0.41	0.32
	Episodic Memory	0.61	0.58	0.47
	Semantic Memory	0.87	0.42	0.61
	Working Memory	0.80	0.26	0.45
	Perceptual Speed	0.08	0.42	0.11
	Visuospatial	0.56	0.56	0.62
Entorhinal Cortex	Global Composite	0.40	0.24	0.20
	Episodic Memory	0.77	0.16	0.42
	Semantic Memory	0.21	0.14	0.07
	Working Memory	0.49	0.97	0.64
	Perceptual Speed	<0.001	0.92	0.23
	Visuospatial	0.96	0.82	0.99
Hippocampus+Entorhinal	Global Composite	0.14	0.52	0.10
	Episodic Memory	0.18	0.73	0.35
	Semantic Memory	0.29	0.13	0.15
	Working Memory	0.68	0.86	0.13
	Perceptual Speed	<0.001	0.87	0.28
	Visuospatial	0.93	0.96	0.93

False discovery rate significance level: p = 0.003

Figure 1

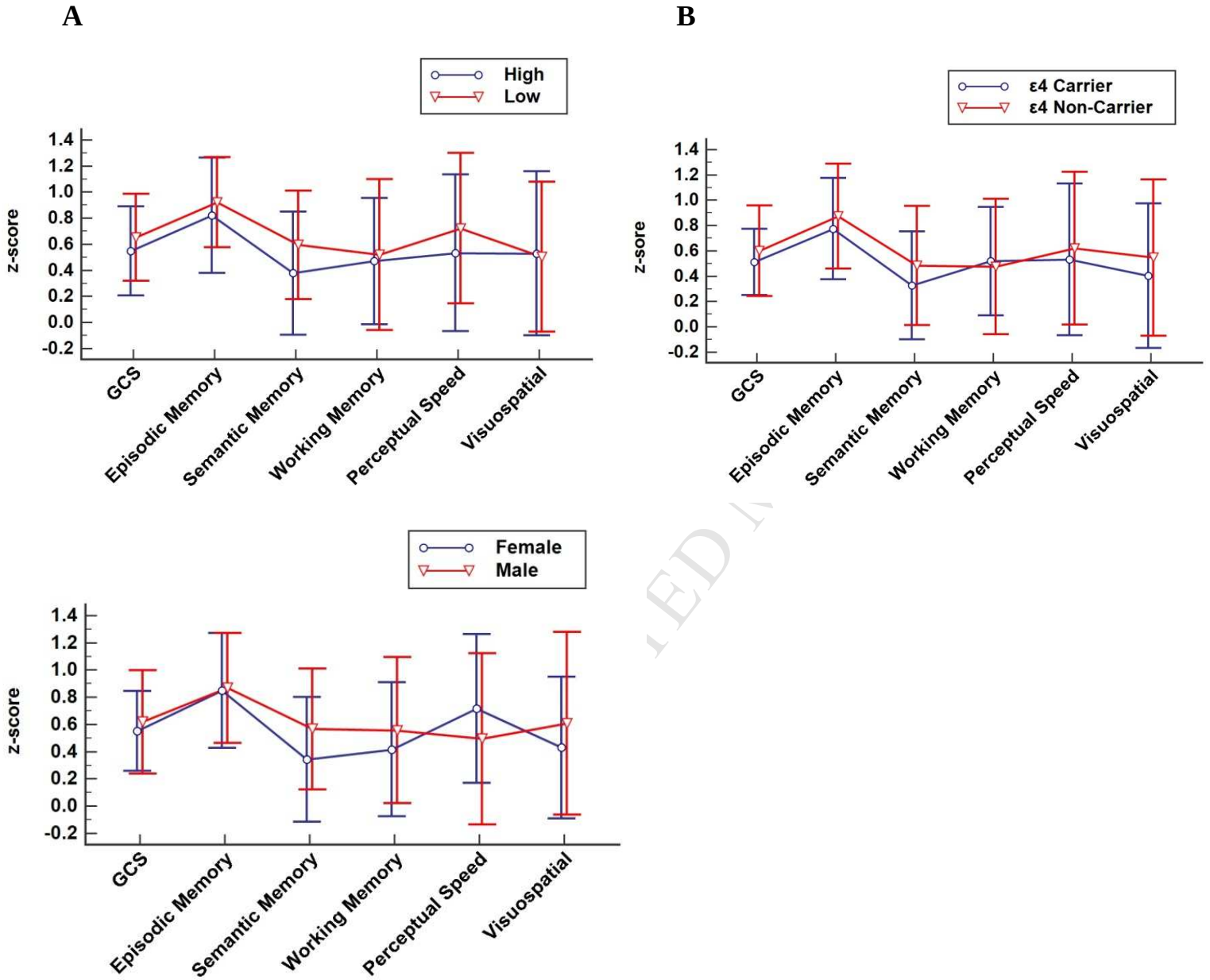
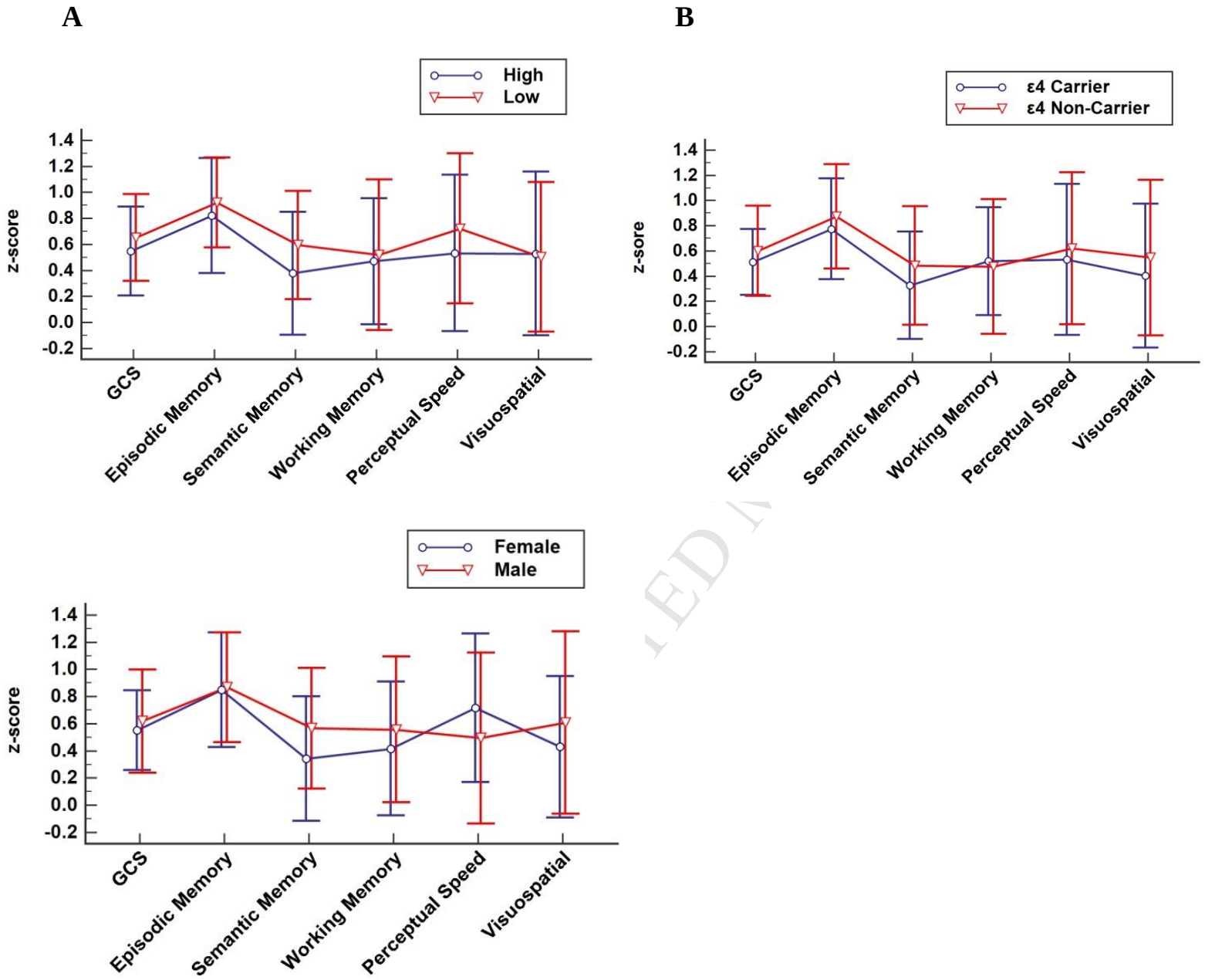

A
High
Low
z-score
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
GCS
Episodic Memory
Semantic Memory
Working Memory
Perceptual Speed
Visuospatial
B
ε4 Carrier
ε4 Non-Carrier
z-score
GCS
Episodic Memory
Semantic Memory
Working Memory
Perceptual Speed
Visuospatial
Female
Male
z-score
GCS
Episodic Memory
Semantic Memory
Working Memory
Perceptual Speed
Visuospatial

Figure Legend

Figure 1. Linear graphs showing relationships between cognitive domains and (**A**) Braak scores, (**B**) APOE ε4 status and (**C**) gender. Note the significant differences between male and female for semantic memory (p=0.006) and visuospatial (p=0.04) scores. Circles and triangles represent mean z-scores, error bars represent standard deviation.

Figure 1


A
High
Low
z-score
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
GCS
Episodic Memory
Semantic Memory
Working Memory
Perceptual Speed
Visuospatial
B
ε4 Carrier
ε4 Non-Carrier
z-score
GCS
Episodic Memory
Semantic Memory
Working Memory
Perceptual Speed
Visuospatial
Female
Male
z-score
GCS
Episodic Memory
Semantic Memory
Working Memory
Perceptual Speed
Visuospatial

Highlights:

1. Braak stage failed to correlate with cognition in non-demented elders.
2. Elderly with preserved cognition show a range of Braak scores.
3. Elderly with extensive NFT and plaque pathology demonstrate brain resilience, reserve or plasticity