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Utilizing semantic intrusions to identify amyloid positivity in mild cognitive impairment

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Abstract

Objective

Semantic intrusion (SI) errors may highlight specific breakdowns in memory associated with preclinical Alzheimer disease (AD); however, there have been no investigations to determine whether SI errors occur with greater frequency in persons with amnestic mild cognitive impairment (aMCI) confirmed as amyloid positive (Amy+) vs those who have clinical symptoms of aMCI-AD with negative amyloid scans (suspected non-AD pathology [SNAP]) or persons who are diagnosed with other brain disorders affecting cognition.

Methods

Eighty-eight participants with aMCI underwent brain amyloid PET and MRI scans and were classified as early AD (Amy+), SNAP (Amy−), or other neurological/psychiatric diagnosis (Amy−). We focused on SI on the Loewenstein-Acevedo Scales for Semantic Interference and Learning (LASSI-L) targeting proactive semantic interference (PSI; old semantic learning interferes with new semantic learning), failure to recover from PSI after an additional learning trial (frPSI), and retroactive semantic interference (new semantic learning interferes with memory for old semantic learning).

Results

SIs on measures of PSI and frPSI distinguished between Amy+ AD and SNAP and other non-AD cases. PSI and frPSI intrusions evidenced moderately high associations with reduced volumes in the entorhinal cortex, superior temporal regions, and supramarginal gyrus. No such associations were observed in cases with SNAP.

Conclusions

SIs on the LASSI-L related to PSI and frPSI uniquely differentiated Amy+ and Amy− participants with aMCI and likely reflect deficits with inhibition and source memory in preclinical AD not captured by traditional cognitive measures. This may represent a specific, noninvasive test successful at distinguishing cases with true AD from those with SNAP.

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Glossary

AD = Alzheimer disease; **aMCI** = amnestic mild cognitive impairment; **Amy+** = amyloid positive; **Amy−** = amyloid negative; **ANOVA** = analysis of variance; **CDR** = Clinical Dementia Rating; **CI** = confidence interval; **DSM-V** = *Diagnostic and Statistical Manual of Mental Disorders, 5th edition*; **ERC** = entorhinal cortex; **FDR** = false discovery rate; **frPSI** = failure to recover from proactive semantic interference; **FTLD** = frontotemporal lobar degeneration; **HVLT-R** = Hopkins Verbal Learning Test–Revised; **LASSI-L** = Loewenstein-Acevedo Scales for Semantic Interference and Learning; **MCI** = mild cognitive impairment; **MMSE** = Mini-Mental State Examination; **PSI** = proactive semantic interference; **ROC** = receiver operator characteristic; **RSI** = retroactive semantic interference; **SNAP** = suspected non-Alzheimer pathology; **Tukey HSD test** = Tukey honestly significant difference test.

There is a critical need to develop disease-modifying treatments for Alzheimer disease (AD) before the occurrence of significant multisystem degeneration.[1–3] Preventing or reducing the accumulation of β-amyloid seems to be a promising target for early therapeutic intervention; however, there is a dearth of cognitive outcome measures associated with this biomarker with sufficient sensitivity and specificity to detect and monitor early cognitive change,[4,5] which is ultimately required by the US Food and Drug Administration for drug approval.

The use of traditional cognitive assessment paradigms poses a major limitation to the field of early detection in that existing instruments are mostly insensitive and not specific to early cognitive deficits associated with AD.[5] We and others have found that the failure to recover from proactive semantic interference (frPSI)[6] may be one such cognitive marker of preclinical AD in adults diagnosed with amnestic mild cognitive impairment (aMCI) and is related to volumetric reductions in selectively AD-vulnerable brain regions.[7–9] Proactive semantic interference (PSI) is the deleterious effect on the learning of new items caused by previous learning of items in the same semantic category. frPSI refers to the persistence of PSI effects even when the subject is given additional opportunities to learn the new items.

Furthermore, even among cognitively normal elders, frPSI has been associated with increased amyloid load in AD-prone regions.[10] Even though intrusion errors on memory tasks have been found to be an important feature in AD,[11–13] semantic intrusions on Loewenstein-Acevedo Scales for Semantic Interference and Learning (LASSI-L) measures sensitive to PSI and frPSI have not been previously investigated in aMCI. Such semantic errors may reflect deficits in source monitoring or filtering previously encoded information[14,15] and in strategic retrieval of such material,[16,17] and we hypothesized that these intrusions might be unique or even specific to early AD.

Methods

Standard protocol approvals, registrations, and patient consents

All participants consented to participate in this Institutional Review Board–approved study.

We recruited 88 participants diagnosed with aMCI (50.6% female) from the 1Florida Alzheimer's Disease Research Center cohort. Mean age of the participants was 72.9 years (SD 7.7, range 56–98 years); the average educational attainment was 14.7 years (SD 3.5, range 6–22 years). The mean Mini-Mental State Examination (MMSE) score was 27.4 (SD 2.0, range 23–30). Our aMCI cohort was subdivided into 3 groups: amyloid-positive (Amy+) participants with aMCI with clinical and MRI findings typical of AD, amyloid-negative (Amy−) participants with aMCI with clinical and MRI findings typical of AD meeting criteria for suspected non-Alzheimer pathology (SNAP), and Amy− participants with aMCI with clinical and MRI findings suggestive of a non-AD diagnosis such as frontotemporal lobar degeneration (FTLD), diffuse Lewy body disease, chronic traumatic encephalopathy, vascular cognitive impairment, sleep apnea, or a primary psychiatric disorder.

All participants were administered a common clinical assessment, which included the Clinical Dementia Rating (CDR) scale[18] and the MMSE.[19] Memory and other cognitive complaints were assessed by a geriatric psychiatrist (M.G.) with formal training in administering the CDR who was blinded to the neuropsychological test results. The 88 participants were all community-dwelling older adults who were functionally independent, had reliable informants, and did not meet criteria for major neurocognitive disorder by DSM-V criteria.[20] The clinician scored the global CDR as 0.5 and considered a diagnosis of mild cognitive impairment (MCI) based on their examination, pending the results of neuropsychological testing if there was a notable history of cognitive decline. Subsequently, a standard neuropsychological evaluation was conducted independently of the clinical evaluation. Testing included the Hopkins Verbal Learning Test–Revised (HVLT-R), Delayed Recall Trial,[21] Delayed Recall from the Logical Memory subtest of the National Alzheimer's Coordinating Center Uniform Dataset,[22] Category Fluency,[23] the Block Design subtest of the Wechsler Adult Intelligence Scales–Fourth Edition,[24] and parts A and B of the Trail Making Test.[25]

In the current study, we measured the occurrence of semantic intrusions on LASSI-L Cued Recall trials susceptible to PSI, frPSI, and retroactive semantic interference (RSI). We postulated that semantic intrusions on competing list-learning tasks sensitive to PSI and frPSI might better highlight inhibitory failures and deficits in source memory that are not

typically detected by raw scores on standard cued recall tasks. Furthermore, we postulated that PSI and frPSI semantic intrusions on the LASSI-L might distinguish individuals with typical Alzheimer pathology (i.e., amyloid positivity) from those with non-Alzheimer pathology. Previous studies suggest that Alzheimer pathology results in a lack of integration of information stored within different cortical regions and between cortical and subcortical regions.[15] We hypothesized that the resulting distortion of source memory represented by semantic intrusions may be typical and possibly unique in the MCI stages of early AD.[17]

MRI visual inspection

All participants described above underwent structural MRI with a Skyra 3T Siemens MRI (Malvern, PA) at Mount Sinai Medical Center, Miami Beach, FL. The MRI scans were evaluated by visual inspection to assist in determining the etiology of cases of aMCI and quantitatively to obtain volumetric data for various brain regions. These assessments were performed with a T2-weighted fluid-attenuated inversion recovery sequence with 5-mm-thick sequential axial slices to assess (1) atrophy in the right and left temporal, parietal, frontal, and occipital lobes; (2) volume of the left and right lateral ventricles and the third ventricles; (3) the occurrence of lacunar infarcts in each hemisphere; (4) large vessel infarcts in each hemisphere; and (5) degree of periventricular and deep white matter hyperintensities within each hemisphere. Using a 3-dimensional magnetization-prepared rapid gradient echo sequence with 1-mm-thick coronal slices, we assessed severity of atrophy in the (6) right and left hippocampus and (7) entorhinal cortex (ERC) using susceptibility-weighted intensity sequences (axial slices 5 mm thick) and (8) sulcal hemorrhages, microhemorrhages, and macrohemorrhages in each hemisphere using an integrated impression of all the above assessments. A global impression was determined for each MRI scan as either consistent with a typical AD diagnosis or consistent with an alternative diagnosis such as FTLD, posterior cortical atrophy, and vascular dementia.

Amyloid imaging

We scanned all participants for a duration of 20 minutes on a Siemens Biograph 16 PET/CT scanner operating in 3-dimensional mode (55 slices per frame, 3-mm slice thickness, 128 × 128 matrix) using the following tracers: NeuraCeq ([F-18] florbetaben; Piramal Imaging, Boston, MA) 300 MBq (78% of the sample) and Amyvid (florbetapir; Eli Lilly, Indianapolis, IN) (22% of the sample). Images were obtained from the top of the head to the top of the neck, and CT data were used for initial attenuation correction and image reconstruction in the coronal, sagittal, and axial planes. As described in our previous work,[8] the florbetaben PET/CT scan, including the outline of the skull, was linearly coregistered (i.e., trilinear interpolation) with 12 *df* onto the T1 image. This registration process ensured that both MRI parcellation and segmentation were the same as the florbetaben PET/CT image. All amyloid scans were read by an experienced reader (R.D.) who was blinded to diagnosis. In a prior study, this reader had high reliability with an independent neuroradiologist and rated 95 amyloid scans of older adults (20.0% cognitive normal, 17.9% pre-MCI,[10] 19% early MCI, 15.8% late MCI, and 27.4% dementia). The same reader rated 41 of these scans as Amy+ and 54 of these scans as Amy−. The concordance between the reader and the independent neuroradiologist was 93.2% for Amy+ scans and 100% for Amy− scans. The 3 cases for which there was disagreement were 3 participants diagnosed as cognitively normal.

Diagnostic criteria for Amy+ aMCI-AD (n = 34)

The following 5 criteria were used to define this group: (1) subjective memory concerns, a progressive decline in memory reported by the participant and/or reliable informant, and a clinical course consistent with a diagnosis of AD; (2) global CDR score of 0.5; (3) ≥1 memory measures 1.5 SD below normal limits relative to age- and education-related norms, as described above and comparable to previous studies; (4) findings of atrophy in medial temporal and/or parietal regions on visual assessment of structural MRI and the absence of non-Alzheimer pathology such as infarcts and of space-occupying lesions; and (5) Amy+ status based on expert visual reading of amyloid PET scan (R.D.) without reference to quantitative amyloid scores, which were not available for all participants.

Diagnostic criteria for Amy− SNAP (n = 29)

Classification criteria included all items described above for Amy+ aMCI-AD except for item 5; the amyloid status was negative on the basis of expert visual reading of the amyloid PET scan.

Diagnostic criteria for Amy− aMCI non-AD (n = 25)

Classification criteria included all items described above for Amy− aMCI-AD except that the clinical course was suggestive of a non-AD diagnosis and the MRI findings were consistent with a non-AD diagnosis. In this group, the following non-AD diagnoses were made: FTLD (n = 3); diffuse Lewy body disease (n = 3); cerebral infarctions (n = 4); chronic traumatic encephalopathy (n = 1); major depression, anxiety, or other psychiatric disorder (n = 9); sleep apnea (n = 1); or undetermined non-AD etiology (n = 4).

The LASSI-L

The LASSI-L is an established cognitive stress test that been validated in both English and Spanish.[6,9] It uses controlled learning and cued recall to maximize storage of an initial list of target words representing 3 semantic categories. The specific elements of the test are described below.

The participant is asked to read aloud 15 words presented individually that are fruits, musical instruments, and articles of clothing (5 words per category). In the unlikely event that the person cannot correctly read the word, the examiner reads the word and asks the person to repeat the word. If a person does not know one of the words (also unlikely), the examiner tells

the person the semantic category to which the word pertains (e.g., "lemon is a fruit") and asks the person to repeat the word. After reading all 15 words, the person is asked to recall the words. After the free recall trial, the participant is presented with each category cue (e.g., clothing) and is asked to recall the words that belonged to that category (LASSI-L A1). Subsequently, there is an additional presentation of the target stimuli for a second learning trial with cued recall to strengthen the acquisition and recall of the list A words. This provides a measure of maximum storage of the to-be-remembered information (LASSI-L A2). A semantically comparable list (i.e., list B) is then presented in the same manner as list A. List B consists of 15 different words from list A; however, all list B words belong to 1 of the 3 semantic categories used in list A (i.e., fruits, musical instruments, and articles of clothing). PSI effects are then measured through free recall of the list B words (LASSI-L B1). List B words are presented a second time, followed by a second category, cued recall trial, which facilitates a measure of recovery from the initial PSI effects (LASSI-L B2). Recovery from PSI is a feature of the LASSI-L that is not assessed by any existing list-learning measure.[5] Finally, short-delayed recall of list A provide an index of RSI. Previous studies have demonstrated robust test-retest reliability of the LASSI-L, and classification of patients of aMCI vs cognitively normal elderly exceeded 90% accuracy.[6,26]

Thus, a unique feature of the LASSI-L is the presentation of a second list of words with shared semantic categories that elicit a considerable amount of proactive semantic interference[5,6] and is considered a cognitive stress test.[5,10] Unlike other memory paradigms, the LASSI-L facilitates the measurement of recovery from PSI effects through a second cued recall trial of competing target words. RSI through cued recall is also assessed. For this study, we focused on semantic intrusion errors made on Cued B1 (subject to PSI), Cued B2 (subject to frPSI), and Short-Delay Cued A (subject to RSI).

MRI quantification

Eighty-eight of the participants described above (34 Amy+ AD, 29 SNAP, and 25 Amy−) with different aMCI etiologies underwent MRI scanning with a Siemens Skyra 3T MRI scanner with parcellation obtained using a 3-dimensional T1-weighted sequence (magnetization-prepared rapid gradient echo) with 1.0-mm isotropic resolution and MRI quantification. Atrophy in Alzheimer signature regions was assessed with FreeSurfer version 5.3 software (surfer.nmr.mgh.harvard.edu).[27,28] For this study, we focused on the hippocampus and ERC, because these areas are affected during early AD stages, and other AD-prone regions such as the precuneus, posterior cingulate, superior temporal lobule, inferior temporal lobule, temporal pole, superior parietal lobule, inferior parietal lobule, supramarginal gyrus, superior frontal lobule, and rostral middle frontal lobule. Given the high degree of association between corresponding structures in the left and right brain hemispheres, homologous structures (e.g., inferior temporal lobules, precuneus) were combined and normalized with the use of intracranial volume.[29] For associations between semantic intrusion errors and brain volumes, we focused on left hemisphere regions because the LASSI-L is a measure that focuses on verbal learning.

Statistical analyses

Demographic variables were analyzed with a series of analysis of variance (ANOVA) and χ^2 models. Following statistically significant results at $p < 0.05$, post hoc tests were conducted with the Tukey honestly significant difference (HSD) test. The number of semantic intrusions on Cued B1, Cued B2, and Short Delay Cued A trials was examined among the 3 groups using ANOVA procedures with post hoc Tukey honestly significant difference tests for comparisons of means. Analysis of covariance models were also used, controlling for baseline differences in demographic variables. Because ANOVA and analysis of covariance models yielded identical results, only the results of ANOVA models with unadjusted means are presented. Receiver operator characteristic (ROC) curves were calculated for the semantic intrusion measures to determine their ability to classify Amy+ cases with MCI from cases with SNAP and to determine their ability to classify Amy+ cases with MCI from Amy− cases without AD. The Youden Index and bootstrapping techniques using 1,000 iterations were also used. Adding demographic measures, intrusion errors, and other LASSI-L measures did not yield any differences in the area under the ROC curve. Correlation coefficient matrices were constructed to examine semantic intrusion errors in Amy+ aMCI and SNAP (Amy−) aMCI groups across AD-vulnerable brain regions. For each separate intrusion measure, we controlled for false discovery rate (FDR).[30]

Data availability

Anonymized data not published within this article will be made available by request from any qualified investigator.

Results

The aMCI non-AD Amy− group members on average were ≥6 years younger than the aMCI Amy+ participants and those with SNAP ($F_{2,86} = 7.43$, $p \leq 0.001$). In addition, the Amy+ group had lower MMSE scores than individuals in the other study conditions ($F_{2,86} = 6.58$, $p = 0.002$). Group differences in primary language in which participants were evaluated (English vs Spanish revealed a greater number of Amy− cases with non-AD MCI who were evaluated in Spanish, $\chi^2 = 8.64$, $df = 2$, $p = 0.013$) HVLT-R, Category Fluency, and Trail Making Part B, and the standard LASSI-L measures did not differ among the 3 study groups. Group differences in both hippocampal and ERC volumes were observed ($F_{2,80} = 3.65$, $p = 0.03$ and $F_{2,80} = 3.27$, $p = 0.03$), remaining so after adjustment for baseline covariates. Amy+ participants had lower hippocampal and ERC volumes than Amy−participants with non-AD MCI . Post hoc exploratory analyses did not reveal group volumetric differences in other AD-prone regions such as the precuneus, posterior cingulate, supramarginal gyrus, inferior and superior parietal lobules, inferior and superior temporal lobules, or rostral middle frontal or superior frontal lobules.

Table 1 Comparison between MCI-AD Amy+, MCI-SNAP Amy−, and MCI non-AD etiologies Amy−

	MCI-AD Amy+ (n = 34)	MCI-SNAP Amy− hippocampal+ (n = 29)	MCI non-AD Amy− (n = 25)
Age[a] (range 56–98) (SD), y	74.41[e] (7.0)	75.14[e] (8.5)	68.31[d] (5.7)
Education (range 6–22) (SD), y	14.91 (3.6)	14.93 (3.4)	14.15 (3.5)
Female, %	50.0	58.6	42.3
English speakers %	69.2	69.2	32.1
MMSE score[b] (range 23–30) (SD)	26.50[d] (2.0)	28.14[e] (1.5)	27.77[e] (2.0)
Total hippocampal volume[c] range (0.00266–0.00715) (SD)	0.00479[d] (0.00098)	0.00509[d,e] (0.00084)	0.00543[e] (0.00076)
Total ERC volume[c] range (0.00111–0.00326) (SD)	0.00189[d] (0.00040)	0.00207[d,e] (0.00043)	0.00217[e] (0.00040)
LASSI-L Cued Recall B1 (range 0–10) (SD)	6.06 (2.0)	4.83 (2.2)	5.88 (2.7)
LASSI-L Cued Recall B2 (range 3–13) (SD)	8.50 (2.2)	8.76 (2.9)	9.20 (2.1)
LASSI-L Cued Recall A3 (range 0–14) (SD)	6.03 (2.3)	6.55 (3.4)	6.56 (2.2)
HVLT-R total (range = 3–30) (SD)	17.03 (5.0)	18.69 (4.5)	17.94 (7.1)
Category fluency (range 13–66) (SD)	35.36 (9.3)	35.69 (9.5)	35.38 (11.6)
Trail Making Part B time (range 47–300 s) (SD), s	164.59 (79.1)	168.55 (83.5)	137.28 (71.1)

Abbreviations: AD = Alzheimer disease; Amy+ = amyloid positive; Amy− = amyloid negative; ERC = entorhinal cortex; HVLT-R = Hopkins Verbal Learning Test–Revised; LASSI-L = Loewenstein-Acevedo Scales for Semantic Interference and Learning; MCI = mild cognitive impairment; SNAP, suspected non-Alzheimer pathology.
[a] p =0.001
[b] $p < 0.01$.
[c] $p < 0.05$.
[d,e] Means with different superscripts are statistically different at $p \leq 0.05$ by the Tukey HSD procedure. When age, Mini-Mental State Examination scores, and testing language were entered into statistical models as covariates, identical results were achieved as with analysis of variance models without these covariates.

There were differences between groups with regard to PSI semantic intrusions ($F_{2,85} = 10.79$, $p < 0.001$), frPSI intrusions ($F_{2,85} = 10.06$, $p = 001$), and RSI intrusions ($F_{2,85} = 6.94$, $p = 0.002$) (tables 1 and 2). Post hoc comparison of means for PSI and frPSI intrusions with the Tukey honestly significant difference procedure indicated that Amy+ participants committed a significantly higher number of PSI, frPSI, and RSI semantic intrusion errors than did participants in the 2 other study groups. We ran additional analyses adjusting for age, MMSE score, and primary education using ERC volume as covariates and obtained similar results.

ROC analyses were conducted for frPSI and PSI intrusions to determine how they distinguished between Amy+ MCI-AD and other diagnostic groups.

Amy+ participants with MCI-AD vs Amy− participants with MCI non-AD

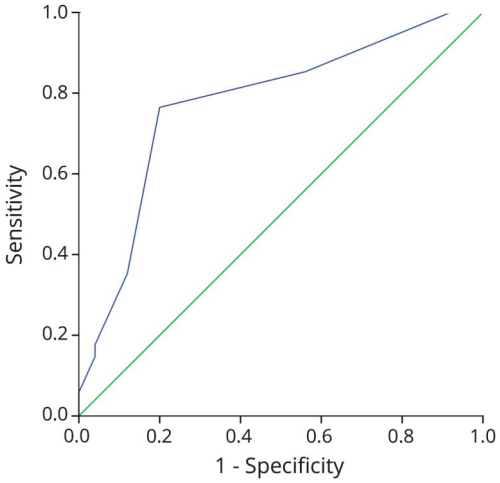
For Cued B2 intrusions (frPSI), ROC analyses for the MCI Amy+ group vs the aMCI non-AD Amy− group yielded an area under the ROC curve of 0.77 (SE 0.06, $p < 0.001$) (figure) with a binomial exact 95% confidence interval (CI) ranging from 0.64 to 0.87. A cutoff of >3 intrusion errors by

Table 2 Comparison of different types of semantic errors between MCI-AD Amy+, MCI-SNAP Amy−, and MCI non-AD etiologies Amy−

	MCI-AD Amy+, (n = 34)	MCI-SNAP Amy− hippocampal+ (n = 29)	MCI non-AD Amy− (n = 25)	*F* test (*p* value)
LASSI-L Cued B1 intrusions sensitive to PSI (range = 0–12) (SD)	6.41[b] (3.6)	3.41[a] (2.8)	3.36[a] (2.2)	10.79 (<0.001)
LASSI-L Cued Recall B2 intrusions sensitive to frPSI (range = 0–11) (SD)	4.97[b] (2.8)	2.62[a] (2.2)	2.44[a] (2.2)	10.06 (0.001)
LASSI-L Cued Recall A3 intrusion sensitive to RSI (range = 0–9) (SD)	6.00[b] (3.6)	3.28[a] (2.9)	3.60[a,b] (2.8)	6.94 (0.002)

Abbreviations: AD = Alzheimer disease; Amy+ = amyloid positive; Amy− = amyloid negative; frPSI = failure to recover from proactive semantic interference; LASSI-L = Loewenstein-Acevedo Scales for Semantic Interference and Learning; MCI = mild cognitive impairment; PSI = proactive semantic interference; RSI = retroactive semantic interference; SNAP, suspected non-Alzheimer pathology.
[a,b] Means with different superscripts are statistically significant at < $p < 0.05$ with the Tukey HSD procedure. When age, Mini-Mental State Examination scores, and testing language were entered into statistical models as covariates, identical results were achieved as with analysis of variance models without these covariates.

Figure ROC curve analyses of frPSI intrusions in distinguishing between Amy+ MCI-AD from Amy− MCI non-AD



Diagonal segments are produced by ties. AD = Alzheimer disease; Amy+ = amyloid positive; Amy− = amyloid negative; frPSI = failure to recover from proactive semantic interference; MCI = mild cognitive impairment; ROC = receiver operator characteristic.

the Youden criteria yielded a maximum sensitivity of 76.5% and a specificity of 80.0%. The Youden J index was 0.5637 with a 95% bootstrap cut point CI after 1,000 iterations of 0.3271 to 0.7554.

For Cued B1 intrusions (PSI), the area under the ROC curve was 0.74 (SE = 0.06; $p < 0.001$) with a binomial exact 95% CI ranging from 0.61 to 0.85. A cutoff of >4 intrusion errors by the Youden criteria yielded a maximum yielded sensitivity of 73.5% (95% CI 55.6–87.1) and a specificity of 72.0% (95% CI 50.6–87.9). The Youden J index was 0.4553 with a 95% bootstrap cut point CI after 1,000 iterations of 0.2043 to 0.6153.

Amy+ participants with MCI-AD vs Amy− participants with MCI-SNAP

For the Amy+ aMCI vs aMCI-SNAP comparisons, for Cued B2 intrusions (frPSI), the area under the ROC curve was 0.75 (SE 0.06, $p < 0.001$) with a binomial exact 95% CI ranging from 0.62 to.85. A cutoff of >3 intrusion errors by the Youden criteria yielded a maximum sensitivity of 76.5% (95% CI 55.8–89.3) and a specificity of 72.4% (95% CI 55.8–89.3). The Youden J index was 0.4888 with a 95% bootstrap cut point CI after 1,000 iterations of 0.2428 to 0.6708.

For the Amy+ aMCI vs aMCI-SNAP comparisons, for Cued B1 intrusions (PSI), the area under the ROC curve was 0.74 (SE 0.06, $p < 0.01$) with a binomial exact 95% CI ranging from 0.61 to 0.84. A cutoff of >4 intrusion errors by the Youden criteria yielded a maximum sensitivity of 73.5% (95% CI 55.6–87.1) and a specificity of 75.9% (95% CI 55.6–87.1). The Youden J index was 0.4939 with a 95% bootstrap cut point CI after 1,000 iterations of 0.2482 to 0.6613.

For all of the above models, entering age, MMSE primary language score, or other LASSI-L variables as covariates in ROC analyses did not improve classification. Finally, the associations between PSI, RSI and frPSI, HVLT-R total recall (a measure not used in the initial diagnosis), and the volume

Table 3 Associations between different semantic intrusion measures and left hemisphere AD-prone regions for Amy+ aMCI

	LASSI-L B1 semantic intrusions	LASSI-L B2 semantic intrusions	LASSI-L A3 short delay semantic intrusions	HVLT-R total memory score
Hippocampus	$r = -0.22$, $p = 0.105$	r = −0.23, $p = 0.100$	$r = -0.18$, $p = 0.153$	$r = 0.33$, $p = 0.031$
ERC	$r = -0.33$, $p = 0.030$	$r = -0.44$, $p = 0.004$[a]	$r = -0.18$, $p = 0.155$	$r = 0.35$, $p = 0.025$
Precuneus	$r = -0.191$, $p = 0.140$	$r = -11$, $p = 0.275$	$r = -0.15$, $p = 0.193$	$r = 0.30$, $p = 0.045$
Posterior cingulate	$r = -0.19$, $p = 0.137$	$r = -0.21$, $p = 0.112$	$r = -0.13$, $p = 0.235$	$r = 0.29$, $p = 0.050$
Inferior temporal	$r = -0.18$, $p = 0.364$	$r = -0.18$, $p = 0.153$	$r = -0.30$, $p = 0.042$	$r = 0.17$, $p = 0.171$
Superior temporal	$r = -0.49$, $p = 0.002$[a]	$r = -0.46$, $p = 0.003$[a]	r = 0.30, $p = 0.044$	$r = 0.26$, $p = 0.076$
Inferior parietal	$r = -0.15$, $p = 0.199$	$r = -0.26$, $p = 0.006$	$r = 0.06$, $p = 0.368$	$r = 0.29$, $p = 0.048$
Superior parietal	$r = -0.12$, $p = 0.245$	$r = -0.11$, $p = 0.269$	$r = -0.15$, $p = 0.196$	$r = 0.10$, $p = 0.299$
Supramarginal	$r = -0.51$, $p = 0.001$[a]	$r = -0.36$, $p = 0.020$	$r = -0.45$, $p = 0.004$[a]	$r = 0.16$, $p = 0.184$
Superior frontal	$r = -0.28$, $p = 0.057$	$r = -0.22$, $p = 0.105$	$r = -0.22$, $p = 0.103$	$r = 0.16$, $p = 0.180$
Rostral middle frontal	$r = -0.06$, $p = 0.367$	$r = 0.10$, $p = 0.285$	$r = -0.11$, $p = 0.277$	$r = 0.16$, $p = 0.192$

Abbreviations: AD = Alzheimer disease; aMCI = amnestic mild cognitive impairment; Amy+ = amyloid positive; ERC, entorhinal cortex; HVLT-R = Hopkins Verbal Learning Test–Revised; LASSI-L = Loewenstein-Acevedo Scales for Semantic Interference and Learning.
Adjusted *p*-values for one-tailed directional tests for each semantic intrusion measure for 11 MRI Regions of Interest.
[a]Correlation coefficients remained statistically significant after adjustment for false discovery rate[30] at $p < 0.05$.

of 11 different regions for Amy+ participants with aMCI were examined. After adjustment for the FDR, statistically significant associations remained between PSI and frPSI and volumes of the ERC, supramarginal gyrus, and superior temporal regions, with correlation coefficients ranging from $r = -0.44$ to -0.51 (table 3). The number of RSI intrusions was correlated with supramarginal gyrus volume ($r = -0.45$). No other associations survived correction for the FDR. No statistically significant associations linked semantic intrusion measures and any MRI regional volume in Amy− cases with SNAP. Similarly, in the non-AD Amy− group, after correction for FDR, we found no statistically significant associations between semantic intrusion measures and any MRI regional volumes.

Discussion

Identifying a reliable and inexpensive clinical marker of amyloid positivity among at-risk individuals would allow recruitment of trial-worthy participants in AD clinical trials much more efficiently than is currently possible. In addition, this may be useful for clinicians who do not have access to amyloid imaging. This study evaluated whether the presence and number of semantic intrusions on a memory test could differentiate between Amy+ and Amy− cases of aMCI. We have provided strong evidence that semantic intrusions related to PSI and frPSI on a cognitive stress test (the LASSI-L) successfully and accurately differentiated patients with aMCI who were Amy+ (and presumably had underlying prodromal AD) from those with aMCI with a clinical course and MRI findings suggestive of AD who were Amy− (classified as SNAP). The severity of these intrusions also differentiated Amy+ patients with aMCI from Amy− patients with aMCI with non–AD-like conditions such as FTLD, vascular cognitive impairment, and other neuropsychiatric conditions. Notably, these findings were obtained even though there were no differences in the severity of memory deficits for each aMCI group (e.g., HVLT-R total score) and after statistical adjustment for demographic and global mental status differences between study groups.

While Amy+ participants with aMCI had lower hippocampal and ERC volumes than the Amy− groups with other non-AD clinically diagnosed pathology, no other reductions in brain volumes in regions vulnerable to AD pathology distinguished the Amy+ aMCI group from the 2 Amy− groups. Even after statistical adjustment for hippocampal and ERC volumetric differences and the other aforementioned covariates, Amy+ participants with aMCI had a significantly greater number of semantic intrusion errors related to proactive interference than did the 2 other nonamyloid diagnostic groups. The current investigation highlights the importance of considering cognitive stress tests such as the LASSI-L, which in other laboratories has outperformed the Free and Cued Selective Reminding Test in MCI-AD groups confirmed by ROC analyses.[31] Indeed, Sánchez et al.[32] recently found that 50% of asymptomatic middle-aged children of parents with late-onset AD had intrusion errors on PSI trials of the LASSI-L and that these errors were associated with lack of connectivity between AD-prone regions on fMRI.

Currently, treatment development for AD is based primarily on using the amyloid hypothesis to identify individuals who are Amy+ and are either preclinical or in a very early stage of the disease. Therefore, the design of clinical trials to prevent or modify the course of AD requires the recruitment of participants who are cognitively normal or are very mildly impaired and are Amy+. The findings in this study suggest that the frequency of semantic intrusions on a memory test such as the LASSI-L would serve well as an inexpensive and readily available clinical marker with potentially high specificity for predicting amyloid positivity among participants being recruited for an AD clinical trial. At the very least, pending additional studies, it could help to screen for people to scan for entry to a therapeutic intervention.

Simons and Spiers[33] postulated that the medial temporal and medial orbital and other frontal regions are responsible for discrete and elaborate representations of to-be-remembered targets involved in the learning process and source memory.[34,35] Recently, the supramarginal gyrus has also been implicated in verbal working memory.[36] These areas work together to reactivate, monitor, and differentiate semantic associations and representations, and damage to this system may lead to semantic intrusion errors.[36–38] Indeed, both PSI and frPSI intrusions in Amy+ participants with aMCI were related to reduced left ERC, superior temporal, and supramarginal gyrus volumes, although among the Amy− groups, there was no association between the number of semantic intrusions and ERC volumes. These semantic intrusions may not be solely related to structural brain loss. Tau deposition and dendritic excitability in persons with early AD likely have a key role in synaptic plasticity, and aberrant dendritic morphology and ion channel activity contribute to hyperexcitability signaling and disruption of neuronal circuits.[38]

While semantic intrusions have been previously reported in early AD[13,14] and have even differentiated AD and vascular groups,[39] this study is the first to provide evidence that semantic errors on cognitive stress tests such as the LASSI-L are more prevalent in patients with aMCI-AD who are Amy+ than they are in patients with SNAP who have the clinical features of AD but are Amy− or other neurological psychiatric conditions that clearly are not related to AD (and confirmed as Amy−). The present investigation confirms our clinical experience that the presence of semantic intrusions on cognitive stress tests distinguishes those who are completely cognitively normal from those with subtle cognitive impairment but who would otherwise be classified as cognitively unimpaired if only correct responses were taken into account. About 75% to 80% of these semantic intrusions consist of words recalled from a competing word list on the LASSI-L, while 20% to 25% of these intrusions were semantically related items that were not on a competing list of target items. These semantic intrusions clearly represent impairment in

inhibition or distinguishing semantically related responses, which may be a pathophysiological feature of early AD.

Strengths of this study included the use of detailed, well-established, and standardized operational criteria in the evaluation and diagnosis of patients with aMCI, the availability of both expert readings of amyloid positivity (with highly reliable independent neuroradiological ratings) and volumetric MRI data for these participants, and adjustment for FDRs to control for spurious errors of inference. Limitations include potential bias of those individuals who are self-selected to participate in the 1Florida Alzheimer's Disease Research Center and undergo amyloid scans and that there were some Amy+ individuals who were not identified by PSI semantic intrusion errors. Just as we were able to assemble a group of patients with SNAP aMCI who presented clinically with an AD phenotype but were Amy–, it would also be beneficial to have larger homogeneous groups of participants with FTLD or diffuse Lewy body disease or participants with cerebrovascular disease who were Amy–. As in any study, the results of ROC analyses always produce the possibility of overfitting the model despite our use of CIs and bootstrapping to obtain a range of cut points. Therefore, these results should be considered preliminary and subject to independent replication. Our ongoing longitudinal studies will further elucidate the predictive utility of these types of measures with regard to progression, disease specificity, and ultimately response to new emerging treatments.

Author contributions

David A. Loewenstein obtained funding, conceptualized the study, analyzed the data, and prepared the manuscript. Rosie E. Curiel revised the manuscript content and was involved in study supervision. Steven DeKosky and Russell M. Bauer revised the manuscript content. Monica Rosselli revised the manuscript content and was involved in study supervision. Salvador M. Guinjoan, Malek Adjouadi, and Warren W. Barker revised the manuscript content. Sindy Goenaga revised the manuscript content and coordinated the study. Todd Golde revised the manuscript content and obtained funding. Maria T. Greig-Custo revised the manuscript content, acquired data, and was involved in study supervision. Kevin S. Hanson, Chunfei Li, Gabriel Lizarraga, and Michael Marsiske revised the manuscript content. Ailyn Peñate revised the manuscript content, acquired data, and was involved in study supervision. Ranjan Duara revised the manuscript content; acquired data, was involved in study supervision, and obtained funding.

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Disclosure

The authors report no disclosures relevant to the manuscript. Go to Neurology.org/N for full disclosures.

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