

RESEARCH ARTICLE

Spatial extent as a sensitive amyloid-PET metric in preclinical Alzheimer's disease

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Abstract

INTRODUCTION: Spatial extent-based measures of how far amyloid beta ($A\beta$) has spread throughout the neocortex may be more sensitive than traditional $A\beta$ -positron emission tomography (PET) measures of $A\beta$ level for detecting early $A\beta$ deposits in pre-clinical Alzheimer's disease (AD) and improve understanding of $A\beta$'s association with tau proliferation and cognitive decline.**METHODS:** Pittsburgh Compound-B (PIB)-PET scans from 261 cognitively unimpaired older adults from the Harvard Aging Brain Study were used to measure $A\beta$ level (LVL; neocortical PIB DVR) and spatial extent (EXT), calculated as the proportion of the neocortex that is PIB+.**RESULTS:** EXT enabled earlier detection of $A\beta$ deposits longitudinally confirmed to reach a traditional LVL-based threshold for $A\beta$ + within 5 years. EXT improved prediction of cognitive decline (Preclinical Alzheimer Cognitive Composite) and tau proliferation (flortaucipir-PET) over LVL.**DISCUSSION:** These findings indicate EXT may be more sensitive to $A\beta$'s role in preclinical AD than level and improve targeting of individuals for AD prevention trials.

KEYWORDS

amyloid, amyloid PET, amyloid staging, cognitive decline, early detection, longitudinal, positron emission tomography, preclinical Alzheimer's disease, tau PET

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Highlights

- A β spatial extent (EXT) was measured as the percentage of the neocortex with elevated Pittsburgh Compound-B.
- A β EXT improved detection of A β below traditional PET thresholds.
- Early regional A β deposits were spatially heterogeneous.
- Cognition and tau were more closely tied to A β EXT than A β level.
- Neocortical tau onset aligned with reaching widespread neocortical A β .

1 | BACKGROUND

Recent clinical trials have demonstrated that anti-amyloid beta (A β) treatments that remove A β pathology slow Alzheimer's disease (AD) progression and clinical decline,^{1–4} but emerging evidence suggests their efficacy was strongest when applied earlier in the disease continuum while tau pathology was in early phases of deposition.² This suggests that anti-A β treatment may be most effective when used preventively by targeting individuals with the first signs of A β , before the spread of tau pathology.^{5,6} Consequently, optimized tools for detecting and tracking the earliest deposits are urgently needed. Since the inception of A β -PET⁷ nearly two decades ago, researchers have traditionally estimated an individual's overall burden of A β pathology using the average level within a global aggregate of neocortical regions. This approach is sensitive to widespread A β in symptomatic stages of AD, but studies correlating A β -positron emission tomography (PET) with neuropathology ex vivo^{8–10} or cerebrospinal fluid/plasma biomarkers^{11–15} in vivo have established that the regional A β deposits that emerge decades earlier at the start of preclinical AD^{9,11,16} often fall below the signal-to-noise thresholds for average neocortical burden. It is therefore worth considering whether quantifying the average A β level in a large neocortical aggregate is the best approach to measure early A β or if an alternative metric like the spatial extent (EXT) of A β may be more sensitive and meaningful at this earlier stage.

Neuropathological staging of amyloidosis established that the first pattern of A β pathology consistent across most people is widespread neocortical A β .¹⁷ It has been less clear how individuals progress from undetectable pathology to widespread neocortical A β , and in vivo PET attempts to describe a stereotyped spatiotemporal sequence to reach widespread neocortical A β have varied greatly across study and sample.^{12,16,18–24} Instead, the emergence of A β pathology appears to be a heterogeneous process^{25–27} that may be best characterized more generally as spread from a few regional A β deposits to widespread neocortical A β . It follows, then, that an optimal early A β PET metric would seek to quantify how far A β has spread throughout the neocortex rather than the average neocortical A β level. Furthermore, it may be how far A β has spread, rather than the A β level that is most relevant to understanding A β 's role in AD pathogenesis, particularly its hypothesized role in driving the spread of tau pathology.

This study introduces a new A β -PET spatial extent (EXT) metric, computed as the percentage of the neocortex with PET evidence of A β pathology. This approach was contrasted with the conventional PET measure of A β level using longitudinal Pittsburgh Compound-B (PIB)-PET data from a cohort of cognitively unimpaired older adults to demonstrate spatial extent's utility for detecting and quantifying A β in preclinical AD. We hypothesize that the flexibility afforded by EXT will enable more sensitive detection of the earliest A β deposits, potentially allowing research and clinical trials to target an even earlier phase of amyloidosis below standard PET detection thresholds. Furthermore, we anticipate that individuals will reach widespread amyloidosis (100% EXT) at moderate to high A β levels beginning in preclinical AD. We propose a new staging schema for amyloidosis, differentiating between an earlier stage characterized by the spread of A β throughout the neocortex and a later stage characterized by already widespread neocortical A β but continued increase in the concentration of neocortical A β level. Finally, we will demonstrate how complementing traditional measures of A β level with A β EXT provides insight into how A β impacts tau proliferation and cognitive decline in preclinical AD. Overall, we aim to demonstrate how this straightforward but fundamental modification to how A β -PET is used and interpreted can improve our understanding of A β accumulation and its role in AD pathogenesis and, in turn, improve AD clinical trial design.

2 | METHODS**2.1 | HABS sample**

A total of 261 cognitively unimpaired older adults (Clinical Dementia Rating = 0, Mini-Mental State Exam $\geq$ 27) were included from the Harvard Aging Brain Study (HABS).²⁸ of whom 209 had up to 8 years of PIB follow-up (median = 5.93 $\pm$ 2.32 years). PIB-PET was acquired at baseline (n = 261), 1.5-year follow-up in a subset (n = 47), Year 3 (n = 197), Year 5 (n = 146), and Year 8 (n = 104). All participants also underwent annual cognitive testing with a median follow-up length of 9.30 $\pm$ 3.33 years. Tau-PET was introduced after the start of HABS, at Year 3 for most participants (tau baseline: n_{Year3} = 112, n_{Year0} = 17, $n_{\text{Year1.5}}$ = 23, n_{Year5} = 32). At least one Flortaucipir (FTP) scan was

available in a subset of 184 participants, and 138 had longitudinal FTP data over a median of 4.89 ± 1.61 years.

Detailed inclusion criteria for HABS were published previously.²⁸ The procedures for this study were approved by the Partners Human Research Committee, the Institutional Review Board for the Massachusetts General Hospital, and Brigham and Women's Hospital. All participants provided informed consent.

2.1.1 | Diversity, equity, and inclusion

HABS has recruited from a diverse nearby geographical region, including urban Boston, surrounding communities, and the New England area. Women are well represented in the sample (57% female-identifying; Table 1). Extensive efforts were made to increase recruitment of minoritized populations, which in this study was defined as all individuals with an ethnoracial background other than non-Hispanic White. In the full sample ($n = 261$), 19.9% were from minoritized populations (15.7% African American, 2.3% Asian, 1.1% Hispanic, and 0.38% mixed race). Further descriptions of the inclusion of women and minorities are broken down by A β status within the main sample and each subsample in Table 1. Analyses included biological sex as a covariate but did not covary for race due to the relatively small sample size once subdivided by race/ethnicity.

2.2 | A β -PET acquisition and processing

Detailed PIB-PET acquisition parameters for HABS were published previously.^{29–31} In brief, full dynamic PIB-PET scans were acquired, and distribution volume ratios (DVRs) were calculated with a cerebellar gray matter reference region. Magnetic resonance imaging data were processed and parcellated with Freesurfer version 6 and co-registered with PET. To measure A β level (LVL), the average PIB DVR was computed across a standard neocortical aggregate of 42 regions from the Desikan–Killiany³² atlas (Figure 1A), as previously reported and well validated.^{28,31,33–38} To aid in interpretation, the average neocortical (NEO) PIB DVR was also translated to the Centiloid (CL) scale³⁹ using the Level 2 CL method^{33,40} (Section S1) to approximate where each individual's A β LVL fell on the CL scale. Individuals were classified as LVL+/- based on a threshold of 1.19 DVR/24 CL. This LVL threshold was previously derived at HABS baseline³¹ using a common Gaussian mixture modeling (GMM) approach that establishes a signal-to-noise threshold beyond which values can be confidently considered to reflect A β rather than noise.

2.3 | A β -PET spatial extent computation

Average PIB DVR was also computed within each of the 42 NEO regions, and EXT was calculated as the percentage of NEO regions of interest (ROIs) that were PIB+. The same GMM approach was used to derive separate signal-to-noise thresholds for PIB+ in each

RESEARCH IN CONTEXT

1. **Systematic review:** The authors reviewed the A β -PET literature regarding early stages of A β accumulation and found extensive evidence of meaningful regional A β signal below traditional PET thresholds but inconsistency across studies in where these early regional A β deposits were found. We hypothesized that neocortical A β -PET spatial extent (EXT) measures might be more informative in early stages of AD, but our literature search found only one recent paper measuring A β -PET EXT.
2. **Interpretation:** Our findings suggest that neocortical EXT is not only a sensitive tool for early A β detection but may also be more strongly associated with tau proliferation and cognitive decline than traditional A β level measures.
3. **Future directions:** We will evaluate the robustness of these findings across different samples and tracers to develop a generalizable EXT-based algorithm for A β quantification that may improve the targeting of individuals for AD prevention trials and provide a new avenue for leveraging A β -PET to understand AD pathogenesis.

ROI (Figure 1B). Additional details about each ROI's distribution and threshold are included in the supplemental material (Table S1, Figure S1), including bootstrapped 95% confidence intervals for each ROI threshold. While the most straightforward method to compute an individual's EXT would be to simply sum the number of PIB+ ROIs in the NEO aggregate, the 42 ROIs vary greatly in size (Table S1) such that a simple sum biases EXT in favor of smaller ROIs. Instead, we computed EXT as the proportion of the NEO that is PIB+ by summing the number of voxels across all PIB+ ROIs and dividing by the total number of voxels in the NEO aggregate (Figure 1C). Expressed at a percentage, EXT values ranged from 0% (no PIB+ NEO ROIs) to 100% (all NEO ROIs are PIB+), with all values in between estimating the proportion of the neocortex with PIB evidence of A β . In addition to these continuous measures of neocortical amyloid LVL and EXT at each time point, within-subjects ordinary least-squares regression was used to extract slopes of EXT and LVL over time. Additionally, standardized z-score versions of both EXT and LVL were computed relative to their respective baseline distributions and used to generate standardized EXT and LVL slopes.

2.4 | Spatial extent thresholds and stages

We recognized that by computing PIB+ separately for each of the 42 ROIs to capture focal A β elevations we are also increasing the risk that some NEO ROIs may be misclassified as PIB+/- due to chance. Consequently, two EXT thresholds were computed using the longitudinal PIB data to allow for some error near the two EXT extremes.

TABLE 1 Sample demographics. Summary demographic data are shown for the full HABS sample, the longitudinal subset with at least one follow-up PIB visit, and the tau subsample.

	Full HABS sample (n = 261)		Longitudinal PIB subsample (n = 209)		Tau subsample (n = 184) ^a	
	BL LVL–	BL LVL+	BL LVL–	BL LVL+	tauBL LVL–	tauBL LVL+
n	192	69	154	55	125	59
Age, years	73.5 (6.32)*	75.7 (5.83)*	73.2 (6.46)*	75.3 (5.36)*	75.8 (6.73)*	78.0 (5.96)*
Education, years	15.8 (3.13)	16.0 (2.92)	15.9 (3.16)	16.6 (2.71)	16.2 (3.19)	16.6 (2.82)
Sex (n, % female)	107 (55.7%)	41 (59.4%)	89 (57.8%)	30 (54.5%)	68 (54.4%)	36 (61.0%)
APOE (n ϵ 4, %) ^b	32/188 (17.0%)*	38/65 (58.5%)*	25/151 (16.6%)	32/51 (62.7%)*	18/125 (14.4%)*	32/53 (60.4%)*
Race (n minority, %)	43 (22.3%)	9 (13.0%)	22 (14.2%)	7 (12.7%)	22 (17.6.2%)	7 (22.9%)
BL LVL DVR	1.08 (0.05)*	1.46 (0.18)*	1.08 (0.05)*	1.46 (0.16)*	1.09 (0.05)*	1.48 (0.19)*
LVL slope			0.009 (0.014)*	0.028 (0.023)*		
PIB follow-up length, years	4.98 (3.23)	4.34 (2.97)	6.21 (2.32)*	5.45 (2.23)*		
Cog follow-up length, years	8.37 (3.37)	7.76 (3.31)	9.27 (2.53)*	8.37 (2.90)*		
BL PACC	0.007 (0.69)	–0.11 (0.70)	0.07 (0.68)	0.001 (0.67)		
PACC slope	–0.04 (0.15)	–0.21 (0.24)	–0.04 (0.09)	–0.23 (0.25)		
MTL FTP SUVR					1.32 (0.15)*	1.54 (0.29)*
TEMP FTP SUVR					1.39 (0.11)*	1.53 (0.24)*
FTP follow-up length, years					3.63 (2.36)*	2.64 (2.26)*

Note: Data are mean (standard deviation) for continuous variables and count (percentage) for categorical variables.

^aSummary data are shown for a tau subsample used in analyses involving tau PET, with data reported relative to tau baseline.

^bAPOE data were missing for eight participants, so the number of APOE ϵ 4 carriers are reported out of the total with APOE data available.

* $p < 0.05$ for the difference between LVL– and LVL+ within the sample, using t tests for continuous variables and χ^2 for categorical variables.

The EXT detection threshold (EXT+ threshold) was set slightly above 0% (detailed in Section 3.2.2 below), at the baseline EXT value beyond which subsequent EXT slopes were consistently positive,³³ indicative of continuing spread of A β to new ROIs rather than reversion of artifactually elevated ROIs back to PIB– at follow-up. A second EXT++ threshold representing widespread neocortical A β was set slightly below 100% EXT at the baseline EXT value beyond which subsequent change in EXT over time was no longer significantly increasing because all (or nearly all) NEO ROIs are already PIB+. These two thresholds were then utilized to define three stages of A β spatial extent: EXT–: no A β or insufficient A β for reliable PET detection; EXT+: spreading A β ; EXT++: widespread neocortical A β .

2.5 | Tau-PET acquisition and processing

FTP-PET acquisition parameters for HABS were published previously.⁴¹ Standardized uptake value ratios (SUVRs) were computed at 80 to 100 min after injection using a cerebellar gray matter reference tissue. Data were partial volume corrected (PVC) using the Geometric Transfer Matrix method.⁴² Analyses were repeated using FTP data that were not PVC, but the pattern of the results was the same. Average FTP SUVR was computed in two aggregates most relevant in the HABS sample: a medial temporal lobe (MTL) aggregate including entorhinal, parahippocampal, and amygdala and a temporal

neocortex (TEMP) aggregate of inferior temporal, fusiform, and middle temporal cortices. GMM thresholds for regional tau positivity were derived for TEMP and MTL FTP SUVR.

2.6 | Cognition

The PACC5^{33,43} version of the Preclinical Alzheimer Cognitive Composite (PACC)⁴⁴ was computed as the averaged z-scores of five tests (Mini-Mental State Examination, Logical Memory Delayed Recall, Free and Cued Selective Reminding Test, Digit Symbol Substitution, Category Fluency).

2.7 | Statistical analysis

All analyses were conducted in R version 3.6.0. T tests and χ^2 tests were used to assess demographic differences between LVL– versus LVL+ (Table 1) and between EXT– versus EXT+ and EXT+ versus EXT++ groups in Section 3.3.4. Analyses first described the distributions of EXT and LVL independently as A β metrics both cross-sectionally and longitudinally before EXT and LVL were directly compared within participants. Since EXT expresses the spread of A β from 0% to 100% of the neocortex, we used logistic growth modeling to estimate the relationship between EXT and LVL. The asymptote was constrained to 100%,

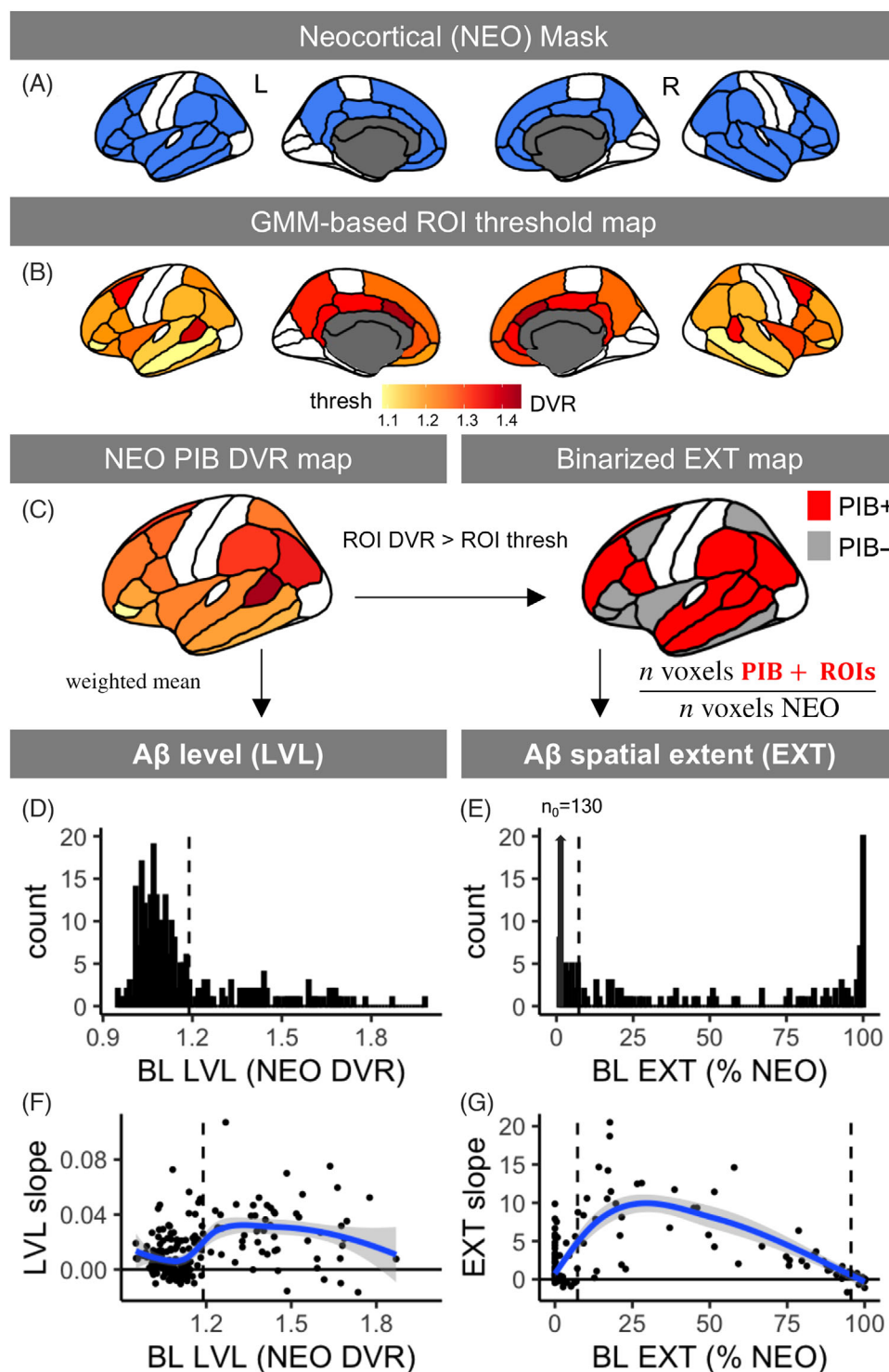


FIGURE 1 Approach. (A) Aβ LVL and EXT metrics were computed within a set of 42 Desikan–Killiany neocortical ROIs typically used for measures of global Aβ burden (blue). (B) A brain map shows the GMM-derived detection thresholds for PIB+ in each NEO ROI, with the color scale indicating each ROI's PIB DVR threshold. (C) As shown in this example participant, computation of both Aβ level and EXT started from each individual's NEO PIB DVR map. Aβ level (LVL) was computed as mean DVR across NEO ROIs, weighted by the number of voxels in each ROI. Each participant's NEO PIB DVR map was binarized relative to the GMM-based ROI threshold map (red: PIB+ ROI, gray: PIB– ROI) and EXT was computed as number of voxels in PIB+ ROIs divided by total number of NEO voxels (both red PIB+ and gray PIB– ROIs). (D) Histogram of distribution of LVL at baseline, with dashed line at GMM-derived LVL threshold (1.19 DVR/24CL). (E) Baseline EXT distribution with dashed line at EXT detection threshold (7.3%). The high frequency of individuals with 0% EXT ($n = 130$) is represented as an arrow rather than a bar to scale the y-axis to properly visualize the non-zero EXT distribution. (F) LVL slope over time plotted against baseline LVL with smoothed LOESS curve. Dashed line represents LVL threshold. (G) EXT slopes are plotted against baseline EXT with the LOESS estimated line (blue). The two dashed lines represent the EXT+ detection threshold (lower) and the EXT++ threshold for widespread Aβ (higher).

TABLE 2 Linear mixed-effects model results testing baseline A β LVL and EXT as predictors of tau proliferation and cognitive decline. Output from a series of linear mixed-effects models are reported testing change in each outcome over time (PACC, MTL FTP SUVR, TEMP FTP SUVR) as a function of baseline A β LVL (Model 1), baseline A β EXT (Model 3), and both baseline A β LVL and EXT in the same model (Model 2).

			β	95% CI	AIC	χ^2
PACC	Model 1: PACC ~ BL LVL $\times$ time	LVL	-0.069***	-0.091, -0.058	3182.3	7.89*
	Model 2: PACC ~ BL LVL $\times$ time + BL EXT $\times$ time	LVL	-0.009	-0.056, 0.037	3178.4	1.87
		EXT	-0.065**	-0.113, 0.018		
	Model 3: PACC ~ BL EXT $\times$ time	EXT	-0.073***	-0.085, 0.053	3176.3	
MTL FTP SUVR	Model 1: MTL FTP ~ BL LVL $\times$ time	LVL	0.007*	0.002, 0.013	-378.78	12.3**
	Model 2: MTL FTP ~ BL LVL $\times$ time + BL EXT $\times$ time	LVL	-0.015	-0.030, 0.001	-387.04	3.84
		EXT	0.024**	0.008, 0.039		
	Model 3: MTL FTP ~ BL EXT $\times$ time	EXT	0.010**	0.004, 0.016	-387.20	
TEMP FTP SUVR	Model 1: TEMP FTP ~ BL LVL $\times$ time	LVL	0.013***	0.008, 0.019	-556.3	16.0***
	Model 2: TEMP FTP ~ BL LVL $\times$ time + BL EXT $\times$ time	LVL	-0.015*	-0.030, -0.0003	-568.37	7.82*
		EXT	0.030***	0.016, 0.045		
	Model 3: TEMP FTP ~ BL EXT $\times$ time	EXT	0.016***	0.011, 0.021	-564.55	

Note: All models included baseline age, sex, education, agexime as covariates, and the random intercept and slope. For each model, the beta estimates and 95% confidence intervals are reported for the BL A β LVL $\times$ time and/or BL A β EXT $\times$ time interaction. LVL and EXT were standardized relative to their own baseline distribution (mean, standard deviation) to allow for comparison of β estimates. Overall model fit was reported using Akaike's information criterion (AIC) to allow for comparison between models, with lower AIC indicating better fit. Finally, χ^2 is reported to formally compare the combined EXT-LVL model (Model 2) to starting models with either LVL (Model 1) or EXT (Model 3) alone. The significant χ^2 between Models 1 and 2 for each outcome indicates that adding EXT significantly improved prediction over LVL alone, while the non-significant χ^2 between Models 2 and 3 for PACC and MTL FTP SUVR indicates adding LVL did not improve prediction over EXT alone. While the χ^2 between Models 2 and 3 was significant for TEMP FTP SUVR, this was due to a negative association between A β LVL and TEMP FTP SUVR. Last, while the χ^2 test does not distinguish between improved fit due to the additional main effect or the interaction with time, the main effects of A β LVL and EXT were not significant in the combined models for any of the three outcome measures, and only the interactions with time are reported. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

and we selected the midpoint and logistic growth rate parameters with the lowest sum of squared error.

To assess EXT's utility for early detection below traditional PET thresholds, separate receiver operator characteristic (ROC) curve analyses were conducted within those who were LVL- at baseline. Each model assessed the sensitivity/specificity of the A β predictors (baseline EXT or LVL) to discriminate between those who progressed from LVL- to LVL+ within 5 years and those who remained LVL-.

To evaluate the spatial heterogeneity of early A β accumulation, additional analyses explored which ROIs were elevated at baseline in the LVL- to LVL+ progressors. To assess whether the ROIs that have been most often classified as early A β regions across nine prior studies^{12,16,18-24} were more vulnerable to early A β , we selected two sets of ROIs: a core set most often (four or five studies) implicated as early (core set: precuneus,^{12,16,20,22,24} posterior/isthmus cingulate,^{12,16,20,22} rostral/caudal anterior cingulate,^{16,18,20,22} medial orbitofrontal^{12,16,20,21,23}) and an expanded core+ set including additional ROIs that have been implicated across three studies: lateral orbitofrontal,^{12,20,23} inferior temporal,^{18,22} middle temporal,²¹⁻²³ inferior parietal.^{16,22,24} Within the LVL- to LVL+ progressors, χ^2 tests were used to compare elevation frequency between the core (or core+) set and all remaining neocortical ROIs. Since many prior studies used bilateral ROIs that did not distinguish between the left and right hemispheres, ROIs were assigned to the core/core+ sets bilaterally. We further evaluated whether ROI elevation frequency differed by

hemisphere (left vs right) and laterality (bilateral vs unilateral) using χ^2 tests.

Next, we evaluated baseline A β LVL and A β EXT as predictors of cognitive decline and tau proliferation. Due to the later introduction of FTP to HABS, analyses relating "baseline" A β and tau over time were conducted using EXT and LVL measured at the PIB scan closest to the tau baseline (± 1 year). We first conducted separate linear mixed effects (LME) models testing baseline A β LVL and EXT as predictors of change over time (A β LVL $\times$ time or A β EXT $\times$ time) in cognition (PACC) or tau (FTP SUVR), while covarying for baseline age, time $\times$ baseline age, sex, and education and included the random effects of participant intercept and slope. Next, we compared A β LVL and EXT directly as predictors of changing tau and cognition by running an additional LME with both baseline A β LVL and EXT in the same model. For each outcome, we compared model fit using Akaike's information criterion (AIC) between models with A β LVL alone (Model 1), A β LVL and A β EXT in the combined model (Model 2), and A β EXT alone (Model 3), with lower AIC indicating better model fit. The models were numbered with the combined model in the center (Model 2) to allow for clearer reporting in Table 2. Finally, χ^2 tests were used to formally test whether adding EXT to the LVL-only model (Model 1 vs Model 2) explained significant additional variance in tau/cognition over time and, likewise, whether adding LVL to the EXT-only model (Model 2 vs Model 3) explained additional variance. Since adding the EXT $\times$ time interaction (or LVL $\times$ time) also required the addition of the EXT (or LVL)

main effect, the number of model parameters increased by 2 in the combined model, and 2 degrees of freedom were used for the χ^2 test.

3 | RESULTS

3.1 | Sample demographics

Table 1 displays the sample demographics grouped by baseline LVL+/- for (1) the full HABS sample, (2) a subsample with longitudinal PIB, and (3) a subsample with at least one FTP scan. In each subsample, LVL+ individuals were slightly older ($t_{full} = 2.67$, $p_{full} = 0.009$; $t_{long} = 2.42$, $p_{long} = 0.017$; $t_{tau} = 2.17$, $p_{tau} = 0.031$) and more likely to be APOE ϵ 4 carriers ($\chi^2_{full} = 39.4$, $p_{full} < 0.001$; $\chi^2_{long} = 37.9$, $p_{long} < 0.001$; $\chi^2_{tau} = 36.7$, $p_{tau} < 0.001$) than the LVL- participants. Participants were slightly older at the tau baseline ($t = 4.26$, $p < 0.001$) since most participants had their first FTP scan at Year 3, but otherwise there were no demographic differences between the full HABS sample, longitudinal subsample, and tau subsample.

3.2 | A β -PET metrics: EXT versus LVL

We first characterized EXT and LVL independently as A β measures, with their baseline distributions shown in Figure 1D/E. LVL exhibited the bimodal DVR distribution characteristic of A β -PET data in non-demented samples of older adults, while EXT had a U-shaped distribution due to the higher frequency of individuals at either end of the EXT scale ($n_{0\%} = 137$ [52.5%], $n_{100\%} = 19$ [7.3%]). Figure 1F/G demonstrate longitudinal changes in EXT and LVL. EXT exhibited fewer negative slopes (16.2%) than LVL burden (22.5%), changes that even after standardization were of lesser magnitude ($M_{EXT} = -0.009$, $SD_{EXT} = 0.012$) than negative LVL slopes ($M_{LVL} = -0.025$, $SD_{LVL} = 0.019$, $t = 4.32$, $p < 0.001$).

3.2.1 | EXT reliability

To assess EXT's reliability and its dependence on the specific ROI thresholds, we generated resampled EXT values based on the bootstrapped 95% CI for each ROI GMM threshold (Figure S2) and computed the correlation between the original EXT value and resampled EXT. Even after varying the ROI GMM thresholds, the resampled EXT distributions remained highly similar to the original EXT (Pearson's r 95% CI: 0.992 to 1), and the rank ordering was largely maintained (Spearman's ρ 95% CI: 0.917 to 0.992).

3.2.2 | EXT thresholds

Using the longitudinal PIB data (Figure 1G), the EXT+ detection threshold was set at 7.3% EXT. Values below this point at baseline were associated with both positive and negative EXT slopes, while above

7.3% slopes were consistently positive. EXT slopes declined back toward no change as individuals approached the EXT maximum of 100% EXT, when all ROIs were PIB+. To allow for some error near 100% EXT, an EXT++ threshold representing widespread neocortical A β was set to 95.6% EXT, the value beyond which subsequent change in EXT over time was no longer significant (Figure 1G).

3.3 | Direct comparison of A β level and spatial extent

Next, we directly compared EXT and LVL within subjects both at baseline (Figure 2A) and longitudinally (Figure 2B). Overall, we observed rising EXT starting well below the LVL+ threshold, followed by the concerted rise in both EXT and LVL as A β spread throughout the neocortex until A β was detected across all regions resulting in an EXT plateau but ongoing increase in LVL. Based on the logistic growth model (midpoint = 1.26 ± 0.002 , logistic growth rate = 0.051 ± 0.001), half of the neocortex was PIB+ (50% EXT) at 1.26 DVR/35 CL. Participants were estimated to reach LVL+ at EXT = 27.8% (95% CI: 26.1 to 29.3). We used our two EXT thresholds to establish an EXT-based staging schema (Figure 2B) for preclinical amyloidosis from EXT- (EXT range: 0% to 7.3%) to EXT+ (7.4% to 95.5%) to EXT++ (95.6% to 100%).

3.3.1 | EXT- stage: A β - based on spatial extent

At baseline, 62.4% of HABS participants were considered A β - based on EXT, compared to 73.6% with the conventional LVL threshold. In the EXT- stage, the range of LVL burden was wide (range = 0.95 to 1.18 DVR/-9.5 to 23 CL, mean = 1.06 DVR/5.6 CL, $SD = 0.04$ DVR/6.0 CL) despite most participants having 0% EXT (130/166, 78.3%). Higher baseline LVL burden in EXT- participants was associated with subsequent longitudinal declines in LVL DVR ($r = -0.34$, $p < 0.001$), indicating higher DVR was more likely to reflect high noise than subthreshold A β . This suggests that any loss of information about subthreshold A β in the EXT- range is likely to be outweighed by EXT's reduction in error.

3.3.2 | EXT+ stage: Spreading A β

On average, individuals reached EXT+ at 1.09 DVR/10 CL. A total of 64 participants were EXT+ at baseline, and none of the 52 participants with longitudinal data reverted to EXT- at any follow-up visit. In the EXT+ group, there was a strong linear relationship between EXT and LVL burden ($r = 0.97$, $p < 0.001$). In this window, an increase of 0.01 DVR corresponds to a 3.2% increase in the proportion of the neocortex with detectable A β and 1 CL to a 2.3% increase in EXT. Based on an average rate of change in EXT of 5.56% per year from this regression, it was estimated to take 16.0 years to go from the onset of detectable A β with EXT to widespread neocortical A β .

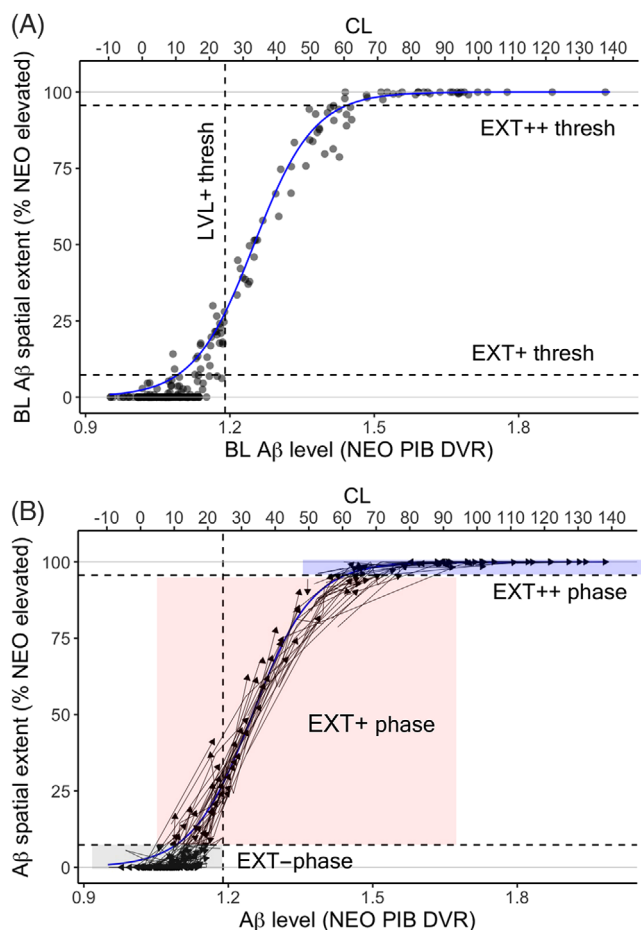


FIGURE 2 Level versus spatial extent (EXT) as A β metrics. The relationship between LVL (x-axis) and EXT (y-axis) is shown both cross sectionally at baseline (A) and longitudinally (B). Dashed lines indicate LVL detection threshold (1.19 DVR/24CL), EXT detection threshold (7.3% EXT), and EXT++ threshold for widespread A β (95.6% EXT). The relationship between EXT and LVL (blue curve) was fit to the logistic growth model with the lowest sum of squared error at baseline. EXT began rising above its detection threshold well below the typical 24CL detection threshold, with elevated EXT detected as low as 8CL. The shaded areas in (B) represent three stages of neocortical amyloidosis based on EXT: EXT- (gray shaded area), EXT+ (spreading phase, red shaded area), EXT++ (widespread phase, blue shaded area). In the EXT+ stage, both EXT and LVL increase as A β spreads throughout the neocortex. In the EXT++ stage, A β is detectable across all (>95.6%) NEO ROIs such that EXT plateaus and further increases in the amount of A β in the neocortex are quantified with A β level. On average, participants reached EXT++ at 1.44 DVR/62CL, though some participants reached EXT++ as low as 1.33 DVR/50CL.

3.3.3 | EXT++ stage: Widespread neocortical A β

Participants reached the EXT++ threshold for widespread A β on average at 1.45 DVR/62 CL. Out of 30 participants who were EXT++ at baseline, 28 (93.3%) remained EXT++ across all available time points. Observed changes in EXT in the EXT++ group were minimal (mean_{EXTslope} = -0.0004, SD_{EXTslope} = 0.0035). Since the neocortical

concentration of A β continues increasing, the EXT++ group exhibited extensive variance in baseline LVL burden (1.44 to 1.98 DVR/60 to 138 CL) and increased in LVL over time (m_{LVLslope} = 0.0255, t = 4.61, p < 0.001).

3.3.4 | Demographic differences by EXT stage

Consistent with the A β -related demographic differences observed between LVL- and LVL+ in Table 1, EXT+ were older than EXT- (M_D = 2.28, p = 0.034) and more likely to be APOE ϵ 4 carriers (χ^2 = 24.8, p < 0.001) but did not significantly differ from EXT++ on age (M_D = -1.71, p = 0.43) or ϵ 4 status (χ^2 = 2.08, p = 0.15). Sex, race, and education did not significantly differ among these three EXT stages. These results support the notion that the EXT+ and EXT++ groups represent earlier and later stages of neocortical amyloidosis, rather than distinct populations.

3.4 | Earlier PET detection of A β with spatial extent

At baseline, 26 participants were EXT+ but fell below the LVL threshold of 1.19 DVR/24 CL, such that 10.0% of the HABS sample would be reclassified as A β + if using EXT rather than LVL to determine A β positivity. Longitudinal PIB follow-up was available for 20 of these EXT+/LVL- participants, and all 20 continued increasing in A β over time, whether measured by EXT slope or LVL slope (Figure 3A).

To establish EXT as a superior method for the early detection of A β and provide further confirmation that EXT+ individuals below the LVL threshold can confidently be considered A β +, we next evaluated EXT's ability to predict progression from LVL- to the gold standard LVL+ at follow-up. As shown in Figure 3B, baseline EXT was a better predictor of progression from LVL- to LVL+ within the next 5 years (AUC = 0.97 [CI: 0.92 to 1]) than baseline LVL burden (AUC = 0.91 [CI: 0.83 to 0.99], DeLong's Z = 2.30, p = 0.02). The 7.3% EXT threshold provided optimized sensitivity (SE = 0.95), specificity (SP = 0.99), and positive predictive value (PPV = 0.95). The LVL threshold could not be lowered to improve sensitivity further than 55% (at 1.16 DVR/20 CL) without increasing the false positive rate (Figure 3A). Selecting a lowered LVL threshold that maximized the Youden index (1.13 DVR/15.5 CL) resulted in higher sensitivity (SE = 85%, SP = 90%) that approached EXT's sensitivity, but added so many false positives that the positive predictive value fell to just above chance (PPV = 57%).

Median time to LVL+ in the EXT+ group was 2.94 years (95% CI: 2.86 to 4.20), and there was a strong inverse relationship between increasing baseline EXT and shorter time to LVL+ (Figure S3). While neither baseline EXT nor LVL were able to detect the seven individuals that progressed to LVL+ at Year 8, 6/7 became EXT+ at an intermediate follow-up compared to only 2/7 whose LVL burden rose above 1.16 DVR/20 CL at an intermediate follow-up prior to reaching the 1.19 DVR/24 CL gold standard.

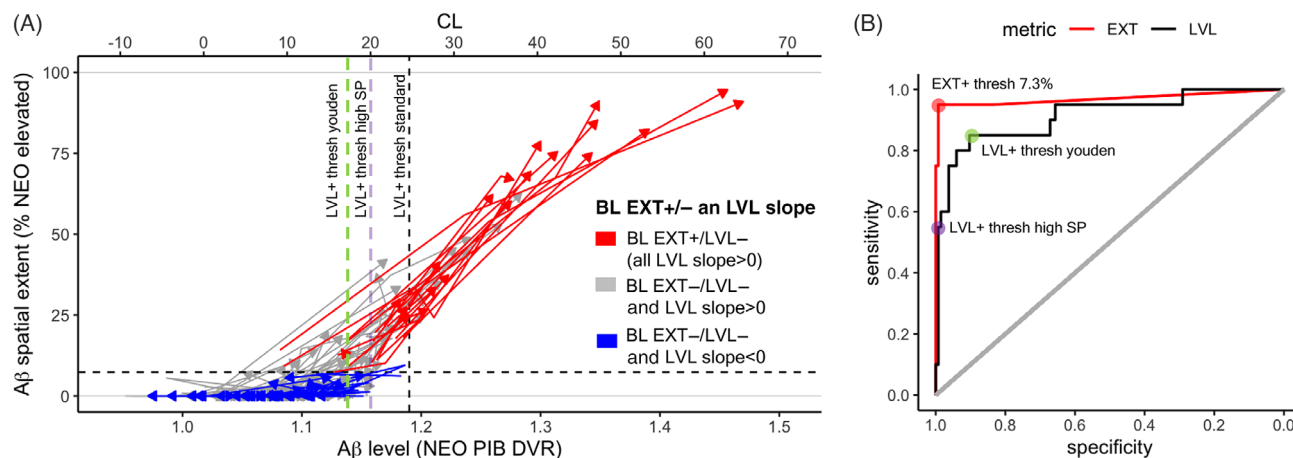


FIGURE 3 Sensitive detection of earliest A β deposits below conventional PET thresholds. (A) To examine EXT's utility below the typical threshold for A β LVL at 1.19 DVR/24CL, we replicated the spaghetti plot showing the longitudinal relationship between EXT and LVL from Figure 2B but zoomed in on the longitudinal subset of 154 individuals below 24CL at baseline. All EXT+ individuals (red arrows) below the LVL threshold continued increasing in A β over time (both EXT slope and LVL slope > 0). EXT- /LVL- participants were split based on their subsequent A β accumulation into those who increased over time (LVL slope > 0; gray arrows) and those who decreased over time (LVL slope < 0; blue arrows) and would therefore be considered false positives if included as A β +. Two alternative LVL thresholds were derived from the ROC analyses in (B) and plotted as vertical dashed lines (purple: LVL threshold that maintains high specificity; green: LVL threshold that maximizes the Youden index), demonstrating that shifting the LVL threshold down to improve detection of the early A β deposits detected with EXT is problematic due to the increased inclusion of false positives with decreasing LVL slope (blue arrows). (B) ROC curves are shown for EXT (red) and LVL (black), depicting the sensitivity and specificity of each metric at varying thresholds for predicting whether an individual that is LVL- at baseline will progress to LVL+ in the next 5 years. EXT reaches high sensitivity (SE = 0.95) while specificity remains excellent (SP = 0.99) at the same 7.3% threshold identified as the EXT detection threshold based on the EXT slope (red point). The sensitivity of LVL only reaches 55% while maintaining the 99% specificity obtained with EXT (at a 1.158 DVR/20CL threshold, purple point). If we select the LVL threshold that maximizes the Youden index (1.126 DVR, green point), sensitivity reaches 85% but specificity decreases to 90% and the positive predictive value falls to 57%.

3.4.1 | Spatial heterogeneity in early A β accumulation

As shown in Figure 4, there was substantial spatial heterogeneity in early regional PIB positivity across the 19 LVL- to LVL+ progressors with EXT > 0. No single ROI was PIB+ across more than 37% of the progressors. The core set of ROIs most often found to be early-accumulating across prior studies was not elevated more frequently across progressors than the remaining neocortical ROIs ($P_{\text{core}} = 0.145$, $P_{\text{other}} = 0.167$, $\chi^2 = 0.582$, $p = 0.446$), nor did expanding to the core+ set alter the significance ($P_{\text{core+}} = 0.173$, $P_{\text{other}} = 0.148$, $\chi^2 = 0.951$, $p = 0.356$). However, there was marked asymmetry in ROI elevations across participants, with elevation frequency twice as high in the left hemisphere as in the right ($P_{\text{left}} = 0.218$, $P_{\text{right}} = 0.103$, $\chi^2 = 19.7$, $p < 0.001$) and twice as likely to be unilateral as bilateral ($P_{\text{uni}} = 0.216$, $P_{\text{bilateral}} = 0.105$, $\chi^2 = 18.0$, $p < 0.001$). Furthermore, there was a trend for more frequent bilaterality in the core+ ROIs than in the remaining neocortical ROIs ($P_{\text{core+}} = 0.394$, $P_{\text{other}} = 0.258$, $\chi^2 = 2.68$, $p = 0.10$).

3.4.2 | Early detection using subsets of neocortical ROIs

We tested whether honing the neocortical aggregate to a smaller set of "early" ROIs might improve the detection of LVL- to LVL+ pro-

gressors, including the core and core+ ROI sets based on existing literature as well as a subset of the most frequently elevated ROIs in the present study. Neither LVL nor EXT computed within any of these potential early A β aggregates was able to achieve the high sensitivity and specificity obtained using neocortical EXT (Tables S2/S3).

3.5 | A β spatial extent and cognitive decline

In the full HABS sample ($n = 261$), we assessed the association between baseline EXT or LVL and change over time on the Preclinical Alzheimer Cognitive Composite (PACC-5 version). As demonstrated in Models 1 and 3 in Table 2 and Figure 5A/B, baseline EXT was comparable to LVL as a predictor of future cognitive decline and had a slightly stronger effect size ($\eta^2_{\text{EXT}} = 0.27$, $\eta^2_{\text{LVL}} = 0.24$; Figure 5A/B) despite the fact that EXT reached ceiling at moderate LVL burden. Higher baseline A β LVL (Model 1, Table 2) alone significantly predicted faster PACC decline, but when baseline A β EXT was added to the model (Model 2), it explained significant additional variance ($\chi^2 = 7.89$, $p = 0.02$) and only EXT remained a significant predictor of PACC change over time (Table 2). Testing in the reverse direction, A β LVL did not explain additional variance ($\chi^2 = 1.87$, $p = 0.40$) not already explained by A β EXT alone (Model 3, Table 2). To clarify why LVL did not explain additional variance in PACC despite EXT's plateau in the EXT++ stage, we evaluated the association between LVL and changing PACC within EXT++

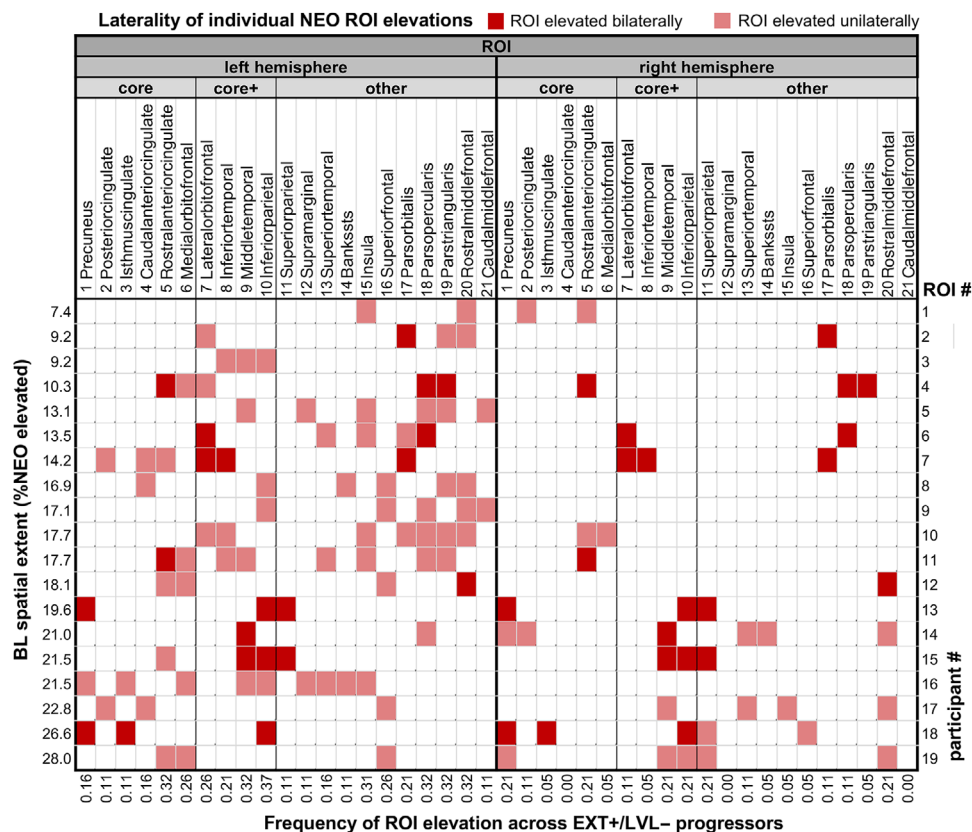


FIGURE 4 ROI elevations in LVL- to LVL+ progressors. A heatmap was used to display the baseline ROI elevations for the subsample of 19 LVL- to LVL+ progressors who were EXT+ at baseline (the final progressor was excluded because no ROIs were elevated at baseline). Each row is a participant, ordered by baseline EXT (reported in the leftmost column). Elevated ROIs are colored red but were further coded based on whether a given ROI elevation in a participant was elevated unilaterally (light red; same ROI in other hemisphere is not elevated) or bilaterally (dark red; same ROI in other hemisphere is elevated). Each column is a ROI subdivided by hemisphere. There is notable asymmetry, with elevations observed more frequently in the left hemisphere than in the right and more frequently unilateral. The frequency of elevation for each ROI across the 19 EXT+/LVL- progressors is reported on the bottom axis, with no single ROI elevated across more than 37% of the progressors. The ROIs were ordered and numbered to reflect both anatomical proximity and their presumed vulnerability to early A β based on past PET studies of early A β accumulation and staging. However, the assumption that some core ROIs (ROIs 1 to 6: precuneus, cingulate, medial orbitofrontal) are more vulnerable to early A β accumulation and would therefore be more frequently elevated than other regions was not supported by our data. If we shift additional ROIs into this core set (core+ ROIs 7 to 10: lateral orbitofrontal, inferior and middle temporal, inferior parietal) we still did not observe a greater frequency of elevation amongst the core+ set (ROIs 1 to 10) than the remaining regions (ROIs 11–21).

individuals. As we observe in Figure 5B, further increases in baseline A β LVL in individuals already at widespread EXT were not associated with changing PACC over time ($n = 30$, $\beta = 0.066$, $SE = 0.085$, $p = 0.447$).

Cognitive decline on the PACC was well characterized by differences between EXT stages (Figure 5C). While EXT- individuals exhibited a mild practice effect in the first 4 years followed by a very slow downward trajectory, the EXT+ group started at the same level as the EXT- group but diverged at follow-up by failing to exhibit a practice effect and then exhibiting significant decline starting at Year 5. Those with already widespread A β at baseline (EXT++ group) declined more rapidly than the EXT- and EXT+ group (Figure 5C), but variance in the rate of cognitive decline in EXT++ individuals was not attributable to higher LVL A β burden.

Finally, we assessed the relationship between EXT/LVL and PACC decline in the baseline LVL- group (Figure 54). Higher baseline EXT ($\beta = -0.099$, $SE = 0.036$, $p = 0.006$) but not LVL ($\beta = -0.033$, $SE = 0.021$,

$p = 0.118$) was associated with faster PACC decline, indicating that the early A β deposits detectable with EXT but falling below a traditional A β -PET threshold are associated with future cognitive decline.

3.6 | A β spatial extent and tau proliferation

Next, we examined the relationship between A β EXT and tau proliferation. Descriptions of this subsample are presented in Table 1, and further details about the EXT and LVL values at tau baseline are reported in Figure 55 and Table 54. As demonstrated in Models 1 and 3 in Table 2 and Figure 5D-I, baseline A β EXT was a modestly stronger predictor of increasing FTP SUVR over time than A β LVL in both the MTL ($\eta^2_{EXT} = 0.09$, $\eta^2_{LVL} = 0.05$) and TEMP ($\eta^2_{EXT} = 0.28$, $\eta^2_{LVL} = 0.19$). Higher baseline A β LVL (Model 1, Table 2) alone significantly predicted faster tau proliferation in the MTL and TEMP, but when baseline A β

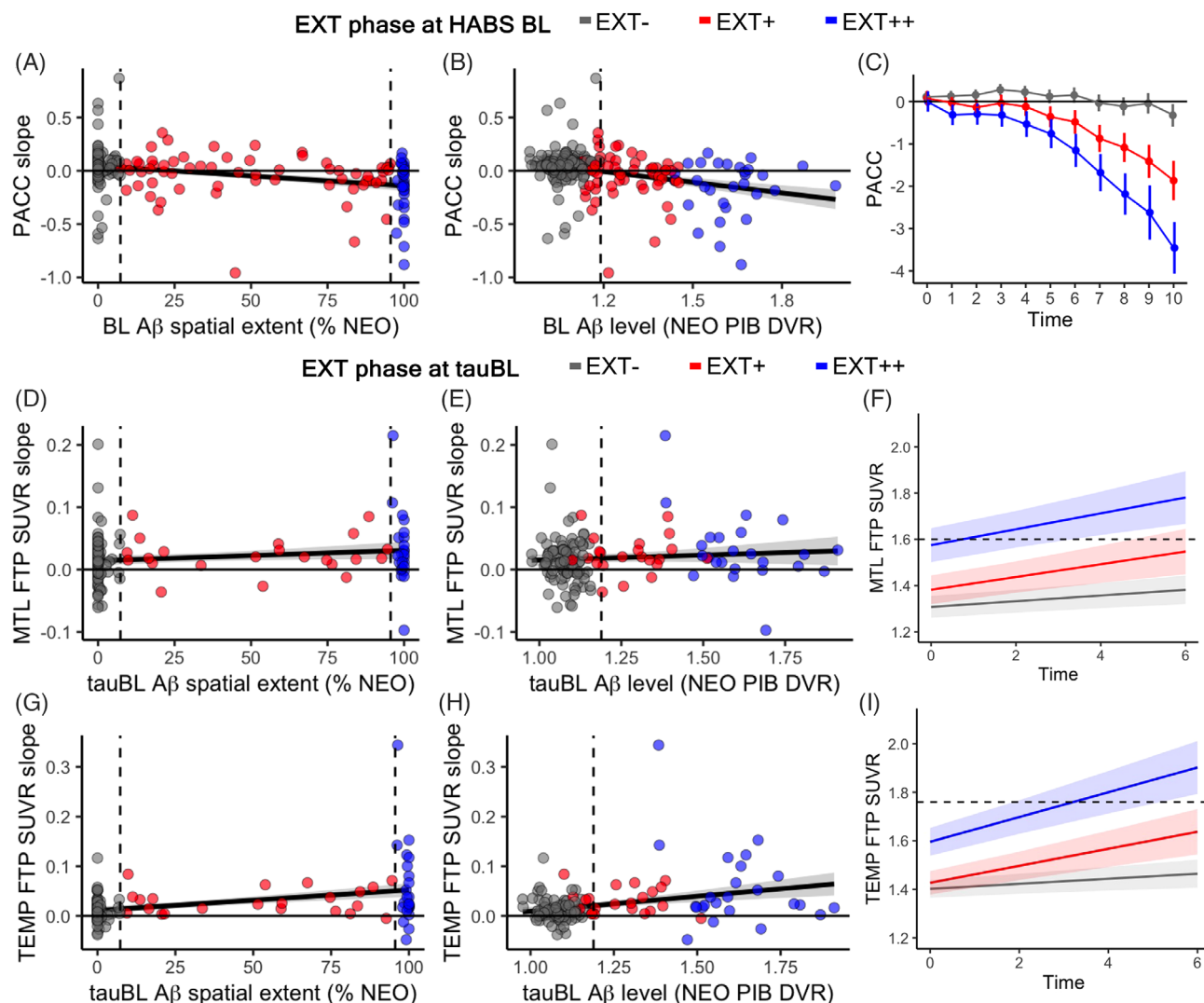


FIGURE 5 Baseline Aβ EXT versus LVL as predictors of future changes in cognition and tau proliferation. Each row displays the association between baseline Aβ EXT and level and subsequent change over time in cognition with PACC (first row) and change in tau in MTL (second row) and TEMP (third row). Since tau PET was introduced later, the first row of plots relating Aβ to PACC used Aβ variables (EXT, LVL, EXT stage) from the main HABS baseline, but in the second and third rows related to tau the PIB scans closest to tau baseline (tauBL) were used to measure Aβ EXT and LVL. All plots are colored based on the EXT stage at baseline (or tau baseline) to aid in interpretation. The first and second columns display scatterplots relating either continuous baseline Aβ EXT (A, D, G) or Aβ LVL (B, E, H) to extracted slopes for PACC, MTL FTP SUVR, and TEMP FTP SUVR. Both higher baseline Aβ EXT and LVL were associated with faster PACC decline (A, B) and faster tau proliferation in the MTL (D, E) and TEMP (G, H). However, higher magnitude of LVL burden was not associated with faster PACC decline (B) or tau proliferation (E, H) once widespread EXT was observed (blue points). The third column displays the estimated marginal means of change over time in PACC (C), MTL FTP SUVR (F), and TEMP FTP SUVR (I) from linear mixed-effects models using baseline (or tau baseline) EXT stage as the predictor. (C) While PACC decline was strongest in EXT++ participants and EXT+ participants after 5 years, a more subtle difference between the EXT+ and EXT- groups was detectable after 2 years of follow-up. The EXT- group exhibited a practice effect, while the EXT+ showed no increase in PACC performance at follow-up due to prior exposure. (F, I) The EXT+ (red) and EXT++ (blue) groups both exhibited a faster increase in MTL and TEMP FTP SUVR relative to the EXT- group (gray) but changed at a similar rate. EXT+ and EXT++ groups instead differed in their tau level at baseline, with EXT++ individuals already exhibiting higher tau.

EXT was added to the model (Model 2), it explained significant additional variance in MTL FTP SUVR ($\chi^2 = 12.3$, $p = 0.002$) and TEMP FTP SUVR ($\chi^2 = 16.0$, $p = 0.003$). In the combined EXT and LVL models (Model 2), only EXT remained a significant predictor of increasing FTP SUVR over time in MTL and TEMP (Table 2), though increasing baseline LVL was significantly associated with decreasing TEMP tau proliferation and a marginally significant decrease in MTL tau over

time. Consequently, Aβ LVL did explain significant additional variance in TEMP FTP SUVR not explained by EXT ($\chi^2 = 7.82$, $p = 0.02$) and marginally significant additional variance in MTL FTP SUVR ($\chi^2 = 3.84$, $p = 0.07$), but in the opposite direction from that expected. As shown in Figure 5E/H, in 32 individuals who had already reached the EXT++ stage (blue) at tau baseline, further increases in the magnitude of Aβ LVL were associated with decreasing change in tau over time in the

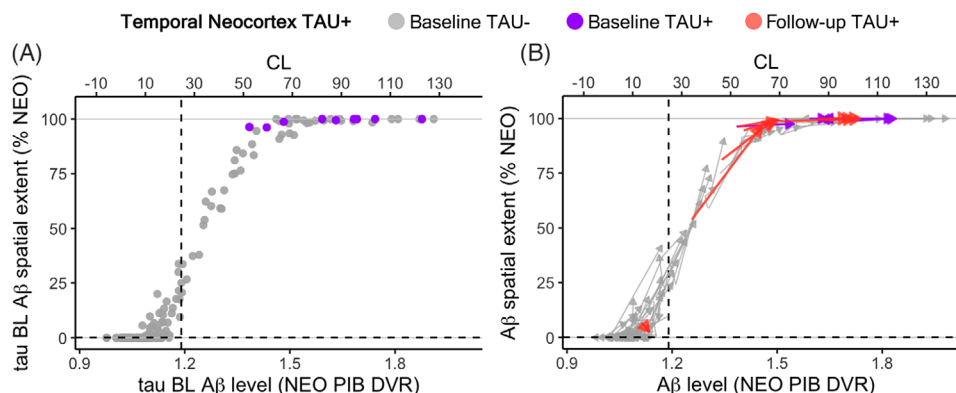


FIGURE 6 Tau positivity aligns with reaching widespread A β EXT. The plots relating A β EXT and LVL from Figure 2 were replicated using cross-sectional PIB data at tau baseline (A) and using longitudinal PIB data from tau baseline onward (B). Additionally, points/lines are colored by tau positivity in the temporal neocortex (TEMP). (A) At tau baseline, all TAU+ individuals (purple) were in the EXT++ stage of widespread neocortical amyloidosis. (B) Of six individuals who were TAU– at tau baseline but reached TAU+ at follow-up (orange), 5/6 were also EXT++ at follow-up (4/6 progressed from EXT+ to EXT++ over this interval, one was already EXT++). While the number of TAU+ individuals is small and replication is needed, these findings suggest a strong temporal alignment between reaching widespread neocortical amyloidosis and the onset of tau in the temporal neocortex.

MTL ($\beta_{\text{MTL}} = -0.023$, SE = 0.010, $p = 0.03$) and TEMP ($\beta_{\text{TEMP}} = -0.025$, SE = 0.013, $p = 0.05$). This suggests that once A β is widespread, the magnitude of the neocortical A β burden has a limited impact on tau proliferation.

As shown in Figure 5F/I, both the EXT+ and EXT++ groups exhibited accelerated rates of MTL and TEMP tau proliferation relative to the EXT– group, but the rate of change in FTP SUVR did not differ between EXT+ and EXT++ groups. The difference in tau between the EXT+ and EXT++ groups was found instead in FTP SUVR at tau baseline, with the EXT++ group already exhibiting higher levels of MTL and TEMP tau than the EXT+ group ($\beta_{\text{MTL}} = 0.19$, SE = 0.063, $p = 0.003$; $\beta_{\text{TEMP}} = 0.17$, SE = 0.050, $p = 0.002$).

3.6.1 | Onset of tau positivity and A β spatial extent

Figure 6 demonstrates the alignment between reaching widespread neocortical A β EXT and the onset of tau positivity in the temporal neocortex. All participants (9/9) that were TEMP TAU+ at tau baseline were also in the EXT++ stage of widespread A β (Figure 6A). Furthermore, of six participants who progressed to TEMP tau+ at follow-up (Figure 6B), five were EXT++ at follow-up, including four participants who progressed from EXT+ to EXT++ over the same interval during which they progressed from TAU– to TAU+.

4 | DISCUSSION

Our findings demonstrate the potential value of a PET measure of neocortical A β EXT when targeting the earliest, preclinical stage of AD. EXT enabled sensitive detection of longitudinally confirmed early A β deposits that at baseline were below typical A β -PET thresholds while avoiding the false positives encountered when lowering the A β

LVL threshold. However, EXT's utility extended beyond early detection alone, providing a continuous quantitative measure of how far A β pathology had spread throughout the neocortex. A β EXT modestly improved prediction of subsequent tau proliferation and cognitive decline relative to A β level, and when they were modeled together, it was EXT rather than level explaining most of the A β -related variance in increasing tau and declining cognition. EXT allowed for the discrimination of an earlier spreading A β stage (EXT+) and a later widespread A β stage (EXT++). While more severe cognitive decline and greater tau pathology were observed after A β is already widespread in the EXT++ phase, individuals in the EXT+ phase nevertheless exhibited significant future tau proliferation and cognitive decline, suggesting they could provide an ideal window for prevention trials seeking to intervene while tau pathology is still limited.

Recent years have seen growing acknowledgment that while some regions may be more vulnerable to early A β deposits^{12,16,18–24} or appear so due to regional variations in non-specific binding,²⁶ there is no universal path to neocortical amyloidosis.⁴² A recent study²⁷ used spatial extent measured within an a priori set of regions previously implicated as early-accumulating, demonstrating how the flexibility conferred by extent could improve detection of A β below standard PET thresholds. Our findings provide further evidence of extent's value for early A β detection and demonstrate that placing regional constraints on neocortical A β pathology adds unnecessary limits that reduce sensitivity. We observed spatial heterogeneity in ROI elevations in progressors below the typical 1.19 DVR/24 CL threshold, and elevations were no less frequent outside the set of regions most often deemed early-accumulating across past studies.^{12,16,18–24} Due to the strong asymmetry in ROI elevations in progressors, the common practice of using bilateral ROIs may have contributed to the underestimation of the frequency of early A β deposits in lateral frontal and parietal regions. Differences in sample and methodology, especially in setting ROI thresholds for positivity, are also likely contributors to the

perception that the core medial regions (precuneus, cingulate, medial orbitofrontal cortex) are more frequently elevated early in amyloidosis, since non-specific binding varies across regions²⁶ and tends to be highest in core medial regions (Table S1, Figure S1). However, given the small sample of progressors, it is possible that elevation frequency in the core medial regions was underestimated in our sample by chance. Critically, our results indicate that a neocortical EXT approach confers the flexibility needed to maximize sensitive detection of early regional A β deposits and avoids biasing detection to specific regions that may represent one of many AD phenotypes.²⁵

This study was not intended to suggest that the use of standard measures of the A β level in the neocortex is incorrect or that EXT should replace A β LVL measures entirely, but instead to introduce the added benefit of complementing traditional A β LVL measures with EXT to further improve A β detection during the earliest stages of amyloidosis and improve our understanding of A β 's role in AD pathogenesis. A β LVL is inherently tied to A β EXT, because as A β spreads to additional regions, the average neocortical A β LVL increases. However, A β level is a function of the concentration of radiotracer binding to A β pathology in regions where it is present, and local increases in concentration without the spread of pathology to new regions would also increase A β LVL. By explicitly measuring the EXT of A β pathology in the neocortex, we were able to uncouple EXT and concentration to provide insight into how A β accumulates and how it impacts tau proliferation and cognitive decline. In the EXT+ group, A β EXT and LVL were very strongly correlated, suggesting that low to moderate burden on typical measures of A β level is primarily a reflection of the spread of a low level of A β pathology throughout the neocortex rather than focal increases in concentration where A β is already present. However, EXT and LVL clearly diverge as measures of neocortical A β pathology in the EXT++ stage, with EXT providing no further information about ongoing A β accumulation while LVL continues to provide an estimate of A β 's neocortical concentration.

Contrary to what we might expect, the EXT ceiling did not weaken detection of an association between A β EXT and tau proliferation/cognitive decline but instead resulted in a slight improvement. Moreover, when pitted against each other, it was EXT rather than LVL that explained the A β -related variance in increasing tau and declining cognition. Instead, our analyses in EXT++ individuals indicated that the weaker association with LVL was related to the observation that further increases in A β LVL once EXT was widespread were not associated with cognitive decline and were actually associated with slower tau proliferation. These findings are consistent with the hypothesis that A β triggers the acceleration of tau proliferation in the MTL and its resulting spread into the temporal neocortex, but that once triggered tau is largely self-propagating.^{45,46} Prior PET studies support the idea that a threshold level of A β must be reached before this triggering event occurs,^{40,47} with emerging support for a threshold around 50 CL.^{40,48,49} Interestingly, we observed a strong alignment between reaching widespread neocortical EXT and the onset of tau PET positivity starting around 50 CL, suggesting it is not some arbitrary amount of A β but instead reflects the A β LVL most commonly associated with reaching widespread neocortical EXT. Thus, while replication in addi-

tional samples with other PET tracers is needed before we can draw conclusions about A β EXT being a stronger predictor of tau proliferation and cognitive decline than traditional A β LVL measures, our findings clearly demonstrate the benefit of complementing our traditional A β LVL metrics with EXT to deepen our understanding of the mechanisms underlying A β 's relationship with other AD biomarkers.

Our findings also support further research to enable EXT metrics to be used in clinical trial design. Anti-A β treatment may be most effective when used preventively by targeting individuals before A β induces the spread of tau pathology.^{5,6,50} The EXT+ phase may be an ideal target for such a trial, allowing inclusion of both individuals at an earlier stage of amyloidosis than is possible with traditional neocortical A β level thresholds and individuals approaching widespread A β pathology (EXT++) who are likely to be on the cusp of accelerated tau proliferation. Our longitudinal analyses in the EXT+ group indicate that both tau proliferation and cognition may be suitable outcome measures to test whether the removal of either or both A β and tau may slow or halt progression along the AD cascade over a reasonably short trial interval. The lack of a practice effect on the PACC in the EXT+ group is consistent with prior studies suggesting that A β accumulation has a subtle but relevant impact on learning^{51,52} prior to accelerated tauopathy that may have prevented EXT+ individuals from benefiting from prior exposure. Clinical trials targeting EXT+ individuals may therefore further optimize the detection of treatment effects on cognition by using composites honed to the more subtle cognitive changes observed in this earlier stage, including measures of learning.

For clinical trials seeking to intervene closer to clinical impairment, the EXT++ group could constitute an effective tool for selecting individuals with widespread A β and likely tauopathy. Additionally, measuring both A β EXT and LVL could help better characterize how anti-A β antibodies remove plaques and in turn may help trials evaluate how different doses or participant characteristics, for example, may impact efficacy.

This study was intended to introduce the potential utility of an EXT-based A β -PET approach, and further research and validation in other samples are under way to ensure these findings are not dependent on the HABS sample or the use of the C11-PIB tracer. While PIB's low noise made it ideal for proof of concept in this first study, it is not commonly used in research and clinical trials due to its short half-life. Replication with the more commonly used F18 tracers is essential to assess how their differing noise characteristics impact different aspects of EXT measurement (ie, accurate ROI positivity thresholds, reliable EXT+/++ stage thresholds) and whether it may weaken EXT's sensitivity for early detection and its association with tau and cognitive decline. Furthermore, HABS is composed predominantly of Caucasian, highly educated individuals with relatively low cardiovascular risk, and future studies with more representative samples are needed to understand how sample demographics may influence both the biological spread of A β and the robustness of PET EXT metrics.

In addition to validating these findings in other samples, future research will center around optimizing our EXT approach. A voxel-wise approach should in theory provide a more accurate estimate of A β EXT, but in practice the high measurement error at the voxel-level makes

establishing accurate voxel-level thresholds of A β positivity challenging and results in increased false positives. Averaging across hundreds to thousands of voxels within the 42 Desikan–Killiany atlas ROIs smooths out this error, but there is a trade-off because A β deposits do not always adhere to these atlas boundaries and focal deposits can be diluted, particularly within large ROIs. Our ongoing research seeks to improve understanding of how noise properties at the voxel level vary across the neocortex based on the tracer, scanner, pipeline, and so forth in order to optimize the development of a voxel-based EXT algorithm for A β quantification. We also restricted our EXT measure to the neocortex to inform the next generation of prevention trials seeking to intervene as early as possible. Prior work combining HABS and ADNI demonstrated that striatal PIB-PET positivity was associated with increased neurodegeneration and disease progression to mild cognitive impairment and AD.⁵³ Future work will examine how including striatum and other later-accumulating cortical and subcortical structures may broaden the utility of an EXT-based approach to all stages of AD.

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CONFLICT OF INTEREST STATEMENT

The authors report no relevant conflicts of interest. Michelle E. Farrell, Emma G. Thibault, J. Alex Becker, Julie C. Price, Rachel F. Buckley, Heidi I. L. Jacobs, and Charles D. Chen have no disclosures. Bernard J. Hanseeuw has served as a paid consultant for Biogen, Eisai, and Roche. Aaron P. Schultz has been a paid consultant for NervGen and Galen-Atlantica. Brian C. Healy has received research support from Analysis Group, Celgene, Bristol-Myers Squibb, Verily Life Sciences, Merck-

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CONSENT STATEMENT

All participants provided informed consent. All experimental procedures were performed in ethical accordance with the Declaration of Helsinki and were approved and monitored by the local Institutional Review Boards.

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