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Not quite PIB-positive, not quite PIB-negative: slight PIB elevations in elderly normal control subjects are biologically relevant.

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Abstract

Researchers employing Pittsburgh Compound B positron emission tomography (PIB-PET) imaging have consistently indentified old normal control (oNC) subjects with elevated tracer uptake, suggesting the presence of beta-amyloid deposition in these individuals. However, a consensus regarding the level at which PIB reveals a biologically meaningful signal does not exist (ie. an appropriate cutoff value for PIB positivity remains unclear). In this exploratory study, we sought to investigate the range of PIB distribution volume ratio (DVR) values present in our oNC cohort (N=75, age range=58-97). oNC subjects were classified based on global PIB index values (average DVR across prefrontal, parietal, lateral temporal and cingulate cortices) by employing two approaches: (1) an iterative outlier approach that revealed a cutoff value of 1.16 (IO-cutoff) and (2) an approach using data from a sample of young normal control subjects (N=11, age range=20-30) that yielded a cutoff value of 1.08 (yNC-cutoff). oNC subjects falling above the IO-cutoff had values similar to AD subjects (“PIB+”, 15%). Subjects falling between the 2 cutoffs were considered to have ambiguous PIB status (“Ambig”, 20%) and the remaining oNC were considered “PIB-“ (65%). Additional measures capturing focal DVR magnitude and extent of elevated DVR values were consistent with the classification scheme using PIB index values, and revealed evidence for elevated DVR values in a subset of PIB- oNC subjects. Furthermore, there were a greater proportion of ambiguously elevated values compared to low values, and these elevated values were present in regions known to show amyloid deposition. The analyses presented in this study, in conjunction with recently published pathological data, suggest a biological relevance of slight PIB elevations in aging.

Keywords

PIB-PET imaging; aging; Alzheimer's disease (AD); beta-amyloid; PIB-positivity; preclinical AD

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1. Introduction

Following the landmark human ^{11}C-labeled Pittsburgh Compound B positron emission tomography (PIB-PET) study in 2004 (Klunk et al., 2004), many researchers have applied amyloid imaging to investigate the relevance of beta-amyloid (Aβ) deposition in cognitively normal elderly "controls" (NC) (Rabinovici and Jagust, 2009). Consistent with data from post-mortem examination, these studies have re-affirmed that many NCs have extensive Aβ deposition. Furthermore, elevated PIB in NCs has been associated with differences in brain structure (Bourgeat et al., 2010; Dickerson et al., 2009; Fotenos et al., 2008; Jack et al., 2008; Mormino et al., 2009; Oh et al., 2010; Storandt et al., 2009) and brain function (Hedden et al., 2009; Mormino et al., 2009; Sheline et al., 2009; Sperling et al., 2009; Vannini et al., 2011), as well as subsequent decline in memory and conversion to AD (Morris et al., 2009; Storandt et al., 2009; Villemagne et al., 2011), suggesting that Aβ in NCs is not benign and may reflect an early stage of AD development.

Although PIB scans from some NC subjects display uptake indistinguishable from a typical AD scan, less-obvious cases exist that show sub-AD levels of tracer uptake. A major difficulty in assessing the relevance of these slightly elevated PIB values is that the conversion between actual plaque burden and tracer uptake is unclear. Comparison of PIB values to Aβ detected in brain tissue (via postmortem examination and brain biopsy) have shown that elevated PIB uptake is present in cases with high quantities of neuritic Aβ plaques, however the concordance between these measurements is inconclusive in subjects with evidence of low Aβ burden (Bacskai et al., 2007; Cairns et al., 2009; Ikonomovic et al., 2008; Leinonen et al., 2008). The ideal approach would involve comparison of postmortem measurements of Aβ with PIB in NCs, but these data are extremely difficult to obtain, and nearly impossible to obtain with a short delay between scanning and autopsy in NC individuals. To our knowledge, one study directly comparing PIB uptake with postmortem levels of Aβ in cognitively normal controls has been published (Sojkova et al., 2011). In this study, 3 of the 6 NC subjects showed slightly elevated pre-mortem PIB values and had a moderate CERAD rating in at least 1 of 3 regions examined at autopsy. Although limited by sample size, this data suggests that low levels of PIB uptake in NCs may reflect the presence of low quantities of Aβ deposition before AD-comparable levels are reached.

Given the limited data in older NCs, it is not surprising that a consensus regarding "PIB+" categorization is not established. For example, some groups have treated PIB as a continuous variable (Mormino et al., 2009; Pike et al., 2007) whereas other groups have dichotomized subjects into PIB- and PIB+ groups (Aizenstein et al., 2008; Dickerson et al., 2009; Fotenos et al., 2008; Jack et al., 2008; Rowe et al., 2010). A cutoff value for group dichotomization is avoided by treating PIB as a continuous variable, however, the skewed distribution of this variable violates the assumptions of least-square regression and it is likely that there are low PIB values that merely reflect noise. Furthermore, there is variability in categorization approaches amongst studies that dichotomize into PIB+ and PIB- groups [hierarchical clustering (Rowe et al., 2010), iterative outlier removal (Aizenstein et al., 2008), etc]. Moreover, classification into PIB+ and PIB- is often dependent on the distribution of PIB values present in the NC group under investigation rather than on a group of subjects lacking Aβ deposition. Therefore, we cannot be confident that resulting PIB- subjects are definitively negative for Aβ.

To this end, we acquired PIB-PET data from a group of subjects 20 to 30 years of age under the assumption that these individuals have no Aβ deposition and therefore the corresponding PET signal should only reflect noise. Although the age at which Aβ deposition begins is unknown, it has been shown that deposition is minimally present in early life. For instance, Braak and Braak reported that only 1 of 61 subjects below age 35 had any evidence of Aβ

(Braak and Braak, 1997). Furthermore, a separate study reported 7 of 114 subjects under age 50 had sparse CERAD ratings; no subjects under age 50 had moderate or frequent plaques (Kok et al., 2009). It is therefore highly unlikely that 20-30 year olds will have appreciable Aβ deposition and therefore are an appropriate representation of true PIB- scans. Thus, the goal of this study was to investigate the presence of slightly elevated PIB values by employing different techniques to define positive scans and evaluate the likelihood that slight elevations in PIB uptake represent biologically significant Aβ deposition.

2. Materials and Methods

2.1 Subjects

2.1.1 Recruitment—All subjects underwent PIB-PET imaging and magnetic resonance imaging (MRI) for this study (table 1). Seventy-five older normal control (oNC) subjects were recruited via advertisements and word of mouth, and 11 young NC (yNC) subjects were recruited via online postings. Eligibility requirements for all NC subjects were no MRI contradictions, living independently in the community, MMSE≥26, normal performance on cognitive tests, absence of neurological or psychiatric illness and lack of major medical illnesses and medications that affect cognition.

Ten age-matched Alzheimer's disease (AD) patients were selected from a larger pool of subjects recruited from the University of California San Francisco (UCSF) Memory and Aging Center that have been scanned with PIB-PET and MRI. AD diagnosis was based on a comprehensive multi-disciplinary evaluation that includes a clinical history and physical examination, a caregiver interview and a battery of neuropsychological tests (Kramer et al., 2003). All AD subjects met NINDS criteria for probable AD (McKhann et al., 1984) and had no significant co-morbid medical, neurologic or psychiatric illnesses.

2.1.2 APOE Genotyping—DNA from blood samples for oNC and AD subjects were analyzed for apolipoprotein E (APOE) polymorphisms using a standard protocol. For statistical comparison between groups, subjects were dichotomized into carriers and non-carriers of the E4 allele. Genotyping was unavailable for 4 oNC subjects and 1 AD patient.

2.2 Image acquisition

2.2.3 PIB-PET—PIB was synthesized at the Lawrence Berkeley National Laboratory's (LBNL) Biomedical Isotope Facility using a published protocol and described in detail previously (Mathis et al., 2003; Mormino et al., 2009). PIB-PET imaging was performed at LBNL using an ECAT EXACT HR PET scanner (Siemens Medical Systems, Erlangen Germany) in 3D acquisition mode. 370 to 555 MBq of PIB was injected into an antecubital vein. Dynamic acquisition frames were obtained as follows: 4 × 15 sec, 8 × 30 sec, 9 × 60 sec, 2 × 180 sec, 8 × 300 sec and 3 × 600 sec (90 minutes total). Ten minute transmission scans for attenuation correction were obtained for each PIB scan. Filtered backprojected reconstructions were performed on the transmission and emission data to judge transmission alignment with each frame of emission data. In the case of misalignment, the transmission image was coregistered to that individual emission frame, and then forward projected to create an attenuation correction file specific to that head position. PET data were reconstructed using an ordered subset expectation maximization algorithm with weighted attenuation. Images were smoothed with a 4mm Gaussian kernel with scatter correction.

2.2.4 Structural MRI—For oNC and yNC subjects, T1-weighted volumetric magnetization prepared rapid gradient echo scans (MPRAGE, axially acquired, TR/TE/TI=2110/3.58/1100ms, flip angle = 15°, 1.00×1.00mm^2 in plane resolution, 1.00mm thickness with 50% gap) were collected at LBNL on a 1.5T Magnetom Avanto System

(Siemens Medical Systems, Erlangen Germany) with a 12 channel head coil run in triple mode. For AD patients, MPRAGE scans were collected on a 1.5T Vision System (Siemens Medical Systems, Erlangen Germany) with a quadrature head coil (coronally acquired, TR/TE/T1=10/7/300ms, flip angle = 15°, 1.00×1.00mm^2 in plane resolution, 1.40mm slice thickness with no gap).

2.3 Image processing

2.3.1 PIB-PET—PIB-PET data were preprocessed using the SPM8 software package (http://www.fil.ion.ucl.ac.uk/spm). Realigned PIB frames corresponding to the first 20 minutes of acquisition were averaged and used to guide coregistration to the subject's structural MRI scan. Distribution volume ratios (DVRs) for PIB images were created using Logan graphical analysis with frames corresponding to 35-90 min post-injection and a gray matter masked cerebellum reference region defined using FreeSurfer software (Logan et al., 1996; Price et al., 2005).

2.3.2 Structural MRI—MPRAGE scans were processed as described previously (Mormino et al., 2009) using FreeSurfer version 4.5.0 (http://surfer.nmr.mgh.harvard.edu/) to derive regions of interest (ROIs) in each subject's native space (Dale et al., 1999; Fischl et al., 2001; Fischl et al., 2002; Segonne et al., 2004). PIB index values were derived by averaging PIB DVR values from the following ROIs: prefrontal, lateral temporal, parietal and cingulate (the entire cingulate cortex was used). Additionally, mean PIB DVR values were extracted from the 68 cortical ROIs defined automatically by FreeSurfer (Desikan et al., 2006).

2.4 Classification approaches

2.4.1 Primary approach—Two methods to define cutoffs using PIB index values were employed: an iterative outlier approach (Aizenstein et al., 2008) and an approach using PIB index values from the yNC group. The iterative outlier approach removes oNC cases from the oNC group until all outliers are excluded (defined as values greater than the upper quartile value plus 1.5 times the interquartile range). Once all outliers are removed from the data set, 2.5% is added to the PIB index of the highest remaining case (corresponding to the upper inner fence of the box-and-whisker plot) and the resulting value is used as the cutoff. A global PIB index value was used in this procedure rather than multiple ROIs (Aizenstein et al., 2008). The yNC derived cutoff was 2 standard deviations above the mean yNC PIB index value.

Subjects were classified into 3 categories based on these 2 cutoffs—subjects classified as high in both methods were labeled “PIB+” and subjects classified as low in both methods were classified as “PIB-.” Cases with incongruent labeling (high in 1 approach and low in the other) were labeled “Ambiguous” (Ambig).

2.4.2 Secondary Approaches—To investigate PIB uptake in a manner unbiased by the pattern of regional deposition (as is the case with the PIB index approach), additional approaches were taken to explore focal DVR magnitude, as well as measures investigating the extent of PIB elevation in each subject. Focal DVR magnitude was investigated by examining the maximum ROI value for each participant (mean DVR values from all 68 FreeSurfer cortical ROIs were investigated). To restrict this analysis to ROIs outside the range shown by PIB-yNCs, ROI-specific cutoff values were determined for each ROI using 2 standard deviations above the young group's mean for that particular ROI. ROIs below the corresponding ROI-specific cutoff were excluded from this analysis for each subject. To explore uptake extent, the distribution of ROI values for each subject was compared against the distribution of ROI values from the yNC group using a Kolmogorov–Smirnov test.

Resulting d-statistics from this test provides a measure of the overall shift in the distribution of ROI values for each subject relative to the young population, thus reflecting the degree to which PIB extent is elevated irrespective of specific spatial location (both rightward and leftward shifts were tested; a rightward shift indicates a greater extent of increased PIB values whereas a leftward shift indicates a greater extent of reduced PIB values). A final measure simultaneously combined focal magnitude and extent. For this approach, the number of "high" ROIs (2 standard deviations above the yNC mean for that ROI) was counted for each subject.

2.4.3 Comparison between ambiguous elevations and analogous decreases— Instances of ambiguously elevated PIB values were directly compared to analogous reductions in oNC subjects using chi-squared and paired t-tests. This analysis was performed using the 3 aforementioned measures of PIB uptake: (1) PIB Index (the number of subjects falling above and below 2 standard deviations from the mean of yNC PIB index values), (2) d-statistics from the KS-tests (the number of subjects showing significant rightward and leftward shifts relative to the young distribution [$p<0.01$]) and (3) high versus low ROIs (the number of ROIs above and below 2 standard deviations from the yNC mean for each specific ROI). PIB+ oNC were excluded from this analysis, enabling direct investigation of subjects falling in the ambiguous range.

2.4.4 Spatial distribution of elevated brain regions—To explore the spatial pattern of heightened PIB uptake in a data driven manner, the percentage of oNCs showing elevated uptake for each ROI was computed and overlaid on a brain schematic. Percentages were re-computed within each oNC group separately to determine whether a pattern of deposition was apparent across different levels of PIB uptake.

2.5 Statistical analyses and graphics

All statistical analyses and plots were completed using R version 2.11 (http://www.r-project.org/). Group differences in demographic variables were determined with Wilcoxon signed rank tests for continuous variables and chi-squared tests for dichotomous variables. Within oNC subjects, multiple regression with planned contrasts (PIB+ oNC versus PIB- oNC; PIB+ oNC versus Ambig oNC; Ambig oNC versus PIB- NC) were used to assess relationships between MMSE and PIB (controlling for age, sex and education). $P<0.05$ was considered a significant difference, and trends corresponding to $p<0.15$ were noted. Brain schematics were designed in GIMP version 2.6 (http://www.gimp.org/).

3. Results

3.1 Group Characteristics

Group characteristics are listed in table 1. There were no significant differences in gender or education across yNC, oNC and AD groups, although there was a trend for a higher frequency of males than females in AD compared to oNCs ($\chi^2 = 2.38$, df = 1, p= 0.123) and a trend for higher education in oNCs than yNCs (W = 291, p= 0.106). yNCs showed significantly lower PIB index values than oNCs (W = 576, p= 0.035) and AD (W = 103, p= 0.0003), and oNC subjects had lower PIB index values than AD (W = 667, p= 7.008e-05). AD patients had lower MMSE scores than yNCs (W = 5, p= 0.0004) and oNCs (W = 19, p= 3.09e-07). MMSE was not different between yNC and oNC subjects.

3.2 Group Classification

The iterative outlier approach revealed a cutoff value of 1.16 (IO-cutoff), whereas the young-derived cutoff was 1.08 (yNC-cutoff; yNC mean=1.04, sd=0.02; figure 1). This

young derived cut off value was virtually identical using an alternative processing stream that did not use Freesurfer ROIs to compute PIB index values (supplemental analysis 1), suggesting stability of this value within our cohort of young subjects. Fifteen percent (11/75) of oNC subjects were above the IO-cutoff (PIB+), 20% (15/75) were below the IO-cutoff but above the yNC-cutoff (Ambig) and 65% (49/75) were below the yNC-cutoff (PIB-). Nine AD patients were PIB+ while 1 AD patient was PIB-.

To investigate influences of noise on classification, test-retest variability values were computed in a subset of 14 oNCs that had undergone follow up PIB-PET scans (supplemental analysis 2). This analysis revealed a mean test-retest variability for PIB index values of 2.63 (2.32)%, suggesting a low amount of variability across multiple measurements within subjects.

AD subjects had greater PIB index values than PIB- oNC ($W = 35$, $p= 1.613e-06$) and Ambig oNC groups ($W = 15$, $p= 0.0004$). There was a trend for AD subjects to have higher PIB index values than PIB+ oNC ($W = 33$, $p= 0.132$), and this relationship became significant after removal of the single PIB- AD subject ($W = 22$, $p= 0.038$).

PIB+ oNC subjects were significantly older than Ambig subjects ($W = 44$, $p= 0.047$) and there was a trend for PIB+ oNCs to be older than PIB- oNCs ($W = 192$, $p= 0.143$). There were no pair wise differences in education and gender across the 3 oNC groups. Controlling for age/gender/education, there was a trend for worse MMSE in PIB+ oNC than Ambig oNC ($t=-1.548$, $p=0.126$).

The APOE4 allele was present in 24% PIB- oNC, 33% Ambig oNC, 55% PIB+ oNC and 67% of AD subjects. There was a significant pairwise difference in APOE4 allele frequency between AD and PIB- oNCs ($\chi^2 = 4.396$, $df = 1$, $p= 0.036$) and a trend for more e4 carriers in PIB+ oNCs than PIB- oNCs ($\chi^2 = 2.498$, $df = 1$, $p= 0.114$). No other pairwise comparison was significant.

3.3 Secondary Measures of PIB Uptake

Three additional measures were derived to investigate PIB uptake in a manner unbiased by the spatial pattern of PIB uptake. The first approach examined the highest ROI value for each subject, restricting the analysis to only those ROIs exceeding an ROI specific cutoff value (cutoffs are listed in supplemental table 1). In this analysis, 12 of 49 PIB- oNCs and 7 of 11 yNCs did not have an ROI that exceeded the young-defined ROI cutoffs, whereas all Ambig oNC, PIB+ oNC and AD subjects had at least 1 high ROI. The ROI with the highest DVR value for each subject is plotted against group status in figure 2A, and reveals an upward trend between maximum ROI value and group status. Of note is 1 yNC subject with a maximum ROI value of 1.44 located in the left caudal anterior cingulate.

To investigate the extent of elevated ROIs irrespective of spatial location, Kolmogorov–Smirnov (K-S) tests were performed to compare the distribution of the 68 ROI values for each individual subject to the distribution across all young NC subjects. Resulting d-statistics from this test are plotted in figure 2B (a d-statistic of 0.19 corresponds to a p-value of 0.01 for the difference between each subject's distribution of ROI values and the yNC group's distribution), and group level histograms are shown in supplemental figure 4. This approach reveals a rightward shift in the distribution of PIB ROI values in Ambig oNC, PIB + oNC and AD groups, reflecting a greater number of elevated PIB ROIs in these subjects.

A final measure of PIB uptake that simultaneously combined aspects of focal DVR magnitude and extent is plotted in figure 2C. In this analysis, the number of ROIs with values at least 2 standard deviations above the yNC ROI-specific mean was tallied for each

subject. This analysis shows a clear stepwise increase across groups. Interestingly, this analysis reveals that many PIB- oNC subjects show substantial numbers of ROIs (>10) with high PIB uptake, suggestive of amyloid accumulation in this group.

3.4 Ambiguous elevations versus analogous decreases

Instances of ambiguously elevated PIB values were compared to analogous reductions for (1) PIB Index (the number of subjects falling above 1.08 versus below 0.99), (2) KS-test d-statistics (the number of subjects showing significant deviation from the young distribution ($p<0.01/d>0.19$) in each direction) and (3) high versus low ROIs (the total number of ROIs above and below yNC ROI-specific cutoffs). This analysis was restricted to data from Ambig oNC and PIB- oNC subjects, since these data contain the ambiguous signals under investigation. Chi-squared tests were used to compare the number of subjects with high versus low PIB index values, as well as the number of subjects showing a significant distribution shifts. A paired t-test was used to contrast the number of high and low ROIs across subjects.

There were significantly more oNC with ambiguously high PIB indices than ambiguously low (15 versus 4 subjects; $\chi^2 = 6.37$, df = 1, p = 0.01; figure 1), a greater number of subjects with a significant rightward than leftward shift in PIB ROI values (23 versus 10 subjects; $\chi^2 = 5.12$, df = 1, p= 0.024) and a trend for more high than low ROIs across subjects (a mean of 8 high ROIs compared to 5 low ROIs per subject; t = 1.69, df = 63, p-value = 0.096).

3.5 Spatial distribution of high ROIs

To investigate the spatial pattern of elevated PIB values, the percentage of oNC with high values for each ROI was plotted on a schematic brain for the entire oNC group, as well as within PIB+, Ambig, and PIB- oNC groups. This analysis revealed that elevated ROIs were most common in association cortices, most notably medial orbital, dorsolateral prefrontal, and temporoparietal cortices (figure 3). Repeating this analysis within groups revealed diffuse elevations in PIB+ oNC subjects and more focal deposition in Ambig oNC subjects (in prefrontal, precuneus and temporoparietal cortices, figure 4A-B). Furthermore, the PIB- oNC group showed evidence for elevated ROIs in dorsolateral prefrontal, medial orbital and temporoparietal cortices (figure 4C). Conversely, low ROIs were diffusely spread across the examined regions and did not reveal a consistent pattern (supplemental table 1 and supplemental figure 5).

4. Discussion

In this study we employed two approaches to classify oNC subjects based on PIB index values. An iterative outlier approach using index values from the oNC group yielded a cutoff value of 1.16 (IO-cutoff). An approach using data from a sample of yNC subjects (age range 20-30, who are likely to lack Aβ deposition and hence provide a good estimate of PIB negativity), revealed a lower cutoff value of 1.08 (yNC-cutoff). oNC subjects falling above the IO-cutoff (PIB+ oNC; 11/75) showed substantial overlap with AD subjects across all examined measures of PIB uptake. Subjects falling between the 2 cutoffs were less obvious and labeled as ambiguous (Ambig oNC; 15/75), and the remaining subjects were considered PIB- oNCs (49/75). Further examination of these oNC groups across different PIB quantification measures consistently confirmed the intermediate status of Ambig oNC subjects, suggesting that this categorization was not merely an artifact of the employed classification approach. Furthermore, ambiguously elevated values were consistently more prevalent than analogous decreases and present in regions known to show amyloid deposition, suggesting that slightly elevated values do not solely reflect noise. Interestingly, a number of PIB- subjects showed evidence of elevation across the different measures,

suggesting that a subset of PIB- subjects may also have elevated Aβ burden. Overall, the analyses presented in this manuscript, in conjunction with recently published pathological data, suggest a biological relevance of slight PIB elevations in oNCs.

4.1 An approach that combines magnitude and extent may be most sensitive

Qualitative comparison across the measures used to quantify PIB reveals that the number of high ROIs has the clearest stepwise progression from young NC to PIB-/Ambig/PIB+ oNC groups and AD, perhaps because the combination of magnitude and extent makes this measure less inherently noisy than the other measures examined. For instance, the maximum ROI approach identified a yNC subject whose value was much higher than all yNC and PIB- oNC subjects, as well as the majority of Ambig oNC subjects (1.44 in the left caudal anterior cingulate). However, the variance in the yNC group was substantially decreased using the total high ROI approach, and this subject's tally fell below all Ambig oNC as well as many PIB- oNC subjects. Therefore, it is possible that multiple ROIs should be considered to reduce noise in PIB classification. On the other hand, a global approach—such as the PIB index approach—may lose sensitivity by averaging too many regions. Overall, it will be necessary to apply these methods to independent cohorts to determine the generalizability of these approaches.

4.2 Slight elevations outnumber slight decreases

An obvious possibility is that slight elevations simply reflect noise amongst subjects lacking Aβ deposition. Noise within PIB-PET scans may be caused by bleed-in effects of nonspecific white matter binding, wash-out effects of neighboring cerebrospinal fluid, co-registration errors between MRI-defined ROIs and PIB PET scans, inaccurate labeling of gray matter ROIs, subject motion and errors during PET acquisition/reconstruction. It is difficult to predict how these factors will interact to affect signal, but one possibility is that these factors would simply produce noise without any bias towards higher or lower values. Thus, a higher prevalence of slight increases compared to slight decreases would support the claim that slightly elevated values reflect a biologically relevant signal. To this end, we contrasted the number of instances of slight elevations to the instances of analogous decreases. We excluded PIB+ oNC subjects to focus on the ambiguous signals present in Ambig and PIB- oNC. Across all 3 measures examined (high versus low PIB index values, right versus leftward shift in ROI distributions, and the total number of high versus low ROIs), there were greater numbers of ambiguous elevations compared to analogous reductions. This asymmetry argues that these slight elevations contain a meaningful signal rather than merely reflecting noise in the data.

4.3 Spatial distribution of elevated regions follows known pattern of amyloid deposition

It is also possible, however, that the noise in the PIB signal is positively biased towards higher cortical uptake because high white matter binding of the tracer is the predominant factor. Thus, we investigated the spatial pattern of elevated regions amongst oNCs by computing the percentage of subjects with high ROIs for the 68 FreeSurfer regions investigated. These percentages were plotted for the entire oNC group, and also separately for PIB+, Ambig and PIB- oNC subjects. This analysis revealed a pattern consistent with known patterns of amyloid deposition as measured with PIB-PET imaging (Fripp et al., 2008; Mintun et al., 2006) and postmortem staining (Braak and Braak, 1991; Thal et al., 2002). This regional specificity is difficult to explain by elevated white matter binding. Specifically, elevated PIB across subjects was seen across multiple association cortices, with the highest percentages in medial orbital, dorsolateral prefrontal, and temporoparietal cortices. Within-group examination revealed diffuse elevation in PIB+ oNC, with a more restricted pattern in Ambig oNC subjects. Elevated uptake was even present for a subset of PIB- oNC subjects (dorsolateral prefrontal, medial orbital and temporoparietal cortices),

suggesting the presence of amyloid deposition in PIB- oNC subjects. Importantly, the pattern of regional elevation across these 3 levels of PIB uptake resembles the stages of amyloid deposition described by postmortem staining studies (Braak and Braak, 1991; Thal et al., 2002). Specifically, our analysis revealed the greatest vulnerability to Aβ burden in neocortical heteromodal regions (prefrontal and temporoparietal cortices), followed by the cingulate gyrus and medial temporal cortex whereas the least vulnerable cortical regions were unimodal sensory cortices. Overall, concordance between the spatial distribution of elevated PIB in this study and established patterns of Aβ deposition further strengthens the claim that these slight elevations are indicative of Aβ deposition.

4.4 Slightly elevated PIB values and postmortem data

Recent research from the Baltimore Longitudinal Study of Aging suggests that slightly elevated PIB indices may correspond to Aβ deposition measured at postmortem examination (Sojkova et al., 2011). The authors describe a series of 6 oNC subjects that underwent both PIB imaging and postmortem examination. Although the PIB values are not directly comparable to the values reported in our study (due to inter-scanner differences, data processing methods, etc), the pattern presented in their research parallels the findings presented in our manuscript. Three cases in the Sojkova series had low to slightly elevated PIB index DVR values (1.01, 1.06 and 1.09; cases B, C and D respectively), as well as postmortem evidence of plaque deposition (all had a CERAD rating of moderate). In contrast, case A had a PIB index of 0.96 and no evidence of plaques at postmortem examination. In addition to reporting global PIB index values, PIB values for 9 ROIs were also examined. Interestingly, the maximum ROI value for the “Aβ -negative” case A was 1.03, whereas the maximum values for “Aβ-ambiguous” cases B-D were 1.16, 1.24, and 1.21 respectively. The PIB profile seen in cases B-D is reminiscent of the Ambig oNC reported in our study. The observation that these 3 ambiguous cases have postmortem confirmation for the presence of amyloid deposition greatly strengthens the argument that slightly elevated PIB values in oNC may reflect a biologically relevant signal.

4.5 Future approaches are needed to establish the relevance of ambiguous cases

The results presented in this manuscript are largely descriptive—it is clear that follow up studies are needed to determine whether ambiguously elevated PIB values represent a biologically meaningful signal. Future studies that directly compare PIB to post-mortem measurements of Aβ in slightly elevated cases are of utmost importance in determining the sensitivity of PIB imaging. It is likely that these studies will primarily draw from patient populations with low levels of Aβ since this type of data is extremely difficult to obtain in cognitively normal individuals. Longitudinal PIB-PET imaging in oNCs will also offer insight into the relevance of Ambig cases. For instance, longitudinal examination of regions showing high uptake will reconcile whether these regional elevations reflect noise or meaningful signal. It is also likely that Ambig oNCs are at risk for transitioning to PIB+, and longitudinal PIB imaging will be able to confirm or refute this speculation. More immediately, it is possible that interpretation of Ambig oNC subjects as PIB- may lead researchers to falsely accept a lack of difference between PIB+ and PIB- oNC. Overall, the potential relevance of these slightly elevated oNC subjects may help guide future studies examining PIB uptake in oNC populations.

4.6 Atrophy correction

A limitation in this study is the lack of atrophy correction to PIB-PET data. In previous publications our group has employed a 2-compartmental atrophy correction procedure to PIB data (Mormino et al., 2009; Rabinovici et al., 2010), however we choose to refrain from this procedure for suspicion that some older subjects may have high levels of atrophy without any amyloid deposition (for instance, atrophy may be due to vascular etiologies

(Raz et al., 2007)). During an initial analysis of this data we applied a 2-compartmental atrophy correction to yNC and oNC PIB scans, and found that the majority of oNC subjects were above the yNC PIB index cutoff (data not shown), and have assumed that this result represents overcorrection. Consequently, the lack of atrophy correction in this manuscript may in fact underestimate the prevalence of slightly elevated levels of amyloid in oNC (ie. the signal from amyloid must first overcome the washout effects of atrophy). Interestingly, this point may be applicable to the PIB- AD case presented in this manuscript (PIB index=1.03). Although this PIB index value falls amongst yNC and PIB- oNC values, this subject has cortical atrophy comparable to the 4 oNC subjects with the lowest PIB index values (0.88, 0.93, 0.96 and 0.98). Thus, it is unclear whether this PIB- AD case is truly Aβ negative, or has evidence for elevated uptake compared to “atrophy-matched” PIB- oNC subjects. The strategy of “atrophy matching” cases to assess slight elevations amongst oNC and patients against subjects with high atrophy that are confirmed to be amyloid-free (via postmortem examination, cerebrospinal fluid, etc) may be a promising alternative to traditional atrophy correction techniques.

A further complication is the possibility that increased signal amongst Aβ-negative subjects may be due to bleed-in from nonspecific white matter binding. A 3-compartmental model that accounts for PIB binding in white matter may address this issue, however, given the increased susceptibility to segmentation and registration errors associated with this approach (Meltzer et al., 1999), we opted to refrain altogether. It is important to note that the influence of nonspecific white matter binding amongst Aβ negative cases would result in a higher young derived cut off value. Although bleed-in effects of white matter may likewise elevate the PIB signal in Aβ-free oNCs, we suspect that the washout effects of CSF in oNCs due to age-related atrophy will have a larger impact than bleed-in effects of white matter (ultimately resulting in underestimation rather than overestimation amongst oNCs). Therefore, it is likely that the lack of atrophy correction in this manuscript underestimates the quantity of elevated PIB values amongst oNCs.

4.7 Arbitrary nature of derived cut offs

In this study, we identified a cut off of 1.08 using data from a cohort of presumably Aβ-free young control subjects. This value was not affected by employing a different image processing pipeline, suggesting stability of this estimate within our young cohort (a situation of low variability due to young age, structural homogeneity, and negative scans of the subjects). This cut off value is below the cut offs typically used in PIB-PET imaging studies, which tend to isolate oNCs with AD-levels of PIB uptake. However, it is likely that the same methods applied to an independent cohort may yield a slightly different cutoff value. Therefore, it is important to keep in mind that the exact number derived from the methods herein represent an arbitrary value that is likely influenced by scanning parameters, image resolution, subject sample, etc, and may not be applicable to data from other laboratories. Thus, we do not posit that our methods circumvent the arbitrariness that accompanies demarcation of a continuous PIB index variable, but rather provides support for a biological relevance in values that fall beneath the levels that are typical in the context of AD.

It is possible that incorporation of test-retest reliability values may minimize the arbitrary nature inherent within PIB positivity cut off values. For instance, we established test-retest reliability in a subset of 14 oNCs scanned an average of 2.77 years apart and found that the average percent difference between PIB index values was 2.63%, consistent with previous test-retest reports (Lopresti et al., 2005). Given this test-retest variability, a more conservative approach would have been to eliminate subjects falling within 2.63% of our young defined cut off (ie: 1.08+/-2.63%=1.05 to 1.11). However, the lack of a gold standard for determining whether these slightly elevated values are indicative of Aβ makes it difficult

to determine whether this sort of approach is optimal, and should be addressed in follow up studies.

5. Conclusions

By applying two distinct cutoff approaches, we indentified 3 groups of oNC subjects with varying levels of PIB uptake. Although the interpretation of this classification scheme is debatable, investigation of Aβ levels in oNCs is of utmost importance in understanding the temporal course of Aβ deposition in AD development, and perhaps will afford an ideal window in which anti-Aβ treatments may be most effective (before widespread amyloid deposition has occurred). Overall, the data presented in this manuscript supports the biological relevance of slightly elevated PIB values in oNC subjects, and future studies of aging and preclinical AD will be able to directly test these claims.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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- Two cutoff approaches were employed to define PIB-positivity in elderly controls.
- A subset of elderly controls have PIB levels similar to Alzheimer's disease.
- A subset of elderly controls have ambiguously elevated PIB values.
- Ambiguously elevated PIB values may reflect early signs of amyloid deposition.

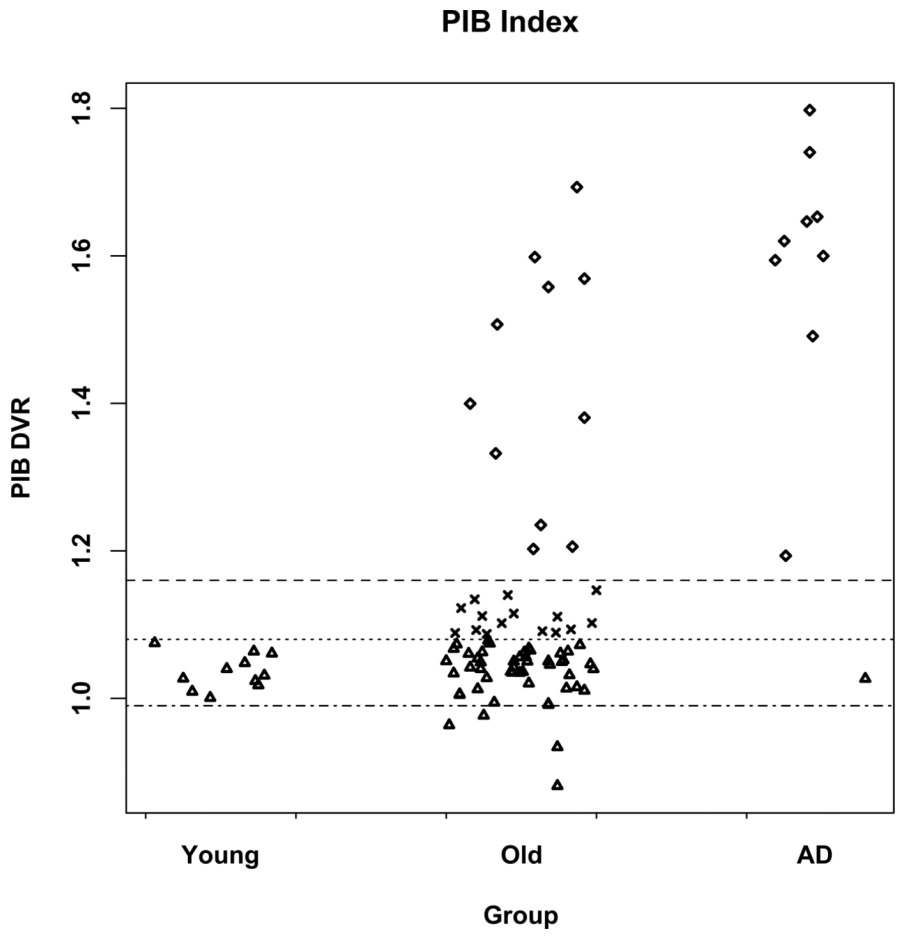

Figure 1. Distribution of PIB index values

Values are randomly scattered on the x-axis to allow visualization of individual subjects. The iterative outlier cut off is shown with a dashed line ("IO-cutoff", 1.16) while the yNC derived cut off is shown with a dotted line ("yNC-cutoff", 1.08). Additionally, an analogous yNC derived low cut off is shown with a dash-dotted line ("yNC-cutoff-low", 0.99). Based on the IO- and yNC-cutoffs, subjects were classified into 3 groups: PIB+ (above IO-cutoff; diamonds), Ambig (below IO-cutoff and above yNC-cutoff; 'x's) and PIB- (below yNC-cutoff; triangles).

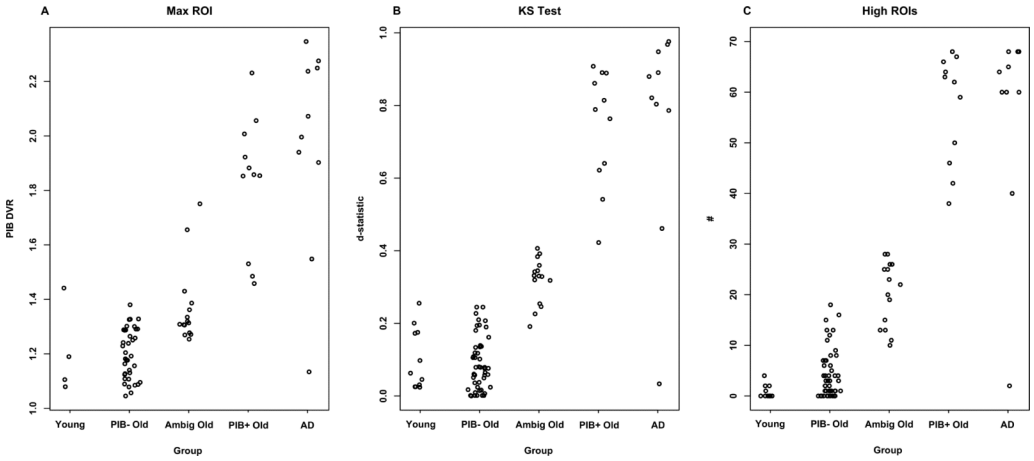

Figure 2. Secondary measures of PIB uptake

(A) Focal PIB DVR magnitude was examined by plotting the maximum ROI value for each subject. (B) Extent of elevated PIB DVR values was assessed using Kolmogorov–Smirnov (K-S) tests. (C) High ROIs were tallied for each subject (as defined by yNC-derived cut off values). For consistency, oNC are plotted using group classification as defined by PIB index cut offs. Across these approaches, Ambig oNC subjects reside between PIB- and PIB+ oNC groups, and the PIB+ oNC group is similar to the AD group.

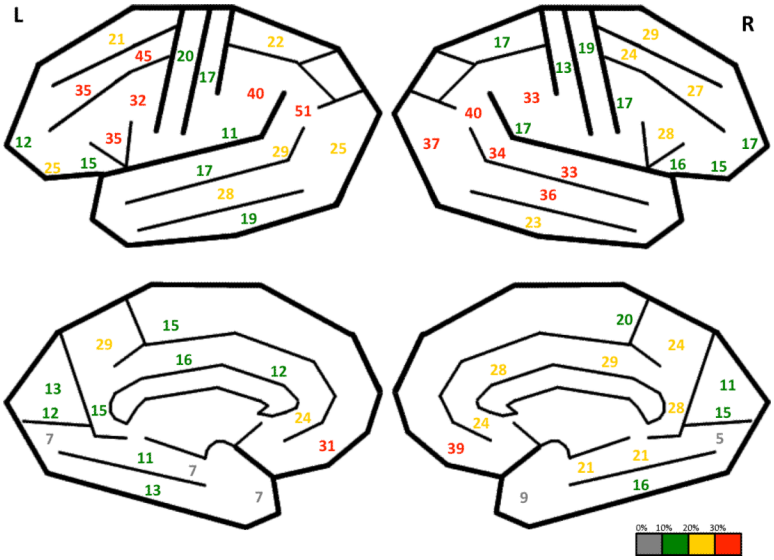

Figure 3. Spatial distribution of elevated PIB values

Schematic showing the percentage of oNC subjects with high PIB values for each cortical ROI. Many subjects have elevated ROI values in association cortices, with the largest percentages present in medial orbital, dorsolateral prefrontal, and temporoparietal cortices. All ROIs listed in supplemental table 1 are represented other than the insula. Text color scheme: red=greater than 30%, yellow=21-30%, green=11-20%, gray=0-10%.

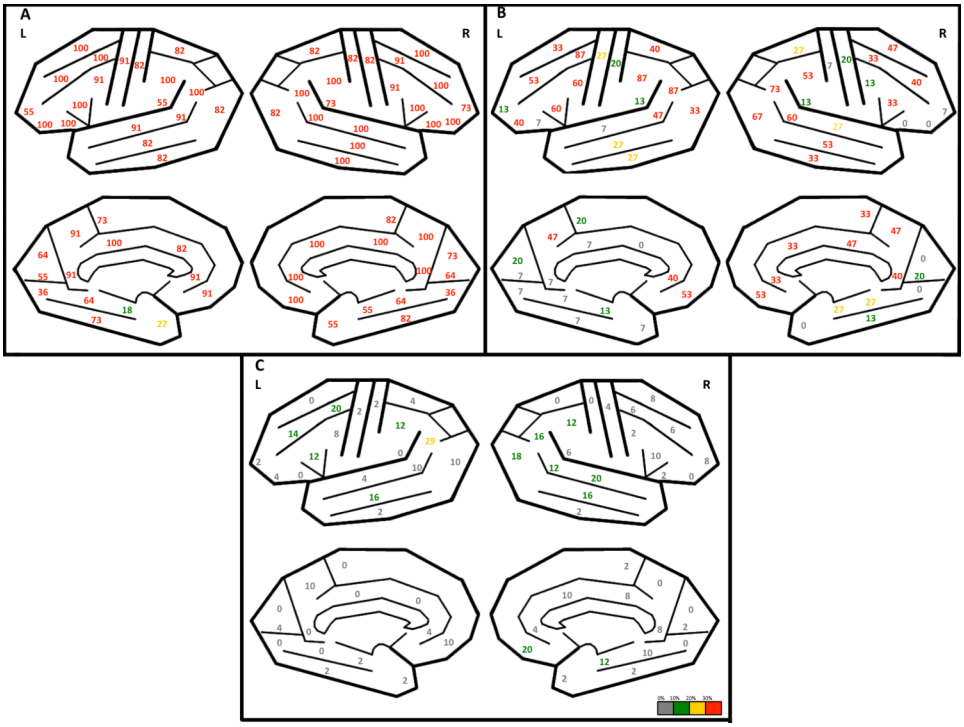

Figure 4. Within group spatial distributions

Percentage of subjects with high ROIs plotted separately for (A) PIB+, (B) Ambig and (C) PIB- oNC groups. Elevated ROIs are present across all regions in PIB+ oNC subjects, whereas Ambig oNC subjects show a pattern of restricted elevation, albeit in areas known to show high levels of amyloid deposition (prefrontal, precuneus and temporoparietal cortices). Interestingly, even the PIB- oNC group shows some evidence of focal deposition in dorsolateral prefrontal, medial orbital and temporoparietal cortices. All ROIs listed in supplemental table 1 are represented other than the insula. Text color scheme: red=greater than 30%, yellow=21-30%, green=11-20%, gray=0-10%.

Table 1

Subject characteristics.

	yNC	PIB- oNC	Ambig oNC	PIB+ oNC	AD
N	11	49	15	11	10
Age	24.5 (3.4)	75.1 (6.6)	72.8 (6.4)	79.2 (8.0)	74.8 (8.7)
Gender	5M	20M	6M	3M	7M
Education	16.2 (1.9)	17.2 (2.0)	17.1 (1.5)	16.5 (2.1)	17.1 (2.8)
APOE (ε4+/-)	NA	11/34	5/10	6/5	6/3
MMSE	28.8 (1.4)	29.1 (1.1)	29.6 (0.6)	28.7 (1.3)	19.5 (8.0)
PIB Index	1.04 (0.02)	1.03 (0.02)	1.11 (0.02)	1.43 (0.17)	1.54 (0.24)

yNC=young normal controls, oNC=old normal controls, AD=Alzheimer's disease patients. Means and standard deviations are reported for continuous variables. APOE status was unavailable for 4 oNC subjects and 1 AD patient.