
Research Articles: Neurobiology of Disease

Phases of hyper and hypo connectivity in the Default Mode and Salience networks track with amyloid and Tau in clinically normal individuals.

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DOI: 10.1523/JNEUROSCI.3263-16.2017

Received: 17 October 2016

Revised: 7 March 2017

Accepted: 8 March 2017

Published: 17 March 2017

Author contributions: A.P.S., K.J., and R.S. designed research; A.P.S., J.C., T.H., B.H., M.L., K.J., and R.S. performed research; A.P.S., J.C., E.M., J.S., W.H., R.B., K.J., and R.S. analyzed data; A.P.S., J.C., T.H., E.M., B.H., J.S., W.H., M.L., R.B., K.J., and R.S. wrote the paper.

This work was supported in part by shared instrumentation grants and the Martinos Center for Biomedical Imaging: S10OD010364, S10RR023401, and 1S10RR019307-01. The research was supported in major part by the Harvard Aging Brain Study (P01 AG036694).

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Cite as: J. Neurosci ; 10.1523/JNEUROSCI.3263-16.2017

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Accepted manuscripts are peer-reviewed but have not been through the copyediting, formatting, or proofreading process.

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Short Title:

Amyloid, Tau, and Functional Connectivity.

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41 **Number of Pages: 29**

42 **Number of Figures: 3**

43 **Number of Tables: 1**

44

45 **Word Counts:**

46 **Abstract: 188/250**

47 **Introduction: 639/650**

48 **Discussion: 1487/1500**

49

50 **Acknowledgements:**

51

52 This work was supported in part by shared instrumentation grants and the Martinos Center for
53 Biomedical Imaging: S10OD010364, S10RR023401, and 1S10RR019307-01. The research was
54 supported in major part by the Harvard Aging Brain Study (P01 AG036694).
55

56

56 **Potential Conflicts of Interest:**

57

58 A. Schultz has been a paid consultant for Janssen Pharmaceuticals and Biogen.

59

60 E. Mormino received funding from NIH grant from NIH grant K01 AG051718. She has also
61 served as a consultant for Biogen, and has received research support from Eli Lilly and Janssen
62 Pharmaceuticals.
63

64

64 B. Hanseeuw received support from the Belgian American Education Foundation (BAEF).
65

66

66 T. Hedden received funding from NIH grants K01 AG040197, P01 AG036694, P50 AG005134,
67 R01 AG053509, and R01 AG034556.
68

69

69 J. Sepulcre received funding from HHG grant K23EB019023
70

71

71 R. Buckley received funding from the Australian National Health and Medical Research Council
72 (NHMRC) and Australian Research Council (ARC) Dementia Research Fellowship APP1105576.
73

74

74 K. Johnson has served as paid consultant for Bayer, GE Healthcare, Janssen Alzheimer's
75 Immunotherapy, Siemens Medical Solutions, Genzyme, Novartis, Biogen, Roche, ISIS Pharma,
76 AZTherapy, GEHC, Lundberg, and Abbvie. He is a site coinvestigator for Lilly/Avid, Pfizer,
77 Janssen Immunotherapy, and Navidea. He has spoken at symposia sponsored by Janssen
78 Alzheimer's Immunotherapy and Pfizer. K. Johnson receives funding from NIH grants
79 R01EB014894, R21 AG038994, R01 AG026484, R01 AG034556, P50 AG00513421, U19
80 AG10483, P01 AG036694, R13 AG042201174210, R01 AG027435, and R01 AG037497 and the
81 Alzheimer's Association grant ZEN-10-174210.
82

83

83 R. Sperling has served as a paid consultant for Abbvie, Biogen, Bracket, Genentech, Lundbeck,
84 Roche, and Sanofi. She has served as a co-investigator for Avid, Eli Lilly, and Janssen Alzheimer

85 Immunotherapy clinical trials. She has spoken at symposia sponsored by Eli Lilly, Biogen, and
86 Janssen. R. Sperling receives research support from Janssen Pharmaceuticals, and Eli Lilly and
87 Co. These relationships are not related to the content in the manuscript. She also receives
88 research support from the following grants: P01 AG036694, U01 AG032438, U01 AG024904,
89 R01 AG037497, R01 AG034556, K24 AG035007, P50 AG005134, U19 AG010483, R01 AG027435,
90 Fidelity Biosciences, Harvard NeuroDiscovery Center, and the Alzheimer's Association.
91

92 **Abstract:**

93 Alzheimer's disease (AD) is characterized by two hallmark molecular pathologies: amyloid $a\beta_1$.
 94 $_{42}$, and Tau neurofibrillary tangles. To date, studies of functional connectivity MRI (fcMRI) in
 95 preclinical AD have relied on associations with in-vivo measures of amyloid pathology. With the
 96 recent advent of in-vivo Tau-PET tracers it is now possible to extend investigations on fcMRI in a
 97 sample of cognitively normal elderly humans to regional measures of Tau. We modeled fcMRI
 98 measures across four major cortical association networks (Default Mode (DMN), Salience,
 99 Dorsal Attention (DAN), and Frontoparietal Control (FPCN)) as a function of global cortical
 100 amyloid (PiB-PET) and regional Tau (AV1451-PET) in entorhinal, inferior temporal (IT), and
 101 inferior parietal (IP) cortex. Results showed that the interaction term between PiB and IT
 102 AV1451 was significantly associated with connectivity in the DMN and salience. The interaction
 103 revealed that amyloid positive ($a\beta+$) individuals show increased connectivity in the DMN and
 104 salience when neocortical Tau levels are low, whereas $a\beta+$ individuals demonstrate decreased
 105 connectivity in these networks as a function of elevated Tau-PET signal. This pattern suggests a
 106 hyper-connectivity phase followed by a hypo-connectivity phase in the course of preclinical AD.

107
 108 **Significance:** This paper offers a first look at the relationship between Tau-PET imaging with F^{18} -
 109 AV1451 and functional connectivity MRI (fcMRI) in the context of amyloid-PET imaging. The
 110 results suggest a non-linear relationship between fcMRI and both Tau-PET and amyloid-PET
 111 imaging. The pattern supports recent conjecture that the AD fcMRI trajectory is characterized
 112 by periods of both hyper- and hypo- connectivity. Furthermore, this nonlinear pattern can

- 113 account for the sometimes conflicting reports of associations between amyloid and fMRI in
- 114 preclinical AD.

115 **Introduction:**

116 By measuring the coordination of time-varying brain activity, functional connectivity
 117 magnetic resonance imaging (fcMRI) can be a sensitive indicator of early network disruption
 118 and may prove useful in tracking the progression of neurodegenerative diseases. fcMRI has
 119 been studied previously in Alzheimer's disease (AD) across a range of asymptomatic and
 120 symptomatic clinical states. It has been well established that, relative to clinically normal (CN)
 121 controls, patients with a clinical diagnosis of AD exhibit wide-spread differences in functional
 122 connectivity across multiple cortical networks (Brier et al., 2012; Chhatwal et al., 2013;
 123 Damoiseaux et al., 2004; Jack et al., 2013; Jones et al., 2016; Petrella et al., 2011a; Schultz et al.,
 124 2014; K. Wang et al., 2007). Even at the stage of mild cognitive impairment (MCI), there are
 125 notable differences compared to clinically normal controls, and these differences are
 126 accentuated when the analysis is focused on MCI who progress to dementia (Petrella et al.,
 127 2011b).

128 The relationship between early AD pathology and fcMRI is more tenuous in preclinical
 129 (asymptomatic) AD (Sperling et al., 2011), where consensus in terms of location, size, and
 130 direction of fcMRI effects has been more difficult to establish. While many studies have
 131 reported decreased functional connectivity with increased amyloid burden ($A\beta$) in the medial
 132 temporal lobe, posterior midline, and parietal regions (Brier et al., 2014; Chhatwal et al., 2013;
 133 Hedden et al., 2009; Sheline et al., 2010b; L. Wang et al., 2013), other studies have reported
 134 regions of both increased and decreased connectivity with elevated amyloid (Lim et al., 2014;
 135 Mormino et al., 2011). One account of these discrepant reports is that there are both hyper-
 136 and hypo-connectivity effects at different points in the preclinical AD spectrum. Utilizing

137 longitudinal amyloid-PET imaging, Jack et. al., 2013 categorized individuals according to
 138 changes in amyloid burden and reported increased posterior DMN connectivity among low
 139 amyloid ($a\beta^-$) who became $a\beta^+$ at follow up whereas patients with AD dementia had reduced
 140 connectivity, suggesting both hyper- and hypo-connectivity effects at different stages of
 141 disease.

142 The focus of these fcMRI studies in preclinical AD have largely been on amyloid status.
 143 The opportunity to examine both hallmark AD molecular pathologies, in particular variations in
 144 regional Tau pathologic burden and/or interactions between regional Tau and amyloid burden,
 145 may provide insight into the nature of the effects of preclinical AD pathology on functional
 146 connectivity. With the recent advent of Tau-PET ligands, we now extend the study of fcMRI in
 147 the context of preclinical AD to PET measures of amyloid and regional Tau burden obtained
 148 with PiB and AV1451 respectively.

149 Prior reports on the relationship between amyloid-PET imaging and tau-PET imaging
 150 (Cho et al., 2016; Johnson et al., 2016; Schöll et al., 2016; Sepulcre et al., 2016; Villemagne, et
 151 al., 2015) have revealed highly significant associations between cortical amyloid-PET and
 152 AV1451 signal in regions associated with Braak stages (Braak, et al., 2006; Braak, et al., 2011).
 153 Notably a pattern of MTL (entorhinal/parahippocampal), fusiform, inferior temporal, parietal,
 154 and posterior midline regions define the standard AD-type pattern of paired helical filament
 155 (PHF) tau as measured by AV1451. In preclinical AD cohorts, this pattern is generally limited to
 156 MTL, fusiform, and inferior temporal regions and is generally lower than MCI/AD, with few
 157 cases of elevated signal outside these regions. Also of interest, low amyloid subjects also have
 158 elevated AV1451 signal in medial and lateral temporal regions, though of smaller magnitude

159 than high amyloid subjects. This suggests that some degree of tau pathology outside the MTL is
 160 present independent of amyloid. We hypothesized entorhinal and inferior temporal would be
 161 good indicators of nascent AD related tau pathology, and inferior parietal was used as a check
 162 against the possibility of relevant low level AV1451 signal associated with more advanced Braak
 163 stages.

164

165 **Methods:**

166 Participants:

167 Ninety-one participants from the Harvard Aging Brain Study (HABS-P01AG036694) with
 168 AV1451-PET, PiB-PET and resting state fMRI collected within 1 year were included in the
 169 present study (MR vs. PiB = 110 ± 72 days; MR vs AV1451 = 129 ± 82 days; PiB vs AV1451 = 81 ± 74
 170 days). All participants were clinically normal at baseline and at the assessment closest to the
 171 AV1451 scan. This classification was determined by Clinical Dementia Rating (CDR) = 0 ((Morris,
 172 1993)), Geriatric Depression Scale (GDS) < 11 (Yesavage et al., 1982), Mini Mental State Exam
 173 (MMSE) > 25 (Folstein et al., 1975), and performance within education adjusted norms for
 174 Logical Memory Story A delayed recall (Wechsler, 1987). All study procedures were approved
 175 by the Partners Healthcare IRB, and all participants provided written informed consent.

176 The sample consisted of 54 Females / 37 Males, aged of 75.78 ± 6.14 years with $15.69 \pm$
 177 2.95 years of education. 32 were APOE ϵ 4 carriers, and 30 participants were categorized as
 178 amyloid positive using a quantitative threshold (20 of whom were ϵ 4 carriers).

179

180 Resting State fMRI Imaging:

181 All data were collected on two matched 3T Trio Tim scanners (Siemens Medical Systems,
 182 Erlangen, Germany) using 12-channel phased-array head coils at the Athinoula A. Martinos
 183 Center for biomedical imaging in Charlestown, MA. Scanner noise was attenuated using foam
 184 earplugs. fcMRI data were acquired using a gradient-echo echo-planar imaging sequence
 185 sensitive to BOLD contrast. Whole-brain coverage, including the cerebellum, was acquired
 186 aligned parallel to the anterior-/posterior-commissure using the following parameters:
 187 repetition time (TR) = 3000ms, echo time (TE) = 30ms, flip angle = 85°, field-of-view (FOV) =
 188 216x216mm, matrix = 72x72, and 3x3x3mm voxels. 124 volumes were acquired in each of two
 189 6:12 minute runs (including 4 dummy volumes – 12 sec.). Instructions were to lie still, remain
 190 awake, and keep eyes open.

191 All resting state data were processed using SPM8 (<http://www.fil.ion.ucl.ac.uk/spm/>).
 192 The first four volumes of each run were excluded to allow for T1 equilibration. Each run was
 193 slice-time corrected, realigned to the first volume of each run with INRIAAlign ([http://www-](http://www-sop.inria.fr/epidaure/software/INRIAAlign/)
 194 [sop.inria.fr/epidaure/software/INRIAAlign/](http://www-sop.inria.fr/epidaure/software/INRIAAlign/); (Freire et al., 2001)), normalized to the MNI 152 EPI
 195 template (Montreal Neurological Institute, Montreal, Canada), and smoothed with a 6 mm
 196 FWHM Gaussian kernel. Following these standard preprocessing steps, additional processing
 197 known to be beneficial for fc-MRI analysis was conducted. These included (sequentially, and in
 198 this order) (1) regression of realignment parameters (plus first derivatives) to reduce movement
 199 artifacts on connectivity and (2) temporal band-pass filtering (second order Butterworth filter)
 200 to remove frequencies outside of the 0.01-0.08 Hz band.

201 Data were then processed with Template Based Rotation (TBR,(Schultz et al., 2014)),
 202 using the same template maps as published in Schultz et al, 2014 (TBR scripts and templates

203 (nifti format) are available at: <http://mrtools.mgh.harvard.edu>). Whole network measures for
 204 the default-mode network (DMN), salience network (SAL - aka ventral attention network),
 205 dorsal attention network (DAN), left frontoparietal control network (FPCN), and right FPCN,
 206 were extracted as described in Schultz et al., 2014. This including averaging all values within a
 207 mask defined on the template maps, at greater than 40% of the max value in the corresponding
 208 template map. Since DMN and salience were represented in a single map as anti-correlated
 209 networks, the salience was defined as less than 40% of the minimum value.

210 As an additional step, (for seed based ROI analyses only), we regressed out the average
 211 signal from white-matter, ventricles, and global signal (plus first derivatives; (Van Dijk et al.,
 212 2010; Vincent et al., 2006)). Note that the operations were conducted in the specified order to
 213 prevent the reintroduction of nuisance variance in the stop band frequencies (Hallquist et al.,
 214 2013). While white matter, ventricle, and global signal were regressed after band-pass filtering,
 215 the signals were taken from the band pass filtered data and so did not inadvertently
 216 reintroduce nuisance variance outside of the stop-band. For seed based analyses we utilized a
 217 set of cluster based seed regions defined on the template maps across the same networks as
 218 described above. Additional details can be found in (Shaw et al., 2015), supplemental, seed
 219 masks for each ROI can be found online at
 220 <http://mrtools.mgh.harvard.edu/index.php?title=Downloads>.

222 Structural MR Imaging:

223 Structural T1 weighted images were acquired as magnetization-prepared rapid gradient-
 224 echo (MPRAGE) with the following acquisition parameters: TR/TE/TI = 2300/2.95/900ms, flip

225 angle=9°, 1.1x1.1x1.2mm resolution, 2X (GRAPPA) acceleration. Notably, this is the same
 226 acquisition used in ADNI2-GO.

227 The structural MRI data were processed with Freesurfer v5.1
 228 (<http://surfer.nmr.mgh.harvard.edu>, (Dale et al., 1999)) and were automatically parcellated
 229 using the Desikan-Killany atlas (Desikan et al., 2006) for cortical regions of interest (ROI), and
 230 the Freesurfer ASEG atlas (Fischl et al., 2002) for subcortical ROIs. Freesurfer automated
 231 segmentation results were manually evaluated to ensure the accuracy of the results (see
 232 <https://www.nmr.mgh.harvard.edu/lab/harvardagingbrain/tools> for additional details).
 233

234 PET Imaging:

235 ¹¹C Pittsburgh Compound B was prepared and PET data were acquired as described
 236 previously (Sperling et al., 2009). ¹¹C PiB PET was acquired with a 8.5 to 15 mCi bolus injection
 237 followed immediately by a 60-minute dynamic acquisition in 69 frames (12x15 seconds, 57x60
 238 seconds).

239 ¹⁸F AV1451 was prepared at Massachusetts General Hospital with a radiochemical yield
 240 of 14±3% and specific activity of 216±60GBq/μmol (5837±1621mCi/μmol) at the end of
 241 synthesis (60min), and validated for human use (Shoup et al., 2013). Images were acquired
 242 from 80-100 minutes in 4 x 5-minute frames after a 10.0 ± 1.0 mCi bolus injection.

243 All PET data were acquired using a Siemens/CTI (Knoxville, TN) ECAT HR+ scanner (3D
 244 mode; 63 image planes; 15.2cm axial field of view; 5.6mm transaxial resolution and 2.4mm slice
 245 interval. PET data were reconstructed, attenuation corrected, and evaluated to verify adequate
 246 count statistics and absence of head motion.

PET images were co-registered to the corresponding T1 image for each subject using a 6 degree of freedom, rigid body registration, and structural ROIs, as determined by Freesurfer, were mapped into native PET space. For both PiB and AV1451 we utilized a cerebellar grey matter reference region from the Freesurfer aseg atlas as previously described (Becker et al., 2011; Chien et al., 2013; Johnson et al., 2016), with AV1451 measures computed as standardized uptake value ratios (SUVRs), and PiB measures computed as distribution volume ratios (DVRs) using the Logan graphical method (Logan et al., 1990), with slopes extracted from the 40-60 min time frame.

Additionally we performed partial volume correction (PVC) using the geometric transform matrix method (GTM-PVC, (Labbe et al., 1998; Rousset, et al., 1998)), as implemented in Freesurfer 6.0, and described in (Greve et al., 2016), using a slightly modified Freesurfer atlas mapped to each participants native structural space, that included ROIs for CSF, white matter, and extra-cerebral structures. The PVC processing was performed assuming a uniform 6mm point spread function.

Based on prior studies we utilized a single PiB measure of global cortical amyloid burden from regions including: bilateral Precuneus, Rostral Anterior Cingulate, Medial Orbito-Frontal, Superior Frontal, Rostral Middle Frontal, Inferior Parietal, Inferior Temporal, and Middle Temporal (simple mean across ROI values), the so called Frontal, Lateral, Retrosplenial (FLR) ROI (Mormino et al., 2014a). PiB measures were used both continuously and dichotomously. The threshold for dichotomization into high and low amyloid groups was 1.2, and was derived via a Gaussian mixture model as described in (Mormino et al., 2014b).

268 For AV1451 we focused our analyses on three structurally defined regions of interest;
 269 entorhinal, inferior temporal, and inferior parietal. Entorhinal cortex was chosen as it is among
 270 the first areas to develop Tau pathology, even in the absence of amyloid. Inferior temporal
 271 cortex (IT) was used as the current best choice of a surrogate marker of early AD related Tau
 272 spread into neocortex (IT AV1451 showed the largest effect size between impaired and non-
 273 impaired individuals as reported in (Johnson et al., 2016)). Inferior parietal (IP) was chosen as a
 274 marker of additional spread of Tau pathology into other regions of cortex, likely associated with
 275 more advanced Braak stages.

276

277 Whole network analysis:

278 We investigated the relationship between each fcMRI network measure and PiB-PET
 279 and AV1451-PET controlling for Age, Sex, average movement (MV – mean movement as
 280 measured by the Euclidean distance between volumes) during the rsfMRI scan, the temporal
 281 SNR measured from the rsfMRI scan, and scanner (two matched Siemens Trio Tim scanners
 282 were used for data collection). For each network we investigated a hierarchical set of models:

- 283 1) $\text{fcMRI} \sim \text{PiB} + \text{Covariates}$
- 284 2) $\text{fcMRI} \sim \text{AV1451} + \text{Covariates}$
- 285 3) $\text{fcMRI} \sim \text{PiB} + \text{AV1451} + \text{Covariates}$
- 286 4) $\text{fcMRI} \sim \text{PiB} * \text{AV1451} + \text{Covariates}$
- 287 5) $\text{fcMRI} \sim \text{PiB Group} * \text{AV1451} + \text{Covariates}$

288 This set of models was designed to look at interactions between measures of amyloid and Tau
 289 pathology, as well as main effects of each molecular marker in the context of collinearity

290 between PiB and AV1451 measures. PiB was also used dichotomously (PiB group) using our
291 previously published threshold of 1.2 DVR units (Mormino et al., 2014a). To help curb effects of
292 positive skew in both the PiB and AV1451 distributions, both measures were log-transformed
293 before being entered into the models (effects were similar with and without log-transforms).

294

295 Node Based Connectivity Analysis:

296 To investigate the possibility of effects localized to particular nodes within a network or
297 effects involving inter-nodal connectivity between networks, we performed a separate
298 exploratory analysis in which each network was broken into a set of constituent nodes (29
299 nodes across the networks analyzed). Time-series data for each node were extracted from the
300 resting state fMRI scans, and a node-to-node connectivity measurement for each pair of nodes
301 was made for each subject. We then ran the models listed above for each node-to-node
302 connection to evaluate localized effects of amyloid and Tau. Data were then visualized using a
303 schema-ball plot (mrtools.mgh.harvard.edu), where the corresponding statistic for each
304 connection is visualized as a color graded line between the corresponding nodes. This resulted
305 in a visualization that represents the sensitivity of node-to-node connectivity on the effect of
306 interest. Additional details can be found in (Shaw et al., 2015), supplemental. We also
307 separated the connections into positive and negative connections based on the mean
308 connectivity between nodes in the sample, and then reverse scored anti-correlations so that
309 higher values in both sets represent connectivity strengths further from 0.

310

311 RESULTS:

312 Relationships between and among PET measures:

313 Similar to our previous report examining AV1451 Tau PET across the spectrum of AD
 314 (Johnson et al., 2016) , there was a significant correlation between the cortical aggregate PiB-
 315 PET measure and regional AV1451 measures. In the present sample we observed significant
 316 correlations with PiB-PET for all three AV1451 regions explored in the current study: entorhinal
 317 (ET: $r=0.46$; $p<0.001$), inferior temporal (IT: $r=0.40$; $p<0.001$), and inferior parietal (IP: $r=0.31$;
 318 $p=0.003$). The AV1451 measures from the three ROI were significantly correlated with one
 319 another; ET by IT ($r=0.69$; $p<0.001$), ET by IP ($r=0.54$; $p<0.001$), IT by IP ($r=0.71$; $p<0.001$). As
 320 with previous reports (Johnson et al., 2016; Sepulcre et al., 2016), the PiB groups showed
 321 significant differences using two-sample t-tests in entorhinal AV1451 ($t(89)=3.80$, $p<0.001$), IT
 322 AV1451 ($t(89)=3.20$, $p=0.002$), and IP AV1451 ($t(89)=2.01$, $p=0.047$).

323 Whole network analyses:

324 Results from the five models (see Methods) are reported in Table 1. We examined the
 325 relationship of AV1451 signal (in the entorhinal, inferior temporal (IT), and inferior parietal (IP)
 326 cortices) as well as global amyloid burden to measures of functional connectivity in 5 cortical
 327 networks, the DMN, salience, DAN, left FPCN, and right FPCN. Of note, we found the strongest
 328 effects using DMN and salience with the interaction of PiB and AV1451 signal in IT. No other
 329 effects survived correction for multiple comparisons (FWE for 15 tests: $p<0.003$), though
 330 marginal effects were present for the interaction between Inferior Parietal Tau and PiB. We did
 331 not observe main effects outside the context of the interaction. For DMN connectivity the PiB
 332 by inferior temporal AV1451 interaction term was significant both when PiB was tested
 333 continuously ($t(82)=-3.616$; $p<0.001$) and dichotomously ($t(82)=-2.494$; $p=0.010$). The same

334 pattern was true of salience connectivity for continuous PiB ($t(82)=-4.774$; $p<0.001$) and
 335 dichotomous PiB ($t(82)=-3.642$; $p<0.001$). Statistical results for all five models are shown in
 336 Table 1.

337 Figure 1 depicts the results of the continuous interaction term (PiB x IT AV1451). Here
 338 the interaction term is well described as a quadratic, suggesting hyper-connectivity associated
 339 with elevated amyloid among participants with low IT AV1451 signal, followed by hypo-
 340 connectivity with increasing IT AV1451 signal in higher amyloid individuals. Figure 1 also shows
 341 that the vast majority of values on the low end of the interaction term are PiB- participants.
 342 Additionally, the inverted U shape of the fit explains the lack of main effects for PiB and
 343 AV1451.

344 To more thoroughly explore the significant interaction between PiB and IT AV1451, we
 345 examined the pattern within the high PiB only ($N=30$). This analysis shows a linear relationship
 346 between increasing AV1451 signal in the inferior temporal cortex, and decreasing functional
 347 connectivity in the DMN (partial $r=-0.42$, $p=0.037$) and salience (partial $r=-0.67$; $p<0.001$)
 348 networks. Examination within the low PiB group ($N=61$) showed weak positive relationships
 349 between FC and IT AV1451 in the DMN (partial $r=0.28$, $p=0.040$) and salience (partial $r=0.29$,
 350 $p=0.030$). Similarly, an analysis of PiB within a median split of IT AV1451 (IT tau > 1.18) shows
 351 a large effect of PiB in the low Tau group ($N=46$) for both DMN (partial $r=0.44$, $p=0.004$) and
 352 salience (partial $r=0.45$, $p=0.003$), whereas the effect of PiB in the high IT-AV1451 ($N=45$) group
 353 was not significant for the DMN (partial $r=-0.14$, $p=0.380$) and only marginally significant for the
 354 salience (partial $r=-0.27$, $p=0.090$). This pattern of simple effects (see Figure 2) shows that the

PiB by AV1451 interaction term in the full model is driven by the positive effect of PiB when IT AV1451 is relatively low, and the negative effect of IT AV1451 when PiB is high.

Node-Based connectivity analysis:

Figure 3 depicts the statistical results for the PiB by IT AV1451 interaction term from Model #4 on the node to node connections (406 pairs in total) thresholded at a liberal exploratory $p < 0.01$ uncorrected in order to reveal the global pattern of the effect. Lines outside the circle represent within network connections, lines inside the circle represent between network connections. The sign of the effects corresponds to whether the direction of the PiB x AV1451 interaction effect was related to increased connectivity (yellow – away from 0 connectivity) or decreased connectivity (purple – towards 0 connectivity). This analysis revealed that nearly all effects of decreasing connectivity are localized to the DMN and salience networks, providing additional support for the specificity of this effect the DMN/salience axis. Of interest, the number of significant connections between salience nodes is smaller than what is observed for DMN and connections between DMN and salience nodes. This may indicate that the PiB by AV1451 interaction effect is most sensitive to the DMN-salience axis as a whole.

DISCUSSION:

We examined the relationship between amyloid burden as measured by PiB-PET, paired helical filament (PHF) Tau burden as measured by AV1451-PET, and network integrity as measured by functional connectivity fMRI (fcMRI) in a sample of clinically normal elderly. The results point to an interaction between amyloid and regional PHF Tau in the inferior temporal

(IT) cortex relating to fcMRI in the default mode, and salience networks. Furthermore, the pattern of this interaction suggests a hyper-connectivity phase among A β + that have low levels of AV1451 binding, and a hypo-connectivity phase as IT Tau pathology accrues in the presence of elevated amyloid burden.

The amyloid cascade hypothesis in its most common formulation posits that amyloid accumulation precedes neocortical Tau accumulation and neurodegeneration (Hardy et al., 1992). As such, molecular AD pathology can be described as a two-phase process of amyloid accumulation followed by continued amyloid accumulation *and* the spread of neurofibrillary tangles into neocortex. The results presented here suggest that these two periods of AD pathology have differential effects on functional connectivity with the early amyloid accumulation phase characterized by increased functional connectivity, followed by the progressive decline of functional connectivity with increased neocortical Tau pathology.

Interpreting hyper-connectivity:

While the effect of Tau in amyloid positive individuals accords nicely with our expectations of decreased connectivity accompanying increased pathology, the evidence for increased connectivity with increased amyloid presents something of a puzzle when taken in context of loss of connectivity later in the disease trajectory.

Evidence for a preclinical hyper-connectivity phase was reported in Jack et al., 2013 where incident a β positivity was cross-sectionally associated with increased posterior DMN connectivity. Increased functional connectivity has also been reported in individuals carrying the apolipoprotein ϵ 4 (APOE ϵ 4) risk allele for AD (Filippini et al., 2009; Sheline et al., 2010a; Westlye et al., 2011). APOE ϵ 4 carriers have also been shown to have increased task related

399 activity during the performance of episodic memory tasks (Huijbers et al., 2015; Oh et al., 2016;
400 Oh et al., 2015; Sperling et al., 2010). Hyper-connectivity has also been observed in psychiatric
401 disorders such as schizophrenia and bipolar disorders (Baker et al., 2014; Whitfield-Gabrieli et
402 al., 2009). Together, these findings indicate that hyper-connectivity likely has pathologic
403 connotations.

404 From a more mechanistic perspective, hyper-connectivity may be an important
405 component of a pathological feedback mechanism that is both being driven by and driving the
406 production of amyloid and the accumulation of amyloid plaques (Busche et al., 2015). Recent
407 studies have linked AD pathology with increased incidence of late-onset seizure disorders and
408 non-convulsive epileptiform discharges (Born, 2015; Palop et al., 2009; Vossel et al., 2013). In
409 turn, non-convulsive epileptiform activity has been linked to hypersynchronous neuronal
410 activity (Khambhati et al., 2015). If correct, early amyloid related hyper-connectivity would
411 signify a disruption to the healthy functioning of large scale neuronal networks.

412 There are alternative accounts for the observed pattern of results. First, it is possible
413 that the effect we see is due to a survival bias such that high amyloid individuals who are
414 clinically normal are more likely to harbor low Tau burden. The data from our, and other labs,
415 suggests that individuals with high amyloid and high Tau burden are unlikely to remain clinically
416 normal (Jack et al., 2016; Johnson et al., 2016). If increased connectivity is protective, one
417 could easily imagine that within this group we are selecting for people with protective
418 endophenotypes. These amyloid resilient individuals could then be responsible for driving the
419 interaction.

420 Second, increased connectivity may be compensatory, and could represent a systems
 421 level response to neuronal injury from pathological insult that allows for the maintenance of
 422 behavioral performance. This would result in a period of hyperactivity until the compensatory
 423 mechanisms are overwhelmed by neuronal loss which would then lead to decreased
 424 connectivity.

425 Third, our interpretation of hyper- vs. hypo-connectivity may be underdeveloped
 426 relative to the dynamic reality of brain connectivity. For instance, a loss of functional
 427 dynamicity could result in the over-representation of specific brain activity states. This reduced
 428 dynamic flexibility would then manifest in our measures as increased connectivity in the
 429 “typical” brain states that form the basis of functional networks.

430 To explicate which of these models best characterizes the network disruption in
 431 preclinical AD will require cross-sectional replication of these results in other samples,
 432 longitudinal assessment of fMRI data, and development of new analytic tools and functional
 433 sequences to more fully understand and measure the dynamic properties of connectivity.

434

435 Interpreting hypo-connectivity:

436 The hypo-connectivity effect is more straight forward, suggesting that the loss of
 437 functional connectivity – as with loss of structure and neuronal death – is more closely
 438 associated with neocortical Tau pathology than with amyloid. Notably this suggests that loss of
 439 connectivity is not directly caused by $A\beta$ toxicity, but rather by Tau pathology, though $A\beta$
 440 toxicity may impair the functioning of these networks resulting in hyper-connectivity.

441

442 Relationship to existing amyloid-fcMRI literature.

443 Our present results also hold implications for prior studies of amyloid and APOE ϵ 4 status
 444 with fcMRI data. Namely, the observed results are likely to be dependent on the specific
 445 makeup of the amyloid positive group. If the amyloid positive individuals are biased towards
 446 low Tau (e.g. young ϵ 4 carriers), there may be an increased likelihood of observing hyper-
 447 connectivity effects. Conversely, if the amyloid positive individuals in a given sample are biased
 448 towards elevated neocortical Tau, then there will likely be a hypo-connectivity effect. If the
 449 amyloid positives are relatively balanced between low and elevated Tau, then there may be no
 450 observable effect of amyloid. Thus, the pattern of hyper-connectivity followed by hypo-
 451 connectivity observed here may help explain the varied effects reported in the literature,
 452 highlighting the need to consider additional pathologies beyond amyloid burden.

453 Additionally, given that there is non-negligible AV1451 signal in low amyloid subjects in
 454 both allocortical (i.e. entorhinal and parahippocampus) and neocortical regions (i.e. fusiform
 455 and inferior temporal), it will be important to consider the effects of PHF-tau on fcMRI in the
 456 absence of amyloid, as it relates to generic aging processes and non-amyloid tauopathies.

457 A great deal of work remains to elucidate the relationship between fcMRI and preclinical
 458 AD pathology, however it appears clear that *in-vivo* Tau imaging will be critical to providing new
 459 insights into the sequence and consequences of the AD pathological cascade. One area of
 460 particular interest will be in discerning how propagation of Tau pathology is related to
 461 functional and structural network architecture. Recent work by (Ossenkoppele et al., 2016)
 462 looking at clinically impaired AD variants found that spatial patterns of Tau pathology across AD
 463 phenotypes mirrored atrophy patterns and metabolic patterns, suggesting non-local spread of

464 Tau, presumably via network connections. Recent work by (Wu et al., 2016) demonstrated that
 465 neuronal activity can modulate the release of Tau, and that this Tau can spread through
 466 extracellular space, providing a potential mechanism for local spread of Tau pathology
 467 associated with hyper-activity. The extent to which the spread of nascent Tau pathology in
 468 preclinical populations mirrors functional network architecture remains to be elucidated.

469 Interpreting Default Mode/Salience effects:

470 The observed effects were limited to default mode / salience network, and were not
 471 observed in dorsal attention or control networks. Given the anti-correlated nature of the
 472 default mode / salience in our dataset, observing the effect in both is not surprising, and
 473 corresponds with previously reported functional connectivity effects across the AD spectrum
 474 (Brier et al., 2012). Furthermore, given the preclinical nature of this cohort, our findings
 475 support the hypothesis that the default mode network is the first affected by nascent AD
 476 pathology, consistent with other reports (Hedden et al., 2009, Lim H et al., 2014, Jones et al.,
 477 2016). Previous work in autosomal dominant AD and APOE4 carriers have also implicated the
 478 DMN and salience networks in younger asymptomatic genetic at-risk individuals (Chhatwal et
 479 al., 2013; Filippini et al., 2009; Machulda et al. 2011).

480 AD is characterized by two primary molecular pathologies, both of which appear to be
 481 necessary for cognitive decline and progression to dementia (Vos et al. 2015). Amyloid- β
 482 deposition begins in heteromodal cortices, largely overlapping the topology of the cortical
 483 connectivity hubs (Buckner et al., 2009), whereas tau pathology accumulates early in the MTL,
 484 in regions strongly connected to cortical DMN regions (Ward et al., 2014). Moreover, episodic
 485 memory is typically the most salient cognitive symptom of early AD, and relies on the interplay

486 of DMN and MTL activity (Miller et al., 2008; Ward et al., 2015). Executive function also
487 commonly declines in concert with episodic memory in aging and early AD, which may
488 particularly implicate interplay between DMN network and salience networks (La Corte et al.,
489 2016). Ongoing work in our group and others seeks to further differentiate the network
490 alterations associated with aging and the earliest alterations specifically associated the
491 molecular pathologies of AD. These observations will guide the use of functional connectivity as
492 an exploratory outcome measure in AD secondary prevention trials, aiming to decrease amyloid
493 accumulation to prevent the spread of tau pathology and cognitive decline associated with AD
494 (Sperling et al., 2014).

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744 **LEGENDS**

745 *Table 1:* Summary of results from statistical models. Robust effects are limited to amyloid by
 746 Inferior Temporal AV1451 interactions for the DMN and salience (SAL) networks. Of note, main
 747 effects outside of context of the interaction term are non-significant. Effects highlighted with
 748 bold/italicized/underlined font survive FWE of $p < 0.0033$ (.05/15 tests); effects highlighted with
 749 bold font are significant at an uncorrected $p < 0.05$.

750

751 *Figure 1:* Visualization of the PiB x IT AV1451 interaction term vs. DMN connectivity (left) and
 752 salience (SAL) connectivity(right). Both networks show a significant quadratic pattern that can
 753 be described as a positive relationship with amyloid when IT AV1451 signal is low, and a
 754 negative association with IT AV1451 signal when PiB signal is high. The significant interaction is
 755 driven in large part by the relatively elevated connectivity seen in high-PiB low-IT AV1451
 756 participants (red points near the middle of the x-axis).

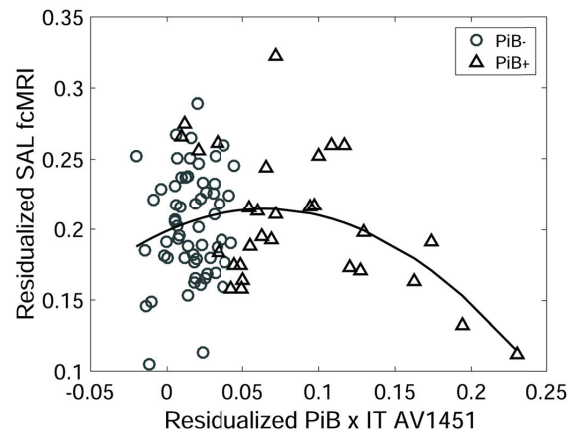
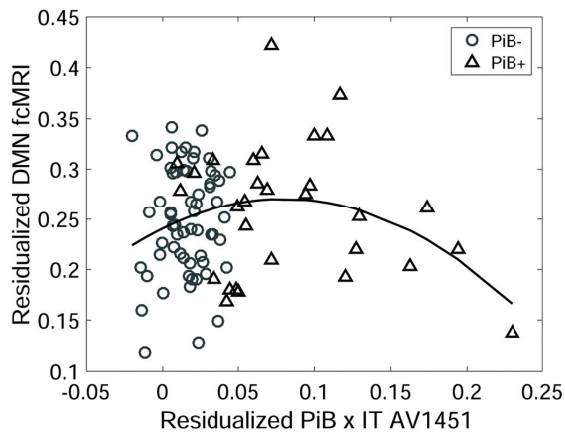
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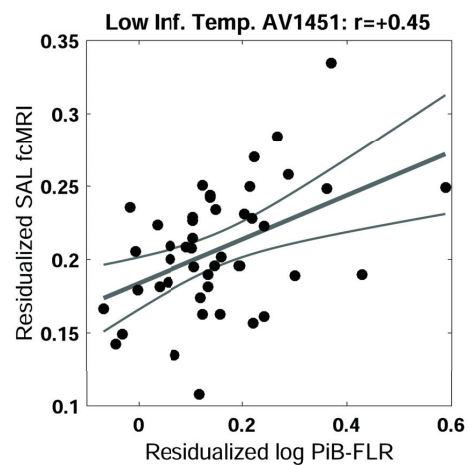
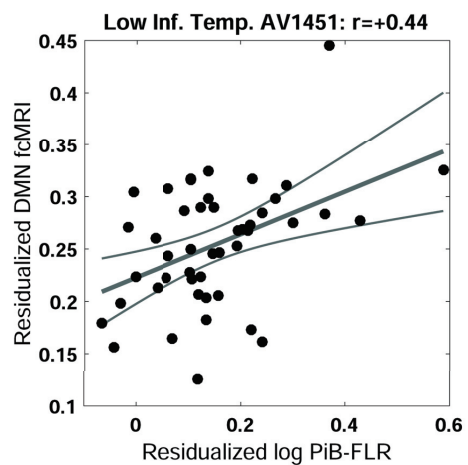
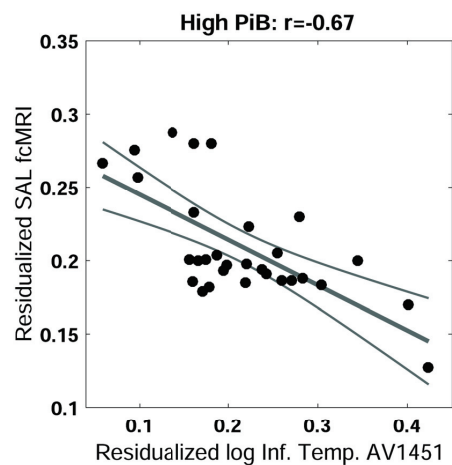
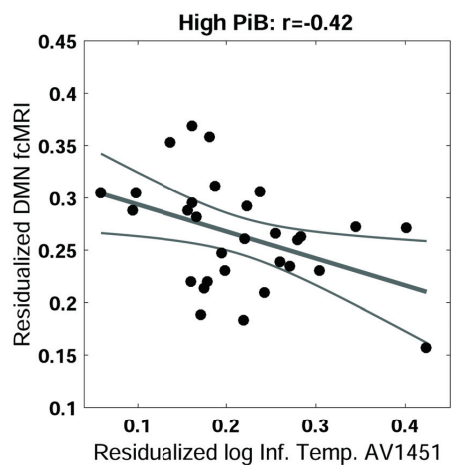
758 *Figure 2:* The top two figures show the relationship between IT AV1451 signal in the high PiB
 759 group (top figures) with DMN connectivity (left) and salience (SAL) connectivity (right). Within
 760 the context of high amyloid, increased levels of IT AV1451 are associated with decreased
 761 connectivity in the DMN and salience networks. The lower two figures show the association
 762 between amyloid burden in the low IT AV1451 signal subjects (median split) with DMN
 763 connectivity (left panel), and salience connectivity (right panel). These two effects, the positive
 764 effect of amyloid when IT AV1451 signal is low, and the negative effect of IT AV1451 signal
 765 when amyloid is high account for observed interaction effect, and the absence of main effects.

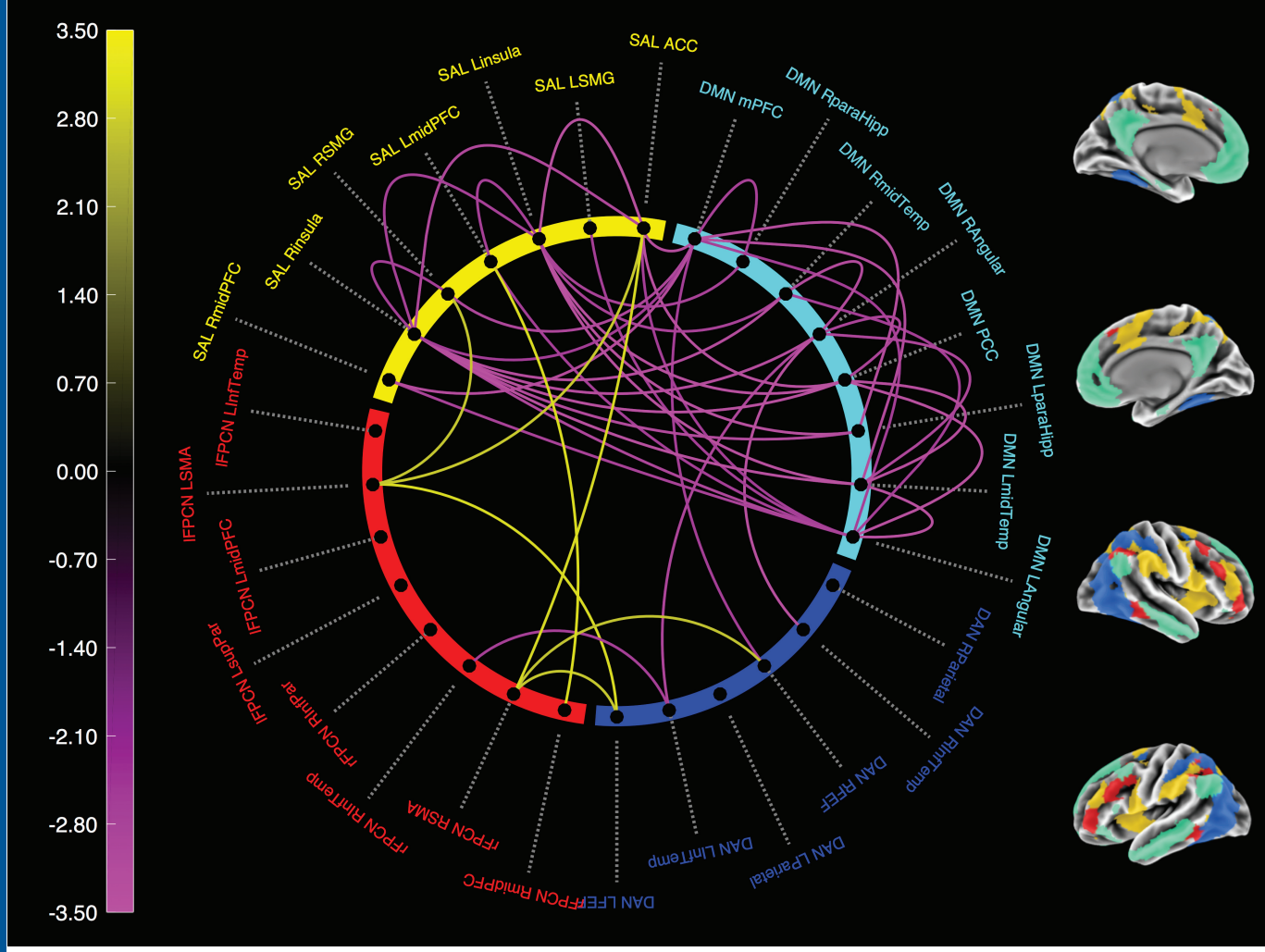
766

767 *Figure 3:* Node level analysis of the IT AV1451 x PiB interaction term on connectivity between
768 each pair of nodes. Lines on the outside of the figure correspond to within network
769 connections. Lines on the inside correspond with between network connections. Purple colors
770 represent decreased connectivity (movement towards 0); yellow colors represent increased
771 connectivity (movement away from 0). Only connections with an effect $p < 0.01$ are shown. The
772 patterns of significant nodes correspond well to the whole network analysis and demonstrate
773 the sensitivity to AV1451 and PiB are largely focused within and between the DMN and salience
774 (SAL).

775







	Models #1 and #2		Model #3		Model #4	Model #5
Entorhinal AV1451	PIB	TAU	PIB	TAU	PIB x TAU	PG x TAU
DMN	t(84)=1.086; p=0.28	t(84)=0.150; p=0.88	t(83)=1.127; p=0.26	t(83)=-0.355; p=0.72	t(82)=-0.226; p=0.82	t(82)=0.628; p=0.53
Saliency	t(84)=0.478; p=0.63	t(84)=-0.770; p=0.44	t(83)=0.902; p=0.37	t(83)=-1.084; p=0.28	t(82)=-0.959; p=0.34	t(82)=-0.110; p=0.91
DAN	t(84)=0.601; p=0.55	t(84)=0.444; p=0.66	t(83)=0.451; p=0.65	t(83)=0.202; p=0.84	t(82)=0.515; p=0.61	t(82)=0.998; p=0.32
Left FPCN	t(84)=1.596; p=0.11	t(84)=1.734; p=0.09	t(83)=0.939; p=0.35	t(83)=1.153; p=0.25	t(82)=0.803; p=0.42	t(82)=1.673; p=0.10
Right FPCN	t(84)=0.501; p=0.62	t(84)=0.951; p=0.34	t(83)=0.099; p=0.92	t(83)=0.809; p=0.42	t(82)=-1.080; p=0.28	t(82)=-0.704; p=0.48
Inferior Temporal AV1451	PIB	TAU	PIB TAU	TAU PIB	PIB x TAU	PG x TAU
DMN	t(84)=1.086; p=0.28	t(84)=-0.141; p=0.89	t(83)=1.197; p=0.23	t(83)=-0.531; p=0.60	<u>t(82)=-3.616; p=0.00</u>	<u>t(82)=-2.494; p=0.01</u>
Saliency	t(84)=0.478; p=0.63	t(84)=-1.308; p=0.19	t(83)=0.972; p=0.33	t(83)=-1.557; p=0.12	<u>t(82)=-4.774; p=0.00</u>	<u>t(82)=-3.642; p=0.00</u>
DAN	t(84)=0.601; p=0.55	t(84)=-0.880; p=0.38	t(83)=0.949; p=0.35	t(83)=-1.145; p=0.26	t(82)=-1.224; p=0.22	t(82)=-1.073; p=0.29
Left FPCN	t(84)=1.596; p=0.11	t(84)=0.601; p=0.55	t(83)=1.468; p=0.15	t(83)=0.083; p=0.93	t(82)=-1.184; p=0.24	t(82)=-0.959; p=0.34
Right FPCN	t(84)=0.501; p=0.62	t(84)=-0.133; p=0.89	t(83)=0.575; p=0.57	t(83)=-0.316; p=0.75	<u>t(82)=-2.237; p=0.03</u>	<u>t(82)=-2.293; p=0.02</u>
Inferior Parietal AV1451	PIB	TAU	PIB	TAU	PIB x TAU	PG x TAU
DMN	t(84)=1.086; p=0.28	t(84)=0.557; p=0.58	t(83)=0.960; p=0.34	t(83)=0.255; p=0.80	<u>t(82)=-1.890; p=0.06</u>	t(82)=-1.450; p=0.15
Saliency	t(84)=0.478; p=0.63	t(84)=-0.381; p=0.70	t(83)=0.612; p=0.54	t(83)=-0.540; p=0.59	<u>t(82)=-2.668; p=0.01</u>	<u>t(82)=-2.369; p=0.02</u>
DAN	t(84)=0.601; p=0.55	t(84)=-0.149; p=0.88	t(83)=0.670; p=0.50	t(83)=-0.337; p=0.74	t(82)=-1.010; p=0.32	t(82)=-0.982; p=0.33
Left FPCN	t(84)=1.596; p=0.11	t(84)=0.594; p=0.55	t(83)=1.476; p=0.14	t(83)=0.145; p=0.89	t(82)=-0.834; p=0.41	t(82)=-0.466; p=0.64
Right FPCN	t(84)=0.501; p=0.62	t(84)=-0.034; p=0.97	t(83)=0.530; p=0.60	t(83)=-0.186; p=0.85	<u>t(82)=-1.968; p=0.05</u>	t(82)=-1.777; p=0.08