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TECHNICAL REPORT

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**Disruption of Task-Free Resting State Network fMRI Correlation Structure in Mild Cognitive Impairment and Alzheimer’s Dementia in ADNI 2**

Kamil A. Grajskia and Steven L. Bresslerb, for the Alzheimer’s Disease Neuroimaging Initiative1

aNuroSci, LLC., West Palm Beach, FL. [kgrajski@nurosci.com](mailto:kgrajski@nurosci.com)

bCenter for Complex Systems and Brain Sciences, Florida Atlantic University, Boca Raton, FL. [kgrajski@nurosci.com](mailto:kgrajski@nurosci.com)

Abstract

Abstract goes here.

Keywords

ADNI, Alzheimer’s disease, Mild cognitive impairment, Resting state networks, Functional correlation, Atlas-based, Seed-based, Biomarker

[[1]](#footnote-1)

# Introduction

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# Materials and Methods

Data used in the preparation of this report was obtained from the Alzheimer’s Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). The ADNI project was launched in 2003 as a public-private partnership, led by Principal Investigator Michael Weiner, MD. The primary goal of ADNI has been to test whether serial magnetic resonance imaging (MRI), positron emission tomography (PET), other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of mild cognitive impairment (MCI) and early Alzheimer’s disease (AD).

## Clinical population

The clinical population in this report is a subset of the ADNI 2 experimental sub-study on resting state fMRI functional connectivity (rsfMRI) (ADNI 2, 3T MRI Technical Procedures Manual, 2016). The complete set of ADNI 2 clinical documents can be found online (ADNI Study Documents, 2016). The ADNI 2 rsfMRI data available for download on the Laboratory of Neuro Imaging (LONI) website as of August, 2016 consisted of 886 scan pairs (structural MRI, rsfMRI) from 220 subjects recorded during multiple visits over the course of the multi-year (and ongoing) ADNI 2 study. The paired series were subjected to a multi-stage image processing and quality assurance procedure. The procedure yielded 98 scan pairs from 98 unique subjects that is age-, education- and gender-matched across three diagnostic conditions (Cognitively Normal, Moderately Cognitively Impaired, Dementia). See Table 1.

**Table 1.** Study demographics, cognitive scores and APOE status.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **CN** | **MCI** | **D** | **P value** |
| Subjects, n | 40 | 38 | 20 |  |
| Women, n (%) | 24 (60) | 19 (50) | 6 (30) | 0.091a |
| Age, yrs., median (min,max) | 73.4 (65, 87) | 73.6 (62, 86) | 74.2 (59, 83) | 0.647b |
| Education, yrs., median (min,max) | 16 (12, 20) | 16 (12, 20) | 16 (12, 20) | 0.438b |
| CDR, median (min,max) | 0 (0, 0.5) | 0.5 (0.5, 1.0) | 0.5 (0.5, 1.0) | <<0.001b |
| CDRSB, median (min,max) | 0 (0, 1.5) | 1 (0, 5.5) | 4 (2, 9) | <<0.001b |
| MMSE, median (min,max) | 29 (27,30) | 28 (22, 30) | 22 (12, 29) | <<0.001b |
| APOEε4=2, n (%) | 1 (2.5) | 6 (16) | 5 (25) | 0.007a |
| *a(Chi-square test); b(ANOVA);* | | | | |

## Image acquisition

The development of ADNI 2 MRI protocol details is discussed elsewhere (Jack, et al., 2008) and the complete set can be found online (ADNI Study Documents, 2016). Of interest in the present analysis are two of the up to eight different scans obtained during each ADNI 2 subject visit. A typical visit sequence consisted of an initial screening visit, a baseline visit, a 3-month, 6-month and 12-month, and annual visit thereafter. The scans of interest here are restricted to the high-resolution T1-weighted 3D volumetric structural scan (Scan #2 in the protocol) and resting state functional scan (Scan #4 in the protocol) obtained during each subject visit. Field mapping scans were also obtained, but they are not used in the present analysis (see next steps).

Whole-brain high-resolution structural images were recorded in 3T MRI scanners of a single manufacturer (Philips Medical Systems: Achieva, Ingenia, Ingenuity, Intera) from 14 unique sites in two configurations (MULTI-COIL (n=16); SENSE-HEAD (n=96)), and fifteen (15) different software versions, the most common being 3.2.1 or higher (n=99). The ADNI structural scan protocol defined a T1-weighted sagittal magnetization-prepared rapid gradient echo (MP-RAGE) scan. The following parameters were extracted from the 98 XML metadata files: a) flip Angle, 9o; b) volume; 256x256x170; c) pixel spacing, 1.0mm; d) slice thickness, 1.2mm. The metadata file entry for TE (3.14 msec) and TR (6.77msec) are inconsistent with the ADNI MRI protocols. As visual inspection indicates that the MRI quality is good to excellent it is considered that these entries are incorrect, inconsistent, or represent some (unknown to these authors) encoding.

The rsfMRI scan protocol defined a T2-weighted sequence. The following parameters were extracted from the 98 rsfMRI XML metadata files: a) flip angle, 80o; b) pixel spacing, 2.79-3.313mm; c) slice thickness, 3.312mm; d) TE=30.0 msec; e) TR=3000 msec; and f) phase-direction (anterior-posterior, n=14; posterior-anterior, n=91). There were nine (9) different fMRI temporal slice order sequences listed in the 886 scan dataset metadata. Only two temporal slice order sequences were admitted to the present 98 scan dataset: a) ascending 48 planes (odd, even), n=91; and b) descending 48 planes (even, odd), n=7. rsfMRI scans were of two durations: a) 140 TRs (“resting state”, n=85); b) 200 TRs (“extended resting state”, n=13).

## Image preprocessing

Structural images and rsfMRI images were downloaded from LONI either in DICOM format (and locally converted using dcm2nii) or in 4DNIfTI format (whereby the DICOM to NIfTI conversion is completed by LONI). Preprocessing and data analysis were performed using a combination of Analysis of Functional Neuroimages (AFNI) software (Cox, 1996), FSL (Smith, et al., 2004; Jenkinson, et al., 2012), and “home-grown” analysis implemented in R and *tcsh* shell scripts. The AFNI Version was AFNI\_16.3.04 (19-Oct-2016). The FSL version (FSLView) was V3.2.0. The R version was Version 3.2.3 (10-Dec-2015). The AFNI Python script uber\_subject.py was used to generate a baseline preprocessing shell script which was manually edited.

For structural images, the sequential preprocessing steps included skull stripping, center alignment to a standard atlas (AFNI: MNI\_avg152), anatomical alignment to the rsfMRI EPI registration base volume (4th EPI overall, 2nd after dropping first two EPIs) and warping to the standard atlas. For rsfMRI EPIs, the preprocessing sequence consisted of the following steps: a) alignment of EPI centers to the standard atlas; b) elimination of the first two EPIs; c) outlier detection and de-spiking; d) application of time-shift correction; e) alignment of each EPI to the base volume, alignment to the anatomical image and warp to standard space; f) generation of anatomical and EPI masks; g) application of spatial smoothing (FWHM = 4.0, 6.0, 8.0, or 10.0); h) scaling each voxel time series to a mean value of 100; i) generation of demeaned motion parameters and motion parameter derivatives; j) generation of motion “censor” masks (motion limit = 0.3-0.5); generation of cerebral-spinal fluid(CSF) and white-matter (WM) segmentation masks and corresponding demeaned voxel time series; k) computation of the global averaged time series signal (GS); and l) deconvolution with either eight regressors (motion+ CSF + WM), nine regressors (8 regressors + GS)), 14 regressors (8 regressors + 6 motion derivatives), or 15 regressors (9 regressors + 6 motion derivatives) to yield a 4D residual error dataset that was input to the next stage of correlation analysis. The deconvolution step included additional regressors to implement a time-series filter with bandwidth 0.01 – 0.1 Hz. [Note: de-spiking and band-pass filtering were added to the preprocessing pipeline once the matched 98 data set was defined.]

The list of high-resolution and rsfMRI ADNI image identifiers and the research-grade (i.e., not for clinical or commercial use) AFNI, R, and other shell scripts have been uploaded to github. Access is available upon request on an “as is” warranty-free basis.

## Quality assurance at the study participant level

The goal of quality assurance is to automate the decision process for inclusion and exclusion of scans in individual and group-level analyses. The main focus of the procedure is to identify and reject scans based on a reproducible quantitative assessment of motion artefacts. The starting database consisted of 886 rsfMRI scans (and their paired structural images) downloaded from LONI. An R package “ADNIMERGE” contained MRI and rsfMRI quality assessments (e.g., comments by ADNI quality reviewers) and related data (e.g., rsfMRI slice order) stored in the tables “mayoadirl\_mri\_imageqc” and “mayoadirl\_mri\_fmri”, respectively (Mayo ADIR, 2011, 2012). The 886-scan set (n=220 subjects) was reduced to 688 scans (n=209) by rejecting all scans that did not conform to the slice order sequences noted above, or could not be opened in the FSL viewer, or had other file-related issues, or had clearly excessive motion based on visual inspection in the FSL viewer, or contained any reviewer comment in either of the available pair of comment fields. Comments typically referenced excessive motion or potential or actual clinical findings.

The eight (8) regressors (six motion, CSF and WM) preprocessing sequence with motion-limit set to 0.5 was applied to each of the 688 structural-rsfMRI pairs. The global average signal of the rsfMRI scan was analyzed as an initial screen for excessive motion. Scans were highlighted for further inspection where any global average time-series amplitude value fell outside the range 96-104 or that the standard deviation of the global average time series exceeded 2.0. The range and standard deviation thresholds were set by an iterative process. In the first iteration, the threshold values were 2-3x wider than those listed above. Flagged scans were visually inspected. The most common cause was clearly evident excessive motion. The threshold values were narrowed until scans were flagged for which it was no longer immediately obvious by visual inspection what drove the excess global signal. This gross motion screening procedure reduced the 688 structural-rsfMRI pairs database to 564 (n=201 subjects).

The 564 scan pairs database was then screened on the basis of statistics generated by the AFNI-based preprocessing pipeline. Scans were rejected according to the following criteria: a) DICE coefficient < 0.71; b) censor fraction > 0.10; c) maximum censored displacement > 1.00; d) average TSNR > 300 or average TSNR < 100; e) degrees of freedom left < 100; and f) global correlation > 0.05. This screening stage reduced the 564 pairs database to 314 (n=145). The full preprocessing pipeline was then rerun with 8 regressors (no GS removal) and 9 regressors (GS removal), but with motion limit value tightened to 0.3. The global average time series and AFNI statistics filters were applied to each preprocessed set. The 8-regressor set yielded 218 scans (n=112 subjects). The 9-regressor set yielded 219 scans (n=112 subjects). The reduction from 314 to ~218 was due almost exclusively (>95% of cases) to failing the criterion (10%) for percentage of scan time slices censored. This procedure yielded a dataset of 211 (n=109 subjects) common to both the 8- and 9-regressor configurations. The final dataset reduction step enforced the rule of a single scan pair per subject. Where multiple candidate scans were present, the latest available was selected. 80%+ of the final dataset consists of data recorded from subjects who were on at least their second ADNI 2 study visit. The final 98 subject, age-, gender-, and education-matched dataset shown in Table 1 was obtained by random selection of subjects (by age or by gender) where needed to achieve statistical balance.

Table 2 shows that the preprocessing performance measures are matched across the three diagnostic groups. “Matched” means that for each performance statistic, the null hypothesis of equal group means is rejected in one-way ANOVA with diagnostic label as a three-level factor. Matched performance statistics are seen across all four preprocessing pipeline configurations (e.g., 8-, 9-, 14- or 15-regressors in the deconvolution stage). In nearly all cases the ANOVA *p*-value was >0.05.

For good measure, *post hoc* pair-wise diagnosis group *t* tests of significance were performed for the statistics that were not >>0.05. These include Censor Fraction, the Max Censored Displacement and the Global Correlation statistics. For the Censor Fraction statistic, the three *P* values (rounded to nearest hundredth) were (CN vs MCI: 0.01; CN vs. Dementia: 0.32; MCI vs Dementia: 0.21) with corresponding Cohen’s effect sizes (0.61, 0.26, 0.38). The null hypothesis (that there is no statistically significant difference in the mean fraction of time slices censored (for motion) in the group MCI vs the group CN) cannot be rejected. The statistical effect size is moderate. The MCI Censor Fraction median value was 1% and that for CN 2%. This difference is not expected to have material impact on the conclusions of this report.

For the Maximum Censored Displacement statistic, the three *P* values were (0.24, 0.03, 0.24) with corresponding Cohen’s effect sizes (0.26, 0.21, 0.03). The null hypothesis (that there is no statistically significant difference in the maximum displacement in remaining time slices after censoring in the group Dementia vs CN) cannot be rejected. The statistical effect size is small. The Dementia Maximum Censored Displacement median value was 0.59 and that for CN 0.49. This difference is not expected to have material impact on the conclusions of this report.

Table 2 includes a list of changes in the Global Correlation statistic as a function of the number of preprocessing pipeline regressors (8-, 9-, 14- or 15). Utilizing the *P*-value as a heuristic, the trend appears to be in the “right direction.” Removing the global signal (8 vs 9 regressors; 14 vs 15 regressors) or adding motion derivative regressors (8 and 9 vs 14 and 15) reduces differences between the means across the three groups. Similarly, TSNR increases with additional regressors. [Note. A rigorous analysis of the role of number and type of regressor (which is beyond the scope of this study) would include a control experiment in which the 8-regressor-plus-GS (9-regressors) is compared to an 8-regressor-plus-random sequence.]

**Table *2.*** Preprocessing summary statistics by diagnostic class.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **CN** | **MCI** | **D** | **P valuea** |
| Censor Fraction, median, (min,max) | 0.02 (0.0, 0.09) | 0.01 (10.0, 0.07) | 0.01 (0.0, 0.07) | 0.029b |
| Avg Censored Motion, median, (min,max) | 0.10 (0.05, 0.14) | 0.09 (0.06, 0.15) | 0.10 (0.05, 0.14) | 0.168 |
| Max Censored Displ, median, (min,max) | 0.49 (0.21, 0.79) | 0.50 (0.25, 0.91) | 0.59 (0.32, 0.89) | 0.077b |
| DICE Coefficient | 0.85 (0.73,0.90) | 0.84 (0.66, 0.89) | 0.84 (0.73, 0.88) | 0.547 |
| 8-Reg Global Correlation, med., (min,max) | 0.02 (0.00, 0.06) | 0.03 (0.00, 0.05) | 0.03 (0.01, 0.06) | 0.515 |
| 9-Reg Global Correlation, med., (min,max) | 0.02 (0.00, 0.05) | 0.02 (0.00, 0.04) | 0.02 (0.01, 0.05) | 0.721 |
| 14-Reg Global Correlation, med., (min,max) | 0.02 (0.00, 0.06) | 0.02 (0.00, 0.05) | 0.02 (0.01, 0.07) | 0.613 |
| 15-Reg Global Correlation, med., (min,max) | 0.02 (0.00, 0.05) | 0.02 (0.00, 0.04) | 0.02 (0.01, 0.05) | 0.757 |
| 8-Reg Avg TSNR, med., (min,max) | 263 (182,323) | 263 (213,342) | 255 (169, 308) | 0.280 |
| 9-Reg Avg TSNR, med., (min,max) | 271 (186,326) | 267 (216,352) | 261 (171, 315) | 0.322 |
| 14-Reg Avg TSNR, med., (min,max) | 286 (194,343) | 284 (229,367) | 274 (181, 332) | 0.401 |
| 15-Reg Avg TSNR, med., (min,max) | 292 (198,347) | 291 (231,377) | 281 (183, 342) | 0.452 |
| *a(ANOVA); bPost hoc pair-wise t tests performed in those cases where ANOVA P value was “near” P = 0.05.* | | | | |

## Seed ROI definition

Seed ROI definitions were drawn from three sources for a total of 196 ROIs. Thirty-six (36) ROI seeds are those listed in Table 2 of the Brier, et al. (2012) study of resting state networks (RSN). Each seed served as the origin for a 6mm radius sphere. The continuous volumetric extent of ROI regions was ~33 voxels. Each ROI was assigned an additional label corresponding to an identified cortical RSN as in Brier, et al. These included: a) Control (CON), 5 ROIs; b) Dorsal Attention (DAN), 8 ROIs; c) Default-Mode (DMN), 9 ROIs; d) Salience (SAL), 7 ROIs; and e) Sensory-Motor (SMN), 7 ROIs. In networking terms, each ROI is a node within an RSN with a quantifiable degree of functional connectivity with other nodes both within RSN and between RSNs (inter-RSN). Thirty-three of thirty-six seeds sampled cortical regions; 2 sampled the posterior cerebellum (left, right); and 1 sampled the medial thalamus.

Sixteen (16) ROI seeds were obtained from the Mackey-Petrides anatomical atlas (Mackey-Petrides, 2014) of the human ventromedial pre-frontal cortex (VmPFC). The volumetric extent (number of voxels) for each ROI was controlled by a probability parameter set to 0.5 and recorded as a brain mask. The volumetrically smallest extent was 33 voxels (Area 25); the largest 243 voxels (Area 14m). Analogously to the grouping of ROI seeds into RSNs, Mackey-Petrides regions were labelled as belonging to one of 8 VmPFC sub-regions each with bilateral extent: a) Area 11m; b) Area 14c; c) Area 14m; d) Area 14r; e) Area 14rr; f) Are 24; g) Area 25; and h) Area 32. In networking terms, each Mackey-Petrides ROI is a node within a VmPFC sub-region with a quantifiable degree of functional connectivity with other nodes both within VmPFC sub-region (intra-Network) and between VmPFC sub-regions (inter-Network).

Finally, the Eickhoff-Ziller probabilistic atlas (Eickhoff, et al., 2005) contributed one hundred forty-three (143) ROI seeds. In AFNI the Eickhoff-Ziller atlas is implemented as the CA\_PM\_18\_MNIA atlas daemon. The 143 ROI seeds consisted of 72 left and 71 right hemisphere cytoarchitectonically identified regions. The continuous volumetric extent (number of voxels) for each ROI was controlled by a floor probability parameter set to 0.5 and recorded as a brain mask. ROI extents varied widely. For example, the Area 1 voxel count was 229 voxels. The Amygdala CM voxel count was 16. Analogously to the grouping of ROI seeds into RSNs, Eickhoff-Ziller regions were assigned to one of fifteen (15) sub-regions each with bilateral extent: a) TE\_3 (2); b) A17.18 (4); c) A44.45 (4); d) Amygdala (6); e) A4.6 (6); f) hIP (6); g) hOC (6); h) Ins (6); i) A1.2.3 (8); i) OP (8); j) Hippocampus (10); k) IPC (14); l) Th (14); l) SPL (14); and m) CBLM (35; left: 18; right:17). Notably, the Eickhoff-Ziller atlas enables analysis of subcortical structures bilaterally: a) amygdala (3 sub-regions); hippocampus (5 sub-regions); and c) thalamus (7 sub-regions). In networking terms, each Eickhoff-Ziller ROI is a node within an anatomical sub-region with a quantifiable degree of functional connectivity with other nodes both within sub-region (intra-Network) and between regions (inter-Network).

For each subject and for each ROI the volumetric average time series was computed and stored for pair-wise correlation analysis. Volumetric correlation maps were obtained by computing the Pearson correlation coefficient between the ROI time series average and all other brain voxels subject to a group mask. The group mask was formed as the union of all (n=98) subject-dependent anatomical brain masks (as registered to the standard template MNI\_avg152T1). [A variation on this procedure (not (yet) done) could be to apply segmentation (e.g., CSF) to this group anatomical mask.]

Prior to inclusion in pair-wise correlation analysis individual average time series were subjected to a final quality assurance check for outliers. For subject *k*, ROI average time series *j* is rejected if any of the following conditions is true: a) the time series is constant; b) the time series standard deviation, σjk > 2.0; or c) the fraction of time points with values in excess of the outlier threshold θjk exceeds 0.05. The outlier threshold is set as θjk = µjk + 2σjk , where µjk is the time series average. This final outlier check arose following the observation that even in images that met the subject-level quality assurance criteria described above, individual ROI time series contained anomalies (e.g., spikes, bursts, and other noise). Roughly 4-8% of ROI time series were rejected on this basis. A systematic analysis of the root causes for these anomalies has not been attempted.

It is possible that the quality assurance criteria described here are too conservative as the data yield is only 11% (98 scans “survive” from a starting set of 886). The strategy here is to start with strict criteria to establish findings as solidly and cleanly as possible. Conclusions could be revisited on an iterative basis wherein particular quality tests are systematically relaxed.

## Statistical analysis

### Volumetric correlation analysis

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### Pair-wise correlation analysis

The goal of pair-wise correlation analysis is to determine whether and to what degree there exist statistically significant differences in correlation structure between the Cognitively Normal, Mild Cognitively Impaired and Dementia clinical group. The core statistical inference method is the *post hoc* *t* test applied pair-wise between clinical subpopulations taking into account large-scale multiple testing (Efron and Tibshirani, 1993; Westfall and Young, 1993; Benjamini and Hochberg, 1995; Nichols and Holmes, 2002; Westfall and Troendle, 2008; Efron, 2004, 2010). Pair-wise correlation analysis is conducted separately on three levels: a) intra-Network (RSN or anatomical region); b) inter-Network (RSNs or anatomical regions); and c) inter-Node (individual RSN nodes or individual anatomical sub-regions).

*Intra-RSN (Intra-Network).* Brier, et al., defined a composite score that provides an aggregate measure of the internal correlation structure of an RSN. For example, the Brier composite score *c* for subject *k* DMN is the simple average of the Fisher z-transformed (*z*) Pearson correlation coefficients (*r*) over the set of 36 possible *within* DMNRSN node pairs. For RSN network X, X ϵ {CON, DAN, DMN, SAL, SMN}, the Brier composite score for subject k is:

(1)

. (2)

Brier, et al., then perform one-way ANOVA and *post hoc* pair-wise *t* tests per RSN network for the three clinical groups (Bonferroni corrected for N=5 tests). The present study adopts a similar approach for analysis of RSN networks in the ADNI 2 rsfMRI data.

The present study further applies the Brier composite score approach to atlas-derived ROI seeds. For the Eickhoff-Ziller and Mackey-Petrides ROIs, the anatomical region labels are analogous to the RSN-level labels. The Eickhoff-Ziller ROIs were grouped into 15 “networks”. For example, in the Eickhoff-Ziller representation of the hippocampus, the intra-hippocampal “network” contains 10 anatomical sub-regions (e.g., nodes), (5 left, 5 right) giving rise to 45 pair-wise intra-RSN correlations, and the thalamus “network” contains 14 (7 left, 7 right) nodes giving rise to 91 pair-wise intra-RSN correlations. For the Mackey-Petrides-derived ROI seeds, in perhaps a bit of overuse of this approach, the 16 sub-regions were partitioned into 8 two-node “networks” such that the sole intra-Network correlation pair is that between corresponding left and right hemispheric anatomical sub-regions.

*Inter-RSN (Inter-Network).* Brier, et al., extended the definition of a composite score to provide an aggregate measure of the correlation between RSNs. For RSN network X and Y, where X, Y ϵ {CON, DAN, DMN, SAL, SMN}, the Brier composite score for subject k is:

(1)

Brier, et al., then perform one-way ANOVA and *post hoc* pair-wise *t* tests per RSN network for the three clinical groups (Bonferroni corrected for N=10 tests). The present study adopts a similar approach for analysis of RSN networks in the ADNI 2 rsfMRI data. The present study further applies the Brier composite score approach to atlas-derived ROI seeds and networks. The 15 Eickhoff-Ziller “networks” give rise to N=105 pair-wise inter-Network tests. The 8 Mackey-Petrides “networks” give rise to N=28 pair-wise inter-Network tests.

*Inter-Node.* Brier, et al., analyzed correlation at the level of RSN node pairs by computing an “expanded seed ROI” for each of the nine (9) DMN nodes. An expanded seed ROI was obtained by sub-sampling the (equally-weighted) group-averaged Fisher z-transformed Pearson correlation maps in the regions surrounding the original seeds subject to a threshold criterion (|z|=0.3). The average time series was obtained from each of the expanded regions. Their pair-wise Pearson correlation coefficients were computed, Fisher z-transformed, and stored. Brier, et al., reported “inter-Node” results only for the DMN node posterior cingulate cortex (PCC) pair-wise with each of the other eight (8) nodes in the DMN. Results comprised a one-way ANOVA with three diagnosis group levels where *p* values were false discovery rate corrected to (*q* = 0.05) and select pair-wise *post hoc* *t* tests were performed for significance and size effects with *p* < 0.05.

The present study completes analyses at the level of RSN and anatomical pairs using correlation matrices derived from original ROI seeds and expanded ROI seeds. The approach taken here to computation of expanded ROI seeds is almost exactly the same as that described by Brier, et al. Brier, et al. implemented equal weighting of diagnosis groups. This was perhaps necessary as the cognitively normal group size was 4X and 10X the other diagnosis groups. In the present study, the maximum ratio is 2:1. Another difference is that in the present study the threshold was lowered to |z| = 0.015 in order to assure >0 volumetric extent for all possible seeds (although even with the lowered threshold there were 6 ROI seeds that yielded no surviving voxels). It is possible that the present expanded seed ROI procedure may have to be revisited. ROI and expanded ROI seed analysis each yield N=630 pair-wise correlation values for the RSN nodes. For the Mackey-Petrides nodes there are N=120 pairs. For the Eickhoff-Ziller there are N=10,153 pairs.

*Further extensions.* The present study extends the work of Brier, et al. (2012). First, it was observed in the present data that the distributions of within-RSN network correlation values have more complex structure (e.g., asymmetric tails) than might be captured by a mean value. Consequently, where Brier, et al., computed a single overall composite score for the nodes comprising an RSN, the present study computes six composite scores per RSN: a) overall mean; b) overall median; c) mean, positive-only values; d) mean, negative-only values; e) median, positive-only values; and f) median, negative-only values.

[A further extension that at the time of this writing is in the pilot stage is to perform (multiple) hypothesis testing directly at the distribution-level using non-parametric approaches, e.g., Wilcoxon tests. Another extension is to treat the question of intra-RSN (intra-Network) internal structure as an N-dimensional M-classification problem, where N is the number of pair-wise correlation values computed for the given RSN (anatomical region) and where M is the number of diagnosis labels (e.g., CN, MCI, D). The experimental approach is to compare the performance of a naïve Bayes classifier against a more sophisticated approach such as support vector machines (SVM), all under a data resampling framework to estimate true performance.]

The second extension of the Brier, et al., approach to intra-RSN network analysis follows the observation during pilot studies here that results not infrequently varied with different subsets of the available data. Observed differences were at times quantitatively minor (e.g., remaining statistically significant with a *p* value < 0.05, though with differences in the group average and *t*-statistics values), or qualitative (e.g., non-reproducible findings of statistical significance). The core question raised by these observations is what should the *p*-value be for the multiple *post hoc* pair-wise *t* tests? How shall the *p* value be adjusted to reflect multiple comparisons?

This study adopts a bootstrap resampling procedure to compute false discovery rate corrected *p* values. Consider as an example the pair-wise group comparison CN vs MCI for a single node pair X and Y. The same procedure is applied when X and Y are RSN composite scores (for intra-RSN or inter-RSN analysis).

1. Step one is to apply the *t*-test using all of the available CN and MCI data and to store the resultant *t* statistic as the sample *t* statistic, .
2. The goal of step two is to estimate the *t* statistic distribution under the null hypothesis that the CN and MCI groups have the same mean. To do this, the CN and MCI data are first resampled (with replacement *per class*) and the means adjusted per equations (3) – (7) below to enforce the null condition (the “null dataset”). A *t* test is then applied to the null dataset with the resultant resampled *t* statistic stored as . The resampling procedure is repeated B times on the null dataset to generate the distribution ,…,, B=10,000.

To establish the null hypothesis condition for the input dataset consisting of n1 scalar values **X** = {x1, x2, …, xn1} from group 1 and n2 scalar values **Y** = {y1, y2, …, yn1} from group 2 apply the following transformation:

+ Z (3)

+ Z (4)

, (5)

and (6) and (7)

Perform all subsequent resampling operations on the dataset consisting of and .

Alternatively, and for good measure, results obtained with the above procedure are compared and contrasted with the Fisher-type permutation approach. In the present running example, permutation means that the *n1 + n2* values are randomly divided (e.g., without replacement) into disjoint sets of size n1 and n2. The two-sample *t*-statistic is computed on the shuffled data set. The procedure is repeated B times to generate the distribution ,…,, B=10,000.

1. The goal of step three is to compute the resampling estimate *p* value, , for the k*th* of N simultaneous hypothesis tests. Step three counts the number of occurrences in {,…,} where and normalizes:

(8)

Step three operates in the same fashion for the distributions obtained either by bootstrap or by permutation.

1. Step four implements the Benjamini-Hochberg false detection rate (FDR) control procedure. The FDR control procedure operates on the set of resampled estimates of values *k = 1,...,N* for the N simultaneous hypothesis tests under consideration, e.g., Eickhoff-Ziller *N* = 10,153. To obtain the FDR-corrected *p* value, *pFDR*, sort the values from smallest to largest. Compute the largest index *imax* for which

. (6)

The null hypothesis is rejected for tests

The above approach to significance testing differs from that of traditional individual hypothesis testing. In individual hypothesis testing, the goal is often to reject the null hypothesis with high probability. In the present case, the goal is to identify a subset of “interesting” cases. That is, it is expected that for many, most, or perhaps even all of the hypothesis tests, the null hypothesis cannot be rejected.

### Multi-dimensional pattern classification

Text here.

# Results

## RSN

Figures 1-5 demonstrate that the CON, DAN, DMN, SAL and SMN RSNs are present in group average correlation maps derived from the ADNI 2 experimental sub-study on resting state fMRI functional connectivity (rsfMRI). The Figures provide initial qualitative visual evidence of possible changes in RSN correlation structure and RSN volumetric extent as a function of clinical progression.

Figures 1-5 show the output of volumetric one-sample *t* tests on group averaged correlation maps. A group average correlation map consists of the group average of individual subject Fisher z-transformed Pearson correlation coefficient maps. The displayed values are in units of the standard normal, *Z*. An *ad hoc* thresholding approach is used to identify clusters for *display purposes only*. An uncorrected voxel level *P*value (one-sided) is used as the cluster defining threshold (CDT), *PCDT*  = 0.025 (*Z*=1.96). Cluster extent threshold, NCT, is set to an arbitrary threshold of 50-100 9mm3 voxels. For RSNs with nodes that are themselves comparatively volumetrically small NCT=50. In comparison, the volumetric extent of a 6-mm radius ROI seed is 33 voxels. Figure color maximum threshold is controlled by *PCMT* = 0.005, one-sided, such that Z ≥ +*PCMT* saturates red; Z ≤ -*PCMT* saturates dark blue. That is, only the “tails” of the normalized distribution of group averaged correlation map values are shown. All of the Figures below are with global signal removed, spatial smoothing of 6-mm and simple ROI seed (i.e., not using the “expanded ROI”).

The AFNI 3dClustSim procedure was used to estimate the probability of false positive clusters. The output of 3dClustSim is Nα = C(*PCDT*, α), where Nα is the smallest cluster size for which the probability of an image subjected to threshold *PCDT* and having a noise-only cluster of size C is less than α. Input parameters for the spatial autocorrelation function (ACF) option were estimated by averaging over the pooled set of individual subject ACF estimates (obtained during the preprocessing stage). The results were: a = 0.569; b = 5.126; c = 13.929. 3dClustSim output was C(*PCDT* = 0.025, one-sided; α = 0.05, two-sided) = 531 = Nα.

Figure 1 shows CON RSN group averaged correlation maps for CN, MCI and Dementia groups for an ROI positioned at the dorsal medial PFC. In the CN group, the correlation (standard normal) Z score range was (-4.41, 4.29) with positive correlation between ROI seed and left and right anterior PFC (cluster size, NV=4185, α<<0.01), and anti-correlation with left and right superior parietal cortical regions (NV=1134, α<<0.01). In the MCI group, the correlation Z score range was (-4.80, 4.66). Cluster size and α value for anterior PFC was (NV=5333, α<<0.01); (NV=841, α<0.01) for superior parietal. The region of positive correlation with PCC (NV=184, α>0.1) fails statistical significance for spatial extent. In the Dementia group, the correlation Z score was (-4.09, 3.91). Cluster size and α value for anterior PFC was (NV=3622, α<<0.01). In the region occupied by the single superior parietal cluster observed in CN and MIC there are two smaller independent: NV=108, α>0.1 (left); and NV=378, α>0.1 (right). Neither cluster on its own meets the criterion for statistical significance. However, intuition suggests that the occurrence of a pair of clusters bilaterally and in approximately equivalent positions is a low probability event.

Figure 2 shows DAN RSN group averaged correlation maps for CN, MCI and Dementia groups for an ROI positioned at the left Visual Area MT. In the CN group, the correlation Z score distribution was long-tailed to the positive side (-4.17, 6.23) with positive correlation in a large single cluster including left and right posterior intraparietal sulcus (NV=7791, α<<0.01), and smaller clusters in right anterior intraparietal sulcus (NV~230, α>0.1), and left and right frontal eye fields (NV~240). Clusters of anti-correlation were with left and right posterior intraparietal sulcus regions (left NV~110; right NV~190) and with PCC (NV~75). In the MCI group, the correlation Z score range (-4.32, 5.89) and the spatial pattern of correlations and anti-correlations appear mainly intact particularly on the right side. Individual cluster sizes corresponding to nominal DAN nodes were in the range 115 (left intraparietal sulcus) to 290 (right anterior frontal cortex). In the Dementia group, the correlation Z score range was (-4.07, 5.84). In the posterior region, the positive correlations form a nearly continuous band (NV=5837, α<<0.01) compared to the discrete zones observed in CN. The anti-correlations with PCC and left and right posterior intraparietal sulcus regions are diminished or absent. Even with *PCDT* >> 0.025 and NC=20 there emerge no additional posterior zones of anti-correlation. A zone of anti-correlation with the ventral anterior cingulate gyrus is observed (not shown in Figure).

Figure 3 shows DMN RSN group averaged correlation maps for CN, MCI and Dementia groups for an ROI positioned at the PCC. In the CN group, the correlation Z score range was (-4.23, 6.00) with positive correlation with PCC (NV=2353, α<<0.01), with medial PFC (NV=1500, α<<0.01) and left (NV=384, α>0.10) and right (NV=461, α<0.10) lateral parietal regions. Anti-correlations were observed with left (NV=174, α>0.10) and right (NV=262, α>0.10) parietal cortical regions (e.g., Visual Area MT), in right inferior frontal gyrus in an area corresponding to Brodmann Area 9 (NV=457, α<0.10), and in right superior parietal lobule (NV=438, α<0.10). In the MCI group, the correlation Z score range was (-4.14, 4.82). Zones of positive correlation were observed with PCC (NV=2275, α<<0.01), with the anterior medial PFC (NV=2979, α<<0.01), left (NV=530, α<0.10) and right (NV=451, α<0.10) inferior temporal regions, and left (NV=412) and right (NV=379) lateral parietal regions. In the Dementia group, the correlation range was (-3.97, 4.26) elements of the basic spatial pattern was maintained (e.g., medial PFC, NV=1078, α<<0.01) though with apparently reduced spatial extent compared to CN (even when *PCDT* > 0.025 and reduced NC). Other than a zone of positive correlation centered around the seed ROI PCC with PCC (NV=1078, α<<0.01) no other positive correlation or anti-correlation cluster were statistically significant.

Figure 4 shows the SAL RSN group averaged correlation maps for CN, MCI and Dementia groups for an ROI at right dorsal ACC. In the CN group, the correlation Z score range was (-4.16, 4.06). The extended zone of positive correlation shown in the Figure includes the ACC and bilaterally zones in the superior frontal gyrus (NV=2700, α<<0.01). Not shown in the Figure is a bilateral pair of zones of positive correlation centered in the region of the left (NV=268, α>0.1) and right (NV=179, α>0.1) insula and putamen. There is a zone of anti-correlation centered in the middle occipital gyrus in an area corresponding to Brodmann Area 18 (NV=445, α<0.10). In the MCI group, the correlation Z score range was (-3.92, 4.13). In the axial section shown a single cluster (NV=3069, α<<0.01) of positive correlation encompasses the PCC, the dorsal ACC and a distributed zone in the right frontal gyrus. The lateral-most cluster shown (NV=670, α<0.025) is the posterior-most portion of a cluster that traverses the interior frontal gyrus from the precentral gyrus and proceeds anteriorly. The zone of anti-correlation (NV=413, α>0.1) in the middle occipital gyrus is in an area corresponding to Brodmann Area 19. In the Dementia group, the correlation Z score range was (-4.29, 3.59). There is a zone of positive correlation located anteriorly (NV=1508, α<<0.01) and laterally (NV=537, α<<0.05).

Figure 5 shows SMN RSN group averaged correlation maps for CN, MCI and Dementia groups for ROI at supplemental motor area. In the CN group, the correlation range was (-4.02, 4.46) with positive correlation shown in the Figure for left (NV=773, α<<0.025) and right (NV=744, α<<0.025) zones located in the superior temporal gyrus and inferior frontal gyrus and precentral gyrus. Not shown is large positive correlation cluster centered around the SMA ROI seed (NV=2082, α<<0.01). In the MCI group, the correlation range (-3.62, 4.17). The large positive correlation cluster centered around the SMA ROI grows to (NV=3888, α<<0.01) and includes the right lateral zone shown in the Figure. There is a zone of positive correlation with the ACC (NV=1286, α<<0.01). There is a left lateral positive correlation cluster (NV=203, α>0.10) that by itself does not meet criteria for statistical significance. In the Dementia group, the correlation range was (-3.94, 4.44). Positive correlation is with left (NV=195, α>0.10) and right (NV=932, α<<0.01) inferior frontal gyrus superior temporal gyrus zones. A possible bilateral region of anti-correlation emerges located in regions with 1-3mm of Brodmann Area 18 (left, NV=108, α>0.10) and 19 (right, NV=119, α>0.10).

The results shown in Figures 1-5 are consistent, but not identical across the set of pre-processing combinations tested. Roughly consistent features included the number and location of individual RSN sub-nodes, the direction of correlation, and progression of changes with clinical severity. Clusters that met the criteria for statistical significance were more robust to processing stream details. Clusters that met only the ad hoc criteria were less robust, for example, such that global signal removal was required for their appearance. A systematic analysis was not conducted, but two features appeared to be readily impacted by choice of pre-processing pipeline. First, with increasing spatial smoothing (ranging from 4mm to 10mm, or when using the expanded ROI approach) smaller, individual RSN node spatial extent and correlation values were suppressed. This was particularly true for anatomically isolated or limited nodes, such as the insula and the putamen in the SAL RSN. The second feature impacted by choice of pre-processing stream was the measured correlation value. For a given correlation pair, positive values tended to become more positive and negative values tended to become more negative subject to whether the global signal was removed or not, whether ROI or “expanded” ROI were applied, and the degree of spatial smoothing.

### Intra-RSN

### Inter-RSN

### Inter-Node

## Atlas

### Intra-Regional

### Inter-Regional

### Inter-Sub-Regional

Text

### ROI pair correlation matrices

Text

## Progressive disruption of probabilistic atlas seed-ROI correlations

Text

### fMRI maps

Text

### ROI pair correlation matrices

Text

# Discussion

## Principal findings

## Correspondence with previous findings

## Extensions to previous findings

## Limitations and next steps

# References

ADNI. (2016). Study documents. <http://adni.loni.usc.edu/methods/documents/>.

ADNI 2. (2016). 3T MRI Technical Procedures Manual. <http://adni.loni.usc.edu/wp-content/uploads/2012/10/ADNI2MRITrainingManual_2014.pdf>.

ADNI 2. (2016). Procedures Manual. <https://adni.loni.usc.edu/wp-content/uploads/2008/07/adni2-procedures-manual.pdf>.

Allen, G. I., et al. (2016). Crowdsourced estimation of cognitive decline and resilience in Alzheimer’s disease. *Alzh & Dementia*. 12:645-653.

Benjamini, Y. and Hochberg, Y. (1995). Controlling the false discovery rate: A practical and powerful approach to multiple testing. *J Roy Statist Soc Ser B* 57:1:289-300.

Brier, M. R., Thomas, J. B., Snyder, A. Z., Benzinger, T. L., Zhang, D., Raichle, M. E., Holtzman, D. M., Morris, J. C. and Ances, B. M. (2012). Loss of Intranetwork and internetwork resting state functional connections with Alzheimer’s disease progression.  *J Neurosci.* 32:26:8890-8899.

Cai, S., Huang, L., Zou, J., et al. (2015). Changes in thalamic connectivity in the early and late stages of amnestic mild cognitive impairment: a resting-state functional magnetic resonance study from ADNI. *PLoS ONE* 10(2): e0115573. Doi:10.137/journal.pone.0115573.

Corder, E.H., Saunders, A. M., Strittmatter, W. J., et al. (1993). Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer’s disease in late onset families. *Science*. 1993:261:921-923.

Cox, R. W. (1996). AFNI: Software for Analysis and Visualization of Functional Magnetic Resonance Neuroimages. *Comp Biomed Res.* 29:3:1762-173.

Eickhoff, S.B., Stephan, K.E., Mohlberg, H., et al. (2005). A new SPM toolbox for combining probabilistic cytoarchitectonic maps and functional imaging data. *Neuroimage*. 25(4):1325-35.

Efron, B. and Tibshirani, R. (1993). An Introduction to the bootstrap. Monographs on Statistics and Applied Probability, vol. 57. New York: Chapman and Hall.

Efron, B. (2004). Large-scale simultaneous hypothesis testing: the choice of a null hypothesis. *J Amer Stat Assoc.* 99:465:96-104.

Efron, B. (2010). Large-scale inference: Empirical Bayes methods for estimation, testing and prediction. IMS Monographs. Cambridge: Cambridge University Press.

Eklund, A., Nichols, T. E., and Knutsson, H. (2016). Cluster failure: Why fMRI inferences for spatial extent have inflated false-positive rates. *Proc Natl Acad Sci (USA).* 113:28:7900-7905.

Greicius, M. D., Srivastava, G., Reiss, A. L., and Menon, V. (2004). Default-mode network activity distinguishes Alzheimer’s disease from healthy aging: Evidence from functional MRI. *Proc Natl Acad Sci USA* 101:13:4637-4642.

Hall, P. and Wilson, S. R. (1991). Two guidelines for bootstrap hypothesis testing. *Biometrics*. 47:757-762.

Jack, C., et al. (2008). The Alzheimer’s disease neuroimaging initiative (ADNI): MRI methods. *J Magn Reson Imaging.* 27(4): 685–691. doi:10.1002/jmri.21049.

Jenkinson, M., Beckmann, C. F., Behrens, T.E., Woolrich, M. W. and Smith, S. M. (2012). FSL. Neuroimage. 62:2:782-790.

Mackey, S. and Petrides, M. (2014), Architecture and morphology of the human ventromedial prefrontal cortex.  *Eur J Neurosci.* 40:2777-796.

Mayo Aging and Dementia Imaging Research (ADIR) Laboratory. (2011). ADNI GO/2 MRI QC procedures. 31-January-2011.

Mayo Aging and Dementia Imaging Research (ADIR) Laboratory. (2012). Data file specifications for image quality assessment. Version 1.0. 31-October-2012.

Nichols, T. E. and Holmes, A. P. (2002). Nonparametric permutation tests for functional neuroimaging: A primer with examples. *Hum Brain Mapp.* 15:1:1-25.

Smith, S.M., Jenkinson, M., Woolrich, M. W., et al. Advances in functional and structural MR image analysis and implementation as FSL. *NeuroImage*. 23(S1):208-19.

Weiner, M. W., Veitch, D. P., Aisen, P. S., Beckett, L. A., Cairns, N. J., Cedarbaum, J., Green, R. C., Harvey, D., Jack, C. R., Jagust, W., Luthman, J., Morris, J. C., Petersen, R. C., Saykin, A. J., Shaw, L., Shen, L., Schwarz, A., Toga, A. W., Trojanowski, J. Q., Alzheimer’s Disease Neuroimaging Initiative. (2014). 2014 Update of the Alzheimer’s Disease Neuroimaging Initiative: A review of papers published since its inception.  *Alzheimer’s and Dementia.* 11:e1-e120.

Westfall, P. H. and Young, S. S. (1993). Resampling-based multiple testing: Examples and methods for *p*-value adjustment. Wiley; New York: 1993.

Westfall, P. H. and Troendle, J. F. (2008). Multiple testing with minimal assumptions. *Biom J.* 50:5:745-755.

# Figures

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**Figure 1.** Seed-based ROI group-averaged correlation maps for CN, MCI and Dementia groups. Results shown for ROI seeds: CON, dorsal medial prefrontal cortex; DAN, left visual area MT; DMN, posterior cingulate cortex; SAL, right dorsal anterior cingulate cortex; and SMN, supplementary motor area. Cluster and display parameters: PCDT=0.025; PCMT=0.005; NC=100. (NC=50 for DAN).

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# Glossary

ACC Anterior Cingulate Cortex

ACF Auto-correlation Function

AD Alzheimer’s Disease

ADNI Alzheimer’s Disease Neuroimaging Initiative

APOE Apolipoprotein E

CDR Clinical Dementia Rating (Global)

CDRSB Clinical Dementia Rating Sum-of-Boxes

CDT Cluster Defining Threshold

CN Cognitively Normal

CON Control RSN

CSF Cerebral-spinal Fluid

dmPFC Dorsal Medial Pre-Frontal Cortex

EPI Echo Planar Image

FDR False Detection Rate

LONI Laboratory of Neu

MCI Mild Cognitive Impairment

MPRAGE Magnetization Prepared Rapid Gradient Echo

MT Middle Temporal (Visual Area 5)

Nα  Cluster extent minimum condition on false-positive level α

NCT  Cluster extent threshold

NV  Cluster extent

PCC Posterior Cingulate Cortex

PCDT Cluster Defining Threshold

PCMT Color Maximum Threshold

ROI Region of Interest

RSN Resting-state Network

SVM Support Vector Machine

rsfMRI Resting State Functional Magnetic Resonance Imaging

WM White Matter

XML Extensible Markup Language

1. Data used in preparation of this report were obtained from the Alzheimer’s Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data, but did not participate in analysis or writing of this report. A complete listing of ADNI investigators can be found at: http://adni.loni.usc.edu/wp-content/uploads/how\_to\_apply/ADNI\_Acknowledgement\_List.pdf [↑](#footnote-ref-1)