Disrupted Brain Connectivity in Alzheimer's Disease: Effects of Network Thresholding

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Abstract. Diffusion imaging is accelerating our understanding of the human brain. As brain connectivity analyses become more popular, it is vital to develop reliable metrics of the brain's connections, and their network properties, to allow statistical study of factors that influence brain 'wiring'. Here we chart differences in brain structural networks between normal aging and Alzheimer's disease (AD) using 3-Tesla whole-brain diffusion-weighted images (DWI) from 66 subjects (22 AD/44 normal elderly). We performed whole-brain tractography based on the orientation distribution functions. Connectivity matrices were compiled, representing the proportion of detected fibers interconnecting 68 cortical regions. We found clear disease effects on anatomical network topology in the structural backbone – the so-called '*k*-core' – of the anatomical network, defined by varying the nodal degree threshold, *k*. However, the thresholding of the structural networks – based on their nodal degree – affected the pattern and interpretation of network differences discovered between patients and controls.

Keywords. brain connectivity, *k*-core, threshold, DTI, tractography, graph theory

1. Introduction

Diffusion imaging has recently been added to several large-scale neuroimaging studies, including the Alzheimer's Disease Neuroimaging Initiative (ADNI), to monitor white matter deterioration using metrics not available with standard anatomical MRI. Diffusion MRI yields measures sensitive to fiber integrity and microstructure, such as the mean diffusivity and fractional anisotropy of local water diffusion [1]; in addition, tractography can be used to infer neural pathways and connectivity patterns, yielding additional, more complex mathematical metrics describing fiber networks.

Despite the enthusiasm for using diffusion imaging to map brain connectivity and how it changes with disease, there is a lack of serious groundwork validating these methods to see if the connections they map are correct and how acquisition and analysis

protocols affect them. Post-processed connectivity data is also affected by the level of thresholding applied to the brain connectivity matrices; thresholding is commonly applied to retain key information on the most crucial subnetworks, while eliminating false positive fibers or connections inaccurately inferred due to noise and imaging artifacts. There is no consensus about what might be the ideal level of thresholding to retain only the most relevant information in post-processed connectivity data. A common approach filters networks based on the nodal degree, leaving only the most highly connected nodes. As this loses information, some groups advocate defining metrics on the entire set of networks at all thresholds, using concepts such as the *Rips filtration* [2].

Here we studied anatomical fiber networks in 44 controls and 22 identically scanned people with Alzheimer's disease (AD) using novel mathematical network metrics derived from the 'structural backbone' – or *k*-core – of the human brain. Based on prior studies [3], we were interested in understanding how the different number of nodes, *N*, in filtered networks from healthy and diseased subjects affects graph theory measures computed from thresholded connectivity matrices. In the end, it would be unwise to infer that AD affects networks in a particular way, if networks filtered differently showed different disease effects. To explore this, we computed the network's structural core using a *k*-core decomposition [4] to find important sets of nodes that are highly and mutually interconnected. The level of the *k*-core, *k*, serves as a threshold to retain nodes in the connectivity matrix with degree *k* or higher. We systematically varied the values of *k* (*k*=1, …, 20) and analyzed the changes in the resulting network measures to understand how they are affected by thresholding the size or degree of the networks (*N, k*). We calculated global measures sensitive to anatomical network topology: the clustering coefficient (CC), characteristic path length (CPL), efficiency (EFF), and nodal degree (NOD) for all 66 subjects at each of the 20 *k*-core levels. All network measures showed group differences that depended heavily on the nodal degree and size of the threshold applied to the network. We aimed to find out which network measures are most and least sensitive to variation in the *N* and *k* levels, in terms of their ability to resolve differences between the healthy and diseased groups.

2. Methods

2.1. Subjects and Diffusion Imaging of the Brain

We analyzed diffusion-weighted images (DWI) from 66 subjects scanned as part of phase 2 of the Alzheimer's Disease Neuroimaging Initiative (ADNI2), a large multi-site longitudinal study to evaluate biomarkers to assist diagnosis and track disease progression. **Table 1** shows subject demographics and diagnostic information; data collection is ongoing. All 66 subjects underwent whole-brain MRI scanning on 3-Tesla GE Medical Systems scanners, at a variety of sites across North America, with the same protocol, which had been optimized for SNR. Standard anatomical T1-weighted SPGR (spoiled gradient echo) sequences were collected (256x256 matrix; voxel size =

1.2x1.0x1.0 mm^3; TI = 400 ms, TR = 6.984 ms; TE = 2.848 ms; flip angle = 11°) in the same session as the diffusion-weighted images (DWI; 256x256 matrix; voxel size: 2.7x2.7x2.7 mm^3; scan time = 9 min). 46 separate images were acquired for each DTI scan: 5 T2-weighted mages with no diffusion sensitization (b_0 images) and 41 diffusion-weighted images (b = 1000 s/mm^2).

Table 1. Demographic information for 44 controls and 22 AD patients scanned with diffusion MRI as part of ADNI. Their ages ranged from 55.7 to 90.4 years.

	Controls	AD	Total
N	44	22	66
Age	72.7 ± 5.9 SD	75.5 ± 10.0 SD	73.6 ± 7.5 SD
Sex	22M/22F	14M/8F	36M/30F

2.2 Image Analysis

Pre-processing and Co-registration

Non-brain regions were automatically removed from each T1-weighted MRI scan, and from a T2-weighted image from the DWI set using the FSL tool "BET" (http://fsl.fmrib.ox.ac.uk/fsl/). Anatomical scans subsequently underwent intensity inhomogeneity normalization using the MNI "nu_correct" tool (www.bic.mni.mcgill.ca/software/). All T1-weighted images were linearly aligned using FSL (with 6 DOF) to a common space with 1mm isotropic voxels and a 220×220×220 voxel matrix. The DWI were corrected for eddy current distortions using the FSL tools (http://fsl.fmrib.ox.ac.uk/fsl/). For each subject, the 5 images with no diffusion sensitization were averaged, linearly aligned and resampled to a downsampled version of their T1-weighted image (110×110×110, 2×2×2mm). b_0 maps were elastically registered to the T1-weighted scan to compensate for susceptibility artifacts or EPI induced distortions.

Tractography and Cortical Extraction

The transformation matrix from linearly aligning the mean b_0 image to the T1-weighted volume was applied to each of the 41 gradient directions to properly re-orient the orientation distribution functions (ODFs). We also performed whole-brain tractography as described in [5] on the sets of DWI volumes. We used a method based on the Hough transform to recover fibers, using a constant solid angle orientation density function to model the local diffusion propagator. The angular resolution of the ADNI data is deliberately limited to avoid long scan times that may increase patient attrition, but the ODF model makes best use of the limited available angular resolution.

Elastic deformations obtained from the EPI distortion correction, mapping the average b_0 image to the T1-weighted image, were then applied to each recovered fiber's 3D

coordinates to more accurately align the anatomy. Each subject's dataset contained ~10,000 useable fibers (3D curves) in total. 34 cortical labels per hemisphere, as listed in the Desikan-Killiany atlas [6], were automatically extracted from all aligned T1-weighted structural MRI scans using FreeSurfer (http://surfer.nmr.mgh.harvard.edu/) [7].

***N*x*N* Matrices Representing Structural Connectivity**

For each subject, a baseline 68x68 connectivity matrix was created, based on 34 right hemisphere ROIs and 34 left hemisphere ROIs. Each element described the estimated proportion of the total number of fibers, in that subject, that passes through each pair of ROIs. We note that various normalizations could be applied (e.g., using the volume or area of the target ROIs, or to turn these counts into densities), but for simplicity we here just used the fiber counts (normalized to the total number of fibers detected in the brain).

2.3 Brain Network Measures

Topological differences in the brain's networks may be analyzed using graph theory, which represents the brain network as a set of nodes and edges. The network's *N* nodes are typically defined as ROIs, usually on the cortex, segmented from anatomical MRI. These network nodes are linked by 'edges' whose weights denote some measure of connectivity between the two regions, such as the density or integrity of fiber tracts in DTI studies [8]. An *NxN* connection matrix may therefore be compiled to describe the network. A square matrix can represent any network of connections, and may also be displayed as a graph, i.e., a discrete set of nodes and edges [8], leading the way for analyses through the branch of mathematics known as graph theory. In our analysis, the matrix entries store the total proportion of fibers connecting each pair of regions (the nodes); these could also be considered as the "weights" of the edges that connect a pair of nodes [8].

From the connection matrices, we applied a threshold by computing the *k*-core for 20 levels of the nodal degree threshold, *k*, using a decomposition algorithm that identifies subsets of graphs (*k*-cores) by recursively removing nodes with degrees lower than *k*, such that *k* serves as a degree threshold for nodes [9]. For a graph $G = (N, E)$ with $|N| = n$ nodes and $|E| = e$ edges, a *k*-core is computed by assigning a subgraph, $H = (B, E|B)$ where set $B \subseteq N$ is a *k*-core of order *k* iff $\forall\, v \in B$: $\text{degree}_H \geq k$, and *H* is the maximal subgraph (most highly connected one) satisfying this property [9]. In other words, to compute the *k*-core of the connectivity matrix, we kept all nodes with a degree *k* or higher. These then become new 68x68 matrices, each being a somewhat thresholded version of the original; weights of nodes that did not satisfy the *k*-cutoff were replaced with zeroes.

We obtained the *k*-core matrices by varying *k* from 1 to 20 for both controls and AD subjects. The global graph theory measures (CC, CPL, EFF, and NOD) were derived from each *k*-core matrix for each subject, to yield four representative network measures at each *k*-level (i.e., each subject had 20 global metrics for CC, CPL, EFF and NOD). NOD was computed as a nodal measure first, and then averaged overall all 70 cortical regions for

each subject to output a global measure. These widely-used measures are detailed in [8], although their use in brain connectivity and AD research is yet to be extensively explored. CC and CPL measures were normalized based on 100 randomized networks of equal size and similar connectivity distribution. We tested for between-group differences using a linear regression, controlling for age and sex, with AD coded as 1 and controls as 0. We tested for differences between groups of controls and AD subjects for CC, CPL, EFF and NOD at each *k*-core value for the brain network. We also tested for within-group differences for network measures EFF and NOD, which were found to be "most significant" in the between-group comparison. For this, we compared every *k*-level across subjects within one diagnostic group with every other *k*-level in that group (i.e., EFF for controls at $k1$=1,2…19 was compared to EFF for controls at $k2$=($k1$+1)…20) using a 2-tailed paired *t*-test. We applied an FDR correction on all (20*20-20)/2 comparisons.

3. Results

The variation in the *k*-core levels (*k*=1, …, 20) affected the networks and, as expected, resulted in changing graph theory measures (CC, CPL, EFF and NOD) in each diagnostic group.

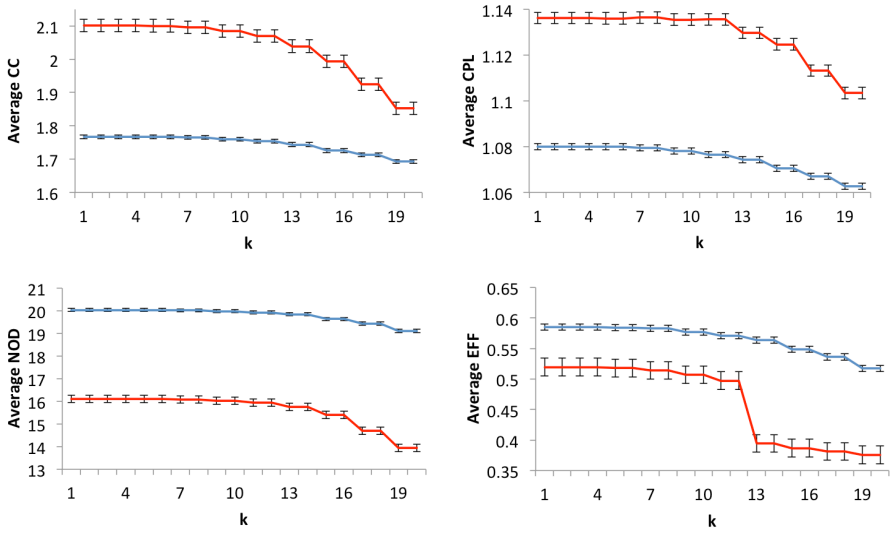

Figure 1. Average and global CC, CPL, EFF and NOD for the whole brain in 44 controls (*blue*) and 22 AD subjects (*red*), based on thresholding the network at *k*=1, …, 20. Error bars show the standard errors.

We performed *between* group comparisons to find out how effect sizes for group differences depended on the network degree threshold. Relative to controls, the AD group had a higher global CC (FDR critical *p*-value=6.26E-03) for the entire range of *k*-core values (*k*=1-20) and a higher global CPL (*p*-value=5.72E-3) for *k*-cores in the range *k*=1-

18. Obtaining a higher CC in AD, relative to controls, may not be entirely intuitive, but the CC can be disproportionately influenced by nodes with low degree [8]. NOD (FDR critical *p*-value=3.65E-05) and EFF (FDR critical *p*-value=6.21E-05) were lower in AD over the whole range of *k*-core values (*k*=1-20), relative to controls. Averaged network measures (**Figure 1**) and *p*-values (**Figure 2**) are plotted.

Furthermore, we tested for *within* group differences in all subjects for NOD and EFF, as these measures showed greatest effect sizes in the diagnostic group comparisons. The results are shown in a 20x20 matrix, where the EFF was calculated from matrices thresholded at each *k*-level. We compared the EFF network measure to the same network measure calculated from the other *k*-levels – always within the same diagnostic group, to avoid incorporating disease effects (**Figure 3**). EFF changed significantly as *k* varied in both controls and AD (FDR critical *p*-value=1.42E-02 for controls and 1.27E-02 for AD). Within-group measures for NOD were not significantly different across any *k*-levels in either group.

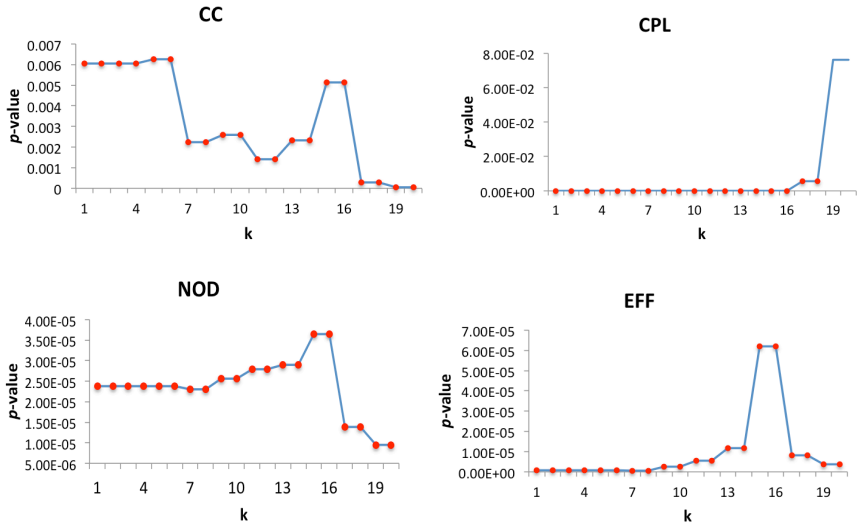

Figure 2. *P*-values from a regression controlling for age and sex, testing for significant differences between AD subjects and controls for whole-brain global CC, CPL, EFF and NOD in AD subjects versus controls. Red points highlight *p*-values that are less than the *p*-value threshold (CC *p*-value=6.26E-03, EFF *p*-value=6.21E-05, NOD *p*-value=3.65E-05 and CPL *p*-value=5.72E-03) that controls the FDR at 5%. This FDR correction allows us to state that the groups truly differ, even though multiple thresholds were tested.

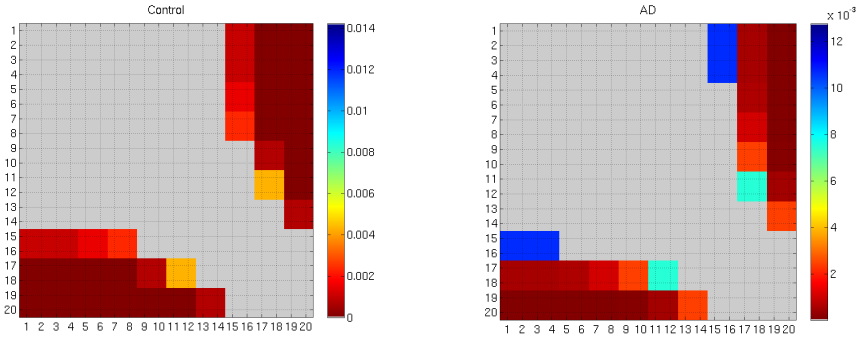

Figure 3. Matrix (20x20) representing the *p*-values from the *within* group comparisons for EFF across all *k*-levels within each group (FDR critical *p*-value=1.42E-02 for controls and 1.27E-02 for AD). A given cell (*x,y*) in this matrix gives the *p*-value for the *t*-test comparing the value of EFF between *k*-cores where the minimum nodal degree is *x* and *y*, respectively. As expected, greatest differences in network measures were found between lowest and highest *k*-levels (red *p*-values).

4. Discussion

Graph theory has been widely used to assess functional and anatomical networks in the brain, but not nearly so much attention has been paid to analyzing network variations due to choices made in analysis methods (i.e., network thresholding) and how they impact network topology comparisons. With the growing interest in connectivity analyses, it is important to understand how stable network measures are, and develop reliable guidelines when applying them to study disease. The interpretation of network breakdown in disease may be somewhat different depending on the criteria used to compare or filter networks.

Here we analyzed brain connectivity in cognitively impaired patients with AD and matched normal controls. We varied the nodal degree threshold applied to the connectivity matrices for both groups by using a wide range of *k*-core values (*k*=1, …, 20). Some network measures - CC, CPL, EFF and NOD - *declined* across all subjects as nodal degree threshold levels were increased. Network measures that showed the greatest differences between diagnostic groups over *k* levels ranging from 1 to 20 are in the following order (i.e., with the greatest size effect and smallest *p*-values): NOD, EFF, CPL, and CC. NOD and EFF were found to have greatest size effects among all measures (FDR critical *p*-value=3.65E-05 and 6.21E-05) (**Figures 1** and **2**). This led us to analyze within-group differences for NOD and EFF; we found that increasing levels of *k* significantly affects the apparent efficiency of the overall network in both controls and AD, while NOD was not affected by varying *k* levels (**Figure 3**).

The decline in all network measures with increasing *k* levels is expected in both diagnostic groups. This is because networks thresholded at higher *k* levels required a greater number of nodes to be connected (e.g., at *k*=20, approximately 30% of the nodes are connected). Similarly, AD is known to disrupt the overall network topology of the

brain [2,3] leading to fewer nodes when compared to controls. This is why NOD had the greatest effect size in the between-group comparisons.

An ideal network threshold for this data is in the range of k=15-18. This includes at least 22-26% of the nodes in each brain network, yielding the 'most significant' effects in both *between* and *within* group comparisons. Ideally, this threshold would tend to suppress noise and some imaging artifacts, removing weak connections while emphasizing stronger connections altered in disease. This range may vary with study-specific parameters.

We studied the effect sizes for the group differences here, to clarify how network filtering parameters influence the differentiation of diseased versus normal groups based on graph theory metrics. Although there is no universal method and no definitive answer as to how networks of different sizes and connectivity densities should be accurately compared and analyzed [10], maintaining these measures consistent across study groups is crucial for obtaining comparable results. Normalizing the network measures using randomized networks with the same number of nodes and connections may make graph metrics more stable with respect to differences in N and k [10]. In the end, methods based on network filtrations may supersede those applied to thresholded networks, if they better detect disease effects on brain connectivity.

References

1. Clerx, P., et al., *New MRI markers for Alzheimer's disease: a meta-analysis of diffusion tensor imaging and a comparison with medial temporal lobe measurements*. **J Alzheimer's Dis.** 2012; 29(2):405-29.
2. Lee H., et al., *Persistent Brain Network Homology from the Perspective of Dendrogram*. **IEEE Transactions on Medical Imaging** 31(12):2267-77.
3. Daianu M., et al., *Breakdown of Brain Connectivity between Normal Aging and Alzheimer's Disease: A Structural k-core Network Analysis*. **Brain Connect.** 2013 [Epub ahead of print].
4. Hagmann, P., et al., *Mapping the structural core of the human cerebral cortex*. **PLoS Biology** 2008;6(7):1479-1493
5. Aganj, I., et al., *A Hough transform global probabilistic approach to multiple-subject diffusion MRI tractography*. **Med Image Analysis** 2011;15(4):414-25.
6. Desikan, R. S., et al., *An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest*. **NeuroImage** 2006; 31(3):968-80.
7. Fischl B., et al., *Automatically parcellating the human cerebral cortex.* **Cerebral Cortex** 2004;14:11-22.
8. Sporns, O., *Networks of the Brain*. Cambridge, MA, 2011;5-31.
9. Alvarez-Hamelin, J. I., et al., *Large scale networks fingerprinting and visualization using k-core decomposition*. Weiss Y, Scholkopf B, Platt J. editors. Cambridge (Massachusetts) 2006; MIT Press 44-55.
10. Wijk, B. C. M., et al., *Comparing Brain Networks of Different Size and Connectivity Density Using Graph Theory*. **PLoS One** 2010; 5(10):e13701.