Published in final edited form as:

Neurobiol Aging. 2015 January ; 36(1): 401–412. doi:10.1016/j.neurobiolaging.2014.06.019.

Weighted brain networks in disease: centrality and entropy in HIV and aging

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Abstract

Graph theory models can produce simple, biologically informative metrics of the topology of resting-state functional connectivity (FC) networks. However, typical graph theory approaches model FC relationships between regions (nodes) as unweighted edges, complicating their interpretability in studies of disease or aging. We extended existing techniques and constructed fully-connected weighted graphs for groups of age-matched HIV positive (n=67) and HIV negative (n=77) individuals. We compared test-retest reliability of weighted vs. unweighted metrics in an independent study of healthy individuals (n=22) and found weighted measures to be more stable. We quantified two measures of node centrality (closeness centrality and eigenvector centrality) to capture the relative importance of individual nodes. We also quantified one measure of graph entropy (diversity) to measure the variability in connection strength (edge weights) at each node. HIV was primarily associated with differences in measures of centrality, and age was primarily associated with differences in diversity. HIV and age were associated with divergent measures when evaluated at the whole-graph level, within individual functional networks, and at the level of individual nodes. Graph models may allow us to distinguish previously indistinguishable effects related to HIV and age on FC.

Keywords

HIV; aging fc-MRI; graph theory; neurodegeneration; centrality

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Introduction

Biologically informative metrics of blood-oxygen level dependent (BOLD) resting-state brain functional connectivity (FC) can be produced using tools from complex network analysis, allowing us to assess the topology of functional relationships in the brain (Sporns, 2013). These techniques are potentially useful to studies of disease and age because many conditions [including human immunodeficiency virus (HIV) and age] have manifested similar FC changes in more standard measures of BOLD time series correlation (Andrews-Hanna et al., 2007; Biswal et al., 2010a; Brier et al., 2012; Greicius et al., 2004; Thomas et al., 2013). Aging (Andrews-Hanna et al., 2007; Biswal et al., 2010b) and disease [including HIV (Thomas et al., 2013), Alzheimer's Disease (AD) (Greicius et al., 2004) and schizophrenia (Orr et al., 2014)] alter average correlations in similar functional systems [including the Default Mode Network (DMN)], though these processes have dramatic differences in disease mechanisms. Graph tools from network analysis may allow us to capture unique topological changes in properties such as node importance (centrality) and graph disorder (entropy) due to age and different neurodegenerative diseases in a novel fashion (Meunier et al., 2009; Supekar et al., 2008). Graph tools allow for the assessment of more information than average correlation alone (Geerligs et al., 2014).

Researchers modeling FC as a collection of nodes (functional regions) joined by edges (FC relationships) have suggested that the brain has topological properties with characteristic resiliency to particular types of insult. One such property is reflected in the brain's hub architecture, where a small number of nodes have a disproportionately high importance (centrality). Compared to random graphs (wherein all nodes have equivalent importance), graphs with this hub structure tend to be more resilient to random attack (i.e., random removal of edges or nodes) but more vulnerable to targeted attack (i.e., targeted removal of hubs) (Achard et al., 2006; Albert et al., 2000; Joyce et al., 2013).

Recent research has suggested that targeted disruption of hubs can track cognitive deterioration associated with neurodegenerative diseases such as AD (Buckner et al., 2009). In contrast, increases in graph randomness, as measured by entropy, have been shown to track changes associated with age (Yao et al., 2013). Age has been associated with increased functional connectivity in particular functional systems (e.g., sensorimotor) and decreased functional connectivity in others (e.g., DMN) (Andrews-Hanna et al., 2007; Biswal et al., 2010b) whereas HIV has been characterized as causing targeted disruption of particular systems (e.g., DMN) (Thomas et al., 2013). How these specific patterns of functional connectivity changes relate to changes in secondary metrics remains unclear. It is possible that HIV and aging may lead to distinct alterations in graph topology, with HIV primarily affecting measures of node centrality (like other neurodegenerative conditions) and age primarily affecting measures of node entropy.

While these metrics may be useful in studies of disease, new network modeling techniques are needed to facilitate comparisons between individuals. Existing graph studies of FC have been difficult to interpret in the context of disease. Graph measures in these studies have been computed based on FC correlation matrices thresholded to yield unweighted graphs with a constant and arbitrary number of connections. Unweighted graph measures of FC

have two closely-related methodological drawbacks which may confound results (Fornito et al., 2013; Sporns, 2013). First, on account of thresholding, small correlations between regions are discarded. Thus, for diseases that manifest as decreased magnitude of correlation values in different parts of the brain, discarding the potentially topologically salient information contained in low magnitude correlations from the diseased individuals may obscure true differences or introduce spurious differences. Second, between-group differences based on unweighted graphs are difficult to interpret because any observed differences are specific to a particular edge-density. Apparent topological abnormalities that manifest in one group for graphs of a given edge density may also be present in the other group but only at a different edge density. Researchers have tried to compare unweighted graphs across a range of thresholds (Achard et al., 2006), but these methods suffer from multiple-comparison challenges (Fornito et al., 2013). In addition, the test-retest stability of unweighted graph measures has not been fully established (Braun et al., 2012; Cao et al., 2014).

Researchers have recently extended graph theory techniques by constructing fully connected weighted graphs that may be particularly suited to revealing subtle effects of disease (Rubinov and Sporns, 2011; 2010). Analysis of three specific weighted graph metrics may allow us to distinguish the effects of HIV and aging in the brain. We include two weighted measures of node centrality (closeness centrality and eigenvector centrality) that capture the relative importance or “hubness” of each node in the brain. Centrality properties may be particularly related to HIV-associated neurodegenerative processes. Diversity is a graph topology measure that quantifies network entropy by measuring how varied edge weights are at a given node. Diversity may be particularly related to the increased variability seen with age.

We compute these three measures at three scales: 1) the global level (all nodes), 2) the resting-state network (RSN) level (within particular RSNs), and 3) the node level. We establish the test-retest reliability of these measures compared to the more typical unweighted measures in a separate data set. We then assess the effects of HIV and age on each of the weighted measures and investigate how graph topology effects are related to changes in edge weights at particular nodes. We hypothesize that HIV and age lead to differentiable effects on graph topology.

Materials and Methods

A. Participant characteristics

We analyzed two separate datasets for this study: 1) a longitudinal cohort of HIV-participants to assess the test-retest reliability of weighted graph measures and 2) a cross-sectional cohort of HIV- and HIV+ participants to assess the effects of HIV and aging. All participants provided informed consent approved by the WUSTL Institutional Review Board.

1. To assess the test-retest reliability of weighted graph measures, we separately analyzed a set of 22 older control participants acquired by the Knight Alzheimer's Disease Research Center at Washington University in St. Louis (WUSTL). These

participants had two imaging sessions on average 3.3 days apart. Individuals with a history of neurologic illnesses, major psychiatric disorders, or active substance abuse were excluded from participation.

2. To assess the effects of HIV and age, we analyzed a total of 144 participants (67 HIV- and 77 HIV+) who had clinical examinations (performed by BMA) and neuroimaging at WUSTL. The serologic status of all HIV+ individuals was confirmed by documented positive HIV enzyme-linked immunoassay and Western blot or detection of plasma HIV RNA. All HIV positive (HIV+) participants had laboratory evaluations (plasma CD4 cell count and plasma HIV RNA viral load [VL]) performed within 3 months of neuroimaging.

B. MRI acquisition and processing

1. Image acquisition—Neuroimaging scans were collected using a 3.0-Tesla Tim-Trio scanner (Siemens, Erlangen, Germany) equipped with the standard 12-channel head coil as previously described (Thomas et al., 2013). High-resolution T1 magnetization-prepared rapid gradient echo (MPRAGE) [echo time [TE] = 16 milliseconds, repetition time [TR] = 2,400 milliseconds, inversion time = 1,000 milliseconds, flip angle = 8°, 256 × 256 acquisition matrix, 1 × 1 × 1 mm voxels] and T2 fast-spin echo (FSE) [TE = 455 milliseconds, TR = 3,200 milliseconds, 256 × 256 acquisition matrix, 1 × 1 × 1 mm voxels] anatomical images were collected to facilitate atlas alignment of resting state blood oxygen level dependent (BOLD) images. Resting-state functional connectivity MRI (rs-fcMRI) scans were collected using a gradient spin-echo sequence (TE = 27 milliseconds, TR = 2.2 seconds, 64 × 64 acquisition matrix, flip angle 90°) that was sensitive to the BOLD contrast (T2* weighting). A total of 36 contiguous, 4.0-mm-thick slices were acquired parallel to the anterior commissure/posterior commissure plane. Each participant had two 6-minute rs-fcMRI scans that were concatenated into a single time series for analysis. During rs-fcMRI scans, participants were instructed to remain still and stay awake.

2. Preprocessing of rs-fcMRI—Data were preprocessed to correct slice-dependent time shifts, eliminate systematic artifactual odd-even slice intensity differences caused by interleaved acquisition, and align frames within and across runs. Image intensity was scaled (1 multiplicative factor applied to all voxels of all frames within each run) to obtain a mode value of 1,000 (Ojemann et al., 1997). This facilitated computation of voxel-wise variance for quality assurance (QA) purposes without affecting correlations computed from the resulting time-series. Atlas normalization consisted of computing and combining 3 affine transforms. rs-fcMRI scans were aligned to the FSE (T2 anatomical scan); the FSE images were aligned to each individual's MPRAGE (T1 anatomical scan), and the MPRAGE was subsequently aligned to a group Talairach atlas. Alignment was computed with six degrees of freedom to account for translation and rotation with respect to x, y, and z axes using a standard method (Thomas et al., 2013). The composition of these 3 affine transforms allowed for transformation of the original rs-fcMRI data to 3-mm³ atlas space. Head-motion correction was included in this resampling step. Additional preprocessing in preparation for correlation mapping included spatial smoothing (6-mm, full-width, half-maximum Gaussian blur in each direction), voxel-wise removal of linear trends over each rsfcMRI scan, and

low-pass filtering in the time domain to retain frequencies below 0.1 Hz. This Gaussian blur step has several favorable properties related to signal-to-noise ratio, but also reduces the impact of minor inter-individual alignment differences. Spurious variance was reduced by regressing nuisance waveforms derived from head-motion correction as well as time series extracted from a-priori regions of interest placed in white matter and cerebrospinal fluid (CSF) (Smyser et al., 2010). We also performed whole-brain global signal regression (GSR). This method effectively removes a significant amount of movement-associated contamination in BOLD data while enforcing a zero mean in correlations (Power et al., 2012).

3. Quality assurance (QA)—QA included measurement of root mean square of head displacement (in millimeters) derived from the motion-correction processing procedure, measurement of the voxel-wise time series standard deviation (sd) averaged over the whole brain, and quantification of the accuracy of alignment between subjects' scans and the group atlas (Smyser et al., 2010). QA exclusion thresholds were empirically determined in a way that maximized the number of included participants while achieving equivalent QA parameter distributions for each group. If an individual frame had intensity ± 3 s.d. from the mean intensity for a subject's BOLD run, this frame was excluded for that subject. On average, a small percentage of frames was excluded for each group (HIV- = 0.6%, HIV+ = 0.9%). If the sd of the mean rs-fcMRI signal for an entire concatenated run was $> 2.2\%$ (after nuisance regression) or if the root mean square movement was > 2 mm, then the individual was excluded from further analysis (Power et al., 2013). A total of 1 HIV+ and 6 HIV- individuals were removed because of excessive movement. Further, we assessed the quality of the alignment of the subject T1 and group atlas using the spatial correlation between the single subject scan and the atlas where 1 represents ideal alignment and 0 represents no alignment. Alignment accuracy did not differ between groups ($p=0.96$), was not associated with age ($p=0.29$), and did not significantly impact the subsequent analyses.

4. Post-processing of functional connectivity—After pre-processing and QA, we extracted the timeseries from each participant's BOLD rs-fcMRI scans using a previously-published library of 160 functionally-defined nodes or regions of interest spanning 6 distinct resting-stated functional networks (RSNs) [default mode (DMN), frontoparietal (PAR), cingular-opercular (CINGO), cerebellar (CER), sensorimotor (SMN)] (Fig. 1) (Dosenbach et al., 2010). All regions of interest were 6mm radius spheres. From these time-series, we constructed a Fisher z-transformed cross-correlation matrix, z , for each participant. Each cell of that matrix, $z_{u,v}$ represents the FC between nodes u and v . We performed timeseries analysis, graph analysis and statistical processing using the igraph package in R (Csardi and Nepusz, 2006).

C. Graphs

We constructed weighted graph representations of FC data for all participants from the longitudinal and cross-sectional cohorts. For the longitudinal cohort, we also constructed unweighted graphs in order to compare test-retest reliability for weighted and unweighted metrics.

1. Weighted Graph Construction from FC data—A weighted graph G is defined as a collection of nodes $\{N\}$ and weight edges $\{W\}$: $G:=\{N,W\}$. Edge weights were based on FC measured between each of the 160 nodes defined above. For each pair of nodes, we computed edge weight between nodes u, v based on the z-transformed correlation between these ($z_{u,v}$):

$$w_{u,v} = \frac{1}{2 - z_{u,v}}.$$

This procedure transformed the distribution of FC correlation values (from [-1,1] to [0,2]) but did not affect the rank order of correlations. The scaling value 2 was chosen experimentally to account for the fact that a z-transformed correlation value can have magnitude > 1 . No edges were excluded from the weighted analysis.

2. Unweighted graph construction from FC data—In the cohort of HIV- individuals scanned twice on average 3.3 days apart, we constructed both weighted graphs (as described above) and also a set of unweighted, binary graphs that spanned a range of edge densities [0,1] (where $edge\ density = \frac{\text{number of edges}}{\text{number of possible edges}}$) by thresholding w for each participant over a range of thresholds. FC relationships above a given threshold were modeled as unweighted, binary edges. Unweighted graphs were constructed for a range of thresholds such that mean degree (k) for these graphs met the following criteria: $\log(N) < k < N$, where $N=160$ nodes. In our sample of healthy controls, we computed graph topology measures at two time points for weighted and unweighted graphs and compared test-retest reliability of these measures.

3. Graph metric definitions—We computed weighted closeness centrality (c), eigenvector centrality (e), and diversity (d) for all participants. In the longitudinal cohort, we also computed unweighted measures of c and e , as d is undefined for unweighted graphs.

a) Closeness centrality (c) provides a measure of integration for each node comparable to local efficiency (Sporns et al., 2007). If a node is more closely correlated with other nodes in the brain, then c for this node is high. In contrast, if a node is less well connected with the rest of the brain, then c is low:

$$c_u = \sum_{v \neq u} \frac{1}{l_{u,v}}.$$

The element $l_{u,v}$ denotes the weighted path length between u and v , where path length is the sum of weights along the strongest connected path connecting u and v for weighted graphs or simply the number of edges along the shortest path connecting u and v for unweighted graphs (Rubinov and Sporns, 2011).

b) Eigenvector centrality (e) captures the assortative structure of the brain network (the extent to which nodes with a high number of highly weighted edges are also connected to each other) by weighting the centrality score for each node u by the centrality scores of

those nodes connected to u . A node has high e (i.e., is more hub-like) if it is highly connected (i.e., has many high-weight FC relationships) with other nodes that are themselves highly connected to other highly connected nodes. A decrease in e signifies that a node has lost its hub-like influence on the other nodes of the whole brain network. When nodes with high e centrality become dysfunctional, this leads to a disproportionately high impact on network efficiency compared to when nodes with lower eigenvector centrality are dysfunctional.

$$e_u = \frac{1}{\lambda} \sum_{v \in M(u)} e_v = \frac{1}{\lambda} \sum_{v \in G} w_{u,v} e_v$$

The set $M(u)$ contains all of the neighbors of u and λ is a constant. Neighbors of u are the nodes to which u is connected. e for any node u depends on the e of each neighbor of u and the weight of connections between u and these neighbors. The above equation reduces to the eigenvalue decomposition of the weighted edge matrix:

$$we = \vec{\lambda} e.$$

Where the u^{th} index of the first eigenvector corresponds to e for the u^{th} node. For unweighted analysis, we substitute the binarized edge matrix for the weighed matrix w .

c) *Diversity* (d) reflects the entropy (or variety) of edge weights at any given node and provides a measure of overall node and network disorder.

$$d_u = \frac{h_u}{\log(k_u)}.$$

Here, k_u corresponds to the degree of node u and h_u is defined as the connection entropy of u :

$$h_u = \frac{- \sum_{v=1}^{k_u} w_{u,v}}{\sum_{l=1}^{k_u} w_{u,l}}.$$

Nodes with a wider range of weights on adjacent edges will have higher diversity. Diversity is undefined for unweighted networks.

D. Data analysis

1. Statistical models—We used general linear models (GLM) to assess main effects of HIV, age, and HIV x age interaction on graph metrics. We also included a main effect of gender to correct for gender differences between groups. Because education level may influence studies of aging by affecting levels of cognitive reserve (Roe et al., 2011), we also evaluated education as a potential confound for each measure.

2. Global graph analysis—We computed values for each of the three secondary measures (c , e , d) for every node (160) of the brain. We computed whole-brain average graph metrics by averaging these values across nodes for each participant (Sporns et al., 2007).

3. RSN-specific analysis—We next restricted our analysis to specific RSNs by constructing RSN composite scores. We studied the following networks: default mode (DMN), fronto-parietal (PAR), cerebellar (CER), occipital (OCC), cingulo-opercular (CINGO), sensorimotor (SMN). DMN and PAR are known to be disrupted in HIV and aging (Thomas et al., 2013). To construct composite scores, we computed node-wise measures (either c , e , or d) and averaged these values over the constituent nodes of each of the six RSNs. For example, average c in the DMN was computed in the following way:

$$c_{DMN} = \frac{1}{DMN} \sum_{u \in DMN} c_u.$$

This allowed us to analyze the metrics in an RSN-specific manner while limiting the total number of statistical tests conducted. Results from network-composite analyses were corrected for false discovery rate (FDR) across 6 networks ($q < 0.05$).

4. Node-level analysis—We finally analyzed graph measures on a node-level basis. We compared the effects of HIV and age on each node for each graph topology measure, assessing whether HIV and age were preferentially associated with changes in nodes with certain graph characteristics (e.g., whether HIV leads to greater effects on more central nodes or whether age primarily alters diversity of nodes with lower diversity scores).

5. Comparison of weighted and unweighted test-retest reliability—We used intra-class correlations (ICC) to compute test-retest reliability of unweighted graph measures and weighted graph measures for the independent set of HIV- participants who were scanned twice approximately 3.3 days apart. We computed test-retest reliability at all three of the above scales (global, RSN-level, and node-level). We directly compared the ICC values for unweighted vs. weighted measures for the 160 nodes as samples in the paired t-tests. Results were FDR-corrected ($q < 0.05$).

6. Primary (edge weight) analysis—We analyzed how average values of time-series FC measures (i.e., graph edge weights) changed with HIV and age. We used network-based statistics (NBS) to correct for family-wise error rate without lowering our statistical power to detect significant differences in edge weights with age and HIV (Zalesky et al., 2010). This procedure consisted of randomly reassigning independent variables 5,000 times and testing each edge for significant effect ($p < 0.005$) of a given factor. We then constructed a distribution of the size of connected units of disrupted edges (called components) and retained only those components in the top 99.999 percentile ($p < 0.001$). For each factor (HIV, age, HIV x age), NBS allowed us to determine the number of adjacent edges that must be disrupted in order for a cluster of disrupted edges to be considered significant. The

permutation test showed that if two or more adjacent edges were disrupted by a factor, this cluster should be considered significant.

7. Overlap of primary and secondary effects—We next computed the overlap of primary (i.e., FC edge weight) and secondary [i.e., graph topology (c, d, e)] effects at the node level. We assessed changes in graph topology for each of the 160 nodes ($p < 0.05$) and defined a node as having a significant effect on secondary measures if it showed either higher or lower values on any of the three topological graph measures. We defined a node as having a significant effect on primary measures if it was connected (i.e., adjacent to) to a disrupted edge (based on thresholds defined in the previous paragraph). We assessed the amount of overlap between primary and secondary effects for both aging and HIV effects by characterizing which nodes showed significant effects on primary measures, secondary measures, both, or neither. We then used a chi-squared test to compare the proportion of nodes with significant effects by each factor on both primary and secondary measures or secondary measures alone.

8. Clinical HIV measures—We performed separate linear regressions on the global and network level graph measures as a function of each available clinical HIV variables (i.e., highly active anti-retroviral therapy (HAART) status, viral load, CD4 count, CD4 nadir and interactions between HAART status and viral load, CD4 count, and CD4 nadir). Each model also included age as a regressor.

Results

A. Establishment of test-retest reliability

Longitudinal cohort demographics—Our longitudinal test-retest analysis included 22 participants collected on average 3 days apart (Table 1). This group was one-third male and older (mean age 61 years old).

Comparison of weighted and unweighted graph metric reliability—We first compared test-retest reliability of weighted vs. unweighted measures in this separate longitudinal cohort of 22 HIV- individuals. All three global measures c_{GLOBAL} (ICC=0.404, $p=0.039$), e_{GLOBAL} (ICC=0.39, $p=0.039$) and d_{GLOBAL} (ICC=0.532, $p=0.014$) were reliable for weighted graphs. However, c_{GLOBAL} was not reproducible in unweighted graphs (Supplemental Fig. 1b) and e_{GLOBAL} was reproducible over only a very narrow range of edge densities in unweighted graphs (Supplemental Fig. 1b). RSN network composites of weighted measures were reproducible for many networks, but less reproducible for unweighted measures (Supplemental Figure 1). Diversity (d) is undefined for unweighted graphs.

We quantitatively compared ICCs at each node for unweighted (Fig. 2, first column) and weighted graphs (Fig. 2, second column). For each node, unweighted ICCs were averaged across thresholds. When values were compared using the 160 nodes as samples, weighted graph ICCs were statistically higher for e (paired t-test (d.f.=159)=10 Λ -16) and c (paired t-test (d.f.=159)=10 Λ -16).

B. Tests of the effects of HIV and age

Cross sectional cohort demographics—Our cross sectional analysis included 144 participants grouped into two cohorts: HIV+ (n=77) and HIV- (n=67) (Table 1). While these groups were matched for age, there were significant differences in gender and education. More than half of the HIV+ individuals were on a stable HAART for at least 6 months and most were virologically controlled. We controlled for differences in gender between groups. However, we did not include education in our final models as there were no education effects on any of the graph measures evaluated.

Global graph effects—We computed global weighted graph metrics (c_{GLOBAL} , e_{GLOBAL} and d_{GLOBAL}) for each participant in our cohort of HIV+ and HIV- individuals. In order to assess the global structure of centrality and entropy changes with HIV, we used three separate models to test the independent effects of HIV, age, and HIV x age interaction on c , e , and d . We observed that HIV and age was independently associated with distinct effects on these measures (Table 2). We observed a main effect of HIV on c_{GLOBAL} ($p=0.0296$), no effects of HIV or age on e_{GLOBAL} , and an age effect as well as HIV x age interaction on d_{GLOBAL} ($p=0.025$) (Table 2).

RSN composite graph effects—We analyzed graph metrics on a finer scale by averaging metrics (c , e , d) within six RSNs to assess the impact of HIV, and aging, and their interaction. We tested all network composite measures (e.g., c_{DMN} , e_{DMN} , etc.) and saw divergent effects of HIV and aging. After FDR correction, we observed that was HIV primarily associated with changes in closeness in the DMN and PAR, but not diversity or eigenvector centrality. In contrast, age was associated with higher diversity in the DMN but not closeness or eigenvector centrality. HIV and age effects were relatively separable at the RSN-level. Our observation that neither HIV nor age affects eigenvector centrality suggests that the global association structure of the brain (wherein high weight nodes tend to associate with other high weight nodes) remains relatively intact on the global and RSN scales (Zuo et al., 2011).

Node level graph effects—Graph measures have certain properties that are not captured by RSN composites but are only observable in node-level maps of group-average topological characteristics (Fig. 3). For example, there was regional variation within RSNs on these measures: certain nodes of the DMN such as the medial prefrontal cortex (black circles) have higher c and d , but others such as posterior cingulate (dashed black circles) have lower c and d . Additionally, node-level values vary between metrics within particular nodes. For instance, nodes with average c in the posterior cingulate cortex (dashed circles) have relatively high e and nodes in the cerebellum (grey circles) have average c and e , but high d .

We conducted an exploratory analysis to quantify how graph properties change at the node level with respect to HIV and age (Fig. 4). This analysis largely confirmed the network composite result presented above. HIV was associated with lower c in nodes comprising the DMN and age was associated with higher d across multiple RSNs (i.e., PAR, CINGO) ($p<0.05$). Regions in other RSNs outside the DMN and PAR varied on several measures

which did not show significant composite score differences above. At the node level, eigenvector centrality was lower with HIV and higher with age. Nodes in the cerebellum were also seen to have higher closeness with age.

We next conducted an exploratory analysis to assess whether nodes with particular characteristics (e.g., high c or e or low d) were more associated with either HIV or age. There was a suggestion that nodes with higher c and higher e tended to be more associated with HIV (c : $p=0.06$, e : $p=0.02$). Nodes with lower d and higher e tended to be more associated with age (d : $p=10^{-7}$, e : $p=0.05$) (Fig. 4, scatterplots). Nodes predominantly had higher e with age, and lower e with HIV (Chi-squared (d.f.=1) = 18.4; $p=10^{-5}$).

Edge weight effects—The observed divergence in secondary effects related to HIV and aging may be driven by divergent patterns of change in average values of FC correlation between nodes (i.e., edge weights). That is, if the two processes affect different edges, this may explain the divergence in effects due to HIV compared to age for higher order measures. We observed that HIV was associated with changes in FC weights of a number of edges connecting frontal and lateral parietal lobes (i.e., regions with significantly different closeness above), including some links between posterior cingulate and medial prefrontal regions of the DMN (Fig. 5). HIV was primarily related to a reduction in edge weight (blue). Because c depends closely on correlation strength, this could explain why networks connected by these edges are most associated with HIV on c . In contrast, age was associated with mixed higher and lower weights, predominantly in temporal and parietal lobes (regions with significant association between c and d measures with age). More total edges were associated with age (186 edges) than HIV (13 edges) (Chi-squared (d.f.=1) = 149.8, $p=10^{-16}$). Interestingly, connections to superior frontal regions were relatively unassociated with age, though these regions were associated with HIV. The wide array of higher and lower edge weights seen with age could explain why we see a large increase in d across multiple RSNs with age.

Overlap of graph topology and edge weight effects—We characterized how edge weight changes (i.e., changes in average correlations) and secondary graph topology changes (i.e., changes in c , e , or d) overlapped for particular nodes (Figure 6). We characterized a node as having primary effects if average correlations along any edge connected to that node were associated with HIV or aging (as shown in Figure 5 above). We characterized a node as having secondary effects if we observed a significant effect ($p<0.05$) of HIV or aging on c , e , or d at that node in the node-level analysis (as shown in Figure 4 above). We first analyzed patterns of overlap using qualitative visualizations and then quantified the extent to which HIV and age were associated with different patterns of overlap.

For our qualitative analysis, each node was characterized as having one of four possible states and was color-coded accordingly (Figure 6). Nodes with both primary and secondary effects (of HIV or age) were color-coded pink; nodes with primary effects but no secondary effects were coded green; nodes with secondary effects but no primary effects were coded orange; nodes with neither primary nor secondary effects were color-coded grey. Many nodes were not associated with either HIV or aging on primary or secondary measures (grey

in Figure 6). However, for demonstrating effects for both primary and secondary measures, a qualitative visual inspection of the data suggested interesting patterns of overlap in the visualization of the HIV effect. For instance, in the parietal lobe (black circle), an area regularly implicated in HIV (Chang et al., 2013; Thomas et al., 2013), we observed that certain nodes demonstrating effects on both primary and secondary measures were surrounded by nodes demonstrating effects only on secondary measures.

We next quantified this observation by computing the ratio of the number of nodes demonstrating significant effects on both primary and secondary measures [i.e., number of nodes for each factor (HIV, age)] which had graph topology effects and also had disrupted edges] to the number of nodes demonstrating significant effects solely on secondary measures. That is, we computed the ratio $\frac{1^{\circ} \wedge 2^{\circ}}{2^{\circ}}$ for the top and bottom graphs in Figure 6 and displayed this ratio in a pie chart. We observed that this ratio was significantly higher for age than HIV (Chi-squared (d.f. =1)=39.8, $p < 10^{-10}$).

Clinical HIV measures—We performed regressions between our graph measures and available clinical HIV variable (i.e., HAART status, viral load, CD4 count, CD4 nadir, and interactions between HAART status and viral load, CD4 count, and CD4 nadir) and age. However, none of these effects were robust enough to survive multiple-comparisons.

Discussion

We applied weighted graph theory tools to analyze FC. These weighted measures effectively simplify statistical comparisons of graph topology by allowing simultaneous modeling of all edges. We showed that graph theory analysis of FC (performed at three scales) reveals previously unobserved pathophysiological differences between HIV and aging. We observed that HIV was primarily associated with lower centrality scores and age was primarily associated with higher diversity scores. Eigenvector centrality was relatively preserved with respect to age and HIV, though exploratory node-level analysis suggested that there may be node-level changes in eigenvector centrality with both HIV and age. Additional exploratory analysis suggested that nodes with lower diversity had greater increases in diversity with age. At a trend-level, nodes with higher centrality measures had greater reductions in centrality with HIV. HIV was associated with a large number of nodes with graph topology changes that had no FC time-series changes, whereas nodes associated with age effects on graph measures also tended to have associated changes on FC time-series measures.

Previous between-group comparisons of graph FC have proceeded by generating graphs for individual participants based on thresholded and binarized FC correlation matrices. Any correlation relationships that superseded an arbitrary threshold were modeled as binary, unweighted edges. As outlined above, this approach has two primary problems: 1) in between-group comparisons, this approach may reveal spurious differences or conceal real differences between groups, 2) the test-retest reliability of these measures has not been well established. Previous attempts at addressing the first issue have generated a range of graphs for each participant (Tijms et al., 2013) but these approaches have still suffered from multiple-comparisons problems and have generally still discarded negative edge-weight information. We addressed this problem by constructing weighted graphs where correlations

were remapped to a positive weight scale. We then used weighted graph metrics on the resulting fully-connected graphs, yielding one set of measurements for each participant which we were able to analyze with standard statistical methods.

We showed that weighted graph measures have improved reliability over the reported reliability of unweighted graph measures (Braun et al., 2012). At the global and RSN levels, weighted graphs were statistically more reliable than unweighted measures. Global and RSN averages are frequently used to compare graph properties in different diseases, and the improved stability of weighted measures demonstrated here suggests that these measures could improve studies of global topology in disease. At the node-level, similar results were seen with weighted measures being more reliable than unweighted measures. In a supplemental analysis (Supplemental Figure 1), we demonstrate that for unweighted measures, it would be impossible to pick a specific edge density such that all graph topology measures were reliable at all scales.

We next applied weighted graph measures to understand how HIV and age differentially affect centrality and graph entropy. The first centrality metric we compared for the HIV+ and HIV- participants in our study was closeness centrality, which reflects the overall level of node integration. Cognition can deteriorate in the HIV+ population but remains relatively intact in healthy HIV- individuals across the age-range analyzed in this study (Jacobs et al., 2012). We observed that HIV (but not age) was associated with lower closeness globally and particularly in the DMN and PAR. These results suggest that DMN and PAR are less integrated with the rest of the brain, possibly making it more difficult for information to be passed from these networks to other networks of the brain and making the brains of HIV+ individuals more susceptible to changes in cognition. Age was not associated with closeness in the global or RSN-level analyses, but node-level closeness analysis suggested that age may affect nodes in the posterior portion of the DMN. Regions of the cerebellum may have higher closeness with age. It may be that lower closeness reflects impairment at these nodes and higher closeness represents potential reorganization with age.

The second centrality measure we evaluated, eigenvector centrality, reflects the global association structure of the brain: the extent to which highly-weighted nodes are also strongly connected to each other. Networks with this structure tend to be more resilient to random attack, but more vulnerable to targeted attacks on highly central nodes (Bullmore and Sporns, 2009). Previous work has shown that eigenvector centrality is relatively preserved with age, regardless of the fact that individual edges may be disconnected in advanced age (Zuo et al., 2011). In our global and network composite analyses, we observed that neither HIV nor aging were associated with changes in eigenvector centrality in any network, suggesting that the structure reflected by this measure may be one of the brain's more robust network organization principles (Zuo et al., 2011). In our node-level analysis, we observed that aging may actually be associated with higher eigenvector centrality in the sensorimotor network which suggests that the network may reorganize over the lifespan to maintain the brain's resilient assortative structure (Fling et al., 2012; 2011; Langan, 2010). However, in the node-level analysis, we also observed that HIV was associated with large effect sizes in nodes with higher eigenvector centrality. It may be that HIV targets the

assortative structure of the brain and leads to losses of network resilience, whereas this structure is relatively persevered with age (Achard et al., 2012; Zuo et al., 2011).

We next evaluated diversity, which reflects the entropy (or variance) of graph edge weights at each node. It has been observed that aging is associated with dedifferentiation in the brain network, causing brain regions to become less specialized (Li and Lindenberger, 1999; Roski et al., 2013). We observed that age was correlated with higher global graph entropy, particularly strong in the DMN. However, there was also a HIV x age interaction wherein HIV+ subjects were associated with higher global entropy per year compared to HIV- controls. This result is similar to previous work that has shown lower levels of FC with age in certain networks (e.g., the DMN) but higher correlations in other networks (e.g., the motor system) (Langan, 2010; Thomas et al., 2013). The observed interaction differs from our previous work that showed independent changes with age and HIV in brain structure (Ances et al., 2012) and rs-fcMRI (Thomas et al., 2013). The effects of age on structure and certain measures of brain function are perhaps not modulated by HIV status. These results suggest that secondary graph measures (in particular, diversity) are better markers of HIV-associated modulation of the age effect. The fact that no average diversity effect was seen for the HIV group suggests that dedifferentiation may not occur as a baseline effect of HIV, but only as an effect of age, with HIV possibly causing deterioration in certain networks.

Next, we analyzed how mean edge weights change at each node, showing that HIV and age were associated with distinct patterns of change in these measures. HIV was generally associated with lower weights in edges connecting the frontal cortex to the rest of the brain (Thomas et al., 2013). This frontal disconnection may underpin the reported decreases in executive function seen with HIV (Carey et al., 2006). We observed that age was associated with mixed higher and lower weight across the brain, though frontal connections were relatively unaltered. This possibly reflects brain-wide reorganization in age (Grady et al., 2006; Roski et al., 2013) and may explain the higher diversity seen in the DMN with age.

When we compared the spatial overlap of graph topology effects (e.g., centrality and entropy measures) and FC time-series effects, we observed more divergences between HIV and aging. In HIV, we observed that a large number of nodes with graph topology effects that were not associated with FC time-series effects. It may be that FC time-series relationships primarily change in a sub-threshold fashion with HIV and that graph topology measures are more sensitive to the aggregate of these changes. Future studies may show that more significant alterations of FC time-series measures only occur in HIV+ individuals with more advanced disease. Many nodes with age associations on graph measures had coincident FC time-series changes, though here again, we observed a number of nodes with only on graph measure effects. Future studies may show that nodes with only on graph metric effects are sites to which FC time-series disruption spreads with time. However, definitively establishing how graph topology can predict and quantify the spread of pathology and whether that pathology primarily targets grey matter or white matter will require additional multimodal longitudinal data.

We made two design decisions in our analysis which impact interpretation of our results. First, we used spherical nodes defined functionally rather than anatomically or on a voxel-

wise basis. While future studies exploring and comparing these node-definition methods need to be conducted, functionally defined nodes likely provide a reasonable summary of the meaningful FC in the brain (Fornito et al., 2013). Second, we did not threshold our FC matrices, meaning that for each participant we retained in our analyses certain edges that may not survive multiple comparisons correction for significant FC correlation. However, some of these low-weighted edges are likely to be topologically important (Sporns, 2013). Weighted graph measures allow inclusion of these edges and simplify comparison of graphs across groups.

Graph topology measures potentially have much power to characterize and disentangle the effects of disease and age on the brain. Our previous work showed that FC time-series measures of BOLD correlations in the DMN change similarly in HIV (Thomas et al., 2013), aging (Onoda et al., 2012) and Alzheimer's disease (AD) (Greicius et al., 2004). We previously suggested that HIV and aging manifest independent but similar BOLD changes. However, our finding may simply have resulted from the fact that simpler measures of BOLD time-series correlation may be ineffective at distinguishing the effects of disorders that manifest similarly in FC. We showed that graph topology measures might be associated with HIV and aging in distinct and characteristic ways.

The neurobiological basis of aging and disease effects on brain function remains incompletely understood. Many studies have demonstrated that aging is associated with decreased functional connectivity, particularly within the DMN (Andrews-Hanna et al., 2007) but other RSNs are also disrupted (Meier et al., 2012). These changes likely reflect changes in underlying neural function. Indeed, age is associated with the accumulation of several sub-clinical changes such as grey matter atrophy (Fox and Schott, 2004) and white matter alterations (Bennett et al., 2009). However, HIV was associated with primarily subcortical dysfunction in the pre-HAART era (Paul et al., 2007) and with cortical and subcortical changes in the post-HAART era (Clifford and Ances, 2013). Pathologic changes have been noted both within the grey and white matter (Hult et al., 2008). Unfortunately, rsfMRI within cortical areas cannot distinguish between changes caused by grey or white matter pathology. This study demonstrates that aging is primarily associated with diffuse changes in network organization (diversity). Conversely, HIV affects primarily closeness centrality in the DMN and PAR suggesting particular changes in integration within these networks. These results are consistent with previous work demonstrating changes within these same regions (Maini et al., 1990, Thompson et al., 2005). Importantly, neither process was associated with changes in eigenvector centrality which suggests that the brain maintains its general organization in the face of both etiologies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We thank Elizabeth Westerhaus for her assistance collecting imaging data.

Funding

This work was supported by grants from the NIH to Dr. Ances (K23MH081786, R01NR12657, R01NR 012907, R01NR014449, R21MH099979). Research reported in this publication was supported by the Washington University Institute of Clinical and Translational Sciences grant UL1 TR000448 from the National Center for Advancing Translational Sciences (NCATS) of the National Institutes of Health (NIH). The content is solely the responsibility of the authors and does not necessarily represent the official view of the NIH.

Definitions

c, e, d	Abbreviations for closeness centrality, eigenvector centrality, diversity
$\vec{c}, \vec{e}, \vec{d}$	Vectors containing graph properties computed for each node of graph
N, W, M	Set of nodes, set of weights, set of nodes connected to a given node (neighborhood)
z, w	Matrix containing relationships between nodes: z-transformed correlations, edge weights
z_{u,v}, w_{u,v}	z-transformed value of correlation between nodes <i>u</i> and <i>v</i> ; edge weight between nodes <i>u</i> and <i>v</i>
primary measures	edge weights computed from Fisher z-transformed Pearson correlation (<i>r</i>) between nodes
secondary measures	graph theory metrics computed from cross-correlation matrices

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Highlights

- Weighted graph theory measures have improved test-retest reliability compared to unweighted measures.
- HIV and aging have similar changes in functional connectivity that can be disentangled using graph theory.

Figure 1. Node placement in the six resting-state functional connectivity (FC) networks: blue=default (DMN); turquoise=fronto-parietal network (PAR); red=cerebellar (CER), yellow=occipital (OCC), magenta=sensorimotor (SMN); green=cingular-opercular (CINGO).

Figure 2.

Intra-class correlations (ICC) for each node. Weighted ICCs for closeness centrality (top row) and eigenvector centrality (bottom row) were greater for weighted graphs than unweighted graphs. Diversity is not presented as it is undefined for weighted graphs. Scatter plots show ICC values for both weighted and unweighted graphs. For both centrality metrics most points fall above the identity line indicating better consistency for weighted graphs.

Figure 3.

Average closeness centrality (top row), eigenvector centrality (middle row), and diversity for each node for HIV+ and HIV- groups. Certain regions belonging to the same resting state network (RSN) [e.g., the posterior cingulate cortex (dashed lines) and the medial prefrontal cortex (black circles)] show differences on the same metrics. Other regions, such as the cerebellum (grey circles) show variability between metrics.

Figure 4.

Node-level values of topology both higher (red nodes) and lower (blue nodes) with HIV (left columns) and age (right columns). Scatter plots show significance of effect ($-\log_{10} p$) plotted against average graph metric derived from the HIV- group for each node. Blue indicates that the node on average had a lower value, red indicates that the node had a higher value. Dashed line indicates trend-level or significant relationship between $-\log_{10} p$ and node-level average metric across nodes. For HIV, nodes with higher e had greater decreases ($p=0.02$). There was a trend toward the same relationship in c ($p=0.06$). For age, nodes with higher e had greater increases ($p=0.05$) and nodes with lower d had greater increases ($p=10^{-7}$).

Figure 5.

FC time-series measures captured by edge weight increased (warm colors) and decreased (cool colors) due to HIV and age. Most HIV-related losses occurred within PAR and DMN, whereas age-related changes are widespread. blue=default (DMN); turquoise=fronto-parietal network (PAR); other networks pictured: red=cerebellar (CER), yellow=occipital (OCC), magenta=sensorimotor (SMN); green=cingular-opercular (CINGO).

Figure 6.

Nodes connected to disrupted edges were said to have primary (1°) effects (green nodes). Nodes with effects on graph measure characteristics were said to have secondary (2°) effects (orange nodes). Nodes may have both 1° and 2° effects (pink nodes) or neither (grey nodes). Black circles indicate the parietal lobe. Pie charts show the relative frequency of nodes associated in each way by HIV and age.

Table 1**Participant demographics**

Demographics information. a) HIV+ and HIV– individuals differed slightly in education and gender. This cohort was assessed cross-sectionally for main effects of HIV and age; b) an independent HIV- group was longitudinally studied to establish test-retest reproducibility of graph metrics. HAART= highly-active antiretroviral therapy.

a)			
	HIV–	HIV+	p-value
N	67	77	
Age	46.8 (20.0)	44.6 (18.5)	0.95
Education (years)	15.8 (3.0)	13.9 (2.6)	0.002
Gender (%male)	57%	87%	<0.001
Log viral load (count / mL), median [IQR]	1.70 [1.70, 3.90]		
CD4+ cell count, median [IQR]	459 [349, 734]		
Nadir CD4+, median [IQR]	279 [349, 735]		
%HAART	60%		

b)	
	Test-Retest Cohort
N	22
Age (years)	61 (6.6)
Education (years)	15 (3)
Gender (% male)	29%
Days between scan	3.3 (4.8)

Table 2**Global graph theory effects**

Global graph theory scores assessed for effects of HIV, age, and HIV $\times$ age interaction. HIV primarily affects closeness centrality. Age primarily affects diversity, with an HIV $\times$ age interaction in diversity.

		HIV	Age	HIV:Age
Closeness Centrality	p-value	0.03	0.35	0.5
	beta	-0.00776	-0.00011	-0.00017
Eigenvector Centrality	p-value	0.272	0.783	0.736
	beta	-0.00502	-0.00001	0.00006
Diversity	p-value	0.921	0.038	0.025
	beta	-0.0042	0	0.00001

Table 3

Resting-state network (RSN) graph theory effects

Resting-state network (RSN) composite scores of graph measures. Closeness centrality was associated with HIV, diversity measures were associated with age. There were trends toward HIV $\times$ age interactions in diversity in certain RSNs.

	Closeness centrality			Eigenvector centrality			Diversity		
	HIV	Age	HIV:Age	HIV	Age	HIV:Age	HIV	Age	HIV:Age
DMN	p-value	0.006	0.227	p-value	0.754	0.968	p-value	0.846	0.00035
	beta	-0.001408	-0.000034	beta	-0.007735	-0.000102	beta	-0.000309	0.000014
PAR	p-value	0.033	0.255	p-value	0.754	0.803	p-value	0.774	0.0527t
	beta	-0.003703	-0.00007	beta	-0.009027	-0.000055	beta	0.000028	0.000006
CER	p-value	0.286	0.168	p-value	0.717	0.803	p-value	0.491	0.263
	beta	-0.022115	-0.000036	beta	0.002883	-0.000013	beta	-0.000884	-0.000015
CINGO	p-value	0.454	0.583	p-value	0.449	0.890	p-value	0.574	0.0527t
	beta	-0.008	-0.000025	beta	-0.005049	-0.000052	beta	-0.000436	0.00001
OCC	p-value	0.552	0.255	p-value	0.273	0.803	p-value	0.491	0.263
	beta	-0.00289	-0.000175	beta	-0.006721	0.000047	beta	-0.000535	-0.000005
SMN	p-value	0.552	0.255	p-value	0.273	0.803	p-value	0.846	0.333
	beta	-0.011797	-0.000275	beta	-0.002816	0.000136	beta	-0.000473	-0.00001