USING BRAIN CONNECTOMICS TO DETECT FUNCTIONAL CONNECTIVITY DIFFERENCES IN ALZHEIMER'S DISEASE

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DEDICATION

This thesis is dedicated, first and foremost, to my parents, Joe and Rosario Contreras, and my brother, Jacob Contreras, for the constant support and for instilling me with the courage to achieve my goals not only through their verbal and emotional encouragement, but through their actions as well. Specifically, my dad, an MTA bus driver for over 35 years, has shown me the importance of a strong work ethic and responsibility to family, how to be compassionate to others you don't know, and what it is to have the spirit of the bear;) My mother, a secretary turned CEO of her own company, has taught me that life will always surprise you and failure is something to never shy away from, but rather an opportunity to better yourself and grow. She taught me to be stubborn but ask for help, and she taught me that, in the end, I want to grow up to be just as beautifully and unapologetically intelligent as she is. Lastly, my little brother gave me the opportunity to learn how to be a mentor and mentee. He continues to inspire me through his persistence and teaches me something new about the world every day. Jacob, I will always love you and try to protect you no matter how old we get. Throughout my academic career, there have been times of discouragement from teachers and peers, but my family would never let me fall into times of despair. Any belief that my goals were too lofty or unrealistic were swiftly wiped away by my family.

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better their families, and each step they took allowed me to achieve my dream of getting a PhD.

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Joey Annette Contreras

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Prodromal Alzheimer's disease (AD) has recently been identified as a disease state where pathophysiological changes may progress despite the absence of significant clinical symptoms. Yet, the specific processes of neural dysfunction occurring during this preclinical phase remain unclear. Resting state fMRI (RS-fMRI) in combination with brain connectomic measurements may be able to provide ways to measure subtle connectivity changes in different neurological disease states. For instance, RS-fMRI scans allow us to determine functionally connected yet spatially distinct brain regions that can then be separated into resting-state networks (RSNs). More recently, the exploration of RSNs in disease states have proved promising since they have been reliably altered when compared to a control population. By using brain connectomic approaches to assess functional connectivity we can evaluate the human connectome from a different and more global perspective to help us better understand and detect prodromal neurodegenerative disease states.

Karmen K Yoder PhD

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LIST OF ABBREVIATIONS

Aβ	Amyloid Beta
AD	Alzheimer's disease
ADNI	Alzheimer's disease Neuroimaging Initiative
ANOVA	Analysis of Variance
APOE	Apolipoprotein E
ASD	Autistic Spectrum Disorder
BET	Brain Extraction Tool
BOLD	Blood Oxygenation Level Dependent
CC	Cognitive Complainers
CCI	Cognitive Complaint Index
CER	Cerebellar Brain Regions
CN	Controls
connICA	Connectivity-based Independent Component Analysis
COWA	Controlled Oratory Word Association
CSF	Cerebral Spinal Fluid
CVLT	California Verbal Learning Test
DA	Dorsal Attention Network
DMN	Default Mode Network
DOF	Degrees of Freedom
DWI	Diffusion Weighted Imaging
EEG	Electroencephalography
EPI	Echo Planar Imaging

FA	Fractional Anisotropy
FC	Functional Connectome/Connectivity
FLIRT	FMRIB's Linear Image Registration Tool
fMRI	Functional Magnetic Resonance Imaging
FP	Fronto-parietal Network
FP-DMN	Fronto-parietal and Default Mode Network
GM	Gray Matter
IADC	Indiana Alzheimer's Disease Center
ICA	Independent Component Analysis
IMAS	Indiana Memory and Aging Study
LIM	Limbic Network
MCI	Mild Cognitive Impairment
MNI	Montreal Neurological Institute
MPRAGE	Magnetization-Prepared Rapid Gradient Echo
MRI	Magnetic Resonance Imaging
NS	Not Significant
PACE	Prospective Acquisition Correction
PET	Positron Emission Tomography
PiB	Pittsburg Compound B
ROI	Region of Interest
RSNs	Resting State Networks
RS-fMRI	Resting State Magnetic Resonance Imaging
RVLT	Rey Auditory Verbal Learning Test

SC	Structural Connectome
SCD	Subjective Cognitive Decline
SM	Somatomotor Network
SPON1	Spondin 1
SUB	Subcortical Brain Regions
TE	Echo Time
TR	Repetition Time
VIS	Visual Network
VA	Ventral Attention Network
WAIS	Wechsler Adult Intelligence Scale
WM	White Matter
WCST	Wisconsin Card Sorting Test

Chapter 1: The Structural and Functional Connectome and Prediction of Risk for Cognitive Impairment in Older Adults

Introduction

The average life expectancy has steadily increased with respect to each previous generation. As the mean age rises, experimental evidence indicates a greater decline of sensory, motor, and cognitive functions in the elderly population [1]. Given that abnormal aging can result from a variety of factors (such as genes,environment or lifestyle), cognitive impairment associated with aging is considered a systemic problem. Therefore, effective prediction of successful aging versus cognitive decline becomes difficult. Aiming to face this complexity, interest in early markers of cognitive impairment has grown, since it is widely regarded as an early marker of dementia. Recently, hope has been placed in identifying biomarkers that predict risk for developing cognitive impairment. Given advances in non-invasive neuroimaging techniques and network science, the emerging field of brain connectomics is increasingly well situated to link cognitive impairment with structural and/or functional network changes. These changes may be reflected in a number of topological features that cover global and local aspects of the brain organization and communicability.

For the reader's convenience, a glossary is presented for terms related to network science. Throughout this review words in italics will refer to items present in Table 1, which includes a glossary of network indices.

Table 1. Glossary for network terms in alphabetical order: network indices

Index	**SC and FC measure**	**Definition**
Cluster	SC and FC	Refers to a group of nodes that have denser connections between each other than to the rest of the network
Edge	SC and FC	Refers to a connection between nodes. Can either be weighted orbinary.
Fractional Anisotropy(FA)	SC	A scalar metric between 0 (indicating unrestricted diffusivity) and 1 that describes the degree of anisotropy of a diffusion process.
Functional Connectome	FC	A comprehensive map of nodes correlated across time (such as, brain regions probed with fMRI) that is studied as a network by means of a network science and graph theory.
Global Efficiency	SC	The overall information transfer efficiency across the whole network. It is defined as the inverse of the average shortest path.
Hub	SC and FC	A highly connected node, highly relevant for efficient network communication that if damaged might be

		especially disruptive for network integrity.
Independent Component Analysis(ICA)	FC	Signal processing method that aims to decompose multivariate signal into additive subcomponents. It is widely used to identify resting-state networks by separating fMRI data into unique spatial and time components.
K-core	SC and FC	A measure to assess the overall connectedness of a network by identifying the most highly interconnected sub-networks within a global network.
Modularity	FC	A measure of the strength of division of a network into modules – groups of strongly connected nodes that are sparsely connected to other modules.
Network	SC and FC	A representation of relations between discrete objects that is described by a Graph Theory. Graphs include a set of nodes joined by a set of connections referred to as edges.
Network Core	SC and FC	Much like a cluster, a network core is a set of nodes that are highly and mutually interconnected.

Node (vertex, point)	SC and FC	An object related to another object, where each of the related pairs of objects is called an edge.
Nodal Degree	SC and FC	The number of edges that connect to a node.
Path/fiber length	SC	Describes the (shortest) path length between two nodes.
Radial Diffusivity	SC	Average of the diffusion tensor eigenvalues along the two minor axes. A metric sensitive to white matter myelination, often used to indicate white matter pathology.
Rich Club	SC and FC	A collection of highly connected hubs, thought to be important for integration of information.
Streamline	SC	A representation of a direct connection obtained through tractography (3D modeling technique to visually represent neural tracts).
Structural Connectome	SC	A comprehensive map of anatomical white matter connections in the brain studied as a network by means of network science and graph theory.

The Human Connectome

The concept of the human brain as a large-scale complex network termed "connectome" was originally introduced in 2005 [2]. The key proposal was to model the brain as a network, with the different gray matter regions acting as nodes, and their structural connectivity (SC) through white matter fiber bundles as edges. As the first drafts of the human connectome became available [3] an increasing interest in applying graph theory to human neuroimaging data (specifically to diffusion weighted imaging (DWI)) emerged. This helped to uncover topological features of the structural connectome such as small world-ness (a network in which there are high levels of local clustering among nodes within that network to form communities and short paths that globally link all nodes of the network), highly connected hubs, and modularity. It also allowed modeling and visualization of the most prominent white fiber tracts constructed from bundles of streamlines connecting different brain regions, an important first step in identifying the basic layout of the brain's SC and its relation to gray matter derived functional connectivity (FC) [4]. Interestingly, the basic idea of the brain as a structural network of connected neurons with functional implications was proposed years, if not decades, before the idea of the connectome. The foundational writings of Santiago Ramon y Cajal were a first antecedent of the neuronal networks we now know [5]. His work and intricate circuit diagrams unveiled individual neurons and their synapses. In comparison, the field of brain connectomics works at a much coarser scale and aims to model and assess the complex interactions between neural populations (as divided by regions) to

understand the behavior of the system overall. In turn, brain connectomics uses network science to handle the complex methodologies needed to understand the dynamic interactions of different brain regions both functionally and structurally and how these interactions might influence cognition.

The Structural and Functional Connectomes

An improvement of diffusion weighted imaging (DWI), Diffusion Tensor Imaging (DTI) allows a full characterization of molecular diffusion in three dimensions of space. This technique can distinguish free (isotropic) and restricted diffusion principally along the myelin sheet of axons, providing data to model white matter fiber tracts. The resulting SC information can be represented as a binary network where nodes represent gray matter regions and edges indicate the presence or absence of fibers connecting those regions. To further elaborate this network representation, it is possible to gather fiber descriptors such as number of streamlines, fiber integrity as measured by fractional anisotropy (FA), the spatial trajectory of fibers, the estimated length of these fibers (in millimeters, fiber length), and composites such as fiber density [3, 6] between pairs of brain regions. This collection of fiber descriptors allows weighted representations of SC (see Figure. 1a, b), which more fully defines the physiological effects and capacity for plasticity changes within the structural connectome. In contrast to structural connections, functional connections refer to statistical dependencies among time series of neuronal activity or blood oxygenation level dependent (BOLD) signals, often expressed simply as linear Pearson correlations. Functional connections are time-dependent and can

fluctuate on time scales as fast as 1-2 seconds (BOLD fMRI; recently closer to a 0.5 sec range) or even hundreds of milliseconds (EEG, MEG). Recent work has shown that functional connections exhibit dynamic changes during rest as well as reconfiguration in the context of different stimuli and tasks [7].

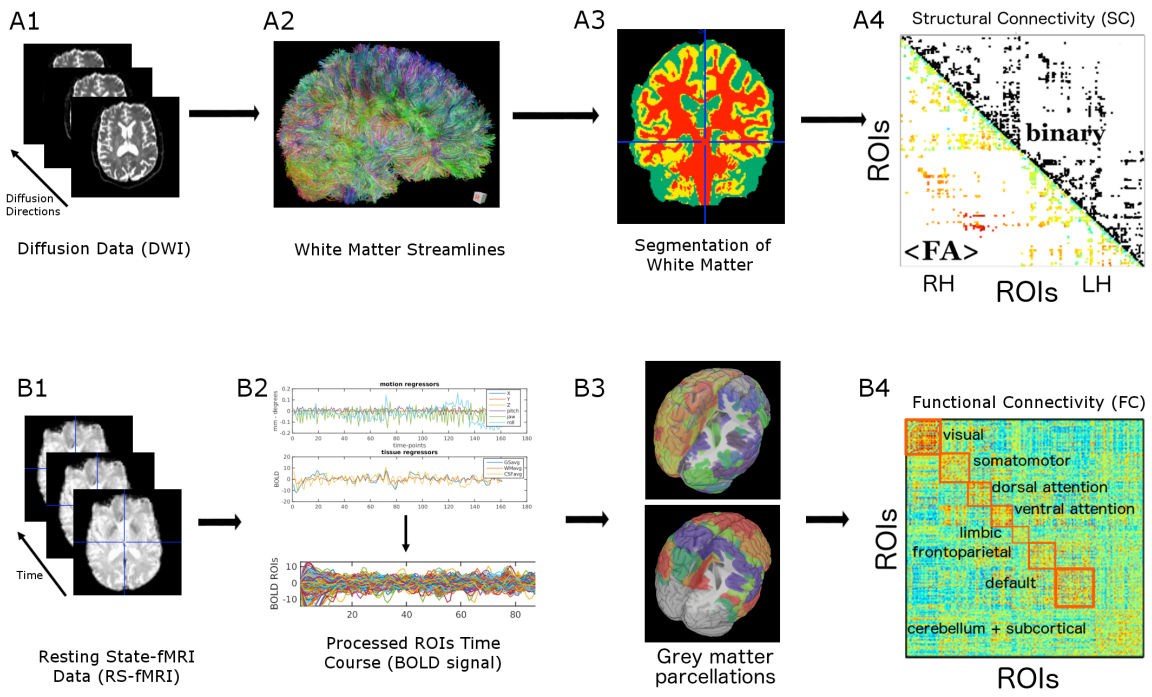

Figure 1. Basic Layout of Functional and Structural Connectivity Processing. (A) Top Panel denotes basic steps for processing of diffusion tensor imaging data. (*A1*) Different gradient directions acquired in the Magnetic Resonance Imaging (MRI) scanner are reconstructed into the full diffusion tensor, which provides magnitude and direction of water diffusion in the white matter; (*A2*) "streamlines" are obtained using tractography and visualized (the more directions allow more accurate tractography); (*A3*) tissue segmentation of T1-weighted high resolution anatomical image allows "streamlines" only within white matter voxels; (*A4*) structural connectivity matrices are generated in binary or weighted form. The binary form is represented in the *upper triangular* matrix elements, where each dark dot indicates the presence of fibers connecting a pair of gray matter regions. The weighted form is represented in the *lower triangular* elements and denotes average fractional anisotropy (FA) value. (*B*) *Lower panels* illustrate basic

processing steps of the resting state BOLD fMRI (RS-fMRI) data. (*B1*) fMRI image “snapshots” allow extraction of time courses for each image voxel and brain region (after averaging); (*B2*) *top* shows regional BOLD signal isolated by regressing out nuisance variables, with time series of all regions of interest (ROI) *shown on the bottom*; (*B3*) *top* shows an example of a gray matter parcellation, whereas *bottom* shows seven most prominent resting-state networks as reported by Yeo et al.; (*B4*) example of a resting-state functional connectivity matrix with rows and columns ordered according to those resting-state networks. *Red squares* highlight each of the RSNs.

Systematic assessment of resting-state FC has led to the concept of the "functional connectome." In a landmark study employing resting-state functional MRI (RS- fMRI), Biswal and colleagues found BOLD signal fluctuations at low frequencies (<0.1 Hz) that revealed patterns of coherent spontaneous fluctuations among spatially remote and distinct brain regions [8]. This seminal work spurred further studies to confirm the existence of synchronous spontaneous fluctuations between different areas in the brain, revealing coherent components that were highly consistent among individuals [8-12]. Low frequency, spontaneous (BOLD) fluctuations that occur at rest are referred to as intrinsic brain activity and its correlated components represent resting state networks (RSNs) [11, 13]. Note that RSNs refer to components or sub-networks within the cortex; hence, the broad term "network" denotes a set of regions or even voxels showing high functional coherence within a sub-network and low functional coherence with the rest of the system. There has recently been significant work demonstrating the validity and biological underpinnings of temporal correlations found in RSNs to infer FC [14-18]. Several studies of the relationships between spontaneous BOLD signals in different and spatially distinct brain regions have identified multiple RSNs [12, 19]. Such analyses allow functionally based assessment of network differences between subjects or clinical groups, while greatly reducing the dimensionality from thousands of voxels to a few prominent (and presumably clinically-relevant) sub-networks. For instance, differential connectivity between subjects with impaired cognition and normal cognition in the default mode network (DMN, one of the earlier [19] and most well-studied

networks) suggests that FC is sensitive to pathology and its assessment may improve diagnostic methods.

The Human Connectome and Normal Aging

Research on structural and functional brain networks has jointly given rise to the field of brain connectomics. Network science and graph theory have furthered this advancement by providing a suitable framework to quantify and give objective meaning to SC/FC or brain connectomics changes. For instance, using these tools, Betzel and colleagues examined how the connectome changes with aging and found that indeed both SC and FC evolve or "re-organize" during aging [20]. According to the study, FC of several RSNs decreased with healthy aging, while others experienced an increase. Changes were also observed in SC, including an overall decrease in the density of anatomical connections between brain regions and an overall loss of white matter fiber tracts as age progressed. This paper added evidence that FC and SC are closely related and encourage further exploration of how age-related changes in SC and FC correlate with changes in behavior and cognition.

Further research on the aging connectome found that the topology of important "hubs" or highly connected regions in the structural connectome of older adults tended to remain similar to the young adult population, with only subtle gender differences in terms of fiber length [21]. The preserved hub regions included subcortical structures such as the thalamus, striatum, and the amygdala, as well as cortical regions such as the anterior cingulate, insula, and precentral gyrus, which were consistent with other adult structural network

studies [22, 23]. This finding indicates that there is some topological network stability across normal aging, which has strong implications for healthy large-scale network communication and might support the preservation of memory and executive functions in normal aging.

Using Connectomics to Study Different Stages of Alzheimer's Disease

As dementia continues to become more prevalent, the field of brain connectomics is quickly evolving to not only study human connectome disruptions that occur as a function of normal aging but disruptions in neurodegenerative disorders as well [24, 25]. Many brain connectome studies have already begun generating results regarding the dysfunction occurring in neurodegenerative disorders. The brain connectomics provides a more systemic view by modeling whole brain networks rather than focusing on a particular region of interest or a relatively small targeted neural circuit. By adopting such approach, brain connectomics can assess organizational changes or disruptions at a whole brain level. AD is often thought of as a disconnection disorder in that a lesion or plaque in one region of the brain can disrupt healthy communication between other brain regions resulting in an eventual overall reduction of connections required for healthy cognitive functioning [26]. As a result, numerous studies have begun to take an extensive look at brain network changes in AD [27-33]. Analyses of SC and FC in AD have given rise to new hypotheses of the underlying pathophysiology of AD (refer to table 2). Further, they can also be applied to discover new correlations with clinical and cognitive symptoms that

cannot be explained from a single region or connection perspective [33]. Table 2 summarizes a list of recent SC and/or FC studies in those at risk and with AD.

Table 2: Summary of brain connectivity studies included in review.

Studies	Diagnosis	Modularity	Network	Main Findings	Significance
Sporns et al., 2005	Healthy Controls	DWI	Structural	Research proposal that introduces the idea of displaying connections between brain regions as matrix	Introduces the term "human connectome" and the research strategy to achieve it
Biswal et al., 1995	Healthy Controls	RS-fMRI	Functional	Confirm the existence of synchronous spontaneous fluctuations between primary and higher order somatosensory areas in the brain	First to identify resting-state networks
Betzel et al., 2014	Healthy aging adults	DWI/RS-fMRI	Structural/Functional	SC and FC evolve/re-organize with healthy aging	Indicates the close relationship between SC and FC and adds evidence to how age-related connectivity changes
Rose et al. 2000 Bozzali et al., 2002 Kiuchi et al., 2009 Ukmar et al.,	MCI and AD	DWI	Structural	Disruption of the white matter in important hub regions: cingulum bundle tract, corpus callosum, and the superior longitudinal fasciculus	Indicates a re-organization of important "hub" regions as a result of disease state

2008					
Douaud et al., 2011	MCI and mild AD	DTI	Structural	MCI show higher FA values in hub region, centrum semiovale region than controls	Diffusion MRI has the ability to detect sensitive changes in SC between disease states
Wang et al., 2015	MCI and AD	DTI/RS-fMRI	Structural/functional	Found disease stage specific changes in FC and SC. Further analysis showed FC changes (>coupling in anterior, <posterior brain regions) were positively correlated with SC changes (< FA, >MD) demonstrating an association between the two	Demonstrate specific patterns of interhemispheric changes between MCI and AD and the relationship between FC and SC
Liu et al., 2014	AD	RS-fMRI	Functional	Patients with severe AD were found to have reduced amplitude fMRI oscillations and reduced strength of FC, specifically in long-distance FC compared to mild AD patients (disease not as progressed)	Progression of disease can be modeled through looking at connectomics
Daianu et al., 2013	MCI and AD	DTI	Structural	Network nodal degree, normalized characteristic path length, and efficiency decreased with AD, while normalized small-worldness	Connectivity metrics can help understand whole brain network breakdown as cognitive

				increased	impairment increases
Daianu et al., 2015	MCI and AD	DTI	Structural	AD patients were found to have an undisturbed structural core network	Demonstrates possible preservation and plasticity in diseased brains
Dzezga et al., 2015	MCI	MRI/PET	Functional	Significant disruptions of whole brain connectivity were found in amyloid-positive patients with and without MCI in typical cortical hubs	Indicate that connectomics can capture early functional consequences of emerging molecular AD pathology before clinical symptoms
Brown et al., 2011	APOE ε4 carriers (at risk)	DTI	Structural	APOE ε4 carriers demonstrated an accelerated age-related loss of mean local interconnectivity compared to non-carriers as well as age-related loss in mean cortical thickness	Highlights a genotype-specific brain network pattern that can potentially affect the rate and/or spatial distribution of AD-related pathology

Wang et al., 2015	AD APOE ε4 carriers and AD APOE ε4 non-carriers	RS-fMRI	Functional	Whole brain graph analyses revealed that APOE ε4 significantly disrupted whole brain topological organization	Highlights how connectomics can help uncover genotype-specific abnormal functional brain network pattern in a preclinical population
Chen et al., 2015	HC (at risk)	DTI/RS-fMRI	Structural/functional	Reduced global efficiency was found in WM and marginally in FC (FC dysfunctions mainly in medial temporal areas) in APOE ε4 carriers	Connectomics helped demonstrate ε4-specific abnormal structural and functional patterns despite no clinical diagnosis, which may potentially serve as biomarkers for early detection before the onset of the disease

The structural connectome of AD patients has shown disruptions in connectivity in the cingulum bundle tract, the corpus callosum, and the superior longitudinal fasciculus [34-37]; for additional information, see [38]). Patients with mild cognitive impairment (MCI), which is considered a prodromal stage of AD, have also been shown to exhibit many of these same disruptions, although not as severely as seen in AD. This further supports MCI as an intermediate state between healthy aging and AD [39-41]; additionally, see review [42]). Another study also found significant abnormalities in a crossing fiber region known as the centrum semiovale (close to an important hub region, [6]) only in MCI patients compared to cognitively healthy age-matched controls [39]. When evaluating whole brain diffusion differences, MCI and AD patients were found to have abnormal FA values when compared to cognitively healthy age-matched controls. FA value refers to the shape of diffusion ranging from 0(indicating less directionality) to 1 (indicating more directionality). Together, this evidence suggests a selective degeneration in crossing fiber pathways. This result is important for two reasons: (a) it demonstrates that diffusion MRI is able to detect subtle changes in the structural connectome between disease states and (b) it highlights the need for further interpretation of both increases and decreases in SC with regards to the underlying neuropathology. Overall, changes in the fiber tracts provide evidence of a disruption of whole brain organization in prodromal and clinical AD through damage to hub regions.

Important FC findings in AD have also been reported. Many studies have already found significant FC changes in AD patients [43, 44]. For instance,

patients with severe AD showed reduced amplitude of fMRI oscillations, as well as reduced FC strength, specifically in long-distance FC connections, when compared to mild and moderate AD patients [45]. A reduction in clustering and modularity (used as a measure of segregation within a network and defined as the degree to which a system's components may be separated into discrete communities/clusters) was also observed in patients with AD compared to cognitively healthy age-matched controls, indicating a less segregated or modular nature of connectivity, and hence a more disorganized way in which brain regions communicate [46-48].

Other metrics have also been used to measure the way different pathways break down in AD. One such metric is the k-core, which is a measure that assesses the overall connectedness of a network by identifying the most highly interconnected subnetworks within a global network [6]. As opposed to looking at a whole connectivity matrix that may include unreliable connections making it difficult to pull out meaningful disease effects, using the k-core can enhance detection of changes that may be caused by the disease. This is accomplished by looking at deeper highly connected subnetworks or cores. In AD patients, k-core analysis revealed that there was a complete loss of all core networks in the left hemisphere indicating a dramatic unilateral change in brain network topology as disease progresses [49, 50]. This finding suggests that there is a loss of healthy organizational structure in the brains of AD patients. Similarly, Daianu and colleagues assessed structural cores of the brain in AD patients and found that structural brain connectivity was affected according to a variety of network

metrics that describe the topological organization of the brain [51]. Disrupted whole brain functional connectivity in cortical hubs was also reported in patients with MCI [48]. Evaluation of other FC measures revealed a similar pattern; nodal degree decreased as the disease progressed, as did path length and efficiency (inversely related to path length and a measure of how efficiently information is communicated between nodes) [50, 51]. It has recently been shown that not all structural core networks are disrupted in AD. Specifically, AD more strongly affected lower connected brain regions (low-degree networks) rather than the brain regions that are highly integrated and connected to other brain regions (known as hubs), leaving this high-degree network better preserved [52]. However, more research is needed to better understand the SC and FC changes in AD and their impact on clinical and cognitive decline.

AD is believed to have a prolonged prodromal and preclinical phase initially characterized by the development of silent pathologic changes, including the development of amyloid-beta (Aβ) plaques and neurofibrillary tangles caused by hyperphosphorylated tau, with no clinical symptoms [53-56]. Thus, cognitively normal individuals with significant amyloid and tau pathology (assessed either by measuring cerebrospinal fluid (CSF) levels or by positron emission tomography (PET) techniques) are at high risk for progression to AD [57-60]. Other changes in brain network structure and function may also be present in the preclinical phase of AD. For instance, one group set out to measure FC in the DMN using independent component analysis (ICA) in a distinct preclinical group (marked by individuals who have cognitive complaints yet have normal psychometric

performances) along with an MCI group and healthy controls. The study found that the preclinical group, identified as informant-verified cognitive complainers (CC), demonstrated lower connectivity in the DMN, a RSN known to be disrupted in AD, compared to cognitively healthy age-matched controls without complaints yet have higher connectivity compared to the MCI group [61]. In another study by the same group, diffusion tensor imaging revealed white matter changes between diagnostic groups as measured by FA values in regions associated with AD. Their main findings revealed lower FA values in parahippocampal WM among the MCI group compared to cognitively healthy age-matched controls, as well as intermediate FA values in the CC group falling between MCI and cognitively healthy age-matched controls without complaints. These studies added evidence to the existence of a preclinical intermediate state using connectivity measures [62]. Another group, Drzezga and colleagues, used CSF measures to show that asymptomatic individuals with increased amyloid burden demonstrated subtle whole brain FC disruptions and hypometabolism (as measured with [^{18}F]fluorodeoxyglucose PET) [48]. While the pathology of this preclinical category is still unclear, these studies indicate that connectomics can capture early functional and structural changes. This may be a consequence of emerging molecular AD pathology.

Genetic markers have also helped identify asymptomatic people with high risk for AD. The major genetic risk factor for late-onset AD is the ε4 allele of the apolipoprotein E (APOE) gene on chromosome 19 [63]. An early study demonstrated that asymptomatic APOE ε4 carriers exhibited an increase in the

magnitude and extent of brain activation during a verbal memory task in brain regions known to degenerate in AD. Furthermore, these same subjects were retested 2 years later and brain activation in the previously identified areas correlated with the degree of memory decline [64]. More recently, cognitively intact carriers of the APOE ε4 allele have been linked to accelerated age-related decline in local interconnectivity of structural brain networks [65], as well as disruption in the functional connectome [66], relative to APOE ε4 non-carriers. Cognitively normal APOE ε4 carriers also show differences in large-scale brain functional networks (i.e., decrease in nodal efficiency in medial temporal areas) and structural connections (aberrant regional topological patterns in temporal lobe and other regions) [67]. Interestingly, Wang and colleagues found reduced efficiency in whole brain topological organization, as well as decreased intramodular connectivity, within the posterior DMN and executive control network in APOE ε4 carriers with AD relative to non-carriers [66]. Impaired functional hubs and their rich club connections with other RSNs were also found in APOE ε4 AD patients indicating differential brain network organization as a function of APOE ε4 variants in AD. These studies explore the connectome by combining different imaging modalities and a network science approach suggest that these tools can help elucidate changes that would otherwise be undetectable in a high-risk, preclinical population.

Recent Developments

While the continued development and refinement of RS-fMRI [68-70] and DWI methods [71] hold great promise for under- standing biological changes

associated with normal aging and dementia so can integrating genomic data. The combination of improved neuroimaging methods to measure biological processes and genetic information from across the genome is likely to provide a more complete picture of health and disease. Genetic analysis may also be incorporated with DWI data. Indeed, the diffusion properties of white matter, placement of fiber tracts, and overall SC networks are thought to have strong heritability [72]. White matter integrity (as measured by FA) was found to show a strong genetic influence, specifically in the bilateral frontal and parietal lobes and the left occipital lobe. Results from a larger family-based study from Kochunov and colleagues supported high heritability of white matter diffusion properties, showing that average measures for FA and radial diffusivity were highly linked to genetics [73]. Overall, these studies suggest that the microstructure of cerebral white matter is at least partially genetically determined. Imaging genetics with DWI data has also been applied to dementia research. Jahanshad et al. evaluated the influence of gene variants or “polymorphisms” on SC and found that variations in a gene known as SPON1 affected SC in an older adult population with varying degrees of cognitive impairment [74]. Furthermore, older adults who carried the variant associated with increased SC had less severe dementia scores and a lower risk of AD. This discovery of SPON1 variants associated with brain connectivity suggests a new neurogenetic pathway with links to dementia severity that can be further explored.

Genomic data may also help elucidate the biological basis for the synchronous low frequency fluctuations that are suggested to give rise to brain

networks using RS-fMRI. One study found that expression of 136 genes (later identified to be involved in ion channel formation) were significantly associated with RS-fMRI measures in humans and significantly associated with axonal connectivity in a rodent model [75]. Additionally, Richiardi and colleagues demonstrated that functional brain networks as defined by RS-fMRI are significantly correlated with gene expression data. Their data revealed that functional networks are associated with a set of genes that code for ion channels and other important synaptic functions (i.e., dopamine neurotransmitter release), further supporting a strong biological foundation of RSNs. The results from these studies provide additional evidence that integrating gene expression and brain connectivity measures can provide insight into the molecular mechanisms underlying not only functional networks but also structural networks. Other studies have found that an altered expression of genes associated with AD can translate to altered FC at the molecular level [76]. These studies integrating RS-fMRI and genomics suggest that the connectome may offer a rich and promising target for genetic analysis.

Another future direction of brain connectomics is through the integration of different technologies, specifically studies that integrate multiple imaging modalities [77]. Multimodal imaging refers to the integration of data from more than one imaging modality, such as DWI, RS-fMRI, EEG, and PET. This integration typically occurs at the level of statistical analysis where each modality has an influence over the end result. Additionally, integration of multimodal imaging techniques has shown significant promise in examining brain disorders.

For example over the last decade, theoretical accounts of ASD (autistic spectrum disorder) have shifted towards emphasizing the breakdown of neural communication and connectivity much like AD. In light of this shift, the use of multimodal imaging has begun to show promise. Studies in ASD show convergence of altered SC and FC within the same regions, including regions of the medial prefrontal cortex and posterior cingulate/precuneus that are core members of specific RSNs [78-80]. Additionally, a convergence in altered function, volume, and connectivity of frontal regions involved in executive function, language, motor, and sensorimotor functions was also found in ASD [81-83]. Finally, a study revealed age-related differences in the coupling of function and structure in healthy controls that did not occur in ASD patients.

Multimodal neuroimaging approaches are also useful for achieving the best possible spatial and temporal resolution (i.e., EEG and fMRI [84]) as well as for using data from one modality to constrain the interpretation of another modality. In the latter case, the purpose of multimodal imaging would not be to merge data but to assist with informed judgment of the data within a broader context. For instance, EEG data measuring participant alertness has been used to inform interpretation of fMRI data [84]. Ultimately, multimodal data integration can yield important insights into brain processes and structures in addition to spatiotemporal resolution complementarity, including a comprehensive physiological view on brain processes and structures.

As a complement to the challenges and opportunities of this technological integration, the area of brain connectomics is also evolving from a theoretical

point of view. Multimodal imaging naturally allows us to elaborate on multiplex or multi-layered networks [85, 86], where one system is not represented by a unique connectivity matrix, but by more than one, attending to different features or to the different nature of their connections (such as a structural and functional connectivity matrix as illustrated in Figure 1).

Finally, another innovation in modeling FC is the emergence of dynamic RS-fMRI. Until recently, most RS-fMRI studies looked at data in a stationary framework to interpret results. While studies employing this framework have uncovered large-scale properties of brain function, the resulting characterization ultimately represents an average across complex spatio-temporal phenomena. Quantifying changes in FC metrics over a longer scan (10 min or longer) may provide greater insight into fundamental properties of brain networks and how they change over time [87]. Recent studies have demonstrated that correlations among brain regions, both within and between networks, indeed evolve over time [7, 88-91]. These results suggest that resting brain activity can be divided into subsets or “communities” of brain regions that strongly interact for a time but that these interactions are not static. For instance, low frequency BOLD signal fluctuations revealed synchronized communities that reoccurred intermittently in time and across imaging sessions during RS-fMRI scans [92, 93]. In addition, these synchronized communities constitute components of previously defined RSNs known to be engaged in sensorimotor or cognitive function. As discussed earlier, altered static FC in RSNs has been found in AD patients. Thus, it would be of great interest to see if using a non-static or “sliding-window” approach

would provide an even more accurate description of FC changes in AD. One of the first reports demonstrating RS-fMRI changes in AD patients beyond the traditional average FC metrics [94] reported impairments in the dynamics of spontaneous activity by examining time varying changes of a modularity metric [95]. Using dynamic FC representations, the authors reported differences in the "dwell time" within different sub-network configurations of the DMN between AD and cognitively healthy age-matched controls. In other words, AD patients spent less time in brain states with strong posterior DMN region contributions and more time in states characterized by anterior DMN region contributions. This observation has the potential to lead to a better understanding of the large-scale characterizations of AD and potentially a more accurate prognostic picture. This early work offers great promise in revealing aspects of dynamic FC at a macroscopic scale.

Conclusion

The increased sensitivity and whole brain perspective of SC and FC measures assessed with diffusion weighted MRI and RS-fMRI, respectively, have already provided numerous insights into the specific disturbances of network organization that occur in the diseased brain. For instance, normal cognitive aging results in changes of brain network features that are mainly localized in hub regions of the frontal, parietal, and occipital lobes. However, it is important to note that, overall, brain networks have been shown to have high topological global efficiency despite aging. While significant topological modification is observed with brain maturation, the general structure of the connectome remains

stable over time. This review has considered changes in both the structural and functional connectome in the context of AD-associated neurodegeneration as the primary example at three distinct stages, i.e., manifest disease, MCI prodrome, and the preclinical at risk stage. At the disease stage, brain connectomic techniques have made it possible to model the structural and functional failing integrity of specific neural subsystems and offered new insights into how degenerative processes may spread through interconnected networks via central regions. Specifically, AD patients have shown disruptions in SC in important hub regions, interhemispheric regions, and fiber crossing regions. These changes are characterized by a reduced network nodal degree, change in white matter path lengths, reflected in FC changes as well such as decreased global efficiency of networks. During the MCI stages, evidence of the impact of intermediate neurodegeneration has been shown using both SC and FC networks features, specifically abnormal loss of white matter and significant changes in functional network organization respectively. At the earliest stage, there is significant evidence of emerging FC changes reflected by differences between at risk populations and cognitively healthy age-matched controls. These changes have been found primarily amid the interconnectivity between major RSNs, as well as differences in whole brain organization and network structure suggesting that connectomics level analysis is sensitive to subtle changes in brain networks before the onset of clinical symptoms. However, research on brain connectomics in disorders of cognitive aging remains at an early stage and new findings on changes in network features in preclinical and prodromal neurodegenerative

diseases including non-AD and mixed types of disease will undoubtedly augment our knowledge in important ways relevant for clinical translation.

The present review also examines more recent developments such as the relationship to genetic markers, other multimodal imaging methods, and the emergence of dynamic FC, which has aided the field in characterizing patient specific abnormalities. The convergence of this information is likely to be beneficial for a design of targeted interventions especially in heterogeneous diseases like AD and dementias of mixed etiology. Selective regional pathology plays an important role in the manifestations of different neurodegenerative diseases; and characterizing the progression to and from these brain regions during the advancement of neurodegeneration is important. Connectomics analysis is likely to be useful for enhancement of clinical trial designs by incorporating information about regional brain networks at baseline and after interventions. Connectomics analyses will also likely contribute to clinical trials by providing a dynamic metric that can (a) help identify subtle changes early in the disease course and (b) provide more comprehensive metrics to assess the impact of targeted therapeutic strategies.

In this review, we have illustrated the emerging utility of brain connectomics in providing new quantitative network features that can be used to detect specific structural and functional disruptions within brain circuitry including identification of white matter tracts and their integrity, the strength of functional connections between regions, and network disruption in unique RSNs. The future of brain connectomics holds significant promise in several areas. First,

connectome studies may permit identification of early pathological changes that can predict disease progression. Second, due to the non-invasive nature of RS-fMRI and diffusion MRI, it is possible to collect data more easily and at more time points to address long-term cognitive and behavioral changes and relate them to changes in the structural and functional connectome. Third, such studies offer insight into the unique characterization of different diseases identifying neurodegenerative signatures based on network features. To elaborate on the idea of a unique signature or “fingerprint,” brain connectomics may be well-suited to reveal individual differences in brain networks and to help adapt therapeutic approaches centered on a person’s baseline topological features and assess how treatment changes network topology, whether positively or negatively in terms of adverse side effects. In this way, connectome analysis may constitute a new approach that can guide the brain repair and recovery of brain networks especially as disease-modifying interventions are developed.

Connectome-wide analyses hold promise of an unbiased means of characterizing brain network disturbances during manifest disease and in early preclinical and prodromal stages before onset of significant observable cognitive differences. However, this promise is likely to be realized with further improvements in imaging technology, statistical methodology, and integration of large-scale multimodal and dynamic data sets. Improving image quality will permit more precision in tracking changes in brain networks with disease progression, as well as to help resolve the directionality of fiber tracts withregards to SC. For SC, this will involve developing more accurate fiber reconstruction,

image registration and tissue segmentation methodologies, as well as defining measures of connectivity that have a clear biological interpretation.

Further development of appropriate statistical methods is needed to allow better handling of complex data models, and the integration of multiple multimodal data sets as existing methodology is limited. Lastly, learning to account for the distinctive nature of dynamic data will be essential for the mapping of BOLD fluctuations in psychological states to their corresponding neurobiological states.

The young field of brain connectomics has been successful in generating evidence for aberrant network properties in early stage neurodegenerative diseases, demonstrating the potential for improved prediction of cognitive impairment, prior to on-set of significant clinical symptoms. However, moreclinical research is needed to refine methodology and validate the initial observations from patients with various neurodegenerative diseases at all stages. Eventually, follow-up studies linking in-vivo connectomics changes to post-mortem analyses of specific proteinopathies underlying many dementing disorders will be important. MRI acquisition techniques and post-processing methods will continue to evolve bringing significant improvement in accuracy and reliability, with improved capacity to map more subtle connectivity changes. All of these developments will facilitate the translation of intriguing research findings on connectome changes in cognitive aging and early stage disease to a level of development where they can contribute along with clinical assessment and other

“-omics” and biomarkers to diagnosis and treatment monitoring as part of an evolving precision medicine.

Chapter 2: Cognitive Complaints in Older Adults at Risk for Alzheimer's disease are Associated with Altered Resting-State Networks

Introduction

Alzheimer's disease (AD) is often recognized as a disconnection disorder where plaques, neurofibrillary tangles, and neurodegeneration of the brain lead to reduced communication and coordination among regions important for cognition [26]. Therefore, brain connectomics studies designed to examine disruptions of connectivity in AD have become increasingly common [27-30, 33, 96]. Brain connectomics is a field of study that uses graph theory to analyze the ensemble of brain connectivity networks, including functional networks. While neurodegenerative diseases are often studied in a focal manner (i.e., a particular region of interest [ROI] or relatively small neural circuit), brain connectomics provides a more systematic view by modeling the entire human brain as a set of networks and assessing whole-brain organizational changes or disruptions.

Functional connectivity (FC) estimates the level of functional coupling of regional brain activity, and is assessed by evaluating time series [97] of brain regions regardless of distance or structural connections [6]. This method involves the resting-state functional magnetic resonance imaging (RS-fMRI) data to measure correlations in low-frequency blood oxygenation level–dependent (BOLD) signal fluctuations between distinct brain regions. Implementation of this technique combined with a gray-matter parcellation (obtained from a separate, structural MRI image) allows the whole-brain FC data analysis. Brain regions can be further organized into resting-state networks (RSNs), which have been shown

to correlate with well-described brain functions and are defined by synchronous neural activity between distinct, spatially separate brain regions [98-101]. Grouping brain regions according to RSNs reduces data dimensionality and provides psychophysiologically relevant results [12]. Several recent studies show that abnormalities in brain networks appear in early stages of AD [40, 102, 103]. Because current evidence indicates that neurodegenerative changes begin before clinical manifestations become apparent [104], it is crucial to identify these changes during preclinical and prodromal stages of disease. Investigating early stages of AD from a FC connectomics perspective may give rise to new hypotheses about underlying pathophysiology that cannot be ascertained using data from isolated regions or circuits [33, 46].

Therefore, this study examined FC differences across the continuous spectrum of early-stage AD, from at-risk and prodromal stages to clinical AD dementia with significant cognitive and functional impairment. Participants included those categorized as experiencing subjective cognitive decline (SCD) [105], as well as mild cognitively impaired (MCI) individuals and those diagnosed with AD. SCD participants reported a significant burden of subjective decline in memory and cognition in the absence of psychometric deficits [105, 106]. As individuals age, there are increased reports of self-perceived memory decline compared with earlier periods in life [107, 108]. However, older adults with SCD have a greater number of complaints and may even subjectively consider their own cognition to be impaired relative to their peers despite objective cognitive test performance (i.e., SCD individuals are only clinically distinguishable from

healthy participants in the number of reported cognitive complaints). Relatively high levels of these memory complaints or concerns have been regarded as relevant to the diagnosis of prodromal AD and are believed to be predictive of subsequent development of amnestic MCI and, at later stages, AD. Therefore, early detection of network changes associated with cognitive complaints could help inform diagnosis and treatment planning and may be useful as a biomarker for enrichment in or as an end point for therapeutic trials.

Because neurodegeneration typically progresses gradually in AD, with subtle transitions between preclinical and prodromal stages rather than discrete changes [109-111], we applied a recently developed analytic framework to identify FC network patterns across early stages of AD. This data-driven methodology, denominated connectivity independent component analysis (connICA) [112] and detailed in the methods section, is distinct from voxel-level and *a priori* region–based independent component analyses (ICAs)[113]. Our approach can uncover inherent and independent FC patterns that represent different functional RSN features within the population (our group cohort). In addition, it includes an ability to test associations of these FC patterns with subjective and psychometric cognitive parameters relevant to AD. The latter is particularly useful when investigating a continuum of states in which stratification into diagnostic groups may not be clear-cut because of the inherent complexity of diagnostic criteria.

Methods

Participants

The Indiana University Institutional Review Board approved the study, and written informed consent was obtained from all participants. Participants were older adults from a larger cohort recruited for a longitudinal study of brain aging and memory (Indiana Memory and Aging Study, herein referred to as IMAS cohort) and were included based on the availability of resting-state imaging data during their first visit. The present cohort included 16 participants with significant cognitive complaints despite cognitive test performance within the normal range (SCD group) [106], 21 participants with amnestic MCI, 8 AD patients, and 13 cognitively normal (CN) controls with minimal cognitive complaints. Further details regarding participant recruitment, selection criteria, and characterization are described in previous reports [106, 114] and in Table 3. No differences were found between groups regarding age and education (one-way analysis of variance [ANOVA]) or gender (chi-squared test).

Neurocognitive variables of interest

All participants underwent a comprehensive clinical assessment and neuropsychological battery. Five cognitive variables were included in the present analysis: (1) the California Verbal Learning Test (CVLT-II) long-delay free recall [115], a measure of episodic memory; (2) the Wisconsin Card Sorting Test (WCST) correct number of categories, a measure of executive function [116]; (3 and 4) a cognitive complaint index (CCI) score from both the participant (“self”) and an informant calculated as the percentage of all items given that was

endorsed as a complaint [106]; and (5) the highest CCI score from either self or informant, labeled CCImax (see Table 4), included to address the diminished self-awareness that may occur in later stages of AD. Significant differences between groups were detected in all cognitive measures in the expected direction using a one-way ANOVA (see Table 3).

MRI data acquisition

All subjects were imaged on a 3T Siemens Tim Trio MRI scanner with a 12-channel head coil array. A detailed anatomical magnetization-prepared rapid gradient echo (MPRAGE) with whole-brain coverage was acquired using a 3D MPRAGE sequence (repetition time [TR]/echo time [TE] = 2300/2.98 ms, 220 sagittal slices with slice thickness = 1.2 mm, 1 × 1 × 1.2 mm voxels) following the Alzheimer's Disease Neuroimaging Initiative imaging protocol [117]. RS-fMRI data were collected with instructions given to subjects to think of nothing in particular while remaining still with their eyes closed. Whole-brain functional images were obtained using a Siemens gradient echo sequence (echo planar imaging [EPI]; 6 minutes 9 sec duration; 161 BOLD contrast-sensitive volumes; TR/TE = 2250 ms/29 ms; 2.5 × 2.5 × 3.5 mm^3 voxels; 39 interleaved axial slices with 3.5 mm thickness and no gap; GRAPPA acceleration factor 2; 3D PACE prospective motion correction [118]).

BOLD preprocessing

RS-fMRI preprocessing procedures, including approach to head motion, were adapted from Power et al. [69]. These included slice timing correction, registration to MPRAGE volume (FLIRT six degrees of freedom [DOFs] and

boundary-based registration), detrending, band-pass filtering (0.009–0.08 Hz), and intensity normalization to mode 1000. In addition to including motion regressors from the realignment and their derivatives, three image quality control measures (framewise displacement, DVARS, and standard deviation) were applied to identify and remove ("scrub") outlier BOLD volumes ("D" referring to temporal derivative of time-courses, "VARS" indicating RMS variance over voxels, DVARS). EPI volumes with an excessive (>50%) fraction of outliers were dropped from subsequent analyses. This resulted in the exclusion of imaging data from seven subjects (one CN, two SCD, and four MCI), with the remaining 58 subjects comprising the final sample. The first five signals obtained from principal component analysis of the three brain tissue compartments were regressed out to address confounding effects of physiologic noise and residual head motion. This procedure relies on eroded masks of the whole-brain gray matter, white matter, and cerebrospinal fluid of the third ventricle, and also includes global signal regression [68].

The T1-weighted MPRAGE volume of each participant was used to extract the brain (FSL "BET") [119] and perform a sequence of transformations (FSL's FLIRT 6 DOFs, FLIRT 12 DOFs, and nonlinear FNIRT) to the Montreal Neurological Institute (MNI) brain template to provide tissue segmentation. The inverse MNI-to-native transformation then enabled functionally derived brain parcellation [120] into 278 cortical and subcortical ROIs to be performed in native RS-fMRI space. Correlation coefficients (Pearson's *r*) between mean BOLD time series of each ROI pair were sorted into a 278 × 278 FC matrix for each

participant and used to quantify the degree of connectivity between the paired ROIs. Brain regions were then further ordered into seven independently well-defined cortical RSNs [12], with subcortical and cerebellar regions added for a total of nine networks (Figure 2). This effectively results in a 9 × 9 FC matrix, where the seven RSNs, subcortical and cerebellar regions comprise the cells on the diagonal, and the remaining “off-diagonal” elements indicate their interactions.

connICA method

connICA [112] is a data-driven method based on ICA to obtain cohort-level independent “FC patterns” while estimating the presence of each pattern in an individual subject. Here, ICA is applied directly onto the functional connectome domain (as opposed to time-series domain at the voxel level). Specifically, ICA input consists of FC profiles of all participants sorted into a single data set matrix with 58 rows (one row per subject) and 38,503 columns corresponding to unique (diagonal and upper triangular elements) of each subject's FC matrix (see Figure 3). These independent patterns extracted from the full FC data set then represent “independent connectome subsystems” that characterize the whole sample and comprise FC profiles of all subjects without any *a priori* group stratification. ICA decomposition into FC pattern matrix was performed with the fastICA algorithm [121], where the number of components was fixed to 15 as guided by voxel-based ICA studies [122]. Owing to a nondeterministic nature of ICA, we performed connICA 100 times and kept only independent component patterns deemed to be “robust”, that is, appearing in at least 75 (out of 100) runs. In

particular, “appearance” required a Pearson correlation coefficient above 0.75 (absolute value) with respect to the first run. This method provides two outputs. The first one “FC pattern,” represents an independent pattern of FC similar to the representation of each subject's FC—a square symmetric matrix with brain regions in rows and columns (and therefore referenced as the FC pattern). However, the FC pattern matrix values are in connectivity units (“loadings”), with their range not restricted to the [−1,1] range, unlike the Pearson correlation coefficient values from the individual FC matrices. The second connICA output is a vector of the signed weights of the FC pattern present on each participant, which quantifies the prominence/presence of the trait in each individual FC matrix as illustrated by Figure 5.

connICA yielded six FC patterns (Figure 4). To establish the biological relevance of these patterns, we organized brain regions into a 9 × 9 matrix as illustrated by Figure. 2. A pattern was considered in further analyses only if the average FC loadings within at least one element included a significant similarity with either one of the canonical RSNs or their interaction (Figure 4, diagonal or off-diagonal elements, respectively). Specifically, the significance testing included the following steps: (1) generating a frequency histogram of FC loadings from the matrices of all six patterns, with each pattern contributing (278*277)/2 values (not including the diagonal); (2) setting a threshold value at the 95^{th} percentile of the FC loading value distribution; (3) calculating the average FC loading for each pattern for every element of the 9 × 9 matrix; and (4) testing a selection criterion that at least one canonically defined RSN cell must have an average FC value

that equals or exceeds the 95th percentile value of 0.72. This significance criterion was met by three out of the initial six FC patterns.

Statistical analysis

The statistical inferences based on multilinear regression analysis were performed using MATLAB [123] function “regress”. The subject weights associated with each FC pattern were entered as a response (dependent) variable in an incremental multilinear regression model with up to five predictors (independent variables). The predictors included nuisance variables, including age, gender, and years of education, and a single neurocognitive variable of interest at a time (CVLT long-delay free-recall score, WCST correct number of categories score, CCI-self score, CCI-informant score, and CCImax score). To assess the level of predictability that each neurocognitive variable of interest added, we compared the R^2 coefficient value of the multiple regression model run with the neurocognitive variable of interest to the R^2 value of the model containing only nuisance variables as predictors (This analysis was later updated using a hierarchical regression model described in Chapter 3. Results can be found in Appendix A)

Finally, the aforementioned cognitive variables that significantly increased the predictability of the chosen FC pattern were identified ($p \leq 0.05$) to extract FC patterns most associated with cognitive performance and subjective cognitive ratings, which would likely be most sensitive to functional brain changes in preclinical and prodromal AD.

Table 3. Subject Demographics for IMAS cohort. The participants of this study were selected from a larger Indiana Memory and Aging Study cohort. Below are the listed means and standard deviations within each group for selected demographic and neurocognitive variables. Between-group differences in age, education, and cognitive variables were tested using a one-way analysis of variance, while chi-squared test was used to detect gender differences between groups (*One-way ANOVA; †Chi-squared).

	CN	**SCD**	**MCI**	**AD**	***P*-value**
N	13	16	21	8	--
Mean age(y)	67.15(5.50)	73.38(7.95)	73.33(8.98)	76.38(8.98)	*NS**
Gender(M/F)	1/12	8/8	9/13	2/6	*NS*†
Mean Education(y)	17.32(1.9)	17.38(1.9)	16(2.8)	16.13(3.7)	*NS**
Mean CVLT-delayed (episodic memory)	44.21(7.66)	43.59(7.41)	29.56(8.1)	9.25(11.75)	*<0.05**
Mean WCST—# of categories (executive function)	4.1(1.19)	3.5(1.41)	2.67(1.49)	1.17(0.41)	*<0.05**
Mean subject CCI score	9.97(4.54)	27.92(16.01)	20.85(11.76)	51.29(28.38)	*<0.05**
Mean informant CCI score	7.7(11.46)	15.82(13.41)	14.54(16.1)	N/A	*<0.05**

Table 4. Neurocognitive variables of interest. The following variables were included in the multilinear regression model as independent variables: (1) Wisconsin Card Sorting Test score to evaluate executive function; (2) CVLT II-delayed score to measure episodic memory, (3) cognitive complaint index score allowing either the participant or a trusted informant to subjectively rate cognitive function. Also listed are affected brain regions, effects in AD, and more details about each score measure.

Assessment	Brain Region Affected	Effect in AD	How it is Measured
Wisconsin Card Sorting Test (WCST)—# of categories	Frontal lobe: dorsolateral prefrontal cortex, orbitofrontal cortex, anterior cingulate cortex; parietal lobe	Executive function declines with the disease progression	Stimulus cards are presented, and the participant is told to match the cards, but not how to do the matching
California Verbal Learning Test (CVLT)-II	Medial temporal lobe: hippocampus, entorhinal cortex, etc.; precuneus; medial prefrontal cortex	MCI: loss of both immediate and delayed episodic memory recall; AD: episodic memory is devastated	16 nouns read aloud over five learning trials. After each trial, the subject is asked to recall as many words as they can in any order—then again after a delay
Cognitive complaint index (CCI) score (Saykin et al., Neurology. 2006)	Negative correlation with the hippocampal volume (Saykin et al., 2006)	Early-stage patients have many complaints. As AD progresses, complaints decrease due to loss of cognitive	Combined score across multiple assessments that allows participant (self) or informant to subjectively

		awareness	rate cognitive function; CCImax: the higher complaint score either self- or informant-provided

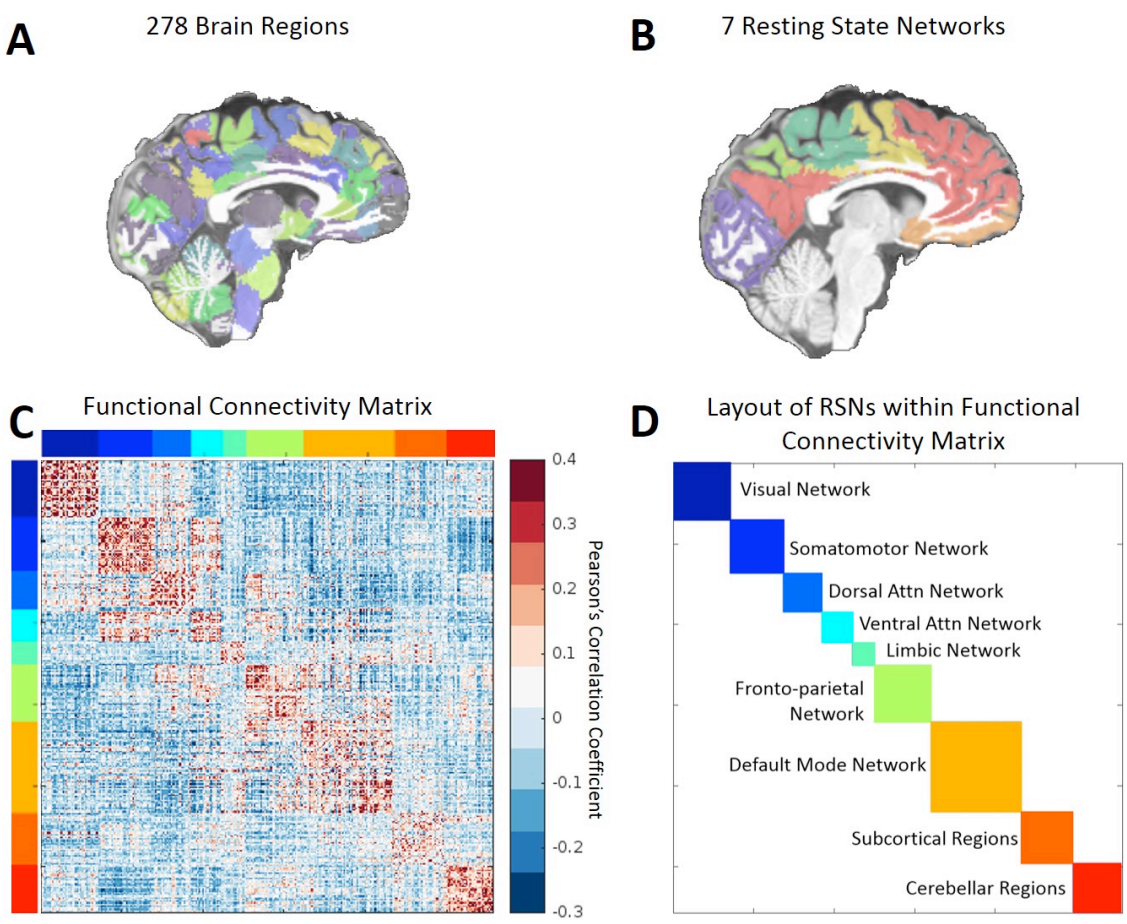

Figure 2. Schematic flow of how brain regions are organized into a functional connectivity matrix and canonical RSNs. (A) The brain parcellation into 278 gray matter regions is based on a functional parcellation by Shen et al., 2013. (B) Brain regions are further organized into canonical RSNs according to Yeo et al., 2011. (C) Group average whole-brain functional connectivity (colorbar shown on the right, Pearson correlation coefficient) based on aforementioned gray matter parcellation. Brain regions were ordered according to RSNs (as denoted by different colors on left and top). (D) Layout of resting-state networks within the functional connectivity matrix color coordinated by specific resting-state networks.

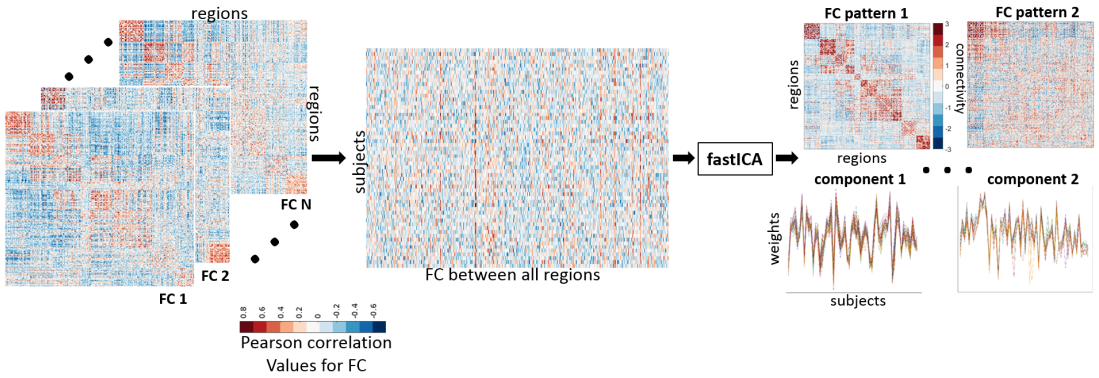

Figure 3. Connectivity independent component analysis (connICA) methodology. Individual functional connectivity (FC) matrices are concatenated into a group matrix where each row corresponds to one subject and columns are the functional connectivity entries of each subject's FC matrix. FastICA extracts components (i.e., FC patterns) associated to the cohort and their relative weights across subjects. Color bars indicate positive (red) and negative (blue) values; Pearson correlation coefficient values for individual FC matrices (left side of figure) and unit-less connectivity weights for the FC patterns (right side of figure).

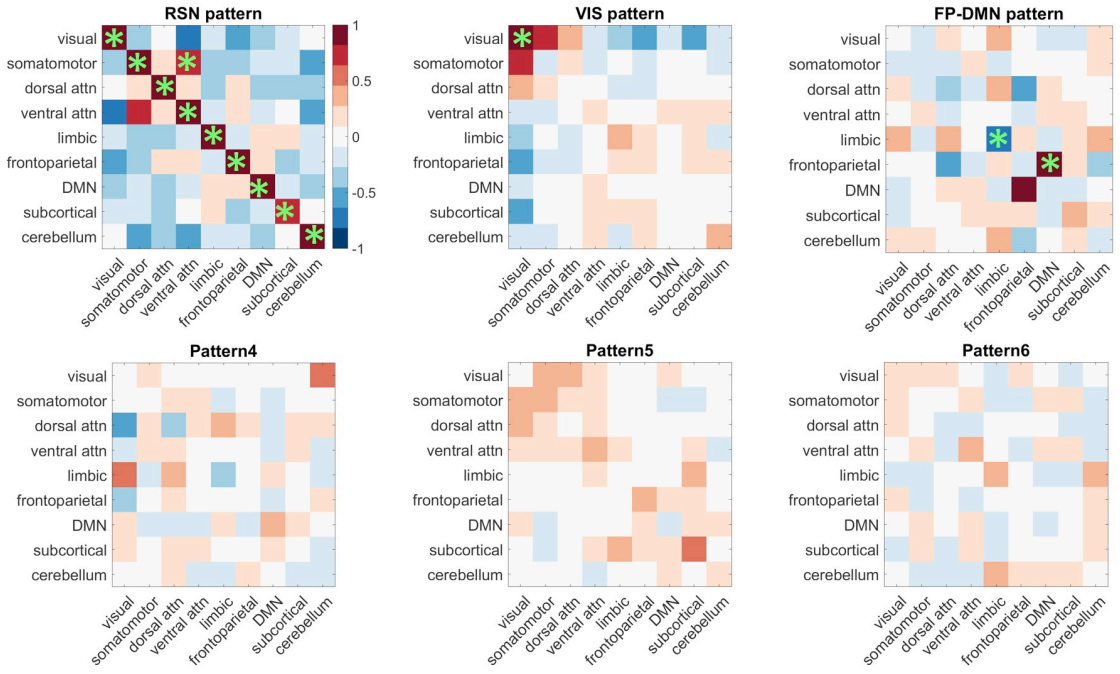

Figure 4. Functional connectivity pattern selection criteria. The six robust patterns generated by connICA are illustrated by matrices of the loading values averaged within each block either within the described RSNs (diagonal) or between RSN pairs (“interaction”; off-diagonal). Green asterisk denotes significant blocks (only the upper triangle of these symmetric matrices is denoted). Only three FC patterns shown in the top row had significant blocks and were considered in subsequent analyses.

Results

Identification of FC patterns

The three FC patterns identified using connICA and passing the pre-specified selection criteria were (1) resting-state network or RSN-pattern; (2) visual network or VIS-pattern; and (3) fronto-parietal and default mode networks or FP-DMN pattern (Figure 5). RSN-pattern primarily captures a within-network coherence of the RSNs introduced by Yeo et al [12][14]. The VIS-pattern was characterized primarily by a significant increase of within-network connectivity in the visual network. Lastly, FP-DMN pattern primarily showed an increased interaction between DMNs and fronto-parietal networks.

Relationship of neurocognitive variables of interest and FC patterns

As expected, the CN group had the highest CVLT-delayed and WCST performance followed by the SCD group, MCI, and AD groups (Table 3). Similarly, CCI scores differed across diagnoses, in part, by definition as cognitive complaints are part of the criteria for SCD and MCI.

The multilinear regression models including age, gender, education, and neurocognitive variables of interest from Table 2 were performed for each of the three identified FC patterns, with significant results summarized in Table 5.

The first significant finding was observed in the model that included age, gender, education, and CCI-self scores as predictors for subject's weights of the RSN-pattern (Figure 6A, top panel). The multiple regression model with only nuisance variables as predictors yielded an R^2 value of 0.11. However, the inclusion of CCI-self score increased the R^2 value to 0.20 (Figure 6A, middle

panel), which was significantly predictive of RSN-pattern weight (Figure 6A, bottom panel). Specifically, we found that a higher level of self-perceived cognitive decline (e.g., more complaints) was associated with a lower subject weight in the RSN-pattern.

The second significant multilinear regression model included age, gender, education, and CCImax scores as predictors for subjects' weights for the VIS-pattern (Figure 6B, top panel). If only nuisance variables were used as predictors for the subjects' weights of VIS-pattern, the R^2 value was 0.01. However, the R^2 value increased to 0.10 with the addition of CCImax as a predictor, with CCImax significantly predictive within the model for VIS-pattern weights (Figure 6B, bottom and middle panels). In this analysis, we observed that a higher maximum complaint score (reported from either the subject or a knowledgeable informant; CCImax) was associated with a lower subject weight score in the VIS-pattern.

The third significant multilinear regression model included age, gender, education, and CVLT scores as predictors for subjects' weights of the FP-DMN-pattern (Figure 6C, top and bottom panels). Using only nuisance variables as predictors yielded an R^2 value of 0.11. CVLT-delayed score increased the R^2 value to 0.14 (Figure 6C, middle panel). In other words, the better the episodic memory performance (i.e., higher CVLT-delayed score), the more that participant exhibited a pattern of increased communication between two important RSNs known to be involved in episodic memory, the DMN and fronto-parietal network.

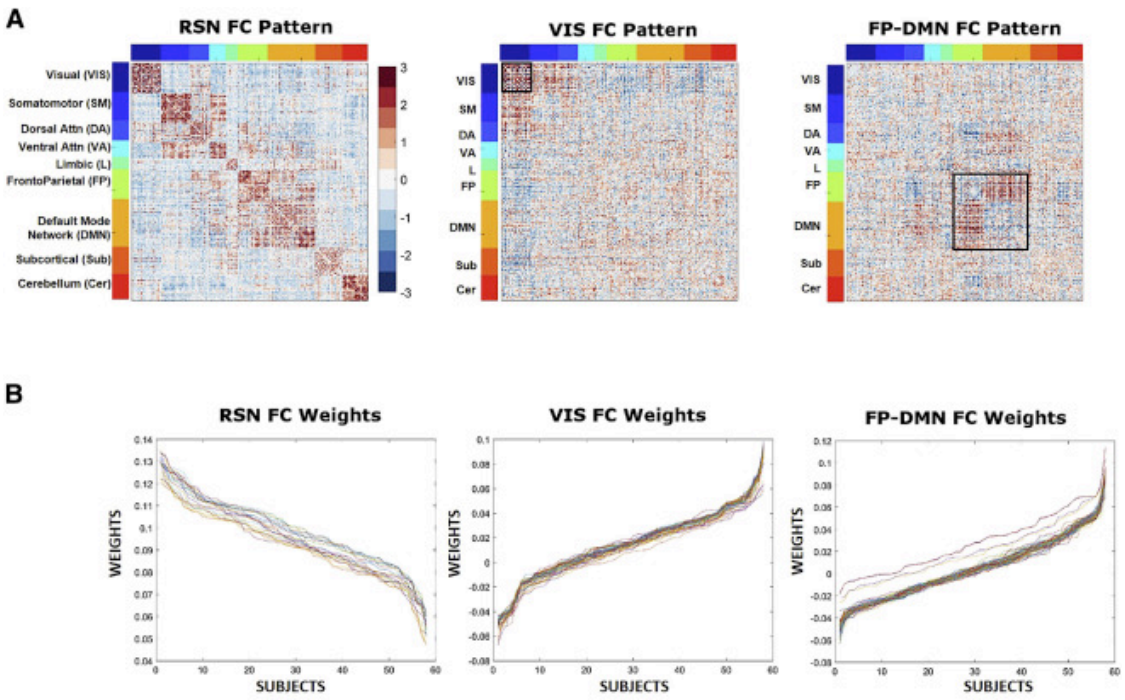

Figure 5. Robust FC patterns and individual weights as obtained by connICA. (A) Visualization of the FC patterns sorted according to Yeo et al. (2011) functional RSNs. (B) Lines represent the quantified presence of each FC pattern on each individual's functional connectome (across all runs), termed as “weights.” All 58 subjects are represented along the x axis and ordered from high to low presence of the corresponding FC pattern within their functional connectome. Each colored line represents a single ICA run.

Table 5. Significance values for multilinear regression models. NOTE. Table shows significantly predictive multilinear regression models for the three FC patterns.

RSNpattern weights=$b_0+b_1.age_1+b_2.sex_2+b_3.educ_3+b_4.cognitivevariable_4$

VISpattern weights=$b_0+b_1.age_1+b_2.sex_2+b_3.educ_3+b_4.cognitivevariable_4$

FP-DMNpattern weights=$b_0+b_1.age_1+b_2.sex_2+b_3.educ_3+b_4.cognitivevariable_4$.

NOTE. Variables included show added significance to individual models. Significance denoted by asterisk ($p < 0.05$).

Pattern	Model F-value	Model *P*-value	Predictor *t*-value	Predictor *P*-value
RSN	3.40	0.015*	--	--
Age	--	--	-1.69	.097
Sex	--	--	0.29	.773
Education	--	--	0.55	.582
*CCIself	--	--	-2.39	.020*
VIS	1.35	0.267	--	--
Age	--	--	0.44	.659
Sex	--	--	0.84	.407
Education	--	--	-0.6	.550
*CCImax	--	--	2.04	.047*
FP-DMN	1.96	0.116	--	--
Age	--	--	-0.23	.823
Sex	--	--	-0.63	.535
Education			2.47	.017
*CVLT-del			0.27	.791

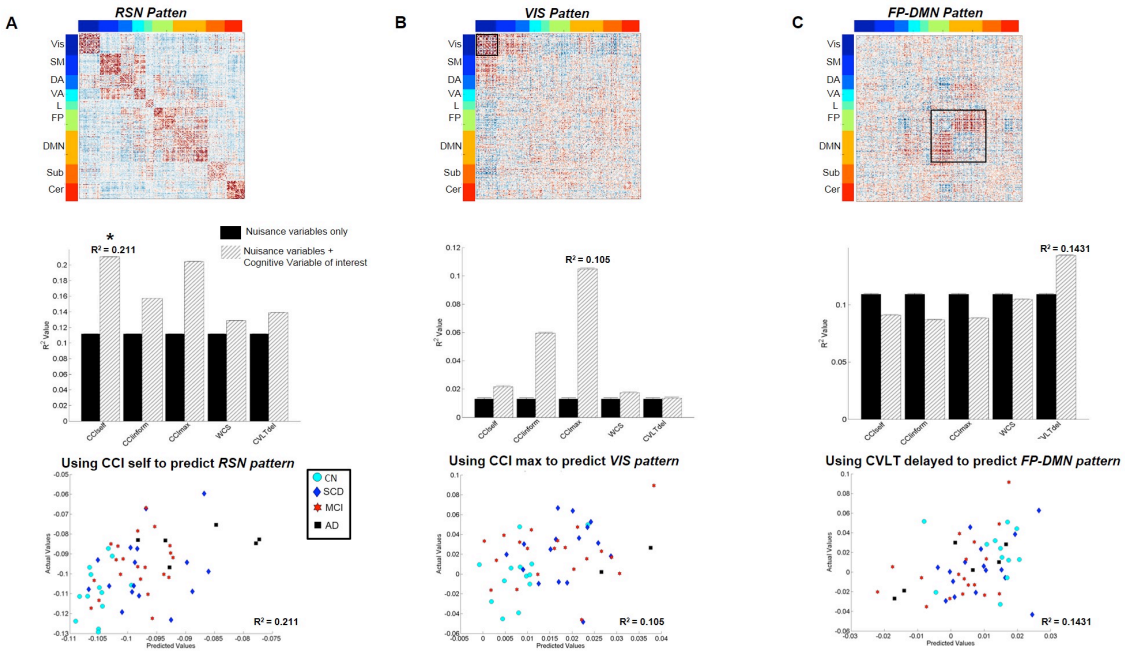

Figure 6. Relationship of FC patterns and neurocognitive variables of interest. Visualization of the three identified FC patterns (top row). The contributions of neurocognitive variables of interest showing significant increase of the baseline R^2 value in the multiple regression models are presented in the bar plots (middle row). A grouped bar plot where black bars indicate the baseline (only age, gender, and education) R^2 value, while the hatched patterned bars indicate when a given neurocognitive variable of interest has been added. The standard error bars were calculated across the 100 ICA runs. R^2 value is shown above the cognitive variable that has the greatest increase from the baseline R^2 value. The scatter plots (bottom row) show actual versus model-predicted subject weights with different symbols indicating group membership. The multilinear regression models include age, sex, education, and one of the neurocognitive variables of interest. The three columns illustrate the relationships of (A) RSN-pattern and CCI-self score; (B) VIS-pattern and CCImax score, and (C) FP-DMN pattern and

CVLT score. These relationships are further detailed in the Results section. Significance denoted by asterisk ($p < 0.05$).

Discussion

ICA is often used to evaluate the spatiotemporal structure of BOLD data and to extract functional connections from voxels' time series. In a similar vein, we applied a novel, data-driven connICA methodology directly onto functional connectome data sets to model and tease apart common underlying FC patterns of healthy and diseased human brains. We successfully extracted independent FC patterns and quantified the degree to which each pattern was present in each individual's functional connectome. Those individual estimations or pattern weights were used as the response variables in multilinear regression models (one per FC pattern) with nuisance covariates and cognitive performance variables included as predictors. This approach allowed a continuous mapping across different stages of AD within functional connectomes.

Of the initial six robust independent FC patterns, three (RSN, VIS, and FP-DMN) were considered biologically relevant and retained for further consideration. All three FC patterns showed associations with neurocognitive status including performance scores. Specifically, we found that SCD measures, considered to be among the earliest indicators of risk for AD, were negatively associated with FC in several important RSNs. These data indicate the potential utility of connectomics approaches in early detection and diagnosis.

The RSN-pattern, which resembles the canonically defined RSNs [12], was negatively associated with CCI-self score after controlling for age, gender, and education. In other words, lower FC within the RSN-pattern was seen in participants with more SCD (i.e., increased complaints). This finding is highly

relevant because in early stages of AD, self and informant concerns may precede clinically significant psychometric deficits in cognition, and cognitive complaints are believed to confer an increased risk of cognitive impairment and progression to dementia [105, 106]. Our finding suggests that disordered brain function, characterized by decreased coherence in major RSNs, may be related to self-perceived cognitive decline. This result further adds to growing literature that SCD may indeed reflect neuronal changes and suggests that RSN coherence may serve as a good biological predictor of decline [105, 106, 124]. If FC patterns could be used to identify individuals in a preclinical state of AD, then this technique has the potential to enhance therapeutic or preventative trials to identify disease-modifying treatments that can stop or slow clinical progression [125, 126].

The VIS-pattern had a strong negative association with CCImax score. In other words, higher levels of SCD (taken from either the subject or the informant) are associated with lower within-network FC in the visual network. This result provides evidence that impairment in brain areas responsible for visual cognition may be associated with early indications of cognitive impairment. This is in agreement with studies that have found similar links between visual impairment and early neurodegeneration [114, 127]. In fact, tests of visual cognition may be particularly sensitive to the effects of AD [128]. Importantly, we may be able to use the integrity of the FC in the visual network as a diagnostic marker to better characterize or identify early sensory impairments caused by early AD that may result in decreased quality of life for patients.

Lastly, features of FC in FP-DMN-pattern, which was characterized by strong connectivity of FC between the fronto-parietal network and DMN, were positively associated with episodic memory. This result is similar to those from previous studies that suggest that interdependent activity of DMN and parietal regions are involved in memory retrieval [129, 130]. Thus, our findings and others suggest that strong connections between brain regions in the fronto-parietal network and in the DMN are important for better episodic memory performance.

There are limitations to the present study. The sample size is modest, and there is unequal sampling across groups (CN, SCD, MCI, and AD). Although we did not analyze results based on group, these factors could affect the relative distribution of neurocognitive scores across the sample. Future studies with significantly larger overall and subgroup samples are warranted. Second, we elected to use a specific brain parcellation scheme [120] and referenced our results to a particular set of RSNs [12]. It is possible that alternative anatomic and functional templates would yield different results. Thus, future studies including multiple brain parcellation schemes and RSN definitions would help determine the validity of our findings. Third, the ICA approach is still being optimized, in particular with regards to the upper limit of components that the algorithm can extract. Fourth, permutation tests and cross-validation of the clinical cohort would enable nonparametric estimation of significance [131] and provide a data-driven approach to multiple testing correction. Fifth, we were unable to incorporate data from clinically relevant biomarkers such as β-amyloid and tau. To determine the impact of hallmark AD biomarkers on FC patterns,

future studies will incorporate plasma and cerebrospinal fluid concentrations of amyloid and tau, as well as positron emission tomography data with radioligands that bind to these proteins.

Finally, the field of brain connectomics has great promise for elucidating the complex relationships between cognitive decline and FC patterns. This approach has strong potential to generate clinically relevant biomarkers for brain function in prodromal AD and other neurodegenerative disorders.

Chapter 3: Cognitive Complaints Associated with Altered Resting State Networks: Replication in an Independent Cohort

Introduction

Network science has enabled the creation of powerful methodologies, which have allowed for a more comprehensive analysis of connectivity of neuronal systems. The studies of changes in whole brain functional connectivity (FC) across neurodegenerative diseases are of particular interest. connICA is a method that allows one to explore the breakdown in whole brain FC, which is described by the degree of synchronous blood-oxygen level dependent (BOLD) signals within the brain. Indeed, alterations in FC are thought to be one of the earliest indicators of neuronal dysfunction in Alzheimer's disease (AD) [132]. In fact, many studies have found aberrant functional connectivity in subjects diagnosed with early mild cognitive impairment (MCI), and studies that examined the FC of canonical resting state networks (RSNs) have shown a breakdown in brain communication along the neurodegenerative spectrum [97, 133-135].

However, as more studies use novel and often complex methodology [136] to discover how brain networks are affected in neurodegeneration, there is an emergent necessity to validate the measurements derived from these data [137]. Validation of these methods is critical for drug efficacy and for establishment of novel and clinically useful biomarkers. Validation of novel approaches is needed to eliminate the possibilities that the results are unique to a single study sample and that the underlying methodology is unstable. Consequently, reproducibility within the brain connectome field is considered

essential, and therefore, increasing studies address this issue[138]. In particular, the disruption of important resting state networks in the earliest stages of AD (i.e., a subjective cognitive decline population) has not been studied widely. Therefore, the aim of this study was to evaluate the reproducibility of the results from our previous study reported in Chapter 2.

To determine if we could replicate our previous finding showing that the number of cognitive complaints (considered to be an early indicator of AD) was negatively associated with positive connectivity within RSNs (specifically, an "RSN pattern"), we applied connectivity based ICA (connICA) to FC data from a separate cohort that was demographically and diagnostically similar to the one presented in Chapter 2 [139]. The connICA method offers distinct advantages. First, it allows us to objectively examine multiple resting state networks at once. Second, we can then relate connectivity within the resting state networks to cognitive variables of interest. Here, we hypothesized that, as reported previously, scores on the cognitive complaint Index (CCI[106], a measure of subjective cognitive decline) are associated with poorer connectivity in canonical resting state networks.

Methods

Participants

The Indiana University Institutional Review Board approved the study, and written informed consent was obtained. The cohort for the current study included a subset of older adults from the Indiana Alzheimers Disease Center (IADC), herein referred to as the IADC cohort. Participants were included based on

availability of resting state fMRI data and included: 25 subjects with significant cognitive complaints despite cognitive test performance within the normal range (Subjective Cognitive Decline, SCD), 14 participants with amnestic MCI, 12 AD patients, and 35 cognitively normal (CN) controls without significant levels of cognitive complaints (CN). The previously published, original cohort consisted of participants in the Indiana Memory and Aging Study (16 SCD, 22 MCI, 8 AD patients, 13 CN).

One-way ANOVA was used to test for differences in age, education and neurocognitive variables between groups, and when appropriate, across cohorts. Chi-squared test was used to assess differences in sex, as well as group membership across cohorts. Demographic information for both the IMAS and IADC cohort are presented in Table 3 and Table 6, respectively.

Neurocognitive Variables

All IADC participants underwent a comprehensive clinical assessment and neuropsychological battery which tested for attention, processing speed, executive function, episodic memory and language (detailed in [140]). Cognitive concerns were assessed using the Cognitive Change Index (CCI), which was completed by both the 1) participant ("self") and 2) an informant [141]. The sum of the first 12 items of the CCI-self and CCI-informant (maximum score = 60) were used as target variables. IADC variables also included 3) an average non-visuospatial executive function composite *z*-score, obtained by averaging the *z*-scores of Trail Making Part B [142] and the Digit span subtests of a version of the Wechsler Adult Intelligence Scale (WAIS); and 4) the Rey Auditory Verbal

Learning Test (RVLT) Long Delay Free Recall, a measure of episodic memory [143] (see Table 7).

Image acquisition and preprocessing

Subjects from IADC cohort were imaged on a 3T Siemens PRISMA MRI scanner with a 64-channel head coil array. Subjects were instructed to think of nothing in particular while remaining still with eyes closed. Resting-state functional magnetic resonance imaging (RS-fMRI) data were collected with the multi-band sequence as detailed in Xu et al. 2013 [144]: gradient echo, echo planar imaging (EPI), multi-band factor of 3, 500 BOLD contrast sensitive volumes resulting in the total scan duration of 10 min 7 sec, repetition/echo time TR/TE=1200ms/29ms, 2.5 mm^3 voxels, 54 slices.

An anatomical T1-weighted scan was acquired with whole brain coverage using a 3D Magnetization Prepared Rapid Gradient Echo (MPRAGE) sequence (220 sagittal slices, 1x1x1.2mm^3 voxels) per the Alzheimer's Disease Neuroimaging Initiative (ADNI-1) imaging protocol[117]. In the IADC cohort, we implemented an accelerated protocol (GRAPPA, R=2) to reduce imaging time from 9:14 sec to 5:12 sec.

Two short (12 sec) spin echo field-mapping scans (TR/TE=1560/49.8ms, one in A-P and one in P-A phase direction) with an imaging volume and voxel size identical to the RS-fMRI EPI were acquired immediately before the resting state scan. These spin echo scans, with an advanced B0 shim mode adjustment, optimized the field homogeneity and facilitated the RS-fMRI EPI volume distortion evaluation and unwarping [145]. This procedure was performed using

FSL's topup/applytopup [145], which yielded improved localization across the whole brain, most notably in ventral and frontal areas.

The resulting unwarped RS-fMRI data were preprocessed within the Matlab framework using FSL as recommended by Power et al [68, 69]: 1) Slice time correction, 2) Motion correction, 3) Tissue segmentation into white matter (WM), gray matter (GM), and CSF using T1-weighted MPRAGE, 4) Co-registration of T1 to RS-fMRI volumes through a sequence of transformations (FSL's FLIRT 6 DOFs, FLIRT 12 DOF's and nonlinear FNIRT), 5) Application of brain parcellation with 278 regions of interest (ROI) [146] in native RS-fMRI space, 6) Use of mode 1000 normalization and linear detrending of the BOLD signal, 7) Inclusion of 18 regressors (6 head motion parameters, mean WM, CSF, and whole-brain tissue-based signals and their 9 derivatives) and scrubbing procedure to exclude extreme head motion outlier volumes (method adapted from [69]), 8) Application of a first-order Butterworth bandpass filter (0.009Hz to 0.08 Hz), and 9) Use of 5 principal components in the WM and CSF tissue BOLD signal to regress them from the GM signal. The connectivity of brain regions in each participant was quantified using correlation coefficients (Pearson's *r*) between mean BOLD time series of each ROI pair, which were compiled into a symmetric 278 x 278 "FC matrix". Brain regions were further ordered into nine blocks: seven well-defined cortical RSNs [147], plus a subcortical and a cerebellar network (Figure 2). Methodological details can be found in Contreras et al.[148] and Amico et al. [149].

connICA Method

connICA [148, 149], a data-driven methodology that uses independent component analysis (ICA) to obtain FC patterns from a cohort, was applied to both IMAS and IADC datasets separately using FSL's FASTICA [121]. ICA input consisted of the FC profiles of all participants sorted into a single data set matrix, with 86 rows (e.g., one row per subject in the IADC cohort) and 38,503 columns corresponding to the diagonal and upper triangular elements of each FC matrix. The number of components was fixed to 15, as suggested by voxel-based ICA studies [150]. Due to the non-deterministic nature of ICA, connICA was performed 100 times, and only independent component patterns that appeared in at least 75 runs were deemed robust. Additionally, we required that each emergent pattern had an average Pearson correlation coefficient above 0.75 with the pattern from the first run. This method provides two outputs: 1) the "FC pattern", which represents an independent pattern of FC with values of connectivity units ("loadings"), and 2) a vector of the signed weights of the FC pattern present in each participant's FC matrix, which quantifies the relative magnitude of the trait in each individual (Figure 3). The output for the IADC and IMAS cohorts were 9 and 6 FC patterns, respectively.

At this stage, we applied additional selection requirements on the emergent FC patterns with the following steps: 1) A frequency histogram of FC loadings from all emergent FC pattern matrices was generated, and the value at the 95^{th} percentile of the distribution was used as a threshold, 2) For each pair of the predefined nine resting state networks (see Image Acquisition and

Preprocessing), we averaged the FC loadings, which resulted in a 9x9 matrix of FC loadings, and 3) We required that at least one block of the resultant matrix have an average value that equals or exceeds the 95th percentile value, which in this case was 0.55. This resulted in 6 and 3 robust FC patterns of interest for the IADC and IMAS cohort (Figure 7 and Figure 5, respectively) (detailed previously in [148]). These robust FC patterns were used for subsequent statistical analyses.

Statistical Analysis

To determine relationships between the FC patterns and cognitive variables, we used hierarchical multi-linear regression analysis (SPSS, version 24; [151]). Subject weights for each emergent robust FC pattern were used as the response (dependent) variable, with up to five predictors (independent variables). Nine models were tested for each FC pattern. Model 1 (null model) included only nuisance variables (age, sex, and years of education). Subsequent models included the three nuisance variable plus one cognitive variable. To assess the significance of each predictor, for each model, we calculated change in R^2 (additive R^2 value)[152] to assess the influence of each cognitive variable relative to model 1.

Table 6. Subject demographics and cognitive scores in IADC cohort. Values are the means and standard deviations within each group for selected demographic and neurocognitive variables

	CN	**SCD**	**MCI**	**AD**	***p-value***	***Posthoc Results***
N	35	25	14	12	--	--
Mean age(y)	65.26(9.05)	70.32(10.42)	69.07(9.56)	69.92(13.53)	*NS*[*]	--
Sex(M/F)	8/27	9/16	8/8	5/7	*NS*[†]	--
Mean Education(y)	16.63(2.34)	16.80(2.63)	16.21(2.16)	14.50(3.48)	*NS*[*]	--
Mean RVLT-delayed (episodic memory)	10.54(2.60)[§≠]	9.80(3.37)[§≠]	4.23(2.83)[ǂ¥≠]	6.25(6.61)[ǂ¥§]	*p<0.001*[*]	*CN,SCD>MCI>AD*
Mean Composite Executive function	0.00(0.65)[§≠]	-0.22(1.39)[§≠]	-0.96(1.30)[ǂ¥≠]	-2.91(1.55)[ǂ¥§]	*p<0.001*[*]	*CN,SCD>MCI>AD*
Mean subject CCI score	26.24(4.53)[§≠]	42.32(10.32)[§≠]	50(16.31)[ǂ¥≠]	41.86(17.18)[ǂ¥§]	*p<0.001*[*]	*CN,SCD>MCI>AD*
Mean informant CCI score	23.26(4.34)[§≠]	27.29(10.12)[§≠]	45.83(20.36)[ǂ¥≠]	71(28.52)[ǂ¥§]	*p<0.001*[*]	*CN,SCD>MCI>AD*

[*]One-way ANOVA; [†]Chi-squared). Post-hoc *t*-tests, $p<0.05$: [ǂ] significantly different from CN, [¥] significantly different from SCD, [§]significantly different from MCI, [≠]significantly different from AD.

Table 7. Matched neurocognitive variables used for IADC cohort.

Assessment	Brain Regions	Relevance for AD	Measures
Executive Function Composite Score	Frontal lobe: Dorsolateral prefrontal cortex, orbitofrontral cortex, anterior cingulate cortex	Executive function declines with disease progression	Combined measure of tests of executive function Averaged z-score of tests, Trail B, Digit Span Forward, Digit Span Backward
Rey Auditory Verbal Learning Test (RVLT) – delayed Recall	1.Medial temporal lobe: Hippocampus, entorhinal cortex, etc. 2.Precuneus 3.Medial prefrontal cortex	MCI: Impairment of delayed episodic memory recall AD: progressive worsening of episodic memory	15 nouns read aloud over five learning trials. After each trial, the subject is asked to recall as many words as they can in any order - then again after a delay
Cognitive Change Index (CCI) – self and informant[153]	Negative correlation with the hippocampal volume[106]	Older adults with preclinical and prodromal AD may report concerns. As AD progresses, complaints often decrease due to loss of self awareness	Self, Informant, and combined scores rate cognitive function

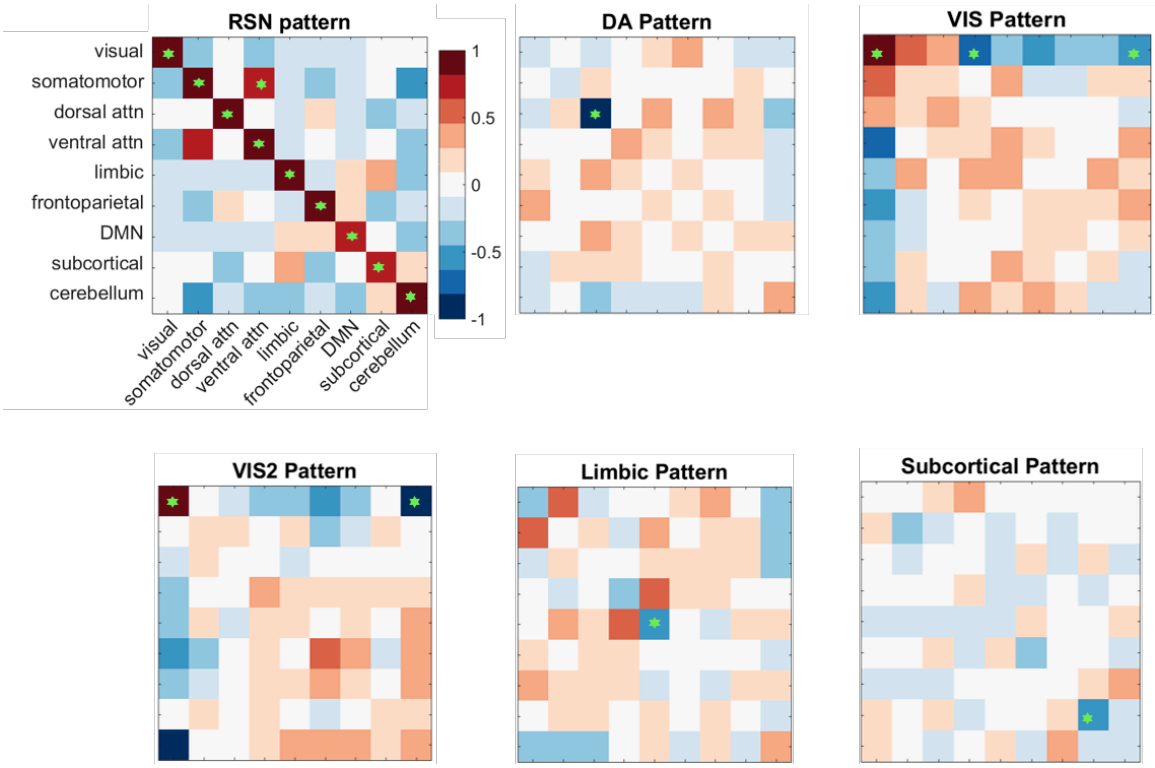

Figure 7. Selection of FC patterns of interest for IADC cohort. The six robust patterns generated by connICA are illustrated by matrices of the loading values averaged within each block either within the described RSNs (diagonal) or between RSN pairs (“interaction”; off-diagonal). Green asterisk denotes significant blocks considered in subsequent analyses (only the upper triangle of these symmetric matrices is denoted).

Results

Subject characteristics and cognitive scores for the IADC cohort are presented in Table 6. Compared to CN and SCD, MCI and AD groups had lower scores on the executive function composite score and RAVLT. By definition, CCI scores differed across diagnoses, as cognitive complaints are part of the diagnostic criteria for SCD and MCI.

No differences were found in age, sex, and education between groups across study cohorts. Group sizes differed across cohorts, with more CN and less MCI participants and in the IADC cohort compared to IMAS.

Demographics and a subset of neuropsychological data from the IMAS cohort are presented in Chapter 2, Table 3.

Results: IADC Cohort

Six robust FC patterns emerged (Figure 7 and 8), and involved: (1) all 9 resting-state networks (RSN pattern), (2) dorsal attention (DA) network, (3) and (4) visual network (VIS and VIS2, respectively), (5) limbic network, and (6) subcortical network. The RSN-pattern captures a within-network coherence of the cortical resting state networks as described by Yeo et al [147] (Figure 2).

There were several cognitive variables that were associated with the RSN weights: CCI self and RAVLT delayed score each significantly increased R^2 value compared to the null model (Model 1a, Table 8). CCI self score had a significant negative association with the RSN-pattern, which was consistent with our prior report [148]. RAVLT-delayed score showed a significant positive association with the RSN pattern (Table 8; Figure 9).

There were no other significant predictors of any neurocognitive variable for any of the five remaining robust FC patterns (data not shown).

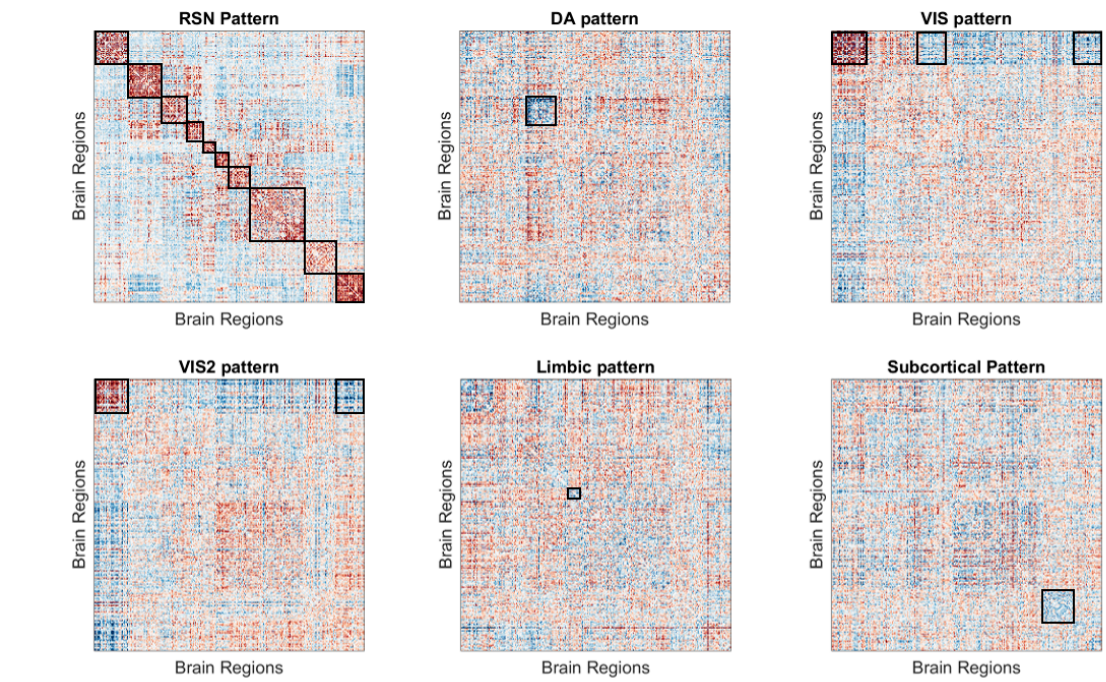

Figure 8. Robust FC patterns of interest for IADC cohort. (1) The RSN pattern captures a within network coherence of the cortical resting state networks as described by Yeo et al. (2) The DA pattern had positive connectivity in the dorsal attention network. (3) The VIS pattern was characterized primarily by a significant within-network connectivity in the visual network. (4) The VIS2 pattern was characterized primarily by the significant positive connectivity in the visual network as well as a significant negative interaction between the cerebellum and visual network. (5) The Limbic pattern was characterized by the negative connectivity within the limbic network and (6) The Subcortical pattern involved negative connectivity within subcortical brain regions.

Table 8. Significance values for hierarchical regression models in IADC cohort applied to the RSN pattern weights. Table shows significantly predictive regression models. Only the variables that added significance to individual models are presented. The asterisk indicates that a model included covariates.

Model Tested (Dependent Variable)	Model *F*-value	R^2 value	R^2 change	Significance of F-change	Model *p*-value	Model coefficients (β)	Predictor *p*-value
Model 1 (covariates only)	1.70	0.07	--	0.18	*NS*	--	--
Age	--	--	--	--	--	-0.19	*NS*
Sex	--	--	--	--	--	0.19	*NS*
Education	--	--	--	--	--	-0.04	*NS*
Model 2	2.37	0.13	0.06	0.05	*0.05*	--	--
*CCI self-score	--	--	--	--	--	-0.25	*0.047*
Model 3	2.14	0.12	0.05	0.07	*NS*	--	--
*CCI informant-score	--	--	--	--	--	-0.24	*NS*
Model 4	1.48	0.08	0.01	0.37	*NS*	--	--
*Executive Function Composite Score	--	--	--	--	--	0.15	*NS*
Model 5	2.57	0.14	0.07	0.03	*0.05*	--	--
*RVLT-delayed Score	--	--	--	--	--	0.28	*0.03*
*included covariates							

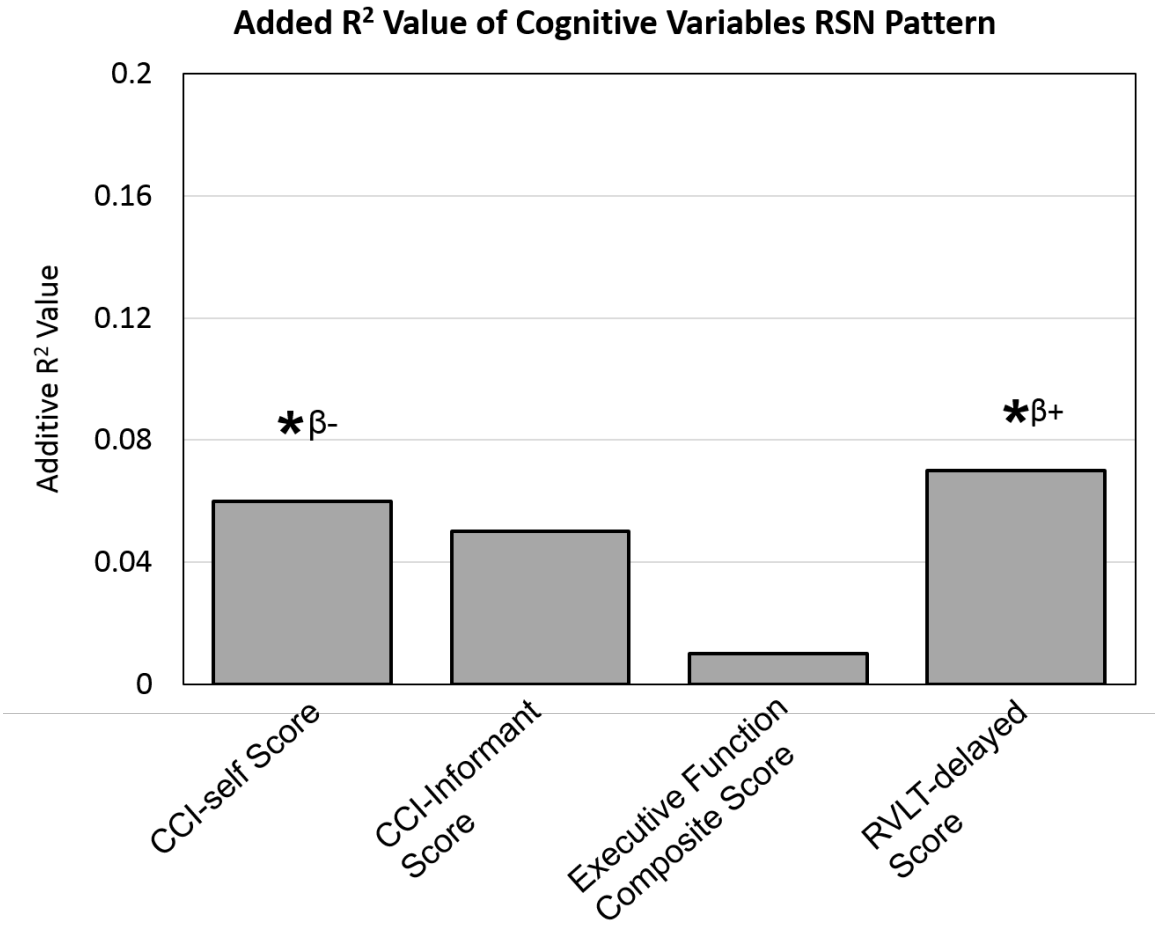

Figure 9. Relationship between RSN pattern weights and neurocognitive variables of interest for IADC cohort. The contributions of neurocognitive variables of interest showing R^2 change from the baseline R^2 value (from null Model 1) in the hierarchical regression models are presented in the bar plots. The cognitive variables that significantly increased the R^2 value (predictability) are denoted by an asterisk. β- and β+ indicate negative and positive directions, respectively, of the variable with the RSN pattern weights.

Discussion

To validate our previously reported findings correlating RSN functional connectivity pattern with one of the earliest signs of cognitive decline (CCI self-score) [139], we performed the same analyses on an independent dataset that included the same diagnostic groups. In this replication, we used a data-driven connectivity based methodology, connICA [112], to analyze functional connectome data across the AD continuum, from healthy to early and late stages of AD. This yielded six robust independent FC patterns that were brought forward for further statistical analyses. Of those six, the RSN FC pattern was replicated in the IADC cohort and found to be significantly associated with cognitive variables, including CCIself. Thus, we replicated our initial result that CCI self-score was negatively correlated with the RSN pattern.

The RSN pattern resembled the canonically defined RSNs [147] and was significantly negatively associated with the CCI self-score and positively associated with RAVLT-delayed score (after controlling for age, gender and education) in a hierarchical linear regression model. We believe the CCISelf-RSN result to be highly relevant for several reasons. The first is the reoccurrence of the robust RSN pattern across cohorts. This pattern captures the functional connectivity organization of the human brain, which is disrupted in many pathological states. Therefore, relating this functional connectivity backbone to cognitive variables of interest in a reproducible manner is important. Secondly, our findings provide additional evidence to support the idea that SCD, the earliest detectable stage of AD [154, 155], may be associated with disordered brain

function, characterized by lower coherence of the RSNs. This supports the hypothesis that subjective cognitive decline reflects neuronal changes and suggests that RSN coherence may serve as a biological predictor of cognitive decline.

We also found that RAVLT-delayed score was positively associated with the RSN pattern weights, which may suggest that a high episodic memory score is associated with higher FC coherence within canonical resting state network blocks. This extends the existing literature to earlier stages, as episodic memory is known to be severely compromised in AD [156-158]. While some studies have focused on episodic memory in relation to the medial temporal lobe and default mode network systems [159-161], alterations of FC in multiple RSNs (in addition to the default mode network, DMN) have been reported in memory-impaired patients [162]. In fact, Castellazzi and colleagues reported prominent alterations FC in MCI and AD patients, including the DMN, as well as frontal, visual, cerebellar, basal ganglia (motor) and anterior insular (or attentional) networks. Other studies have reported similar results that implicate multiple brain networks in addition to the DMN [161, 163-165]. Overall, our study provides additional evidence that healthy FC in many RSN networks is important for a performance of episodic memory tasks, and that abnormal FC in these networks may be related to memory deficits.

The challenges of reliability and reproducibility of resting state network and rsfMRI findings have been recognized for some time [166, 167]. These challenges consist of the known inter- and intra- individual variability that exists

within a person's functional connectome [168, 169]. These types of variance can be caused by differences in instructions given to participants (for example, eyes closed, open or fixated on a crosshair), subjects' head motion during imaging (often more problematic in clinical groups), differences in scan duration (e.g., a reported range of 5-15 min), and the inherent differences between scanner platforms, head coils, and pulse sequences.

Additionally, a particular methodological and conceptual limitation of resting-state fMRI data analyses should be noted. For instance, studies have identified "task-positive" and "task-negative" networks in both resting state and task-based conditions [19, 170]. The "negative" or "anti-correlated" networks are thought to hold functional significance in domains of attention, higher cognitive control, and perhaps consciousness [171], and as such, could be central to disorders associated with cognitive impairment [172]. Yet, outstanding questions remain regarding anti-correlated data, as certain methodological steps (i.e. inclusion of a global signal regression to the preprocessing of resting state fMRI data) are thought to induce spurious negative correlations [173]. We overcame this concern by assessing our functional connectome matrices with and without global signal regression (GSR) to determine if the number of anti-correlations increased with the inclusion of GSR (it did not).

Lastly, it has been shown that FC patterns in RS-fMRI exhibit dynamic behavior on the scale of seconds with varying spatiotemporal structure [174]. In fact, FC states have been shown to have a strong relationship with ongoing cognition [89, 175, 176] and, as such, analyses using averaged RS-fMRI data

should be interpreted with caution, with the knowledge that brain states may be changing during the acquisition. Furthermore, methodological considerations such as the choice of regional brain parcellations (anatomical vs. functional, finer vs. coarser) and amount of variance retained or removed during the preprocessing likely influence the strength of measured FC.

Despite the aforementioned challenges, our ability to replicate our initial novel results in two independent cohorts acquired on different platforms is encouraging, and suggestive of the validity of the connICA approach.

There were several specific limitations to this study. Diagnostic group proportions were significantly different across the two cohorts. However, between-group analysis of each diagnostic group across the IADC and IMAS cohorts showed no significant differences in age, sex and education (e.g., there were no differences in age, sex and education between the CN samples from IMAS and IADC cohorts). A larger cohort would help strength results; in particular, the inclusion of more MCI and AD subjects could improve the distribution of neurocognitive scores across the entire cohort. Additionally, we cannot know whether the SCD subjects will develop AD; however, it should be noted that longitudinal data collection is underway, and will be available for future analyses. As this was cross-sectional data, longitudinal follow-up will be particularly useful in confirming diagnosis. Although we included age, sex, and education as model covariates, it would be instructive to investigate the specific roles these factors played in the current results. Lastly, we applied two parcellations to our data, an anatomic parcellation from Shen et al [120] and a

functional grouping of RSN networks from Yeo and colleagues [12]. The exploration of other methods of parcellation would allow us to evaluate which approaches may be the most appropriate to address specific scientific questions.

In conclusion, network science has become an important tool in neuroscience research. The application of novel analytical approaches to functional imaging of the brain has led to fundamental insights within the AD field [177-180], and increasingly has led to new potential methods for early detection of AD [177, 181-183]. Therefore, it is imperative that the replicability of these methods be thoroughly investigated. This is necessary to ensure sound and effective development of objective biomarkers for early detection of AD, which is important for timely diagnosis of and intervention.

Chapter 4: Mapping Verbal Fluency onto Resting State Networks across the Alzheimer's Disease Spectrum

Introduction

Assessment of semantic memory via verbal fluency measures has long been an important component of clinical neuropsychological evaluation in diagnosing Alzheimer's disease (AD) [184, 185]. Indeed, animal naming (which requires the subject to generate as many animal names as possible in 60 seconds) and letter fluency are common tests of phonemic fluency. Poor performance on these tasks has been shown to be a prominent clinical feature in prodromal AD. Progressive deficits in verbal fluency during the course of AD make these cognitive tests useful markers of progression of AD [186].

In addition to the many cognitive problems exhibited by AD patients and those at risk for AD, functional abnormalities in resting state networks (RSNs) have also been described using resting-state functional magnetic resonance imaging (RS-fMRI) [187-189]. In RS-fMRI, oscillations of spontaneous neuronal activity (inferred from blood oxygenation level dependent (BOLD) signal fluctuations) in the absence of external stimuli are used to define brain regions that work together. These networks are thought to mediate the cognitive functions that are deficient in AD patients and examination of these RSNs may provide insight into the neural bases of the disease. AD patients have been shown to have disrupted connectivity within the default mode network (DMN) (consisting of the posterior cingulate cortex and precuneus, the medial prefrontal cortex and angular gyrus), which are thought to be involved in high-level and self-

referential cognition [190]. Decreased RS-fMRI functional connectivity (FC) between the left thalamus and left frontal areas has been associated with AD patients' immediate/delayed scores on an auditory verbal learning test [191]. Additionally, RS-fMRI FC values between the thalamus and temporo-parietal regions positively correlate with patients' scores on the Mini Mental State Examination [192]. Moreover, the degree of disconnection can be attributed to stage of the disease, meaning that patients diagnosed as having mild cognitive impairment (MCI) have more preserved functional connectivity than those who have progressed to AD [193]. These results are based on *a priori* hypotheses using seed-based connectivity methods. In the present study, we adopted a novel data-driven global connectome-based approach to study how functional connectivity relates to verbal fluency performance among individuals along the AD spectrum. Examining verbal fluency with an objective global sampling method has the potential to identify aberrant patterns of resting state FC across brain regions and networks that have not previously been explored or associated with verbal fluency performance.

Few studies have examined the functional neural correlates of verbal fluency using a global network approach. The neural basis of verbal fluency has traditionally been examined using region- or voxel-based approaches with fMRI [194-196]. However, region-based methods are often spatially restrictive, and do not evaluate possible effects outside of the *a priori* specified brain regions. For example, previous RS-fMRI studies of verbal fluency have been limited to subcortical areas and well-known language areas, such as the DMN [188],

fronto-parietal cortex, and Broca's and Wernike's language areas [197]. Therefore, the aim of this study was to examine if verbal fluency performance can be associated with RSNs known to be disrupted in AD, including areas outside of traditionally studied language regions in the brain. To do so, we applied brain connectomics to provide data-driven large-scale analyses of RS-fMRI data. Specifically, we employed a recently developed connectome analytical framework, connICA, which has been used to quantitatively relate patterns of resting state functional connectivity (FC) [149] and relevant continuous cognitive variables [148].

In this study, we used connICA to examine how verbal fluency measurements map onto resting state FC patterns. To maximize the dynamic range of our data, we studied subjects across the entire AD spectrum, including the earliest stage of AD, before clinical symptoms become manifest. To test the validity of our findings, we applied the same methodology to a diagnostically- and demographically-similar dataset from the "IMAS cohort" reported in [148].

Methods

Participants

The Indiana University Institutional Review Board approved the study, and written informed consent was obtained for each participant from both cohorts. The first cohort included a subset of older adults from the Indiana Alzheimer's disease Center (IADC), herein referred to as the IADC cohort. Participants were included in the present study based on availability of resting state imaging data: 22 with subjective cognitive decline (SCD) despite cognitive test performance

within the normal range, 12 participants with amnestic mild cognitive impairment (MCI), 11 AD patients and 29 cognitively normal controls with minimal cognitive complaints (CN). The replicate cohort consisted of participants in the Indiana Memory and Aging Study (IMAS): 16 in the SCD group, 22 participants with amnestic MCI, 8 AD patients, and 13 CN controls. Other data from this group have been published previously [148].

One-way ANOVA was used to test for differences in age, sex, education, and neurocognitive variables between groups, and when appropriate, across cohorts. The Chi-squared test was used to assess putative differences in gender, as well as in group membership across cohorts. Demographic information for both the IMAS and IADC cohort are presented in Table 9 and Table 10, respectively [148].

Neurocognitive Variables

All participants underwent a comprehensive clinical assessment and neuropsychological battery which tested for attention, processing speed, executive function, episodic memory, and language (detailed in: [140]). Data from the IADC cohort included: 1) a verbal fluency composite *z*-score, which was an average of the *z*-scores of the following tasks: animal and vegetable list generation and C-F-L letter task [198]; 2) animal naming raw score; 3) vegetable task raw score; and 4) C-F-L letter task raw score.

The IMAS cohort data included: 1) verbal fluency composite score obtained by averaging the raw, *z*-scored tasks: animal fluency, controlled oral

word association (COWA) letter fluency [199], and Boston naming task [200]; and 2) animal naming raw score.

Image acquisition and preprocessing

Subjects from the IADC cohort were imaged on a 3T Siemens PRISMA MRI scanner with a 64-channel head coil array. Whole brain RS-fMRI data were collected; subjects were instructed to think of nothing in particular while remaining still with eyes closed. We implemented the multi-band sequence as detailed in [201]: gradient echo sequence, echo planar imaging (EPI), scan duration 10 min 7 sec, 500 BOLD contrast sensitive volumes, multi-band factor of 3, repetition/echo time TR/TE=1200ms/29ms, isotropic 2.5 mm^3 voxels, 54 interleaved axial slices. The IMAS cohort was imaged on a 3T Siemens Tim Trio MRI scanner with a 12-channel head coil array using: a product gradient echo sequence, echo planar imaging, scan duration 6 min 9 sec, 161 BOLD contrast sensitive volumes, repetition/echo time TR/TE=2250ms/29ms, 2.5x2.5x3.5 mm voxels, 39 interleaved axial slices covering the whole brain, GRAPPA acceleration factor 2, 3D PACE prospective motion correction.

Subjects from both cohorts were scanned with a T1-weighted whole brain anatomical 3D Magnetization Prepared Rapid Gradient Echo (MPRAGE) sequence (220 sagittal slices, 1x1x1.2mm^3 voxels) following the Alzheimer's Disease Neuroimaging Initiative (ADNI-1) imaging protocol. In the IADC cohort, we implemented an accelerated protocol (GRAPPA, R=2) to reduce imaging time from 9:14 sec (IMAS dataset) to 5:12 sec.

Two short (12 sec) spin echo field-mapping scans (TR/TE=1560/49.8ms, one in A-P and one in P-A phase direction) with an imaging volume and voxel size identical to the RS-fMRI EPI were acquired immediately before the resting state scan. These spin echo scans, with an advanced B0 shim mode adjustment, optimized the field homogeneity and facilitated the RS-fMRI EPI volume distortion evaluation and unwarping [145]. This procedure was performed using FSL's topup/applytopup [145], which yielded improved localization across the whole brain, with the most notable improvements in frontal areas.

The resulting unwarped RS-fMRI data were preprocessed within the Matlab framework using FSL as recommended by Power et al. [68, 69]: 1) slice time correction, 2) motion correction, 3) tissue segmentation of white matter (WM), gray matter (GM), and CSF using T1-weighted MPRAGE, 4) co-registration of T1-weighted MPRAGE to RS-fMRI volumes through a sequence of transformations (FSL's FLIRT 6 DOFs, FLIRT 12 DOFs and nonlinear FNIRT), 5) application of brain parcellation with 278 regions of interest (ROIs) [120] in native RS-fMRI space, 6) use of mode 1000 normalization and linear detrending of the BOLD signal, 7) Inclusion of 18 regressors (6 head motion parameters, mean WM, CSF, and whole-brain tissue-based signals and their 9 derivatives) and scrubbing procedure to exclude extreme head motion outlier volumes (method adapted from [69]), 8) application of a first-order Butterworth bandpass filter (0.009Hz to 0.08 Hz), and 9) use of 5 principal components in the WM and CSF tissue BOLD signal to regress them from the GM signal. The connectivity of brain regions in each participant was quantified using correlation coefficients

(Pearson's *r*) between mean BOLD time series of each ROI pair, which were compiled into a 278 x 278 "Functional Connectivity matrix". Brain regions were further ordered into nine blocks: seven well-defined cortical RSNs (Yeo et al., 2011), plus blocks of subcortical and cerebellar regions (Figure 2). Details can be found in [112, 139].

connICA method

connICA [148, 149], a data driven methodology that uses independent component analysis (ICA) to obtain FC patterns from a cohort, was applied to both IMAS and IADC datasets separately using FSL's FASTICA [121]. ICA input consisted of the FC profiles of all participants sorted into a single data set matrix, with 86 rows (one row per subject) and 38,503 columns corresponding to the diagonal and upper triangular elements of each FC matrix. The number of components was fixed to 15, as suggested by voxel-based ICA studies [150]. Due to the non-deterministic nature of ICA, connICA was performed 100 times, and only independent component patterns that appeared in at least 75 runs were deemed robust. Additionally, we required that each emergent pattern had a Pearson correlation coefficient above 0.75 with respect to the pattern from the first run. This method provides two outputs: 1) the "FC pattern", which represents an independent pattern of FC with values of connectivity units ("loadings"), and 2) a vector of the signed weights of the FC pattern present in each participant's FC matrix, which quantifies the relative magnitude of the trait in each individual (Figure 3). The outputs for the IADC and IMAS cohorts were 9 and 6 FC patterns, respectively.

At this stage, we applied additional selection requirements on the emergent FC patterns with the following steps: 1) a frequency histogram of FC loadings from all emergent FC pattern matrices was generated, and the value at the 95th percentile of the distribution was used as a threshold; 2) for each pair of the nine network blocks, we averaged the FC loadings, which resulted in a 9x9 matrix of FC loadings; and 3) for interpretability reasons, we required that at least one canonically defined RSN cell had an average value that equaled or exceeded the 95th percentile, which in this case, was 0.55. This resulted in 6 and 3 robust FC patterns of interest for the IADC and IMAS cohort, respectively (Figure 7 and Figure 4, respectively) (detailed previously in [148]). These robust FC patterns were used in subsequent statistical analyses.

Statistical Analysis

To determine relationships between the FC patterns and cognitive variables, we used a hierarchical multi-linear regression analysis (SPSS, version 24; [151, 152]). Subject weighting from the emergent robust FC patterns was used as the response (dependent) variable, with up to five predictor (independent) variables. Nine models were tested for each FC pattern. Model 1 (null model) included only nuisance variables (age, sex, and years of education). Subsequent models included the three nuisance variable plus one cognitive variable. To assess the significance of each predictor for each model, change in R^2 (additive R^2 value)[152] was calculated with respect to model 1.

Results

No differences were found in age, sex, and education between groups across cohorts. Group membership differences were found across cohorts. The IADC cohort had proportionally more CN and fewer MCI subjects compared to the IMAS cohort.

Subject characteristics and cognitive scores for the IADC cohort are presented in Table 9. Compared to CN, MCI and AD groups had lower scores on language fluency score. The SCD group had lower scores compared to AD only, and AD had lower scores compared to MCI. Compared to CN and SCD, MCI and AD groups had lower scores on both animal and vegetable fluency, with AD performing worse than MCI. There were no differences in letter fluency scores across CN, SCD, and MCI. Letter fluency was significantly lower in AD compared to all other groups.

Demographic and a subset of neuropsychological data (namely, available language fluency measures) from the IMAS cohort are presented in Table 10. Compared to CN, SCD, and MCI, the AD group had lower verbal fluency composite scores. Animal naming scores were significantly lower in MCI and AD groups compared to CN and SCD groups.

Results: IADC Cohort Only

Six FC patterns met specified criteria (displayed in Ch. 3, Figure 8). However, only the weights of two patterns (RSNs and subcortical network) were significantly associated with any of the analyzed neurocognitive variable.

The verbal fluency composite score was significantly negatively associated with RSN weights, and significantly increased R^2 value compared to the null model (Model 1). Both animal and letter fluency significantly increased R^2 value (Table 11; Figure 10).

For the subcortical weights, letter fluency significantly increased R^2 value compared to the null model (Figure 11; Table 12), and had a positive correlation with subcortical pattern weights.

Results: IMAS cohort Only

As reported previously [148] three FC patterns were identified using the same specified criteria as described in Chapters 2 and 3: 1) RSN pattern, 2) VIS-pattern, and 3) frontal parietal/default mode network (FP-DMN) pattern (Figure 5). We tested verbal fluency and animal fluency as potential predictors of FC pattern.

Similar to the IADC data, the RSN weights for the IMAS cohort were significantly predicted by both verbal fluency composite scores and animal fluency scores (Figure 12; Table 13).

Table 9. Subject demographics and verbal fluency scores in the IADC cohort. Values are the means and standard deviations within each group for selected demographic and neurocognitive variables.

	CN	**SCD**	**MCI**	**AD**	***p*-Value**	***Post hoc Results***
N	35	25	14	12	--	--
Mean age(y)	65.26(9.05)	70.32(10.42)	69.07(9.56)	69.92(13.53)	*NS*[*]	--
Sex(M/F)	8/27	9/16	8/8	5/7	*NS*[†]	--
Mean Education(y)	16.63(2.34)	16.80(2.63)	16.21(2.16)	14.50(3.48)	*NS*[*]	--
Verbal Fluency (composite score)	0.00(0.65)[§≠]	-0.16(0.89)[≠]	-0.73(1.33)[‡≠]	-2.75(1.16)[‡¥§]	*p<0.001*[*]	*CN>MCI>AD; SCD>AD*
Animal Fluency	23.06(3.96)[§≠]	22.04(4.89)[§≠]	18.29(5.66)[‡¥§]	8.45(6.15)[‡¥§]	*p<0.001*[*]	*CN,SCD>MCI >AD*
Vegetable Fluency	15.54(3.70)[§≠]	16.04(3.63)[§≠]	12.43(4.43)[‡¥§]	5.64(4.43)[‡¥§]	*p<0.001*[*]	*CN,SCD>MCI >AD*
Letter Fluency	30.89(6.51)[≠]	29.80(8.13)[≠]	29.07(11.47)[≠]	13.70(8.93)[‡¥§]	*p<0.001*[*]	*CN,SCD,MCI >AD*

[*]One-way ANOVA; [†]Chi-squared. Post-hoc t-tests, $p<0.05$: [‡] significantly different from CN, [¥] significantly different from SCD, [§]significantly different from MCI, [≠]significantly different from AD.

Table 10. Subject demographics and verbal fluency scores in the IMAS cohort. Values are the means and standard deviations.

	CN	**SCD**	**MCI**	**AD**	***p*-Value**	**Posthoc Results**
N	13	16	21	8	--	--
Mean age(y)	67.15(5.50)	73.38(7.95)	73.33(8.98)	76.38(8.98)	*NS**	--
Gender(M/F)	1/12	8/8	9/13	2/6	*NS*†	--
Mean Education(y)	17.32(1.93)	17.38(1.9)	16(2.8)	16.13(3.7)	*NS**	--
Verbal Fluency (composite score)	-0.10(1.51)$^{\neq}$	0.38(.98)$^{\S\neq}$	-1.18(1.96)$^{¥\neq}$	-2.98(2.57)$^{‡¥\S}$	*p<0.001**	CN>AD;SCD> MCI>AD;
Animal Fluency	25.88(5.87)$^{\S\neq}$	23.73(6.20)$^{\S\neq}$	19.00(4.28)$^{‡¥\S}$	11.33(2.42)$^{‡¥\S}$	*p<0.001**	CN,SCD>MCI >AD

∗One-way ANOVA; †Chi-squared. Post-hoc t-tests, p<0.05: ‡ significant difference from CN, ¥ significant difference from SCD, §significant difference from MCI, ≠significant difference from AD.

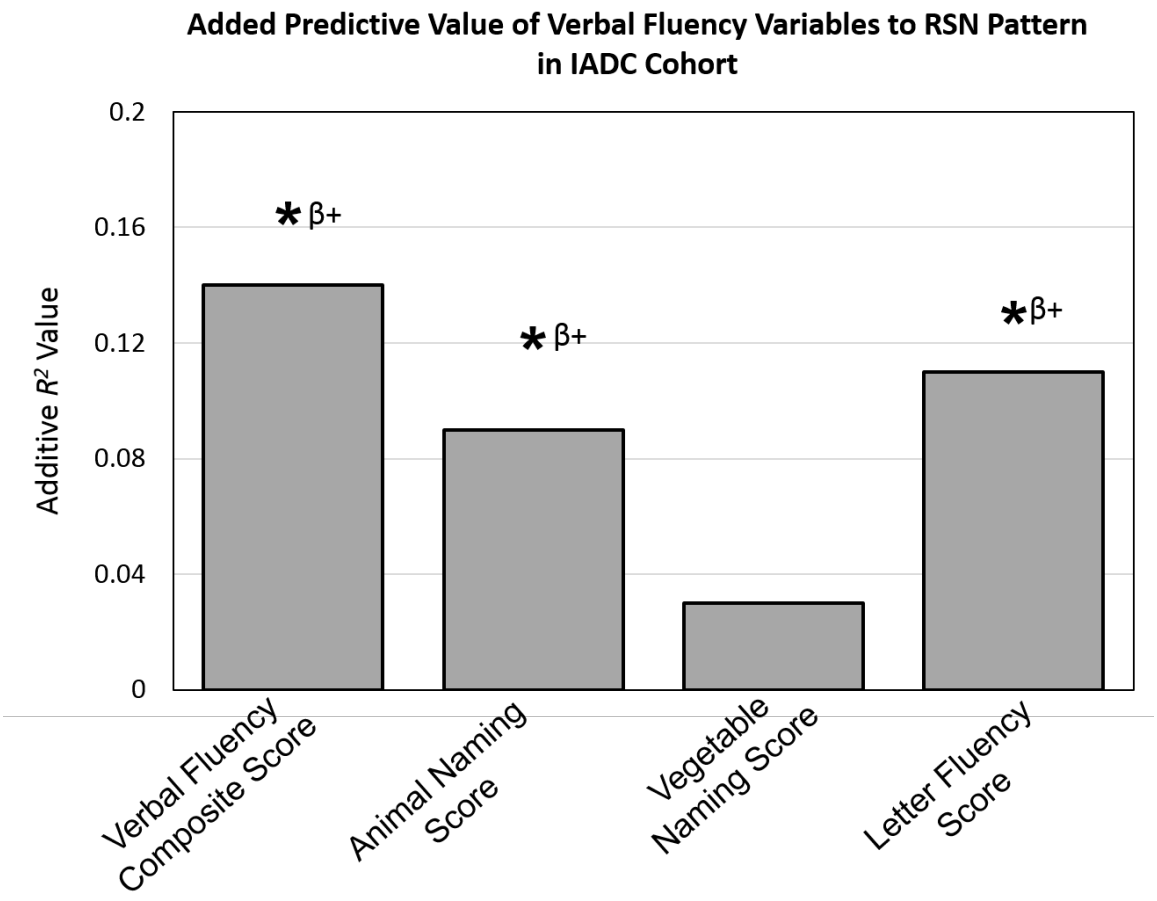

Figure 10. Relationship of the RSNs pattern weights and verbal fluency measures for IADC cohort. Bar plots display additive R^2 with inclusion of verbal fluency measures. *Actual *p-values* presented in table 11. β+ indicates a positive relationship between RSN weights and the neurocognitive score.

Table 11. Model data from hierarchical regressions from IADC cohort using RSN pattern weights. Model 1a is the "null" model with age, sex, education as predictors. Models 2a-5a include the null variables, plus an additional cognitive score. Significance is set at *p<0.05*.

Model Tested	*F*-Value for Model	R^2 Value	R^2 Change	Sig *F*-Change	Model *p*- value	Model β-coefficient	*p*-value for predictor
Model 1a	1.70	0.043	--	0.176	*NS*	--	--
Age	--	--	--	--	--	-0.19	*NS*
Sex	--	--	--	--	--	0.19	*NS*
Education	--	--	--	--	--	-0.05	*NS*
Model 2a	4.26	0.21	0.14	0.00	*p<0.005*	--	--
Verbal Fluency Composite Score	--	--	--	--	--	0.49	*0.00*
Model 3a	3.14	0.16	0.09	0.01	*0.02*	--	--
Animal Fluency Score	--	--	--	--	--	0.35	*0.01*
Model 4a	1.70	0.10	0.03	0.13	*NS*	--	--
Vegetable Fluency Score	--	--	--	--	--	0.19	*NS*
Model 5a	3.70	0.19	0.11	0.47	*0.01*	--	--
Letter Fluency Score	--	--	--	--	--	0.38	*p<0.005*

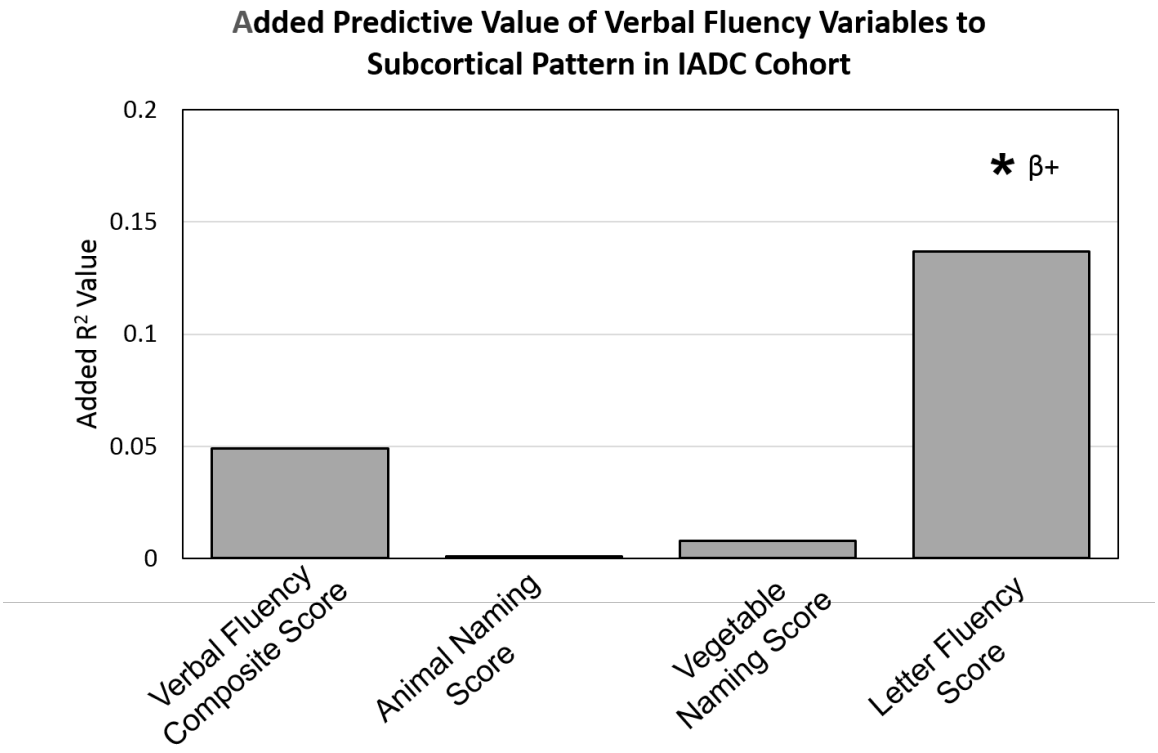

Figure 11. Relationship of the subcortical FC pattern weights and verbal fluency measures for IADC cohort. Bar plots display R^2 change for inclusion of verbal fluency measures. *Actual *p-values* presented in table 12. β+ indicates a positive relationship between RSN weights and the neurocognitive score.

Table 12. Model data from hierarchical regressions from the IADC cohort using Subcortical (SUB) pattern weights. Model 1b is the "null" model with age, sex, education as predictors. Models 2b-5b include the null variables, plus an additional cognitive score. Significance is set at *p<0.05*.

Model Tested	*F*-Value for model	R^2 value	R^2 change	Sig *F* – change	*p*-value for model	Model β-coefficient	*p*-value
Model 1b	0.22	0.103	--	0.065	*NS*	--	--
Age	--	--	--	--	--	0.01	*NS*
Sex	--	--	--	--	--	0.10	*NS*
Education	--	--	--	--	--	-0.02	*NS*
Model 2b	1.03	0.06	0.05	0.07	*NS*	--	--
Verbal Composite Score	--	--	--	--	*NS*	0.25	*NS*
Model 3b	0.18	0.01	0.00	0.81	*NS*	--	--
Animal Fluency	--	--	--	--	--	0.04	*NS*
Model 4b	0.30	0.02	0.01	0.47	*NS*	--	--
Vegetable Fluency	--	--	--	--	--	0.09	*NS*
Model 5b	2.80	0.15	0.14	0.00	*0.03*	--	--
Letter Fluency	--	--	--	--	--	-0.41	*p<0.005*

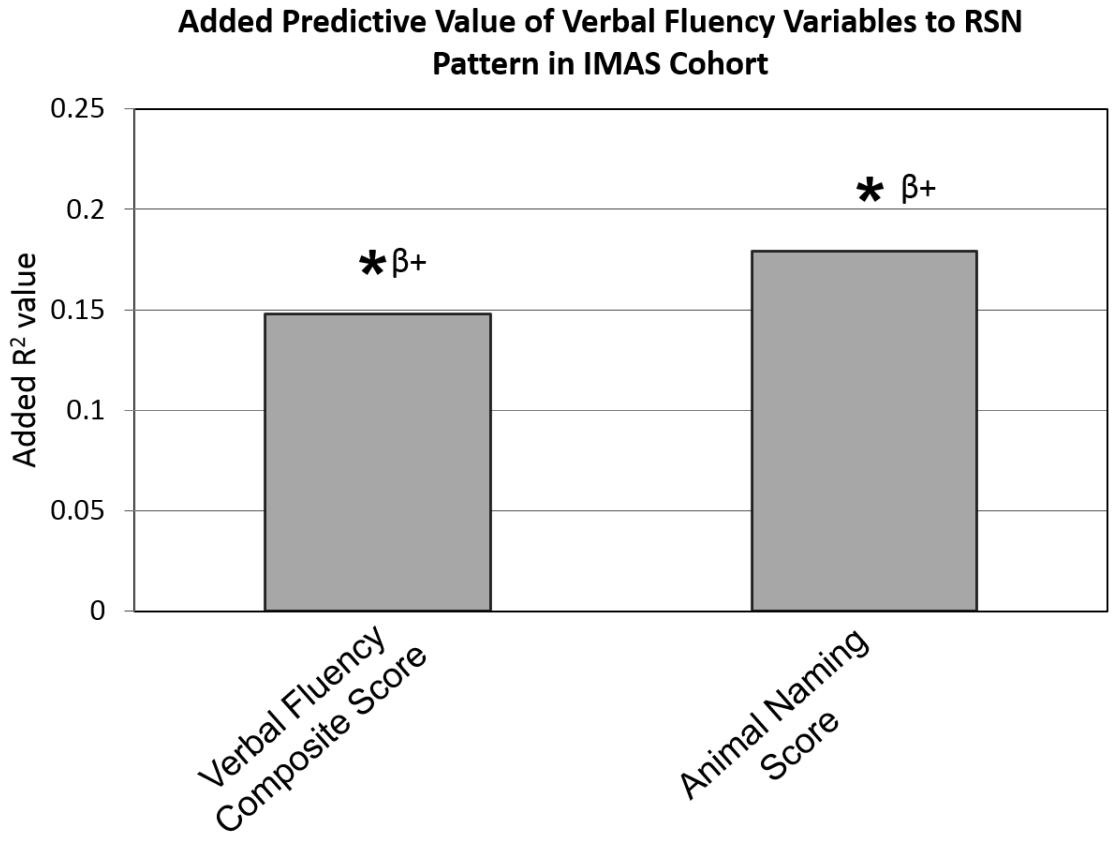

Figure 12. Relationship of the RSN FC pattern weights and verbal fluency measures for IMAS cohort. Bar plots display R^2 change for inclusion of verbal fluency measures. *Significance set at $p<0.05$. β+ indicates a positive relationship between RSN weights and the neurocognitive score.

Table 13. Model data from hierarchical regressions from IMAS cohort using RSN pattern weights. Model 1c is the "null" model with age, sex, education as predictors. Models 2c-3c include the null variables, plus an additional cognitive score. Significance is set at *p<0.05*.

Model Tested	F-Value for model	R^2 value	R^2 change	Sig F – change	*p*-value for model	Model β-coefficients	*p*-value for predictor
Model 1c	2.42	0.19	--	0.08	*NS*	--	--
Age	--	--	--	--	--	-0.32	*NS*
Sex	--	--	--	--	--	0.27	*NS*
Education	--	--	--	--	--	0.10	*NS*
Model 2c	3.86	0.33	0.15	0.01	*p<0.005*	--	--
Verbal Fluency Composite Score	--	--	--	--	--	2.62	0.01
Model 3c	4.43	0.36	0.18	0.01	*p<0.005*		--
Animal Fluency	--	--	--	--	--	2.96	0.01

Discussion

As the disease develops, AD progressively affects brain areas beyond the medial temporal region, and consequently, cognitive changes become evident in domains in addition to memory including language. Therefore, we evaluated RS-fMRI in relation to the verbal fluency performance domain, hypothesizing that it would show a relationship to RS FC patterns, as was previously reported for other cognitive functions across prodromal and dementia stages of AD [148, 202, 203].

This study used an analytical framework to identify FC network patterns across the continuum from cognitively normal older adults controls to early stage of AD. Specifically, we applied a data-driven connectivity methodology directly to functional connectome data. Using two independent diagnostically and demographically similar cohorts, we found that verbal fluency measures were positively correlated with the RSN and subcortical patterns in both samples.

Assessment of verbal fluency has long been an important clinical measure used in diagnosis of AD and other dementias [199]. While fluency scores are useful indicators of general verbal functioning, it is important to understand how performance in these tasks can be reflected back to global brain patterns/networks affected by AD. Previous findings have limited the investigation of verbal fluency deficits to frontal brain networks, that play a major role in executive function [204-206]. Yet, verbal fluency may be influenced by other RSNs [207, 208]. In fact, tests of verbal fluency engage multiple processes, including behavioral strategy, retrieval of words from semantic or phonological

memory, visualization of semantic/phonological representations, inhibition of off target words, and articulation of the retrieved word [205]. Therefore, our finding that the RSN pattern (which includes the set of canonically-defined RSNs) was significantly associated with verbal fluency composite score supports a growing body of evidence that language fluency is subserved by multiple brain networks [209, 210].

Our other main finding was the relationship of the subcortical pattern to letter fluency scores. Specifically, letter fluency was found to be positively associated with the subcortical pattern weights, which represent FC data within thalamus, basal ganglia, hypothalamus, and midbrain structures as well as some parahippocampal brain regions. Prior reports suggest that letter fluency may be preserved through prodromal stages of AD, only being affected at the stage of an AD diagnosis [211-213]. Similarly, as neurodegeneration results in progression to AD, subcortical areas become severely atrophied [214]. Given that we know subcortical brain regions to be most affected in AD populations, it was not surprising to find that lower letter fluency scores coincided with lower connectivity within a subcortical network of brain regions.

Final comments concern limitations of the study, as well as future directions. Diagnostic group membership was significantly different across the two cohorts. Specifically, the IADC cohort had proportionally more CN and fewer MCI subjects compared to the IMAS cohort. However, between-group analysis of each diagnostic group across the IADC and IMAS cohorts showed no significant differences in age, sex and education (e.g., there were no differences in age, sex

or education between the CN samples from IMAS and IADC cohorts). The inclusion of additional MCI and AD subjects could help more closely reproduce specific patterns between cohorts and could better balance the distribution of neurocognitive scores across the samples. Another limitation was that we were unable to analyze letter fluency and vegetable fluency scores in the IMAS sample. Therefore, the IMAS language composite score was comprised of slightly different tests than the IADC cohort. Additionally, although we report associations between FC patterns and cognitive variables, we cannot establish causality. Finally, while the functional connectomes in both groups were analyzed in the same manner, the two cohorts were acquired on different Siemens 3T scanners. Also, scan duration differed between cohorts, and longer RS-fMRI acquisition times can improve data quality [215]. The RS-fMRI acquisition in the more recent IADC cohort was superior, both spatially (smaller and isotropic voxels) and temporally (almost twice as fast sampling). These factors could account for differences in quantity and appearance of FC patterns between cohorts. However, the similarity of results between cohorts gives us confidence in the relationships reported herein.

The present analyses were conducted across the continuum from CN to AD. In future studies, with larger samples, it would be useful to determine if particular FC patterns are associated with specific disease states. Specifically, connICA could be applied to the functional connectomes of individual stage specific samples (i.e., CN, SCD, MCI, and AD separately). This would provide a window into the possibility of unique progression of FC patterns over the course

of AD, and may clarify how connectivity among RSNs degrades overtime. Furthermore, we may be able to link decline on specific cognitive assessments to changes in FC in the context of specific stages of AD.

In summary, we identified two FC patterns that were significantly associated with verbal fluency measures in two independent samples. This is consistent with the concept that the brain's basal activity may be more relevant to cognitive tasks than previously assumed [216-218]. The data-driven methodology we employed derives patterns of brain activity related to cognitive variables. The ability to extract FC patterns from an entire cohort may reveal significant findings that may be hidden by conventional between-group analysis approaches. Neuropsychological assessments, while often designed to measure a single facet of brain function, require and recruit multiple cognitive processes. Therefore, using a whole-brain connectomic approach can elucidate other circuits related to cognition that might be affected by disease progression. Ultimately, we believe it may be advantageous to integrate RS-fMRI data with neuropsychological data in clinical settings. This could provide valuable insight into the transitions between stages of AD, and may aid in the evaluation of potential therapies intended to slow progression, as well as generate clinically relevant biomarkers for brain function in cognitive tasks affected in prodromal AD.

Chapter 5: Conclusions and Future Directions for Brain Connectomics to Study Alzheimer's Disease

Summary and Discussion

Increasingly, Alzheimer's disease has been interpreted as a disconnection syndrome to explain its symptomatology [26, 219, 220]. In this framework, AD pathology results from a breakdown of the brain's effective connectivity between multiple neuronal systems, as opposed to pathophysiology within one neuronal system. Early studies using electrophysiological approaches provided evidence to support the disconnection syndrome hypothesis [221-223]. This has since been expanded to include structural and functional neuroimaging data [26, 224-228]. In part because neuroscience has benefitted from tremendous advances in advanced imaging, connectomics and large-scale neuroinformatics initiatives are emerging to help better integrate neuroimaging data with patients' cognitive performance. In this work, we have taken steps to expand knowledge along several lines.

First, we reviewed studies that examined changes in the structural and functional connectome in the context of both normal and abnormal aging (Chapter 1, [139]). Until the emergence of brain connectomics, previous studies had investigated neurodegenerative diseases mainly by focusing on specific brain regions. Additionally, many studies examined the progression of AD in discrete stages, which may be a limitation in some contexts given the multidimensional and continuous nature of AD progression. Finally, we suggested that using new methodologies within the field of connectomics might

provide a way to overcome some of these limitations, and may lead to novel biomarkers for detection and progression.

As such, we incorporated a novel connectivity-based method that enabled quantitative assessment of whole brain functional connectivity (FC) patterns in canonical resting state networks while treating AD as a continuum (Chapter 2, [139]). This approach allowed us to relate pertinent cognitive variables (used in the diagnosing and staging of AD) to functional connectivity patterns, in a manner unrestricted by between-group analysis and assumptions of fixed stages of AD. Here, we reported several novel findings: 1) the more subjects reported cognitive complaints or concerns, the lower the functional connectivity among important canonical resting state networks; 2) this result was replicated in an independent diagnostically similar cohort; and 3) verbal fluency performance was positively correlated with functional connectivity among RSNs.

The first finding was important for several reasons. We were able to examine the functional connectivity of multiple RSNs across all early stages of AD. Most prior connectivity studies of AD studies were focused on the default mode network (DMN) due to the strong overlap between brain regions known to be part of the DMN and amyloid and tau burden, the two hallmark pathologies of AD [214, 229-231]. Yet, the topography of network dysfunction and the topography of amyloid and tau do not consistently spatially overlap [232]. There are regions with functional connectivity disruptions that are not strongly affected by amyloid or tau, and, conversely, there are brain regions with amyloid and tau depositions where functional connectivity disruptions are not prominent.

Therefore, we decided to examine all potential RSNs in a comprehensive and unbiased manner. Secondly, we showed that self and informant-reported cognitive complaints are significantly associated with abnormal functional connectivity. Until recently, little attention was directed to those who reported cognitive concerns but who test within normal ranges on cognitive performance scales. The research reported here contributes to a growing body of evidence of neuroimaging biomarkers suggesting abnormal brain structure and function in these individuals. Our data suggest self-reported, or subjective, complaints are associated with biophysical changes. In order to strengthen these results, we replicated our finding in an independent, but diagnostically similar, cohort (Chapter 3). Given the challenges of reproducibility [233-239], confidence in our methodology and findings were strengthened by observation of the same pattern of results in an independent cohort. Lastly, because verbal fluency has been an important component of clinical assessment for diagnosing AD [199], and limited prior research has examined how performance on these tasks is reflected by global brain network changes, our study provided a new promising direction for further investigation (Chapter 4). Specifically, the finding that verbal fluency composite scores were associated with the RSN pattern (which resembles canonically-defined RSNs) adds to a growing literature that language fluency performance is dependent on a diversity of brain networks beyond classical language areas [209, 210].

Future Directions

Our understanding of large-scale changes in neural behavior associated with early stages of AD has been expanded through these studies employing RS-fMRI and novel analysis. They establish a basis from which new research questions can be addressed. Specifically, in order to advance the field, several future directions should be considered: 1) acquisition of longitudinal data to confirm SCD conversion to AD in order to determine if the negative association between cognitive complaints and FC within RSNs could be an early predictive biomarker of AD; 2) examination of resting state networks across normal aging; and lastly, 3) better assessment of when structural changes occur with regards to functional changes, and to what end that this relationship affects cognition along the AD continuum.

To address the first point, we are currently collecting longitudinal data on the subjects in our IADC cohort sample and therefore, will be able to track whether patients identified as SCD progress to MCI or AD.

Secondly, the examination of these resting state networks in a normal aging population has yet to be thoroughly investigated and should be a next step with additional analyses of RS-fMRI neuroimaging data in cohorts of healthy participants as they age. Future studies should examine functional connectomes across time in CN participants. FC values may change significantly within RSNs in those who are developing age related pathology. Similarly, interactions among RSNs may be altered. This could be assessed by computing the mean FC values of brain regions within and between the RSNs from a normal control functional

connectome across time, with comparisons to the baseline scan. Additionally, FC patterns can be extracted across different time points using connICA (for Methods, please refer to Chapters 2-4) to examine if they remain constant for individuals that retain a CN diagnosis over time. These results can be compared to individuals that have had progressive changes including diagnostic conversion (i.e. progressing from CN to SCD, MCI or AD, or SCD to MCI, etc). This would help to clarify how FC patterns relate to neurodegeneration.

Another important future direction to consider would be how best to integrate structural and functional connectivity data to elucidate how their relationship may change along the AD continuum. In our data collection protocol, in addition to RS-fMRI data, each participant undergoes a diffusion weighted imaging scan. This yields data that can describe white matter integrity and connectivity. These data can be processed into the same matrix format (described previously) to create a unique structural connectome for each participant. Future studies could therefore address integration of the structural and functional connectomes. To date, there have been limited studies that have combined RS-fMRI and DTI data to examine the structure-function (SC-FC) relationship in AD. However, some neuroimaging studies have demonstrated that the structural and functional networks, or connectomes, of the human brain share many important topologic features, including modularity (a measure of segregation within a network and defined as the degree to which a system's components may be separated into discrete communities/clusters), and the existence of highly connected brain regions (hubs) [240-242]. This would suggest

an intricate relationship between the structural and functional networks of the human brain. Additionally, SC-FC coupling has been found to strengthen with age rather than remain constant [243, 244], which suggests that direct white matter connectivity plays an increasingly important role in constraining brain-wide coherence and synchrony [245]. This would indicate that variations in SC-FC coupling in AD could be expected. Therefore, we could hypothesize that patients who progress in diagnosis towards AD may have aberrant coupling between SC and FC; specifically, AD subjects would have significantly less SC-FC connections than individuals who age normally. Future analyses could include patients that have converted to AD, to determine if structural connections between regions that are part of the RSNs are weakened or disrupted compared to controls. Structural connections can be described by multiple metrics, such as fractional anisotropy (i.e., white matter tract integrity), fiber count (or fiber presence/absence), or shortest path distance among regions. Examining these SC metrics would help determine if SC changes accompany any observed FC changes. Results could be compared to a cognitively normal population. This type of analysis could also be used to compare the SC-FC relationships between CN and prodromal AD samples (SCD, MCI). Such studies will be helpful for deepening our understanding of the dynamic structural functional relationships in relation to cognition and pathophysiology.

Taken together, advanced neuroimaging tools have the potential to detect incipient pathological changes when neuropsychological testing indicates little or no impairment. RS-fMRI and brain connectomics are likely to increase the ability

of clinicians to diagnose during early stages of AD. By using RS-fMRI with connectomic metrics, we have been able to identify brain networks that can be viewed as a type of phenotype, or “fingerprint.” These fingerprints may help bridge the gaps between genetics, molecular pathology, and behavioral outcomes. Thus, brain connectomics is poised to make an important contribution toward unifying the many areas of neuroscience of aging and dementia by providing a common conceptual framework and a common toolset to help address contemporary challenges in the field.

Appendix A

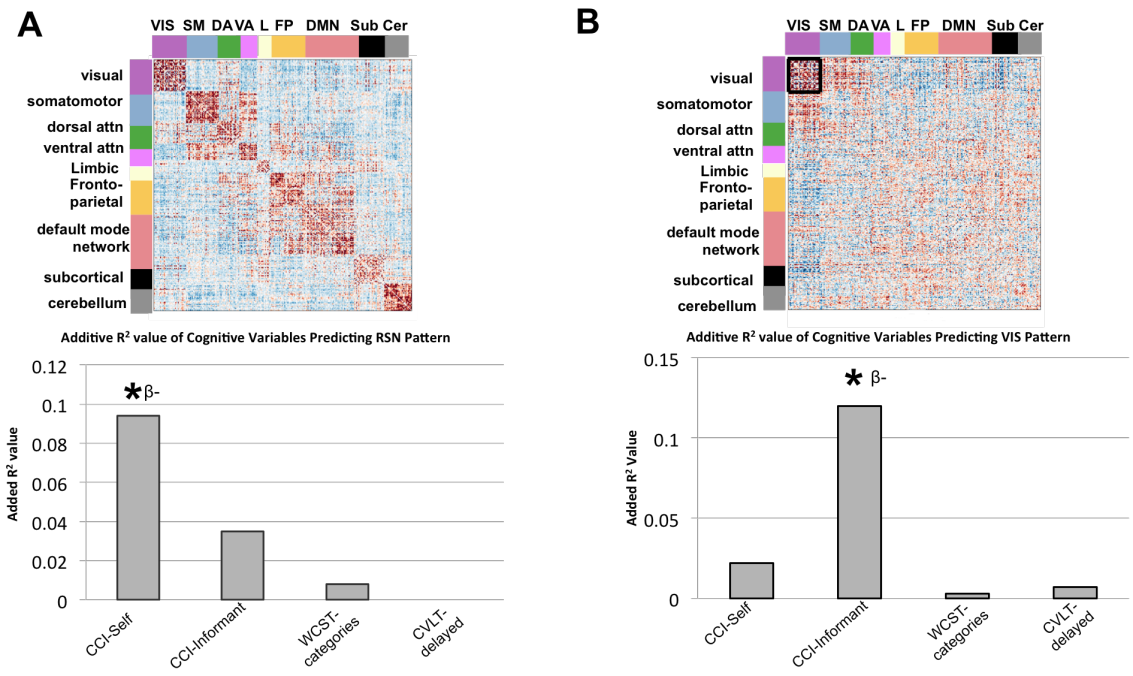

Figure A-1: Robust FC patterns of interest and their relationship with cognitive variables using a hierarchical linear regression in IMAS cohort. (A) Upper panel shows visualization of RSN FC pattern. Lower panel shows contributions of neurocognitive variables of interest via R^2 change from the baseline R^2 value (from null Model) using RSN pattern weights as the dependent variable. (B) Upper panel shows visualization of VIS FC pattern. Lower panel shows R^2 change for neurocognitive variables from R^2 value of null model using VIS pattern weights as the dependent variable.

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Curriculum Vitae

Joey Annette Contreras

Education

Indiana University — Aug '12-Sep '17
PhD Medical Neurosciences

California State University,Los Angeles — June '06-Mar '10
MS Biology
M.S. Biology

University of California Irvine — Aug '02-June '06
B.S. Psychology
Minor: Biology

Research Experience

Ph.D. Candidate — April '13-Sep '17
Dr. Andrew Saykin's Laboratory, Dept of Radiology, IUSM at IUPUI

- Use diffusion weighted Imaging and resting state fMRI to assess potential brain network differences in prodromal neurodegenerative populations within the field of brain connectomics
- Obtain expert knowledge in fMRI image processing and data analysis using UNIX/LINUX and MATLAB

Project Manager /Research Associate — Oct '11-July '12
Dr. Fred Sabb's Laboratory at UCLA, Semel Institute

- Worked with a team on project BrainTest.org to determine how best to analyze neuroimaging data from a Task Switching paradigm

Staff Research Associate — Oct '10-Oct '11
Dr. Robert Bilder's Laboratory at UCLA, Consortium for Neuropsychiatric Phenomics

- Performed neuropsychiatric characterization of research subjects
- Administered/scored a battery of neuropsychological assessments in healthy controls and patients with schizophrenia, bipolar disorder, ADHD, and 22Q Deletion Syndrome

Research Assistant/ UCLA Prep Scholar — Sept '09-Sept '10
Dr. Carrie Bearden's Laboratory at UCLA, Department of Neuroscience

- Assisted with acquisition and processing of MRI data, administered fMRI tasks and cognitive tests
- Manually traced amygdalae for volumetric analysis

Laboratory Researcher/Volunteer — June '09-Aug '09
Dr. Caleb Finch's Laboratory at USC, Department of Neuroscience

- Prepared radioactive probe for progesterone receptors for in-situ hybridization in a rat model of AD
- Gained experience with laboratory techniques such as cell culture, northern blotting, and PCR

M.B.R.S. Rise Scholar/Graduate Student May '07-Mar '10
Dr. Amelia Russo-Neustadt's lab at CSULA, Department of Biology

- Prepared prospectus, thesis, and dissertation for my own research project: "Can exercise prevent the effects of chronic unpredictable stress on growth factor expression in the hippocampus?"

MHIRT Scholar June '06-Sept '06
Minority Health and International Research Training (MHIRT), UCI, Department of BioSciences
Dr. Gina Quirarte's laboratory at UNAM/Instituto de Neurobiologia, Mexico

- Trained rats in an inhibitory avoidance learning task, administered cycloheximide injections (a memory-impairment protein synthesis inhibitor)
- Prepared work for publication in the journal of Neurobiology of Learning and Memory

Research Assistant Jan '06-June '06
Dr. Larry Cahill's Laboratory at UCI, Center for Neurobiology of Learning and Memory

- Input data
- Explained and conducted experimental procedure to participants and addressed any questions afterward

Scientific Service

Brain and Beyond Outreach liaison Oct '15-Oct '16
IUSM Medical Neurosciences Program

- Present and interact with local schools (K-12) to convey knowledge about the field of neuroscience and the careers available to students

EPSP Chair- MedNeuro Graduate Student Rep Aug '15 –May '17
IUSM Medical Neurosciences Program

- Arrange meetings for neuroscience graduate students to present current and past research
- Organize career development workshops and meetings
- Organize social events and activities for medical neuroscience graduate students
- Act at general liaison between students and Medical Neuroscience administration
- Act as part time representative for MedNeuro Program at IBMG, GPSG, and IUSM council meetings.

MORE Programs Student Advisory Committee, Chair Sep '07-June '09
Minority Opportunities in Research (MORE) CSU Los Angeles

- Arranged meetings and assigned tasks for committee members
- Planned tasks for the coming year and oversaw them to completion.
- Organized spring academic seminar talks
- Organized and planed workshops for MORE student body to help promote successful transition to PhD program

Publications

Book Chapters:

Zárate, C.B. Ureña Guerrero, M.E., Lliberia, M.P., Camins, A.Topicos De Actualizacion En Neurobiologia: Procesos Cognitivos y Mecanismos de Neurodegeneración. 2008 Universidad de Guadalajara, Centro Universitario de Ciencias Biológicas y Agropecuarias.

Peer-Reviewed Manuscripts:

Contreras, J.A., Goñi, J., Risacher S.L., Amico, E., Yoder K., Dzemidcic M., West, J.D., McDonald, B.C., Farlow, M.R., Sporns, O., Saykin, A.J.(2017) Cognitive complaints in older adults at risk for Alzheimer's disease are associated with altered resting state networks. *Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring*, DOI: 10.1016/j.dadm.2016.12.004

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Li, H., Fang, S., Goñi, J., **Contreras, J.A.**, Liang, Y., Cai, C., West, J.D., Risacher, S.L., Wang, Y., Saykin, A.J., Shen, L. (2015) Integrated Visualization of Human Brain Connectome Data. IEEE VIS.

Johnson, P. L., Federici, L. M., **Contreras, J.**, Fitz, S. D., Molosh, A., Truitt, W., & Shekhar, A. (2013). Serotonin Transporter Deficient Rats Exhibit Increased Panic and Amygdala Responses to Panicogenic Hypercapnic Stimuli and Enhanced Acquisition and Disrupted Extinction of Conditioned Fear. *Biological Psychiatry*, 73(9), 251S-251S.

Arnulfo Díaz-Trujillo, **Joey Contreras**, Andrea C. Medina, Gerardo A. Silveyra-Leon, Anaid Antaramian, Gina L. Quirarte, Roberto A. Prado-Alcalá. Enhanced inhibitory avoidance learning prevents the long-term memory-impairing effects of cycloheximide, a protein synthesis inhibitor. *Neurobiology of Learning and Memory*. (2008)

Non Peer-Reviewed Manuscripts:

Contreras, J.A, Russo-Neustadt, A. Thesis: Can Exercise Prevent the Effects of Chronic Unpredictable Stress on Growth Factor Expression on the Hippocampus. 2009 California State University of Los Angeles Library.

Manuscripts in Preparation:

JA Contreras, J Goñi, SL Risacher, K Yoder, M Dzemidzic, JD West, E Tallman, BC McDonald, MR Farlow, L Apostolova, O Sporns, AJ Saykin. Language Fluency Predicts RSN Connectivity Pattern

JA Contreras, S Fortunato, A Avena-Koenigsberger, SL Risacher, JD West, E Tallman, BC McDonald, MR Farlow, B Mathews, LG Apostolova, J Goñi, O Sporns, AJ Saykin. Resting state network modularity along the prodromal late onset Alzheimer's disease continuum.

JA Contreras, SL Risacher, JD West, Y Wu, Y Wang, JR Murrell, M Dzemidzic, MR Farlow, F Unverzagt, B Ghetti, BR Matthews, KA Quaid, O Sporns, AJ Saykin, J Goñi . Structural connectivity changes in hereditary diffuse leukoencephalopathy with spheroids: A Case Study.

TJ Hohman, D Tommet, S Marks, **JA Contreras**, R Jones, D Mungas. Evaluating Alzheimer's disease Biomarkers as Mediators of Age-Related Cognitive Decline.

L Huang, S Fang, **JA Contreras**, JD.West, SL Risacher, Y Wang, O Sporns, AJ Saykin, J Goni, L Shen, for the Alzheimer's Disease Neuroimaging Initiative. Brain Explorer for Connectomic Analysis

Invited Presentations
JA Contreras. "Language Fluency Predicts Resting State Network Connectivity Pattern" Indianapolis Chapter of the Society for Neuroscience, Indianapolis IN. April 9 2017

JA Contreras. "Using Brain Connectomics to Characterize Neurodegeneration" MedNeuro Student Research Symposium, IU School of Medicine, Stark Neuroscience Program, Indianapolis IN. December 12 2016

JA Contreras. "Using Brain Connectomics to Detect Functional Connectivity Differences in Alzheimer's Disease" Stark Neuroscience Seminar, IU School of Medicine, Indianapolis IN. October 6 2016

JA Contreras*, J Goñi*, SL Rishacher, E Amico, M Džemidžić, JD West, BC McDonald, MR Farlow, O Sporns, AJ Saykin. *Cognitive Complaints in Older Adults at Risk for Alzheimer's Disease Are Associated with Altered Resting State*

Networks. Alzheimer's Association International Conference, Toronto, Canada. July 24-28 2016

JA Contreras, "Imaging above the SPINEs: Using Brain Connectomics to Characterize Different Prodromal States of AD" SPINES Symposium, University of Chicago, Chicago IL. October 16 2015

Abstracts

JA Contreras, J Goñi, SL Risacher, K Yoder, M Dzemidzic, JD West, E Tallman, BC McDonald, MR Farlow, L Apostolova, O Sporns, AJ Saykin. *Language Fluency Predicts RSN Connectivity Pattern*. Submitted for Alzheimer's Association International Conference. London, Great Britain. July 2017

JA Contreras, S Fortunato, A Avena-Koenigsberger, SL Risacher, JD West, E Tallman, BC McDonald, MR Farlow, B Mathews, LG Apostolova, J Goñi, O Sporns, AJ Saykin. *Resting state network modularity along the prodromal late onset Alzheimer's disease continuum.* International School and Conference on Network Science. Indianapolis, IN. June 2017

JA Contreras, SL Risacher, M Dzemidzic, JD West, BC McDonald, MR Farlow, B Mathews, LG Apostolova, J Brosch, B Ghetti, J Goñi, O Sporns, AJ Saykin. *Resting state network profiles of Alzheimer's disease and Frontotemporal Dementia: A Preliminary Examination.* Society for Neuroscience Conference. San Diego, CA. November 2016

EJ Chumin, ME Halcomb, **JA Contreras**, M Džemidžić, J Goñi, KK Yoder. *Preliminary assessment of the influence of corticostriatal structural connectivity on striatal [11C]raclopride availability in social drinkers and non-treatment seeking alcoholics*.11th International Symposium on Functional NeuroReceptor Mapping of the Living Brain. Boston, MA. July 2016.

JA Contreras, J Goñi, SL Rishacher, E Amico, JD West, E Chumin, BC McDonald, MR Farlow, O Sporns, AJ Saykin. *Mapping Cognitive Complaint Index onto Resting State Networks in Prodromal Stages of Alzheimer's disease*. Indy SfN. Indianapolis, IN. March 2016

M Halcomb, E Chumin, **JA Contreras**, J Goñi, M Džemidžić, KK Yoder. *Altered within- and between network functional connectivity as a function of drinking status*. 39th Annual RSA Scientific Meeting. New Orleans, LA. June 2015

EJ Chumin, **JA Contreras**, M Džemidžić, ME Halcomb, J Goñi, KK Yoder. *Differential structural connectivity in non-treatment seeking alcoholics*. 39th Annual RSA Scientific Meeting. New Orleans, LA. June 2015

A Avena-Koenigsberger, O Sporns, B Corominas-Murtra, R Hawkins, **JA Contreras**, J Goñi*. Generalized Search Information: an information theoretical*

approach to network communication through ensembles of shortest-paths. Zaragoza, Spain. June 2015

SL. Risacher, L Shen, J Goñi, **JA Contreras**, S Gao, PM Thompson, CR Jack Jr., MW Weiner, AJ Saykin. *Association of Eye Disease with Increased Diffusivity in the Sagittal Stratum*. Alzheimer's Association International Conference. Washington, D.C. July 2015.

JA Contreras, SL Risacher, JD West, Y Wu, Y Wang, JR Murrell, M Dzemidzic, MR Farlow, F Unverzagt, B Ghetti, BR Matthews, KA Quaid, O Sporns, AJ Saykin, J Goñi . *Characterizing neurodegeneration in the human connectome: A network science study of hereditary diffuse leukoencephalopathy with spheroids*. Indiana Alzheimer Disease Center Scientific Symposium: Network Science and Alzheimer's disease. Indianapolis, IN. March 2015.

JA Contreras, SL Risacher, JD West, Y Wu, Y Wang, JR Murrell, MR Farlow, F Unverzagt, B Ghetti, BR Matthews, KA Quaid, O Sporns, AJ Saykin, J Goñi. *Mapping Neurodegeneration in the Human Connectome: A Network Science Study of Hereditary Diffuse Leukoencephalopathy with Spheroids* The Fourth Annual Indiana CTSI Disease and Therapeutic Response Modeling Symposium, Indianapolis, IN. November 2014.

JA Contreras, Y Wang , SL Risacher, BC McDonald, JD West, E Tallman, S Gao, MR Farlow, AJ Saykin. *Altered Default Mode Network Connectivity in Older Adults with Subjective Cognitive Decline and Mild Cognitive Impairment* Indiana Alzheimer's Disease Center (IADC) Scientific Symposium on Alzheimer's Disease, Indianapolis, IN. March 2014.

JA Contreras, Y Wang, SL Risacher, BC McDonald, JD West, S Gao,MR Farlow, AJ Saykin. *Altered Salience Network Connectivity in Older Adults with Subjective Cognitive Complaints* Society for Neuroscience 43rd Annual Meeting, San Diego, CA. November 2013

JA Contreras, A Bato, E Congdon, KH Karlsgodt, RA Poldrack, ED London, TD Cannon, RM Bilder, FW Sabb. *The Effect of WM capacity on the Neural Correlates of Task Switching in a Large Community Cohort*. Cognitive Neuroscience Society, Chicago, IL. April 2012

C Chow, **JA Contreras,** JS Ho, C Bearden. *Do Deficits in Theory of Mind and Impulsivity Result in Behavioral Problems in the 22qDS Population*. Consortium of Neuropsychiatric Phenomics Retreat, Los Angeles, CA. June 2010

JA Contreras, A Russo-Neustadt. *Can exercise prevent the effects of chronic unpredictable stress on growth factor expression in the hippocampus.* ABRCMS Conference, Orlando, FL. November 2008

JA Contreras, GL Quirarte, RA Prado-Alcala, AC Medina. *Enhanced Inhibitory Avoidance Training Protects Against the Amnesic Effects of Protein Synthesis Inhibition.* AAAS Conference, San Francisco, CA. February 2007

JA Contreras, GL Quirarte, RA Prado-Alcala, AC Medina**.** *Enhanced Inhibitory Avoidance Training Protects Against the Amnesic Effects of Protein Synthesis Inhibition.* SACNAS Conference, Tampa Bay, FL. October 2006

Certifications

CITI training	(IUSM) 2016
HIPAA certified	(IUSM) 2013
SCID certified	(UCLA) 2010
MRI safety certified	(UCLA) 2009

Awards

Indianapolis Chapter of Society for Neuroscience Travel Award	Spring'17
Larry Kays Fellowship Award	Fall'16
SPINES (Summer Program in Neuroscience) Travel Award	Fall'15
IUPUI Graduate-Professional Educational Grant Travel Award	Spring'15-'16
Indiana Clinical and Translational Science Institute Grant Recipient	2015-2017

- TL1 Program, TL1 TR001107 (A. Shekhar, PI)

Grant Recipient from National Institute of Aging	Fall '15

- For Advanced Psychometrics Methods Workshop attendance

UCLA PREP Scholar	2009-2010
RISE to PhD Graduate Scholar	2007-2009
AMAGEN Award Scholar	Winter'07

- For MHIRT Neuroscience Research and AAAS conference funding.

MHIRT Research Scholar	Summer'06
Deans Honor List	Spring'06

Funding

Indiana Clinical and Translational Science Institute Grant Recipient	2015-2017

- TL1 Program, TL1 TR001107 (A. Shekhar, PI

Stark Neurosciences Research Institute Fellowship)	2013-2014

Professional Memberships

ISTAART Member	2016-2017
SfN Student Member	2013-2014
SACNAS Student Member	2006-2010, 2015

AAAS Student Member 2006-2010
ABRCMS Student Member 2006-2010

Ad-Hoc Reviewer
Brain Imaging and Behavior

Professional Development

Extracurricular Courses:
PBHL-B 670 Topics in Public Health Fall 2015 at IUPUI
Course Topic(s): STAT METH BRAIN IMAGING
GRAD-G 667 Tools & Tech – Translational Research (3 CR) Fall 2015 IUPUI

Conferences/Workshops

Connectivity Course: Structural and Functional Brain Connectivity via MRI and fMRI. Martinos Center, Boston, MA. October 26-30 2015

Advanced Psychometrics Methods in Cognitive Aging Research Conference. University of Washington Friday Harbor Laboratory. September 13-18 2015.

Chicago Symposium on Translational Neuroscience, “Brain Imaging Biomarkers in Neurological Disease”, University of Chicago Medicine, Chicago IL. May 8 2015

Fourth Biennial Conference on Resting State Brain Connectivity. Cambridge, MA. September 11-13 2014.