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Functional connectivity and graph theory in preclinical Alzheimer's disease

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ABSTRACT

Alzheimer's disease (AD) has a long preclinical phase in which amyloid and tau cerebral pathology accumulate without producing cognitive symptoms. Resting state functional connectivity magnetic resonance imaging has demonstrated that brain networks degrade during symptomatic AD. It is unclear to what extent these degradations exist before symptomatic onset. In this study, we investigated graph theory metrics of functional integration (path length), functional segregation (clustering coefficient), and functional distinctness (modularity) as a function of disease severity. Further, we assessed whether these graph metrics were affected in cognitively normal participants with cerebrospinal fluid evidence of preclinical AD. Clustering coefficient and modularity, but not path length, were reduced in AD. Cognitively normal participants who harbored AD biomarker pathology also showed reduced values in these graph measures, demonstrating brain changes similar to, but smaller than, symptomatic AD. Only modularity was significantly affected by age. We also demonstrate that AD has a particular effect on hub-like regions in the brain. We conclude that AD causes large-scale disconnection that is present before onset of symptoms.

1. Introduction

Alzheimer's disease (AD) is the most common form of dementia (Reitz et al., 2011) and has a long preclinical period wherein pathology accumulates in the absence of overt symptoms (Braak et al., 2011; Price and Morris, 1999; Price et al., 2009). The pathological hallmarks of AD are the accumulation of amyloid beta (Aβ) plaques and tau neurofibrillary tangles (Blennow et al., 2006; Holtzman et al., 2011). The diagnostic specificity of these pathologic changes has led to the elucidation of biomarkers with a proposed progression (Jack et al., 2011). Less is known, however, about how this pathology affects large-scale brain function at different stages of the disease.

The staging of AD can be quantified either clinically or pathologically. A well-validated clinical method is the Clinical Dementia Rating (CDR) (Morris, 1993). Because of the relatively long preclinical stage (Price and Morris, 1999; Price et al. 2009) some individuals with normal cognition harbor AD pathology. Cerebrospinal fluid (CSF) levels of tau and Aβ may assist in staging of cognitively normal individuals with preclinical AD pathology (Jack et al., 2012; Sperling et al., 2011). It is hypothesized that by the time clinical symptoms are detected, at least some AD-related irreversible neurologic damage has developed. The effect of this preclinical pathology is unclear and a greater understanding of these early brain changes would inform the pathophysiology and be relevant to treatment trials targeting the early stages of AD.

Resting state functional connectivity magnetic resonance imaging (rs-fcMRI) measures the temporal correlation of the blood oxygen level-dependent (BOLD) signal between different brain regions (Biswal et al., 1995). Regions that are functionally related,

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or co-activated during a cognitive task, tend to be temporally correlated at rest (Beckmann et al., 2005; Smith et al., 2009). Groups of temporally correlated regions are termed resting-state networks (RSNs). The first RSN implicated in AD pathophysiology was the default-mode network (Greicius et al., 2004). By the time AD symptoms develop, widespread changes in functional connectivity are present throughout the brain (Wang et al., 2007). Although debate exists as to the exact scope of changes that occur in early AD (Zhou et al., 2010), widespread loss of functional connectivity is present by mild AD (CDR 1) (Brier et al., 2012).

A potential shortcoming of the previously mentioned studies is that they consider changes in the correlation structure without investigating these in the context of larger whole-brain networks. For example, some studies consider a large number of pairwise correlations (Wang et al., 2007) and other studies average over sets of pairwise correlations as a data reduction strategy (Brier et al., 2012). In both settings, the dynamics of the network as a whole are obscured either by exclusion (in the case of the former) or by averaging (in the case of the latter).

Graph theory is a mathematical technique that is capable of assessing the properties of systems that can be modeled as sets of vertices (i.e., brain regions) and edges (i.e., functional connections). Graph theory allows for a summary of whole network properties with respect to segregation (termed "clustering coefficient") and integration (termed "path length"). This method has been applied to rs-fcMRI. The first application demonstrated that the brain exhibits small-world character (Salvador et al., 2004). Small-world character occurs when there are many short range connections between related areas and relatively few long range connections between less related areas (Watts and Strogatz, 1998). This results in an efficient organization that reduces the cost of maintaining many connections but also allows for efficient movement of information (Latora and Marchiori, 2001). This efficient organization is lost with aging (Achard and Bullmore, 2007) wherein the functional connectivity network structure becomes less modular (Meunier et al., 2009).

The application of graph theory to rs-fcMRI data in AD has provided conflicting results (Tijms et al., 2013). One rs-fcMRI study found that AD resulted in decreased clustering coefficient, but no change in average shortest path length (Supekar et al., 2008). However, another study showed that AD decreased average path length, but did not change clustering coefficient (Sanz-Arigita et al., 2010). A third study noted increased average path length in patients with amnestic mild cognitive impairment (MCI) (Wang et al., 2013a). These studies can be interpreted as a disruption in small-world behavior (Sanz-Arigita et al., 2010; Supekar et al., 2008; Wang et al., 2013a) albeit by different mechanisms. These disparate findings (with respect to mechanism of small-word disruption) could result from differences in methodology, relatively small sample sizes, and variability in the clinical definition of AD. Further, while these studies have investigated the effects of AD (Ciftçi, 2011; Sanz-Arigita et al., 2010; Supekar et al., 2008) and amnestic MCI (Wang et al., 2013a), none have investigated graph measures in cognitively normal individuals with biochemical evidence of AD.

The present study investigates the changes in graph theory measures on rs-fcMRI in a large, well-characterized sample using both CSF biomarkers and clinical examination. The effect of different levels of cognitive impairment on classic graph theory measures of functional connectivity and whether additional measures more fully capture brain dynamics in AD are examined. Finally, graph theoretical measures in cognitively normal individuals who have AD pathology (preclinical AD) as assessed by CSF biomarkers are investigated.

2. Methods

2.1. Subjects

Data were examined from participants enrolled in studies of memory and aging at the Charles and Joanne Knight Alzheimer's Disease Research Center at Washington University in St. Louis. Participants were aged 43–89 years and in good general health, having no serious illnesses (e.g., end-stage renal disease requiring dialysis) that would preclude participation, or medical contraindications to either CSF or magnetic resonance imaging studies. Participants taking psychoactive medication or with severe psychiatric conditions were excluded. The Washington University in St. Louis Human Research Protection Office approved all procedures. After informed consent, each participant had clinical examinations, neuropsychological testing, lumbar puncture (LP), and neuroimaging studies. Only participants who had neuroimaging performed within 1 year of LP and clinical assessment were included.

2.2. Clinical assessment

Experienced clinicians conducted semi-structured interviews with the participants and a knowledgeable collateral source. The CDR was used to determine the presence or absence of dementia and, when present, to stage the severity (Morris, 1993). Participants were scored as either CDR 0, 0.5, or 1, corresponding to cognitive normality, MCI /very mild dementia, or mild dementia. All CDR $>$ 0 participants in this study were considered to have symptomatic AD, a term that encompasses "MCI because of AD" (Sperling et al., 2011) and "AD dementia" (McKhann et al., 1984). The accuracy of that clinical diagnosis is previously supported by the presence of pathologic AD in 93% of cases that come to autopsy (Berg et al., 1998), including those who meet criteria for MCI (Storandt et al., 2006). Findings from an independent neuropsychological assessment are provided in supplemental Table 1 and further support the distinction of CDR 0 from AD.

2.3. CSF analysis

As previously described, CSF (20–30 mL) was collected by LP using an atraumatic 22-gauge Sprotte (Geisingen, Germany) spinal needle in the morning after overnight fasting (Fagan et al., 2006). CSF was collected in 50 mL polypropylene tubes and was free of visible blood contamination. After collection, the tube was immediately placed on ice and was gently inverted within 1 hour of collection to avoid possible gradient effects. The sample was briefly centrifuged at low speed (2000 *g*, 15 minutes, 4 °C) to remove cellular debris. The sample was aliquoted (500 μL) into polypropylene tubes and frozen at −84 °C. CSF was analyzed for tau and $A\beta_{42}$ by plate-based enzyme linked immunoabsorbent assay (INNOTEST; Innogenetics, Ghent, Belgium) according to the manufacturer's specifications.

2.4. National Institute of aging classification of preclinical AD

Recently proposed criteria by the National Institute of Aging proposes subdividing preclinical AD (i.e., CDR 0) into 3 categories based on cognitive and biomarker status (Jack et al., 2012; Sperling et al., 2011). Stage 0 is defined as no evidence of AD: normal cognition without amyloid pathology or neurodegeneration. Stage 1 is defined as normal cognition and the presence of amyloid pathology but without neurodegeneration. Stage 2 is defined by normal cognition with both amyloid and neurodegeneration pathology. Stage 3 is defined as both amyloid