FUNCTIONAL CONNECTIVITY OF ENTORHINAL CORTEX

IN ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is a progressive neurodegenerative disease characterized by prominent memory impairment, executive dysfunction, language, construction, and visuospatial deficits. In AD, the accumulation of neurofibrillary tangles neuritic plaques, and other associated neuropathology, results in widespread disruption of cortical connections. The entorhinal cortex (EC) is a region of cortical gray matter in the medial temporal lobe important in memory processing, and has been identified as the first structure affected in AD. The current study investigated the functional connectivity of the EC in AD and normal control (NC) subjects using functional connectivity magnetic

resonance imaging (fcMRI). Additional goals of the study were to examine relationships between EC functional connectivity, EC volume, and neuropsychological measures of episodic memory and global cognitive ability.

Nine NC and seven AD subjects were imaged using a 3.0 Tesla magnetic resonance scanner while resting quietly. Compared to the NC group, AD subjects exhibited significantly reduced functional connectivity with the EC in prefrontal cortex (BA 47, 10, 6, 9, & 8), right superior temporal areas (BA 22 & 39), right fusiform gyrus (BA 37), and right perirhinal/entorhinal cortex (BA 35) extending into the hippocampus. Areas of significantly increased functional connectivity in AD subjects included bilateral inferior frontal gyrus (BA 47), left middle frontal gyrus (BA 46), left entorhinal/parahippocampal cortex (BA 28), and the left putamen. No significant relationships were detected among EC functional connectivity, EC volume, and cognitive measures.

The findings of reduced EC connectivity in frontal and temporal association areas in AD are consistent with what is known about the progression of pathophysiology of AD, and provide support for the use of fcMRI in examining cortical connectivity patterns. Increased EC connectivity in prefrontal cortex may reflect the presence of compensatory mechanisms in the neural connections of AD patients. The lack of correlations among EC connectivity, EC volume, and neuropsychological measures suggests that more complex relationships among the variable may exist than was hypothesized. Future research investigating the relationships between functional integrity and structural volume, and how these variables relate to cognitive performance is needed.

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CHAPTER ONE

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder whose cognitive deficits typically manifest late in life, and are characterized by prominent memory impairment with rapid forgetting, executive dysfunction, and difficulties with language, construction, and visuospatial skills (Welsh-Bohmer & Warren, 2006).

AD is the most common form of dementia today, accounting for approximately 70 % of all dementing illnesses, with a national prevalence rate estimated to be 11.1 % of people over the age of 70 years (Plassman et al., 2007). In 2004, it was the seventh leading cause of death for adults in the United States (Heron, 2007). Currently, the number of people in the United States who are 65 years of age or older is about 37 million, and this number is projected to double by the year 2040 (Federal Interagency Forum on Aging-Related Statistics, 2006), placing a significant and growing demand on healthcare for dementia patients that will have serious economic and medical implications. Recent trends in clinical practice (Petersen et al., 2001) and research (Wierenga & Bondi, 2007) emphasize the need for techniques that aid in early detection of AD, thus, allowing for earlier intervention and improvement in the quality of life for these individuals and maintenance of function.

The neuropathology of AD is characterized grossly by frontotemporal atrophy and microscopically by loss of synapses (Davies, Mann, Sumpter, & Yates, 1987). The two cardinal microscopic features are the abnormal accumulation of neurofibrillary tangles (NFTs) and neuritic plaques (NPs) (Braak & Braak, 1991). The buildup of NFTs and NPs in the basal forebrain appears to result in the degeneration of neurons in the cholinergic pathway and an overall reduction of acetylcholine (Whitehouse et al., 1982), a neurotransmitter essential to memory processing. Though the pathophysiology of AD is not fully understood, it targets medial temporal lobe (MTL) structures early in the disease process before progressing to temporal, frontal, and parietal association cortices (Braak & Braak, 1991). Histological studies examining the distribution pattern of NFTs indicate that the transentorhinal and entorhinal cortices are the first areas to become affected in AD, before spreading to the hippocampus and amygdala in the early stages (Braak & Braak, 1991; Gomez-Isla et al., 1996). These MTL structures play a crucial role in episodic memory functioning (Eichenbaum, Yonelinas, & Ranganath, 2007; Squire, Stark, & Clark, 2004), and the early deterioration of neurons in this area is consistent with the initial deficits of learning and memory seen in AD.

A diagnosis of definite AD can only be made by examining brain tissue and documenting the presence of sufficient levels of NFTs and NPs. The clinical diagnosis of AD by McKhann et al. (1984) criteria achieves an 87% accuracy rate

(Ranginwala, Hynan, Weiner, & White, 2008). With the aid of a comprehensive neuropsychological assessment, a diagnostic accuracy rate of 89% can be achieved for mildly impaired individuals (Salmon et al., 2002).

On the other hand, preclinical detection of the disease process with neuropsychological measures is difficult (Goldman et al., 2001). The neuropathological changes characteristic of the AD brain begin long before a clinically observable decline in cognition occurs (Braak & Braak, 1991, 1998; Price, Davis, Morris, & White, 1991; Selkoe, 2002; Whitehouse et al., 1982). Thus, more sensitive tools may be required to detect preclinical markers of the disease. The advent of brain imaging technology has allowed researchers to modify and extend research on histological and animal studies in characterizing early structural and functional changes and how they progress over time, in an attempt to identify potential biomarkers that may allow for earlier detection of AD.

Research on aging and AD indicates that the entorhinal cortex (EC) is particularly sensitive to AD pathology, while remaining relatively unaffected by the normal aging process (Gomez-Isla et al., 1996; Raz, Rodrigue, Head, Kennedy, & Acker, 2004; Rodrigue & Raz, 2004; Small, Tsai, DeLaPaz, Mayeux, & Stern, 2002), making this structure a likely candidate as a potential disease biomarker. In support of this idea, structural magnetic resonance imaging (MRI) studies have found significant reductions in EC volume in individuals with mild

cognitive impairment (MCI) (Devanand et al., 2007; Dickerson et al., 2001; Killiany et al., 2002; Pennanen et al., 2004; Whitwell et al., 2007; Xu et al., 2000), which is characterized by significant cognitive dysfunction in the absence of functional impairment (Petersen et al., 1999). Moreover, there is evidence that the EC atrophies at a faster rate than the hippocampus in patients with MCI (Du et al., 2003). In reviewing the literature on pathology in AD, it is evident that functional changes in the EC precede the cell loss and resultant volumetric changes detectable in structural MRI studies (Davies et al., 1987; Selkoe, 2002; Small et al., 2002), highlighting the importance of examining functional differences in the EC of patients with AD.

Functional connectivity MRI (fcMRI) is a relatively new technique used to examine functional synchrony, or coherence, among brain regions. Brain areas that are functionally related show significant spontaneous fluctuations in neural activity that are correlated during the resting state (Biswal, Yetkin, Haughton, & Hyde, 1995; Cordes et al., 2000; Lowe, Mock, & Sorenson, 1998). Researchers employing fcMRI to investigate functional coherence in AD have found significant changes in connectivity patterns with the hippocampus (Allen et al., 2007; He et al., 2007; Li et al., 2002; K. Wang et al., 2007) when compared to healthy elderly subjects. Additionally, researchers studying the default mode network, a set of brain regions that are consistently active together during rest periods of task-dependent fMRI studies (Greicius, Krasnow, Reiss, & Menon,

2003; Raichle et al., 2001), have demonstrated a disruption in functional coherence of this network in AD (Buckner et al., 2005; Greicius, Srivastava, Reiss, & Menon, 2004; Greicius, Supekar, Menon, & Dougherty, 2008; He et al., 2007; K. Wang et al., 2007). These studies demonstrate the utility of fcMRI in detecting the degradation of functional networks in AD.

Though the EC has been identified as the first structure affected in AD, resting-state functional connectivity of the EC has not been investigated in this disease. Furthermore, the nature of the relationship between functional connectivity and structural volume in this disease has not been established. The goal of the present study is to conduct a pilot investigation of the effect of AD on functional coherence of the EC using fcMRI, and examine the correspondence of this coherence to EC volume. Additionally, the relationship of functional and structural changes to memory test performance will be examined. The results of this study will increase our understanding of the relationship between functional integrity and cognitive performance.

CHAPTER TWO

Review of the Literature

Due to the widespread disruption of cortical connections in this disease, some have conceptualized AD as a disconnection syndrome (De Lacoste & White, 1993). De Lacoste and White (1993) presented a model predicting that the initial pathology seen in AD occurs in the EC and results in damage to this structure. This damage leads to a disconnection of the hippocampal formation from the rest of the cerebral cortex. As the disease progresses and neuropathology spreads, further disruption along corticocortical connections becomes apparent. The interconnections of the EC are described below.

Neuroanatomy of the Entorhinal Cortex

Corticocortical connections. The MTL is composed of several structures that together form the hippocampal formation and the parahippocampal region. The hippocampal formation includes the dentate gyrus (DG), areas CA1-3, and the subiculum (Witter, Wouterlood, Naber, & Van Haeften, 2000). The cortical area adjacent to and surrounding the hippocampal formation comprises the hippocampal region, and includes the presubiculum, parasubiculum, and entorhinal, perirhinal, and parahippocampal (or postrhinal in nonprimates) cortices (Witter et al., 2000). The EC (Brodmann area 28) lies in the parahippocampal gyrus, ventromedially to the periamygdaloid cortex and rostromedially to the presubiculum (Amaral & Insausti, 1990; Insausti, Tunon,

Sobreviela, Insausti, & Gonzalo, 1995). It extends laterally to the medial bank of the collateral sulcus, where it is bordered by the perirhinal cortex. Cytoarchitecturally, the EC can be divided into six cellular layers, and has been subdivided into lateral and medial areas based on connectivity patterns (Kerr, Agster, Furtak, & Burwell, 2007).

Current understanding of the corticocortical projections of the EC has been advanced through histopathological studies of animals, primarily the rat, cat, and monkey (Amaral & Insausti, 1990; Burwell, 2000), in which it has been shown that the EC is highly interconnected with other MTL structures and areas of frontal, temporal, and parietal cortices. The memory system of the MTL is one of the most studied networks in the brain; as such, the interconnections of the EC within this region are well documented (Amaral & Insausti, 1990; Burwell, 2000; Insausti et al., 1998; Kerr et al., 2007; Witter et al., 2000). Multimodal sensory information from association cortex in the frontal, temporal, and parietal lobes reaches the EC through the perirhinal and parahippocampal cortex (Burwell, 2000). Research on non-human primates indicates that perirhinal cortex receives visual object information from the superior temporal sulcus and parahippocampal cortex, while somatosensory input is received from the insular cortex. This information is then projected to the lateral EC (Kerr et al., 2007). Parahippocampal cortex receives visuospatial input from posterior parietal cortex and area V4, while somatosensory information comes from insular cortex, and

auditory information from the auditory association cortex. This multimodal sensory information is projected primarily to the medial EC (Kerr et al., 2007). The EC then projects to the DG through a collection of fibers arising mainly from layer II, called the perforant path, which is the primary source of cortical input to the hippocampus (Witter, 2007). Efferents from the EC also connect to the subiculum, presubiculum, and parasubiculum (collectively called the subiculum complex) and areas CA1 and CA3 (Amaral & Insausti, 1990). Completing the circuit, the EC receives output back from CA1 and the subiculum complex, which it forwards to the perirhinal and parahippocampal cortices. In primates, information from the presubiculum is projected through the hippocampal commissure to the EC of the contralateral hemisphere, accounting for the most prominent link between the MTLs of the left and right side of the brain (Amaral & Insausti, 1990).

Subcortical reciprocal connections with the EC are found in the claustrum, amydgala, nucleus accumbens, hypothalamus, caudate and putamen of the basal ganglia, and dorsal and ventral regions of the thalamus (Amaral & Insausti, 1990; Kerr et al., 2007). The septal complex of the basal forebrain also projects to the EC, and is considered to be the primary cholinergic innervation of the MTL.

Neocortical projections to the EC come from the superior temporal gyrus, and orbitofrontal, dorsolateral frontal, medial frontal, and retrosplenial cortices. Reciprocal projections of the EC have been found with pyriform, frontal, insular,

temporal polar, cingulate, parietal, and occipital regions (Amaral & Insausti, 1990; Kerr et al., 2007). These long-range cortical and subcortical connections through the EC provide the hippocampal formation extensive influence over frontal, temporal, and parietal regions of the brain (Amaral & Insausti, 1990).

Functional role. The prevailing view of MTL function is that it supports processing of episodic, or declarative, memory (i.e., memory for facts and events). The development of a model for MTL-dependent memory functions began with a description of H.M., who underwent bilateral surgical resection of the anterior MTLs as treatment for intractable epilepsy (Scoville & Milner, 1957). Following surgery, H.M. was unable to form new episodic memories, and was thus rendered profoundly amnesic. Subsequent studies of amnestic patients and the development of animal models of human amnesia led to the discovery of structural correlates of episodic memory functioning. Squire and Zola-Morgan (1991) summarized these studies and proposed a model of MTL function. In their paper, they describe a series of lesion studies in monkeys that demonstrate the importance of the hippocampal formation in episodic memory functioning. Larger lesions, involving the hippocampal formation and surrounding areas of entorhinal, perirhinal, and parahippocampal cortices resulted in more extensive memory deficits, indicating that these regions are also necessary for episodic memory functioning, independent of the hippocampal formation. Different pieces of sensory information about an event travel from association cortex in frontal,

temporal, and parietal lobes to the MTL circuit, where they are bound together, or consolidated, to represent a whole event. Information then leaves the MTL system through the EC and subiculum complex, and is projected back to association cortex. As evidenced by amnestic patients who have the ability to recall old memories, the information that is consolidated in the MTL memory system is not stored there long-term, but instead is stored in relevant association cortex networks (Squire et al., 2004).

Current efforts to further elucidate the structural components of memory processing attempt to determine the differential contributions of structures within the MTL. The results of these efforts have been unclear. Some researchers propose that the different components of the episodic memory network serve distinct functions (Daselaar, Fleck, Dobbins, Madden, & Cabeza, 2006; Eichenbaum et al., 2007; Murray, Bussey, & Saksida, 2007; Witter & Moser, 2006; Yonelinas et al., 2007), while others maintain that the different structures are more likely to overlap in their functional contributions (Squire et al., 2004; Stark, Bayley, & Squire, 2002). One theory focuses on the distinction between recollection and familiarity, two components of recognition memory that are proposed to be separate processes (Eichenbaum et al., 2007; Yonelinas et al., 2007). Recollection requires the retrieval of specific information about an event, such as when it happened, or where it happened, and thus could be described as a relational or associative process (Eichenbaum et al., 2007). Sensory information

flows from association cortex through the hippocampal region and converges in the hippocampus. Based on this anatomical model, some researchers propose that the function of the hippocampus is to consolidate different kinds of sensory information related to a single event (Squire et al., 2004). Therefore, relational memory processes, such as recollection, are dependent on the hippocampus. Familiarity, on the other hand, refers to knowing that an event was previously encountered, but without the contextual information. Proponents of this theory posit that familiarity depends on cortical areas that surround the hippocampus, and are earlier in the path of information flow (Eichenbaum et al., 2007). Some studies indicate that familiarity depends predominately on the EC (Yonelinas et al., 2007). Others propose that the perirhinal cortex is important for familiarity, while the EC cortex plays a role in both familiarity and recollection (Eichenbaum et al., 2007).

Despite supporting evidence, a study by Stark, Bayley, and Squire (2002) presents an opposing view on the different functions within the MTL structures. They used an associative learning task to examine relational learning, and a recognition memory task to investigate familiarity in patients with circumscribed hippocampal lesions. Patients were equally impaired on both tasks, suggesting that the hippocampus is important in both familiarity and recollection, or relational learning, tasks. The conflicting findings on the role of the hippocampus, entorhinal, and perirhinal cortices in familiarity and relational learning indicate

that there is not a simple division of labor in memory processing among MTL structures (Squire et al., 2004).

More recently, the EC has been found to be critically involved in spatial navigation in rats. Hafting, Fyhn, Molden, Moser, and Moser (2005) discovered neuronal spatial maps in the dorsocaudal part of the medial EC composed of "grid" cells. The grid-like firing pattern of these cells is dependent on the spatial environment and the animal's location in that environment. The function of these spatial maps is not fully understood, though it suggests that the EC is important in keeping track of position, direction, and distance in space. The presence of grid cells in humans has not yet been established, though they may have interesting implications for AD. The grid cells were found primarily in layer II of the EC, an area that is especially targeted by AD pathology. Deterioration of these neurons may relate to the spatial navigation deficits noted in AD (deIpolyi, Rankin, Mucke, Miller, & Gorno-Tempini, 2007) and MCI (Hort et al., 2007).

Entorhinal Cortex in Healthy Aging

A cross-sectional stereological study by Gomez-Isla, et al. (1996) examined the number of neurons in the EC of healthy elderly and individuals with AD. The researchers found that neuron number, volume, and cell density of the EC remained stable when comparing healthy elderly individuals in their 60s to healthy elderly individuals in their 90s. In the group with AD, EC neuron number and volume were significantly reduced, and cell density was significantly reduced

in layer II of the EC, compared to healthy elderly. In addition, when comparing very mildly impaired subjects with AD to individuals with moderate AD and a control group, a significant reduction was found in EC neuron number in those with mild AD, especially in layers II and IV, with a more pronounced reduction in moderately impaired AD subjects. These findings indicate that the EC has already been substantially affected by the pathology of AD at a stage when cognitive dysfunction is just beginning to emerge, and yet remains relatively stable throughout the normal aging process.

The finding that the EC is relatively impervious to normal aging has been replicated using structural MRI as well. Raz, Rodigue, Head, Kennedy, and Acker (2004) compared the effects of aging on the hippocampus and EC over a span of 5 years. Using a sample of healthy adults between the ages of 26 and 82 years, the researchers demonstrated that the volume of the hippocampus shrank over two times as much as the EC, and correlated more strongly with age than the EC. Additionally, shrinkage in the hippocampus occurred at a faster rate than in the EC. Contrary to the results found by Gomez-Isla et al. (1996), Raz et al. found that volume of the EC was associated with age in elderly adults. When comparing adults less than 50 years of age to those 50 years old and older, EC volume remained stable in the younger adults; however, the older group exhibited a mild degree of volume loss. Hippocampal shrinkage was evident in both groups, though to a greater degree in the older group. While this evidence of EC volume

decline in older adults may appear to be in conflict with previous findings (Gomez-Isla et al., 1996), the authors note that the broad screening measures used to assess cognitive decline may not be sensitive enough to detect early cognitive changes indicative of mild impairment. The possibility that some individuals with mild cognitive dysfunction were included in their sample cannot be ruled out, and could account for the association between EC volume loss and age found in the older adults. An important implication of this study is that age has a differentially negative impact on the hippocampus as compared to the EC.

Further evidence that the EC may be uniquely sensitive to neurological changes associated with preclinical AD was reported by Rodigue and Raz (2004). Using structural MRI, the researchers investigated longitudinal change in the volumes of EC, hippocampus, and prefrontal cortex (PFC) across a wide age range of healthy adults (26 to 82 years of age), and the relationships of these volumes to memory test performance. Consistent with prior research, results indicated that the rate of EC volume loss did not correlate with age, while the hippocampus and PFC exhibited greater shrinkage across the age span. Interestingly, greater losses of EC volume over a five-year time period predicted a decline in memory performance after accounting for the effects of age, as opposed to hippocampus and PFC volume shrinkage that had no relationship to memory scores over and above the effects of age.

One of the challenges for preclinical markers of disease is to differentiate preclinical AD from healthy aging. The biggest risk factor of AD is advancing age (Keller, 2006). Therefore, the EC's relative resistance to the effects of healthy aging may allow for easier detection of structural or functional changes associated with very early AD. Though both EC and the hippocampus are known to be involved in AD, a review of the literature indicates a higher potential for discovering early AD biomarkers in the EC as compared to the hippocampus, due to the difficulty in differentiating the effects of normal aging from pathological changes in the hippocampus in very early stages of the disease. These studies highlight the importance of investigating changes in structural and functional integrity in healthy aging and AD.

Structural MRI of Entorhinal Cortex in Alzheimer's Disease

The investigation of structural changes in AD have typically focused on the hippocampus, due to its high degree of involvement in AD, central role in episodic memory processing, and distinctive boundaries easily identifiable on magnetic resonance (MR) images. Though AD-related pathological changes are noted even earlier in the EC, the difficulty in distinguishing the structural boundaries in MRI has precluded many from investigating this structure. However, recent publications of established protocols for identifying EC boundaries on MR images have resulted in several volumetry studies comparing structural changes in the EC and hippocampus in AD (Goncharova, Dickerson,

Stoub, & deToledo-Morrell, 2001; Insausti et al., 1998; Killiany et al., 2002; Pruessner et al., 2002). A review of early studies investigating these two structures confirmed the consistent finding of volume loss in both, and furthermore, indicated that the EC, in combination with temporal neocortex, provided the most accurate prediction of conversion to AD in patients with MCI (Chetelat & Baron, 2003). However, many of these studies employed cross-sectional designs, and the use of inconsistent sampling procedures and MRI tracing protocols have led to some conflicting results. Recent studies using longitudinal designs have extended our understanding of the EC and hippocampus volume in AD (deToledo-Morrell et al., 2004; Dickerson et al., 2001; Killiany et al., 2002), although the predictive utility of these structures in determining who will develop dementia is still unclear.

The most consistent findings among MRI studies of MTL volume pertain to the ability of brain structure measurements to differentiate between healthy elderly, patients with AD, and those at risk for developing the disease by virtue of having MCI or subjective memory complaints. Although there has been at least one study that found no discriminative ability in the volumes of the EC or the hippocampus (Xu et al., 2000), the majority of findings indicate that the volume of the EC differentiates between healthy elderly and MCI with more sensitivity and specificity than the volume of the hippocampus (Dickerson et al., 2001; Killiany et al., 2002; Pennanen et al., 2004). These finding may be explained by

the EC's earlier involvement in AD compared with the hippocampus. Interestingly, when attempting to discriminate between MCI and AD, some researchers found the volume of the hippocampus to have the best discriminative power (Dickerson et al., 2001; Pennanen et al., 2004), while others concluded that the EC was the most sensitive (Killiany et al., 2002).

Several research groups have examined the predictive utility of volumetry in the conversion of the cognitively impaired to AD, though the data appear to be unclear. By measuring baseline volumes of EC and the hippocampus, and then following participants over time, investigators can compare which structures are the best predictors of cognitive decline. DeToledo-Morrell's laboratory (Dickerson et al., 2001) followed a group of individuals with either MCI or subjective cognitive complaints for a period of time ranging from 12 to 77 months. Over the period of observation, approximately half the participants progressed to a diagnosis of AD. The researchers found baseline EC volume to be significantly smaller in converters compared to non-converters. Furthermore, volume of the EC, but not the hippocampus, was a significant predictor of progression to dementia in this sample.

Another longitudinal study conducted by Killiany et al. (2002) compared baseline volumetry of EC and the hippocampus in four groups of individuals: healthy elderly, individuals with mild AD, people with cognitive decline (termed "questionable," indicated by a standard functional assessment interview), and

those from the questionable group that converted to AD over a period of three years. Contrary to the previous study (Dickerson et al., 2001), in this investigation neither the EC nor the hippocampus could differentiate between the questionable group and those that converted to AD, although both structures were associated with a greater likelihood of progressing to dementia. Interestingly, the EC discriminated between all the other groups with greater sensitivity and specificity than the hippocampus.

There are several methodological differences between these two studies that might account for the conflicting results. One potential explanation pertains to differences in protocols used to trace the regions of interest (ROIs). To investigate this possibility, deToledo-Morrell et al. (2004) conducted another investigation using similar methodology to their previous study. This time, their sample of those at risk for developing AD included only individuals diagnosed with MCI, rather than those with subjective cognitive complaints who have no cognitive impairment on neuropsychological testing. Over the course of three years, 10 of their 27 subjects met criteria for a diagnosis of AD. Using the same tracing protocols as before, they found similar results to their previous study, in that baseline EC volume was a better predictor of conversion to AD than the volume of the hippocampus. Additionally, reanalyzing their data using the same tracing protocol as Killiany et al. (2002) did not change the outcome of the study. Therefore, the authors concluded that sampling differences are more likely to

account for the inconsistent findings than variability in MRI volumetry techniques.

Volumetric changes in the EC and hippocampus have been the focus of several longitudinal investigations. However, many studies have neglected to include ROIs outside of the temporal lobe; therefore, the specificity of findings related to these two structures has not been determined. Whitwell et al. (2007) compared brains from a group of individuals with stable MCI (across three years) to a group with MCI that progressed to AD within an 18-month time period. The groups were examined using voxel-based morphometry (VBM), which provides a voxel-by-voxel comparison of regional concentrations of gray matter, allowing for a comprehensive assessment of differences between groups throughout the entire brain. When compared to healthy control subjects, the group with stable MCI showed no significant gray matter differences. In contrast, those that progressed to AD exhibited significantly less gray matter concentration in the anterior temporal lobe, including the EC, parahippocampal gyrus, hippocampus, amygdala, fusiform gyrus, and inferior and middle temporal gyrus. Additional reductions were noted in the parietal lobe, frontal lobe, basal forebrain, and insula. A similar pattern of reduced gray matter concentration was observed in this group when directly compared to the stable MCI group, with additional reductions in the precuneus, and anterior and posterior cingulate. These findings are consistent with

patterns of neuropathology found in AD, spreading from the MTL to association cortices in temporal, frontal, and parietal regions of the brain.

Overall, fairly consistent findings of reduced volume in the EC and hippocampus in AD have been established. However, the utility of these changes in predicting future decline in those at risk for developing dementia is still under review, due to difficulties in obtaining consistent samples across studies. Research investigating functional changes in MTL structures may be more useful in this regard.

Functional Connectivity in Alzheimer's Disease

Functional connectivity magnetic resonance imaging (fcMRI) is a relatively new method of examining the integrity of functional networks that involves the investigation of spontaneous low frequency fluctuations of neural activity using functional MRI. Functional connectivity MRI or resting-state MRI, examines the intrinsic coherence among functionally related brain regions during rest, as opposed to standard fMRI studies that use a task to stimulate changes in neuronal activity. As in a task-related design, these low frequency fluctuations reflect modulation of the blood oxygen level dependent (BOLD) signal (Peltier & Noll, 2002). The BOLD signal is an indirect index of neural activity that results from an overall localized decrease in deoxyhemoglobin relative to oxyhemoglobin in the blood following glucose metabolism (Bandettini & Wong, 1994).

Biswal, Yetkin, Haughton, and Hyde (1995) were the first to demonstrate that areas of the brain that function together in networks also exhibit coherence in spontaneous low frequency signal changes in FMRI. They found that low frequency BOLD fluctuations in the left somatomotor cortex correlated with the same region in the contralateral hemisphere and additional motor regions in the absence of a motor task. These findings have been replicated (Cordes et al., 2001; Cordes et al., 2000; Lowe et al., 1998), and additional neurocognitive and sensory networks have also been studied using fcMRI, including cerebellar (Allen et al., 2005), visual (Cordes et al., 2000; Lowe et al., 1998), auditory (Cordes et al., 2000), language (Cordes et al., 2000), attention (Fox, Corbetta, Snyder, Vincent, & Raichle, 2006), memory (Allen et al., 2007; Kahn, Andrews-Hanna, Vincent, Snyder, & Buckner, 2008; Li et al., 2002; Vincent et al., 2006), and default mode (Fox et al., 2005; Greicius et al., 2003; Greicius et al., 2004; Greicius et al., 2008; He et al., 2007; K. Wang et al., 2007) systems. Additionally, the disruption of resting state coherence has been investigated in multiple patient populations, including multiple sclerosis (Lowe et al., 2008), autism (Kennedy & Courchesne, 2008; Turner, Frost, Linsenbardt, McIlroy, & Muller, 2006), attention-deficit/hyperactivity disorder (Uddin et al., 2008), chronic pain (Baliki, Geha, Apkarian, & Chialvo, 2008), and AD (Allen et al., 2007; Greicius et al., 2004; Greicius et al., 2008; He et al., 2007; Li et al., 2000; K. Wang et al., 2007).

Standard methods of fcMRI studies involve identifying a "seed" region, or primary ROI, averaging the temporal waveforms of all the voxels in that region, and cross-correlating that average waveform with all other voxels in the brain. After applying appropriate thresholds, significant areas of brain activity in functionally related regions are identified. As mentioned above, fcMRI focuses on coherence among low frequencies between brain regions, and pre-processing analyses involve filtering out high frequency fluctuations. To further investigate the frequencies related to the pattern of functional connectivity, Cordes et al. (2001) conducted a study to examine the temporal patterns that contribute to the cross-correlation between ROIs. By applying a spectral analysis to the cross-correlation coefficient, they were able to determine that, on average, only low frequencies of less than 0.1 Hz contribute significantly to intrinsic interregional coherence. Therefore, most researchers employing fcMRI methods focus only on frequencies less than 0.1 or 0.08 Hz (Fox & Raichle, 2007).

Spontaneous oscillations examined with fcMRI are presumed to reflect neural connections, though little objective evidence for this exists. However, a recent study combining fcMRI and diffusion tensor imaging (DTI) aims to clarify this issue (Greicius et al., 2008). DTI is a noninvasive measure of white matter tracts that allows for the estimation of structural connectivity in the brain. Greicius, Krasnow, Reiss, and Menon (2008) applied DTI and fcMRI to three regions of the brain involved in the default mode network, including posterior

cingulate cortex/retrosplenial cortex (PCC/RSC), medial prefrontal cortex (MPC), and MTL. A comparison of the two resulting maps showed that the results are similar, but with important differences. The DTI map reflected connectivity between MPFC and PCC/RSC, as well as between MTL and PCC/RSC, areas that animal studies have shown to be directly connected. White matter tracts between MPFC and MTL regions could not be established, which the authors attribute to the presence of an indirect connection between the two regions, via the uncinate fasiculus, rather than a direct connection. Alternatively, the fcMRI map reflected resting state coherence among all three regions. These results suggest that fcMRI maps primarily reflect anatomical connectivity, though they are not direct representations.

fcMRI of the medial temporal lobe. Most of what is currently known about the structural and functional connectivity of the episodic memory system and MTL comes from animal research. However, new imaging techniques provide the opportunity for examination of neural networks in the human brain. Rombouts, Stam, Kuijer, Scheltens, and Barkhof (2003) demonstrated functional synchrony between right and left hippocampi in a small group of three young adults. A more detailed study used fcMRI to determine how closely the memory system of humans mirrors that of the monkey. In a sample of 55 young adults, Kahn, Andrews-Hanna, Vincent, Snyder, and Buckner (2008) identified a total of 16 different seed regions within the left hemisphere of the MTL, including eight

across the length of the hippocampus, three in the perirhinal/entorhinal cortex, and five in the parahippocampal cortex. The authors found distinct patterns of spontaneous coherence. Seed regions in the head of the hippocampus and posterior end of the perirhinal/entorhinal cortices showed similar patterns of connectivity to the anterior inferior temporal sulcus (aITS), while seed regions in the body of the hippocampus and the posterior parahippocampal cortex overlapped in coherence with the inferior parietal lobule (IPL), retrosplenial cortex, inferior posterior cingulate, and ventral medial prefrontal cortex, suggesting the presence of two separate but parallel pathways within the MTL. In an attempt to validate their findings, the research group used a second sample of 45 young adults to correlate seed regions in the aITS and IPL with those in the parahippocampal cortex, perirhinal/entorhinal cortices, and hippocampus. They found stronger correlations between the aITS and the anterior head of the hippocampus, perirhinal/entorhinal cortices, and anterior portion of the parahippocampal cortex, relative to the IPL. In contrast, the IPL showed greater correlations with the posterior parahippocampal cortex, relative to the aITS.

With this method, Kahn, Andrews-Hanna, Vincent, Snyder, and Buckner (2008) were able to establish the existence of two separate pathways linking distinct subregions of the MTL that merge in the hippocampus, as has been found in the monkey. The pathway that includes the IPL, retrosplenial cortex, posterior cingulate cortex, and ventral medial prefrontal cortex has been found to be

involved in memory tasks (Vincent et al., 2006), as well as the default network (Greicius et al., 2003). The authors note that these findings provide support for the theory that the primary function of the hippocampus is to integrate spatial information from the parahippocampal cortex with nonspatial sensory information from the perirhinal cortex (Eichenbaum et al., 2007). However, it is important to note that fcMRI cannot establish the direction of functional connectivity pathways. Therefore, the presence of unidirectional and bidirectional coherence pathways among regions (e.g. between the hippocampus and the perirhinal/entorhinal cortices) has not been examined here, thus precluding conclusions about distinct and separate functions occurring in these regions. Additionally, future studies exploring differences in patterns of coherence among the perirhinal and entorhinal cortices with other subregions of the MTL will be useful in determining the functional and structural connectivity patterns of the human episodic memory system.

fcMRI of the MTL in elderly adults and Alzheimer's disease patients. FcMRI has also been used to examine functional changes in the MTL in healthy older adults and patients with AD, with a primary focus on the hippocampus. However, unlike the proliferation of research focused on structural changes in the MTL, only a few studies have investigated potential corresponding changes in the functional integrity of this region. Li et al. (2002) examined low frequency fluctuations in the left and right hippocampi in elderly adults, subjects with MCI,

and patients with AD. They found functional coherence between hippocampi in the two hemispheres to be significantly reduced in those with MCI, as compared with elderly, and even further reduced in the group with AD. Furthermore, the degree of coherence correlated positively with cognitive performance on the Mini Mental State Exam (Folstein, Folstein, & McHugh, 1975). Though conclusions are limited by the restricted approach of examining functional connectivity only between the hippocampi, it is an important study in that it was the first to demonstrate the usefulness of fcMRI in detecting functional differences in AD and MCI, and to establish a relationship between cognitive performance and spontaneous coherence in these populations.

Extending these findings, two additional studies examined functional coherence between the hippocampus and the rest of the brain in AD patients (Allen et al., 2007; L. Wang et al., 2006). In elderly adults, Wang et al. (2006) found low frequency fluctuations in the right hippocampus to correlate with regions that overlap with the default mode network, including medial prefrontal cortex, ventral anterior cingulate cortex, orbital frontal cortex, posterior cingulate cortex, precuneus, left inferotemporal cortex, and right inferior parietal cortex. Cross correlation of the left hippocampus revealed asymmetry in the coherence maps, with similar but fewer regions correlating with the left hippocampus, and to a lesser magnitude and spatial extent. Compared to this group, patients with mild or very mild AD exhibited reduced coherence with the right hippocampus in

medial prefrontal cortex, ventral anterior cingulate cortex, right cuneus and precuneus, right inferotemporal cortex (including inferior temporal gyrus and perirhinal cortex), right superior temporal gyrus, and middle temporal gyrus. A reduction in the right posterior cingulate cortex was also observed, though it did not meet full criteria for significance. The only difference noted in connectivity of the left hippocampus in the AD group was significantly increased coherence with the right dorsolateral prefrontal cortex.

Allen et al. (2007) found a similar pattern of reduced functional connectivity with the hippocampi in AD, though to a greater degree and with a notable exception. Their sample of patients with mild AD showed reduced coherence with parietal, occipital, and limbic regions, as well as with basal ganglia and cerebellum. However, a complete loss of connectivity between the hippocampus and the frontal lobes in AD patients was found, contrary to the increase in functional synchrony between the left hippocampus and the right dorsolateral prefrontal cortex reported by Wang et al. (2006). The authors attribute this absence of connectivity with frontal regions to an overall greater severity in cognitive decline in their sample of individuals with AD as compared to the sample studied by Wang et al. , consistent with previous findings that functional coherence declines as cognitive deficits increase (Li et al., 2002).

These studies demonstrate the utility of fcMRI in detecting functional in vivo changes in the MTL with AD. Despite the central role of the EC in episodic

memory processing, and the knowledge that the EC is affected earlier in the course of the AD than the hippocampus, spontaneous low frequency coherence of this structure has not been examined in this population. The goal of this pilot study is to examine functional connectivity of the EC with the rest of the brain in AD using fcMRI. In particular, potential disruptions in connectivity with the hippocampus will be investigated, and the relationship of low frequency synchrony to structural volume and memory performance will be examined.

CHAPTER THREE

Goals and Hypotheses

Goal1: Examine functional coherence of the EC in the brain, and specifically with the hippocampus, another MTL structure crucial for episodic memory processing prominently affected by AD pathology, in AD and NC.

Hypothesis 1: Functional coherence between the EC and hippocampus will be significantly lower in participants with AD compared to NC.

Exploratory Analysis: Functional coherence between the EC and the rest of the brain will be analyzed to investigate the overall pattern of functional connectivity of the EC.

Goal 2: Intestigate the volume of the EC in a control group of healthy elderly adults (NC) and individuals with AD.

Hypothesis 2: The average volume of the EC will be significantly reduced in participants with AD compared to NC.

Goal 3: Examine functional coherence as it relates to structural volume.

Hypotheisis 3: Functional connectivity of the EC will exhibit a positive relationship with structural volume in the overall group of combined participants.

Goal 4: Examine functional coherence and structural volume of the EC as it relates to performance on neuropsychological measures of episodic memory.

Hypothesis 4: Significant positive correlations will be found between functional coherence of the left EC with the hippocampus and measures of verbal recall. Structural volume of the EC will show significant positive correlations with measures of verbal recall.

Exploratory Goal: Examine the relationship of functional coherence and structural volume of the EC with a global measure of cognitive ability.

CHAPTER FOUR

Methodology

Participants

Twenty participants were recruited from the Alzheimer's Disease Center (ADC) at the University of Texas Southwestern Medical Center (UTSW). The sample included 10 patients with mild probable AD ranging in age from 56 to 83 years (6 females and 4 males, mean age = 69.8 years), and 10 healthy control subjects (NC) ranging in age from 59 to 83 years (5 females and 5 males, mean age = 73.5). Participants in the groups were selected to be similar in age, gender, and education. The UTSW institutional review board approved the study, and all participants gave informed consent for their participation. A clinical diagnosis of AD was made using the National Institute of Neurological and Communication Disorders and Stroke/AD and Related Disorders Association (NINCDS/ADRDA) criteria (McKhann et al., 1984). All patients underwent physical and neurological examinations, and neuropsychological assessment. Functional decline was assessed with the Clinical Dementia Rating Scale (Morris, 1997). All NC participants had CDR scores of 0, while CDR scores for AD patients ranged from 0.5 to 1.0, indicating mild disease. All participants were English-speaking and were screened for other brain disorders such as stroke, unstable medical conditions, and contraindications for the MRI environment.

Neuropsychological Assessment

Participants underwent a neuropsychological evaluation that assessed multiple cognitive domains. For the purpose of this study, a measure of recall, Logical Memory-Story A of the Wechsler Memory Scale-Revised (WMS-R) (Weschler, 1987), was selected from the battery in order to examine the relationship between episodic memory and MRI measures of functional connectivity of the EC. The WMS-R is a standardized battery of tests designed to measure different aspects of memory functioning, originally published in 1945 and revised in 1987. The Logical Memory Immediate Recall subtest involves the presentation of two short stories (i.e., Story A and Story B), with immediate recall following the presentation of each story. A delayed free recall trial is administered 30 minutes following the conclusion of immediate recall. For the purpose of this study, raw scores of immediate and delayed recall of Story A will be used.

Additionally, a measure of global cognitive ability, a total score (Chandler et al., 2005) derived from the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) Neuropsychological Battery (Morris et al., 1989), was selected to investigate the relationship between EC and global cognitive functioning. The set of tests developed for the CERAD are used to detect cognitive impairment in AD. The battery consists of the MMSE and six additional subtests designed to measure aspects of language and memory functioning: Verbal Fluency-Animals, 15-item Boston Naming Test, Constructional Praxis, Word List Memory, Word

List Recall, and Word List Recognition. The CERAD total score was computed following the methods of Chandler et al. (2005) by adding Verbal Fluency, 15-item Boston Naming Test, Word List Learning, Constructional Praxis, Word List Recall, and Recognition Discriminability subtests.

Magnetic Resonance Imaging

Data acquisition. Imaging was performed on a 3T Phillips MRI scanner. High-resolution T1-weighted structural images were acquired using a standard three-dimensional (3D) whole-brain MP-RAGE sequence using sensitivity encoding (SENSE) to reduce susceptibility artifacts in the images (TR = 2000 ms, TE = 30 ms, flip angle = 12°, FOV = 256mm, sagittal slices, thickness = 1 mm). Functional T2*-weighted images were acquired using a single-shot gradient echo planar pulse sequence using SENSE with a TE of 30 ms, matrix size of 64 × 64, and spatial resolution of 3.44 × 3.44 × 4.00 mm. This time series was composed of 205 images acquired for each of 37 axial slices taken in an interleaved fashion. To ensure stability of the MR signal, the first 5 images were not included in the analyses. During the functional scan, participants were at rest in a darkened scanner room, and followed the instructions to lie still with their eyes closed.

Structural volume measurements. Structural ROIs were manually traced on the high resolution images, in the left and right hemispheres of each participant, using the procedures described below.

The EC was identified based on the method described by Killiany and colleagues (2002). This method measures the mid-region of the structure and excludes the anterior and posterior edges, which are difficult to discern, to increase the reliability of the tracings across subjects. Tracing began on the coronal plane in which the junction of the mammillary bodies and the fornix was evident. The outside edge of the rhinal sulcus and the inferior surface of the subiculum formed the boundaries of the EC. These landmarks were used to trace the EC on the two adjacent anterior and posterior slices to cover the extent of the mid-region of the structure.

Hippocampal tracings were performed on coronal slices, following the methods of Slavin and associates (2007). Tracing began on the most posterior slice in which the tail of the hippocampus and the fornix was visible, and was continued on each slice, moving anteriorly, until the amygdala became apparent. These boundaries included regions CA1 through CA4, the subiculum, and dentate gyrus.

Functional connectivity analyses. Image processing and analysis were performed using Analysis of Functional NeuroImage (AFNI) software (Cox, 1996). First, images were transformed into the coordinate system of Talairach and Tournoux (1993), to aid in anatomical localization and allow the combination of data across subjects and comparisons between groups. Next a series of preprocessing steps were taken to increase the signal-to-noise ratio in the images.

Differences in slice-dependent time shifts were corrected, and motion correction was applied by using a 3D-registration algorithm to align the images through time. Data was temporally band-pass filtered to retain only frequencies between 0.08 Hz and 0.009 Hz. This accomplishes the removal of high frequency fluctuations as well as very low frequency oscillations associated with scanner drift (Smith et al., 1999). Finally, linear trends and additional sources of spurious variance were removed following the procedures of Fox and colleagues (2005). Cardiac pulsations and respiratory cycles can result in neural fluctuations aliased in the BOLD signal at low frequencies (Biswal, DeYoe, & Hyde, 1996). These physiological sources of noise can be substantially reduced by regressing out signals from brain regions where this noise is most prominent. Changes in respiration rate and depth result in BOLD signal fluctuations throughout the brain, which can be represented in a global signal created by averaging voxels across the brain (Birn, Diamond, Smith, & Bandettini, 2006). An additional source of respiratory-related noise is prominent in white matter tissue (Wise, Ide, Poulin, & Tracey, 2004), while cardiac pulsations are reflected in the BOLD signal changes of voxels in cerebral spinal fluid (CSF) (Birn et al., 2006). Therefore, the neural signal from a voxel in the lateral ventricle, and the signal from a voxel located in deep white matter, as well as the global signal, were removed using linear regression methods.

To create seed regions for functional connectivity analyses, the high resolution tracings of both structures were resampled to the functional resolution in each subject and edited to include only gray matter within the boundaries of the structures. The pre-processed BOLD time series was extracted from the seed region in the EC and averaged to create a reference function for the left EC. Resampled EC seed regions ranged in size from one to two voxels across individuals. The resampled hippocampal tracing was created for the use of a mask during the target analysis of functional connectivity between the left EC and hippocampus (described below). Hippocampal ROIs ranged in size from 6 to 26 voxels upon resampling.

Statistical analyses. Volumetric measurements of the left EC in AD and NC subjects were compared using a *t* test with equal variances not assumed.

For the whole brain analysis, the EC reference function was cross-correlated with the BOLD signal time series of all other voxels in the brain to produce a correlation map. To enable group comparisons, correlation coefficients were transformed to z values using Fisher's r-to-z transformation (Zar, 1996, as cited in Vincent et al., 2006). Within-group *t* tests were conducted to identify brain regions in the AD group and NC group that exhibit correlations with the left EC that are significantly different from zero. A between-group *t* test was used to identify brain regions that demonstrate a significant group difference in functional connectivity with the EC. To protect against type I error typical of multiple

comparisons, two thresholds were applied to the output from these *t* tests. First, all voxels whose t value do not exceed $\alpha = 0.01$ were excluded from further analysis. Then, Monte Carlo simulations were used to determine the probability of falsely detecting clusters of various sizes. An overall (i.e., over the entire three-dimensional image volume) significance level of $p < 0.05$ was used, and a minimum cluster size that would occur with a probability of less than 0.05 for each comparison was identified. Clusters that exceeded this cutoff were retained.

For the target analysis between the EC and the hippocampus, the average z value of all voxels within the hippocampus was extracted from the unthresholded whole brain analysis for each individual. Significant functional connectivity between these two regions was examined in each group separately using single-sample *t* tests on group averages of z values. A group comparison was also made using a two-sample *t* test.

Correlational analyses were conducted to examine the relationships between EC connectivity with the hippocampus and EC volume. Additional correlational analyses were used to investigate the relationship between EC volume and measures of episodic memory and global cognitive ability. These same neuropsychological measures were correlated with EC connectivity. Depending on the normality of the distributions of these variables, either Pearson product moment or Spearman rank correlation analyses were conducted.

CHAPTER FIVE

Results

Demographic Characteristics

Of the 20 participants recruited for this study, four subjects were eliminated due to significant signal loss in the MTL on the functional scan. The remaining sample of AD subjects included 7 participants aged 56 to 76 years (mean age 69.6 years), with 4 males and 3 females. They had an average education level of 15.7 years, and an average MMSE score of 24.6 with scores ranging from 21 to 28. The sample included one left-handed female. The NC sample consisted of 9 participants (5 males and 4 females) aged 59 to 83 years (mean age 73.8 years), with an average education level of 15.7 years, and an average MMSE score of 29.6 (scores ranged from 29 to 30). The NC sample included three left-handed subjects, two females and one male. The groups did not differ significantly in age [$t(14) = 1.00$, $p > .05$] or education [$t(14) = -0.05$, $p > .05$]. See Table 1 for a display of demographic information.

Table 1. ***Demographic Variables***

	AD (n = 7)	NC (n = 9)
Gender M/F	4/3	5/4
Age *M* (*SD*)	69.6 (6.8)	73.8 (9.2)
Education *M* (*SD*)	15.7 (1.2)	15.7 (2.3)
MMSE *M* (*SD*)	24.6 (2.6)	29.6 (0.5)

Hypothesis One

Hypothesis one stated that functional connectivity between the EC and hippocampus would be significantly reduced in participants with AD compared to NC. Across both groups, MTL signal dropout was more prevalent in the right hemisphere, thus, fcMRI analyses were performed using only the left EC seed. Significant functional connectivity was detected between these two regions in the NC group, with an average z value of 0.4 (SD = 0.31) [$t(6) = 3.60$, $p < .05$]. However, no significant functional connectivity was observed in AD patients, who had an average z value of 0.2 (SD = 0.31) [$t(8) = 1.83$, $p > .05$]. A comparison of the two groups did not detect significantly different levels of functional connectivity between the EC and hippocampus [$t(14) = 1.00$, $p > .05$].

See Figure 1 for a graph of individual z transformed values in NC and AD subjects.

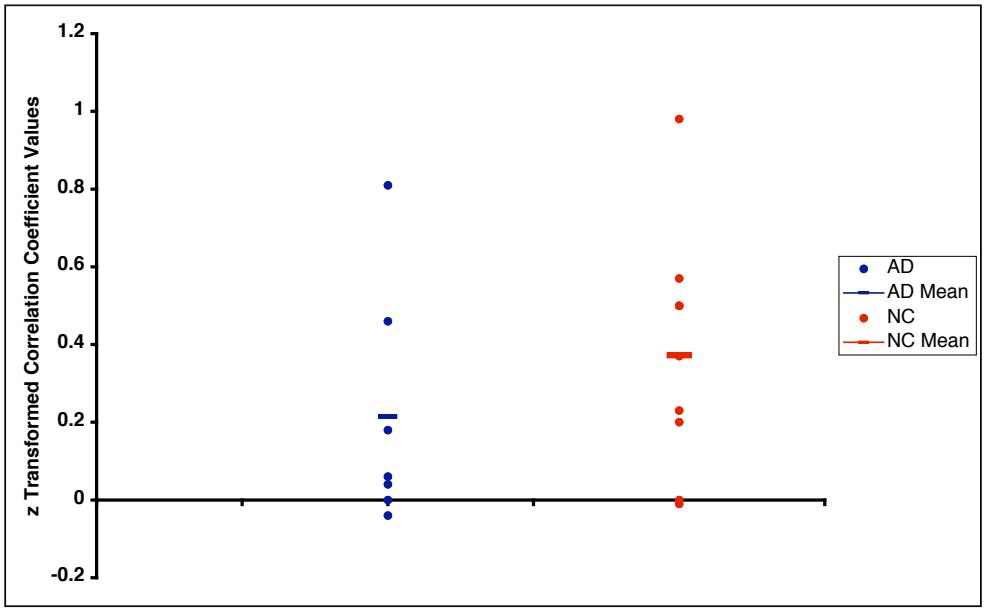

Figure 1. z transformed values measuring functional connectivity between left EC and left hippocampus in AD patients and NC individuals.
Blue circles represent AD subjects, red circles represent NC subjects.

An exploratory analysis examined functional connectivity of the EC with the rest of the brain to identify a) the pattern of whole brain coherence in each group, and b) significant differences between the groups. In the normal control group, the left EC showed significant functional connectivity with a large number of brain areas in frontal, parietal, limbic, and temporal regions. Frontal areas of connectivity included prefrontal cortex and primary motor areas. Parietal areas

included bilateral inferior parietal lobule, primary somatosensory cortex, precuneus, and superior parietal lobule. Functional connectivity with the occipital lobe was found in the right cuneus, left lingual gyrus, and left middle occipital gyrus. Temporal lobe brain regions included bilateral middle temporal gyrus, auditory cortex areas, and the right hippocampus. The limbic regions of anterior cingulate cortex, posterior cingulate cortex, and the parahippocampal gyrus also showed functional connectivity with the EC. Additional areas of functional coherence included the insula, basal ganglia, and cerebellum (Figure 2). For a complete table of these brain regions and associated Brodmann areas see Table 2. AD patients showed patterns of functional connectivity in frontal, parietal, temporal, occipital, and cerebellar regions, though substantially reduced across the brain, most notably in frontal and temporal areas (Figure 3). See Table 3 for a complete list of these brain regions.

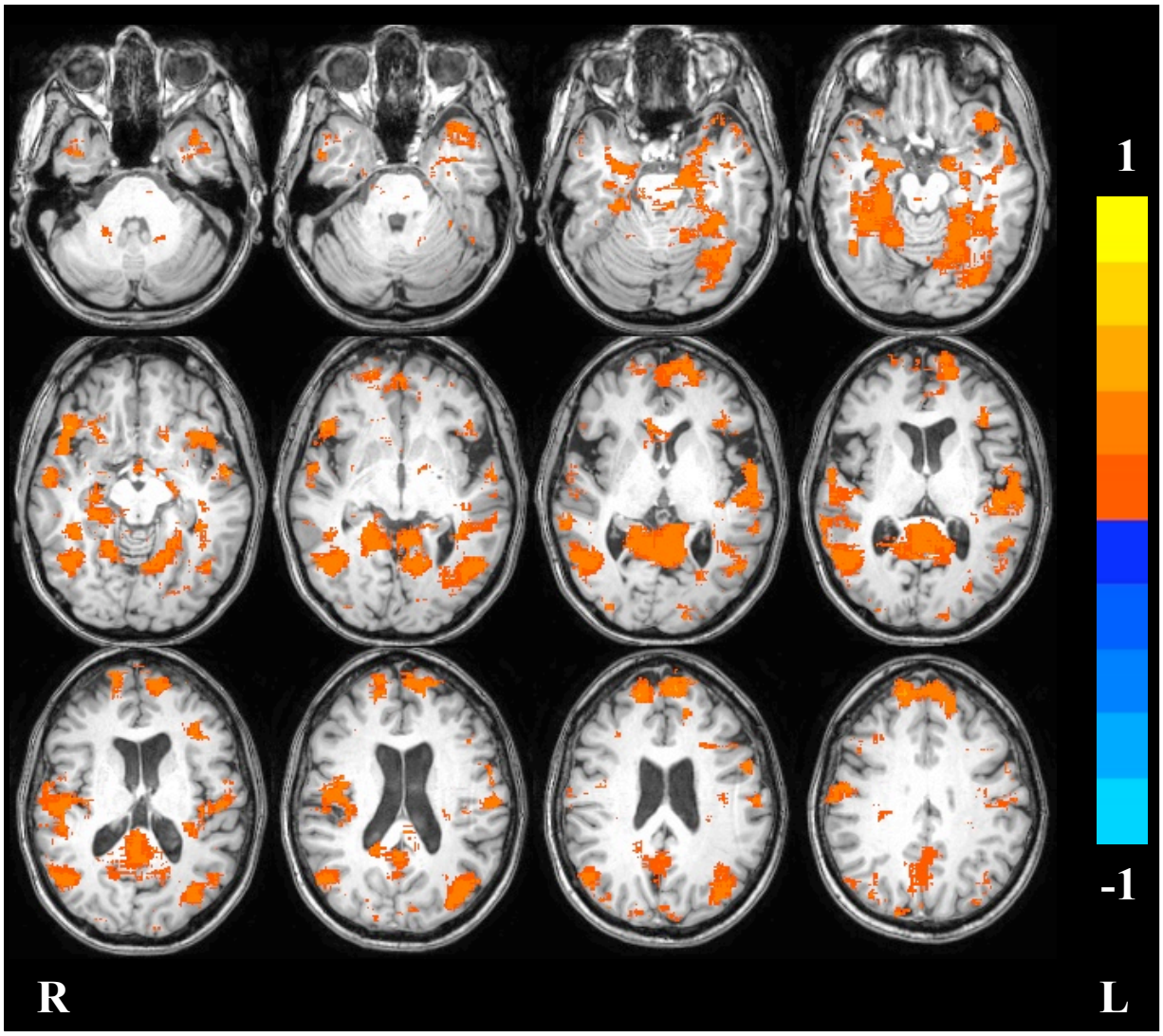

Figure 2. Functional connectivity of left EC in control participants.

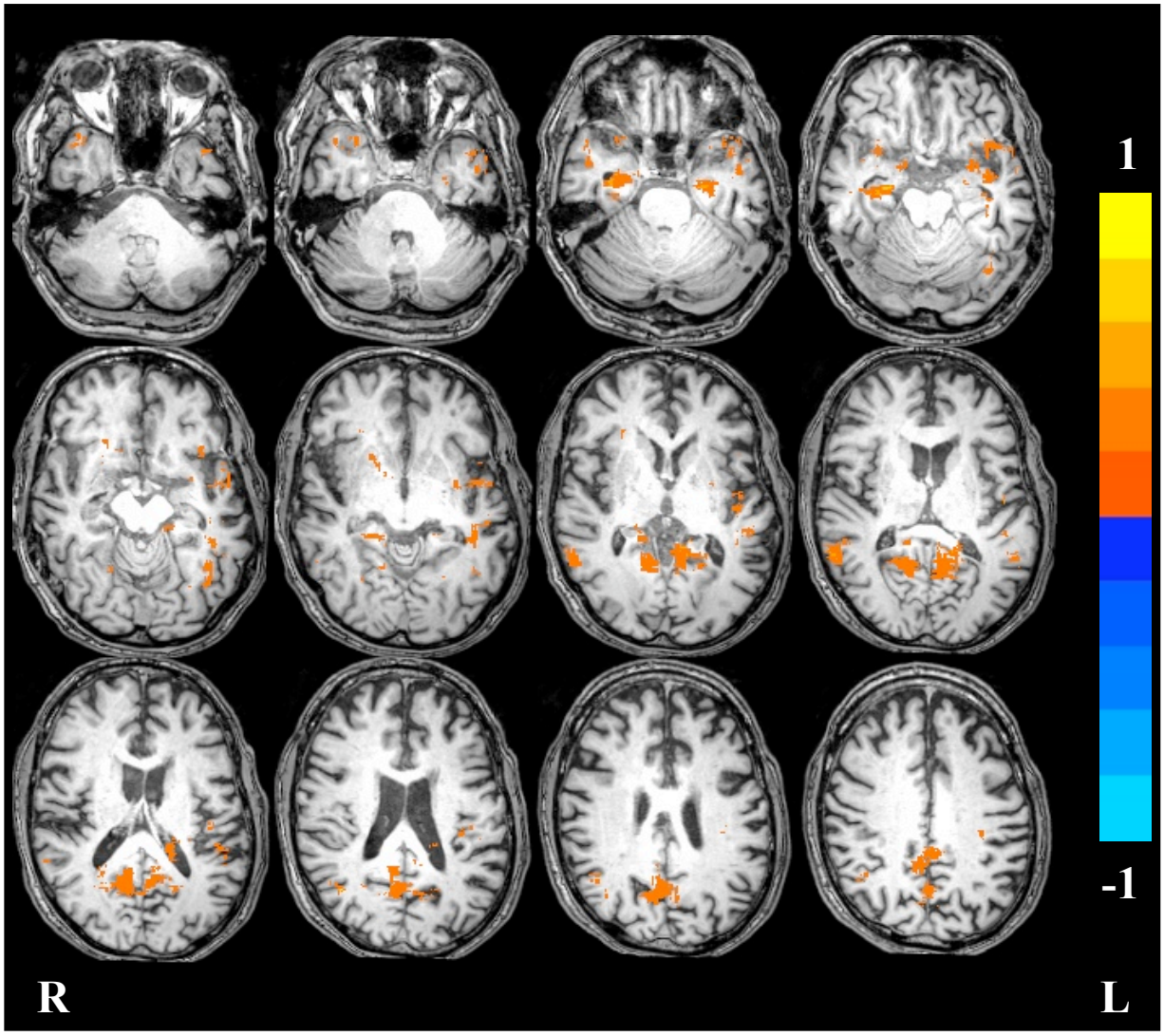

Figure 3. Functional connectivity of left EC in patients with AD

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Table 2. ***Brain regions demonstrating functional connectivity (Local Maxima) with the Left EC in NC participants***

Brain Region	BA	Side	x	y	z	t Value
Frontal						
IFG		L/R	-34/46	28/28	5/-6	8.28/19.60
MedFG	6	L/R	-10/3	-25/-22	53/65	10.24/8.65
MidFG	6	L/R	-23/30	-13/-4	62/61	12.04/7.54
	8	R	26	22	45	7.44
	9	R	41	16	31	4.88
	11	L/R	-35/27	35/32	-11/-12	4.19/4.78
	47	R	45	38	-10	3.37
SFG	10	L/R	-13/14	67/65	6/14	7.12/8.13
ParG	31	L	-2	-18	46	4.25
PrG	6	L/R	-53/50	-4/-3	6/36	10.83/7.91
	4	R	18	-31	64	12.50
Parietal						
IPL	40	L/R	-41/62	-28/-25	39/31	3.39/4.46
PoG	3	L	-24	-28	63	10.28
	40	L	-35	-35	54	5.19
	7	L	-11	-51	66	3.62
	5	R	33	-42	61	5.80
PCun	39	L/R	-38/43	-63/-65	37/37	4.70/4.29
	7	L/R	-24/25	-58/-76	51/46	4.57/3.69
	31	L/R	-7/3	-47/-67	34/23	3.74/3.40
SPL	7	L/R	-30/25	-67/-59	48/45	6.28/4.11
Occipital						
Cuneus	19	R	17	-80	30	5.01
	18	R	5	-83	24	4.50
LgG	18	L	-21	-78	-10	4.76
MOG	18	L	-25	-90	6	4.50

Table 2, Continued

Brain Region	BA	Side	x	y	z	t Value
Temporal						
MTG	37	L	-47	-59	2	6.99
	22	L/R	-55/64	-39/-34	3/5	4.61/6.14
	21	R	51	1	-27	7.42
	39	L/R	-45/47	-55/-63	12/20	3.84/5.85
STG	22	L	-47	8	-4	3.73
	38	R	33	3	-15	8.65
TrG	41	R	56	-16	12	5.80
HC		R	29	-30	-3	3.45
Limbic						
ACC	32	L/R	-11/3	45/40	-5/6	3.66/3.8
PCC	23	L	-4	-32	22	5.20
PHG		L	-21	-16	-12	6.19
Amg		R	27	-6	-18	7.08
Uncus	28	L	-26	7	-19	7.73
Insula	13	L/R	-29/45	-28/8	14/-4	5.50/3.96
Basal Ganglia						
GPl		L	-17	0	-3	3.61
Caudate		L	-31	-35	4	4.53
Cerebellum						
		L/R	-4/28	-57/-32	2/-21	10.25/6.05
		L/R	-36/15	-60/-55	-22/-11	6.51/6.06
		L	-10	-49	-29	5.40
		L	-4	-55	-20	3.93
		R	23	-49	-32	4.43

Abbreviations: BA, Brodmann Area; x, right/left; y, anterior/posterior; z, superior/inferior; L, left; R, right; IFG, inferior frontal gyrus; MedFG, medial frontal gyrus, MidFG, middle frontal gyrus; ParG, paracentral gyrus; PrG, precentral gyrus; SFG, superior frontal gyrus; IPL, inferior parietal lobule; PoG, postcentral gyrus; PCun, precuneus; SPL, superior parietal lobule; LgG, lingual gyrus; MOG, middle occipital gyrus; STG, superior temporal gyrus; TrG, transverse gyrus; HC, hippocampus; ACC, anterior cingulate cortex; PCC, posterior cingulate cortex; PHG, parahippocampal gyrus; GPl, globus pallidus (lateral). Threshold *t* value = 2.75, $p < .01$.

Table 3. ***Brain regions demonstrating functional connectivity (Local Maxima) with the Left EC in AD patients***

Brain Region	BA	Side	x	y	z	t Value
Frontal						
IFG	47	L	-35	15	-11	6.59
SubG	34	R	16	4	-12	5.44
Parietal						
PCun	7	R	12	-69	36	4.24
Occiptal						
FuG	19	R	26	-55	-8	3.79
LgG	19	L	-25	-61	1	4.63
Temporal						
MTG	20	L	-51	-38	-12	4.20
STG	38	R	33	7	-19	11.98
HC		R	3	29	-14	12.41
Limbic						
CC	31	L/R	-2/10	-32/-46	35/3	6.73/5.70
PCC	30	L	-8	-55	9	7.31
Insula	13	L	-42	-18	7	6.60
Basal Ganglia						
Putamen		R	22	12	-6	4.67
Cerebellum						
		R	12	-44	-2	4.43
		L	-25	-64	-12	4.56

Abbreviations: BA, Brodmann Area; x, right/left; y, anterior/posterior; z, superior/inferior; L, left; R, right; IFG, inferior frontal gyrus; SubG, subcallosal gyrus; PCn, precuneus; FuG, fusiform gyrus; LgG, lingual gyrus; MTG, middle temporal gyrus; STG, superior temporal gyrus, HC, hippocampus; CC, cingulate cortex; PCC, posterior cingulate cortex. Threshold t value = 2.97, $p < .01$.

Following correction for multiple comparisons by applying t value (t = 2.51, $p < .025$) and cluster size (43 voxels) thresholds, no brain regions with significantly different levels of EC connectivity between the groups were detected. Applying less stringent thresholds ($t = 2.15$, $p < .05$, cluster size = 15 voxels) resulted in the detection of significant differences among the groups. Compared to the NC group, AD patients exhibited reduced functional connectivity in bilateral prefrontal cortex, right temporal, and bilateral cerebellar areas. Areas of significantly increased functional connectivity in AD patients relative to control subjects were found in bilateral inferior frontal gyrus, left middle frontal gyrus, left parahippocampal gyrus, and left putamen (see Figure 4, Tables 4 and 5).

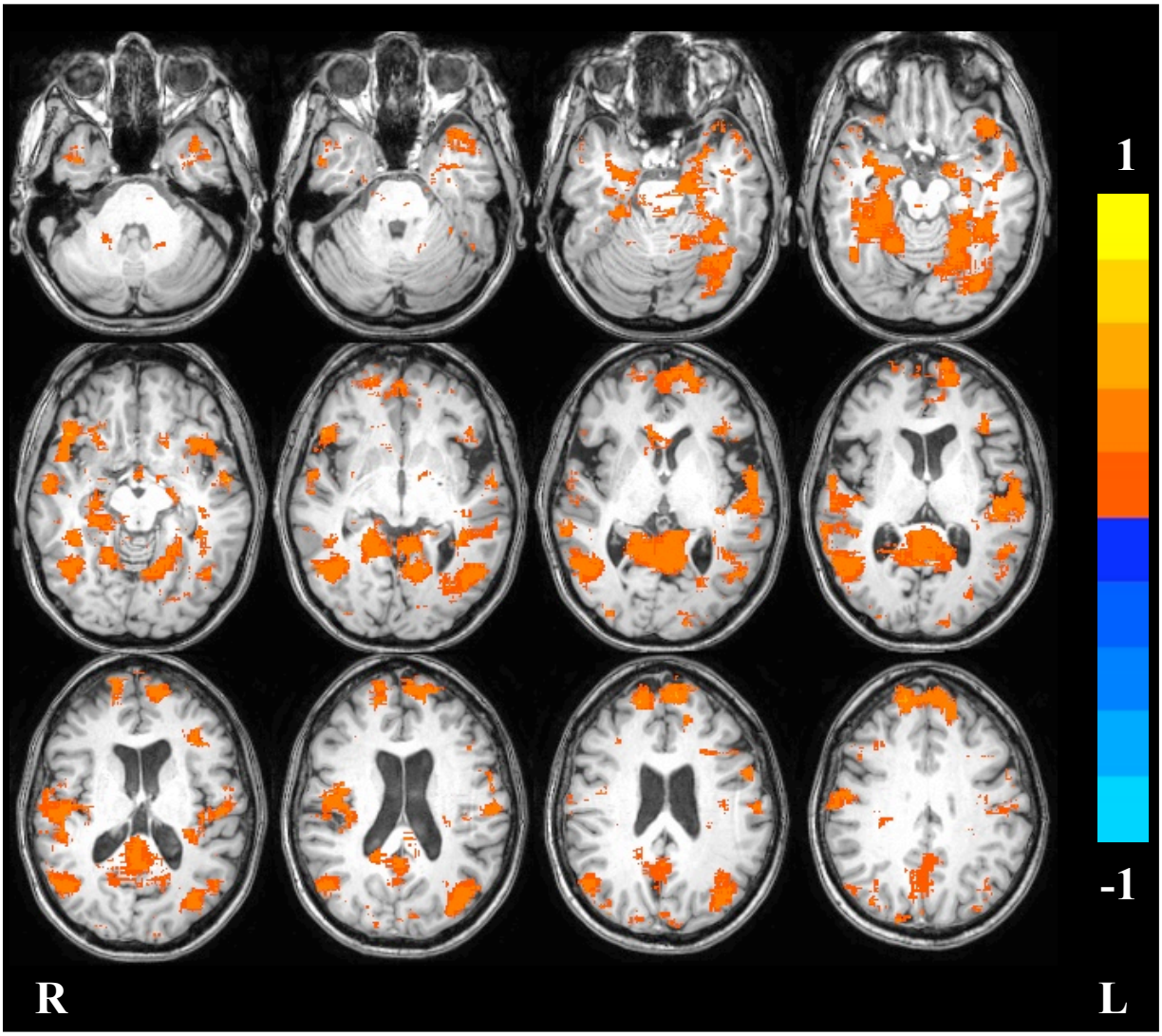

Figure 4. Group comparison of functional connectivity of the EC.

Yellow/red areas indicate brain regions with significantly reduced EC functional connectivity in AD patients compared to controls. Blue areas represent areas with significant increases in functional connectivity in AD patients relative to NC participants.

Table 4. ***Brain regions demonstrating reduced functional connectivity (Local Maxima) with the Left EC in AD patients compared to NC participants***

Brain Region	BA	Side	x	y	z	t Value
Frontal						
IFG	47	L	-44	21	-14	3.90
MedFG	10	R	15	57	1	3.43
MidFG	6	L	-35	10	57	3.24
	10	L	-36	56	18	2.97
SFG	6	L	-4	8	60	3.34
	9	L	-21	54	35	3.24
	8	R	23	28	45	3.33
Temporal						
STG	22	R	55	1	5	3.01
	39	R	49	-59	28	2.69
FuG	37	R	46	-59	-17	2.91
PHG	35	R	23	-26	-11	3.69
Cerebellum						
		L/R	-30/28	-37/-44	-26/-16	4.52/2.86

Abbreviations: BA, Brodmann Area; x, right/left; y, anterior/posterior; z, superior/inferior; L, left; R, right; IFG, inferior frontal gyrus; MedFG, medial frontal gyrus; MidFG, middle frontal gyrus; SFG, superior frontal gyrus; STG, superior temporal gyrus; FuG, fusiform gyrus; PHG, parahippocampal gyrus. Threshold t value = 2.15, $p < .05$.

Table 5. ***Brain regions demonstrating reduced functional connectivity (Local Maxima) with the Left EC in AD patients compared to NC participants***

Brain Region	BA	Side	x	y	z	t Value
Frontal						
IFG	47	L/R	-48/32	34/17	-4/-21	4.57/4.54
MidFG	46	L	-43	40	14	4.20
Temporal						
Uncus	28	L	-25	-11	-27	3.30
Basal Ganglia						
Putamen		L	-19	14	-3	3.33

Abbreviations: BA, Brodmann Area; x, right/left; y, anterior/posterior; z, superior/inferior; L, left; R, right; IFG, inferior frontal gyrus; MidFG, middle frontal gyrus. Threshold t value = 2.15, $p < .05$.

Hypothesis Two

Hypothesis two stated that the average volume of the EC would be significantly smaller in AD subjects compared to NC. All subjects were included in the analyses. The groups were compared on three measures of EC volume: left hemisphere, right hemisphere, and total volume (see Table 6). No significant differences were detected between the groups on any of the three measures [$t(14) = 0.02$, $p > .05$; $t(14) = 0.15$, $p > .05$; and $t(14) = 0.09$, $p > .05$, respectively].

Table 6. ***EC Volume Measurements***

	AD (n = 10)	NC (n = 10)
Left EC *M* (*SD*)	147.3 (40.0)	147.7 (51.7)
Right EC *M* (*SD*)	146.1 (39.0)	149.7 (54.5)
Total EC *M* (*SD*)	293.4 (68.0)	297.3 (104.9)

Note: EC volumes are in cubic millimeters.

Hypothesis Three

Hypothesis three stated that functional connectivity between the EC and hippocampus would be positively related to the structural volume of the EC in the combined group of all subjects. For this analysis, the relationship between left EC volume and connectivity of left EC with the left hippocampus was examined using Spearman rank correlation analysis. No significant correlation was detected (r_s = .05, p > .05).

Hypothesis Four

Hypothesis four stated that significant positive correlations would be found between functional connectivity of the EC and measures of verbal recall, and between volume of the EC and measures of verbal recall. To investigate the

relationship of functional coherence of the EC and hippocampus to memory test performance, individual average z values were correlated with raw scores on LM I Story A and LM II Story A of the WMS-R (Wechsler, 1987) using Spearman rank correlation analysis. No significant relationships were detected (r_s = -.13, and r_s = .07 respectively, $p > .05$). Volume of the left EC was also correlated with LM I Story A and LM II Story A. No significant relationships were detected (r_s = .37, and r_s = .17, respectively, $p > .05$).

As an exploratory analysis, the relationship of EC functional connectivity and EC volume to CERAD total score (Chandler et al., 2005) was examined with Spearman rank correlation analysis. No significant correlations were detected (r_s = .16, and r_s = .30, respectively, $p > .05$). See Table 6 for a display of group and total sample median scores on these measures.

Table 7. ***Neuropsychological and Functional Connectivity Measures***

	AD *Mdn* (*IQR)*	NC *Mdn (IQR)*	Total Sample *Mdn (IQR)*
LM I A	6.0 (4.0)	14.0 (8.5)	9.0 (8.0)
LM II A	4.0 (5.0)	15.0 (9.0)	7.0 (10.7)
CERAD Total	76.0 (18.0)	98.0 (7.5)	89.5 (22.7)
EC z value	0.1 (0.5)	0.4 (0.4)	0.2 (0.5)

Abbreviations: Mdn, median; IQR, interquartile range.

CHAPTER SIX

Discussion

The overall purpose of the present study was to examine functional changes in the EC associated with AD using fcMRI, and to investigate the relationship of these functional changes to memory performance and global cognitive ability. This study represents the first investigation of functional connectivity of the EC in AD patients.

Hypothesis One

The first hypothesis predicted that functional connectivity between the left EC and hippocampus would be significantly lower in AD patients relative to NC. Significant coherence with the hippocampus was found among NCs, a finding that is consistent with existing literature (Kahn et al., 2008). No significant functional connectivity between these regions was found in participants with AD. Despite these disparate outcomes from single-sample analyses, no significant differences were found in a direct comparison of the groups.

There are multiple factors that could account for this outcome. Most notably among them is the lack of power due to small sample size. The data from four subjects were not used in these analyses due to substantial signal loss in the EC as a result of inhomogeneities in the magnetic field, likely resulting in a substantial loss of power.

Another factor that may have influenced these results is the method of data acquisition. Due to the small size of the EC, and the close proximity of this structure to the hippocampus, a smaller voxel size may have allowed for better detection of BOLD signal in these regions. The acquisition parameters used in this investigation were optimized for whole brain coverage with a sampling rate that attempts to minimize the aliasing effects of physiological noise. Collecting data with a smaller voxel size provides a better resolution of the functional data, though often restricts acquisition to only parts of the brain, and thus, prohibiting whole brain analysis, or requiring a longer sampling rate thereby increasing the presence of spurious physiological noise in the data.

In the present study, functional connectivity of the EC with the hippocampus was examined by correlating the average time series of the EC seed with the whole brain, and transforming these correlation coefficients to z values using Fisher's r-to-z transformation (Zar, 1996, as cited in (Vincent et al., 2006). These z values were averaged across the length of the hippocampus in each person. A study by Kahn et al. (2008) investigated whole brain functional connectivity of the EC and subregions of the hippocampus in healthy adults. The findings indicate different patterns of whole brain functional connectivity in subregions of the hippocampus, with anterior hippocampal regions displaying coherence patterns similar to that of the EC. These results indicate the possibility of the presence of different connectivity patterns between the EC and subregions

of the hippocampus. By averaging across the hippocampus in the present study, significant differences between the two groups may have been obscured. Future studies investigating functional connectivity between the EC and different regions of the hippocampus in AD patients may be warranted.

The whole brain exploratory analysis established patterns of connectivity with the EC in both groups. In the NC group, areas showing significant functional connectivity with the EC included sensory association cortex in parietal, occipital, and temporal lobes, and subcortical and cerebellar regions.

Areas of coherence were found bilaterally in the inferior frontal gyrus, medial frontal gyrus, and middle frontal gyrus regions of the prefrontal cortex. This finding is consistent with animal studies showing neocortical projections from the frontal regions to the EC (Amaral & Insausti, 1990; Kerr et al., 2007). Functional connectivity was also found in bilateral primary motor cortex (precentral gyrus).

Functional coherence in the parietal lobe was observed in left primary somatosensory cortex (postcentral gyrus), as well as in bilateral inferior parietal lobule, a multimodal sensory association area. This area has been shown to have connections to the hippocampal formation in monkeys, and is thought to be involved in spatial navigation (Clower, West, Lynch, & Strick, 2001). Additional areas of significance in the parietal lobule include bilateral SPL, which plays a role in forming spatial relationships (Naito et al., 2008), and bilateral precuneus,

an area involved in visuospatial imagery and episodic memory retrieval (Cavanna & Trimble, 2006).

Occipital regions showing significant connectivity are associated with visual processing, including the right cuneus, which contains portions of primary visual cortex, and left middle occipital gyrus. In animal studies these regions have been shown to have reciprocal connections to the EC (Kerr et al., 2007).

Functional connectivity with the EC was noted in several temporal regions, including bilateral middle temporal gyrus, which plays a role in multimodal sensory integration (Convit et al., 2000). Bilateral superior temporal gyrus (STG) and the right transverse gyrus, areas of auditory processing, were also correlated with the EC, as well as the right hippocampus, left uncus, right amygdala, and left entorhinal cortex region of the parahippocampal gyrus. Coherence in the left posterior cingulate was also noted, which is consistent with existing functional connectivity literature examining the MTL (Fox et al., 2005; Greicius et al., 2008).

Bilateral insula were observed to have significant coherence with the EC, This structure processes somatosensory information and projects it to parahippocampal cortex, where it is then sent to the EC (Kerr et al., 2007).

In sum, the overall pattern of connectivity includes primary sensory cortical regions and sensory association cortex, as well as brain regions involved in motor processing including primary motor cortex, basal ganglia, and the

cerebellum. These findings are consistent with animal literature (Kerr et al., 2007) indicating that the EC receives different pieces of sensory information about events that are projected to the MTL memory system where they are consolidated into a whole before being projected back to association cortex (Squire & Zola-Morgan, 1991). Much of what is known about neural networks has been gained from animal studies. The results of this study provide evidence of similarities between human and animal brains, and confirm the utility of fcMRI as a useful method to study connectivity of the human brain.

In contrast to findings in controls, AD participants exhibited a much more restricted pattern of functional connectivity with the EC. Interestingly, no functional coherence was observed in the EC or the parahippocampal gyrus, reflecting reduced interregional connectivity in this area. Connectivity was observed with other areas of episodic memory processing, including the right hippocampus and right precuneus. These findings may indicate that, in individuals with mild AD, functional degradation of the EC initially results in a breakdown of intraregional processing.

Statistical comparison of the two groups found no significant differences when applying strict threshold criteria to control for multiple comparisons. The use of less stringent thresholds revealed significant reductions in frontal, temporal, and cerebellar areas. Consistent with Allen et al. (2007), this study found the biggest reductions in prefrontal cortex of AD patients. Contrary to their

findings, areas of increased activity in AD patients relative to NC subjects were found bilaterally in the inferior frontal gyrus region of the prefrontal cortex and left dorsolateral prefrontal cortex. Increased functional connectivity or activation in prefrontal cortex in AD patients has been evident in other imaging studies of AD patients, including positron emission tomography (PET) (Horwitz et al., 1995), fcMRI (L. Wang et al., 2006), and task-dependent fMRI studies of memory (Yetkin, Rosenberg, Weiner, Purdy, & Cullum, 2006). These findings have been interpreted as evidence of a compensatory process in an effort to redistribute neural resources (Grady et al., 2003). In an fMRI study by Grady et al. (2003) investigating semantic and episodic memory, AD subjects were found to recruit more prefrontal cortex regions during the memory tasks compared to control subjects. Furthermore, greater activation of these areas was associated with better memory task performance in AD patients. In this study, increased coherence was also found in the putamen of AD patients. Since the putamen receives many of its afferents from cerebral cortex, this may also represent a compensatory process in an effort to offset reduced input.

Reduced functional connectivity in AD subjects was also found in the right perirhinal and entorhinal regions of the parahippocampal gyrus extending into hippocampus. An unexpected finding was *increased* coherence in a small region of the entorhinal and parahippocampal portion of the parahippocampal gyrus. This finding is highly unusual, and may be due to the high degree of

variability in connectivity in the EC among the AD patients. Taken together, this can be interpreted as reduced interhemispheric connectivity of the EC in AD patients, while intrahemispheric coherence may remain intact at the mild stage of the disease.

A consistent finding in functional connectivity studies investigating coherence with the MTL in AD patients is reduced connectivity with the ipsalateral superior temporal gyrus (STG) (Allen et al., 2007; K. Wang et al., 2007), an area important in language and speech processing. The present study found reduced coherence in subjects with AD in the right STG. MRI studies investigating volumetric changes in AD have found reductions in the STG (Chetelat & Baron, 2003) and those with MCI that progress to AD (Karas et al., 2008). These findings suggest a particular vulnerability of this temporal lobe region to AD pathology, and are consistent with neuropathological studies demonstrating the abnormal accumulation of amyloid deposits in this region (Braak & Braak, 1991).

Reduced functional coherence was also noted bilaterally in the cerebellum of AD patients in this study. Microscopic neuropathological changes associated with Alzheimer's disease have been documented in the cerebellar cortex (Braak, Braak, Bohl, & Lang, 1989). Posterior cerebellar volume is significantly reduced in AD patients (Thomann et al., in press), and reduced functional connectivity with this structure and the hippocampus has previously been found (Allen et al.,

2007). The findings from this study are consistent with research documenting the disruption of cerebellar networks in AD.

Hypothesis Two

Hypothesis two stated that EC volume would be significantly reduced in AD subjects. Interestingly, no significant difference in volume was found. Research findings indicate that functional changes in the EC precede the cell and volume loss typically found in AD patients (Davies et al., 1987; Selkoe, 2002; Small et al., 2002). The level of disease in participants with AD in this study ranged from very mild to mild. The similarity in EC volume in the NC and AD subjects may indicate that at this level of disease, functional changes are more evident than structural differences.

Hypothesis Three

Hypothesis three predicted a significant positive relationship between EC volume and EC functional connectivity with the hippocampus. No significant relationship was found. The relationship of functional connectivity to structural volume has not been previously investigated. Research has shown that pathological changes in AD result in the loss of cells (Davies et al., 1987; Gomez-Isla et al., 1996), which eventually leads to a reduction of structural volume in AD. Additionally, it has been shown that functional changes, such as the breakdown of synapses (Selkoe, 2002) occurs earlier in the disease process than structural changes. The NC and AD subjects in this study were very similar in

volume measurements of the EC. This lack of variability in volume between the groups, combined with significant differences in functional connectivity are consistent with research indicating earlier decline of function compared to volume of the EC. Furthermore, the similarity in volume between the groups may explain the lack of a relationship between volume and functional connectivity in the EC. It is possible that a significant relationship between volume and functional connectivity does not emerge until later stages of the disease when reductions in volume become apparent.

Hypothesis Four

Hypothesis four predicted significant correlations between episodic memory test performance and functional connectivity with EC and hippocampus.A significant positive relationship between a global cognitive measure, the CERAD total score, and coherence between EC and hippocampus was also predicted. These hypotheses were not supported. One possible reason for this is, again, the small sample size and large amount of variability in functional connectivity of the EC. Alternatively, it is possible that the relationship between functional connectivity and cognitive ability is different for AD patients and healthy elderly adults. By combining these groups it is possible that important relationships among these factors were obscured. Unfortunately, the low sample size in this study prohibits further investigation of this. Future studies clarifying the correspondence of cognitive abilities to functional integrity of the EC will be

useful in understanding how physiological changes in the brain translate into behavioral functional impairment.

Volume of the EC was also predicted to correlate positively with episodic memory measures and global cognitive ability, though no significant relationships were found. As noted above, there was little variability between the groups in volume of the EC, with no apparent reductions in AD subjects. The stability of EC volume among AD patients in this sample may explain the lack of a relationship between neuropsychological measures and volume.

Limitations

As mentioned above, two limitations to this study are the small sample size and large voxel size. Low power resulting from a small sample size makes detection of significant differences between groups difficult. Despite the small sample size used in this pilot study, unique patterns of connectivity were detected in NC and AD subjects. Replication and extension of these findings with a larger sample size will be useful in furthering our understanding of functional connectivity in AD.

A smaller voxel size would allow for a larger number of voxels to be maintained in the EC seed region, and thus, provide a better sampling of connectivity in that region. Several subjects in this study had EC seeds that were one voxel in size, which may have limited the ability to measure connectivity. However, research on the effect of seed size has not been conducted, and no

apparent pattern related to the size of the EC seed regions was observable in these subjects.

An additional limitation of this study was the sampling procedure. Participants were recruited from the UTSW ADC, and thus, were not randomly selected. Overall, they were generally highly educated, and all were Caucasian, with the exception of one African American subject. While this may limit generalizability to some degree, it is important to note that the connectivity findings are similar to other fcMRI studies of MTL functioning in AD (Allen et al., 2007; Wang et al., 2006).

Additionally, a total of four left-handed subjects were included in the sample, one AD patient and 3 NC participants. The effect of handedness on fcMRI studies has not been investigated, yet differences in cortical organization between left and right handed individuals have been documented (Hertz-Pannier et al., 1997; Hines, Chiu, McAdams, Bentler, & Lipcamon, 1992). However, functional MRI results of these subjects were examined individually, and no apparent differences were found in coherence patterns as compared to right-handed subjects

Conclusions and Future Directions

Results of this study examining functional coherence on EC in healthy elderly participants are quite similar to findings from animal studies showing that the EC is highly interconnected to primary sensory cortex and sensory association

cortex in frontal, temporal, parietal, and occipital cortex, indicating a similar organization among humans. Compared to NC subjects, patients with AD demonstrated reductions in frontal and temporal cortices. This pattern of reduced connectivity in the AD group is consistent with what is known about the progression of pathophysiology in AD, which begins in the EC and spreads to areas in frontal and temporal cortex resulting in mild changes in cortical association areas (Braak & Braak, 1991). Pathophysiological studies of AD indicate that the neurofibrillary tangles and neuritic plaques tend to be distributed in a particular pattern, with accumulations beginning in the transentorhinal and entorhinal cortices, and then spreading to the hippocampal formation, and cortical association areas. Only in the late stages of the disease does the pathology spread to superior parietal and occipital regions. Following this pattern of pathology, AD subjects demonstrated reduced connectivity in frontal and temporal association cortices, while no differences were observed in parietal or occipital regions. However, due to the small sample size and limited corrections for multiple comparisons, replication of these findings is needed, and caution is warranted in the interpretation of results.

Future studies that include people at risk for AD, as well as patients with more advanced levels of disease, will help expand our knowledge on the pattern of cortical connectivity with the EC across the spectrum from healthy aging to AD. Additionally, studies using a higher resolution of functional data would allow

for the investigation of differences in patterns of connectivity among the subregions of the hippocampal formation and parahippocampal gyrus, thus, providing information about the interconnections of the MTL in AD and healthy aging. Multiple fcMRI studies of AD have identified reduced connectivity between the MTL and prefrontal cortex (Allen et al., 2007; L. Wang et al., 2006). Future studies investigating functional connectivity of prefrontal cortex in AD would provide a better understanding of the diversity of function in this area and its cortical connections. Due to the small sample size in this study, little information was gained about the relationship of functional connectivity of the EC and episodic memory functioning. Future research investigating the relationship of functional integrity to cognitive performance is needed.

BIBLIOGRAPHY

Allen, G., Barnard, H., McColl, R., Hester, A. L., Fields, J. A., Weiner, M. F., et al. (2007). Reduced hippocampal functional connectivity in Alzheimer disease. *Archives of Neurology, 64*(10), 1482-1487.

Allen, G., McColl, R., Barnard, H., Ringe, W. K., Fleckenstein, J., & Cullum, C. M. (2005). Magnetic resonance imaging of cerebellar-prefrontal and cerebellar-parietal functional connectivity. *Neuroimage, 28*(1), 39-48.

Amaral, D. G., & Insausti, R. (1990). Hippocampal formation. In G. Paxinos (Ed.), *The Human Nervous System* (pp. 711-755). San Diego: Academic Press.

American Psychiatric Association. (2000). *Diagnostic and Statistical manual of mental dosrders* (4th edition, text revision). Washington, DC: Author.

Baliki, M. N., Geha, P. Y., Apkarian, A. V., & Chialvo, D. R. (2008). Beyond feeling: chronic pain hurts the brain, disrupting the default-mode network dynamics. *Journal of Neuroscience, 28*(6), 1398-1403.

Bandettini, P. A., & Wong, E. C. (1994). Magnetic resonance imaging of human brain functiong. *Neurosurgery Clinics of North America, 35*(6), 662-672.

Birn, R. M., Diamond, J. B., Smith, M. A., & Bandettini, P. A. (2006). Separating respiratory-variation-related fluctuations from neuronal-activity-related fluctuations in fMRI. *Neuroimage, 31*(4), 1536-1548.

Biswal, B., DeYoe, A. E., & Hyde, J. S. (1996). Reduction of physiological fluctuations in fMRI using digital filters. *Magnetic Resonance in Medicine, 35*(1), 107-113.

Biswal, B., Yetkin, F. Z., Haughton, V. M., & Hyde, J. S. (1995). Functional connectivity in the motor cortex of resting human brain using echo-planar MRI. *Magnetic Resonance in Medicine, 34*(4), 537-541.

Braak, H., & Braak, E. (1991). Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathol, 82*(4), 239-259.

Braak, H., & Braak, E. (1998). Evolution of neuronal changes in the course of Alzheimer's disease. *Journal of Neural Transmission. Supplementum, 53*, 127-140.

Braak, H., Braak, E., Bohl, J., & Lang, W. (1989). Alzheimer's disease: amyloid plaques in the cerebellum. *Journal of the Neurological Sciences, 93*(2-3), 277-287.

Buckner, R. L., Snyder, A. Z., Shannon, B. J., LaRossa, G., Sachs, R., Fotenos, A. F., et al. (2005). Molecular, structural, and functional characterization of Alzheimer's disease: evidence for a relationship between default activity, amyloid, and memory. *Journal of Neuroscience, 25*(34), 7709-7717.

Burwell, R. D. (2000). The parahippocampal region: corticocortical connectivity. *Annals of the New York Academy of Sciences, 911*, 25-42.

Cavanna, A. E., & Trimble, M. R. (2006). The precuneus: a review of its functional anatomy and behavioural correlates. *Brain, 129*(Pt 3), 564-583.

Chandler, M. J., Lacritz, L. H., Hynan, L. S., Barnard, H. D., Allen, G., Deschner, M., et al. (2005). A total score for the CERAD neuropsychological battery. *Neurology, 65*(1), 102-106.

Chetelat, G., & Baron, J. C. (2003). Early diagnosis of Alzheimer's disease: contribution of structural neuroimaging. *Neuroimage, 18*(2), 525-541.

Clower, D. M., West, R. A., Lynch, J. C., & Strick, P. L. (2001). The inferior parietal lobule is the target of output from the superior colliculus, hippocampus, and cerebellum. *Journal of Neuroscience, 21*(16), 6283-6291.

Convit, A., de Asis, J., de Leon, M. J., Tarshish, C. Y., De Santi, S., & Rusinek, H. (2000). Atrophy of the medial occipitotemporal, inferior, and middle temporal gyri in non-demented elderly predict decline to Alzheimer's disease. *Neurobiology of Aging, 21*(1), 19-26.

Cordes, D., Haughton, V. M., Arfanakis, K., Carew, J. D., Turski, P. A., Moritz, C. H., et al. (2001). Frequencies contributing to functional connectivity in the cerebral cortex in "resting-state" data. *AJNR. American Journal of Neuroradiology, 22*(7), 1326-1333.

Cordes, D., Haughton, V. M., Arfanakis, K., Wendt, G. J., Turski, P. A., Moritz, C. H., et al. (2000). Mapping functionally related regions of brain with

functional connectivity MR imaging. *AJNR. American Journal of Neuroradiology, 21*(9), 1636-1644.

Cox, R. W. (1996). AFNI: software for analysis and visualization of functional magnetic resonance neuroimages. *Computers and Biomedical Research, 29*(3), 162-173.

Daselaar, S. M., Fleck, M. S., Dobbins, I. G., Madden, D. J., & Cabeza, R. (2006). Effects of healthy aging on hippocampal and rhinal memory functions: an event-related fMRI study. *Cerebral Cortex, 16*(12), 1771-1782.

Davies, C. A., Mann, D. M., Sumpter, P. Q., & Yates, P. O. (1987). A quantitative morphometric analysis of the neuronal and synaptic content of the frontal and temporal cortex in patients with Alzheimer's disease. *Journal of the Neurological Sciences, 78*(2), 151-164.

De Lacoste, M. C., & White, C. L., 3rd. (1993). The role of cortical connectivity in Alzheimer's disease pathogenesis: a review and model system. *Neurobiology of Aging, 14*(1), 1-16.

deIpolyi, A. R., Rankin, K. P., Mucke, L., Miller, B. L., & Gorno-Tempini, M. L. (2007). Spatial cognition and the human navigation network in AD and MCI. *Neurology, 69*(10), 986-997.

deToledo-Morrell, L., Stoub, T. R., Bulgakova, M., Wilson, R. S., Bennett, D. A., Leurgans, S., et al. (2004). MRI-derived entorhinal volume is a good

predictor of conversion from MCI to AD. *Neurobiology of Aging, 25*(9), 1197-1203.

Devanand, D. P., Pradhaban, G., Liu, X., Khandji, A., De Santi, S., Segal, S., et al. (2007). Hippocampal and entorhinal atrophy in mild cognitive impairment: prediction of Alzheimer disease. *Neurology, 68*(11), 828-836.

Dickerson, B. C., Goncharova, I., Sullivan, M. P., Forchetti, C., Wilson, R. S., Bennett, D. A., et al. (2001). MRI-derived entorhinal and hippocampal atrophy in incipient and very mild Alzheimer's disease. *Neurobiology of Aging, 22*(5), 747-754.

Du, A. T., Schuff, N., Zhu, X. P., Jagust, W. J., Miller, B. L., Reed, B. R., et al. (2003). Atrophy rates of entorhinal cortex in AD and normal aging. *Neurology, 60*(3), 481-486.

Eichenbaum, H., Yonelinas, A. P., & Ranganath, C. (2007). The medial temporal lobe and recognition memory. *Annual Review of Neuroscience, 30*, 123-152.

Federal Interagency Forum on Aging-Related Statistics (2006). *Older Americans Update 2006: Key indicators of well-being.* Washington, DC: U.S. Government Printing Office.

Folstein, M. F., Folstein, S. E., & McHugh, P. R. (1975). "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *Journal of Psychiatric Research, 12*(3), 189-198.

Fox, M. D., Corbetta, M., Snyder, A. Z., Vincent, J. L., & Raichle, M. E. (2006). Spontaneous neuronal activity distinguishes human dorsal and ventral attention systems. *Proceedings of the National Academy of Sciences of the United States of America, 103*(26), 10046-10051.

Fox, M. D., & Raichle, M. E. (2007). Spontaneous fluctuations in brain activity observed with functional magnetic resonance imaging. *Nat Rev Neurosci, 8*(9), 700-711.

Fox, M. D., Snyder, A. Z., Vincent, J. L., Corbetta, M., Van Essen, D. C., & Raichle, M. E. (2005). The human brain is intrinsically organized into dynamic, anticorrelated functional networks. *Proceedings of the National Academy of Sciences of the United States of America, 102*(27), 9673-9678.

Goldman, W. P., Price, J. L., Storandt, M., Grant, E. A., McKeel, D. W., Jr., Rubin, E. H., et al. (2001). Absence of cognitive impairment or decline in preclinical Alzheimer's disease. *Neurology, 56*(3), 361-367.

Gomez-Isla, T., Price, J. L., McKeel, D. W., Jr., Morris, J. C., Growdon, J. H., & Hyman, B. T. (1996). Profound loss of layer II entorhinal cortex neurons occurs in very mild Alzheimer's disease. *Journal of Neuroscience, 16*(14), 4491-4500.

Goncharova, II, Dickerson, B. C., Stoub, T. R., & deToledo-Morrell, L. (2001). MRI of human entorhinal cortex: a reliable protocol for volumetric measurement. *Neurobiology of Aging, 22*(5), 737-745.

Grady, C. L., McIntosh, A. R., Beig, S., Keightley, M. L., Burian, H., & Black, S. E. (2003). Evidence from functional neuroimaging of a compensatory prefrontal network in Alzheimer's disease. *Journal of Neuroscience, 23*(3), 986-993.

Greicius, M. D., Krasnow, B., Reiss, A. L., & Menon, V. (2003). Functional connectivity in the resting brain: a network analysis of the default mode hypothesis. *Proceedings of the National Academy of Sciences of the United States of America, 100*(1), 253-258.

Greicius, M. D., Srivastava, G., Reiss, A. L., & Menon, V. (2004). Default-mode network activity distinguishes Alzheimer's disease from healthy aging: evidence from functional MRI. *Proceedings of the National Academy of Sciences of the United States of America, 101*(13), 4637-4642.

Greicius, M. D., Supekar, K., Menon, V., & Dougherty, R. F. (2008). Resting-State Functional Connectivity Reflects Structural Connectivity in the Default Mode Network. *Cerebral Cortex*.

Hafting, T., Fyhn, M., Molden, S., Moser, M. B., & Moser, E. I. (2005). Microstructure of a spatial map in the entorhinal cortex. *Nature, 436*(7052), 801-8-6.

He, Y., Wang, L., Zang, Y., Tian, L., Zhang, X., Li, K., et al. (2007). Regional coherence changes in the early stages of Alzheimer's disease: a combined

structural and resting-state functional MRI study. *Neuroimage, 35*(2), 488-500.

Heaton, R., Grant, I., & Matthews, C. (1991). *Comprehensive norms for an expanded Halstead-Reitan Battery: Demographic corrections, research findings, and clinical applications*. Odessa: Psychological Assessment Resources.

Heron, M. (2007). Deaths: Leading causes for 2004. *National vital statistics reports : from the Centers for Disease Control and Prevention, National Center for Health Statistics, National Vital Statistics System, 56*(5), 1-95.

Hertz-Pannier, L., Gaillard, W. D., Mott, S. H., Cuenod, C. A., Bookheimer, S. Y., Weinstein, S., et al. (1997). Noninvasive assessment of language dominance in children and adolescents with functional MRI: a preliminary study. *Neurology, 48*(4), 1003-1012.

Hines, M., Chiu, L., McAdams, L. A., Bentler, P. M., & Lipcamon, J. (1992). Cognition and the corpus callosum: verbal fluency, visuospatial ability, and language lateralization related to midsagittal surface areas of callosal subregions. *Behavioral Neuroscience, 106*(1), 3-14.

Hort, J., Laczo, J., Vyhnalek, M., Bojar, M., Bures, J., & Vlcek, K. (2007). Spatial navigation deficit in amnestic mild cognitive impairment. *Proceedings of the National Academy of Sciences of the United States of America, 104*(10), 4042-4047.

Horwitz, B., McIntosh, A. R., Haxby, J. V., Furey, M., Salerno, J. A., Schapiro, M. B., et al. (1995). Network analysis of PET-mapped visual pathways in Alzheimer type dementia. *Neuroreport, 6*(17), 2287-2292.

Insausti, R., Juottonen, K., Soininen, H., Insausti, A. M., Partanen, K., Vainio, P., et al. (1998). MR volumetric analysis of the human entorhinal, perirhinal, and temporopolar cortices. *AJNR. American Journal of Neuroradiology, 19*(4), 659-671.

Insausti, R., Tunon, T., Sobreviela, T., Insausti, A. M., & Gonzalo, L. M. (1995). The human entorhinal cortex: a cytoarchitectonic analysis. *Journal of Comparative Neurology, 355*(2), 171-198.

Kahn, I., Andrews-Hanna, J. R., Vincent, J. L., Snyder, A. Z., & Buckner, R. L. (2008). Distinct cortical anatomy linked to subregions of the medial temporal lobe revealed by intrinsic functional connectivity. *Journal of Neurophysiology, 100*(1), 129-139.

Karas, G., Sluimer, J., Goekoop, R., van der Flier, W., Rombouts, S. A., Vrenken, H., et al. (2008). Amnestic mild cognitive impairment: structural MR imaging findings predictive of conversion to Alzheimer disease. *AJNR. American Journal of Neuroradiology, 29*(5), 944-949.

Keller, J. N. (2006). Age-related neuropathology, cognitive decline, and Alzheimer's disease. *Ageing Res Rev, 5*(1), 1-13.

Kennedy, D. P., & Courchesne, E. (2008). The intrinsic functional organization of the brain is altered in autism. *Neuroimage, 39*(4), 1877-1885.

Kerr, K. M., Agster, K. L., Furtak, S. C., & Burwell, R. D. (2007). Functional neuroanatomy of the parahippocampal region: the lateral and medial entorhinal areas. *Hippocampus, 17*(9), 697-708.

Killiany, R. J., Hyman, B. T., Gomez-Isla, T., Moss, M. B., Kikinis, R., Jolesz, F., et al. (2002). MRI measures of entorhinal cortex vs hippocampus in preclinical AD. *Neurology, 58*(8), 1188-1196.

Li, S. J., Biswal, B., Li, Z., Risinger, R., Rainey, C., Cho, J. K., et al. (2000). Cocaine administration decreases functional connectivity in human primary visual and motor cortex as detected by functional MRI. *Magnetic Resonance in Medicine, 43*(1), 45-51.

Li, S. J., Li, Z., Wu, G., Zhang, M. J., Franczak, M., & Antuono, P. G. (2002). Alzheimer Disease: evaluation of a functional MR imaging index as a marker. *Radiology, 225*(1), 253-259.

Lowe, M. J., Beall, E. B., Sakaie, K. E., Koenig, K. A., Stone, L., Marrie, R. A., et al. (2008). Resting state sensorimotor functional connectivity in multiple sclerosis inversely correlates with transcallosal motor pathway transverse diffusivity. *Human Brain Mapping*.

Lowe, M. J., Mock, B. J., & Sorenson, J. A. (1998). Functional connectivity in single and multislice echoplanar imaging using resting-state fluctuations. *Neuroimage, 7*(2), 119-132.

McKhann, G., Drachman, D., Folstein, M., Katzman, R., Price, D., & Stadlan, E. M. (1984). Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology, 34*(7), 939-944.

Morris, J. C. (1997). Clinical assessment of Alzheimer's disease. *Neurology, 49*(3 Suppl 3), S7-10.

Morris, J. C., Heyman, A., Mohs, R. C., Hughes, J. P., van Belle, G., Fillenbaum, G., et al. (1989). The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part I. Clinical and neuropsychological assessment of Alzheimer's disease. *Neurology, 39*(9), 1159-1165.

Murray, E. A., Bussey, T. J., & Saksida, L. M. (2007). Visual perception and memory: a new view of medial temporal lobe function in primates and rodents. *Annual Review of Neuroscience, 30*, 99-122.

Peltier, S. J., & Noll, D. C. (2002). T(2)(*) dependence of low frequency functional connectivity. *Neuroimage, 16*(4), 985-992.

Pennanen, C., Kivipelto, M., Tuomainen, S., Hartikainen, P., Hanninen, T., Laakso, M. P., et al. (2004). Hippocampus and entorhinal cortex in mild

cognitive impairment and early AD. *Neurobiology of Aging, 25*(3), 303-310.

Petersen, R. C., Smith, G. E., Waring, S. C., Ivnik, R. J., Tangalos, E. G., & Kokmen, E. (1999). Mild cognitive impairment: clinical characterization and outcome. *Archives of Neurology, 56*(3), 303-308.

Petersen, R. C., Stevens, J. C., Ganguli, M., Tangalos, E. G., Cummings, J. L., & DeKosky, S. T. (2001). Practice parameter: early detection of dementia: mild cognitive impairment (an evidence-based review). Report of the Quality Standards Subcommittee of the American Academy of Neurology. *Neurology, 56*(9), 1133-1142.

Plassman, B. L., Langa, K. M., Fisher, G. G., Heeringa, S. G., Weir, D. R., Ofstedal, M. B., et al. (2007). Prevalence of dementia in the United States: the aging, demographics, and memory study. *Neuroepidemiology, 29*(1-2), 125-132.

Price, J. L., Davis, P. B., Morris, J. C., & White, D. L. (1991). The distribution of tangles, plaques and related immunohistochemical markers in healthy aging and Alzheimer's disease. *Neurobiology of Aging, 12*(4), 295-312.

Pruessner, J. C., Kohler, S., Crane, J., Pruessner, M., Lord, C., Byrne, A., et al. (2002). Volumetry of temporopolar, perirhinal, entorhinal and parahippocampal cortex from high-resolution MR images: considering the variability of the collateral sulcus. *Cerebral Cortex, 12*(12), 1342-1353.

Raichle, M. E., MacLeod, A. M., Snyder, A. Z., Powers, W. J., Gusnard, D. A., & Shulman, G. L. (2001). A default mode of brain function. *Proceedings of the National Academy of Sciences of the United States of America, 98*(2), 676-682.

Ranginwala, N. A., Hynan, L. S., Weiner, M. F., & White, C. L., 3rd. (2008). Clinical criteria for the diagnosis of Alzheimer disease: still good after all these years. *American Journal of Geriatric Psychiatry, 16*(5), 384-388.

Raz, N., Rodrigue, K. M., Head, D., Kennedy, K. M., & Acker, J. D. (2004). Differential aging of the medial temporal lobe: a study of a five-year change. *Neurology, 62*(3), 433-438.

Reitan, R., & Wolfson, D. (1993). *The Halstead-Reitan Neuropsychological Test Battery: Theory and Clinical Interpretation* (2nd ed.). Tucson, AZ: Neuropsychology Press.

Rodrigue, K. M., & Raz, N. (2004). Shrinkage of the entorhinal cortex over five years predicts memory performance in healthy adults. *Journal of Neuroscience, 24*(4), 956-963.

Rombouts, S. A., Stam, C. J., Kuijer, J. P., Scheltens, P., & Barkhof, F. (2003). Identifying confounds to increase specificity during a "no task condition". Evidence for hippocampal hile this may place some limits on tconnectivity using fMRI. *Neuroimage, 20*(2), 1236-1245.

Salmon, D. P., Thomas, R. G., Pay, M. M., Booth, A., Hofstetter, C. R., Thal, L. J., et al. (2002). Alzheimer's disease can be accurately diagnosed in very mildly impaired individuals. *Neurology, 59*(7), 1022-1028.

Scoville, W. B., & Milner, B. (1957). Loss of recent memory after bilateral hippocampal lesions. *Journal of Neurology, Neurosurgery and Psychiatry, 20*(1), 11-21.

Selkoe, D. J. (2002). Alzheimer's disease is a synaptic failure. *Science, 298*(5594), 789-791.

Slavin, M. J., Sandstrom, C. K., Tran, T. T., Doraiswamy, P. M., & Petrella, J. R. (2007). Hippocampal volume and the Mini-Mental State Examination in the diagnosis of amnestic mild cognitive impairment. AJR. *American Journal of Roentgenology, 188*(5), 1404-1410.

Small, S. A., Tsai, W. Y., DeLaPaz, R., Mayeux, R., & Stern, Y. (2002). Imaging hippocampal function across the human life span: is memory decline normal or not? *Annals of Neurology, 51*(3), 290-295.

Smith, A. M., Lewis, B. K., Ruttimann, U. E., Ye, F. Q., Sinnwell, T. M., Yang, Y., et al. (1999). Investigation of low frequency drift in fMRI signal. *Neuroimage, 9*(5), 526-533.

Squire, L. R., Stark, C. E., & Clark, R. E. (2004). The medial temporal lobe. *Annual Review of Neuroscience, 27*, 279-306.

Squire, L. R., & Zola-Morgan, S. (1991). The medial temporal lobe memory system. *Science, 253*(5026), 1380-1386.

Stark, C. E., Bayley, P. J., & Squire, L. R. (2002). Recognition memory for single items and for associations is similarly impaired following damage to the hippocampal region. *Learning and Memory, 9*(5), 238-242.

Talairach, J., & Tournoux, P. (1993). *Referentially Oriented Cerebral MRI Anatomy, Atlas of Stereotaxic Anatomical Correlations for Gray and White Matter*. New York, Thieme Medical Publishers, Inc.

Turner, K. C., Frost, L., Linsenbardt, D., McIlroy, J. R., & Muller, R. A. (2006). Atypically diffuse functional connectivity between caudate nuclei and cerebral cortex in autism. *Behav Brain Funct, 2*, 34.

Uddin, L. Q., Kelly, A. M., Biswal, B. B., Margulies, D. S., Shehzad, Z., Shaw, D., et al. (2008). Network homogeneity reveals decreased integrity of default-mode network in ADHD. *Journal of Neuroscience Methods, 169*(1), 249-254.

Vincent, J. L., Snyder, A. Z., Fox, M. D., Shannon, B. J., Andrews, J. R., Raichle, M. E., et al. (2006). Coherent spontaneous activity identifies a hippocampal-parietal memory network. *Journal of Neurophysiology, 96*(6), 3517-3531.

Wang, K., Liang, M., Wang, L., Tian, L., Zhang, X., Li, K., et al. (2007). Altered functional connectivity in early Alzheimer's disease: a resting-state fMRI study. *Human Brain Mapping, 28*(10), 967-978.

Wang, L., Zang, Y., He, Y., Liang, M., Zhang, X., Tian, L., et al. (2006). Changes in hippocampal connectivity in the early stages of Alzheimer's disease: evidence from resting state fMRI. *Neuroimage, 31*(2), 496-504.

Wechsler, D. (1987). *Wechsler Memory Scale-Revised (WMS-R)*. San Antonio: The Psychological Corporation.

Wechsler, D. (1987). *Wechsler Adult Intelligence Scale-Revised (WAIS-R)*. San Antonio: The Psychological Corporation.

Welsh, K. A., Butters, N., Mohs, R. C., Beekly, D., Edland, S., Fillenbaum, G., et al. (1994). The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part V. A normative study of the neuropsychological battery. *Neurology, 44*(4), 609-614.

Welsh-Bohmer, K., & Warren, L. (2006). Neurodegenerative dementias. In D. Attix & K. Welsh-Bohmer (Eds.), *Geriatric neuropsychology: Assessment and intervention* (pp. 56-88). New York: The Guilford Press.

Whitehouse, P. J., Price, D. L., Struble, R. G., Clark, A. W., Coyle, J. T., & Delon, M. R. (1982). Alzheimer's disease and senile dementia: loss of neurons in the basal forebrain. *Science, 215*(4537), 1237-1239.

Whitwell, J. L., Petersen, R. C., Negash, S., Weigand, S. D., Kantarci, K., Ivnik, R. J., et al. (2007). Patterns of atrophy differ among specific subtypes of mild cognitive impairment. *Archives of Neurology, 64*(8), 1130-1138.

Wierenga, C. E., & Bondi, M. W. (2007). Use of functional magnetic resonance imaging in the early identification of Alzheimer's disease. *Neuropsychology Review, 17*(2), 127-143.

Wise, R. G., Ide, K., Poulin, M. J., & Tracey, I. (2004). Resting fluctuations in arterial carbon dioxide induce significant low frequency variations in BOLD signal. *Neuroimage, 21*(4), 1652-1664.

Witter, M. P. (2007). The perforant path: projections from the entorhinal cortex to the dentate gyrus. *Progress in Brain Research, 163*, 43-61.

Witter, M. P., & Moser, E. I. (2006). Spatial representation and the architecture of the entorhinal cortex. *Trends in Neurosciences, 29*(12), 671-678.

Witter, M. P., Wouterlood, F. G., Naber, P. A., & Van Haeften, T. (2000). Anatomical organization of the parahippocampal-hippocampal network. *Annals of the New York Academy of Sciences, 911*, 1-24.

Xu, Y., Jack, C. R., Jr., O'Brien, P. C., Kokmen, E., Smith, G. E., Ivnik, R. J., et al. (2000). Usefulness of MRI measures of entorhinal cortex versus hippocampus in AD. *Neurology, 54*(9), 1760-1767.

Yetkin, F. Z., Rosenberg, R. N., Weiner, M. F., Purdy, P. D., & Cullum, C. M. (2006). FMRI of working memory in patients with mild cognitive

impairment and probable Alzheimer's disease. *European Radiology, 16*(1), 193-206.

Yonelinas, A. P., Widaman, K., Mungas, D., Reed, B., Weiner, M. W., & Chui, H. C. (2007). Memory in the aging brain: doubly dissociating the contribution of the hippocampus and entorhinal cortex. *Hippocampus, 17*(11), 1134-1140.