Functional Resting State Connectivity in Individuals At-Risk for Alzheimer's Disease

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FUNCTIONAL RESTING STATE CONNECTIVITY IN INDIVIDUALS AT-RISK FOR ALZHEIMER'S DISEASE

by

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ABSTRACT
FUNCTIONAL RESTING STATE CONNECTIVITY IN INDIVIDUALS AT-RISK FOR ALZHEIMER'S DISEASE

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Resting state functional magnetic resonance imaging studies have examined the connectivity between the hippocampus (HIPP) and the posterior cingulate (PC) in individuals with Alzheimer's disease (AD), mild cognitive impairment (MCI), and younger individuals at risk for AD. The present study aimed to examine the functional connectivity between these two memory structures and targets of AD neurodegeneration in cognitively intact elders at risk for AD (positive for ApolipoE protein (ε4) and family history of dementia), MCI, and healthy controls. Seeds and regions of interest were defined in the bilateral hippocampus and posterior cingulate, and the time courses were cross-correlated to generate a value of functional connectivity between two structures for comparisons across groups. Results indicate the presence of greater functional connectivity between the left HIPP and PC in healthy elders at risk compared to patients with MCI and healthy controls and a general reduction in functional connectivity between bilateral HIPP and PC in patients with MCI. This marker of increased functional connectivity, during the resting state of the brain, found in cognitively intact elders at risk compared to cognitively intact controls and symptomatic patients with MCI might be an important diagnostic tool to identify those most vulnerable for the development of AD.

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder and a subtype of dementia. Individuals with AD show hallmark impairment in episodic memory and other behavioral changes in cognitive functioning, personality, and overall ability to perform activities of daily living (Khachaturian, 1985). In 2000, the estimated worldwide prevalence of dementia was more than 25 million, with 50-60% of those suffering from AD. The number of cases of dementia is predicted to increase to 63 million in 2030 and to 114 million by 2050 (Wimo, Jonsson, & Winblad, 2006). The number of dementia cases specific to AD is expected to double every 20 years (Brookmeyer, Gray, & Kawas, 1998) with a threefold increase in developed countries over the next 50 years (Carr, Goate, Phil, & Morris, 1997). There is a worldwide estimate of financial support for dementia of up to $159 billion (Wimo et al., 2006), with the annual cost of Alzheimer's in the United States alone estimated to be $100 billion (Small, Rabins, Barry, Buckholtz, Dekosky, Ferris et al., 1997). Therefore, identification of a biomarker that is sensitive to both the latent and prodromal characteristics of AD, predictive of future decline, would have enormous public health significance. There is a continued need to examine the characteristics of asymptomatic and symptomatic individuals at high risk for AD in order to predict and prevent who will develop this disease.

Aging, AD, and predicting cognitive decline

A number of theories have emerged attempting to explain brain activity patterns in the aging population. The theory of compensation, for example, argues that as an

individual ages, the brain is no longer able to perform tasks as easily and therefore recruits neural activity from other areas in the brain to maintain equal performance (Becker, Mintun, Aleva, Wiseman, Nichols & DeKosky, 1996). The theory of dedifferentiation argues that as the brain ages, it loses its specificity to perform tasks and therefore activates more diffuse areas of the brain, possibly to maintain equal performance; or conversely, possibly making performance less efficient (Voss et al. 2008). The recent Scaffolding Theory of Aging and Cognition (STAC) suggests that the brain undergoes constant compensation throughout life, fueled by frontal activation (Park & Reuter-Lorenz 2009). Finally, Stern's theory of cognitive reserve postulates that it is certain characteristics of each individual, specifically intelligence, which provides each individual with the cognitive reserve necessary to achieve good performance under high cognitive demand into older adult life. He further argues, that when an individual achieves his or her "break point," the cognitive reserve capacity is tapped out and no longer able to support "normal" cognitive demands (Stern, 2009). These theories each provide additional ways to think about and understand the aging process in a healthy population. What is necessary now, however, is to use these theories to help understand and explain the neurodegenerative disease process of dementia, specifically, AD.

The specific profile of cognitive decline provides a way to characterize and diagnose AD, but these cognitive changes are manifestations of the underlying neurodegeneration that precede and give rise to the behavioral changes. Pathologically, AD is characterized by the presence of amyloid plaques and neurofibrillary tangles and by a loss of large cortical neurons in the hippocampus, entorhinal cortex, and association areas of the neocortex (Arnold, Hyman, Flory, Damasio, & Van Hoesen, 1991). This

atrophy leads to marked size reductions in certain regions, specifically the hippocampus, which can be observed using neuroimaging techniques, such as functional magnetic resonance imaging (fMRI). Additional techniques can be used to examine other neurophysiological consequences of the neurodegeneration in AD. For example, positron emission tomography (PET) has been used to quantify the estimated amount of beta amyloid deposition in select brain regions that are susceptible to AD (eg Mormino, Kluth, Madison, Rabinovici, Baker et al., 2008; Hedden et al., 2009). It appears, however, that the structural changes observed (i.e. atrophy and beta amyoid deposition) follow the functional changes of the brain (Wierenga & Bondi 2007).

Despite the advances in neuroimaging technology, currently only a tentative diagnosis of probable AD can be made based on clinical, laboratory, and neuroimaging evidence. Prior to diagnosis, one should also consider the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) or Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria based on the observation of cognitive decline during a patient's lifetime (Knopman et al., 2001). There have been some risk factors identified that appear to predispose some elders to eventually develop AD.

One well-established risk factor for AD is the presence of at least one copy of the ε4 allele of the Apolipoporitein E (ApoE). This allele presents in three different allele polymorphisms; ε2, ε3, and ε4, with the ε4 copy conferring the greatest risk of developing AD. The odds ratio (OR) of developing AD without a copy of the ε4 allele (ε2/ ε2 or ε2/ ε3) is 0.6. For individuals that carry one copy of the ε4 allele (ε2/ ε4 or ε3/e4) the OR is found to be 2.6 and 3.2, respectively. The probability of developing AD

increases to OR = 14.9 if the individual is homozygous (ε4/ ε4) (Ferrer, Cupples, Haines, Hyman, Kukull, et al., 1997). Of the individuals with AD, the ε4 allele is thought to be present in 40% (Saunders et al., 1993) of those cases.

Another known risk factor for developing AD is having a first-degree family history (FH) of dementia (e.g., Lehtovirta, et al., 1996; Strittmatter, et al., 1993), such that an individual's risk of developing AD increases if one or more parent or sibling had dementia. ApoE ε4 status and family history alone, however, do not completely account for the prediction of cognitive decline. In fact, many individuals carry the ε4 allele(s) or have a family history of dementia, yet never develop AD. It is during this high risk preclinical stage, *before* the individual declines, where we need to identify what neurophysiological signatures are being produced causing some to develop AD and some to remain cognitively stable.

Mild cognitive impairment (MCI) is often considered a transitional, diagnosed, stage between healthy aging and AD. Patients with MCI show cognitive decline specifically in learning and memory (Dickerson & Sperling, 2008). Because individuals with a diagnosis of MCI already are demonstrating cognitive decline, MCI is considered an even greater risk for developing AD than the genetic risk factors (presence of ε4 and FH) (Teipel, Born, Ewers et al., 2007). Given that one of the hallmarks of AD is memory loss, the degree to which MCI can be used to predict conversion to AD is strong (Knopman et al., 2001). However, currently no test can accurately determine which patients with MCI will convert to AD and which patients will remain only mildly impaired; nor can any observation of high risk individuals be made to identify those who will develop MCI and/or AD.

Neuroimaging and AD-vulnerable brain regions

Task-activated fMRI is a neuroimaging technique that is presumed to directly link specific cognitive activity to neurophysiological changes, such as functional cerebral hemodynamics. fMRI studies, based on blood oxygen level dependent (BOLD) contrast, have shown that cognitively intact older individuals that carry the ε4 allele demonstrate a greater degree of activation in the right hippocampus than in the left hippocampus during an episodic memory task (Han et al., 2007) and encoding task (Bondi et al., 2005). Other studies comparing across allele status have found reduced activation in the right hippocampus in ε4 carriers compared to ε3 carriers during an episodic encoding task, but no difference in the left hippocampus (Trivedi, 2006). Interestingly, the left hippocampus has been suggested to be more sensitive to novelty distinction that the right hippocampus (Lind et al., 2006a). We have recently shown that the left hippocampus, in individuals at risk (ε4+FH+) and with MCI, shows a greater degree of activation during a famous name discrimination task, which is thought to tax the semantic memory network (Woodard et al., 2009). Understanding the roles of the left and right hippocampi individually may provide valuable differences in Alzheimer's disease characteristics.

Task findings involving these networks in elders diagnosed with MCI suggest that there might be a downward trajectory of activation as a result of this disease process. For example, whereas in cognitively intact elders at risk have been shown to hyperactivate the hippocampus, patients with MCI have been shown to have a decrease in activation of the right hippocampus during episodic encoding compared to controls (Johnson, 2006a). Even within a varying degree of severity of MCI diagnosis, the trajectory is apparent; Celone et al. (2006) found that less impaired MCI patients demonstrated hyperactivation

in the hippocampus, where as more impaired MCI patients demonstrated hypoactivation, similar to mild AD patients, in the hippocampus during an associative memory task. Additionally, Dickerson et al. (2005) found greater hippocampal activation in patients with mild MCI as compared to controls during encoding in a face-name associative memory task. In contrast, subjects diagnosed with AD displayed hippocampal hypoactivation during the task. Therefore, it appears that increased fMRI activation in the hippocampus during the prodromal stages of AD might be predictive of future cognitive decline (Bookheimer et al. 2000; Dickerson et al. 2004; Lind et al., 2006b; Petrella et al., 2997; Sperling et al., 2003).

The posterior cingulate is another memory structure that appears to be a target of the disease process in AD and has been found to be anatomically connected to the hippocampus in primate studies (Kobayashi & Amaral, 2003). Although some studies evaluating the posterior cingulate in patients with MCI have found evidence for decreased activation, during recognition tasks, compared to controls (e.g. Johnson et al., 2006a), our previous findings suggest that patients with MCI, as a well as those at high-risk for AD (+ε4+FH) demonstrate a hyperactivation of the posterior cingulate during semantic memory processing (Leveroni et al., 2000). This observation of increased activation in the posterior cingulate of patients with MCI compared to controls is consistent with the findings of Petrella et al., (2007) who also found patients with AD to have an even greater amount of activation in the posterior cingulate.

Along with other regions, the posterior cingulate has been identified as being involved in the default mode network (DMN). The default mode network, in contrast to task-induced activations, is "activated" during the brain's resting state in the absence of a

specific cognitive demand, and is deactivated during a task. The DMN is thought to play a role in maintaining subconscious levels of the environment, reviewing acquired knowledge, and planning future events (Binder et al., 1999; Raichle et al., 2001). There appears to be several structures, including the posterior cingulate, that overlap with the DMN (activate during rest) and the presumed semantic memory network, which is activated during a task (Binder et al., 2009). Decreased deactivation of DMN regions, specifically of the posterior cingulate, has been found in individuals with MCI and even more decreased (less deactivation) in AD (Rombouts et al., 2005); however, few research studies examining the functional resting state status of the brain networks have been done in asymptomatic individuals who are at high risk for AD.

Resting State Activity and Connectivity in AD

The functional resting state of the brain is measured by spontaneous low frequency fluctuations in BOLD signal patterns across anatomical regions. A correlation of these low frequency fluctuating time courses, generated by their spontaneous activity, can be used to establish the degree of functional connectivity between regions. An examination of spatial activation and coherence via time course cross-correlations reveal that similar spontaneous BOLD fluctuations occur in structures that are functionally related (Fox & Raichle, 2007). Thus, regions in the DMN are thought to be spatially related and spontaneously communicate with each other in the absence of any specific demand for a task (Sorg et al., 2008). Examination of functional resting state connectivity might be an even more useful technique for observing the initial functionally related changes that occur in AD and prior to behavioral manifestations (Fleisher et al., 2009).

The functional connectivity between the right hippocampus and the posterior cingulate has been found to be disrupted in AD (Wang, Zang et al., 2006), and others have found asymmetric connections between the left hippocampus to the posterior cingulate in AD (Zhang et al., 2009). Additionally, significantly lower correlations within the bilateral hippocampi have been found in AD compared to healthy controls, which was significantly higher than MCI, such that there appears to be a systematic degradation of this inter-hippocampal functional connectivity as one progresses from healthy control to MCI and then AD (Li et al., 2002). Another study examining the functional connectivity between the posterior cingulate and the hippocampus in patients with MCI found that a lack of functional connectivity was associated with bilateral hippocampal atrophy (Sorg et al., 2008). The loss of cortical tissue in the hippocampus is a hallmark of AD and may be causing disruption in the memory circuits. Together, the functional connectivity between the hippocampus and the posterior cingulate has been shown to be disrupted in elders with AD (Wang, Zang et al., 2006) and MCI (Sorg et al., 2008), and increased in young- (Fleisher et al., 2009) and middle-aged adults at risk for AD (Filippini et al., 2009); however, its integrity in cognitively intact elders at risk for AD remains elusive.

Purpose and Hypotheses

The goal of this study is to characterize the relationship between functional task activation and the functional connectivity during the resting state between the hippocampus (HIPP) and posterior cingulate (PC) in mild cognitive impairment (MCI) patients and cognitively intact elders at-risk for AD. We anticipate the observed state of the functional connectivity integrity in cognitively intact elders at risk and patients with MCI will follow the observed task-related disease trend (Bookheimer et al. 2000;

Dickerson et al. 2004; Lind et al., 2006b; Petrella et al., 2997; Sperling et al., 2003). Specifically, we anticipate that MCI patients and cognitively intact elders at-risk, compared to healthy elders with no risk factors, will demonstrate hyperactivation in the HIPP and PC during the semantic memory task, consistent with previous literature (Woodard et al., 2009). Additionally, because the elders who are at-risk for AD are cognitively intact and demonstrate hyperactivation during task demands, we anticipate that they, as observed in younger populations (Filippini et al., 2009; Fleisher et al., 2009), will demonstrate increased functional connectivity between the bilateral hippocampi and between the bilateral hippocampi and PC compared to normal controls and those with MCI. Conversely, the MCI patients, because of their diagnosed memory problems and evidence of disrupted functional connectivity (Li et al., 2002; Sorg et al., 2008), will have reduced functional connectivity between the bilateral hippocampi and between the bilateral hippocampi and PC compared to normal controls and those at-risk.

Methods

Participants

Fifty-seven participants between the ages of 65 and 85 comprised the final sample and were divided into three groups: At-Risk, MCI, and Control, which were all matched for age, education and gender to form groups of equal size (n = 19 each). The cognitively intact participants (At-Risk and Control groups) were recruited from a larger sample of 459 community-dwelling adults who were recruited via newspaper advertisements. Following telephone screening, 92 participants met study inclusion and exclusion criteria, and 81 participants agreed to undergo ApoE genotyping from blood samples, a neuropsychological evaluation, and an fMRI scanning session. The At-Risk group (n =

19) was formed based on the presence of at least one APOE ε4 allele and a family history of dementia. As part of the initial screening procedure, each potential participant was asked about his or her family history of dementia. A positive family history was defined as a report of a clear clinical diagnosis of Alzheimer's disease or reported confusion, or judgment problems without a formal diagnosis of Alzheimer's disease prior to death in a first-degree relative. Therefore, all individuals included in the At-Risk group had a positive family history (FH+) and all of them had at least one copy of the ε4 allele (ε4+); 18 were ε 3/ ε 4 and one was ε 4/ ε4. The Control group (n = 19) was formed based on the absence of the APOE ε4 allele and the absence of a family history of Alzheimer's disease. The Control group participants had no family history of dementia and did not possess an APOE ε4 allele (1 ε2/ ε3; 18 ε3/ ε3). At-Risk and Control participants were required to perform within normal limits on neuropsychological testing, which included the: Mini-Mental State Examination (Folstein et al., 1975), Mattis Dementia Rating Scale-2 (DRS-2); (Mattis, 1988; Jurica et al., 2001), Rey Auditory Verbal Learning Test (RAVLT) (Rey, 1958), Geriatric Depression Scale (GDS) (Yesavage et al., 1983) and Lawton Activies of Daily Living (LADL) (Lawton and Brody, 1969). ApoE genotype was determined using a PCR method (Saunders et al., 1996). DNA was isolated with Gentral Systems Autopure LS for Large Sample Nucleic Acid Purification.

The majority of MCI participants were recruited from the Memory Disorders Clinic at the Medical College of Wisconsin. To be included in the MCI group, participants met Petersen criteria (Petersen et al., 2001): (i) memory complaint preferably corroborated by an informant; (ii) objective memory impairment by neuropsychological testing; (iii) normal general cognitive functioning; (iv) intact activities of daily living; and

(v) not demented. MCI participants were also evaluated by a neurologist with expertise in dementia to rule out other possible bases for the memory impairment. All MCI participants obtained a modified Hachinski ischemia score < 4.

Measures

Imaging. The imaging portion of the study lasted approximately one hour, during which anatomical images were acquired and an event-related functional MRI fame discrimination semantic memory task was performed. During this task, participants viewed a total of 60 names on a screen (30 famous and 30 unfamiliar). They were given a button box and asked to make a right index finger button press if the name was famous, or make a right middle finger button press if the name was unfamiliar (described in Douville, et al., 2005). By design, this task is relatively easy, allowing for accurate performance across all groups. Following the acquisition of functional task data, the participant was instructed to relax while the scanner collected five minutes of resting state brain activity.

Image Acquisition. Event-related fMRI was conducted on a General Electric Signa Excite 3.0 Tesla short bore scanner equipped with a quad split quadrature transmit/receive head coil. Echoplannar images were collected using an echoplannar pulse sequence (TE = 25 ms; flip angle = 77 degrees; field of view (FOV) = 24 mm; matrix size = 64 x 64). Thirty-six contiguous axial 4-mm-thick slices were selected to provide coverage of the entire brain (voxel size = 3.75 x 3.75 x 4 mm). The interscan interval (TR) was 2 secs. High-resolution, three-dimensional spoiled gradient-recalled at steady-state (SPGR) anatomic images were acquired (TE = 3.9 ms; TR = 9.5 ms; inversion recovery (IR) preparation time = 450 ms; flip angle = 12 degrees; number of

excitations (NEX) = 2; slice thickness = 1.0 mm; FOV = 24 cm; resolution = 256 x 224). Foam padding was used to reduce head movement within the coil and each subject wore ear plugs to reduce noise.

Region Identification

Hippocampal Region of Interest (ROI). Left and right hippocampal volumes were manually traced on T1-weighted SPGR images to determine the hippocampal regions of interest (ROI) by two raters blinded to participant group membership. Starting with a segmented mask of the medial temporal region created using SPM 5 and overlaid on the anatomical image, raters erased non-hippocampal regions on sagittal views. Using coronal views, the mask was further refined by excluding the fimbria and alveus and retaining the hippocampus (uncal apex, cornu ammonis, subiculum, gyrus of retzius and fasciola cinerea). Hippocampal volumes were normalized by dividing by the total intracranial volume. The intra-class correlation coefficient (ICC) for the two raters was 0.88.

The high resolution anatomically traced hippocampal ROIs were used to select the functional seed voxels. To account for partial voluming of the hippocampus in the echoplanar images (i.e. image voxels only partially occupied by hippocampal tissue), voxels were selected based on having 80% of inclusive hippocampal tissue in the voxel, using the 3Dfractionize function in AFNI. This ensures that the voxels entered into analyses are representative of individual hippocampal anatomy, and not ventricular or non-hippocampal BOLD signals. Due to significant hippocampal atrophy, partial voluming would be of most concern for individuals in the MCI group (see Figure 1).

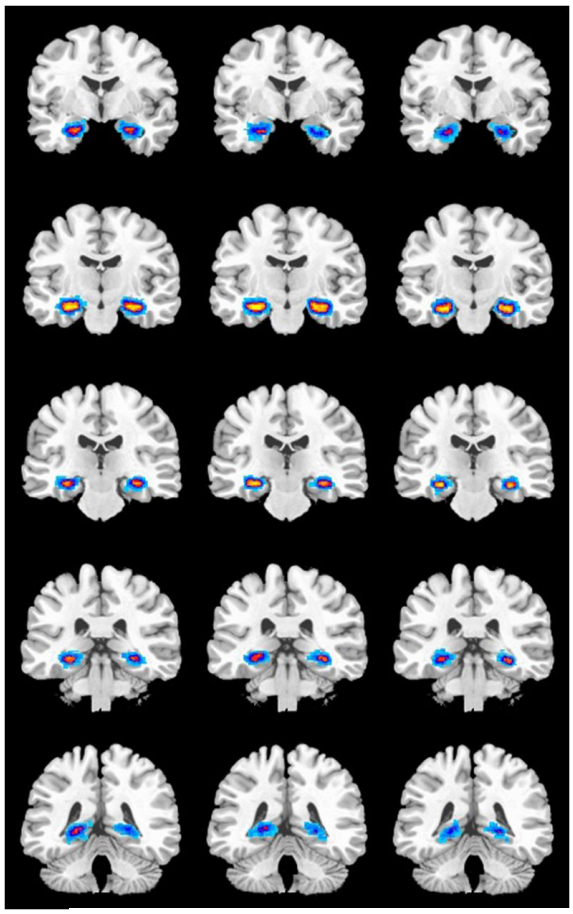

Figure 1. Location of the hippocampal seed regions by group. Voxels were included if they had at least 80% tissue occupancy. The color indicates the degree of overlap. Warm colors illustrate greater overlap, while cool colors illustrate less overlap. For the Control group, the average left hippocampal seed volume was 2.509 ml and 2.125 ml for the right hippocampal seed. For the At-Risk group, the average left hippocampal seed volume was 2.535 ml and 2.158 ml for the right hippocampal seed. For the MCI group, the average left hippocampal seed volume was 2.034 ml and 1.815 ml for the right hippocampal seed.

Posterior Cingulate (PC) Region of Interest. The posterior cingulate region of interest was derived from our semantic memory fame discrimination task spatial extent analysis. In this spatial extent analysis we created statistical parametric maps to identify voxels where the area under the curve (AUC) for famous names differed significantly

from the AUC for unfamiliar names. An individual voxel probability threshold (p = 0.001) was coupled with a minimum cluster volume threshold of 0.28 ml. This combination of individual voxel probability and minimum cluster size thresholds is equivalent to a whole-brain family-wise error threshold of $p < 0.05$ based on 3000 Monte Carlo simulations (Forman et al., 1995). From this spatial extent analysis, any voxel deemed 'activated' by the Famous-Unfamiliar name subtraction in at least one of the three groups contributed to the posterior cingulate ROI (see Figure 2).

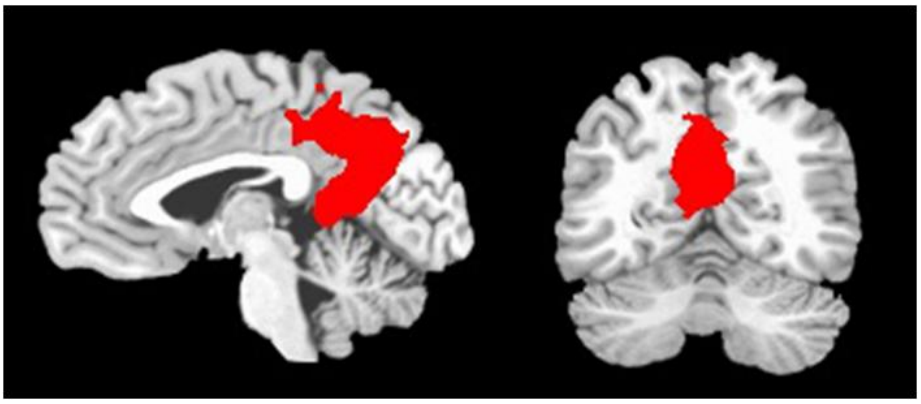

Figure 2. Location of the posterior cingulate region of interest. The posterior cingulate ROI was formed by conjoining the activation maps for each subject. It included Brodmann areas 7 and 31, was located at *x = 1, y = -56, z = 27,* and the total volume was 16.6 ml.

Within this group posterior cingulate ROI, each individual's cluster of the 10 most activated voxels was overlaid to create a consensus region. Within this consensus region, a center of mass was chosen for both the left and right areas of the posterior congulate, which were centered on (x = +/-1, y = -55, z = 25). Finally, the 10 voxels around this center of mass, for the left and right areas respectively, were combined to create the final posterior cingulate seed (see Figure 3).

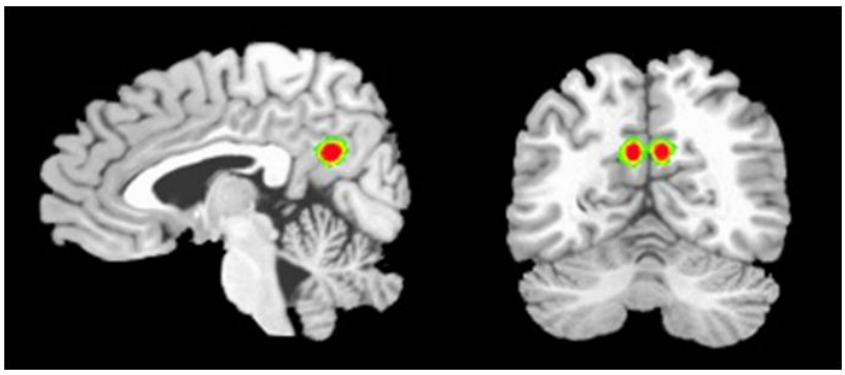

Figure 3. Location of the posterior cingulate seed. The posterior cingulate seed was derived by overlapping each subject's cluster of the 10 most activated voxels within the posterior cingulate region of interest and combining 10 voxels around the center of mass for the left and right areas. The final posterior cingulate seed was centered on x = +/-1, y = -55, z = 25, and 1.125 ml in volume. The warmer colors indicate a greater degree of overlap, while the cooler colors indicate less overlap.

Analyses

Pre-processing. The resting state data was first pre-processed using AFNI (Cox, 1996) and FSL software (Smith et al., 2004). The first step in this process was to realign the anatomical scans to the resting echo-planar images (EPI) using AFNI. Cardiac and respiratory artifacts were then removed from the data using the procedure of Beall and Lowe (2007), which included: 1) registering the subject's time series standard deviation map to a template standard deviation map and using this registration to bring a respiration template into the individual subject's data coordinates. This step was then repeated for cardiac data; 2) performing a slice-wise independent components analysis (ICA) on the resting state time series, to identify stationary sets of voxels whose activations vary together over time and are maximally distinguishable from other sets; 3) identifying ICA components that match the spatial patterns of respiratory and cardiac templates slice-wise and extracting their time series; 4) plotting the frequency spectra of the cardiac and

respiratory components, and selecting frequency bands to filter them out of the time series; and 5) using these filtered time series in the RETROICOR program to create phase files, which were then used to regress physiologic noise out of the time series data. Once the physiological artifacts were removed, the time series were motion corrected using AFNI 3dvolreg and an additional second order motion correction of the data was performed using the motion parameters from the output of the 3dvolreg.

Connectivity Analyses. To assess the functional connectivity between the hippocampus and posterior cingulate at rest, the average seed time series were extracted from the preprocessed resting state data to use as reference time series. These reference time series were then individually cross-correlated to each voxel in the brain. This whole brain analysis was exploratory and will be the focus of future study, whereas the focus of the present study will remain on the hippocampi and posterior cingulate functional connectivity. The voxelwise correlation coefficients were then converted to t-scores, and then normalized to z-scores using the procedure of Lowe, Mock, and Sorenson (1998). These z-scores were averaged for all voxels within each hippocampal anatomical ROI and the functional posterior cingulate ROI identified from activation in the semantic memory task. Because hippocampal atrophy in the MCI group complicated voxelwise interpretation of group differences, the resulting subject z-maps were also transformed to Talairach space for voxelwise group analysis and blurred with a 6mm full-width half-maximum (FWHM) Gaussian filter. Groups were then compared using one-way analysis of variance (ANOVA). Significant group differences were followed by Fisher's post hoc analyses.

Results

Task-related Analysis

There were no group differences in performance on the fame discrimination. The spatial extent analysis confirmed that the At-Risk group activated the left and right hippocampi, and that both hippocampi and the posterior cingulate are among the regions activated during the fame discrimination task. The At-Risk and MCI groups demonstrated greater activation in the posterior cingulate compared to the Control group. The At-Risk group showed greater activation in the left hippocampus compared to the MCI and Control groups, while the right hippocampus showed no group differences in activation.

ROI Connectivity Analysis

Inter-Hippocampal Connectivity. The one-way ANOVA examining functional connectivity between the right hippocampal seed and left hippocampal ROI revealed a significant group difference: $F\,(2, 54) = 3.66$, $p = .032$, $\eta^2 = 0.12$). Post hoc comparisons revealed that the MCI patients demonstrated reduced functional connectivity compared to elders At-Risk ($p = .037$) and Controls ($p = .015$) (Figure 4). There were no connectivity differences between the healthy elders At-Risk and Controls. To see if the inverse of this comparison was true, a one-way ANOVA comparison of the connectivity between the left hippocampal seed and the right hippocampal ROI was conducted. This ANOVA revealed a significant group difference: $F\,(2, 54) = 5.00$, $p = .010$, $\eta^2 = .16$). MCI patients had reduced connectivity compared to those At -Risk ($p = .003$) and Controls ($p = .031$) (Figure 5). There were no differences between healthy elders At-Risk and Controls.

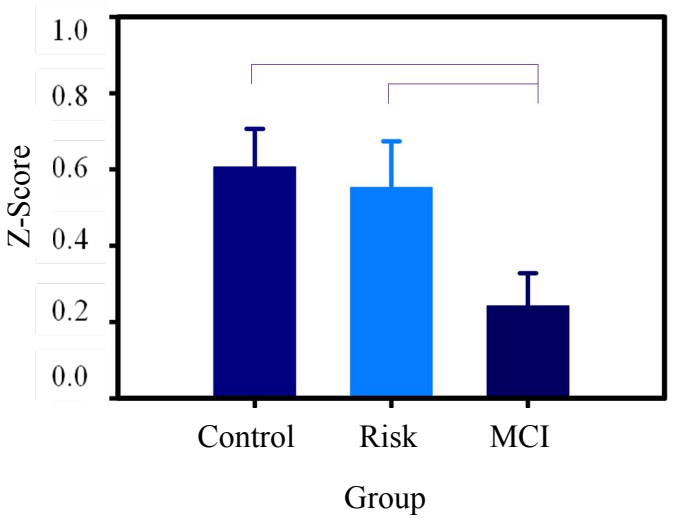

Figure 4. Functional connectivity between the right hippocampal seed and the left hippocampal ROI. The MCI group showed significantly reduced functional connectivity between the right hippocampal seed and the left hippocampal seed compared to the Control and Risk groups. Error bars represent the standard error of the mean (SEM) and significant contrasts are denoted by the gray horizontal bars above the error bars.

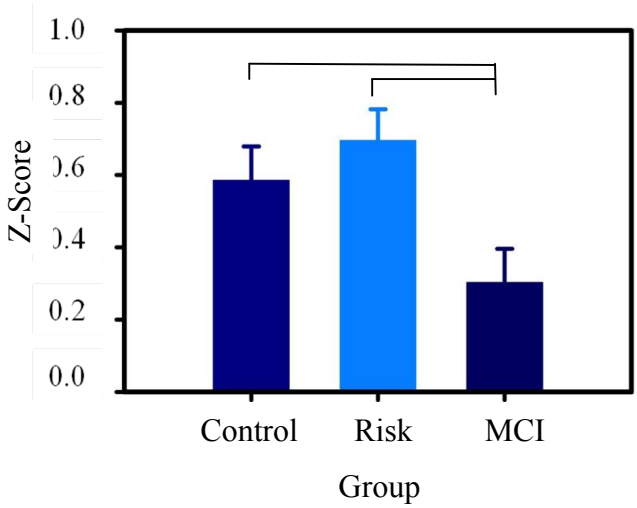

Figure 5. Functional connectivity between the left hippocampal seed and the right hippocampal ROI. The MCI group showed significantly reduced functional connectivity between the left hippocampal seed and the right hippocampal seed compared to the Control and Risk groups. Error bars represent the standard error of the mean (SEM) and significant contrasts are denoted by the gray horizontal bars above the error bars.

HIPP-PC Connectivity. To examine the functional connectivity between the posterior cingulate and the bilateral hippocampi, two separate one-way ANOVAs were conducted. The first one-way ANOVA revealed a significant difference between the functional connectivity of the posterior cingulate seed and right hippocampal ROI, F (2, 54) = 4.14, p = .021, $\eta^2 = 0.13$). Post hoc tests showed that the patients with MCI had significantly less functional connectivity compared to healthy elders At Risk (p =.02) and Controls (p =.02) (Figure 6). There was no significant difference in this connectivity between elders At-Risk and Controls. Interestingly, there were no significant group differences in the connectivity between the posterior cingulate seed and left hippocampal ROI (p = .13) (Figure 7).

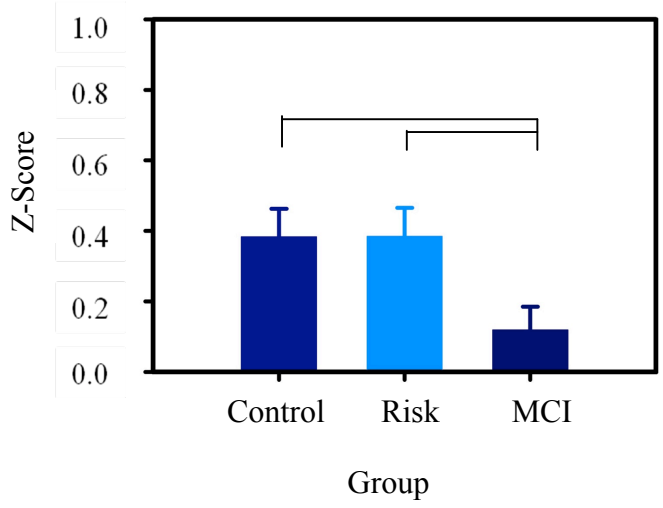

Figure 6. Functional connectivity between the posterior cingulate seed and the right hippocampal ROI. The MCI group showed significantly reduced functional connectivity between the posterior cingulate seed and the right hippocampal seed ROI compared to the Control and Risk groups. Error bars represent the standard error of the mean (SEM) and significant contrasts are denoted by the gray horizontal bars above the error bars.

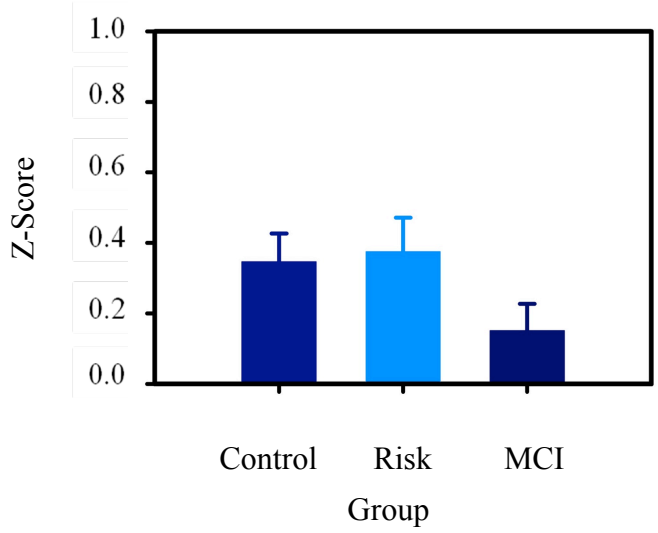

Figure 7. Functional connectivity between the posterior cingulate seed and the left hippocampal ROI. No group differences were observed in the functional connectivity between the posterior cingulate seed and the left hippocampal ROI. Error bars represent the standard error of the mean (SEM) and significant contrasts are denoted by the gray horizontal bars above the error bars.

The connectivity between the right hippocampal seed and the posterior cingulate ROI was examined by a one-way ANOVA and was significantly different across groups: F (2, 54) = 3.71, p = .031, $\eta^2 = 0.12$). Post hocs revealed that elders with MCI had significantly less functional connectivity between the right HIPP seed and the PC compared to elders At-Risk (p =.009), but not compared to Controls. There were no differences between elders At-Risk and Controls (Figure 8). In other words, the right HIPP seed - PC connectivity was greater in elders At-Risk compared to elders with MCI. A one-way ANOVA revealed a significant group difference between the amount of functional connectivity between the left hippocampal seed and the posterior cingulate ROI: F (2, 54) = 5.11, p = .009, $\eta^2 = 0.16$). A post hoc analysis revealed that elders At-Risk had significantly greater functional connectivity compared to Controls (p =.044) and

patients with MCI (p = .003) (Figure 9). There were no significant differences between Controls and patients with MCI.

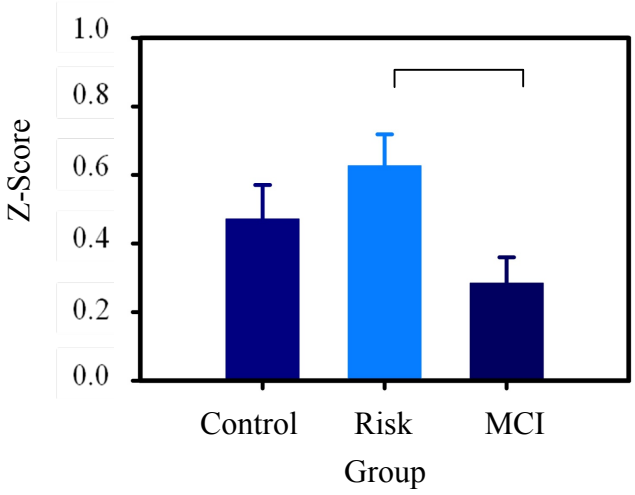

Figure 8. Functional connectivity between the right hippocampal seed and the posterior cingulate ROI. The Risk group showed significantly higher functional connectivity between the right hippocampal seed and the posterior cingulate ROI compared to the MCI group. Error bars represent the standard error of the mean (SEM) and significant contrasts are denoted by the gray horizontal bars above the error bars.

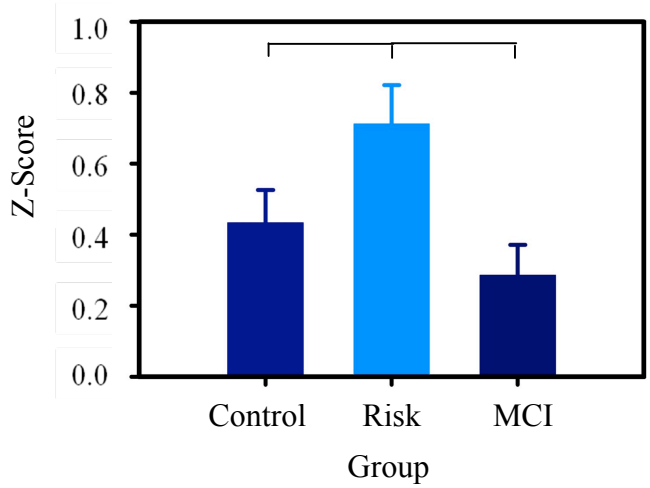

Figure 9. Functional connectivity between the left hippocampal seed and the posterior cingulate ROI. The Risk group showed significantly higher functional connectivity between the left hippocampal seed and the posterior cingulate ROI compared to the MCI and Control groups. Error bars represent the standard error of the mean (SEM) and significant contrasts are denoted by the gray horizontal bars above the error bars.

Discussion

In this study, we examined the level of resting state functional connectivity between the bilateral hippocampi and the posterior cingulate in healthy elders without risk factors for AD, healthy elders with high-risk factors (+ε4+FH) and patients with a diagnosis of MCI. We found reduced functional connectivity between the left and right hippocampi in patients with MCI compared with cognitively intact high-risk and control elders. The reduction in functional connectivity in MCI is consistent with our prediction and with previous literature (Li et al., 2002). Given that the hippocampus is central to memory processing, damage to the network here might compromise the rest of the system. Deficient hippocampal connectivity at rest in MCI may therefore be a primary reason that these individuals exhibit memory impairment. Furthermore, functional impairment of the hippocampus or its connections with other regions may cause other functional impairments due to lost connectivity.

To our knowledge, this is the first assessment of interhippocampal connectivity at rest in individuals at risk for AD. Previous findings suggest a downward trajectory of interhippocampal degradation from healthy controls, reduced in MCI, and reduced even more so in AD (Li et al., 2002). We anticipated that the level of interhippocampal connectivity at rest would be increased in elders at-risk compared to controls, because of their increased task-related activation. However, our data did not support this hypothesis. The similarity in the functional connectivity observed between the hippocampi of controls and intact elders at-risk suggests that those at-risk functionally recruit brain areas during task performance for unknown reasons other than lost interhippocampal connectivity. Other factors responsible for this recruitment need investigation.

In addition to the interhipppocampal comparisons, we assessed the functional connectivity between the hippocampi and the posterior cingulate, another memory-related region. Following the literature suggesting a general decrease in the functional connectivity between the hippocampi and the posterior cingulate as the disease progresses in MCI and AD (Wang, et al., 2006; Zhang et al., 2009), we anticipated similarly reduced connectivity in our MCI sample. The results supported this hypothesis, compared with controls and intact at-risk elders. Interestingly, the right hippocampal seed to the posterior cingulate ROI functional connectivity was reduced in patients with MCI compared to those at risk, but not controls, whereas the functional connectivity between the posterior cingulate seed and the right hippocampal ROI was reduced in patients with MCI compared to those at risk and controls.

Although the findings comparing the patients with MCI and controls are inconsistent across the seed and ROI comparison regions, the trend of decreased functional connectivity between the right hippocampus and the posterior cingulate, regardless of seed and ROI comparisons, was consistently found in elders with MCI compared to elders at risk. A disruption in the connectivity between the right hippocampus and the posterior cingulate has been observed in patients with AD (Wang et al., 2006). Likewise, this finding indicates that the functional connectivity between the right hippocampus and posterior cingulate is consistently greater in elders at risk compared to patients with MCI, which is consistent with previous research examining the influence of the risk factors on middle-aged individuals (Fleisher et al., 2008). To our knowledge, this is the first study examining the influence of genetic risk factors on the functional connectivity between the hippocampi and posterior cingulate in *elders*. It

appears the increased functional connectivity observed in those at risk is foreshadowing the disease trend observed in task-related studies (Bookheimer et al. 2000; Dickerson et al. 2004; Lind et al., 2006b; Petrella et al., 2997; Sperling et al., 2003). That is, perhaps the change in resting brain function in DMN underlies or otherwise influences the recruitment evidenced in the active brain states induced by task performance. Future studies should investigate this possibility. If accurate, resting connectivity may be importantly predictive of cognitive decline at an earlier stage than task-induced activation can predict.

We also anticipated that the functional connectivity between the left hippocampus and the posterior cingulate would be reduced in patients with MCI, and increased in elders at risk, compared to controls based on previous literature (Zhang et al., 2009). While the functional connectivity between the posterior cingulate seed and the left hippocampal ROI was not significant across groups, the functional connectivity between the left hippocampal seed and the posterior cingulate ROI was significantly heightened for elders at high-risk compared with patients with MCI and controls. Consistent with our findings, Filippini et al., (2009) found bilateral hippocampi and posterior cingulate connectivity, representative of the DMN, was increased in young individuals at risk for AD. Increased functional connectivity between the left hippocampal seed and posterior cingulate ROI, as observed in the present study, is also commensurate with our previous findings that demonstrated hyperactivation in the left, but not the right, hippocampus (Woodard et al., 2009). That is, the present study extends the findings of Woodard et al. (2009) by not only implicating the left hippocampus, but also finding that the integrity of

its functional connectivity with related regions may be useful in distinguishing between cognitively intact elders at risk and those not at risk.

While it is important that we observed greater functional connectivity between the right hippocampus and the posterior cingulate in elders at risk compared to patients with MCI, it is arguably more remarkable that we observed greater functional connectivity between the *left* hippocampus and the posterior cingulate in elders at risk compared to patients with MCI *and* controls. That is, the distinction between MCI and cognitively intact elders at risk can be observed behaviorally, whereas the findings in the present study indicate that underlying functional changes while the brain is at rest can be observed and used to distinguish between elders at risk and elders not at risk, before symptoms emerge. Thus, taken together, task-related hyperactivation and resting state connectivity data suggest that the left hippocampus and its functionally connected structures (the posterior cingulate as observed here, but possibly others as well) may be useful as early biomarkers of AD.

Limitations and future directions.

A potential limitation to the present study regards the criteria used to select seed regions. Because resting state functional connectivity analysis is relatively new, there is not a general consensus on the best technique. Some researchers may have chosen more liberal criteria, for example selecting voxels that included at least 55% tissue occupancy. Others may have chosen more conservative criteria for selecting the regions (for example selecting voxels that included at least 89% tissue occupancy). The compromise of at least 80% tissue occupancy is a strength of the present study. Additional strengths of the present study include the manually traced hippocampal regions of interest (versus

automated programs, which often include additional structures, or exclude targeted tissue), the functionally derived posterior cingulate region of interest, and acquiring a sample of brain activity during a true resting state acquisition.

The relationships of the functional connectivity between the bilateral hippocampi and posterior cingulate are meaningful and provide more information in understanding the early disease process of AD. Future studies should expand the results of the region of interest analysis to include other regions, particularly other regions in the default mode network. This will provide a more comprehensive picture of the functional connectivity status of the resting brain in elders at high-risk for AD and patients with MCI. Additionally, a voxelwise analysis of the functional connectivity between default mode network regions while at a resting state would supplement the findings of the region of interest analysis. Finally, a longitudinal analysis is important and would allow us to observe functional connectivity changes over time, thereby providing a better understanding of the disease process as it affects the functional integrity of the brain. Furthermore, after enough of the subjects in our data decline, we can look back at the resting state functional connectivity data and examine any distinguishable differences that might have been present before symptoms emerged. This will provide predictive clues about which cognitively intact elders will most likely decline so that preventative strategies can be employed.

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