Reduced Hippocampal Functional Connectivity in Alzheimer Disease

Greg Allen, PhD; Holly Barnard, MA; Roderick McColl, PhD; Andrea L. Hester, PhD; Julie A. Fields, BA; Myron F. Weiner, MD; Wendy K. Ringe, PhD; Anne M. Lipton, MD, PhD; Matthew Brooker, BS; Elizabeth McDonald, RN; Craig D. Rubin, MD; C. Munro Cullum, PhD

Objective: To determine if functional connectivity of the hippocampus is reduced in patients with Alzheimer disease.

Design: Functional connectivity magnetic resonance imaging was used to investigate coherence in the magnetic resonance signal between the hippocampus and all other regions of the brain.

Participants: Eight patients with probable Alzheimer disease and 8 healthy volunteers.

Results: Control subjects showed hippocampal functional connectivity with diffuse cortical, subcortical, and cerebellar sites, while patients demonstrated markedly reduced functional connectivity, including an absence of connectivity with the frontal lobes.

Conclusion: These findings suggest a functional disconnection between the hippocampus and other brain regions in patients with Alzheimer disease.

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ALZHEIMER DISEASE (AD) is the most common type of dementia, characterized by memory impairment progressing to globally impaired cognition. As the human lifespan has increased, so too has the prevalence of AD and widespread concern about this disease, encouraging the search for new modes of early identification and intervention. Recently, neuroimaging techniques have proved to be useful disease-monitoring tools, prompting further research into their utility for early detection.

An extensive review of the neuroimaging literature concluded that volume loss within the hippocampus most reliably discriminates between healthy control subjects and patients with early AD. Furthermore, hippocampal atrophy correlates significantly with decline in cognitive status in patients with AD and mild cognitive impairment, and may in fact be a predictor of conversion from mild cognitive impairment to AD. This is consistent with the decline in declarative memory characteristic of AD. Thus, neuroimaging techniques for assessing and monitoring hippocampal function may be greatly useful in the evaluation of AD. A new approach to investigating brain function that holds promise for the study of AD is functional connectivity magnetic resonance imaging (FCMRI).

Functional connectivity magnetic resonance imaging is a technique that enables the in vivo examination of functional connections in the brain. It is based on the finding that brain regions that are functionally related show correlated low-frequency fluctuations in the MRI signal that arise from the same blood oxygenation level–dependent origins as task-related functional magnetic resonance imaging signal changes. Functional connectivity magnetic resonance imaging exploits these correlations to create an image of task-independent functional connectivity. This technique has been used to demonstrate connectivity between homologous regions of the right and left hemisphere (eg, motor, visual, and auditory cortices) and other functionally related brain regions (eg, thalamus and hippocampus, Broca and Wernicke areas, and cerebellum, thalamus, and cerebrocortical sites). When applied to patients, FCMRI can be used to examine dysfunction at the level of neurofunctional networks. For instance, an FCMRI study comparing patients with AD and mild cognitive impairment with healthy age-matched controls demonstrated decreases in the functional synchrony of the right and left hippocampi along the spec-
trum from healthy to AD. However, this investigation was limited to an examination of connections between the two hippocampi. In contrast, FC-MRI was used to examine hippocampal connectivity with the rest of the brain in a mixed group of subjects with mild or “very mild” AD. This study reported reduced connectivity between the right hippocampus and several brain regions and increased connectivity between the left hippocampus and the right prefrontal cortex in those with AD. The goal of the present study was to examine a more homogeneous group of subjects with mild AD to gain further insight into the effects of the disease on hippocampal functional connectivity. We hypothesized an overall breakdown in the synchronous activity of neural circuits involving the hippocampus.

**METHODS**

Eight patients with probable AD (Table 1) were recruited from the Alzheimer’s Disease Center and the Mildred Wyatt and Ivor P. Wold Center for Geriatric Care at the University of Texas Southwestern Medical Center. Alzheimer disease was diagnosed by a neurologist or geriatrician using National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer’s Disease and Related Disorders Association criteria. Eight healthy volunteers (Table 1) with no history of developmental, psychiatric, or neurological disorders were recruited from the community. All subjects were right-handed. Before participation, the study was described to the subjects and their caregivers. Written informed consent was then obtained from subjects and a family member or legal representative. The University of Texas Southwestern Medical Center institutional review board approved the complete experimental protocol.

All subjects were administered a brief neurocognitive battery to ensure a mild level of cognitive impairment in the AD group and to rule out impairment in controls (Table 1). This included the Mini-Mental State Examination, the Alzheimer Disease Assessment Scale–cognitive subscale, and the Third Edition. All controls performed in the average to logical memory subtest from the Wechsler Memory Scale–Third Edition.

The magnetic resonance images were acquired at 1.5 T using the standard quadrature birdcage RF head coil (GE Medical Systems, Milwaukee, Wisconsin). A time series of 100 echo-planar image volumes was acquired at 16 axial slice locations through the whole brain while subjects were at rest in a darkened scanner room. Sensory stimulation was limited to the noise of the scanner, which was dampened by earplugs and noise-reducing headphones. The echo-planar image data were acquired with a single-shot gradient-recalled pulse sequence (sequential slice acquisition; repetition time, 2000 milliseconds; echo time, 45 milliseconds; flip angle, 90°; matrix, 64×64; field of view, 24 cm; thickness, 7 mm; and gap, 0.5 mm). High-resolution images of the entire brain (3-dimensional spoiled grass pulse sequence: repetition time, 30 milliseconds; echo time, 5 milliseconds; flip angle, 45°; matrix, 256×256; field of view, 24 cm; and thickness, 2.0 mm) were also acquired.

All analyses were conducted with the use of Analysis of Functional NeuroImages (AFNI) software. First, all data were normalized to the Talairach grid, which aided in anatomical localization and allowed the combination of data across subjects and comparison between groups. Subsequent preprocessing steps included slice-timing correction, detrending, motion correction, and temporal filtering. Early FC-MRI studies demonstrated that coherence in blood oxygenation level–dependent signal fluctuations occurs at low frequencies. Thus, a low-pass filter removed all frequencies of greater than 0.08 Hz. The data were then spatially smoothed with a 3-dimensional gaussian tapering function (5-mm full width at half maximum) to increase the signal-noise ratio.

For each subject, right and left hippocampal “seed volumes” were identified. To ensure that seed volumes were restricted to intact hippocampal tissue in each subject, these volumes were defined as 5 contiguous voxels (at echo-planar image resolution) in the body of the hippocampus. Preprocessed time series echo-planar image signal data were then extracted from these voxels. These data were averaged to create right and left hippocampal signal time courses for each subject, which were used as reference functions for correlation with fluctuations in the magnetic resonance signal in all other brain voxels. The least-squares fit coefficients from these calculations were entered into the group analyses. This same procedure was also conducted using a control seed volume placed in the primary visual cortex.

Within-group 2-tailed t tests identified sites where the AD or control data were significantly different from 0, while unpaired between-group 2-tailed t tests identified sites of significant group difference. The output from these t tests was thresholded using a voxel-cluster-size method. First, all voxels whose t value did not exceed a = .025 were excluded from further analysis. Then, Monte Carlo simulations were used to determine the probability of falsely detecting clusters of various sizes. Our goal was an overall (ie, over the entire 3-dimensional image volume) significance level of P < .01. Thus, we identified the minimum cluster size that occurred with P < .01 for each comparison. For the left hippocampus, the size for controls was 2004 µL; for patients with AD, 1898 µL; and for controls vs those with AD, 2637 µL. For the right hippocampus, the size for controls was 2004 µL; for patients with AD, 2004 µL; and for controls vs those with AD, 2742 µL. Clusters that exceeded this cutoff were retained.

**RESULTS**

**NEUROPSYCHOLOGICAL TESTING**

Control subjects performed within normal limits on the Alzheimer Disease Assessment Scale–cognitive subscale and the Mini-Mental State Examination, while subjects with AD were mildly impaired (Table 1). On the logical memory subtest from the Wechsler Memory Scale–Third Edition, all controls performed in the average to

| Table 1. Characteristics of the 8 Subjects With AD and the 8 Healthy Control Subjects |
|---------------------------------|-----------------|-----------------|-----------------|
| **Characteristic**             | **Subjects**    | **Control**     | **P Value**     |
| Age, y                         | 77.5 ± 6.0      | 77.5 ± 7.1      | . . .           |
| Educational level, y           | 13.1 ± 2.0      | 14.3 ± 5.3      | .57            |
| Male to female ratio           | 2.6             | 4.4             |                |
| MMSE score                     | 23.6 ± 1.8      | 26.9 ± 2.2      | < .01          |
| ADAS-cog score                 | 21.4 ± 3.4      | 10.8 ± 3.9      | < .001         |
| Logical memory, % retention    | 42.0 ± 30.1     | 80.2 ± 12.3     | < .01          |

Abbreviations: AD, Alzheimer disease; ADAS-cog, Alzheimer Disease Assessment Scale–cognitive subscale; MMSE, Mini-Mental State Examination.

a Data are given as mean ± SD unless otherwise indicated.
b Values refer to the results of 2-tailed t tests comparing groups.
above average range on immediate and delayed recall trials (mean scaled scores, 11.6 and 12.0, respectively) and all retained more than 60% of learned information (mean, 80%), ruling out the isolated memory deficit characteristic of mild cognitive impairment.29

HIPPOCAMPAL FUNCTIONAL CONNECTIVITY: WITHIN-GROUP FINDINGS

In controls (Figure 2A), both hippocampi showed extensive functional connectivity with frontal, parietal, occipital, and temporal sites. Connectivity with other limbic regions was also observed, as was connectivity with basal ganglia and cerebellum. In contrast, participants with AD (Figure 2B) showed a much more restricted pattern of connectivity, with a complete absence of connectivity with the frontal lobes.

Figure 1. Left hippocampus seed volume from a single subject overlaid on representative coronal slices.

REDUCED HIPPOCAMPAL FUNCTIONAL CONNECTIVITY IN AD

Compared with patients with AD, control subjects demonstrated significantly greater connectivity of the hippocampus throughout the cerebral cortex, limbic areas,
Figure 2. Connectivity with the left hippocampus in control subjects (A) and in subjects with Alzheimer disease (AD) (B), and increased connectivity in controls vs subjects with AD (C).
subcortical regions, and cerebellum (Table 2 and Table 3 and Figure 2C). While the within-group analysis of subjects with AD indicated a lack of hippocampal-frontal connectivity, the group comparison emphasized much more diffuse reductions in connectivity. In contrast, when the FCMRI analyses were conducted with a primary visual cortex seed volume, no group differences were observed. There were no regions of increased hippocampal connectivity in participants with AD.

**COMMENT**

The pathway between the hippocampus and neocortical regions includes the entorhinal cortex and other medial temporal lobe structures.20 Because these areas are some of the first affected in AD, this disease has been thought...
to involve a breakdown in connectivity between the hippocampus and the rest of the brain. In recent years, neuroimaging studies have sought to identify such disconnections. The first of these, which examined activation during a facial, delayed, match-to-sample task, found reduced functional connectivity between the right prefrontal cortex and hippocampus in patients with AD. Similarly, Wang et al. found disrupted connectivity between the right hippocampus and several brain regions in subjects with AD, while connectivity between the left hippocampus and the prefrontal cortex was relatively increased. In contrast to the study by Wang et al., our findings indicate a more extensive disruption of hippocampal connectivity in AD, with no regions of increased connectivity and an absence of hippocampal-frontal connectivity. The most likely explanation for this difference is a greater disease severity in our sample, supporting the notion that hippocampal connectivity declines progressively during the disease. When we examined functional connectivity with the primary visual cortex, no group differences were observed, lending support to the specificity of the hippocampal connectivity differences. Dysfunctional circuitry connecting the hippocampus with other brain regions is a likely contributor to deficits in learning and memory and other areas of cognition characteristic of AD, and FCMRI of the hippocampus may ultimately provide an in vivo marker of abnormal hippocampal function in this population. If shown to have adequate sensitivity and specificity, hippocampal FCMRI may also prove useful in the diagnosis and monitoring of AD progression.

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Correspondence: Greg Allen, PhD, Department of Psychiatry, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd, Dallas, TX 75390-9127 (Greg.Allen@UTSouthwestern.edu).

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