

Adam Gazzaley, Scott A Small

INTRODUCTION

The application of neuroimaging technology to study Alzheimer's disease (AD) has been steadily increasing over the last two decades. To date, the majority of neuroimaging contributions to understanding the pathophysiology and clinical course of AD have utilized structural magnetic resonance imaging (MRI) and positron emission tomography (PET). Influenced by pathological data, which reveal that the earliest disease manifestations are in medial temporal lobe (MTL) structures such as the hippocampus and entorhinal cortex,¹ structural MRI studies have largely focused on volumetric measures of the MTL. Starting in the early 1990s, MTL volumes were shown to distinguish age-matched normal controls and AD patients, including those with very mild forms of the disease where the diagnosis of dementia was not yet conclusive.^{2,3} Further studies using quantitative volumetric measures have now demonstrated that MTL volumes predict progression to AD from mild states of memory impairment⁴⁻⁷ and correlate with impaired memory performance in AD patients,^{8,9} thus supporting the contention that MTL volumetric measures are both clinically and biologically relevant. The application of structural MRI has continued to advance as other measures have complemented the volumetric studies. For example, the use of diffusion-weighted (DWI) MRI, sensitive to the random motion of water in the brain, revealed an increase in the apparent diffusion coefficient (ADC) in the hippocampus of AD patients that predicts progression to AD from mild impairment.^{10,11}

Despite considerable data on anatomical changes accompanying AD, less is known of concomitant physiological alterations. Over the last two decades, and until very recently, studies that have explored physiological changes in AD have used functional tomographic techniques, specifically PET and single photon emission computed tomography (SPECT), for molecular imaging. These techniques generate three-dimensional brain maps of radionuclide distribution reflecting biochemical and physiological processes. The first studies, in the early 1980s, revealed regional changes in both oxygen and glucose metabolism in AD patients^{12,13} that have been confirmed to be reductions primarily localized to the temporal, parietal, and posterior cingulate cortex.^{14,15} In agreement with the volumetric MRI studies of the MTL, functional tomographic techniques have been shown to have both high sensitivity and high specificity for differentiating AD patients from healthy older individuals and those with mild cognitive impairment,^{16,17} as well as predicting progression of the disease.¹⁸⁻²⁰

In contrast to the two decades of AD research using structural MRI, PET and SPECT, it was only as recently as 1999 that functional MRI (fMRI) appeared in the scientific literature as a research tool to study AD.²¹ fMRI, first developed in the early 1990s,²² has been used predominantly by neuroscientists to examine the neural basis of cognitive and behavioral processes, and only recently has it been applied to study patients with neurological disease. Its widespread availability, non-invasiveness, high spatiotemporal resolution, and reasonable cost, especially when

compared with PET scanning, have all contributed to its increasing popularity. As with PET and structural MRI research, fMRI studies of AD have focused on two overlapping objectives: understanding the basic biological mechanisms and pathophysiology of AD, and the development of an effective diagnostic tool – a clinical biomarker. The development of fMRI as a biomarker is anticipated to influence clinical management of AD in three significant ways: differentiating healthy aging and AD, enhancing diagnostic specificity when evaluating a patient with dementia, and monitoring the biological progression of AD for the purposes of drug development and drug testing. In this chapter, we will review studies that have initiated this process and paved the way to achieve our clinical goals in AD management as well as expanding our understanding of the disease process.

DIFFERENTIATING HEALTHY AGING AND AD

At long last, we are on the cusp of offering effective treatment for AD. Even the most effective treatment, however, is anticipated to halt and not reverse neuronal dysfunction. Therefore, treatment will be most effective when administered to patients in the earliest stages of disease. We know that AD begins in the hippocampal formation before spreading to other areas of the brain²³ and the pathological hallmarks of AD – plaques and neurofibrillary tangles – have been identified during postmortem evaluation in individuals without dementia.²⁴ Corresponding to this anatomical pattern of progression, AD presents as mild forgetfulness years before the onset of dementia.^{25–27} Unfortunately, as a wide range of animal studies have established, normal aging itself also targets the hippocampal formation.²⁸ Thus, by blindly assigning the diagnosis of early AD to any older individual with hippocampal-dependent memory decline, our sensitivity for detection will reach 100%, but our specificity will be unacceptably low. Imaging will enhance our ability to detect AD

as early as possible only when it can distinguish AD from normal aging.

As discussed above, structural MRI studies of MTL volumes have already been somewhat effective in this goal. However, it is hypothesized that physiological changes will precede the development of gross atrophic changes, especially given the extent of tissue loss necessary for consistent MRI detection. Thus, it is anticipated that the identification of functional biomarkers using fMRI will aid in the identification of preclinical AD and lead to earlier treatment.

Over the last few years, research efforts have attempted to identify early evidence of physiological dysfunction by using fMRI to compare regional brain activity in four different populations: healthy older individuals, older individuals with risk factors for AD, those with mild cognitive impairment (MCI), and mild AD patients. Healthy older adults who are considered to reflect ‘normal’ aging, are normal–high cognitively performing individuals (compared with age-matched norms), with no neurological or psychiatric disease and minimal accompanying medical disorders. Individuals at higher risk for AD are frequently defined as healthy older adults who possess at least one apolipoprotein E (*APOE*) $\epsilon 4$ allele on chromosome 19 and/or a significant family history. Genetic studies have identified an association between the presence of the $\epsilon 4$ allele and late-onset AD, which begins after age 60.^{29,30} MCI is considered a transitional stage between healthy aging and very mild AD.³¹ MCI often refers to older individuals with complaints of a decline in their cognitive abilities, objective evidence of cognitive performance deficits out of proportion to that expected for age, and failure to meet commonly accepted criteria for dementia. However, this definition is not fully specified or agreed upon and varies greatly between studies. When memory loss is the predominant feature, MCI is defined as amnesic MCI (aMCI) and has been revealed to be a prodromal state of AD.^{32,33} Indeed AD pathology has already accumulated in many of these individuals and thus it often reflects

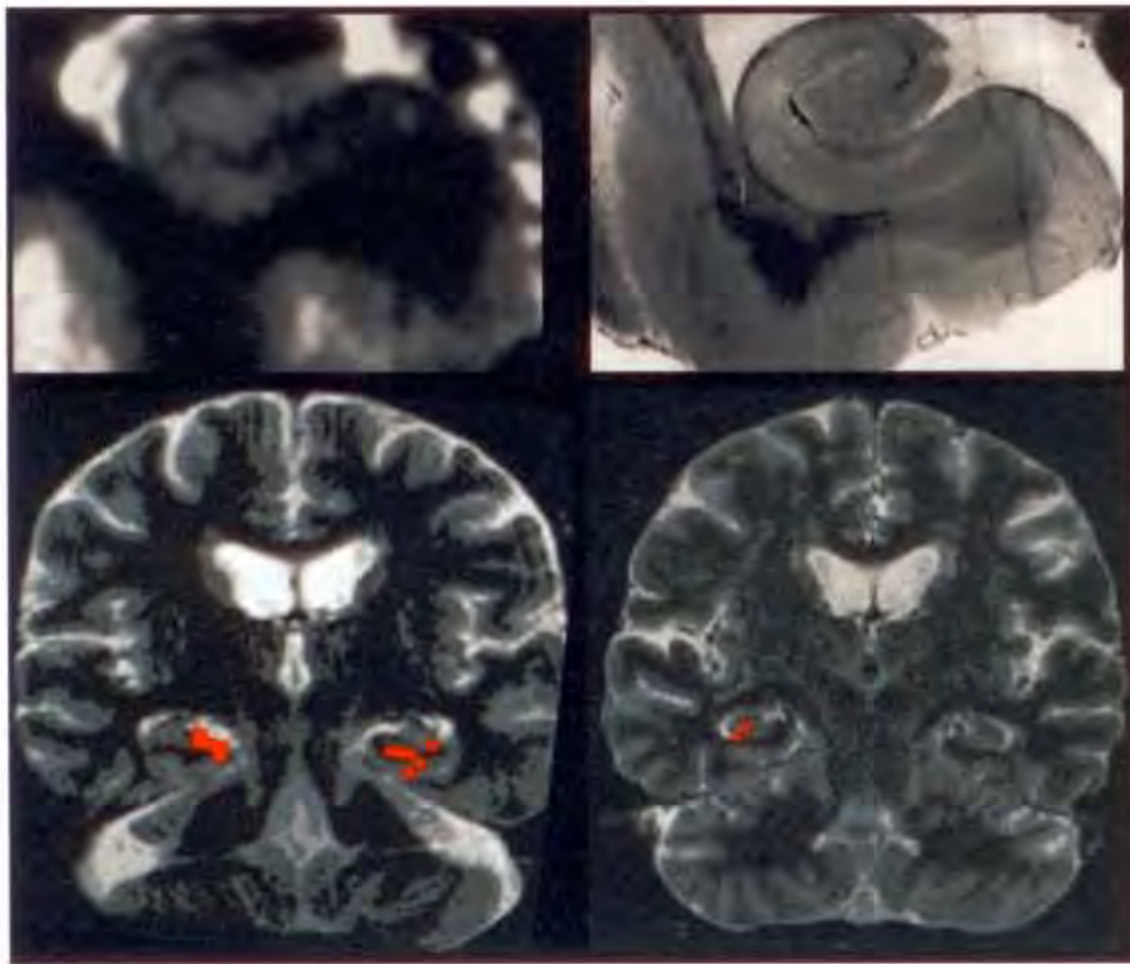


Figure 3.1 The BOLD response in the hippocampal formation induced by a cognitive 'stress test' dissociates Alzheimer's disease from age-matched controls. Top: Comparison of a high-resolution MRI image (left panel) with a postmortem histological slice (right panel) demonstrates that MRI can, in principle, visualize individual hippocampal subregions. Bottom: When viewing unfamiliar faces, the BOLD response in the hippocampal formation is diminished in an Alzheimer's disease patient (right panel) compared with an age-matched control (left panel). Whether this difference in BOLD signal reflects a disease-related change in vascular or neuronal physiology remains unknown. Adapted from Small et al.²¹

early-stage preclinical AD.³⁴ Mild, probable AD is defined by commonly applied criteria for dementia and the exclusion of an identifiable cause other than AD. fMRI studies of these populations have focused largely on comparisons of blood oxygen level-dependent (BOLD) activation patterns during a cognitive task, although there have been alternative approaches that study deactivations and chronic metabolism.

Alterations in regional activations

Most fMRI studies directed at achieving clinical goals are based on the experimental designs pioneered by cognitive neuroscientists that use behavioral tasks to probe neural function with the BOLD signal as a dependent measure.²² The BOLD signal is an indirect measure of neural activity that is dependent on the blood flow-mediated relationship between neural activity and the concentration of deoxyhemoglobin within the surrounding microvasculature. When a neural event occurs anywhere in the brain, there is a local blood flow increase³⁵ that results in a decrease in the concentration of paramagnetic deoxygenated hemoglobin

in the microvasculature surrounding the activated region.³⁶ This local increase in the ratio of non-paramagnetic oxygenated hemoglobin to paramagnetic deoxygenated hemoglobin^{37,38} results in the detection of an increase in the BOLD signal.³⁹ This increase in regional BOLD signal is thus usually interpreted as an increase in neural activity.

The most common approach for studying AD has been to compare the degree of regional activation (BOLD signal magnitude and anatomical extent) while subjects representing various populations perform a task considered to tap into a cognitive process compromised by the disease. Based on this logic, the majority of studies have focused on memory tasks and fMRI BOLD signal changes localized to MTL structures.^{21,40-44} Four of these studies compared the magnitude of MTL activation during tasks that involved visual memory encoding in healthy older adults and patients with mild AD.^{21,40,42,43} The results of these studies were consistent with each other and identified a decrease in MTL activation in mild AD patients compared with healthy older controls (Figure 3.1), mirroring the findings of MTL atrophy using volumetric MRI techniques.^{2,3} These studies established

that alterations in the magnitude of the BOLD signal in MTL structures during a memory task might constitute another indicator of early AD. However, they did not determine whether detectable functional changes precede gross structural changes as hypothesized, and still leave uncertainty as to the nature of the relationship between atrophy and changes in BOLD signal.

To truly assess the practical usefulness of fMRI as a diagnostic tool for AD, it is necessary to study individuals with the subtlest indication of dysfunction to determine whether it is possible to identify those who may have preclinical AD that is undetectable by other techniques. Small et al²¹ studied several older adults with isolated memory impairment and identified a subset who had a similar pattern of decreased activity in hippocampal regions as AD patients. Comparable to this finding, a study of healthy older adults, MCI patients and early AD patients revealed a decrease in MTL activation during memory encoding in both the MCI and AD patients relative to the older controls.⁴⁰ However, these studies did not incorporate a longitudinal assessment, and so it is not possible to determine that the presence of decreased MTL activation predicts subsequent clinical course. A longitudinal fMRI study of 32 MCI patients revealed that a larger extent of activation of an MTL structure, the parahippocampal gyrus, was associated with greater clinical impairment (based on Clinical Dementia Rating score) at baseline and subsequent decline after a 2.5-year follow-up.⁵ This increase may be the result of a compensatory response to accumulating AD pathology. As Dickerson et al⁵ point out, there are numerous differences between studies that could account for such disparities with the Machulda and Small findings:^{21,41} different population selection, data analysis methods, and subject performance.

Compensatory increases in brain activity have also been proposed to explain observations of increased regional activity in studies of normal aging⁴⁵ and studies of individuals without cognitive impairment who are at

increased genetic risk for AD.^{46–49} Carriers of the *APOE* ϵ 4 allele, compared with non-carriers, have increased BOLD signal in multiple brain regions on memory tasks (hippocampus, parietal, and prefrontal cortex^{46,48}) and a letter fluency task (parietal cortex⁴⁹). These results suggest that older adults at increased genetic risk for AD may compensate for preclinical pathology by exerting additional cognitive effort to achieve comparable levels of performance that is then detected as increases in regional brain activity. Additionally, *APOE* ϵ 4 allele carriers were found to generate the same activity pattern as non-carriers on an attention task, suggesting that the compensation is not merely a reflection of increased difficulty, but might have some specificity for the cognitive demands of the task.⁴⁷ However, these results are not entirely straightforward to interpret, as there are many differences in the precise regions of activity increases in these studies, as well as findings of identifiable regions of decreases (inferior temporal cortex⁴⁹ and hippocampus⁴⁸).

In summary, comparability between these studies is difficult owing to numerous methodological differences and a high degree of individual variability that exists in both older controls and subtly impaired populations. Although an unequivocal conclusion is not yet possible, there is an accumulation of evidence that the earliest detectable changes may be regional compensatory activity increases, followed by decreases in activity as regions become increasingly damaged by AD pathology. The need for longitudinal fMRI studies with large numbers of subjects is necessary to confirm these inconsistent findings.

Alternative fMRI approaches

Not all fMRI studies focus on comparing areas of activation during a cognitive task. Two alternative fMRI approaches have been utilized to investigate brain differences between AD and normal aging: mapping deactivation patterns across the whole brain and mapping basal oxygen metabolism within

the hippocampal formation. The former relies on the presence of regional deactivations identified during cognitive tasks in young subjects. These regions of deactivation are consistently located in the posterior cingulate cortex, ventral anterior cingulate cortex, and inferior parietal cortex, and reflect greater activity during a rest period than during a task period (the rest period is often a baseline in fMRI cognitive experiments). These regions that are most active during rest have been proposed to constitute a 'default-mode network' involved in monitoring internal states that is suspended during goal-directed behavior.⁵⁰ Two studies have investigated alterations in the default-mode network in early AD. Greicius et al⁵¹ used independent component analysis to reveal decreased resting-state activity in the posterior cingulate cortex and hippocampus, which they interpreted as a reflection of disrupted connectivity between these two regions. Lustig et al⁵² revealed a failure of deactivation in AD, such that the posterior cingulate cortex decreased in activity in young adults soon after the onset of a semantic classification task, but remained active in AD patients. It is still unclear, however, how these changes in deactivation in the posterior cingulate relate to reductions in resting metabolism in the same region, a hallmark of AD patients studied with PET/SPECT.¹⁵

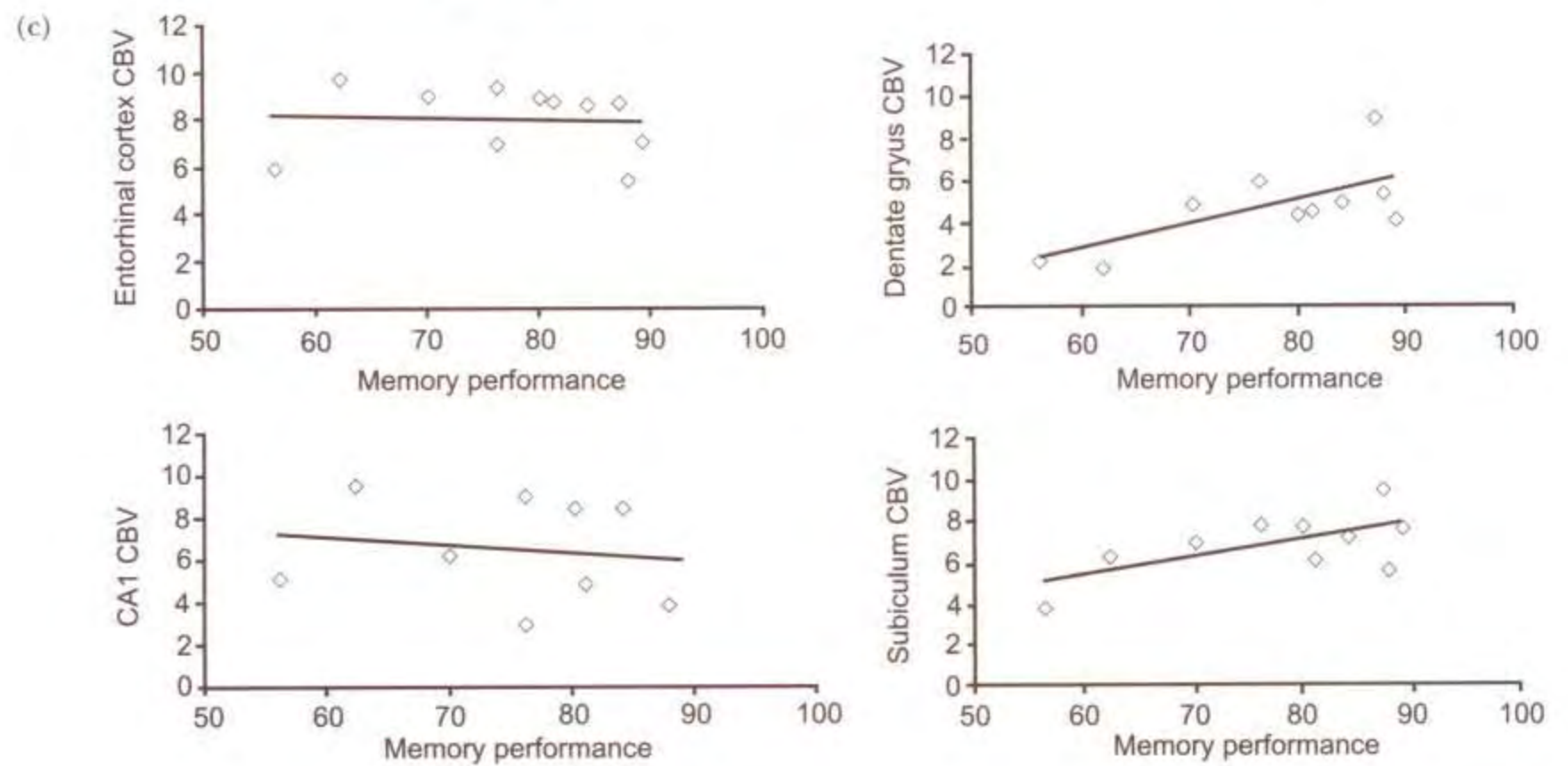
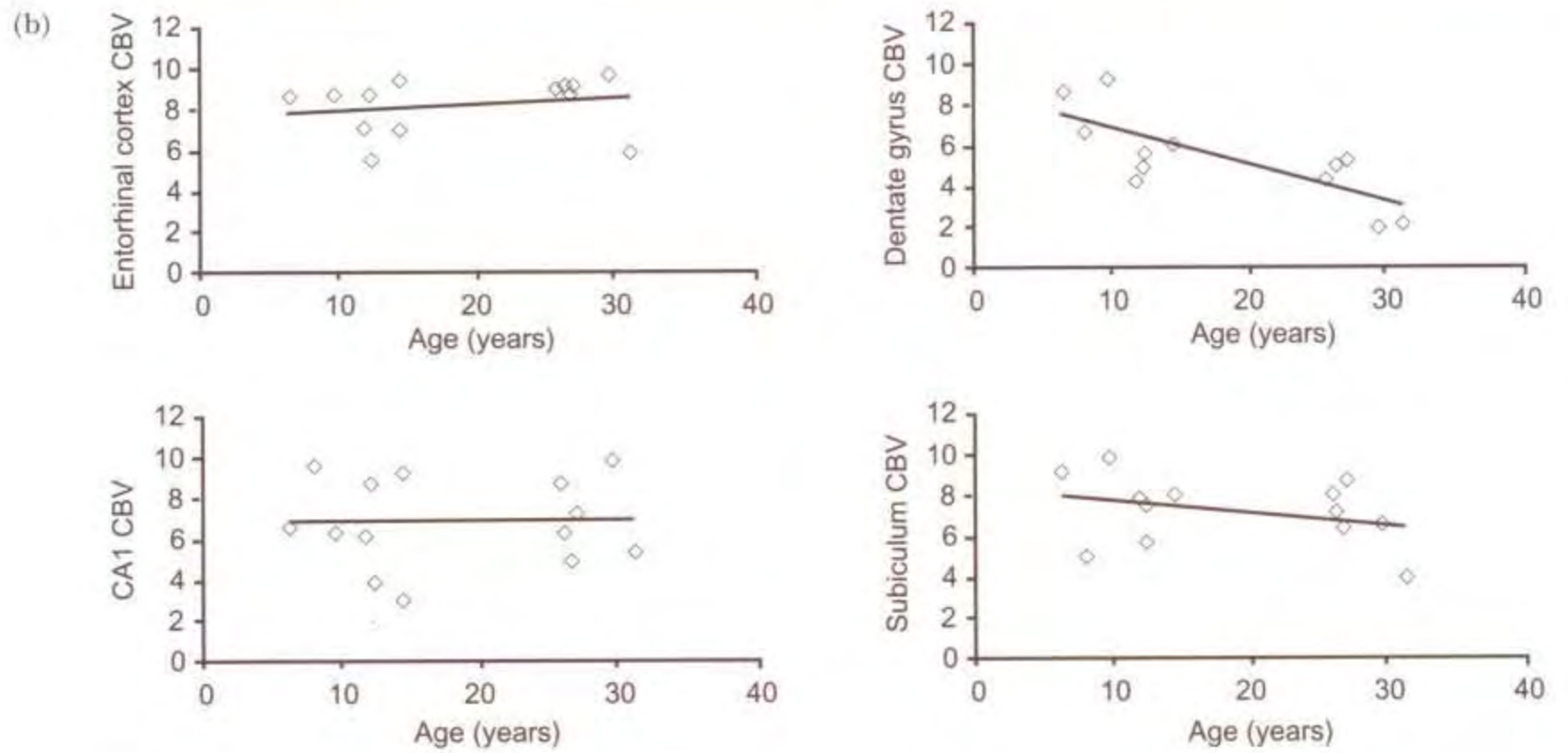
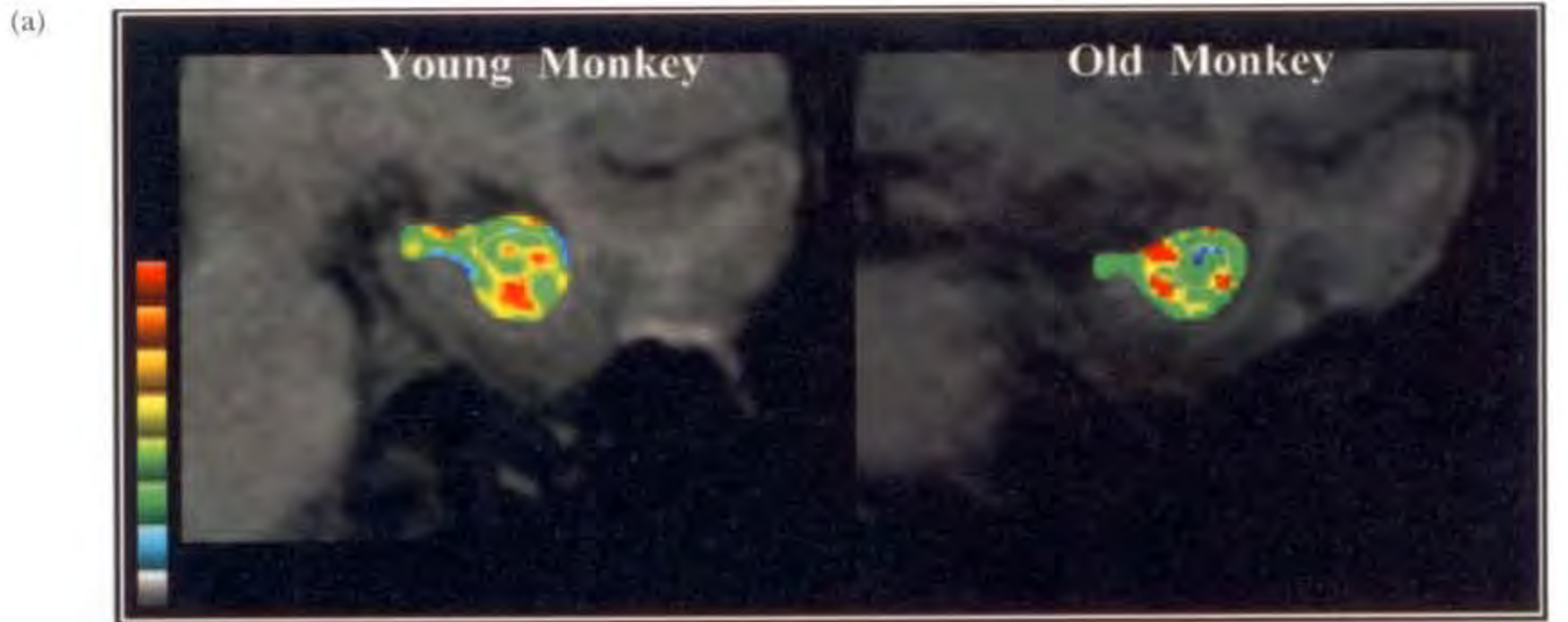
Using fMRI to investigate patterns of basal metabolism within a singular structure – the hippocampal formation – is a second approach that dissociates AD from normal aging. The hippocampus is a complex structure organized into separate but interconnected subregions: the entorhinal cortex, the dentate gyrus, the CA subfields, and the subiculum.^{53,54} Each hippocampus subregion houses a distinct population of neurons unique in their molecular expression profiles. It is this molecular uniqueness that accounts for why each hippocampal subregion is differentially targeted by mechanisms of dysfunction.²⁸ Thus, although both early AD and normal aging cause hippocampal dysfunction, they are predicted to target different

subregions of the hippocampal circuit. In order to test this prediction, an imaging technique requires submillimeter spatial resolution in order to visualize the diminutive hippocampal subregions.⁵⁵ Motivated by this need, a number of studies have relied on correlates of basal oxygen metabolism – either cerebral blood volume or deoxyhemoglobin content – to investigate the hippocampal circuit in AD and in aging.^{56,57} As evidenced by extensive PET and SPECT studies, almost any cause of brain dysfunction manifests as defects in basal metabolism (for reasons discussed in Chapter 1), and relying on the basal state allows a significant enhancement in spatial resolution.

Indeed, using these variants of fMRI, studies have shown that AD and normal aging target different hippocampal subregions:^{56,57} The entorhinal cortex is the hippocampal subregion most vulnerable to AD, while the dentate gyrus is relatively spared; in contrast, the dentate gyrus is most vulnerable to normal aging, while the entorhinal cortex is relatively spared (Figure 3.2). This anatomical double dissociation, and the ability to visualize it in living subjects, forms the basis of a large-scale epidemiological study, in which cerebral blood volume maps of the hippocampal formation will be generated in hundreds of healthy elders, who will then be followed prospectively. This study will test the prediction that healthy elders with entorhinal-predominant dysfunction are harboring the earliest stages of AD.

SPECIFICITY OF THE AD DIAGNOSIS

As a number of epidemiological studies have documented, the diagnostic sensitivity of AD, when presented with a demented patient, is quite high.⁵⁸ In fact, if we were to blindly assign the diagnosis of AD to every patient whose clinical evaluation suggests dementia, our sensitivity could well reach 100%. Our failure is reflected in poor diagnostic specificity – the ability to correctly diagnose the cause of dementia when presented with a



patient who has a non-AD etiology. The diseases that are incorrectly diagnosed as AD are typically within the general category of neurodegeneration, a list that includes dementia with Lewy bodies (DLB), frontotemporal lobe dementia (FTD), corticobasal ganglionic degeneration, progressive supranuclear palsy, Parkinson's disease with dementia, and prionopathies.⁵⁹ Imaging will improve our specificity, and our overall diagnostic accuracy, when it can positively diagnose both AD and non-AD causes of dementia.

One way to achieve the goal of increasing diagnostic specificity is to image the histological markers upon which neuropathologists rely to distinguish the neurodegenerative processes. In this regard, the field of *in vivo* imaging has entered an exciting new era, as evidenced by a couple of human PET studies^{60,61} and more recent mouse MRI studies,^{62–66} showing that amyloid plaques, one of the hallmarks of late-stage AD, can be detected in living subjects.

Relying on regional patterns of dysfunction is a second imaging approach that can distinguish between the neurodegenerative causes of dementia. This approach relies on a time-honored tenet in clinical neuroscience, which assumes that diseases will differentially target separate populations of neurons, and therefore separate regions of the brain. This view is partly supported by anatomical observations,

showing that in a differential manner AD targets the medial temporal lobes, FTD targets the prefrontal cortex, DLB targets the basal ganglia and the occipital lobes, and corticobasal ganglionic degeneration targets the basal ganglia and the posterior parietal lobes or the premotor cortex. By the time a neurodegenerative process causes dementia, it has spread to large areas of the brain, and for this reason spatial resolution is not really a consideration. Furthermore, most sources of signal are likely to capture regional patterns of dysfunction.

A single fMRI study has attempted to exploit these regional patterns of dysfunction to distinguish between early FTD and AD at a stage at which they are indistinguishable by gross cerebral atrophy.⁶⁷ The investigators utilized a working memory task, the verbal *n*-back task, and parametrically varied the information load that the patients experienced. The study revealed that, compared with AD patients, FTD patients showed significantly decreased frontal and parietal cortex activation and a reduced linear increase in activation with load in frontal regions. This study reveals the promise of using fMRI to distinguish between neurodegenerative diseases when structural MRI is not contributory.

MAPPING THE CLINICAL COURSE

Although we have entered the era of pharmacological intervention for AD, this era has just begun and we do not yet have truly effective therapeutics that alter the underlying disease process. Thanks to the insights gained into the molecular biology of AD, there are now many pharmacological agents under development. Accelerating the development of effective anti-AD drugs is the third way in which imaging will impact AD. The ability to longitudinally test drug efficacy in an affected individual is always a more powerful approach than cross-sectional comparisons. Longitudinal drug testing is particularly important when testing the effect of a drug on a slowly progressing disease – as is the case in AD. In general, most imaging approaches will likely prove useful in drug

Figure 3.2 Alzheimer's disease and normal aging cause brain dysfunction by affecting basal metabolic rates. Compared with BOLD, MRI correlates of basal oxygen metabolism are more quantitative and can be generated with higher spatial resolution. (a) Cerebral blood volume (CBV) is one of three correlates of basal oxygen metabolism that can be measured with MRI. CBV maps of the hippocampal formation are shown for a young and an old rhesus monkey. (b) CBV was estimated from individual hippocampal subregions in 14 rhesus monkeys covering the age span. Among all hippocampal subregions, the dentate gyrus was the hippocampal subregion most vulnerable to the aging process. (c) Age-related changes in memory best correlate with age-related changes in dentate gyrus CBV. Adapted from Small et al.⁶⁶

development. Of course, specific modalities might be better suited to a particular mechanism of action – for example PET imaging of amyloid plaques might be the modality of choice for testing an immunotherapy directed at reducing plaque load, and hemodynamic imaging might be the best modality for detecting changes in synaptic strength.

Several fMRI studies have explored the impact of cholinesterase inhibitor treatment, the main therapeutic intervention for AD, on the pattern of brain activity in patients with AD⁶⁸ and amnesic MCI.^{69,70} Rombouts et al⁶⁸ studied the effects of a single dose of rivastigmine on AD patients and revealed increases in activation in the bilateral fusiform gyrus during face encoding and in the prefrontal cortex during a working memory task. Two studies have investigated the impact of cholinesterase inhibitor treatment on MCI patients using different agents: galantamine⁷⁰ and donepezil.⁶⁹ Goekoop et al⁷⁰ revealed an increase in BOLD signal in multiple brain regions for both an episodic memory task and a working memory task after 5 days of galantamine treatment, rather than with a single dose. The study by Saykin et al⁶⁹ showed that frontal activity increased from levels recorded at the beginning of the study after 5 weeks of donepezil treatment compared with unmedicated, age-matched controls. This increase correlated with improved task performance. Although these studies are encouraging for the use of fMRI in pharmacological evaluation, there are several limitations that should be accounted for in future studies. For example, none of the studies utilized a placebo control or a method to evaluate the influence of increasing cholinergic levels on the vascular system in order to determine whether the BOLD changes truly reflected neural activity rather than vasodilatation.

CONCLUSIONS

The dovetailing of advances in pathophysiology and in imaging technology is moving us closer to achieving our clinical goals of AD detection, diagnosis, and drug development.

In fact, we have gained enough theoretical insight and technical sophistication that our current array of imaging tools are adequate to capture key elements of AD pathology. What is still lacking, in most cases, is empirical validation. As has been the case for structural MRI and functional tomography, fMRI studies will have to more conclusively establish the sensitivity and specificity for differentiating AD patients from healthy older individuals and the ability of fMRI to predict disease progression. Within the next few years, the neurobiological assumptions and the practical utility of fMRI will be rigorously tested – either by prospective epidemiological studies in humans or, more mechanistically, in animal models of disease.

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