Episodic memory on the path to Alzheimer’s disease
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This review is focused on specific circuits of the medial temporal lobe that have become better understood in recent years for their computational properties contributing to episodic memory and to memory impairment associated with aging and other risk for AD. The layer II neurons in the entorhinal cortex and their targets in the dentate gyrus and CA3 region of hippocampus comprise a system that rapidly encodes representations that are distinct from prior memories. Frank neuron loss in the entorhinal cortex is specific for AD, and related structural and functional changes across the network comprised of the entorhinal cortex and the dentate/CA3 regions hold promise for predicting progression on the path to AD.

Introduction
Disorders that impair mental capacities are among the most feared outcomes of aging. Dementia, with Alzheimer’s disease (AD) accounting for the vast majority of cases, exacts a staggering toll on individuals, families, and society. Much progress is being made in understanding the pathophysiology of AD, which ultimately causes massive neurodegeneration and a devastating loss of mental faculties. Still, the slow progression of AD runs its course over many years, and biomarkers relevant to the pathophysiology of the disease appear long before a clinical diagnosis. As recently reviewed elsewhere [1,2,3–5], new data coming from the Alzheimer’s Disease Neuroimaging Initiative and other sources support the existence of AD pathology in the brain for at least a decade before clinical diagnosis is typically made. Defining the earliest boundary of AD, when interventions could be most effective and beneficial, is a topic of great interest and yet a significant challenge.

Although the devastation of dementia is clinically unmistakable in the end, the earliest symptoms are barely noticeable to patients, families and physicians. Failures in the ability to remember events of daily living are easily dismissed as normal aging. In clinical practice, the diagnosis of AD is only made when such memory loss accelerates and other cognitive and behavioral symptoms emerge, reaching criteria for dementia as a progressive syndrome [6]. New criteria to define an earlier boundary for AD are under investigation for research purposes, combining evidence for the hallmark of episodic memory impairment with supportive biomarkers indicative of the pathophysiology or topography of AD in the brain [4,7–8]. Here, we focus on specific components of the neural circuitry essential for episodic memory that have become better understood for their computational properties and for how dysfunction in this network contributes to memory loss (see Figure 1). New opportunities for in vivo assessment of biomarkers within this circuitry may contribute to the set of measures that are clinically useful for early AD diagnosis and to assess disease modification in therapeutic trials.

Distinctive effects of early AD and aging on neurodegeneration in the medial temporal lobe
The structures of the medial temporal lobe (MTL) in the mammalian brain are crucial for memory functions that give us a record of our experience, in acquiring new facts and preserving information about the events in our lives, the latter commonly referred to as episodic memory. This system, comprised of the hippocampus and its interconnections with cortex, undergoes changes during aging in circuits that are highly susceptible to neurodegeneration in AD. Here, however, a clear distinction exists between the effects of aging and AD. We have known for awhile that by the time of AD diagnosis, marked neuronal loss has already occurred in the entorhinal cortex (EC), the brain’s interface between the hippocampal formation and neocortex. In their classic study using unbiased stereological methods, Gómez-Isla et al. [9] reported that brains from mild AD patients (with a Clinical Dementia Rating of 0.5) exhibited more than 50% loss of layer II entorhinal neurons, while numbers of those neurons were maintained in healthy aged brains. Since then, stereological studies have widely confirmed that numbers of neurons are largely preserved in the MTL system over the course of aging in humans and other species (e.g. primate and rodent) [10–14], while significant neuronal loss affecting EC is already evident in the earliest stages of clinically diagnosed AD [15,16]. These findings are consistent with structural changes in the volume of EC, not only over its
Role of the perforant path and its targets in episodic memory

The computational functions contributing to episodic memory have become better defined for subregions and circuits in the MTL. The input from layer II of EC to the DG and CA3 is especially crucial for encoding distinctive representations of experiences that share overlapping elements with prior memories, a process referred to as pattern separation. Consistent with earlier predictions based on its anatomy and physiology, studies in laboratory animals have confirmed that the ability of the hippocampus to assign distinct representations to similar inputs emerges in the DG and CA3 regions [31–34]. This feature of information encoding is due, at least partly, to the fact that relatively few dentate gyrus granule cells, among the very large number of granule cells, are activated by a pattern of cortical input.

The generality of this function has been extended from studies of the encoding properties of DG and CA3 neurons in rodents to a corresponding function in humans localized to DG/CA3 with high-resolution fMRI [35,36]. Moreover, studies of memory performance, both in animal models of aging and elderly human subjects, have shown substantially reduced pattern separation, with changes in behavioral performance coupled to altered function in the DG/CA3 network in the aged brain [37,38,39*,40–42,43*]. Relative to age-matched controls, a progressive worsening of this condition is detected in amnestic mild cognitive impairment (aMCI) patients [44*], who have memory impairment greater than would be expected for their age and who are at increased risk for AD. Thus, dysfunction in this subsystem of the MTL appears to make a significant contribution to the clinical condition affecting memory on the path to AD. Although task-related activation using fMRI has potential limitations as a biomarker, functional connectivity measures in resting state fMRI may be more amenable to clinical applications ([45*], but see [46]) and can be used to assess functional connectivity in the EC/DG/CA3 circuit [47*], complementing structural approaches using DTI and tractography as described above.

Apart from a distinctive effect of AD on frank degeneration of EC neurons, aging itself could confer vulnerability to AD in its effects on those neurons most susceptible to degeneration in the disease. Aged rats that do not suffer from neuron loss in the MTL nonetheless have a decrease in the synaptic input from EC specifically affecting the layer II connections in animals that age with memory loss [48]. Together with a weakened perforant path input, a condition of excess activity associated with the CA3 neurons in those animals shifts the EC/DG/CA3 network from pattern separation to pattern completion, which is mediated by CA3 recurrent associative connections [40]. Thus, rather than creating

 clinical course but also on the path to AD before diagnosis [17,18*,19,20]. Still, the overall atrophy of a region is likely to underestimate the magnitude of degeneration that affects a specific subset of neurons. As expected, a structural measure of cortical thinning, which could reflect layer-specific neuron loss in EC, shows greater sensitivity in structural MRI compared to differences in the overall volume of EC [21,22**,23,24*,25**].

Given the evidence just described, the use of improved methods in diffusion tensor imaging (DTI) and tractography applied to the perforant path would seem to hold promise as an early biomarker with great specificity for AD. Because the perforant path, which innervates the dentate gyrus (DG) and CA3 region of the hippocampus, is comprised of the projections from layer II entorhinal neurons, measuring the integrity of those connections could have particular sensitivity for the earliest neurodegeneration in AD. Ex vivo imaging of this pathway in the human brain was recently conducted to validate tractography methods with histological procedures [26*]. In another recent application of in vivo microstructural DTI, imaging of the perforant path provided evidence of decreased integrity in older subjects compared to young adults [27**]. Notably, as a control, the temporo-occipital path, showed no corresponding difference between the age groups, suggesting a circuit-specific basis for signal change. To the extent that such methods prove sensitive to neurodegeneration in EC, a biomarker based on perforant path integrity might perform even better than structural measures of EC itself. Moreover, the specific neurons that give rise to this pathway are closely tied to the pathophysiology of AD in both descriptive studies of AD brains and experimental research [15,28,29*,30*].
distinctive representations, CA3 neurons are more likely to retrieve previously encoded information. This condition of network dysfunction is consistent with behavioral and fMRI data in humans with age-related memory impairment. In behavioral tasks that tax pattern separation, errors in memory judgments exhibit a shift from responses indicative of good pattern separation to greater pattern completion [38,39], and during task performance, fMRI reveals excess activation in the MTL that is restricted to the DG/CA3 region [43]. Such observations made in aged individuals are also further magnified in aMCI [44].

Some of the features described above resemble conditions that can be caused or augmented by the pathophysiology of AD [49]. Consistent with the view that synaptic failure precedes neuronal loss in the degenerative cascade of AD brain, degradation of perforant path connections (from layer II EC neurons) occurs in the brains of AD mouse models overexpressing amyloid precursor protein (APP) [50,51]. Excess hippocampal excitability has also been observed in APP transgenic mice with changes in many molecular markers affecting DG/CA3 circuitry [52]. To the extent that greater hippocampal activation is not compensatory in a beneficial sense, there is growing concern that excitation in the brain, particularly in the hippocampus, could be a driver on the path to AD [53,54,55,56].

**Apolipoprotein E (apoE) and vulnerability in the EC/DG/CA3 network**

Apart from aging itself, apoE is the most important known genetic risk factor for sporadic AD, with the apoE4 allele conferring dramatically increased risk in a dose-dependent manner. A memory/MTL phenotype is characteristic of apoE4 carriers with AD [57]. Studies of asymptomatic apoE4 carriers also report alterations in the EC/DG/CA3 network before an AD diagnosis. In comparison to older heterozygote carriers and non-carriers, ε4 homozygote carriers have greater deficits in episodic memory tasks [58], and ε4 carrier status is associated with greater longitudinal decline in memory performance evident by the sixth decade of life [59]. Consistent with data on memory performance, apoE4 carriers exhibit reduced structural measures of EC, both in cross-sectional comparisons and longitudinal assessments [60,61,62]. It is possible that this morphometric change reflects neuronal degeneration in EC, which is uncharacteristic of normal aging, and instead could represent a signature of prodromal AD in affected individuals.

Another finding described in many studies of ApoE4 carriers is increased fMRI activation in the hippocampus [63–66], a phenomenon that has often been interpreted as compensatory recruitment to support memory performance. In contrast with that view, recent research has demonstrated a detrimental loss of hippocampal inhibitory function in animal models used to study ApoE4. Andrews-Zwilling et al. [67] report that ApoE4 causes age-dependent and tau-dependent impairment of GABAergic neurons in the hilus of the hippocampal formation and that this loss of inhibitory function underlies learning and memory deficits both in ApoE4 knock-in mice and in transgenic mice producing neurotoxic fragments of ApoE4. Contrary to the notion that increased hippocampal activation is beneficial, as discussed in reference to fMRI findings, memory was improved by treatment with the GABA_A receptor potentiator, pentobarbital, in ApoE4 mouse models. Similar to the benefit reported in these ApoE models, improvements have been obtained in memory-impaired aged rats by targeting hippocampal overactivity associated with neurocognitive aging [68].

In the context of the hippocampal subsystem considered here, it is particularly noteworthy that the impact of ApoE4 on inhibitory neurons in mice models was regionally restricted within the hippocampal formation, with an effect observed on interneurons in the hilus that was not seen in CA1. In a recent report examining morphometry within subregions of the hippocampus, ApoE4 carrier status during aging was specifically associated with volume loss in the CA3/DG relative to non-carriers [69,70]. The prominent effect on EC and its targets in ApoE4 carriers could not only account for greater episodic memory impairment but also set a background contributing to AD vulnerability.

**Conclusion**

The ability to make an earlier diagnosis of AD will probably depend on a set of measures that together provide high discrimination for the disease and are clearly relevant to the clinical condition of those on the path to dementia. Memory impairment is a recognized hallmark of AD that shows progressive worsening and has undisputed clinical significance for patients. Here, we have focused on specific components of MTL circuitry that have become better understood in recent years for their computational properties contributing to episodic memory. Although aging affects the condition of this system apart from AD, EC is a site of neurodegeneration that does not occur in healthy aged brains. In addition, structural and functional measures of the EC/DG/CA3 network register a progressive change on the path to AD, yielding biomarkers topographically relevant to the core clinical presentation of memory loss that could be suited for clinical trials of therapies aimed at modifying disease progression.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest


This review article from the AD Neuroimaging Initiative (ADNI) provides an update of current progress as well as plans to include recruitment of a new cohort of early MCI with milder episodic memory impairment to study the earliest stages of AD.


Report of the working group charged by the National Institute on Aging and Alzheimer’s Association with revising the 1984 criteria (referred to as the NINCDS-ADRDA criteria) for AD dementia. Use of biomarker data increases confidence for a diagnosis of probable dementia of the Alzheimer’s type.


An international working group proposes clinical and biological criteria to encompass the full spectrum of progression in AD focused on the clinical feature of episodic memory loss and supportive biomarkers.


Progression from MCI to AD diagnosis over a three year span was examined in two cohorts using automated structural MRI tools and comparing those results to ones obtained using cerebrospinal fluid (CSF) data and positron-emission tomography (PET) measures of brain metabolic activity. In a combined model that included individual CSF, PET, and MRI measures that best predicted disease progression, the MTL MRI factor outperformed cellular and metabolic measures to predict temporal progression of disease.


The authors report that thinning of EC and inferior temporal gyrus in MCI and AD individuals predicted longitudinal hippocampal volume loss in individuals positive for pathophysiological biomarkers of amyloid and tau.


This study tested the accuracy of potential biomarkers, including MRI, CSF, and neuropsychological tests in predicting AD. On the prediction of true conversion from MCI, models with a maximum of 4 predictors performed marginally (and non-significantly) better than a number of single predictors, including right EC thickness, which was the top ranked single predictor. The results confirm an independent contribution of different biomarkers to the prediction of AD, but that in a follow up of up to 3.3 years, current biomarker models still have limited predictive accuracy.

25. Holland D, McEvoy LK, Dale AM: Alzheimer’s Disease

- Neuroimaging Initiative: unbiased comparison of sample size estimates from longitudinal structural measures in ADNI. Hum Brain Mapp 2011, in press [epub ahead of print].

This is a survey of the ADNI database using structural brain scans to estimate longitudinal change and sample sizes needed to detect such change. Of all the measures publicly available in ADNI, entorhinal cortical thinning quantified by the Quaco method proved to be the most powerful change biomarker in both MCI and AD patients.

The authors applied structural and diffusion imaging on the perforant path and importantly validated those measurements histologically, providing the necessary evidence to support the accuracy and reliability of the structural imaging data.


The authors used microstructural diffusion imaging to measure the integrity of the perforant path projecting from layer II of the EC to the hippocampus. By this method, older adults showed degradation when compared to young adults, and measures among the individuals in the aged group correlated with delayed recall performance in verbal memory.


The authors showed that EphB2 receptors in AD-related synaptic dysfunction in the DG, reporting similar impairment in hAPP mice and mice with experimentally reduced EphB2. Increasing EphB2 levels in the DG of hAPP mice rescued DG synaptic function/plasticity and improved performance in hippocampal-dependent tasks. The results noted a novel therapeutic target but also demonstrate that a relatively localized remediation of DG function in the hAPP model has significant behavioral benefit.


Selective overexpression of mutant APP targeting layers II/III of EC in mice led to Aβ deposition and downstream neuropathological abnormalities in the hippocampal formation, consistent with the EC as a key locus affected early in the disease process to induce hippocampal network dysfunction.


In a continuous recognition memory task, nondemented older adults did not differ from young subjects in judgments for items that were ‘new’ (first presentation) or ‘old’ (repeated presentation), but had reduced accuracy for items that were ‘similar’ to those previously presented. Instead of making the accurate ‘similar’ judgment, older adults were more likely than young to identify such items as ‘old’, a profile consistent with a loss of pattern separation.


Healthy older adults show impaired memory performance in a task that was designed to tax pattern separation. Importantly, there was no age difference for crucial test materials that varied in perceptual similarity. When those materials were used in fMRI, impaired pattern separation performance was found to be associated with increased fMRI activation in the DG/CA3 region, suggesting that dysfunction in that subregion of the hippocampus affects memory processes in the aged brain.


Individuals with MCI show impaired performance in a pattern separation task, and a corresponding increase in DG/CA3 activity compared to normal age-matched controls. The EC, by contrast, showed decreased activation during task performance.


This study shows the possibility of using functional connectome, which is made up of resting state fMRI signal fluctuations that are correlated across neural networks, to serve as a biomarker for pathological processes in the brain, such as in AD.


Reliability of task-related fMRI in individuals who were cognitively normal or impaired was assessed over a 4-6 week repeated scanning interval. A reliable signal response was found across both block and event-related designs in the hippocampus, but task-related deactivation in the precuneus demonstrated greater variability.


This work combined multimodal structural and functional methodologies to identify changes in the hippocampal network that can be used to predict age-related memory performance. As stimulus similarity was incrementally varied, fMRI signals in the DG/CA3 were linked to loss of pattern separation performance in older adults with young adults showing better behavioral performance and more comparable activations across the range of stimulus similarity. Among the aged subjects, the fMRI data were also correlated with worse recall performance. Moreover, among the aged subjects this condition in the CA3/DG was significantly correlated with DTI measures indicating loss of perforant path integrity.


The authors place AD mouse models in the asymptomatic phase of AD. They review evidence that these models possess signatures of synaptic dysfunction that are also characteristic of brain aging, which could blur the boundary between aging and asymptomatic AD in man. An important conclusion, however, is that interventions in these models should be...
considered in the context of disease modification on the path to AD rather than treatments that would be effective in the disease as it is currently clinically diagnosed.


The authors studied normal and mildly impaired individuals over a 2 year period. Among those with mild impairment, initial hippocampal activation in fMRI at baseline predicted both subsequent loss of such activation and the most rapid cognitive decline, suggesting that hippocampal hyper-activation may be an aberrant condition rather than serving a beneficial compensatory function.


Presymptomatic carriers of presenilin-1 mutation showed increased activation in the hippocampus during a memory encoding task; these changes in hippocampal fMRI patterns, which preceded cognitive decline, may be useful as a preclinical biomarker for AD.


The authors compared the disease phenotypes in ApoE4 carriers and non-carriers with diagnosed AD, reporting that e4 carrier status modulates both the cognitive and neuroanatomic phenotype of AD. This important study of mild AD patients, all with CSF molecular profiles consistent with AD, showed dissociations in performance on neuropsychological assessments and structural MRI. Carriers had greater impairment on memory measures of MTL function with corresponding greater atrophy in those cortical areas, while non-carriers had greater impairment on non-memory measures with greater cortical atrophy outside the MTL.


Carriers with and without an apoE4 allele were followed for approximately 5 years, with the former showing greater acceleration in memory decline by an age of 60 years.


Asymptomatic ApoE4 carriers and non-carriers at approximately 60 years of age had high-resolution MRI scans repeated at a two-year interval. In a cortical unfolding analysis, ApoE4 carriers showed a significant loss of cortical thickness in the subiculum and EC relative to non-carriers. No change in cognitive performance was detected in either group over the two-year test interval.


An age-dependent reduction in glutamic acid decarboxylase-positive neurons in the hilus in ApoE4 relative to ApoE3 knock-in mice at 16 mo of age was associated with a substantial reduction in GABAergic synaptic function. Further studies in the ApoE4 knock-in model and a model with ApoE4 toxic cleavage fragments provide correlational and mechanistic support for behavioral impairment tied to the status of hippocampal inhibitory neurons in the hilus. The results point to a specific localized vulnerability in the MTL to ApoE4.

