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Neural and Cognitive Substrates of Alzheimer's Disease-Related Navigation Deficits

by

Amy deIpolyi

DISSERTATION

Submitted in partial satisfaction of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Neuroscience Graduate Program

in the

GRADUATE DIVISION

of the

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO

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Dedication and Acknowledgments

First and foremost, I would like to thank my mentor, Dr. Lennart Mucke, for his guidance and perseverance over the last three years. Dr. Mucke has shown me how I might be a better scientist and a better person. He spent countless hours working with me and advising me even when he was at his busiest. I could always count on his honesty, which I have found to be an extremely rare and precious trait in science and medicine. He never failed to act with my best interest at heart and delivered invaluable constructive feedback. I am indebted to him for his energy and dedication to me and lack adequate words to express my appreciation.

I would also like to thank my co-mentor, Dr. Bruce Miller, for his support and encouragement. When I was searching for a thesis project, Dr. Miller provided the resources, connections, and environment I needed to do the kind of research I wanted to do. He set up my co-mentorship with Dr. Mucke and made it possible for me to conduct human research in the Memory and Aging Center. Dr. Miller always looked for ways to support me. I have always admired his leadership and insight and feel privileged to have been part of his group.

Dr. Jennifer LaVail served as my committee chair and has been a solid rock of support ever since I met her five years ago. She met with me countless times to discuss science and even more personal matters. Dr. LaVail was sympathetic when I needed encouragement, and honest when I deserved critical feedback. She led my large and diverse thesis committee with an assurance than inspired optimism in the other committee members that in turn renewed my own confidence. I am so thankful that I happened to meet her during medical school so that I had an opportunity to work with her for so long.

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I would also like to thank the other members of my thesis committee who have helped shape my dissertation. Dr. Loren Frank was an obvious choice for my committee, with his expertise in spatial navigation. Fortunately for me, he was willing to be on the committee and, in fact, agreed to it immediately when I asked him. He has contributed so much to my thinking about spatial cognition and made numerous suggestions that helped formulate more effective tests of navigation in both humans and mice.

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Dr. Carol Barnes agreed to be part of my thesis committee when I essentially ambushed her in a small meeting we had when she was visiting the Gladstone Institute. In the few minutes I spent with her in that meeting, I was immediately certain that I wanted to work with her, even if only for a short time from afar. I greatly appreciate that she agreed to help on such short notice, as I admire her as a scientist and leader in neuroscience.

Dr. Maria Luisa Gorno-Tempini served as an adjunct thesis committee member. When I joined the Memory and Aging Center nearly four years ago, I identified Dr. Gorno Tempini as the person I wanted to work with on my human project. I have always respected her as a scientist and knew that I could learn a great deal about how to study human cognition from her. When one of my committee members could not make a thesis committee meeting, Dr. Gorno Tempini stepped up and joined the group, providing

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invaluable expertise and suggestions. She taught me essential experimental techniques and has worked closely with me to improve the analysis and interpretation of the human data. She always makes time for me, even if it means meeting late into the night. I am so thankful that she agreed to work with me.

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and was so lucky when she had time to help me. I am indebted to these members of the Mucke Lab for their support and contributions.

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I thank the members of the Mucke Laboratory and Gladstone Institute that made my time in graduate school as positive and fun as it could be. I thank Kimberly Scearce-Levie and Erik Roberson for their advice and input; I often asked for their opinion on how to improve my science and to interpret data and they were always insightful and helpful. I thank Jeannie Chin and Luke Esposito for teaching me many experimental skills and helping me find things in lab; they answered all of my questions, even after I had been a member of the lab for two years. I thank Catherine Massaro and Jason Cheng for countless discussions over lunch, coffee, and late-night experiments – discussions that only fellow students could have. In addition, I am lucky that there were so many students in the Finkbeiner Lab, who reminded me every day that I was in graduate school in addition to working in a research institute.

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love of learning and of doing well. I also thank my brother, Jeff, for his love and his trust in me. I know Jeff will fight for me whenever he has a chance.

My mother and father are my indefatigable heroes, and I dedicate this thesis to them. They were always the first people I would call when something went wrong or right. They always took my side and helped me to see the brighter side of situations. They have always believed in me, especially when it seemed like no one else did, including myself. They take pleasure in making me happy and always look for ways to do so. Whatever I need, they have always provided. Without them, I would be lost. I am truly the luckiest daughter in the world.

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Neural and Cognitive Substrates of Alzheimer's Disease-Related Navigation Deficits

Amy deIpolyi

Abstract

Navigation impairments are common among Alzheimer's disease (AD) patients and have a devastating impact on daily functioning. To understand the neural and cognitive bases for these impairments, I studied spatial disability in human patients and a transgenic mouse model of AD. Mice were tested with a specialized maze that distinguished allocentric (world-based) hippocampus-dependent strategies from egocentric (self-based) striatum-dependent strategies. I also measured AD-related pathology in the hippocampus and striatum of these mice. Whereas almost all nontransgenic mice used allocentric strategies, more than half of transgenic mice engaged egocentric strategies. In transgenic mice, the hippocampus had significantly lower levels of synaptic activity-dependent proteins and higher levels of amyloid-β (Aβ) than the striatum, which was relatively spared, paralleling the strategy alterations. Transgenic mice that used hippocampus-independent strategies learned better than those that persistent engaged the hippocampus. Therefore, flexibility in selecting learning strategies may impact the severity of navigation deficits in AD. Encouraging transgenic mice and potentially AD patients to engage hippocampus-independent strategies could alleviate some spatial disability. To study humans, I used a route-learning task to test mild AD and mild cognitive impairment (MCI) patients, and voxel-based morphometry to assess structural changes in brain regions critical for human navigation. AD and MCI patients recalled having seen landmarks, but not when or where they saw them. About half of the patients got lost on

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the route, though traditional measures of disease severity did not predict who got lost. Patients who got lost had significant atrophy in putative neural components of the human navigation network, including the right posterior hippocampus. Thus, distinct patterns of atrophy may yield specific cognitive deficits. Knowing what neural regions are damaged by disease may predict which patients are at risk for navigational disability. In summary, parallel investigations in transgenic mice and human patients suggested a central role of hippocampal dysfunction in AD-related spatial disability and elucidated mechanisms that could be potential therapeutic targets.

Jennifer LaVail, Ph.D., Committee Chair

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Chapter 1. Introduction

Alzheimer's disease (AD) is a debilitating neurodegenerative illness characterized by severe cognitive impairment [3]. AD patients commonly complain of spatial disability and getting lost in new and, as the disease progresses, familiar environments. The purpose of my thesis work was to determine which of the multiple processes underlying spatial cognition contribute to spatial disability in AD by studying both human patients and AD mouse models, and to investigate neural correlates by comparing behavioral measures with structural and biochemical changes. My central hypothesis was that particular cognitive mechanisms underlying spatial impairment in AD can be isolated behaviorally and related to specific patterns of neuropathology. My specific aims included:

***Specific Aim 1*: Determine the cognitive deficits underlying spatial disability in genetic mouse models of AD and the biochemical correlates of those deficits.**

a. Determine how the use of different spatial strategies relates to navigation impairment in AD mouse models.
b. Relate biochemical and histological changes in different brain regions to specific cognitive deficits in mouse models of AD.

***Specific Aim 2*: Determine the cognitive and neuroanatomical variables that distinguish AD patients with navigation deficits from patients without navigation deficits.**

a. Assess spatial cognitive ability in AD patients with a real-life navigation test.
b. Relate atrophy of various brain structures to spatial deficits in human patients.

AD is the most common cause of dementia, affecting 3% of people 65–74 years old, 19% of people 75–84, and 47% of people over 85 [54,77]. AD causes severe cognitive and behavioral deficits [3]. One common complaint is spatial disability [125], affecting at

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least 39% of AD patients [78]. This spatial disability has a profound effect on daily activity [156]. Understanding how pathological changes in the brains of AD patients relate to spatial impairment may allow us to predict the kinds of behavioral deficits patients will likely have, to generate behavioral outcome measures that can be followed before and after prospective treatments, and to develop successful behavioral therapies.

The purpose of this dissertation project is to elucidate the specific cognitive mechanisms and neural substrates in human patients and in a mouse model. To develop effective and robust measures of AD-related spatial impairments, I have applied concepts from previous work on human and animal navigation. In both humans and mice, I focused on specific brain regions that are critical for navigation and used or adapted navigation tests that had been applied to other questions. In this introduction I will first define AD and mild cognitive impairment (MCI). I will then review what is already known about the neural and cognitive mechanisms thought to underlie navigation and the behavioral tests of these mechanisms, to justify the tests that I used. I will also discuss what is already known about navigation in mouse models and human patients and conclude with a description of outstanding questions related to AD-related navigation impairment.

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The Diagnosis of AD and Mild Cognitive Impairment (MCI)

To isolate spatial deficits from other types of cognitive deficits, I studied mild AD and mild cognitive impairment (MCI) patients. AD is characterized by progressive cognitive decline together with a deterioration of activities of daily living. The earliest symptoms involve short-term memory loss. As the disease progresses, impairments

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extend to other cognitive domains, such as language, attention, and frontal executive function (decision-making and planning) [3]. The core diagnostic features of probable AD, all of which must be satisfied, include: 1) dementia (progressive cognitive decline, beyond what might be expected from normal aging, that interferes with daily functioning), established by clinical examination including neuropsychological examination; 2) deficits in memory and at least one other cognitive domain; 3) gradual onset after age 40 with gradual progression; and, 4) cognitive deficits are not accounted for by other systemic disorders or brain diseases [125]. Mild AD refers to patients who are functionally impaired, but do not perform as poorly on cognitive tests as patients with more advanced disease.

MCI is considered to be a transitional state from normal aging to AD, in that the great majority of MCI patients develop AD within 6 years [157]. The key difference between AD and MCI is that MCI patients are not functionally impaired. All of the following criteria must be met for the diagnosis of MCI: 1) memory complaint; 2) objective memory impairment (e.g., performance on a delayed memory test that is at least one standard deviation below the mean for the subject's age and education); 3) intact activities of daily living; 4) being relatively normal on other cognitive tasks; 5) and, not being demented [158].

Typically, studies of AD and MCI include cognitively normal age-matched controls. However, the concept of "normal aging" is complicated by the cognitive changes known to occur in elderly subjects. Numerous studies have shown that elderly people experience decline in behaviors thought to depend on the hippocampus and prefrontal cortex [26]. Though some have suggested that normal aging and AD are part of a continuum with the

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same underlying process [22], evidence suggests that the changes induced by normal aging are distinct from those characteristic of AD and MCI. Age-related cognitive decline is more likely due to impairments of neural plasticity, including alterations of dendritic morphology, disrupted cellular connectivity, and changes in calcium regulation and gene expression [26], rather than the extensive cell loss characteristic of AD [66]. Regional patterns of atrophy are also thought to be qualitatively different, with normal aging associated with more frontal atrophy, and AD and MCI associated with greater medial temporal atrophy [76,145]. Furthermore, MCI patients are far more likely to develop AD than normal elderly subjects [157]. Healthy elderly subjects are impaired in navigation tests [26,131,132], though the differences in spatial learning between normal elderly subjects and AD patients are far more pronounced than the differences between normal elderly subjects and young controls [133].

The Spatial Navigation Network

To investigate the neural substrates of AD-related spatial impairment, I focused on a network of brain structures thought to be critical for different aspects of navigation. For the mouse study, I concentrated on the hippocampus and the striatum. These regions are thought to play complementary roles in navigation, and while the hippocampus is affected severely by AD, the striatum is relatively spared. When assessing human AD patients, I focused on the possible contribution of the hippocampus, parahippocampal gyrus, and parietal cortex to navigation deficits because of their putative roles in human spatial cognition and because they are affected early in AD, with medial temporal structures affected earliest, parietal cortex next, and frontal cortices much later in the

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disease [97,181,191]. Before describing how navigation is tested, I will describe the roles that these regions are thought to play in spatial cognition.

The hippocampus

The study of spatial navigation arguably began with the hypothesis formulated by Tolman in 1948 that rats have "cognitive maps" of the environment, which was based on the observation that rats could devise short-cuts in well-learned mazes [195]. For several years after the discovery by Scoville and Milner in 1957 that lesions of the medial temporal lobe in humans caused memory dysfunction [182], the possible role of the hippocampal formation in spatial memory was the focus of research. The discovery of hippocampal place cells in 1971 [142] led to the hypothesis by O'Keefe and Nadel in 1978 that the hippocampus houses the cognitive map [143]. This hypothesis has inspired a vast body of work both with lesion models and electrophysiological recordings that suggest a central role of the hippocampus in spatial mapping.

Hippocampal place cells have been important models for our understanding of the precise roles the hippocampus may play in navigation. Place cells are pyramidal cells, primarily in the dorsal CA1 and CA3 subregions and also in the dentate gyrus (DG), that fire selectively when the animal is in a particular place [139]. While distal cues control the firing of place cells, local intramaze cues exert little or no influence [14,41,163]. In the rat, place cell firing is often controlled by visual cues [162]. Place fields are not disturbed by turning off the lights, implying that idiothetic cues can serve to maintain place fields, though stable distal visual cues may be used to form them initially [14]. Although most studies of place cells have been conducted in the rat, some primate cells are better characterized as "spatial view cells," firing when the monkey looks at a

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particular place as opposed to actually being there [172,173]. More recently, an intra-operative recording experiment of a human neurosurgical patient navigating a virtual town showed that humans also have hippocampal place cells and spatial view cells [51]. One caveat of the human and nonhuman primate data, however, is that the subjects were not freely moving; restraint can dramatically alter place fields. For example, place cell firing characteristics differ between situations in which a rat is freely ambulating and situations in which a rat is being moved within a car, even if the rat controls the car's movement [193]. Because primate studies are not conducted under unrestricted conditions, primate and rodent place cells are only loosely comparable.

Hippocampal lesion studies have complemented the electrophysiological characterization of place cells and have shown that hippocampal lesions disrupt the use of distal cues when navigating, but not the use of local cues or beacons [135,155]. The hippocampus is, therefore, critical for allocentric spatial mapping, incorporating geometric features of stable environmental landmarks into a map. Furthermore, the dorsal part of the hippocampus, compared to the ventral aspect, in rats is critical for navigation, suggesting a gradient of function [137], though the role of the ventral hippocampus is not well understood. The dorsal hippocampus has higher-resolution spatial scaling than the ventral hippocampus [119], which may be important for affective processes in navigation, including reward processing [174].

In addition to allocentric mapping, the hippocampus is also required for the use of allocentric strategies when confronting navigation problems. For example, in tasks that can be solved by either world-based allocentric strategies or self-based egocentric strategies, hippocampal inactivation in the rat prevents the use of allocentric strategies,

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whereas hippocampal activation with glutamate infusion promotes the use of allocentric strategies [147,149].

Human studies of the hippocampus primarily include assessment of neurological patients and functional imaging experiments in subjects navigating through virtual environments or recalling routes from real-life places. Functional magnetic resonance imaging (fMRI) studies have consistently shown that the hippocampus is activated during navigation and that activation is correlated with good spatial performance [25,110,111]. Furthermore, human subjects that engage allocentric strategies to solve a virtual maze have greater activation in the hippocampus than subjects who use egocentric strategies, a result that parallels studies in rodents [86]. Studies of human patients with hippocampal lesions have shown selective deficits in allocentric rather than egocentric spatial processing [25,101,185]. Activation studies have also revealed robust activation of the posterior aspect of the right hippocampus when navigating through virtual environments, in contrast left hippocampal activation during verbal episodic memory tasks, suggesting that the right hippocampus plays a greater role in navigation than the left hippocampus [25,101,185]. Furthermore, the right posterior hippocampus is larger in London taxi cab drivers than in people without navigation expertise, and this enlargement correlates with the number of years spent on the job [114,115]. Though there is little evidence for left-right lateralization of hippocampal function in rodents, the posterior hippocampus of humans may correspond to the dorsal aspect of the hippocampus in rodents with respect to its critical role in spatial navigation.

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The parahippocampal gyrus

Some controversy exists as to whether the locus of navigation in humans is truly the hippocampus proper, with some arguing that the parahippocampal gyrus (PHG), particularly on the right, may be even more important in people [1,175]. In support of this view, lesions sparing the hippocampus proper but affecting the PHG seem to result in profound navigation impairments [11,18,73,175]. However, whereas the hippocampus plays a critical role in complex cognitive mapping (i.e., encoding interrelations between numerous landmarks), the PHG seems to be more involved in object-location associations, recalling landmarks (but not complex routes), object location in spatial arrays, and passive processing of scenes [111]. In line with this notion, the PHG is activated only when the environment is rich with landmarks, but not when navigating through empty rooms and corridors [113]. These data suggest that the hippocampus proper and the PHG play important roles in navigating that are distinct but may partly overlap; damage to either region can result in navigation impairment.

The parietal cortex

Whereas the hippocampus is critically involved in allocentric spatial mapping, the parietal cortex appears to be more important for egocentric processes and the translation of information between reference frames and across modalities [71]. Some researchers have argued that parietal dysfunction may underlie AD-related navigation impairment [141]. Situated between the primary somatosensory regions surrounding the central sulcus and the visual areas of the occipital cortex, the posterior parietal cortex (PPC) serves as a critical associative area. Most work on the PPC has been done in monkeys, though one study in rats showed that lesions to the associative PPC of rats causes deficits

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in the use of proximal but not distal landmarks in a navigation task [180]. Interestingly, women tend to use more local environmental cues when navigating than men and to activate the PPC more than the hippocampus [72,178]. Overall, the PPC is involved the construction of spatial representations of the environment in relation to the body.

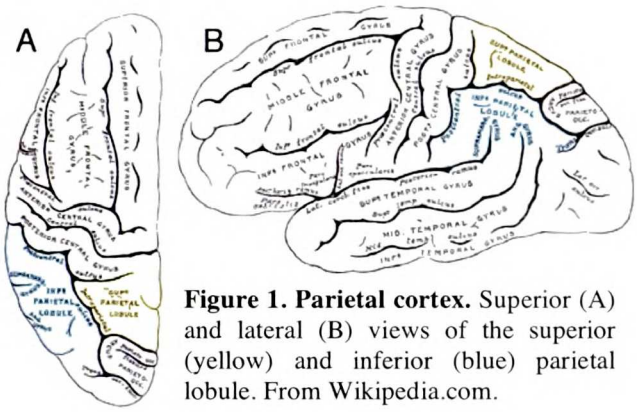

Figure 1. Parietal cortex. Superior (A) and lateral (B) views of the superior (yellow) and inferior (blue) parietal lobule. From Wikipedia.com.

The PPC is divided into the inferior parietal lobule (IPL) and the superior parietal lobule (SPL) by the intraparietal sulcus (IPS) (Fig. 1). The SPL receives primarily somatosensory input and only some visual input, whereas the IPL receives visual input predominantly, and some somatosensory input [171]. The SPL projects to the primary motor cortex and is more involved in the control of arm movements, whereas the IPL projects to the ventral premotor and prefrontal cortex and is more involved in the control of eye movements and visual attention. The IPL is especially important for egocentric spatial localization, has more bimodal and cross-modal responses, and contains the anterior intraparietal area (AIP) and the ventral intraparietal area (VIP), regions that lie along the lateral bank of the IPS [4]. The AIP is involved in visuo-motor transformations and contains visual-, motor-, and bimodal visual-and-motor-responsive neurons. The VIP contains polymodal neurons responsive to visual, tactile, vestibular, and auditory input, and encodes this information in head-centered coordinates [23]. The VIP also receives input from superior temporal areas involved in the perception of biological motion. Thus, the IPL is involved in representing

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objects in near extrapersonal space relative to the self and in controlling how those objects are acted upon to achieve goals. In human navigation studies, activity in the right IPL correlates with accuracy of navigation [111]. Lesions to the IPL, particularly on the right, can cause spatial neglect [171].

The striatum

The striatum (caudate-putamen) also plays a critical role in egocentric spatial processes. There appears to be a triple dissociation in the type of learning mediating by the hippocampus, PPC, and striatum, wherein the hippocampus is dedicated to processing distal cues, the PPC to encoding local cues, and the striatum to mediating cue responses and navigating toward beacons [37,48,49,74,98,122,123,150,211]. The striatum, particularly the dorsolateral aspect, is critical for simple stimulus-response learning and habit-formation [55,103,148,217].

The striatum is also involved in the use of egocentric, response strategies. In rats, inactivation of the striatum causes a shift from egocentric to allocentric spatial strategies, whereas striatal activation with glutamate infusion promotes egocentric strategy use [147,149]. Parallel studies of navigation through a virtual maze show that humans that engage egocentric strategies have enhanced striatal activity relative to humans that engage allocentric strategies [86]. Typically, the hippocampus is engaged early during the learning of a new environment; overtraining leads to a switch to engagement of the striatum [32,147,149]. However, wild-type mice tend to maintain the same strategy irrespective of the amount of training, with C57BL/6 mice consistently preferring allocentric strategies [154]. Human virtual reality tasks have also revealed striatal

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activation during general navigation that may be related to a role in motor learning and planning [110] and to route learning [75].

The frontal cortex

Functional imaging studies have also consistently reported activation in the frontal cortex that may be related to more generalized cognitive functions. Prefrontal areas may be involved in formulating novel routes and, more generally, in motor planning [110]. Furthermore, frontal areas are important for domain-general episodic memory: the dorsolateral prefrontal cortex is critically involved in memory for the temporal order of events [28,29,105,130]. Frontal areas are also involved in choice monitoring and in spatial and nonspatial working memory [64,94,120,146,159,198].

In summary, many neural structures underlie normal spatial navigation in humans and animals. Damage to any of these structures could lead to deficits in spatial navigation. However, components of the navigation network are specialized for specific cognitive processes, and lesions in particular areas lead to distinct types of deficits. By employing selective behavioral tests, one could isolate which component may be causing spatial impairment. In both humans and mice, I have focused on the hippocampus because of its critical role in spatial mapping and because of its early dysfunction in AD. In the human patients, I also investigated the potential role of parietal dysfunction in navigation deficits, concentrating on the IPL. In the mouse, I focused on the striatum to assess the possibility that spatial processes dependent on regions less affected by disease are spared.

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Tests of Navigation in Rodents

Specific Aim 1a was to assess navigation in mouse models of AD. To isolate the contributions of the different components of the navigation network to AD-related spatial deficits, I used tests that distinguished between the different processes thought to be mediated by each region. I will now describe the testing of navigation in mice to justify the specific tests I used.

Morris Water Maze

The seminal development in the study of navigation was the Morris Water Maze (MWM) [134-136]. Though initially developed for rats, the MWM is now the gold standard for reporting navigation deficits in rats and mice [42]. The MWM is a circular tank filled with opacified water. The task is for the rodent to find an escape platform. There are countless variations in the MWM protocol used by different laboratories, including physical maze parameters (tank size, platform size and shape, water temperature) and the actual training procedure. The two most widely used training protocols are visible and hidden platform training [40]. For visible, or cued, platform training, an obvious beacon is placed on the platform. The visible platform protocol is meant to control for procedural aspects of the MWM, and to show that a particular intervention does not interfere with swimming, visual acuity, or any other factor that might influence performance without necessarily affecting learning per se. For hidden platform training, the platform is entirely submerged, and the rodent must learn its location relative to the extra-maze cues. Swimming to a beacon is not disrupted by hippocampal lesions and is most effectively solved with egocentric strategies. According to standard protocols, the hidden platform location remains constant throughout several

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days of training so that the rodent can form a representation of that location relative to distal cues. Thus, the hidden platform task is best solved by allocentric strategies. A probe trial is often administered after the conclusion of hidden platform training to test retention of the learned place.

There are a few noteworthy manipulations of the MWM used to assess particular kinds of memory, learning, or strategy. For example, a cued probe trial can be used with rodents previously trained in both hidden and visible platform tasks [17,48,52,88,123,140,150,180]. In this trial, also called a place-cue competition test, the learned spatial location is contrasted to the location specified by the visible cue. This probe is sensitive to the kinds of landmarks the rodent prefers to use when navigating, as opposed to merely testing the ability of a rodent to use one or the other kind of cue. Hippocampal lesions promote the use of a beacon strategy, and striatal lesions promote allocentric strategies dependent on distal cues [48,49,88,123]. In another variation of the MWM, the location of the hidden platform is changed between blocks of trials. While the spatial representation is formed over many days the standard MWM protocol, this manipulation addresses rapid learning and working memory.

The MWM has multiple benefits. It is a standard and robust test, and animals are motivated to learn because they are averse to being in water. However, though aversive training can lead to more rapid learning, the assessment of learning and memory with the MWM could be confounded by differences in motivation and in anxiety levels. Also, the MWM is a relatively difficult task that requires many days for mice, in particular, to learn. Also, it may not be possible to control completely which distal cues are available to rodents; thus, it may be difficult to replicate some findings in different laboratories. On

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the other hand, the great flexibility in the training protocol allows researchers to find optimal conditions to measure learning deficits.

Visual acuity test

A potential difficulty in interpreting MWM results is that the visible platform task may not be sensitive to deficits that could affect performance in the hidden platform task. For instance, visual acuity impairments limit the use of distal cues during the hidden platform training, but spare cued platform training performance [165]. Conversely, animals could be impaired in the visible platform task in spite of normal visual acuity. Thus, additional experiments can be informative to rule out some potential performance-related confounds.

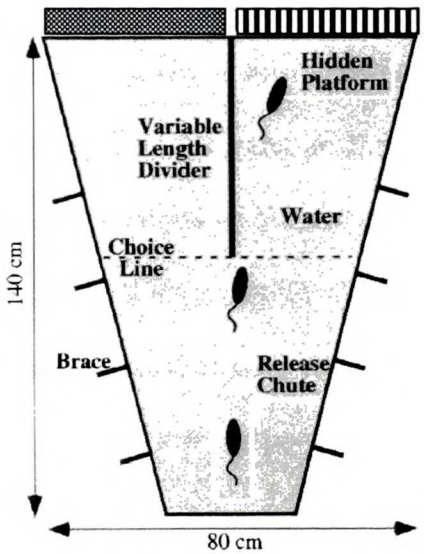

Figure 2. Prusky's visual acuity task, aerial view. See text for description. From [164].

A robust water maze has been developed to test visual acuity in rodents, in which a hidden platform is located at the end of one of two arms (Fig. 2) [164]. The location of the hidden platform is varied pseudo-randomly between trials, but a placard with vertical black and white gratings is always on the wall above the platform. The other arm contains a placard with a finely checkered pattern that is below the visual acuity of the rodent. After reaching a criterion performance, the vertical gratings are made narrower in increments until the rodent fails to perform at criterion, which is defined as the rodent's visual acuity. This test provides both the visual acuity of each rodent and a measure of

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learning. In addition to visual acuity, motor abnormalities that confound swimming difficulties can be ruled out with assays of motor function, including the rotarod test [40].

Radial arm and labyrinthine mazes

Besides the MWM, a number of other mazes have been used to study rodent navigation, typically as dry-land mazes with appetitive rewards. The most commonly employed test is the radial arm maze (typically eight arms), in which rodents must find a reward, typically food, at the end of one or more arms. Different variations of the radial arm maze test distinct aspects of navigation. For example, baiting the same or different arms between trials tests win-stay and win-shift strategy use, respectively [88,122]. If the reward constantly shifts between different arms, then the rodents must employ a strategy to visit the arms without returning to an arm already visited before finding the reward; this type of protocol focuses more on spatial working memory [199]. Other navigation tests include labyrinths that are administered as either dry-land or water mazes, including the Biel Maze of six T-shaped choices [2,87,202]. Hippocampal lesions cause deficits in learning the radial arm maze and other labyrinthine mazes.

Relative to the MWM, the radial arm maze and other labyrinthine mazes are not as flexible for varying the protocol to study how different kinds of landmarks are used in navigation. However, the appetitive stimuli typically used may minimize the extent to which anxiety confounds measurements of learning. More importantly, these mazes can be administered repeatedly to look at behavior before and after an intervention. To this end, rodents are typically trained to some criterion level of performance before an intervention (usually pharmacological) is given. The same behavior can be tested again after the intervention. In contrast, rats can only be trained to find a single location within

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the MWM once; otherwise, there are significant carry-over effects. Changing the platform to a new location to train the rats repeatedly can produce confounds of reversal effects. Thus, the MWM can often only be administered once, which increases the sample size needed to measure group differences.

Naturalistic Paradigms

Some studies have employed more naturalistic paradigms, taking advantage of the fact that rodents like dark places and digging, to investigate the neural substrates of navigation. For example, the circular platform test has been used to assess spatial memory behaviorally and electrophysiologically and has revealed deficits in aged rats [9,10,176]. The circular platform task is conceptually identical to the MWM, in that it tests rodents' ability to locate a goal (one escape hole located along the periphery of a circular table lined with other false holes) relative to distal cues. Also, a cue can be used during training as a control to assess acquisition of a cued navigation test, similar to the cued MWM [8].

Foraging tests have also been used to study dead reckoning, the ability to calculate one's current position relative to some starting location. Dead reckoning, also referred to as path integration, is thought to be a fundamental process required for the formation of cognitive maps [60,61]. Rodents are tested on a table-top with multiple holes around the perimeter: one hole is the rodent's home location, and the others are empty. Rodents emerge from the home location to forage for food, making circuitous routes until food is found. Once they find food, they take a direct route home that indicates the extent to which they have updated their spatial location relative to the starting location. Using this paradigm, studies have shown that lesions to hippocampus or the PPC result in deficits to

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dead reckoning [68,109,179,203,204,209,210]. The entorhinal cortex may also be critical for path integration, which may be mediated by grid cells [126].

The cross maze and other tests of spatial strategy

Tests such as the MWM primarily measure a rodent's ability to apply allocentric or egocentric strategies to navigation problems that typically require one or the other strategy. Other behavioral paradigms that allow rodents to use two or more spatial strategies to solve problems, on the other hand, reveal preferences for particular strategies. One such approach was discussed above—specifically, the use of cued MWM probe trials to put into competition a rodent's preference for distal cue versus a beacon (place-cue competition test). Some researchers have also analyzed the paths taken by rodents while navigating the MWM to determine the strategies they are using. This seems most successful in identifying rodents using allocentric strategies [24,89].

The cross maze task directly contrasts allocentric and egocentric spatial strategies [6,10,12,31,32,39,106,147,154,169]. In this paradigm, a cross-shaped maze is used, with four arms of equal length constructed so that the rodent can see out of the maze to the distal extra-maze cues. During training, the rodent is released from the south arm, the north arm is blocked, and the reward (typically an appetitive reward in a dry-land tank) is located in one of the other arms consistently between trials. Rodents are either trained to criterion or to a set time point and then are given a probe trial in which they are released from the north arm while the south arm is blocked. Rodents returning to the location dictated by the distal cues are called allocentric, or "place," learners, and rodents that have learned to turn in a particular direction will visit the opposite arm and are thus labeled egocentric, or "response," learners. Whereas hippocampal inactivation disrupts

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allocentric strategy use, striatal inactivation disrupts egocentric strategy use [31,149]. Interestingly, the relative cholinergic tone in the hippocampus and striatum, is associated with strategy use. Rats typically engage hippocampus-dependent allocentric strategies at first and transition to egocentric strategies with overtraining without any pharmacological manipulation [81,170]. Acetylcholine levels in the hippocampus and striatum parallel these behavioral changes, and even baseline acetylcholine release predicts which strategy the rodent will engage [32,124]. The eight-arm radial maze can also be manipulated to contrast the rodent's preference for "place" versus cue-driven strategies [118].

Other spatial strategy tests contrast the reliance of rodents on particular kinds of cues. For example, in one paradigm, after being disoriented, rats must locate a reward in one of four corners of a rectangular tank with visible cues on the wall. Even though the visible cues clearly distinguished the four corners, rats strongly preferred the geometrical information provided by the walls of the room and visited the target corner and the geometrically identically opposite corner with equal frequency [34]. Human children below the age of four perform like rats, ignoring the visible cue, whereas human adults use both the geometric and visible cues to locate the reward [79,80]. In another test, rodents searched for food buried under wood chips; landmarks were scattered throughout the tank. Rats were able to use a configuration of landmarks to locate a goal, even when the identity of the landmarks were altered, as long as the geometric relationships between the landmark locations was constant [70]. Rats will not use landmarks to navigate to a reward unless their spatial position remains constant during training [15]. These data suggest a critical role for the stable spatial location of landmarks, although other studies show that rats do attend to featural changes of landmarks, a process possibly linked to

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perirhinal cortical function [27], and that some animal species prefer featural properties of landmarks to their geometric information [196].

In summary, the navigation assays most commonly used directly test the ability of rodents to apply specific spatial strategies. For example, the hidden platform MWM task typically assesses whether a rodent can use an allocentric strategy. In contrast, strategy preference tests allow animals to solve the same problem with multiple solutions, and thus may be more ecologically relevant, since real-life navigation problems can often be solved in a number of equally successful ways. Furthermore, strategy preferences can often reveal more subtle spatial abnormalities than outright testing of particular spatial processes. For example, some studies report that beacon navigation is not impaired by striatal lesions, though when given a choice, striatum-lesioned rats will use distal cues rather than a beacon-strategy [48,49].

For these reasons, I chose the cross maze test to assess spatial learning and strategy use in transgenic mice. Because it distinguishes hippocampal and striatal contributions to navigation, I could relate relative levels of dysfunction in these regions to spatial strategy use and performance. I also observed contexts in which the transgenic mice, which are frequently impaired, performed at normal levels.

The amyloid-β (Aβ) hypothesis

To understand how AD causes cognitive and behavioral impairments, transgenic (TG) animal models have been developed to measure critical pathogenic mediators. Experimental models of AD allow for the testing of specific hypotheses regarding the

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most critical molecular factors involved and are necessary for the development of better treatments.

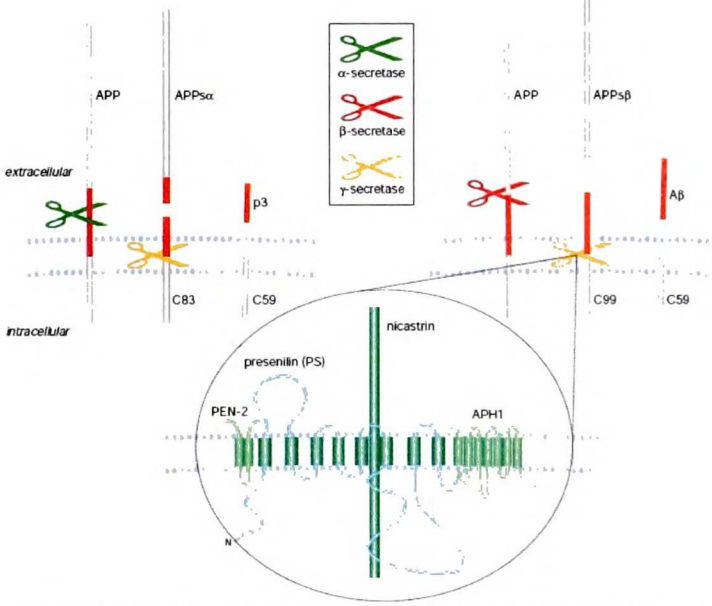

Figure 3. Aβ processing. When hAPP is cleaved by β-, then γ-secretase, Aβ is formed. γ-secretase is a multi-protein complex including APH1, PEN-2, nicastrin, and presenilin. From [93].

The primary animal models of AD are TG mice (Table 1) that have been created based on the "amyloid-β (Aβ) hypothesis," which postulates that some form of Aβ assembly is critically responsible for AD pathogenesis (Fig. 3). This hypothesis is based on a variety of observations, including that Aβ accumulates in AD brains and that all known mutations resulting in autosomal dominant AD are located in the human amyloid precursor protein (hAPP) gene or in genes encoding proteins responsible for hAPP metabolism, including presenilin 1 (PS1) and 2 [183]. When hAPP is cleaved by α-secretase, secreted hAPP (shAPP) is released. When it is cleaved by β-secretase and by γ-secretase, a multiprotein complex that includes PS1, Aβ is released (Fig. 3). The γ-secretase cleavage step also determines whether $A\beta_{40}$ or the more pathogenic $A\beta_{42}$ is formed. Mutations that result in familial AD result in increased absolute or relative levels of $A\beta_{42}$, which has a strong propensity to self-aggregate. Although the most pathogenic assembly state of Aβ remains a matter of active study and debate, much evidence suggests that soluble Aβ oligomers are more

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Model	Genetics	Aβ Plaques	NF Tangles	Neuronal Loss	Synaptic Deficits	Astrogliosis	Neuritic Dystrophy	MWM Deficits	Radial Arm Maze Deficits	Circular Platform Spatial Deficits	Refs.
APP-Transgenic Mice											
PDAPP	hAPP V717F PDGF mini-promoter	Y	N	N	Y	Y	Y	Spatial reference memory; serial spatial memory	Y	Y	[33,50,63,85]
Tg2576	hAPP K670N, M671L Hamster PrP promoter	Y	N	N	N *	Y	Y	Spatial memory acquisition & retention; cued MWM	Y	Y	[84,100,161,208]
APP23	hAPP K670N, M671L Thy-1 promoter	Y	N	Y †	Y	Y	Y	Spatial memory acquisition & retention			[96,186,189]
TgCRND8	hAPP K670N, M671L, V717I Hamster PrP promoter	Y	N	N		Y	Y	Spatial memory acquisition & retention			[38,90]
J20	hAPP K670N, M671L, V717I PDGF mini-promoter	Y	N	N	Y	Y	Y	Spatial memory acquisition & retention; cued MWM			[138,152]
Compound Transgenic Models											
APP+PS1	hAPP Tg2576 hPS1 M146L	Y	N	N		Y	Y	Spatial memory acquisition		Y	[5,67]
APP+ TGF-β1	hAPP J20 TGF-β1	Y	N	N	Y	Y	Y				[214-216]
APP+ ApoE	hAPP J20 hApoE3, hApoE4 NSE promoter	Y ‡	N	N	Y	Y	Y	Spatial memory retention deficit in hAPP/ApoE4 mice			[166,167]
APP+tau	hAPP Tg2576 Tau P301L	Y	Y	Y		Y	Y				[107,168]
3xTg-AD	hPS1 M146V hAPP K670N, M671L Tau P301L	Y	Y	Y	Y	Y	Y	Spatial memory acquisition & retention			[16,144]

* Synaptophysin levels increased in older Tg2576 mice
† Neuronal loss in CA1
‡ Similar Aβ levels in hAPP/ApoE3 and hAPP/ApoE4 mice

Table 1. Classic TG models of AD. Based on [69,104].

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critical in the pathogenesis of AD-related neuronal dysfunction than the fibrillar Aβ deposited in the brain parenchyma in the form of amyloid plaques. hAPP mice with high cerebral levels of soluble Aβ develop behavioral and electrophysiological deficits well before they develop neuritic plaques; furthermore, functional deficits in older mice do not correlate with plaque burden [83,138,151,152].

Although autosomal dominant AD accounts for only a small fraction of all AD cases, the brains of patients suffering from either familial or sporadic AD are indistinguishable with respect to neuropathological markers, supporting the use of TG models based on familial AD [69]. The histopathological hallmarks of AD include Aβ plaques, neurofibrillary lesions, and tau hyperphosphorylation. Interestingly, even though tau-related lesions may relate better to cognitive dysfunction, no tau mutations have been discovered that cause AD. Consequently, most work on AD has focused on manipulating amyloid pathways by expressing mutant forms of hAPP in the mouse. Familial AD-mutant transgenic mice recapitulate the amyloid pathology of AD, but do not typically have neurofibrillary lesions or neuronal loss.

The primary mouse models of AD contain familial AD-mutant hAPP transgenes. Although they do not develop neurofibrillary tangles or substantial neuronal loss, these models recapitulate many aspects of the disease, including amyloid pathology, synaptic deficits, and spatial memory deficits (Table 1). Beyond hAPP mice, compound TG models have also been created to assess the effect of other modifiers on amyloid pathways. For example, crossing hAPP mice with TGF-β1 mice has revealed a role for cytokines and inflammation in the pathogenesis of AD. Interestingly, TGF-β1 accelerates amyloid deposition in cerebral blood vessels while decreasing parenchymal deposition

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within the brain [214,216]. Crossing hAPP TG mice with apolipoprotein E (ApoE) knockout mice expressing either human ApoE3 or ApoE4 showed that ApoE4/hAPP mice were more susceptible to the effects of hAPP/Aβ than ApoE3/hAPP mice; ApoE4 but not ApoE3 hAPP mice were behaviorally impaired even though the Aβ levels were similar in both groups [167]. Crossing hAPP mice with presenilin mutants leads to an acceleration or exacerbation of amyloid pathology [5,67,69]. More recently, a triple TG mouse was developed with mutant hAPP, PS1, and tau [16,144]. This model recapitulates tau pathology in addition to amyloid pathology and spatial memory deficits and therefore reflects more AD-related changes than hAPP single transgenic models. However, no tau mutations have been reported to cause AD and no AD patient has all three mutations, whereas the single transgenic mouse models are based on actual families with AD.

While TG mice are the standard models used for studying AD, some research has employed *Drosophila*, *C. elegans*, or the sea lamprey [69]. These species have been used primarily because of the ease with which they are bred and the power of the genetic tools available. However, their utility may be limited by the vast differences in neuroanatomy compared with humans [69]. In contrast, mouse models recapitulate many aspects of AD and allow for sophisticated behavioral testing that can reveal cognitive deficits associated with region-specific pathology [69].

I chose to use a TG mouse model of AD to look at the relationships of AD-related pathology to navigation impairment. Specifically, I used the J20 line, which is an hAPP TG line with high levels of $A\beta_{42}$. Focusing on the hippocampus and striatum, I assessed the levels of synaptic activity-dependent proteins in addition to Aβ levels because activity-dependent proteins relate better to cognition and behavior (Aim 1b).

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Navigation in TG Mice

Most of studies on navigation in mouse models of AD have employed the MWM. hAPP models of AD have impairments in the acquisition and retention of spatial reference memory in the MWM, and are additionally impaired on other spatial tasks, including the radial arm maze and the circular platform task [91,104] (Table 1). Behavioral deficits in hAPP mice are more closely related to neuronal alterations in synaptic activity-dependent proteins than Aβ levels or deposition. Because I was interested in histological alterations that are linked to behavioral alterations, I chose to focus on these markers rather than on plaques, which are undetectable at 3 months of age, minimal at 6–7 months of age, and unrelated to behavioral deficits in J20 mice [152] and a variety of other hAPP transgenic models [82,104,208]. In contrast, levels of synaptic activity-dependent proteins correlate well with spatial memory deficits [152]. Interestingly, some lines of hAPP mice have deficits in the visible platform MWM [104,152,208], though no study has directly addressed the possibility of visual acuity impairment. Compound TG models have revealed disease modifiers that interact with hAPP/Aβ. ApoE4/hAPP but not ApoE3/hAPP mice are significantly impaired on the MWM probe trial, and human ApoE4 is sufficient to cause hidden platform MWM acquisition deficits in aged hAPP-NTG females [167].

Beyond the assessment of gross navigational ability, some attempts have been made to investigate the search strategies of hAPP mice. For instance, TgCRND8 mice use chaining strategies rather than allocentric strategies in the MWM to find the platform [89], while PDAPP mice similarly seem to adopt looping strategies rather than allocentric

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spatial strategies [24]. To assess spatial strategy preference, however, different strategies must be equally available to the mice. It is not clear in these studies that the hAPP mice ever learned the location of the hidden platform. Thus, one cannot conclude that the hAPP mice prefer egocentric strategies, because it is also likely that they have simply failed to develop allocentric strategies. In a study of Tg2576 mice, almost all TG mice employed egocentric strategies in the cross maze [128]. This finding is surprising because AD typically yields pathology earliest in the hippocampus and tends to spare other regions including the striatum. One would expect hAPP mice to behave similarly to mice and rats with hippocampal inactivation, which leads to more random strategy use [129,149]. The authors concluded that striatal function may be enhanced in Tg2576 mice, similar to rodent models of glutamate-induced striatal activation [147]. However, Tg2576 mice do not have enhanced fronto-striatal plasticity, and no other data support the notion that striatal function is upregulated in hAPP mice.

The questions of how strategy use is altered in hAPP mice and how strategy use relates to navigation performance and neuropathological changes in particular brain regions are unsolved. To elucidate mechanisms of AD-related navigation impairment in the J20 hAPP TG mice, I focused on spatial strategy alterations and how these alterations related to learning and to molecular changes in the striatum and hippocampus.

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Tests of Navigation in Humans

Two-dimensional spatial tests

My first aim in the human project (Aim 2a) was to assess actual navigation ability. The principal difficulty in assessing human navigation is lack of access to a large enough

environment. Consequently, researchers have typically administered two-dimensional tests thought to relate in some way to spatial cognition in general. Examples include pencil-and-paper tests, such as the Money Road Map test, the Rey Osterrieth complex figure test, the pentagon copy test, and the Buschke controlled learning task, among others [13,45,47,65,95,112,201]; table-top tests of memory for the locations of objects in an array [101,112,187,188,205]; and various map-based tests of geographical knowledge in which subjects are asked to recall routes from familiar environments from memory [116,127,192]. In addition, there are some computerized two-dimensional spatial tests that are essentially digitized array tests, such as the Paired Associates Learning task, in which subjects must remember the locations of six unnamable figures displayed on a monitor [43,58,190].

The primary benefit of these tests is that they can be administered in a small space and are easily reproducible. The drawback is that they are poor markers of one's ability to navigate through an actual three-dimensional environment [111]. For instance, in table-top array tests, subjects typically take an aerial perspective which is rarely if ever possible when actually navigating. Furthermore, for two-dimensional tests, all information is available in one field of view, whereas much of the information necessary for accurate navigation is hidden from one particular point of view and must be accessed through active exploration. Lacking from two-dimensional tests is movement through the environment and thus the concomitant visuoperceptual feedback (e.g., optic flow) and idiothetic cues (proprioceptive and vestibular feedback) that are critical for accurate navigation. Most convincing is the double dissociation observed between impairments on two-dimensional spatial tasks and deficits in three-dimensional navigation tests.

Specifically, some patients perform normally on table-top array and geographical knowledge tests but nonetheless suffer from topographical memory deficits [73,121]. Conversely, some patients with normal navigation ability fail at visual memory and geographical knowledge tests [112]. Clearly, despite the ease and convenience of administering these tests at the bedside and in the laboratory, two-dimensional spatial memory tests do not adequately address navigation ability.

Real-life navigation tests

Some studies have instead employed real-life navigation tasks. These typically consist of bringing subjects along a novel route (e.g., through hallways in clinic) and testing various aspects of their memory for that route afterwards. These tests have been applied to the study of AD patients and cognitively normal elderly subjects [35,36,133,153] and to stroke patients [197]. Among these, only the study in stroke patients examined the neural regions involved in particular navigation subtests and reported that accurately navigating the route involved the right hippocampal formation, right PPC, and right temporal lobe, whereas memory for the order in which landmarks were encountered involved the dorsolateral prefrontal cortex [197].

In addition to navigation ability, there have been some studies of spatial strategy use in real-life settings. As mentioned above, subjects have been tested in rectangular rooms that put the geometric information provided by the configuration of the distal walls in conflict with featural information on the wall. These studies indicate that human children use geometric cues and ignore obvious visual information to reorient and locate a reward [79,80]. Another study from the same group tested subjects' memory for the location of objects versus stable features of a room by asking people to point to them with their eyes

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closed [206]. They found that humans tend to encode the locations of distal geometric environmental features allocentrically, whereas object locations were encoded egocentrically.

Real-life navigation tests have also been applied to assess dead reckoning in humans. Subjects typically traverse a simple route actively or passively with or without visual and other sensory information, and are then asked to either return or point to the starting location or to retrace their steps [53]. One study showed that right but not left temporal lobectomy patients were impaired at dead reckoning [213]. Some groups have also tried to use real-life driving tests to assess navigation and driving ability [56], although driving a car certainly entails numerous capabilities beyond navigation and is not sensitive to the specific neural and cognitive underpinnings of navigation deficits. Thus, the few attempts to use real-life navigation tests were limited by the space available for testing and the relative inability to control all possible variables in the environment.

Virtual navigation tests

A promising development in human navigation research is the use of virtual environments to test subjects. Virtual environments offer the distinct advantage of providing full control over all environmental variables and, consequently, a potentially greater level of reproducibility than real-life navigation tests. Immersive environments are not typically used because they are cumbersome and cause motion sickness [99,108]. In contrast, virtual environments on a computer monitor may actually be more effective than immersive displays [62]. Two major drawbacks to virtual navigation are that there are no idiothetic (vestibular, tactile, and proprioceptive) cues and the field of view presented on the computer monitor is narrower than in real life.

In spite of these disadvantages, navigation in virtual environments approximates real navigation, and knowledge imparted during exploration of virtual environments transfers to the actual emulated environment [57]. The subject's feeling of "presence" is the critical factor in determining how well real navigation is approximated, and level of presence is determined by how realistic the environment appears and how many landmarks are available to subjects as they navigation [25,111]. Whereas abstract patterns do not enhance a subject's ability to navigate through otherwise empty rooms and corridors, familiar objects do enhance wayfinding [177].

Virtual environments have typically fallen into three categories: virtual MWMs, labyrinths, and towns. Virtual MWM tests have revealed navigation impairments in traumatic brain injury patients [184], human hippocampalectomy patients [7], and elderly subjects [131]. Elderly subjects are also much worse at solving virtual labyrinths than younger people [132]. Another virtual labyrinth study showed that men typically engaged the hippocampus when navigating, whereas women had greater activity in the PPC, and that men solved the maze more rapidly [72]. Virtual towns appear to approximate real-life navigation better than the virtual MWM or labyrinths [25,111]. Virtual tasks have been used in electrophysiological recordings of human epilepsy patients navigating through town as a "taxi driver." These tests showed that the human hippocampus contains places cells, that the human PHG contains spatial view cells [51], and that human cortical oscillations may coordinate sensory and motor brain activity to facilitate spatial learning and planning [30].

Tests of learning the layout of virtual towns while "walking" through them have typically been used in conjunction with functional neuroimaging to reveal a network of

neural regions active during navigation, including the right hippocampus, caudate, PPC, and frontal cortex [110]. Functional imaging of subjects navigating a virtual museum revealed a critical role for the PHG in encoding information about navigationally-relevant objects [92]. Similar tests have been applied to right and left temporal lobectomy patients to reveal that the right hippocampus is required for spatial navigation, whereas the left hippocampus may be more involved in verbal episodic memory [25,185].

Drawing on animal work, there are also virtual navigation studies of human spatial strategy preference. For instance, in line with data suggesting that men engage the hippocampus and women activate the PPC, a virtual MWM test that included conditions in which local cues were or were not available showed that women depended heavily on local cues. In contrast, men were able to solve the task using the distal geometrical cues of the room alone [178]. These data parallel results in rat studies suggesting different spatial strategies and abilities in male and female mice, depending on the females' hormone status [207,212]. Elderly subjects also tend to rely heavily on proximal cues, whereas younger subjects uses distal geometric features of the environment to navigate a virtual MWM [131].

Virtual environments have also been used to assess allocentric versus egocentric strategy preference, in line with animal work. In a virtual radial arm maze, allocentric strategists who used distal cues had greater hippocampal activation, whereas egocentric strategists who used turning strategies had enhanced caudate activation, similar to rodent studies [86]. Furthermore, patients with medial temporal lobe lesions are selectively impaired at applying allocentric strategies, although not all patients switch to more adaptive egocentric strategies [19]. Another group compared neural activity while

traversing a well-learned route to activity while navigating a novel town. Accurate wayfinding was correlated to activity in the right posterior hippocampus, whereas accurate route following was associated with caudate activity [75]. Interestingly, early Huntington's disease patients do not have route-following deficits in spite of caudate dysfunction, but do have enhanced hippocampal activity that is thought to serve a compensatory role [200].

Virtual navigation tasks, in combination with functional imaging and studies of human neurological patients, have therefore revealed that many of the neural regions involved in nonhuman mammal navigation are also critical for human navigation. However, the prior experience of subjects with computer and particularly computer games may confound the analysis, particularly in studies reporting that men perform better than women and that young people perform better than elderly subjects. With respect to AD, this is particularly concerning because demented patients may not understand the virtual environment as well as age-matched controls.

In summary, it has been challenging to develop reproducible tests of human spatial navigation. Two-dimensional spatial tests are not well correlated with actual navigation ability. Virtual tasks, particularly those that emulate complex towns and spaces rather than the simple MWM, could emerge as the gold standard for demonstrating reproducible navigation deficits in patient populations. However, application of virtual tasks to demented subjects may not be feasible, though real-life hallway navigation tasks have been successfully applied in this population. Thus, I chose to use a hallway navigation test, similar to those already used to assess AD and stroke patients, and developed various

subtests to isolate different aspects of spatial cognition, with an emphasis on testing processes related to hippocampal and parietal function.

Navigation in AD Patients

Though about 39% of AD patients have problems with wayfinding [78], little work has been done to characterize the specific spatial deficits involved. In a study with the Buschke controlled learning task, spatial deficits correlated better with right hippocampal atrophy, and verbal memory deficits correlated with left hippocampal atrophy [45], though the applicability of this two-dimensional test to navigation is uncertain. Some groups have used real-life hallway navigation tasks. One such study found that AD patients cannot navigate to a location in a hospital or return to the starting point, and reported that AD patients had difficulty formulating effective plans [153]. Other groups have used real-life hallway navigation tests to show that AD patients cannot learn a new route and could not identify the locations of places displayed in photographs [35,36,133]. These studies reported that navigation performance was unrelated to global memory impairment, though close examination of their data reveals that AD patients fail to remember whether they had seen landmarks, implying that the deficits are more generalized than a selective mapping impairment. However, because advanced cases of AD were included, these studies may have missed more selective spatial impairment that might characterize milder cases of AD. These studies do show, however, that spatial navigation deficits can be studied in AD patients, although they did not investigate the specific aspects of the navigation network that are affected by AD.

One key question is whether the spatial disability of AD patients is a manifestation of a generalized neurodegenerative process or of damage to specific neural networks and thus specific cognitive processes. Only a few studies have addressed this question. One study identified a subgroup among a population of AD patients that became lost and wandered in familiar environments [44]. This subgroup scored significantly worse on tests of parietal function than the other AD patients, while there was no difference between the groups on a generalized test of mental status. Similarly, spatial disorientation in AD is more related to tests of memory and visuoconstructive functions, but not disease severity, attention, or language impairment [78]. Other groups have focused on the potential contribution of visuoperceptual impairment in AD-related navigation deficits, and showed that early AD and MCI patients perform poorly on tests of optic flow; performance on navigation tasks related better to other visuospatial and perceptual tests than to tests of verbal memory [117]. Based on this observation and reports that a subgroup of AD patients that demonstrate visual impairments and metabolic abnormalities in the occipital and parietal cortices [102,160], these investigators argued that impairments in visual motion processing may underlie general spatial deficits in AD and that damage to the occipital and parietal cortices may cause these impairments [141]. However, they provide no neuroanatomical evidence to support these claims.

Histopathological and neuroimaging work has revealed that the hippocampus is typically affected earlier by AD than the neocortical regions thought to be involved in visuoperceptual processes. According to the pathological staging worked out by Braak and Braak, neurofibrillary tangles and neuropil threads are deposited earliest in the medial temporal lobe and then later in association cortices [20,21]. Studies of gray matter

atrophy reveal similar patterns of neuronal loss [194]. Voxel-based morphometry (VBM) analyses of structural MRI data revealed that full-blown AD is associated with atrophy of the hippocampus, PPC, caudate, frontal cortex, insula, and the temporal and cingulate gyri, but that mild AD is characterized by atrophy of the hippocampus bilaterally [59]. Atrophy of the hippocampus and entorhinal cortex precedes onset of overt dementia [46]. Parietal atrophy distinguishes AD from normal controls at all stages of disease, including mild dementia [181]. Cerebral blood flow is reduced in the medial temporal and parietal lobes of mild AD patients [191]. Even asymptomatic subjects at high risk of developing familial AD present with decreased parieto-temporal regional glucose metabolism [97]. In contrast, the frontal cortices are affected much later in AD, distinguishing AD-related deficits from the structural and functional changes characteristic of normal aging [76].

Because the hippocampus and other medial temporal structures are affected earliest in AD, impairment of hippocampus-dependent allocentric spatial mapping may underlie AD-related navigation impairments, at least early in the disease. In addition to testing specific cognitive processes, future studies must collect neuroanatomical data to gain some understanding of possible anatomical correlates. I therefore focused on the hippocampus and PPC to elucidate which of these regions was related to navigation disability in AD patients (Aim 2b).

Questions Addressed and Plan of Thesis

The goals of this work were to determine which of the multiple processes underlying spatial cognition contribute to AD-related spatial disability in human patients and in mouse models and to investigate neural correlates of spatial impairment by comparing

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behavioral measures to structural and biochemical changes. In mice, I used the cross maze task to assess spatial strategy use and learning (Chapter 2). This maze was designed specifically to distinguish between spatial strategies with distinct neuroanatomical substrates. I then quantified reductions in synaptic activity-dependent proteins and Aβ levels in the brain regions thought to be critical for these strategies. In humans, I assessed navigation by testing patients with a real-life hallway navigation task with a variety of subtests intended to target different spatial cognitive processes, including mapping, dead reckoning, and landmark recognition among others (Chapter 3). I then measured atrophy in navigation-related brain regions to correlate volumetric changes with behavioral outcome measures.

Questions for mouse study

For the mouse study, I focused on the relationship between variation in spatial strategy use and spatial learning. Most studies of TG mouse models of AD have used the hidden platform MWM task to assess spatial memory. While the MWM is a robust and standard test, it does not allow mice to engage alternative strategies to successfully navigate to the goal: the only efficient strategy is to use the distal cues.

Instead, I used the cross maze task, which can be successfully solved with alternative strategies that depend on either the hippocampus (allocentric) or striatum (egocentric). My hypothesis was that, because our TG manipulation causes hippocampal dysfunction, the TG mice would behave similarly to wild-type rodents with hippocampal lesions, and prefer egocentric strategies. The cross maze task also provides a measure of learning (the number of trials to reach criterion). I tested whether TG mice that engage either allocentric or egocentric strategies were better or worse at learning the task. I

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hypothesized that mice engaging hippocampus-dependent allocentric strategies would be more impaired than those engaging hippocampus-independent egocentric strategies. Such a finding would relate to real-life navigation problems encountered by human patients; spatial problems can usually be solved by a number of different strategies. Using inappropriate strategies could reveal spatial cognitive deficits, but engaging adaptive strategies could hide or circumvent some spatial impairments.

After characterizing spatial strategy in the TG mice, I tested how robust and reproducible strategy alterations were. First, I conducted repeated probe trials to show that individual mice engage the same strategy consistently. I also used an independent test of spatial strategy, the cued MWM probe trial, to test whether mice that are egocentric in the cross maze are also egocentric in another task. Finally, as a tertiary goal, I adapted a visual acuity task to ascertain whether the deficits observed in J20 mice in the visible platform MWM task (and, therefore, potentially in the hidden platform task also) are due to visual deficits. Such an assessment has not been done previously in any other TG mouse model of AD.

Once I assessed mice behaviorally, I measured different forms of pathology in the hippocampus and striatum, focusing primarily on levels of synaptic activity-dependent proteins which relate better to learning and memory than levels of Aβ, though Aβ levels were also measured. First, I wanted to test the assumption that the hippocampus is affected more severely than the striatum in our hAPP mice. Additionally, I sought to determine whether the striatal function was upregulated as others have suggested, unaltered, or depressed. Hippocampal impairments in the context of striatal sparing could explain why many TG mice use egocentric rather than allocentric strategies.

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My second goal was to identify factors that predict which TG mice engaged allocentric versus egocentric strategies. One possibility was that the extent of reductions of activity-dependent proteins in the hippocampus correlates with increased likelihood of adopting an egocentric strategy. Studies showing that relative hippocampal and striatal acetylcholine release predict strategy use support this hypothesis. However, about half of human hippocampalectomy patients persist in using allocentric strategies, even though they are ineffective at solving a maze that could be solved by either allocentric or egocentric strategies. Therefore, measures of neuropathology in the hippocampus and striatum may not predict which strategy TG mice use. In this case, hippocampal dysfunction may lead to a more random strategy preference. By comparing hippocampal levels of Aβ and activity-dependent proteins in allocentric versus egocentric TG mice, I hoped to distinguish between these two possibilities.

Questions for the human study

For the human study, my primary goal was to determine which factors were associated with an increased likelihood of having difficulty navigating. One potential explanation for navigation deficits in AD is that they are a manifestation of generalized cognitive decline. In other words, AD patients could have poor memory overall and simply do not remember where they have been. My first objective was to prove that the likelihood of navigation deficits was not related to measures of general intellectual function or measures of other forms of cognition. To test this hypothesis, I focused on MCI and mild AD patients, because it is more feasible to isolate independent cognitive deficits earlier in the disease. If spatial cognition is an independent domain and navigation impairments are dissociable from other types of cognitive deficits, then I

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would find that performance on the hallway task does not correlate well with other standard neuropsychological variables. I expected to find this, given the work done previously in AD patients. However, this still leaves open the question: what is different about AD patients with navigation disability?

To address this question, I measured volumetric differences in brain regions critical for navigation. My hypothesis was that particular patterns of atrophy may be related to spatial disability. Specifically, I considered the evidence that the hippocampus and PPC are involved early in AD, and that these regions, especially in the right hemisphere, are central to human navigation. I sought to determine whether patients with navigation deficits had more atrophy of the right-lateralized navigation network than patients without spatial impairment. One might expect that all AD patients have some level of neuropathology; the topological patterns of this pathology could determine which cognitive capacities are likely to be lost or impaired. No other study has assessed navigation in human AD and MCI patients and correlated this to neuroanatomical variables.

The human project is also the first of its kind to study navigation impairment in MCI, which is defined by a lack of functional impairment. By including MCI patients, I tested whether navigation-related outcome measures were sensitive to early cognitive deficits. Considering the evidence that hippocampal atrophy occurs before the onset of overt dementia and that the hippocampus is critical for navigation, navigation tests could be more sensitive than other neuropsychological tests in detecting early impairments. As a long-term goal, not addressed in this thesis, it would be interesting to know if poor performance on navigation tests predicts which MCI patients go on to develop AD.

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In summary, by conducting behavioral experiments in mice and human patients targeted at particular spatial processes and relating impairments to regional patterns of atrophy and hAPP/Aβ-induced neural dysfunction, I sought to elucidate the neural and cognitive mechanisms of AD-related navigation disability. Revealing some of the underlying causes of spatial impairment may lead to potential avenues for therapeutic intervention.

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Chapter 2. Altered Navigational Strategy Use and Visuospatial Deficits in hAPP Transgenic Mice

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Abstract

Navigation deficits are prominent in Alzheimer's disease (AD) patients and transgenic mice expressing familial AD-mutant hAPP and Aβ peptides. To determine the impact of strategy use on these deficits, we assessed hAPP and nontransgenic mice in a cross maze that can be solved by allocentric (world-based) or egocentric (self-based) strategies. Most nontransgenic mice used allocentric strategies, whereas half of hAPP mice were egocentric. At 3 months, all mice learned the cross maze rapidly; at 6 months, only allocentric hAPP mice were impaired. At 3 and 6 months, hAPP mice had reduced hippocampal Fos expression, which correlated with cross maze learning in older mice. Striatal pCREB expression was unaltered in hAPP mice, suggesting striatal sparing. We conclude that egocentric strategy use may be an earlier indicator of hAPP/Aβ-induced hippocampal impairment than spatial learning deficits. Persistent use of allocentric strategies when egocentric strategies are available is maladaptive when there is hippocampal damage. Interventions promoting flexibility in selecting learning strategies might help circumvent otherwise debilitating navigational deficits caused by AD-related hippocampal dysfunction.

Key words: Alzheimer's disease; spatial memory; navigation; hippocampus; striatum; mouse model; Aβ peptide; strategy; human amyloid precursor protein; hAPP

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1. Introduction

Spatial problems can be approached by different strategies that determine whic environmental cues are used to navigate. Allocentric strategies encode locations relativ to a world-based map and rely on distal geometric cues. Egocentric strategies encod locations relative to the current position of the navigator and rely on idiothetic and loca environmental cues [23,30]. Rats typically engage allocentric strategies when learnin locations and switch to egocentric strategies after overtraining [9], whereas C57BL/ mice typically use allocentric strategies persistently in spite of overtraining [50,64].

Many neural structures subserve navigation [36,37,43,52,53,57,73,75]. Allocentri strategies are impaired by hippocampal lesions and promoted by hippocampa stimulation, whereas egocentric strategies are impaired by large striatal lesions an promoted by striatal stimulation [8,9,46,47,58,60]. More restricted striatal lesions hav revealed that the dorsomedial striatum may contribute to allocentric learning, and th dorsolateral striatum to egocentric strategies [16,83].

Alzheimer's disease (AD), the most common cause of dementia, results i progressive cognitive decline [1,20,27]. AD-related spatial disability is common [48 affecting roughly 39% of patients [28], and profoundly impairs daily activities [22,65 While it is well established that AD affects the hippocampus more than the striatu [7,54,66], the mechanisms underlying AD-related spatial disabilities are poorl understood, and why navigation deficits vary in presentation in patients with simila disease severity is unknown.

Amyloid-β (Aβ) peptides, which play key roles in AD pathogenesis [72,76,78], elic diverse behavioral alterations when produced in neurons of human amyloid precurso

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protein (hAPP) transgenic mice [39]. Here we used hAPP mice to test the hypotheses tha hAPP/Aβ affects hippocampus-dependent learning strategies more than striatum-dependent strategies and that innate proclivity to use one or the other strategy determines the severity of AD-related learning deficits. To ascertain whether alterations in spatia learning and strategy use are related to alterations in hippocampal or striatal function, we assessed baseline levels of synaptic activity-dependent proteins in these regions, which are thought to reflect tonic neuronal activity [42,77]. We first determined whethe strategy alterations in the hAPP mice as a group related to regional patterns of neura dysfunction. Next, we assessed whether measures of hippocampal and striatal functio predicted strategy preference and spatial learning on an individual basis among hAPP mice.

2. Methods

2.1. Transgenic Mice

We analyzed four groups of nontransgenic (NTG) and heterozygous hAPP mal C57BL/6 mice from line J20, which produces hAPP carrying the Swedish (K670N M671L) and Indiana (V717F) familial AD (FAD) mutations (hAPP770 numbering directed by the platelet-derived growth factor (PDGF) β-chain promoter [55]. Behavio was tested at 3 months or 6–7 months of age (Table 1). During testing periods, mice wer housed singly and kept on a 12-h light/dark cycle, with free access to food and water They were tested during the light cycle. Brains were obtained for immunohistochemica analysis 2 days after testing. Mice were left undisturbed in their home cages for the 2 days before sacrifice to ensure that activity-dependent proteins were at baseline levels

One NTG mouse from Group 4 died for unknown reasons after the cross maze trainin and was not further analyzed. All experiments were approved by the Committee o Animal Research (University of California, San Francisco).

Group	Age	NTG	hAPP	Behavioral Tests
1	3 mos.	20	18	Cross maze, MWM, Cued MWM Probe Trial
2	6-7 mos.	10	9	Visual acuity acquisition & performance
3	6-7 mos.	30	22	Cross maze with repeated probes, visual acuity acquisition
4	6-7 mos.	19	17	Cross maze, MWM

Table 1. Groups of mice analyzed in this study. The age at testing and numbers of mice in eac of the four groups of J20 mice are presented. Behavioral tests conducted in each group are liste in chronological order. MWM: Morris water maze.

2.2. Cross maze test

We adapted a standard cross maze paradigm used to assess spatial strategy in rodent [2-4,8,9,40,58,60,64] (Fig. 1A). In this task, rodents learn to navigate to the end of one o four arms; a probe trial administered after reaching criterion distinguishes allocentri from egocentric strategists. The cross maze is learned quickly, allowing for rapi assessment of mice and rats. Training to criterion reduces variability of the strateg measured at probe. Furthermore, because allocentric and egocentric strategies are equall effective, the cross maze provides an unbiased measure of preferred strategy for eac individual. The cross maze we used consisted of 4 clear Plexiglas arms, each 30.5 x 10 20 cm, filled to a depth of 8 cm with opaque water. At the end of one or more arms, a escape platform (10 cm x 5 cm) was submerged 1 cm below the surface of the water. A appropriate, arms were blocked with clear Plexiglas doors. During the experiment, dist cues visible to the mice included a door, a shelving unit with a computer, three posters o the wall, and a curtained rack holding the mouse cages.

Pretraining consisted of 3 sessions (1 on day 1, and 2 on day 2) of 4 trials each wit 1-min rests between trials. For every trial, mice were released from the south arm

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Pretraining consisted of forced trials in which the hidden platform location alternated between the east and west arms. The north arm and the arm opposite from the arm with the platform were blocked. Once mice reached the platform, they remained there for 2 sec and were then placed in their home cage for 1 min before the next trial.

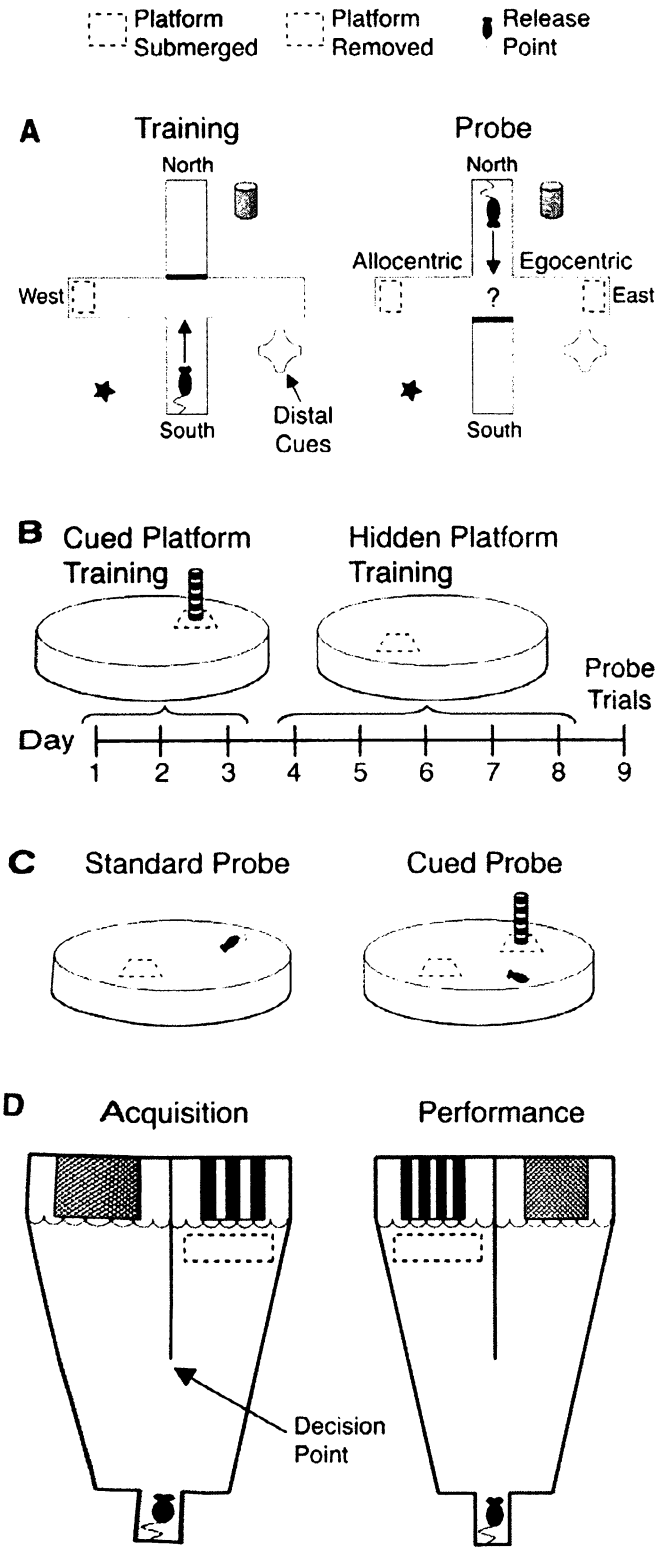

Figure 1. Behavioral paradigms. (A) For cross maze training (left), the escape platform was hidden in the west arm, mice were released from the south arm, and the north arm was blocked. For the probe trial (right), platforms were placed at both east and west ends of the maze, mice were released from the north arm, and the south arm was blocked. Swimming to the platform in the west arm in the probe trial was interpreted as putative evidence for allocentric learning, i.e., that the mouse had learned to locate the platform in the west arm by its spatial relationship to distal cues in the room (indicated by the shapes around the maze). Swimming into the east arm was interpreted as evidence for egocentric learning, i.e., the mouse had learned to locate the platform by turning to its left at the corner. (B) Morris Water Maze (MWM) protocol consisting of cued and hidden platform training. (C) MWM training was followed by two probe trials. For the standard probe trial (left), the hidden platform was removed and the swim path of the mice was recorded for 60 sec. The cued probe trial (right) was carried out similarly, except that the visible cue used during cued platform training (B), was placed 180° from where the hidden platform used to be. (D) Bird's eye view of the water maze used for visual acuity testing. See Materials and Methods for details.

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On the third day, mice received 1 trial to assess any natural biases to a particular side. Mice were released from the south arm while the north arm was blocked and both the east and west arms were available and contained escape platforms. Starting on the third day, they were then trained against their bias in a single session of 4 trials per day (inter-trial interval: 1 min) until they reached criterion, defined as 4 consecutive correct trials. Mice were released from the south arm while the north arm was always blocked. Both the east and west arms were available, but only 1 arm contained the platform. Mice remained on the platform for 5 sec before they were removed. We scored mice as incorrect if they entered the final 15 cm of the wrong arm.

Once mice performed correctly in 4 consecutive trials, we administered a probe trial in which they were released from the north arm while the south arm was blocked. Both the east and west arms contained hidden escape platforms. Mice that chose the correct platform based on the distal cues in the room were categorized as allocentric learners, whereas mice that chose the opposite platform were categorized as egocentric learners (Fig. 1A). For each mouse, we recorded the number of trials required to reach criterion during training and the platform choice during the probe trial.

After the first probe trial, Group 3 received further training as described above in order to assess the consistency of their strategy choice. Additional probe trials were given after 10, 30, and 50 training trials. If mice made a mistake in the 4 trials preceding the probe, we continued to administer training trials until they were correct in 4 consecutive trials.

Alternative methods to assess spatial strategy in rodents include dry-land mazes containing different kinds of landmarks [10,44,74], analysis of search paths in the MWM

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[33], and modified MWM probe trials [15-17,32,45]. We used the last of approaches to confirm our cross maze results by an independent method.

2.3. Morris Water Maze

The water maze consisted of a circular tank (122 cm diameter) filled with op water. A square escape platform (14 cm x 14 cm) was submerged 1 cm below the su of the water and, thus, hidden from view. Our protocol consisted of cued and h platform training [14]. During both training phases, ample distal cues were availab the mice, including a door, shelving unit, 3 posters on the wall, and a curtained ra cages. For cued platform training, we attached a black-and-white striped pole (2. diameter and 15 cm height) to the center of the platform. The platform was placed i center of one of the 4 quadrants of the pool, and the platform location was cha between sessions (cued platform training) or kept constant (hidden platform trai Mice were released along the edge of the tank facing the wall of the pool, from p located in the 3 quadrants that did not contain the platform. Mice that did not fin platform within 60 sec were guided to it by the experimenter. Mice stayed o platform for 8–10 sec before being returned to their home cages between trials. days of cued platform training were followed by 5 days of hidden platform training 1B). Each day, mice received 2 sessions of 2 trials (cued platform training) or 3 (hidden platform training) with 12 min between trials and 3 h between sessions. A setup from Ethovision (Noldus, The Netherlands) was used to record the swim pat escape latency of each mouse.

The morning after the last day of hidden platform training, mice received a 6 probe trial for which the platform was removed. Mice were released from a point

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opposite from the location of the hidden platform during training. After this stan probe trial and a 15-min rest, Group 1 was given one more training trial to reinforce hidden platform location and was then tested in a 60-sec cued probe trial to perfor place-cue competition test (Fig. 1C). A black and white striped pole visually identica that used for cued platform training was placed in the center of the quadrant opposit the quadrant in which the platform was located during hidden platform training, bu platform was present in the pool. Mice were released along the edge of the pool, fa the wall, at a point halfway between the pole and the location where the platform use be hidden. The same distal cues present during the training were available during both hidden and cued probe trials.

2.4. Visual Acuity Test

The task developed by Prusky et al. [71] was adapted for mice (Fig. 1D). We us tank 30.5 cm tall, 72 cm long and 40.6 cm wide at the wider end. After emerging f the release chute, mice could swim into either of 2 arms of the tank that were 20.3 wide and 35.6 cm long from the decision point to the end. At the end of one of the a an escape platform (7.6 x 12.7 cm) was hidden 1 cm below the surface of opaque w At the end of both arms were slots in which we placed square placards (17.8 cm sid One had a fine black-and-white checkered pattern on it, and the other had broader ver black and white stripes. The platform was always located beneath the striped placard. side on which the platform and striped placard were located was varied pseudo-rando between trials.

Mice received one pretraining session consisting of 10 forced-choice trials 10 apart. During pretraining, the incorrect arm with the checkered placard was blocked

transparent wall and mice learned to swim to the striped placard to escape. Mic remained on the platform for 5 sec before they were removed from the water and place in their home cages.

The visual acuity test consisted of an acquisition phase, in which mice were trained t criterion, and a performance phase, in which visual acuity was determined. Mice fror Group 2 completed both phases. Mice from Group 3 were sacrificed fc immunohistochemical analysis 2 days after reaching criterion in the acquisition phase Each day, during both phases of the test, mice received 1–2 sessions, consisting of 1 blocks of 1 to 3 trials, with 10 min between blocks and 2–3 h between sessions. Afte release, mice were allowed to swim until they found the escape platform. They wer scored as incorrect if they entered the wrong arm and passed through the decision poir (Fig. 1D). Mice remained on the platform for 5 sec before they were removed. If mic were correct on the first trial, they were returned to the home cage to await the next tria if they were incorrect, they were immediately released again from the chute. Mice tha continued to choose the incorrect arm in the third trial were returned to their home cag Thus, since mice received 1–3 trials per block and 10 blocks per session, they receive 10—30 trials per session. Compared with what has been reported for rats [71], hAPP mic showed greater variability in their performance in this paradigm (data not shown). W therefore modified the criteria used by Prusky. Mice were considered to reach criterion they were correct in the first trial of at least 9 of 10 blocks in 1 session, at least 8 of 1 blocks in 2 consecutive sessions, or at least 7 of 10 blocks in 3 consecutive sessions.

The relationship between the thickness of the black and white stripes and visu acuity was calculated from the distance between the place in the tank at which mice ha

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to select one of the two arms and the end of the arms. During the acquisition phase, w used a placard set corresponding to 0.09 cycles per degree (cpd), well within the visu acuity limit previously reported for C57BL/6 mice [24,71]. Once mice of Group reached criterion at the 0.09 cpd visual acuity level, they were moved on to th performance phase. We increased the visual acuity level by using placards wit increasingly narrower black and white stripes (corresponding to 0.10, 0.21, 0.30, 0.3 0.40, 0.44, 0.48, 0.51, 0.55, 0.60, and 0.64 cpd). Once mice reached criterion, they wer tested with the next higher visual acuity placard. Visual acuity was scored as the la visual acuity level at which mice were able to reach criterion within 5 consecutiv sessions.

2.5. Immunohistochemical analyses

Mice were anesthetized with chloral hydrate and perfused transcardially with salin The left hemibrain was snap frozen and stored at –80°C. The hippocampus and caudat putamen (striatum) were microdissected from the whole hemibrain on ice for $A\beta_{1-x}$ an $A\beta_{1-42}$ measurements by ELISA [34,35,55]. The right hemibrain was fixed in 4 paraformaldehyde at 4°C for 2 days, transferred into 30% sucrose in PBS for overnigl incubation at 4°C, and then sectioned coronally with a sliding microtome. Sections (3 μm) were collected in cryoprotectant, stored at –20°C, and immunostained with tl standard avidin-biotin/peroxidase method. After quenching endogenous peroxida activity and blocking nonspecific binding, hippocampal sections were incubated wi rabbit anti-calbindin D-28K (CB) (1:15,000, Swant, Bellinzona, Switzerland) or rabt anti-Fos (1:10,000, Ab-5, Oncogene Research Products, San Diego, CA), and striat

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sections with rabbit anti-phosphorylated CREB (pCREB) (1:300, Cell Signaling, Beverly, MA). Primary antibody binding was detected with biotinylated goat anti-rabbit antibody (1:200, Vector Laboratories, Burlingame, CA) and diaminobenzidine as the chromagen.

BioQuant Image Analysis (R&M Biometrics, Nashville, TN) was used to determine the integrated optical density of CB immunoreactivity (CB-IR) in 2 coronal sections (300 μm apart) per mouse between –2.54 and –2.80 mm from bregma. We averaged the integrated optical density of 2 areas (0.04 mm^2 each) in the molecular layer of the dentate gyrus and of 2 areas (0.04 mm^2 each) in the stratum radiatum of the CA1 region. Since CA1 levels of CB are not significantly altered in hAPP mice from line J20, we normalized the level of CB-IR in the dentate gyrus to CA1 CB-IR and presented the data as relative CB-IR levels [63].

The number of Fos-positive granule cells in the dentate gyrus was counted manually in a series of coronal hippocampal sections (~300 μm between sections) throughout the anteroposterior extent of the hippocampus [63]. Metamorph (Molecular Devices Corp., Sunnyvale, California) was used to estimate the relative number of pCREB-IR cells in the dorsomedial and the dorsolateral striatum in 2 striatal sections (300 μm apart) at a level just anterior to the fornix. The average of cells counted per region and section in each mouse was used for statistical analyses.

2.6. *Statistics*

Statview 5.0 (SAS Institute, Cary, NC) was used to perform statistical analyses. We assessed differences between means by Student's t test or by one-way or two-way ANOVA followed by Tukey/Kramer or Fisher's PLSD post hoc tests. Bar graphs represent means ± SEM. Bivariate tabular analyses comparing expected and observed

frequencies were performed with χ^2 tests. Correlations were assessed by simple regression analysis. Dentate gyrus CB-IR was calculated relative to CA1 CB-IR. The numbers of Fos-IR and pCREB-IR cells are presented as values relative to the mean of NTG controls. The relative numbers of Fos-IR cells used in regressions with behavioral data are shown as the natural log of the total numbers of cells counted in each mouse. Null hypotheses were rejected at the 0.05 level.

3. Results

3.1. Neuronal expression of hAPP/Aβ affects strategy choice of mice in the cross maze

The cross maze task can be solved by allocentric or egocentric navigational strategies, and the type of strategy used by individual mice can be probed by releasing them from the arm opposite to that used for training (Fig. 1A). We hypothesized that hAPP mice would make less use of allocentric strategies as a result of hAPP/Aβ-induced

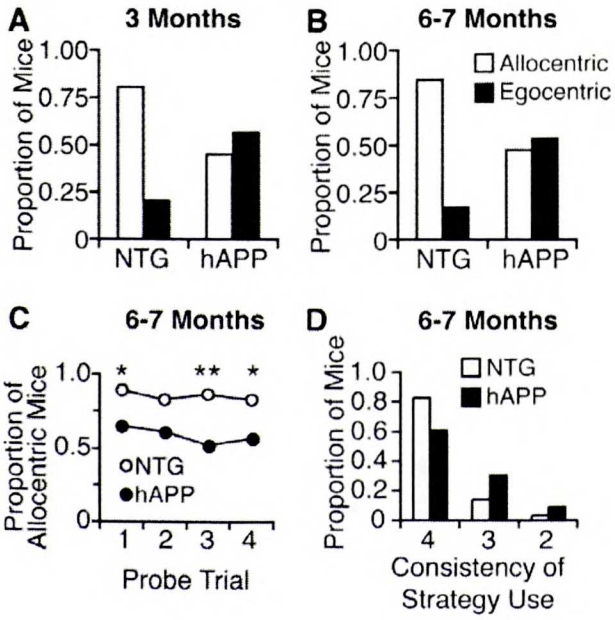

Figure 2. Cross maze testing reveals a persistent increase in egocentric strategy use in hAPP mice. The learning strategy of mice was assessed in the cross maze at 3 months (A) or 6–7 months (B) (3 months: 20 NTG, 18 hAPP; 6–7 months: 19 NTG, 17 hAPP). (C) Another group of 6–7-month-old mice (30 NTG, 22 hAPP) was probed repeatedly after 10, 30, and 50 additional training trials following the first probe trial. *$p<0.05$, **$p<0.01$ by χ^2 tests. (D) Consistency of their strategy use during the repeated probe trials. The x-axis indicates in how many of the 4 trials mice used the same strategy. There was no significant difference between NTG and hAPP mice in the numbers of mice that maintained the same strategy or switched strategies by χ^2 test ($p>0.05$).

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hippocampal impairment. At 3 and 6–7 months of age, the proportion of mice showing evidence for an allocentric strategy on the probe trial was significantly smaller in hAPP mice than in NTG controls (Fig. 2A and B). The genotype effect on strategy use was significant by χ^2 tests for both the younger (χ^2=5.15, p<0.025) and the older (χ^2=5.57, p<0.025) groups.

Whereas rats transition from allocentric to egocentric strategies with prolonged cross maze training, C57BL/6 mice do not [9,50,64]. To rule out the possibility that the hAPP mice were simply more random and made more mistakes at the first probe trial, we continued cross maze training in one cohort of mice and conducted multiple probe trials. The continued training and repeated probe trials revealed that the reduced allocentricity of hAPP mice was robust and persistent. At all additional probe trials, only 50–65% of hAPP mice were allocentric compared with 80–90% of NTG mice (Fig. 2C). Most hAPP mice and NTG controls chose the same strategy for all 4 probe trials (Fig. 2D), indicating that strategy use was consistent across time and training. NTG and hAPP mice did not significantly differ in the frequency with which they chose the same or different strategies across the 4 probes ($\chi^2 = 3.80, p > 0.10$).

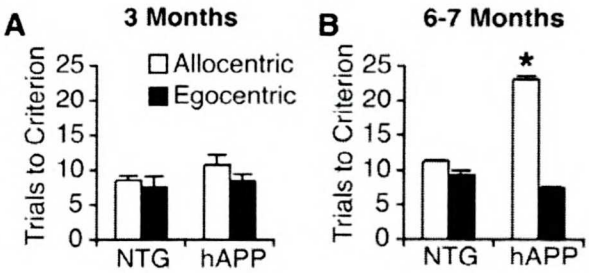

Figure 3. Allocentric, but not egocentric, hAPP mice show age-dependent learning deficits in the cross maze. The ability of NTG and hAPP mice to acquire the cross maze task was assessed at 3 months (A) or 6–7 months (B) (3 months: 20 NTG, 18 hAPP; 6–7 months: 49 NTG, 39 hAPP). *p<0.05 vs. all other age-matched groups by Tukey-Kramer post hoc test.

3.2. Allocentric, but not egocentric, hAPP mice have learning impairments in the cross maze

To assess the relationship between strategy use and spatial learning, we analyzed the number of trials to reach criterion in the cross maze. We hypothesized that hAPP mice persisting in hippocampus-dependent allocentric strategies would be more impaired because of hAPP/Aβ-induced hippocampal dysfunction. At 3 months of age, all mice were able to learn the location of the escape platform in the cross maze independent of their strategy preference (Fig. 3A). At 6–7 months, a two-way ANOVA revealed significant main effects of genotype ($p=0.001$) and strategy ($p<0.0001$) on trials to criterion, and a significant interaction between genotype and strategy ($p<0.0001$). Allocentric hAPP mice required more than twice as many trials as NTG mice to reach criterion, whereas egocentric hAPP mice performed as well as NTG controls (Fig. 3B).

3.3. Addition of local cue during probe trial reveals difference between allocentric and egocentric hAPP mice in MWM test

After determining the navigational strategy preference of mice in the cross maze, we examined whether strategy preference was related to performance in the MWM. The cued component of the MWM can be learned by egocentric strategies, whereas learning the spatial component of this task enforces the engagement of allocentric strategies [45,51,61]. At 3 months, hAPP mice had no difficulties learning to locate the cued platform but swam longer distances than NTG controls before reaching the hidden platform ($p<0.01$ by repeated measures ANOVA) (Fig. 4A). In spite of the latter deficits, 3-month-old hAPP mice performed as well as age-matched NTG controls in the standard

MWM spatial probe, indicating that they successfully applied allocentric strategies to learn the location of the hidden platform (Fig. 4B). Strategy use in the cross maze had no relationship to the performance of the hAPP mice in the cued or hidden platform MWM tasks or in the spatial MWM probe trial ($p>0.05$ by repeated measures ANOVA), similar to previous reports [56].

To assess the dependence of NTG and hAPP mice on distal versus local cues, 3-month-old mice received another hidden platform training trial after the standard spatial probe trial followed by a cued probe trial for which the platform was removed from the target quadrant and a visible cue was placed in the opposite quadrant (Fig. 1C). Similar to the cross maze, the cued MWM probe trial is a place-cue competition

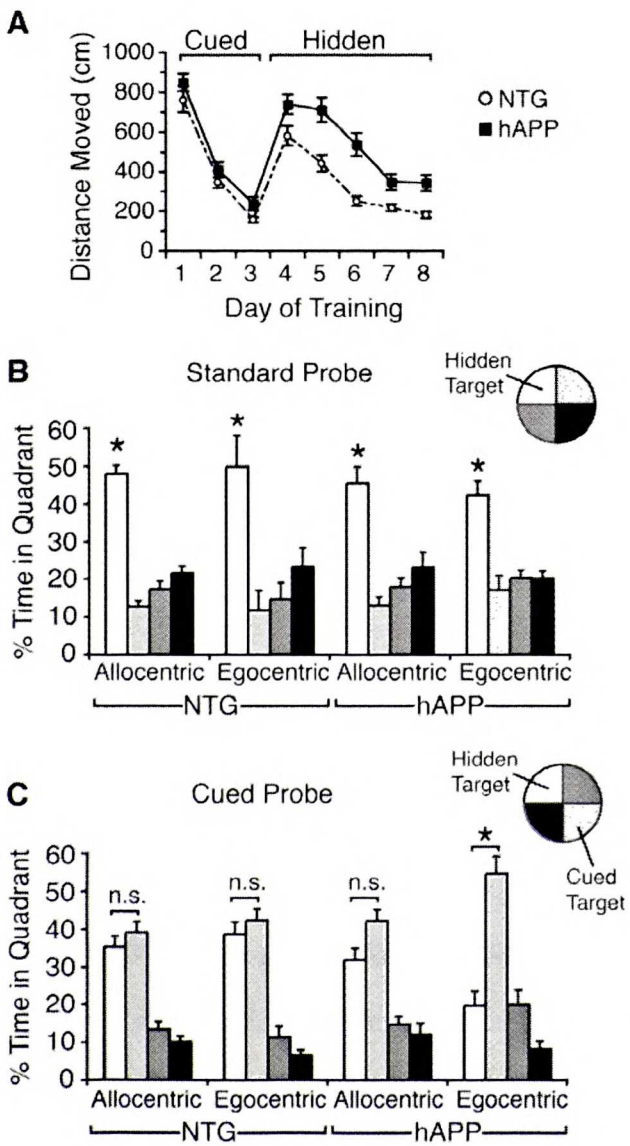

Figure 4. MWM performance of 3-month-old mice. (A) 3-month-old mice were tested in the MWM (20 NTG, 18 hAPP). Learning curves for NTG (dashed line) and hAPP (solid line) mice represent decreases in the total distance mice swum before they found the platform or were placed on it during training. (B) Standard MWM probe trial (Fig. 1C), during which the platform was removed from the target quadrant and swim paths were monitored for 60 sec. Mice were grouped by genotype and by predominant strategy use established in the cross maze test. *$p<0.05$ by one-sample t tests against the null hypothesis of chance (25%). (C) Performance of the same mice in a subsequent cued probe trial (Fig. 1C). *$p=0.001$ by paired Student's t test.

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test that distinguishes preferences for allocentric versus egocentric strategies [15-17,32,45]. Mice that use hippocampus-dependent allocentric strategies spend more time in the quadrant dictated by the distal cues whereas mice that use egocentric strategies spend more time near the cue. We hypothesized that hAPP mice that were egocentric in the cross maze would spend more time near the cue than hAPP mice that were allocentric in the cross maze.

In the cued MWM probe trial, all mice showed a small bias toward the quadrant in which the visible cue was placed, similar to previous studies in wildtype rats [16,32]. Compared with NTG mice, hAPP mice spent significantly less time in the original target quadrant that had contained the hidden platform during training (35% ± 2.5% vs. 25 ± 2.8%, p=0.011). This difference was caused by the egocentric hAPP mice, which spent significantly less time in the original target quadrant and more time in the cued quadrant than all other groups (Fig. 4C).

At 6–7 months, hAPP mice were impaired in both the spatial and the cued components of the MWM as well as in the spatial MWM probe trial (data not shown), consistent with previous studies of hAPP mice at this age [39,63,80]. Since these older hAPP mice never acquired a preference for the target quadrant in the first place, we did not test their performance in the cued probe trial.

3.4 Normal visual acuity in hAPP mice

To exclude the possibility that decreased visual acuity accounts for the difficulties of the older allocentric hAPP mice in acquiring the cross maze (Fig. 3B) and cued MWM tasks, we assessed 6–7-month-old mice in a visual acuity test (Fig. 1D). hAPP mice and NTG controls had a similar visual acuity of roughly 0.50 cpd (Fig. 5A), which is

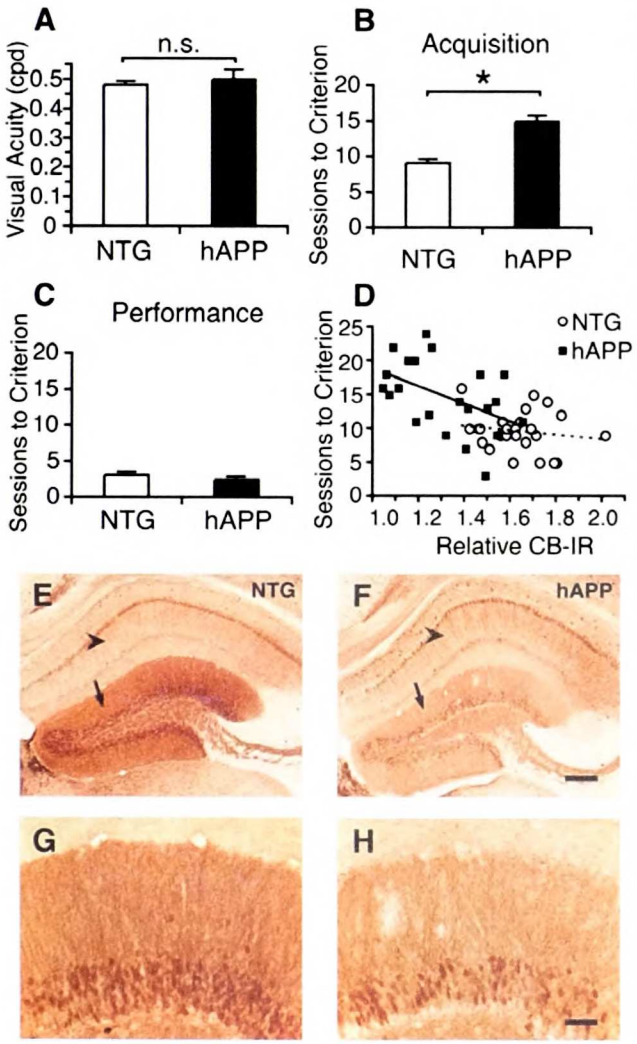

Figure 5. hAPP mice have normal visual acuity, but show learning deficits in the visual acuity test. Six–7-month-old mice (40 NTG, 31 hAPP) were trained in the visual acuity task. (A) A subset of these mice (10 NTG, 9 hAPP) went on to receive a full visual acuity assessment during a performance phase, revealing comparable visual acuity in both groups. (B) When mice were first trained in this task with wide gratings corresponding to visual acuity levels of 0.09–0.1 cpd, hAPP mice required nearly twice as many sessions as NTG controls to reach criterion. *$p<0.0001$ by unpaired Student's t test. (C) After hAPP mice acquired the task, they performed as well as NTG controls in subsequent training with narrower and narrower gratings (corresponding to increasingly higher levels of visual acuity). A repeated measures one-way ANOVA for the number of sessions required to reach criterion at visual acuity levels from 0.10 to 0.55 cpd revealed no significant effect of genotype ($p=0.506$). (D) The number of sessions required to reach criterion in the acquisition phase of the test correlated negatively with relative calbindin immunoreactivity (CB-IR) levels in the molecular layer of the dentate gyrus in hAPP mice (solid line: $R^2=0.22$, $p=0.03$), but not in NTG controls (dashed line: $R^2=0.03$, $p=0.41$). Dentate gyrus CB-IR is presented here as levels relative to CB-IR in CA1. (E–H) Examples of coronal hippocampal sections from NTG (E and G) and hAPP (F and H) mice, depicting CB depletion in the dentate gyrus (arrow) but not CA1 (arrowhead) of the hAPP mouse. Scale bars, 200 μm (E and F) and 50 μm (G and H).

consistent with values reported for other NTG C57BL/6 mice [24,69-71]. However, hAPP mice required almost twice as many sessions as NTG controls to reach criterion in the acquisition phase of this task (Fig. 5B). After successful acquisition of the task, hAPP and NTG mice required a similar number of sessions to reach criterion at each level of

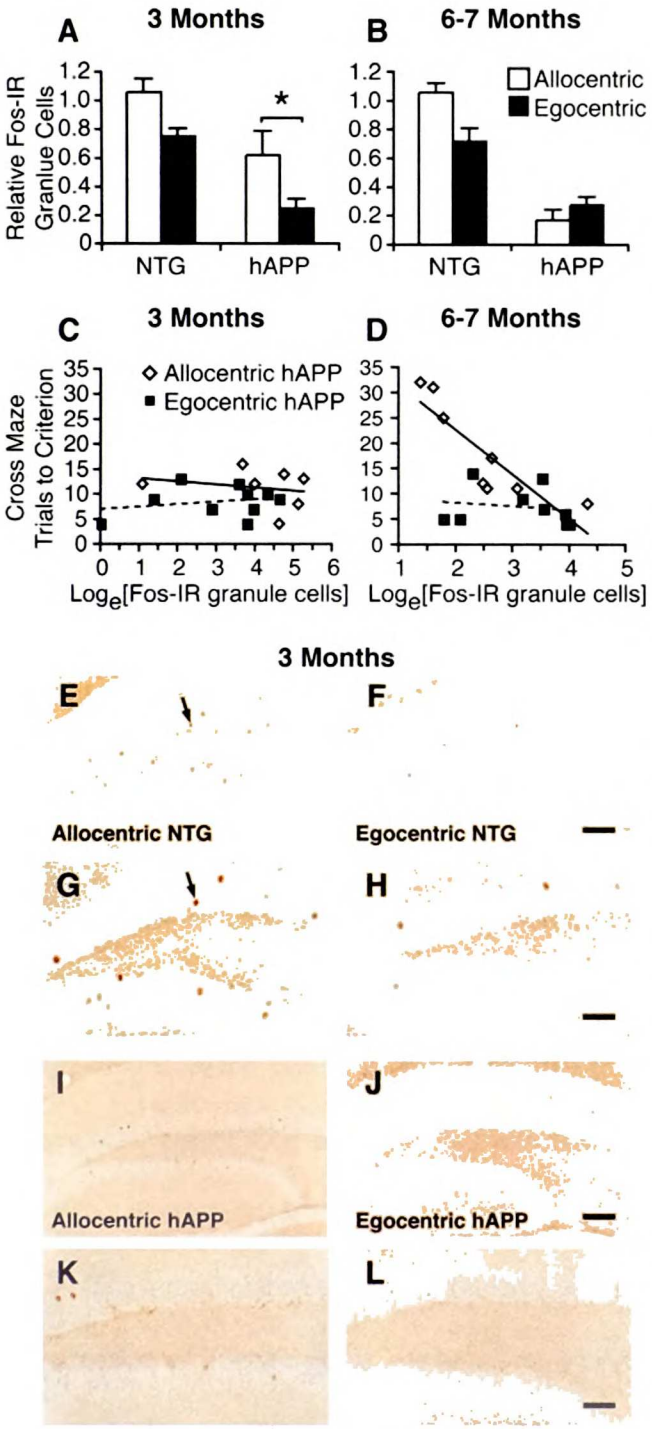

Figure 6. Neuronal Fos expression in the dentate gyrus is greater in allocentric than in egocentric mice and correlates with cross maze learning in allocentric hAPP mice. (A and B) The number of Fos-IR granule cells in the dentate gyrus was determined in hAPP mice and NTG controls at 3 months (A) and 6–7 months (B) (3 months: 20 NTG, 18 hAPP; 6-7 months: 18 NTG, 17 hAPP). For each age group, the mean level of Fos-IR cells in NTG mice was defined as 1. In the younger mice, a one-way ANOVA revealed a main effect of genotype ($p=0.0005$) and strategy ($p=0.009$), but no interaction between them. In the older mice, there was a main effect of genotype ($p<0.0001$) but not strategy ($p=0.38$) and a significant interaction between them ($p=0.024$). *$p<0.05$ by Tukey-Kramer post hoc test. (C and D) To examine by linear regression analysis the relation between learning efficiency and Fos expression in the dentate gyrus, we transformed the number of Fos-IR granule cells to a natural log scale and related these data to the number of trials required by allocentric (solid line) and egocentric (dashed line) hAPP mice to reach criterion in the cross maze at 3 months (C) and 6–7 months (D). Only 6–7-month-old allocentric hAPP mice showed a significant negative correlation between these variables ($R^2=0.75$, $p=0.01$). (E–L) Examples of coronal hippocampal sections stained for Fos in an allocentric NTG mouse (E and G), an egocentric NTG mouse (F and H), an allocentric hAPP mouse (I and K), and an egocentric hAPP mouse (J and L), depicting Fos-IR granule cells (arrow). Scale bars, 100 μm (E, F, I, and J) and 50 μm (G, H, K, and L).

visual acuity (Fig. 5C), indicating that hAPP mice have normal visual acuity but significant deficits in associating a visual cue with an outcome of escape.

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3.5. Relationship of learning deficits to alterations in synaptic activity-dependent proteins in the hippocampus and striatum of hAPP mice

To explore the relationship between hAPP/Aβ-induced behavioral deficits and specific brain regions, we measured expression of neuronal activity-dependent proteins in the hippocampus and striatum. We assessed baseline levels of synaptic activity-dependent proteins in these regions, which reflect tonic neuronal activity [42,77], focusing on Fos and calbindin (CB) in the dentate gyrus and on activated, phosphorylated (p) CREB in the striatum because learning deficits of hAPP mice in the MWM correlate tightly with reductions in baseline levels of Fos and CB in the dentate gyrus [63]. In addition, navigational strategy choice relates closely to Fos levels in the dentate gyrus in NTG rats and mice [12,64] and to pCREB (but not Fos) levels in specific subfields of the striatum in rats [12].

Ability to learn the visual acuity task correlated with CB levels in the dentate gyrus in hAPP mice but not in NTG controls, with higher CB levels associated with fewer sessions to reach criterion (Fig. 5D). In addition, there was a trend in hAPP mice for a negative correlation between Fos expression in the dentate gyrus and learning in the visual acuity task (R^2=0.18, p=0.054).

We hypothesized that the reduced allocentricity of hAPP would be associated with a reduction of dentate gyrus Fos levels as a group. Regardless of strategy preference, hAPP mice had lower Fos levels in the dentate gyrus than NTG controls at 3 (Fig. 6A) and 6–7 (Fig. 6B) months of age, paralleling strategy alterations. On an individual level, strategy preference itself was related to Fos expression in both groups of NTG mice and in 3-month-old hAPP mice, with Fos levels being lower in egocentric than allocentric mice

(Fig. 6A and B). In older hAPP mice, Fos levels were severely reduced in both egocentric and allocentric mice (Fig. 6B). Interestingly, there was a robust negative correlation between the number of Fos-positive granule cells and the number of trials to criterion in the cross maze test in the older allocentric hAPP mice but in none of the other groups (Fig. 6C, D and data not shown).

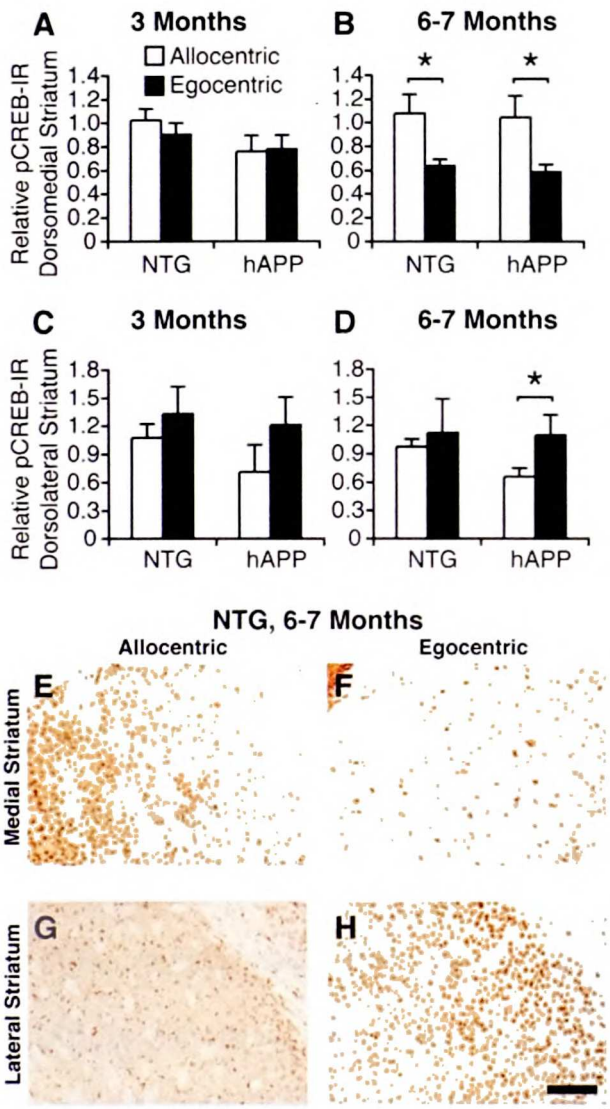

Figure 7. Reduction of neuronal pCREB expression in the dorsolateral striatum of allocentric, but not egocentric, hAPP mice. The levels of neuronal pCREB immunoreactivity were compared in the dorsomedial (A and B) and dorsolateral (C and D) striatum of hAPP mice and NTG controls at 3 months (A and C) or 6–7 months (B and D) months (3 months: 20 NTG, 18 hAPP; 6–7 months: 18 NTG, 17 hAPP). For each brain region and age, the mean of readings obtained in NTG mice was defined as 1. (A and B) In the dorsomedial striatum, levels of pCREB-IR cells were similar in all groups at 3 months (A) and higher in allocentric than egocentric NTG and hAPP mice at 6–7 months (B). A two-way ANOVA for dorsomedial striatal pCREB-IR cells in 6–7-month-old mice revealed a main effect of strategy ($p=0.026$), but not genotype ($p=0.83$). *$p<0.05$ by Fisher's PLSD post hoc test. (C and D) In the dorsolateral striatum, levels of pCREB-IR cells tended to be higher in egocentric than allocentric mice. This trend was stronger in hAPP mice than in NTG controls and reached significance only in 6–7-month-old hAPP mice (D). *$p<0.05$ by Fisher's PLSD post hoc test. (E–H) pCREB-immunostained coronal sections of the medial (E and F) and lateral (G and H) striatum for 6–7-month-old allocentric (E and G) and egocentric (F and H) NTG mice, depicting more striking examples of differences between allocentric and egocentric mice. Scale bar, 100 μm.

Because egocentric hAPP mice were not impaired at solving the cross maze at any age, we hypothesized that striatal levels of activity-dependent proteins would be spared. Previous studies in NTG rodents have related neuronal CREB activity in the dorsolateral

striatum to egocentric strategy preference, whereas the dorsomedial striatum is thought to be critical for allocentric learning [16,83]. Expression of hAPP/Aβ had no significant effect on striatal pCREB levels at 3 or 6–7 months of age (Fig. 7). At 6–7 months, levels of pCREB-positive cells in the dorsomedial striatum were higher in allocentric than egocentric hAPP and NTG mice (Fig. 7A and B). For both ages and genotypes, levels of pCREB-positive cells in the dorsolateral striatum tended to be higher in egocentric than allocentric mice, although this difference reached statistical significance only in the older hAPP mice (Fig. 7C and D).

3.6. Aβ levels are higher in the hippocampus than the striatum of hAPP mice and comparable in allocentric and egocentric hAPP mice

To determine the regional patterns of pathology in the brains of hAPP mice, we assessed Aβ levels in the hippocampus and striatum. Since behavioral alterations in hAPP mice are plaque-independent and the presence of plaques confounds the measurement of soluble Aβ levels [39,55,63,80], we used 3-month-old mice without plaques to measure hippocampal and striatal levels of $A\beta_{1-x}$ (approximates total Aβ) and $A\beta_{1-42}$. Levels of $A\beta_{1-x}$ and $A\beta_{1-42}$ and $A\beta_{1-42}/A\beta_{1-x}$ ratios were higher in the hippocampus than the striatum (Fig. 8). Egocentric and allocentric

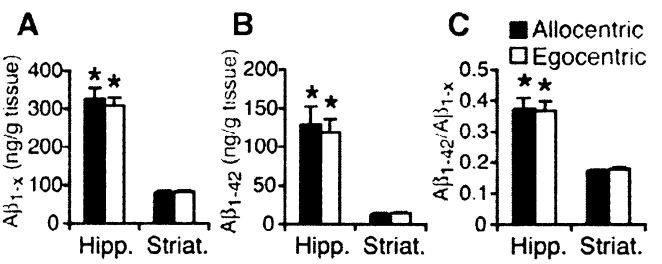

Figure 8. Aβ levels in hippocampus and striatum of hAPP mice at 3 months of age. We used ELISAs to measure Aβ levels in the whole hippocampus (Hipp.) and whole striatum (Striat.) of 3-month-old hAPP mice. Of these mice, 8 were allocentric and 10 were egocentric. $A\beta_{1-x}$ levels (A), $A\beta_{1-42}$ levels (B), and $A\beta_{1-42}/A\beta_{1-x}$ ratios (C) were all significantly lower in the striatum. ANOVA revealed no relationships between strategy and Aβ levels or $A\beta_{1-42}/A\beta_{1-x}$ ratios. *$p<0.001$ vs. striatum by paired t test.

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hAPP mice did not differ in hippocampal or striatal levels of $A\beta_{1-x}$ and $A\beta_{1-42}$ or in $A\beta_{1-42}/A\beta_{1-x}$ ratios, consistent with the comparable age-dependent depletion of Fos and CB in the dentate gyrus of these mice.

4. Discussion

Our study demonstrates that neuronal expression of hAPP/Aβ is sufficient to alter navigational strategy use in hAPP mice. At 3 and 6–7 months of age, over half of the hAPP mice used egocentric strategies to solve the cross maze task, whereas most NTG controls used allocentric strategies. Notably, only the hAPP mice that used allocentric strategies showed learning deficits in this test; those that used egocentric strategies performed as well as NTG controls. These results may be relevant to spatial disabilities of patients with AD since Aβ accumulates to high levels in AD brains and many navigational challenges encountered in people's lives can be solved by allocentric or egocentric strategies, similar to the cross maze task used here. Individuals who are able to engage hippocampus-independent strategies may be less spatially impaired, whereas those who continue to engage the hippocampus may perform worse. Thus, differences in strategy preferences may help explain some of the heterogeneity in spatial disability among both hAPP mice and patients with AD.

Like humans [29,30,79], rodents have a natural inclination to use allocentric strategies when confronted with navigational tasks that can be solved by allocentric or egocentric strategies [9,10,64]. This inclination may reflect the greater efficiency and flexibility of allocentric strategies, which appear to depend in good part on the hippocampus and the dorsomedial striatum [12,16,60,83]. While highly adaptive under

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normal circumstances, this navigational strategy preference could become maladaptive when one or both of these brain regions are altered by disease.

Several lines of evidence suggest that Aβ-induced impairments of the hippocampus might play a particularly important role in AD-related alterations in navigational strategy. In rats, lesions of the hippocampus or the dorsomedial striatum can shift the navigational strategy preference from allocentric to egocentric in the cross maze and impair learning during cued and hidden platform training in the MWM [15-17,60,83]. hAPP mice and humans with AD have marked reductions of CB in the dentate gyrus; in hAPP mice, these alterations correlate with deficits in the MWM and with the depletion of other synaptic activity-dependent proteins, including Fos [11,62,63]. The current study confirmed the depletion of Fos and CB in the dentate gyrus of hAPP mice and revealed that pCREB expression in the dorsomedial striatum is well preserved. Thus, the shift from allocentric to egocentric strategy preference in hAPP mice is more likely caused by hAPP/Aβ-dependent impairments of the hippocampus than of the dorsomedial striatum.

The findings that hAPP/Aβ expression decreased allocentric strategy use among hAPP mice and that a proportion of hAPP mice persistently adhered to an allocentric strategy even though that strategy was disadvantageous relative to the behavioral task the mice were required to solve might be viewed as a paradox. Notably, it is consistent with results obtained in human studies. Only half of human patients with medial temporal lobe resections engage egocentric strategies in a virtual radial maze even though persistent use of allocentric strategies led to learning impairments [5]. The hippocampus is also critical for cognitive flexibility. For instance, hippocampal lesions in rats impair the ability to solve navigational tasks when task demands change [13], suggesting a crucial role of the

hippocampus in applying adaptive spatial strategies. In our cross maze experiment, strategy alterations appeared at earlier ages in hAPP mice than spatial learning deficits, paralleling the early presence of dentate gyrus Fos depletions. Hippocampal dysfunction could impair the ability to choose appropriate strategies independent of determining which particular strategy each mouse prefers. These data, together with previous studies, may help explain why there was no relationship between hippocampal levels of Aβ and strategy use in the hAPP mice. High levels of Aβ in the hippocampus are sufficient to deplete synaptic activity-related proteins severely in the dentate gyrus [11,62,63, and current study] which may disable the hippocampus, causing a more proximal effect on strategy use.

A shift from allocentric to egocentric strategy preference can result not only from lesions of the hippocampus or dorsomedial striatum [60,83], but also from increased activation of the dorsolateral striatum [49,58]. Importantly, hAPP mice had no increase in neuronal CREB activity in the dorsolateral striatum. The finding that egocentric hAPP mice performed at NTG levels in the cross maze, but not better, also suggests that striatal function is not enhanced in hAPP mice. Consistent with this interpretation, a study of fronto-striatal long-term potentiation showed no differences between hAPP mice and NTG controls [49]. Lastly, stimulation of the dorsolateral striatum with glutamate caused nearly all treated rats to engage egocentric strategies [58], in contrast to the partial increase in egocentric strategy use found here. Taken together, the above findings suggest that the increased use of egocentric strategies in hAPP mice is more likely due to impairments in the hippocampus than to an aberrant overstimulation of neurons in the dorsolateral striatum.

Interestingly, Fos expression in the dentate gyrus correlated with learning ability in the cross maze only in 6–7-month-old allocentric hAPP mice but not in any of the other groups. Since Fos depletion in hAPP mice clearly worsened with age, it is conceivable that the learning ability of allocentric hAPP mice was more critically dependent on remaining granule cell activity at 6–7 months than at 3 months. Since egocentric strategies are relatively independent of the dentate gyrus [8,9,16,60,83], it is perhaps not surprising that the learning ability of egocentric hAPP mice in the cross maze was unrelated to neuronal Fos expression in the dentate gyrus. What is remarkable, though, is that egocentric 6–7-month-old hAPP mice had no learning deficits in the cross maze, which, like many spatial tasks in daily life, can be solved by allocentric or egocentric strategies. In contrast, both allocentric and egocentric hAPP mice were impaired in the MWM, which requires allocentric strategies. Thus, whether neuronal expression of hAPP/Aβ results in spatial disability may depend not only on the extent of neuronal dysfunction but also on the range of strategies by which specific tasks can be solved and on the ability of the brain to circumvent focal deficits by the engagement of alternate strategies and brain regions.

The observation that strategy preference of hAPP mice in the cross maze was unrelated to cued or hidden MWM learning implies that spatial learning ability and strategy use are dissociable processes. Other studies have reported that rats engaging allocentric strategies perform better in tasks of allocentric spatial learning [45]. Our data, however, are consistent with previous work in C57BL/6 x SJL mice showing that strategy preference in the MWM place-cue competition test is unrelated to cued or hidden MWM performance [56]. Furthermore, we found that strategy use was altered before spatial

learning ability in the cross maze, supporting the dissociation between learning ability and strategy preference. We did, however, find a relationship between strategy use in the cross maze and strategy in the MWM place-cue competition test. In contrast to the cued and hidden MWM tasks, the place-cue competition task allows mice to choose between two equally successful strategies. These findings are further support that the spatial learning and strategy impairments and alterations that are revealed depend on the demands of the experiment.

In spite of normal visual acuity, 6–7-month-old hAPP mice were impaired in the cued MWM task, implying more generalized cognitive deficits that could also involve the striatum. These mice were also impaired at solving the visual acuity task that requires that they associate a visible cue with escape. The dorsolateral striatum is critical for stimulus-response learning and underlies beacon-oriented navigation [21,59]. However, the cued MWM task also engages allocentric processes [26,81]. Lesions of the hippocampus in C57BL/6 mice cause deficits in cued MWM learning, suggesting that the hippocampus plays some role in these tasks traditionally thought to be striatum-selective [41]. In our study, the number of sessions to reach criterion in the visual acuity task was correlated with dentate gyrus CB levels, further implicating hippocampal dysfunction in the deficits in beacon-oriented navigation. In spite of the cued MWM deficits, older hAPP mice that employed egocentric strategies performed as well as controls in learning the cross maze task, indicating that striatum-dependent learning is spared.

One potential caveat in our study is that we used a water version of the cross maze, whereas most previous studies have employed dry-land cross maze tasks. One could argue that stressful conditions, such as cold water immersion, could alter strategy use and

spatial learning, and that hAPP/Aβ does not have a primary effect on cognition, but rather induces a high level of stress that in turn affects behavior. In support of this argument are reports that stress can impair spatial learning [18,38]. Applying dry-land versions of water maze tasks can also alter spatial strategy preferences [25,82]. However, the effects of stress on cognition may only manifest in situations of extraordinary stress. For instance, water testing is not sufficient to block allocentric strategy preference in rats, whereas fifty foot-shocks prior to behavioral testing shift rats toward egocentric strategies [38]. Another study showed that spatial learning is impaired in rats by stress when the task is difficult (six-arm radial maze), but not when the task is relatively simple (four-arm radial maze) [18]. Also, place-cue competition tests performed in the MWM have revealed a remarkably consistent roles for the hippocampus and striatum in allocentric and egocentric learning [15-17,32,45], suggesting that water maze strategy tasks have neuroanatomical substrates similar to dry-land tasks.

In our study, we employed a simple two-choice paradigm in the cross maze and pretrained animals extensively to minimize the potential confound of stress on cognition. Furthermore, previous studies in our lab have shown that hAPP mice of the line J20 do not spend more time in the closed arms of the elevated plus maze, consistent with the interpretation that the hAPP mice are not characterized by increased stress levels [11], similar to other mouse models of AD [80]. The NTG C57BL/6 mice in our water maze study performed almost identically to wildtype C57BL/6 mice assessed in dry-land cross maze tasks, with the greater majority of mice engaging allocentric strategies at early and late probe trials, suggesting that the water and dry-land tasks are assessing similar cognitive mechanisms [9,64]. However, future studies should further address differences

and similarities in the neuroanatomical substrates of dry-land versus water versions of the cross maze task.

Another potential caveat in our study is that most previous work investigating the neuroanatomical substrates of cross maze learning was performed in rats, and there may be interspecies differences in the relationship of the hippocampus and striatum to spatial mechanisms. For example, as discussed above, whereas rats transition from allocentric to egocentric strategies over time, C57BL/6 mice persistently engage allocentric strategies [9,50,64]. However, available data reveals a remarkably consistent relationship of the hippocampus to allocentric processes and the striatum to egocentric processes across species. As in rats [12], sustained hippocampal expression of Fos characterizes mouse strains that predominantly use allocentric strategies compared with mouse strains that do not [64]. Furthermore, just as in rats, hippocampal inactivation with lidocaine in mice just prior to the cross maze probe trial blocks the expression of allocentric strategies to a similar extent [50,60]. Although additional studies of cross maze learning in mice are necessary to compare rat and mouse data more fully, mouse studies have with parallels even in human navigation. Humans who use allocentric strategies to solve a virtual eight-arm radial maze activate the hippocampus, whereas those who employ egocentric strategies activate the striatum [6,31]. Finally, our finding that only about half of hAPP mice engage egocentric strategies parallels observations of strategy alterations in humans with medial temporal lobe resections [6], further supporting the idea that hippocampal and striatal roles in navigation are well preserved across species and tasks.

Why hAPP mice and human hippocampalectomy patients do not all adopt egocentric strategies remains an intriguing question. Edelman and colleagues have pointed out that

neural systems are characterized by extensive degeneracy, 'the ability of elements that are structurally different to perform the same function or yield the same output' [19]. The concept of degeneracy holds that distinct neural networks may perform the same function, allowing one network to compensate for loss or damage of another [67,68]. The ability to engage alternative neural networks is context-dependent, influenced both by environmental factors and by the organism's particular internal states [19]. Further elucidation of the precise environmental conditions and internal states that allow mammals to engage alternative neural networks when preferred ones are unavailable is of fundamental interest and might culminate in the development of better therapeutic strategies for patients with damage to some, but not other, neural systems.

The results of repeated probe trials during prolonged cross maze training demonstrate that mice do not readily change their navigational strategy preference, regardless of whether they are allocentric, egocentric, hAPP, or NTG. Rats in natural learning contexts tend to use allocentric strategies early in training and switch to egocentric strategies with overtraining [9]. However, consistent with our findings is a previous report showing that NTG C57BL/6 mice are predominantly allocentric at both early and later time points in cross maze training [64]. It is therefore all the more remarkable that the neuronal expression of hAPP/Aβ so profoundly altered the predominant strategy preference in hAPP mice. It will be interesting to determine if anti-Aβ treatments can prevent or reverse this effect. Complementary behavioral or pharmacological approaches might focus on promoting the more effective engagement of strategies and brain regions that are still relatively well preserved, particularly in individuals who use strategies involving brain regions that are severely impaired by disease.

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Acknowledgments

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Chapter 3. Spatial Deficits in Alzheimer's Disease Reflect Right-Sided Atrophy of the Human Navigation Network

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Abstract

Navigation deficits are common among Alzheimer's disease (AD) patients, although the underlying cognitive and neural mechanisms are unknown. We hypothesized that navigation deficits in AD reflect selective spatial cognitive deficits, rather than generalized cognitive decline, and are related to damage to brain regions critical for navigation. We tested 13 mild AD and 21 MCI patients with a hallway navigation task and on various aspects of their route memory. Group differences and correlations between gray matter volumes and accuracy in the spatial tasks were investigated using voxel-based morphometry (VBM) considering components of the previously described spatial navigation network (posterior hippocampus and parietal regions) as a region of interest. Mild AD and MCI patients performed as well as controls in recognizing whether they had seen particular landmarks. However, when asked to draw the route or find landmark locations on a map, both patient groups were significantly impaired. One-half of AD patients and one-quarter of MCI patients got lost when finding their own way on the route, compared with less than one-tenth of controls. Patients who did and did not get lost did not differ on any other neuropsychological test including the Mini-Mental Status Exam. A VBM group analysis revealed that patients who got lost had significantly reduced right posterior hippocampal and right posterior parietal volumes compared to patients that did not get lost and age-matched controls. VBM covariate analysis revealed that right posterior hippocampal and right posterior parietal volume correlated significantly with subjects' ability to identify locations on a map of the route, whereas memory for the order in which landmarks were encountered correlated with volume of a region in the dorsolateral prefrontal cortex. We conclude that the navigation disability

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characteristic of AD and MCI is due to a specific impairment of the spatial cognitive domain and is determined by the extent to which AD-related atrophy impacts the right-lateralized human navigation network. Navigation tests may be valuable early markers of AD and could predict which MCI patients will develop AD.

Keywords: Alzheimer's disease; spatial cognition; mild cognitive impairment; hippocampus; parietal cortex

Abbreviations: AD = Alzheimer's disease; BA = Brodmann's area; BNT = Boston Naming Test; CDR = Clinical Dementia Rating; CVLT-MS = California Verbal Learning Tests – Mental Status Version; IPL = inferior parietal lobule; MCI = mild cognitive impairment; MMSE = Mini-Mental Status Exam; MRI = magnetic resonance imaging; PHG = parahippocampal gyrus; RLT = route-learning test; ROI = region of interest; SPM = statistical parametric mapping; VBM = voxel-based morphometry; VOSP = Visual Object and Space Perception Battery

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Introduction

Spatial navigation deficits occur in roughly half of Alzheimer's disease (AD) patients [42,70] and have a profound effect on daily activity [31,74]. Patients tend to get lost in new and, as the disease progresses, familiar environments. In spite of the frequency of spatial disability, its neural and cognitive bases are unknown. One possibility is that navigation impairment is merely one manifestation of the generalized cognitive decline seen with AD. Alternatively, spatial cognition could be independently impaired in some patients, with impairment related to the particular neural regions affected by the disease. We sought to distinguish between these two possibilities and to determine whether atrophy of specific neural structures relates to the likelihood of navigation deficits.

AD results in a characteristic regional pattern of neuropathology, with medial temporal and posterior cortical structures affected earlier than other regions. Quantification of senile plaques and neurofibrillary tangles indicates that the entorhinal region and hippocampal formation are affected at earlier stages than other neocortical areas [14,15]. Structural magnetic resonance imaging (MRI) studies also show gray matter loss in the medial temporal region earlier than frontal cortical regions in most but not all patients [21,32,86]. Later in the disease, atrophy and pathology becomes widespread and severe. The selective early damage may account for the selective cognitive deficits seen in AD but not with normal aging.

Numerous imaging and lesion studies have revealed a consistent set of neural regions comprising a spatial navigation network in humans [17,52]. Of these regions, the hippocampus and parahippocampal gyrus (PHG) are preeminent, and are thought to play critical roles in incorporating distal cues into allocentric (world-based) spatial maps

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[4,11,13,17,28,29,34,41,43,56,66,76,81]. The hippocampus and PHG are activated during navigation of virtual environments [29,41,44,45,52,56], while lesions to these regions result in deficits in allocentric mapping and navigation [2,8,11-13,30,68,72,82]. It has been hypothesized that the right posterior hippocampus and right PHG are critical for navigation, whereas left-sided hippocampal regions are thought to be more important for episodic memory [17,27,55,57] in conjunction with frontal areas, particularly the dorsolateral prefrontal cortices [19,75,88].

In addition to medial temporal structures, posterior parietal regions are also important in human navigation though they are more involved in egocentric (self-based) spatial processes such as the processing of local cues [47]. Posterior parietal structures are involved in visuoperceptual processes critical for navigation, such as the perception of optic flow [25] and the translation of information between allocentric and egocentric reference frames [5,10,41,51,77,80,83]. Posterior parietal lesions, particularly on the right, can cause topographical agnosia and disorientation [8,51,83,84]. Lateral and inferior regions of the parietal cortex including the inferior parietal lobule (IPL), situated along the temporo-parietal junction, participate prominently in egocentric perspective-taking and spatial cognition [71,84,91]. In contrast, more medial parts of the parietal cortex seem to play more general roles in spatial and nonspatial episodic memory [18].

Because the hippocampus, PHG, and lateral inferior posterior parietal cortex play critical roles in human spatial navigation and are among the earliest regions damaged by AD [16,32,79,86,89], we focused on these regions as the potential mediators of AD-related spatial navigation deficits. We hypothesized that damage to these regions, particularly on the right, would increase the likelihood of navigation deficits. We

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concentrated on mild cognitive impairment (MCI) and mild AD patients in order to isolate specific cognitive deficits and their anatomical underpinnings. To study spatial cognition, we used a real-life route-learning task (RLT), because table-top tests may not be sensitive to actual navigation deficits [40,53,54,61]. Other groups have used a similar RLT to demonstrate AD-related navigation deficits [22,23,65,73]. We adapted this paradigm and included the most robust measures of navigation deficits, such as the Photograph Location test in which subjects identify the location of a photographed landmark on a map. Because of the putative role of the hippocampus in episodic memory, we also added an order memory test in which subjects were asked which places were encountered first along the route. We characterized the performance of mild AD and MCI patients on these navigation-related tests and identified a group of patients that failed to navigate the route. Using voxel-based morphometry (VBM), we assessed whether patients that became lost had particular patterns of atrophy in the human navigation network in comparison to patients that did not get lost, and investigated whether the extent of atrophy correlated with other measures of spatial ability.

Material and methods

Subjects and neuropsychological screening

We recruited 21 patients with MCI (12 men, 9 women) and 13 patients with AD (9 men, 4 women) through the University of California, San Francisco (UCSF) Memory and Aging Center clinic and Alzheimer's Disease Research Center. We included patients who had Mini-Mental Status Exams (MMSE) scores of 20 and greater. Twenty-four

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cognitively normal age-matched controls were recruited through the UCSF Alzheimer's Disease Research Center. We obtained written informed consent from all participants and all experiments were approved by the Committee on Human Research (UCSF). Twelve of 13 AD patients, all 21 MCI patients, and 21 of 24 controls were right-handed. The AD group included 11 patients diagnosed with probable AD and 2 patients diagnosed with possible AD. The mean ages of each group were 73.6 years for the mild AD group, 73.1 years for the MCI group, and 68.0 years for the normal controls (Table 1). Ten mild AD patients, 12 MCI patients, and 21 normal controls received an MRI within one year of behavioral testing and were included in the VBM analyses. All subjects received a general medical and neurological assessment in clinic and a standard detailed neuropsychological screening to assess other major cognitive domains, as described previously [37]. We first assessed whether diagnostic category had a main effect on the measures included in the neuropsychological screening with one-way ANOVA, followed by Tukey/Kramer post hoc tests comparing each patient group to the normal controls and to each other, rejecting null hypothesis at the 0.05 level. Testing revealed patterns of impairment typical of mild AD and MCI (Table 1). In a second analysis we tested whether deficits in the RTL-Forward task (defined below) were associated with greater general cognitive impairment. For this purpose, we combined all AD and compared patients who made at least one error on the RLT-Forward task to patients that made no errors with Student's t tests and rejected null hypotheses at the 0.05 level.

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Parameter	Mild AD	MCI	Normal
Age (Years)	72.9 (11.2)	71.9 (8.7)	67.7 (7.3)
Education (Years)	17.1 (3.2)	16.9 (2.8)	17.0 (2.4)
Geriatric Depression Scale	2.9 (2.3)	7.3 (4.5)	3.1 (3.7)
Mini-Mental Status Exam Score (0-30)**	22.6 (2.0) †◊	27.3 (2.3) †	29.5 (0.7)
Clinical Dementia Rating (CDR)			
CDR Total (0-3)**	0.8 (0.3) †	0.5 (0.5) †	0.1 (0.2)
CDR Box Score (0-18)**	4.9 (1.5) †◊	2.4 (3.3) †	0.2 (0.5)
Verbal Memory: CVLT-MS			
Trial 1 Correct (0-9)*	2.7 (1.6) †	3.9 (1.6)	5.0 (0.1)
Trial 2 Correct (0-9)*	4.5 (2.0) †	4.7 (1.7)	7.5 (0.7)
Trial 3 Correct (0-9)*	5.1 (1.0) †	5.5 (1.6)	8.0 (1.4)
Trial 4 Correct (0-9)*	5.0 (1.4) †	5.7 (1.9)	7.0 (1.4)
Trials 1-4 Total (0-36)**	17.4 (4.8) †	19.7 (6.2) †	27.5 (3.5)
30-Sec Delay (0-9)	2.8 (2.1)	4.3 (2.0)	5.0 (2.8)
10-Minute Delay (0-9)*	1.4 (1.8) †	2.8 (2.7)	4.5 (2.1)
# Recognized (0-9)	7.4 (2.1)	7.3 (1.5)	8.5 (0.7)
# False Positives (0-18)*	6.2 (3.9) †◊	2.3 (2.3)	3.7 (4.0)
Executive Function			
Backwards Digit Span (0-7)**	3.2 (1.5) †	4.2 (1.2) †	5.4 (1.3)
Design Fluency # Correct (0-30)**	3.2 (1.8) †◊	7.9 (3.9) †	10.8 (2.8)
Modified Trail-Making (lines/min)**	7.0 (8.3) †◊	20.3 (15.1)	30.2 (14.1)
Stroop Correct (1 min) (0-100)**	13.7 (11.4) †◊	33.9 (15.2) †	54.7 (8.4)
Language			
# Correct D Words (1 min)*	10.0 (3.6) †	14.1 (5.8)	18.0 (5.1)
# Correct Animal Words (1 min)**	10.5 (3.8) †	14.1 (6.4) †	25.0 (6.1)
BNT Total (0-15)*	11.8 (3.3) †	12.2 (3.4) †	14.4 (0.9)
Visuospatial Memory			
Modified Rey Copy (0-17)*	13.0 (5.1) †	13.9 (3.5)	16.1 (1)
Rey 10-Minute Recall (0-17)**	3.3 (5.1) †◊	7.5 (4.7) †	13.4 (2.9)
Modified Rey Recognition (0-1)*	0.6 (0.5) †	0.8 (0.4)	1.0 (0.2)
VOSP Number Location (0-10)**	7.1 (2.6) †◊	8.9 (1.5)	9.6 (0.7)

*$p<0.05$; **$p<0.005$ significant effect of diagnosis by one-way ANOVA
†$p<0.05$ compared to normal controls by Tukey/Kramer post hoc test.
◊$p<0.05$ compared to MCI patients by Tukey/Kramer post hoc test.
CVLT-MS = California Verbal Learning Tests – Mental Status Version; BNT = Boston Naming Test; VOSP = Visual Object and Space Perception Battery.

Table 1. Demographics and performance on standard neuropsychological tests of patient groups presented as means (standard deviations). We used one-way ANOVA followed by Tukey/Kramer post hoc tests to reveal effects of diagnosis on performance on different neuropsychological tests.

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Route-learning task

To test navigation ability in a real-life context, we adapted a route-learning test (RLT) used previously [22,23,65,73]. Subjects were taken on a novel route through one floor of the Ambulatory Care Center where the Memory and Aging Center clinic is located (Fig. 1). Although all of the patients had visited the clinic before, none had traversed the particular hallways that comprised the route. The route consisted of six turns. The outside perimeter of the Ambulatory Care Center is made of windows; thus, two of the hallways had windows with views to distal cues in San Francisco. Otherwise, the hallways wound through areas with no view outside. During the first trip along the route, the experimenter noted the number of other people encountered.

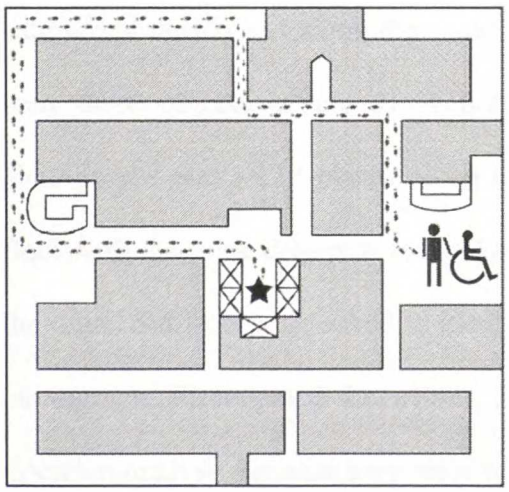

Figure 1. Route-Learning Test. Bird's-eye view of clinic floor, with route drawn in footprints. Experimenters brought the subjects on the route in a wheelchair, starting near the elevators in this position marked with a star. The route consisted of six decision points that were later tested. The perimeter of the building is composed of glass windows. This map was given to patients without the route drawn in for the Map Drawing test.

After traversing the route once, subjects were asked to give directions to repeat the route again in the forward direction (**RLT-Forward**). At each of the six turns, subjects were asked, "Left, right, or straight?" and could respond verbally or by pointing. The experimenter noted how many errors each subject made. If the subject made an error, the experimenter disregarded the subject's direction and took the correct turn instead. After this second experience on the route, subjects were taken to a quiet place in the

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waiting room and were first asked to draw the route on a map (**Map Drawing**). If they failed to draw the route correctly, they were shown four possible route drawings and asked to identify which was correct. Subjects were scored three points if they drew the route correctly, two points if they failed to draw the route but recognized it correctly, and one point if they failed both subtests.

Next, subjects viewed three sets of ten photographs to test their memory for objects and places along the route. Each of these subtests was scored out of a possible ten points. All pictures of places along the route were of objects within the hallways, as opposed to distal cues visible through the windows. The first set of photographs included five that were from places along the route, taken from the perspective of someone traversing the route in the forward direction, and five that were from other places on campus that were similar but clearly not along the route. Subjects were asked to identify whether they had seen these objects or places or not (**Landmark Recognition**). The second set of photographs was all of places along the route. Subjects were also shown a map of the floor with the route drawn in red and three locations identified with large numbers along the route. Subjects were asked to identify which of the three locations were shown in the photograph (**Photograph Location**). Eight subjects were excluded from the Photograph Location analysis because they were tested with a different set of stimuli for this subtest. The third set of stimuli contained ten pairs of photographs from the route, side-by-side. Subjects were asked which place or object was encountered first (**Order Memory**).

After completing the photograph tests, subjects were taken in the wheelchair to the end of the route and asked to give directions to traverse the route in the reverse direction. At the same six turns tested in the forward direction, the experimenter asked, "Left, right,

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or straight?" and subjects answered either verbally or by pointing. The experimenter noted how many errors were made (**RLT-Reverse**) and brought the patient along the correct route. Once reaching the beginning of the route, subjects were asked to keep in mind the location of the starting point, and that they would be taken along the route again, but this time asked at four locations to point directly back to the starting point. This task was added to assess dead reckoning, or the ability to keep one's position relative to a starting place continuously updated (**Dead Reckoning**).

Statistical analyses of behavioral data were performed with Statview 5.0 (SAS Institute, Cary, NC). We assessed differences between means by Student's t test or by one- or two-way ANOVA followed by Tukey/Kramer post hoc tests. Bar graphs represent means ± SEM. χ^2 tests were used to perform bivariate tabular analyses comparing expected and observed frequencies. We assessed correlations by simple regression analysis. Null hypotheses were rejected at the 0.05 level.

Structural MRI acquisition and voxel-based morphometry

T1-weighted structural images were obtained at the San Francisco VA Magnetic Resonance Unit using a 1.5-T SIGNA system (General Electric, Milwaukee, WI) and a volumetric 3D spoiled fast gradient echo sequence (FSPGR, TR/TE = 27/6 ms) with 0.9 mm in-plane resolution and 3.0 mm thick axial sections. All scans were acquired within one year of behavioral testing. Images were preprocessed and analyzed using SPM2 (http://www.fil.ion.ucl.ac.uk/spm/) and standard procedures [6,35,36]. For all VBM analyses, we created and used a study-specific template and entered age, gender, and total intracranial volume into the design matrix as nuisance variables. We accepted a level of

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significance of $p<0.05$ corrected for multiple comparisons when considering the whole brain and $p<0.001$ uncorrected in a priori regions of interest (ROI). ROIs were defined using the AAL Brain Atlas [87] and applied to the SPM dataset WFU Pick Atlas [58,59] (http://www.fmri.wfubmc.edu/). Two types of statistical analyses were performed:

1) Group analysis: We compared gray matter volumes of patients that made at least one error on the RLT-Forward task (6 AD, 5 MCI) to patients that did not get lost (4 AD, 7 MCI) and normal controls (19, excluding the 2 subjects that made one error on RLT-Forward). The ROI for this analysis included hippocampus, PHG, and IPL bilaterally because of the critical roles of these regions in human navigation [38,53].
2) Covariate analysis: In these analyses no group effect was considered but a covariate-only design was instead used to correlate voxel-wise gray matter volumes with accuracy in the Photograph Location and the Order Memory tasks in each subject. Sixteen patients (8 AD, 8 MCI) and 21 controls were included in the Photograph Location analysis and twenty-two patients (10 AD, 12 MCI) and 21 controls were included in the Order Memory analysis. For the Photograph Location analysis, we used the same ROI used in the group analysis, including the hippocampus, PHG, and IPL bilaterally. For the Order Memory analysis, we used the hippocampus and frontal gray matter as our ROI because of the putative role of these regions in episodic, autobiographical, and order memory [17,19,20,24,49,63,81,82,88].

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Results

Mild AD and MCI patients have spatial deficits in spite of intact landmark recognition

We used a real-life hallway navigation task to characterize navigation deficits in mild AD and MCI patients (Fig. 1). We found that one-quarter of MCI patients and one-half of mild AD patients made at least one error recapitulating the route in the forward direction (RLT-Forward), consistent with previous reports indicating that roughly half of AD patients have navigation deficits [42,70]. In contrast, only 2 of the 24 age-matched cognitively normal controls made an error (Fig. 2A). When tested in the reverse direction, one-half of MCI patients and three-quarters of mild AD patients made at least one error, in contrast to control subjects that made no errors. All but two (1 AD, 1 MCI) of the 34 patients that got lost in the RLT-Forward also got lost on the RLT-Reverse, whereas the two normal controls that got lost on the RLT-Forward made no mistakes on the RLT-Reverse. The number of visits prior to testing and the number of people encountered in the hallways did not significantly impact the likelihood of getting lost in the forward or reverse directions among AD or MCI patients ($p>0.05$, unpaired t tests). These data were therefore not included in any further analyses.

Analysis of the other RLT measures revealed that AD and MCI patients remembered having seen landmarks along the route, but failed to recall when or where they saw them (Fig. 2B-F). MCI and mild AD patients performed no worse than age-matched controls and well above chance in the Photograph Recognition task. In contrast, when asked to identify which of three locations displayed on a map corresponded to a photographed

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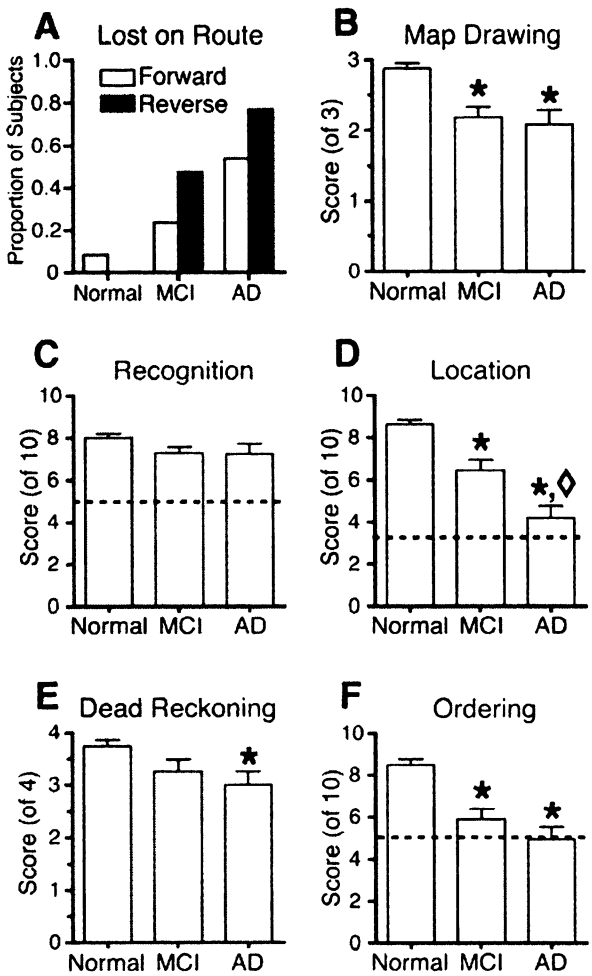

Figure 2. MCI and mild AD patients recognize landmarks that they have seen, but fail at tests of spatial mapping and order memory. (A) Many patients failed to navigate the route in the forward and reverse directions. The proportion of patients making at least one error in each task is presented. χ^2 analyses revealed that the distributions were significant in the forward (χ^2=9.29, p<0.01) and reverse (χ^2=24.60, p<0.001) directions. (B) MCI and mild AD patients also performed poorly on the Map Drawing test (p<0.0001, one-way ANOVA). Only a few patients were able to draw the route correctly, and MCI patients performed as poorly as AD patients. (C) Both patient groups performed at normal control levels on the Photograph Recognition test, indicating that they recalled having seen the photographed places along the route (p>0.05, one-way ANOVA with diagnosis as the main effect). All three groups performed significantly better than chance (p<0.001 by one-group t tests against the null hypothesis of a score of 5). (D) MCI and mild AD patients performed significantly worse than controls on the Photograph Location test (p<0.0001, one-way ANOVA). In contrast to MCI patients and controls, AD patients performed no better than chance (p>0.05 by a one-group t test against the null hypothesis of a score of 3.33) (E) Patients were also mildly impaired in the Dead Reckoning test (p=0.02, one-way ANOVA), although, on average, both groups retained their heading on three of four trials, indicating a less severe impairment than in the other tests. (F) Both patient groups were severely impaired at the Order Memory test (p<0.0001, one-way ANOVA), and neither group performed significantly better than chance (p>0.05, one group t tests against the null hypothesis of a score of 5). * p<0.05 compared with controls, by Tukey/Kramer post hoc tests; ◊ p<0.05 compared with MCI patients, by Tukey/Kramer post hoc test. Dotted lines indicate chance level.

location (Photograph Location), mild AD and MCI patients performed significantly worse than normal controls. Furthermore, MCI patients performed as poorly as mild AD patients when asked to draw the route on a map (Map Drawing), and both patient groups performed significantly worse than controls. There was a significant though milder deficit in the Dead Reckoning task among both AD and MCI patients; the patients still kept their bearing in a majority of the Dead

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Reckoning trials (Fig. 2D). Overall, mild AD and MCI patients had significant navigation and mapping deficits, even though they recalled having seen the places along the route.

In addition to spatial mapping impairments, MCI and mild AD patients had severe order memory deficits. Both patient groups performed significantly worse than controls and obtained scores not significantly different than chance (Fig. 2F). Interestingly, Photograph Location and Order Memory scores did not significantly correlate with one another (R^2=0.12, p>0.05), such that performance on one task was not a good predictor of the other. These data suggest that these two tests reflect separable cognitive processes (spatial mapping and episode ordering) that could be independently affected by disease.

Overall disease severity does not determine which patients have navigation deficits

To determine whether navigation deficits were a result of specific impairments to the spatial cognitive domain rather than to generalized cognitive decline, we used the MMSE and other standard neuropsychological tests of patients that did and did not get lost on the route. We defined getting lost as making at least one error on the RLT-Forward and analyzed data for the subjects included in the VBM analysis. We found that MMSE and CDR scores did not differ between mild AD and MCI patients that did and did not get lost on the RLT-Forward (Table 2). Similarly, measures of verbal, nonverbal, and working memory, language, executive function, and visuospatial memory also did not distinguish patients with spatial impairment from patients without spatial impairment. These data suggest that navigation deficits are not a product of generalized cognitive decay and instead represent selective damage to the spatial domain.

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Parameter	Not Lost	Lost
Age (Years)	76.3 (4.9)	69.7 (12.2)
Education (Years)	16.6 (2.0)	17.6 (4.0)
Geriatric Depression Scale	5.3 (5.5)	6.0 (4.2)
Mini-Mental Status Exam Score (0-30)	26.5 (3.1)	24.3 (3.0)
Clinical Dementia Rating (CDR)		
CDR Total (0-3)	0.5 (0.4)	0.7 (0.4)
CDR Box Score (0-18)	2.2 (1.9)	4.0 (2.5)
Verbal Memory: CVLT-MS		
Trial 1 Correct (0-9)	3.4 (1.5)	3.0 (2.0)
Trial 2 Correct (0-9)	5.0 (1.2)	3.8 (1.9)
Trial 3 Correct (0-9)	5.9 (1.6)	4.9 (0.8)
Trial 4 Correct (0-9)	6.0 (1.5)	4.8 (1.4)
Trials 1-4 Total (0-36)	20.3 (4.9)	16.4 (4.8)
30-Sec Delay (0-9)	4.0 (1.9)	2.7 (2.4)
10-Minute Delay (0-9)	2.8 (2.3)	0.8 (1.5)
# Recognized (0-9)	7.9 (1.4)	6.4 (2.6)
# False Positives (0-18)	3.4 (4.8)	5.9 (2.5)
Executive Function		
Backwards Digit Span (0-7)	3.8 (1.5)	3.3 (1.5)
Design Fluency # Correct (0-30)	7.1 (5)	4.5 (2.0)
Modified Trail-Making (lines/min)	15.0 (19.3)	13.6 (11.8)
Stroop Correct (1 min) (0-100)	26.6 (12.2)	18.6 (14.8)
Language		
# Correct D Words (1 min)	12 (5.3)	13.6 (4.7)
# Correct Animal Words (1 min)	13.8 (5.5)	12.0 (5.9)
BNT Total (0-15)	13.3 (2.2)	11.3 (3.8)
Visuospatial Memory		
Modified Rey Copy (0-17)	14.6 (2.1)	11.4 (6.2)
Rey 10-Minute Recall (0-17)	5.6 (5.4)	5.1 (6.1)
Modified Rey Recognition (0-1)	0.8 (0.5)	0.6 (0.5)
VOSP Number Location (0-10)	9.0 (1.7)	7.1 (2.6)

CVLT-MS = California Verbal Learning Tests – Mental Status Version; BNT = Boston Naming Test; VOSP = Visual Object and Space Perception Battery.

Table 2. Standard neuropsychological measures of lost and not lost patients presented as means (standard deviations). We combined all patients who received MRI scans within one year of testing and grouped them by their performance on the forward route-learning task. Lost patients were defined as those that made at least one error repeating the route in the forward direction and not lost patients made no errors. Unpaired t tests revealed no significant difference in any neuropsychological parameter between patients that did or did not get lost ($p>0.05$).

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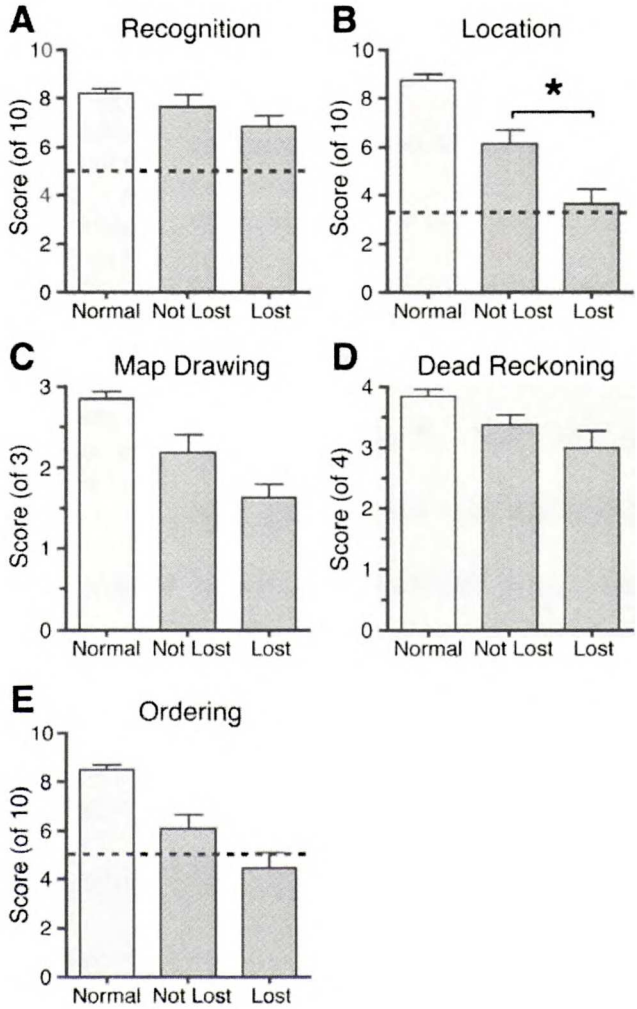
Similar to other neuropsychological measures, patients who got lost did not have significantly lower Landmark Recognition scores (Fig. 3A). However, Photograph Location scores did differ significantly between patient groups, with lost patients performing significantly worse than patients who did not get lost ($p<0.05$, unpaired t test) (Fig. 3B). There were trends for patients who got lost to have lower Map Drawing scores ($p=0.059$, unpaired t test) and lower Order Memory scores ($p=0.069$, unpaired t test) that did not reach significance (Fig. 3C-E). Also, χ^2 analysis revealed no difference in the number of AD and MCI patients in the lost versus not lost groups ($\chi^2=0.73$, $p>0.05$). Overall, these data suggest that navigation deficits are not a product of impairments to other cognitive domains, and show that patients

Figure 3. Behavioral characteristics of lost and not lost subjects included in VBM analyses. A subset of subjects analyzed with the RLT also received an MRI within one year of testing and were categorized as "lost" if they made at least one error in the RLT-Forward task. Among the VBM patients, Photograph Recognition (A) scores did not significantly differ between patients that did and did not get lost ($p>0.05$, unpaired t tests). However, patients that got lost performed significantly worse on the Photograph Location task ($p=0.01$, unpaired t test) (B). These patients also were worse at Map Drawing (C), though this trend did not reach significance ($p=0.059$, unpaired t test). Patients who got lost did not significantly differ from patients that did not get lost on the Dead Reckoning (D) or Order Memory (E) tests ($p>0.05$, unpaired t tests). *$p<0.05$ by unpaired t test.



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who get lost fail on mapping tests in spite of normal landmark recognition.

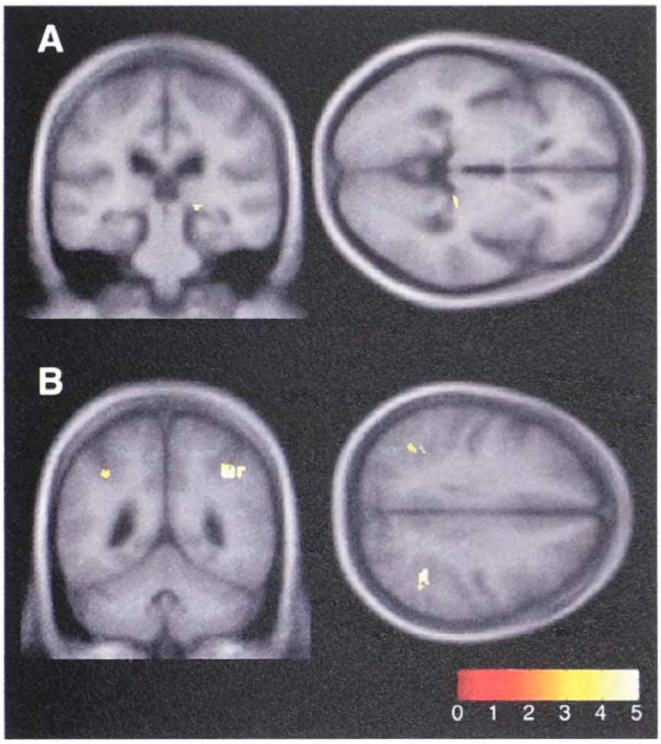

Figure 4. VBM group analysis reveals right-sided hippocampal and posterior parietal atrophy in patients that got lost on the route. Using an ROI analysis at the uncorrected 0.001 level that included the hippocampus, parahippocampal gyrus, and inferior parietal lobule (IPL) bilaterally, we found voxels in the right posterior hippocampus (A) and bilateral IPL (B) that were significantly reduced in patients that got lost compared with patients that did not get lost and age-matched controls. Parietal losses were predominantly right-sided. Whole-brain unbiased analysis with an FWE-corrected p value of 0.05 did not reveal any additional neural regions. Significant regions are displayed on the study-specific template.

VBM reveals greater atrophy of navigation-related structures in patients with navigation deficits and shows correlations between volumes of these regions and some spatial tasks

In the VBM group analysis we compared AD and MCI patients who made at least one error on the RLT-Forward test to patients who did not get lost on the route and to normal controls (Table 3). Mild AD and MCI patients that got lost showed significantly greater atrophy in the right posterior hippocampus ($p<0.001$ uncorrected) (Fig. 4A and Table 3) and in the IPL bilaterally, but predominantly on the right ($p<0.05$ corrected for whole brain) (Fig. 4B). Thus, the RLT may isolate specific neural components of the human navigation network.

We then performed a VBM covariate analysis using data from all subjects regardless of diagnosis or performance in the RTL task, to investigate whether regions within the

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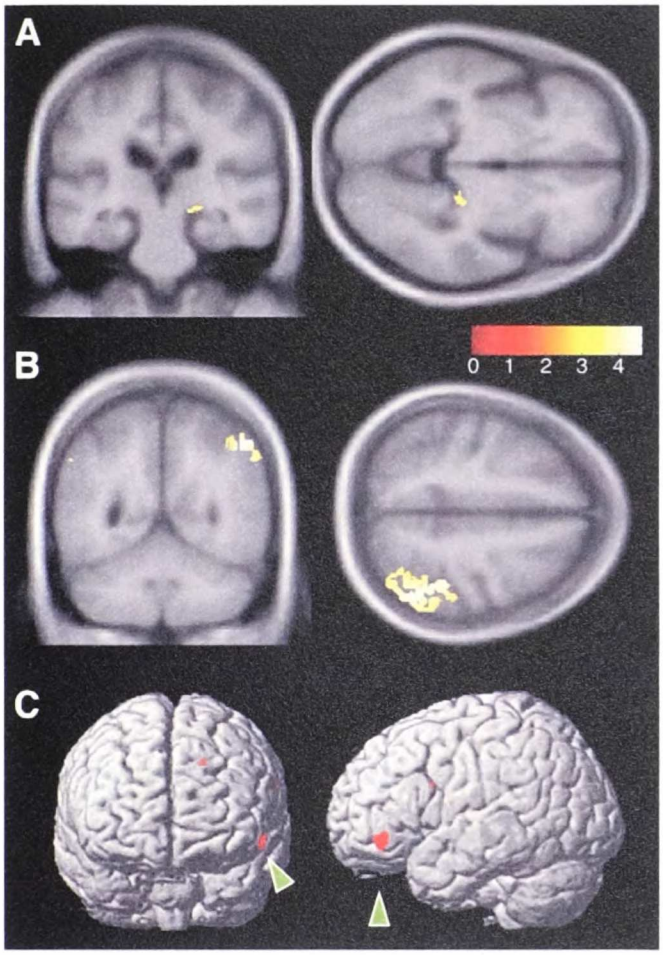

Figure 5. VBM covariate analyses reveal that Photograph Location scores correlate significantly with volumes of regions in the right posterior hippocampus and parietal cortex, whereas Order Memory scores correlate with volumes of regions in the dorsolateral frontal cortex. For the Photograph Location correlation analysis, we used an ROI analysis at the uncorrected p=0.001 level including the hippocampus, parahippocampal gyrus (PHG), and inferior parietal lobule (IPL) bilaterally. Significant voxels, presented on the study-specific template, were found in the right posterior hippocampus (A) and right IPL (B). For the Order Memory correlation analysis (C), we used an ROI analysis at the uncorrected p=0.001 level including hippocampal, parahippocampal, and frontal gray matter bilaterally. Significant regions are presented in red on a 3D rendered image from a single normal subject. Order Memory scores correlated with the volume of a portion of the inferior frontal gyrus (indicated by green arrows), but not with any part of the hippocampus or PHG. Whole-brain unbiased analyses corrected for multiple comparisons at the p=0.05 level did not reveal any additional neural regions in either analysis.

navigation network correlated significantly with performance on the Photograph Location test. We also investigated whether volumes in the navigation network or frontal areas correlated with accuracy in the Order Memory tests. We found volumes that significantly correlated with Photograph Location scores in the right posterior hippocampus (Fig. 5A and Table 3) and IPL, predominantly on the right (Fig. 5B and Table 3). Order Memory scores correlated significantly with the volume of regions in inferior frontal gyrus, particularly on the left, and the left superior frontal gyrus, but not in any part of the hippocampal or parahippocampal regions (Fig. 5C and Table 3). These data, together with the Photograph Location and Order Memory findings, suggest that distinct patterns of atrophy result in

distinct patterns of cognitive deficits, rather than global atrophy resulting in generalized cognitive decline. Specifically, damage to right-sided medial temporal and posterior parietal regions were associated with spatial cognitive deficits, whereas damage to frontal regions including the inferior frontal gyrus was associated with order memory impairments.

Anatomic Region	BA	x, y, z (mm)*	T-Value	Z-Score
Lost Patients vs. Not Lost Patients and Controls				
Right IPL	40	**37, -50, 39**	5.00	4.31
	40	46, -39, 52	4.42	3.91
	40	43, -39, 49	4.16	3.73
Left IPL	40	-57, -46, 21	4.07	3.66
	40	-35, -56, 42	3.93	3.56
Right Hippocampus		20, -29, -4	3.96	3.57
		18, -33, 2	3.35	3.10
Photograph Location Correlation Analysis				
Right IPL	40	50, -56, 48	4.61	4.01
Left IPL	40	-50, -48, 25	3.92	3.51
	40	-47, -54, 37	3.56	3.24
Right Hippocampus	28	21, -26, -7	3.66	3.32
Order Memory Correlation Analysis				
Left Inferior Frontal Gyrus	47	-56, 41, -10	4.45	3.97
	9	-63, 10, 25	3.48	3.22
Left Superior Frontal Gyrus	9	-18, 56, 41	3.63	3.34
Right Inferior Frontal Gyrus	47	57, 41, -7	3.35	3.12

* Montreal Neurological Institute coordinates
Boldface: Also significant when corrected for multiple comparisons ($p<0.05$)
BA = Brodmann's area; IPL = inferior parietal lobule

Table 3. Results of voxel-based morphometry (VBM) analyses. The most significant voxels of each cluster are presented. Each VBM analysis was performed with a defined region of interest at an uncorrected p value of $p<0.001$.

Discussion

Using a real-world navigation task and VBM analyses, we have shown that AD-related navigation deficits are not merely a manifestation of generalized cognitive decline, but

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represent selective impairment of spatial cognition that relates to particular patterns of neural atrophy. We tested a group of mild AD and MCI patients using an objective test of spatial function that can be administered in clinic and found that half of AD patients and a third of MCI patients got lost on a novel route. Patients that got lost did not perform significantly worse in other cognitive domains, demonstrating that navigation deficits are not merely a manifestation of generalized cognitive decline but represent a selective impairment of spatial cognition. Using VBM, we found that AD-related navigational skills related to specific patterns of neural atrophy in the right posterior hippocampus and inferior parietal lobule. We conclude that many MCI and mild AD patients suffer from specific spatial navigation deficits independent of other cognitive domains, and that spatial deficits reflect particular patterns of neurological damage.

MCI and mild AD patients recognized landmarks that they encountered along the route, but failed to incorporate these locations into a map. Both patient groups' performances on the Photograph Recognition subtest were comparable to controls. This is in contrast to previous reports using a RLT in AD patients, in which AD patients failed to recall having seen places along the route [22,23,65]. Most likely, the fact that we studied MCI and mild AD patients accounts for the difference between these reports and our study. Landmark recognition may engage different neural regions than cognitive mapping, including more posterior medial temporo-occipital regions [3,50,62,77], and may only be lost in more advanced stages of AD as atrophy spreads.

We used an RLT because actual navigation engages different neural regions than table-top array or pencil-and-paper tasks and neurologically-lesioned patients have revealed a double dissociation between navigation and these two-dimensional tests

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[40,53,54,61]. In our study, performance on the modified Rey Osterrieth figure test did not predict which patients got lost on the route. Because AD tends to affect the medial temporal lobe earliest, actual navigation tasks may be a more sensitive early marker of AD-related neuropathological changes than other two-dimensional spatial and visuoperceptual measures. Consistent with this notion, MCI patients performed poorly on multiple tests of spatial navigation. One-quarter of them failed to navigate the route in the forward direction, and one-half failed in the reverse direction. They performed as badly as AD patients on the Map Drawing and Order Memory tests, and nearly as badly on the Photograph Location test. The severity of their deficits is striking when one takes into account that these patients do not show serious functional impairment. Our data suggest that navigation tests are especially sensitive to early stages of cognitive decline. Future studies could address whether the RLT measures accurately predict which of the MCI patients convert to AD.

We used VBM to assess whether damage to neural regions critical for human navigation accounted for AD-related spatial deficits. We found that patients that got lost had significant volumetric reductions in the right posterior hippocampus and right posterior parietal cortex compared to patients that did not get lost and age-matched controls. Order memory was instead related to regions in the frontal gray matter, particularly the dorsolateral prefrontal cortex, that has previously been shown to be involved in order memory and both spatial and nonspatial episodic memory [19,20,49,63]. Furthermore, spatial mapping scores and order memory scores did not correlate with each other. Together, these data imply a selectivity of specific cognitive tests to particular neural regions.

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Previous studies of human navigation have implicated hippocampal, parahippocampal, and parietal areas. Patients with hippocampal and medial temporal lesions including the PHG are significantly impaired at spatial navigation tests, particularly when the lesion is on the right [1,2,7-9,11-13,17,48,72]. These deficits appear to be specific for allocentric spatial processing. Interestingly, our study revealed that atrophy in the right posterior hippocampus was related to navigation deficits in AD and MCI. A previous study showed that this region is enlarged in London taxi cab drivers, and that the enlargement correlates with the number of years on the job [57]. Our data complement this study and suggest that the right posterior hippocampus is critical for navigation.

Parietal lesions are more associated with egocentric processing of local landmarks and perspective rather than allocentric mapping. Our finding that RLT impairment was strongly related to parietal atrophy is consistent with a previous report that AD-related navigation deficits correlate with other tests of parietal function [26], and with the notion that much of the spatial disability characteristic of AD may be related to visual perceptual losses such as deficits in the processing of optic flow [46,60,69,85]. Interestingly, a previous functional MRI study on spatial attention suggested a particularly important role for the right posterior parietal cortex. This study reported significantly activation during a spatial attention task in nearly identical regions to those we found to be most significant in the right IPL and intraparietal sulcus [67].

Our finding that both hippocampal and posterior parietal atrophy related to spatial disability implies that both regions are typically damaged in patients with navigation deficits. As has been shown in AD and other disease models, different neural regions can

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compensate for one another if one region is damaged [33,90]. Atrophy of both the hippocampus and posterior parietal cortex may make both allocentric and egocentric strategies unavailable, resulting in frank spatial impairment. Future studies could apply behavioral tests that are more specific for particular spatial processes to tease apart the relative contribution of the hippocampus and parietal cortex. For example, virtual navigation tasks can distinguish between subjects using distal versus local cues [64,78] and allocentric and egocentric strategies [12,43,45]. Because the hippocampus plays a greater role in processing distal cues, whereas the parietal cortex is more involved in processing local cues, such a task might isolate patients with selective damage to the hippocampus or parietal cortex. Previous studies have shown that men prefer to use distal cues and activate the hippocampus while navigating, whereas women prefer local cues and activate parietal regions [39,78]. Though we had too few subjects to address the role of gender in AD-related navigation impairment, male and female patients could be affected differently by distinct patterns of atrophy.

Overall, our VBM analyses implicate a right-lateralized pattern of atrophy involving key regions in the putative human navigation network as the potential neural substrate of AD-related spatial disability. We found that order memory correlated with the volume of a region in the dorsolateral frontal cortex whereas spatial mapping tests were related to right posterior hippocampal and parietal volumes. Therefore, diffuse atrophy does not account for the specific spatial cognitive deficits discussed above; rather, damage to particular regions results in corresponding cognitive impairments. Consistent with this notion, getting lost in the RLT did not relate to performance in tests of other cognitive domains. Our study suggests that whether or not a patient has navigation deficits depends

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on the regional pattern of atrophy that is particular to each patient, and, furthermore, that knowing which regions are affected in a patient may predict which cognitive domains are affected and which symptoms the patient is likely to have.

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Chapter 4. Conclusions and Future Directions

The goal of this thesis project was to investigate and understand the bases of the navigation deficits characteristic of Alzheimer's disease (AD). I tested human patients and a transgenic mouse model to determine what aspects of spatial cognition are particularly disrupted by AD, and to correlate these impairments to measures of neural injury – atrophy of brain regions in the human patients and alterations of synaptic activity-dependent protein levels in the mice. I found that not all spatial processes were disrupted by AD. In human patients, the ability to map landmarks to particular locations was selectively disrupted while landmark recognition was left intact. Furthermore, measures of mapping did not correlate with measures of order memory, suggesting that these cognitive processes can be affected by disease independently. In the mouse, I discovered that although allocentric strategy use was impaired, egocentric strategies were still effective and available to transgenic mice.

Structural and biochemical analyses revealed patterns of neural injury that correlated with these behavioral findings. In patients, atrophy to right-sided structures critical for navigation in normal humans was related to an increased likelihood of getting lost while learning a novel route. Volume of regions in the right posterior hippocampus and right posterior parietal cortex correlated with mapping deficits, whereas volume of regions in the prefrontal cortex correlated with order memory. These results indicate that patterns of neural injury may predict the profile of symptoms a particular patient will encounter. In the mice, hippocampal levels of activity-dependent proteins were significantly lower and Aβ levels higher in transgenic mice compared to nontransgenic mice, whereas the

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striatum was relatively spared. Hippocampal dysfunction likely underlies altered strategy use in the transgenic mice. Interestingly, dentate gyrus Fos levels correlated with spatial learning among older transgenic mice, further highlighting the structure-function relationship between hippocampal damage and spatial disability.

Another theme evident in both the human and mouse projects was that navigation measures revealed deficits early in disease. In the humans, mild cognitive impairment (MCI) patients performed as poorly as AD patients in many spatial tasks, and often not significantly better than chance. In contrast, MCI patients had significantly higher Mini-Mental Status Exam scores and lower Clinical Dementia Rating Box scores than AD patients. The finding of severe navigation impairments in these MCI patients is surprising, in light of the lack of functional impairment among these patients, and suggests that navigation tests could be early markers of cognitive decline in people who are not yet demented. In the mice, I was able to dissociate learning from strategy preference in the cross maze task. Interestingly, there were no learning impairments in the younger transgenic mice in spite of diminished hippocampal levels of synaptic activity-dependent protein levels. In contrast, the younger transgenic mice adopted egocentric strategies to a greater extent than their nontransgenic counterparts. At older ages, transgenic mice were characterized by both altered strategy use and impaired spatial learning. These data suggest that strategy use could be an even earlier indicator of navigation impairment than spatial learning per se.

Both the human and mouse projects shed light on the source of heterogeneity in spatial disability characteristic of patients and mouse models. Among patients, measures of general intellectual function and other cognitive domains did not relate to navigation

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impairment, demonstrating clearly that overall disease severity does not determine which patients have spatial disability. However, volumetric measurements of particular regions did relate to navigation impairments, suggesting that variation in regional patterns of atrophy could explain some of the variation in spatial disability. In the mouse study, transgenic mice that engaged egocentric strategies learned as quickly as nontransgenic controls, whereas older transgenic mice that engaged allocentric strategies were significantly impaired. Because most navigation problems in the real world can be solved by a number of different strategies, it is possible that variation in strategy selection could explain some of the variation in spatial disability.

However, many of the forces driving the heterogeneity of strategy use in the context of hippocampal dysfunction remain obscure. Measures of activity-dependent proteins and Aβ levels did not predict which transgenic mice engaged particular strategies. This finding paralleled observations of humans with hippocampal lesions: half of those subjects used egocentric strategies, and the other half persisted in using allocentric strategies even when they were ineffective. No study has revealed what variables determine each subject's strategy preference after a neurological lesion. From previous work, we know that some brain regions can compensate for other damaged regions. An organism's ability to engage alternative neural structures depends on both environmental factors and the internal states of the organism, including developmental events not easily measured experimentally. Future studies aimed at understanding the conditions that allow the engagement of alternative neural networks could lead to potential therapeutic interventions for patients with damage to some, but not other brain regions, as is the case in AD and MCI.

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The mouse study suggested that one might counteract some of the spatial disability among patients by promoting the use of hippocampus-independent strategies. Critical for this interpretation is confirming that human patients have similar strategy alterations as the mice. Future work could address whether AD patients prefer egocentric strategies by testing patients with tasks that, similar to the cross maze, distinguish between different strategies. One possibility is to apply virtual reality navigation tasks to patients. Though virtual reality tasks have some confounds related to experience with computer use, preliminary work suggests that such tests can be applied successfully in elderly and even demented subjects. Additionally, there are some reports that humans, including neurological patients, can apply spatial knowledge learned in virtual reality to the actual environments emulated. It is possible that some patients could benefit from experience in virtual realities modeled on their daily environments, particularly if processes that are likely spared in AD – such as repetitive route learning – were targeted and ingrained. The mouse data furthermore suggests that emphasizing certain kinds of cues in patients' environments – particularly beacons – may improve patients' ability to navigate through novel places.

Another outstanding question is how traits that are known AD-modifiers impact spatial disability. For instance, we know that ApoE4 genotype augments AD-related deficits. Might ApoE4 subjects have earlier strategy alterations than ApoE3 subjects? Even more interesting, potentially, is the relationship of gender to spatial disability. We know that cognitively normal women tend to engage egocentric strategies, use local cues, and activate the parietal cortex, whereas men engage allocentric strategies, use distal cues, and activate the hippocampus. One might expect that men with AD may suffer from

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navigation deficits earlier than women, and that women may be more liable to engage alternative strategies once hippocampus-dependent strategies become ineffective or unavailable. Furthermore, previous work suggests that women may be more susceptible to the deleterious effects of the ApoE4 genotype. Future human studies that tease apart allocentric and egocentric processes could assess the interaction between gender, ApoE genotype, and disease.

In summary, this thesis project elucidated many of the neural and cognitive substrates of navigation impairment caused by AD. Parallel investigations in transgenic mice and human patients suggested a central role of hippocampal dysfunction in AD-related spatial disability. Hippocampus-dependent spatial processes, including cognitive mapping and allocentric strategy use, were impaired and correlated with measures of hippocampal integrity in both species. While some egocentric processes, including use of local landmarks, may also be impaired, many other egocentric processes, such as beacon-navigation and motor strategy use, are spared, suggesting domain-specific deficits that reflect regional patterns of neural damage. By studying both species, we have gained insight into future avenues of investigation and potential therapeutic interventions.

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