

Place Cell Physiology in a Transgenic Mouse
Model of Alzheimer's Disease

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I, Ming Yi, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Abstract

Alzheimer's disease (AD) is a multifactorial neurodegenerative disorder characterized by progressive cognitive impairments (Selkoe, 2001). Hippocampal place cells are a well understood candidate for the neural basis of one type of memory in rodents; these cells identify the animal's location in an environment and are crucial for spatial memory and navigation. This PhD project aims to clarify the mechanisms responsible for the cognitive deficits in AD at the hippocampal network level, by examining place cell physiology in a transgenic mouse model of AD. I have recorded place cells in tg2576 mice, and found that aged (16 months) but not young (3 months) transgenic mice show degraded neuronal representations of the environment. The level of place cell degradation correlates with the animals' (poorer) spatial memory as tested in a forced-choice spatial alternation T-maze task and with hippocampal, but not neocortical, amyloid plaque burden. Additionally, pilot data show that physiological changes of the hippocampus in tg2576 mice seem to start as early as 3 months, when no pathological and behavioural deficits are present. However, these changes are not obvious at the neuronal level, but only at the hippocampal network level, which represent hippocampal responses to environmental changes. Place cell recording provides a sensitive assay for measuring the amount and rate of functional deterioration in animal models of dementia as well as providing a quantifiable physiological indication of the beneficial effects of potential therapies.

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Introduction

Chapter 1 Alzheimer's Disease: Clinical Features

Alzheimer's disease (AD) is a multifactorial neurodegenerative disorder characterized by progressive cognitive, language and behavioural impairments, eventually leading to dramatic loss of most cortical and subcortical functions and ultimate death (Selkoe, 2001). This chapter discusses the clinical characters of AD, including its epidemiology, risk factors, clinical syndrome, diagnosis and treatment.

1 Epidemiology

AD is named after Alois Alzheimer, who in 1907 first reported the case of a middle-aged woman who developed memory deficits and progressive loss of cognitive abilities accompanied by morbid jealousy (Alzheimer, 1907). Nowadays, AD is the most common type of dementia, constituting approximately 60-70% of all cases. It occurs in mid- to late-life, affecting 7–10% of individuals >65 years of age and 40% of persons >80 years (McKhann et al, 1984; Evans et al, 1989). The prevalence of this disease is increasing because of significant shifts in life expectancy and demographic parameters.

The vast majority cases of AD occur in sporadic form after 65 years of age, lacking alterations in the known set of genetic markers, which suggests either environmental causes or a combination of environmental and predisposing genetic determinants. However, around 5% of AD cases are early-onset (<65 years of age) and have a definite genetic element, whereby they are inherited in an autosomal dominant fashion. Genetic linkage analyses have led to the identification of three genes associated with such familial AD (FAD) – amyloid β -protein precursor (APP) on chromosome 21 (Goate et al, 1991), presenilin-1 (PS-1) on chromosome 14 (Sherrington et al, 1995), and presenilin-2 (PS-2) on chromosome 1 (Levy-Lahad et al 1995; Rogaev et al, 1995). Nevertheless, to some extent, the sporadic late-onset cases of AD also have a genetic component. The most prominent genetic risk factor is the apolipoprotein E (apoE) gene in chromosome 19 (Strittmatter et al, 1993; next section). Because all these genes potentially influence the processing of APP protein and the production of β -amyloid, which is the core component

of amyloid plaques, their functions and mutations will be discussed in the next chapter.

Both clinically and pathologically, FAD and sporadic AD are highly similar or often indistinguishable. This general phenotypic similarity suggests that FAD and sporadic AD may share the same pathogenic pathway leading to the stereotypic neuropathology, that the role of the mutated genes is simply to accelerate the progression of the disease, and that information about FAD is likely to be directly relevant to the pathogenesis of the common, sporadic AD (Hardy and Higgins, 1992).

2 Risk factors

There are behavioural, dietary, environmental and genetical factors that may affect the risk of AD (Table 1.1). Age is the single most potent risk factor, with the prevalence rate increasing dramatically with age (Breitner and Welsh, 1995). Representative prevalence rates are: 5-7% at age 75-85 years; 15-17% at 80-85 years; 35% at 85-90 years; and 50% at 90-95 years (McKhann et al, 1984; Evans et al, 1989). Other risk factors may include low education levels, head injury, consumption of high-calorie diets, and so forth (Mattson, 2004; Blennow et al, 2007; Table 1.1). In contrast, regular consumption of homocysteine-related vitamins, antioxidants, could reduce the risk of AD (Blennow et al, 2007; Table 4.1).

Most AD cases are idiopathic, but to some extent, these cases also have a genetic component. The most prominent genetic risk factor is the apoE4 allele of apoE gene (Strittmatter et al, 1993). Of its three forms ($\epsilon 2$, $\epsilon 3$ and $\epsilon 4$), the presence of $\epsilon 4$ has been shown to increase the likelihood of developing sporadic AD and to accelerate the onset of familial AD, whereas $\epsilon 2$ has a protective effect (Corder et al, 1993; Saunders et al, 1993).

Table 1.1 Key risk factors and protective factors for AD

<p>Primary risk factors: age; family history; genetic markers such as apoE $\epsilon 4$; trisomy 21; mutations in presenilin-1 and -2; female gender after 80 years of age; cardiovascular risk factors such as hypertension, diabetes, obesity, and hypercholesterolemia</p> <p>Possible risk factors: head injury; depression; progression of Parkinson-like signs in older adults; lower thyroid-stimulating hormone within the normal range; hyperhomocysteinemia; folate deficiency; hyperinsulinemia; low educational attainments</p> <p>Possible protective factors: apoE $\epsilon 2$; regular fish consumption; regular consumption of omega-3 fatty acids; high educational level; regular exercise; non-steroidal anti-inflammatory drug therapy; moderate alcohol intake; vitamins C, E, B6, and B12 and folate intake</p>
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Adapted from Desai and Grossberg, 2005

However, the apoE alleles are neither necessary nor sufficient for the development of AD.

Some humans homozygous for the $\epsilon 4$ isoform still show no Alzheimer symptoms in their ninth decade of life and beyond. Conversely, a great many humans develop AD without harboring $\epsilon 4$ alleles. Thus, it is neither specific nor sensitive enough to be used as a diagnostic test.

3 Clinical features of Alzheimer's disease

The primary manifestations of AD include cognitive impairment, neurological abnormalities and neuropsychiatric disturbances (Emilien et al, 2004). Cognition refers to higher brain functions such as learning and memory. Early stage of AD is characterized by cognitive dysfunction including amnesia (loss of memory), aphasia (impairment of language), apraxia (impairment of motor tasks despite normal motor functions) and agnosia (impairment of recognition despite intact sensory functions). Neurological abnormalities include loss of more fundamental neural functions such as impairment of walking and incontinence and occur in the late phases of the illness. Neuropsychiatric alterations are common, though are not necessarily present in all patients and may vary over the course of the illness. Common symptoms include depression, personality change, delusions and hallucinations. Secondary effects of AD include progressive difficulties with instrumental activities of daily living such as driving and cooking, with eventual loss of the ability to do basic daily tasks such as feeding and toileting.

3.1 Pattern of cognitive deficits in AD

Amnesia, aphasia, apraxia and agnosia form the core cognitive dysfunction in AD and are involved in the diagnosis criterion of AD. Determining the progression of AD is important not only for its diagnosis and staging, but also for identifying the corresponding pathogenesis. The majority of patients with sporadic AD exhibit clinical signs during the

Table 1.2 Four-level classification of memory function in aged humans

Levels	Characterization
Normal	Peak performance preserved during senescence
Age-related changes	A decline from the hypothetical peak with cognitive functioning still within the range of same-age peers
Mild cognitive impairment (MCI)	Forgetfulness and significantly worse performance than same-age peers without necessarily experiencing a disruption of daily living activities
Dementia (clinical AD)	A significant deterioration which reaches a level of severity that interferes with daily living activities and becomes consistent with the diagnostic criteria for dementia

Adapted from Mesulam, 1999

seventh decade, whereas individuals with inherited AD often become demented in midlife. The precise onset of clinical AD is very difficult to discern. In general, subtle, intermittent difficulty with the acquisition and retention of new information is the earliest and most salient manifestation of AD (Small et al, 2003; Grundman et al, 2004). Many patients have purely episodic memory impairment with insidious onset for a number of years. When clinical neuropsychological tests are used to evaluate memory in AD patients, recall and recognition performance are impaired in both the verbal and nonverbal domains (Albert, 1996). Several factors contribute to this disruption, including deficiencies in encoding and storing new information and increased sensitivity to disruptive effects of proactive interference.

Impairment in spatial cognition is a common symptom in early AD (Monacelli et al, 2003) and some variants of mild cognitive impairments (Mapstone et al, 2003). More than one-third of patients with AD exhibit visuospatial disorientation with prominent deficits in judging spatial relations (Cogan, 1985) and constructional capacities (Henderson et al, 1989). Patients often complain of “getting lost”, first in unfamiliar environments, subsequently in familiar places as well. Such topographical disorientation in AD has been ascribed to impaired visuospatial function (damage to parieto-occipital regions), to topographical agnosia (damage to parietal lobe) and to an impaired memory for places (damage to the hippocampus and the parahippocampal regions).

Difficulty in speech and language may be also present in early AD (Emilien et al, 2004). Word-finding difficulty is frequently noted and speech output is often diminished. Later in the disease impairment of word comprehension, neologisms and paraphasia occur, which reflects a breakdown in semantic memory function, and a decline in executive functions.

Impairment of other cognitive functions becomes increasingly prominent during the course of the disease. In more affected patients, semantic and remote memory deficits are frequent, whereas procedural memory remains relatively preserved until the very late stages of the disease (Albert, 1996). Apraxia is present in most patients with mild to moderate AD, resulting from damage to parietal and frontal association areas, whereas visual agnosia is common in more advanced stages and results from damage to the visual association areas. The progressive implication of multiple cognitive domains reflects the accumulation of pathological changes in the frontal, temporal and parietal lobes.

3.2 Mild cognitive impairments

The introduction of the concept of “mild cognitive impairment” (MCI) stems from two

aspects of research. Firstly, whereas cognitive impairments characterize AD, a decline in cognitive performance, including difficulties with memory, learning, speed of performance, recall accuracy, and problem solving is also common in the aged population. To evaluate memory function in the aged population, a four-level classification has been suggested (Mesulam, 1999), after a stage of peak performance is reached at some point during the life span. Secondly, it is clear that the neuropathological changes in AD start from the entorhinal cortex, progress to the hippocampus, and affect the neocortex later (Braak and Braak, 1991). However, significant neocortical pathology is already present by the time dementia is clinically diagnosed.

Therefore, AD may be associated with a prolonged preclinical phase, when the cognitive dysfunction is relatively subtle and insufficiently severe for a diagnosis of dementia (Almkvist et al, 1998). This prompted efforts to identify the clinical manifestations of AD in the earliest stage, when neuropathology is primarily restricted in the medial temporal lobe. This is why the concept of MCI generates a great deal of interest. It generally refers to a transitional clinical state of cognitive changes between normal aging and AD, when the subjects have a memory impairment that is out of proportion to that expected for age yet do not meet commonly accepted criteria for clinically probable AD (Petersen et al, 2001; Arnaiz and Almkvist, 2003). As for the relationship between aging, MCI and AD, there is still no consensus if the MCI group represents an inevitable feature of normal aging, an accompanying syndrome of an underlying disease, a separate entity, or simply an early-stage dementia. Also unclear is whether all subjects with MCI will develop AD, or whether the absence of MCI excludes subsequent AD. But one generally accepted conclusion is that individuals with MCI are at an increased risk for developing AD (Dawe et al, 1992; Arnaiz and Almkvist, 2003). Approximately 12% of cases of MCI convert to AD each year (Petersen et al, 1999). Thus MCI may represent a possible prodromal stage of AD (Petersen et al, 1999; Petersen and Morris, 2005).

Despite intense research, the boundary area between aging and the very early stage of AD remains poorly delineated, because the transition from normal cognition to AD is gradual and many of the early cognitive signs of dementia overlap with modifications in normal aging and there are no definitive early diagnostic markers for AD. The current MCI diagnosis requires the presence of memory complaint. One often-used criterion for MCI include complaints of memory problems, memory performance below age-based reference norms, normal performance in other cognitive domains, absence of impairment in instrumental and basic activities of daily living and no diagnosis of dementia (Petersen et al,

1997; Petersen, 2000). However it has been recognized that there are several clinical subtypes of MCI that involve other cognitive domains, either in isolation or in combination with amnesia, such as language, attention, motivation, affect and executive function (Bozoki et al, 2001; Mapstone et al, 2003). Moreover, elderly subjects with MCI involving domains other than memory are two or more times as likely to develop AD within 2-5 years compared to those with memory impairment alone (Bozoki et al, 2001). The subtypes attest to the heterogeneous nature of MCI with several causes leading to the same symptoms.

3.3 Relationships between normal aging and AD

Because of the similarities of cognitive decline in normal aging and AD, it is important to understand the relationship between these two states. Two frameworks have been proposed that differ as to whether aging and AD represent a neuropathological continuum or are dichotomous (Buckner, 2004). Within a unitary factor framework, cognitive decline falls along a single continuum so that only “quantitative” differences separate AD from healthy aging, with the former representing the acceleration or advanced stage of the same processes that lead to more minor cognitive changes in aging. This notion stems from the fact that neurofibrillary tangles and senile plaques, the two neuropathological hallmarks of AD, occur frequently in the brains of nondemented elderly individuals and follow the same pattern of hierarchical vulnerability (Price and Morris, 1999; Morris et al, 2001).

An alternative point of view (multiple factor framework) proposes that aging and AD are not a continuum but are distinct processes that target different brain systems and may independently vary in their level of progression across individuals. Within a multiple factor framework, cognitive decline in nondemented aging may be mild because some individuals, while being influenced by certain aging processes, are spared from the most devastating changes caused by AD. This hypothesis is supported by considerable evidence showing that normal aging and AD can be differentiated both anatomically and clinically (Petersen et al, 1999; Buckner, 2004; Grundman et al, 2004).

Pathologically, subjects of normal aging do not show pathology associated with AD (Buckner, 2004; Mattson, 2004). West et al reported that normal aging is related with losses in the hilus of the fascia dentate and the subiculum but not the dentate granule cell layer, CA3 or CA1, whereas AD is correlated with significant neuronal loss in CA1 in addition to the hilus and subiculum (West et al, 1994; West et al, 2000; West et al, 2004). Similarly, neurologically normal elderly individuals do not show neuronal loss in any layer of the entorhinal cortex, whereas in subjects with very mild AD (using a criterion similar to that

of MCI), there was already a quite extensive loss of neurons in the entorhinal cortex, as much as 50% of the neurons from layer II of entorhinal cortex. In severe AD, almost 90% of the neurons in layer II of entorhinal cortex are missing, with many presumably now having degenerated as end-stage NFT (Gómez-Isla et al, 1996; Price et al, 2001). Finally, Xu et al (2000) reported that measures of hippocampal volume, in addition to the volume of the entorhinal cortex, were able to discriminate between persons with mild AD and MCI, and those who were cognitively normal. Thus normal aging and AD bear different patterns of structural changes, which suggests that these two are not part of a continuous spectrum and do not share a common pathological substrate.

Clinically, age-related memory impairment in humans is different from that seen even in early AD as well. Older adults free from dementia often show difficulties on tasks that stress attention and executive abilities, whereas early AD is characterized by an impairment in declarative memory although deficits on executive function can be present (Buckner, 2004). This dissociation may arise from separate age-associated influences on frontal-striatal circuits and on the medial temporal lobe memory system (Buckner, 2004). Additionally, the alterations in memory associated with early AD are substantially different from those associated with age-related changes in memory (Albert, 1996). Although subjects of normal aging may show significant declines in delayed recall performance, it simply results from the fact that it takes older individuals longer to learn new information, but once learned, it is retained well over numerous delay intervals (Albert, 1996).

4 Diagnosis of Alzheimer's disease

There are no tests that definitively establish the diagnosis of AD in living subjects except brain biopsy. Current diagnosis of AD is most often based on the criteria formulated by the National Institute of Neurologic and Communicative Disorders and Stroke – Alzheimer's Disease and Related Disorders Association (McKhann et al, 1984; Table 1.3), according to which the diagnosis is classified as definite (clinical diagnosis with histologic confirmation), probable (typical clinical syndrome without histologic confirmation), or possible (atypical clinical features but no alternative diagnosis apparent; no histologic confirmation). Establishment of the diagnosis, which is most challenging early in the course of the illness, is based on clinical history, physical examination, neuropsychological testing, brain imaging, and a variety of assessments designed to exclude other causes of dementia. The clinical diagnostic accuracy for AD depends on the stage of the disease and can reach 90% in mid or late stages if standard clinical criteria are followed (Burns et al,

2002). However, since no neuroimaging or laboratory markers now exist for reliable presymptomatic diagnosis of AD, the diagnosis is often forced to wait until individuals become symptomatic.

Clinical manifestation

The classic clinical features of AD are an amnesic type of memory impairment, deterioration of language, and visuospatial deficits. Changes in executive functions also show significant declines early in the disease. Motor and sensory abnormalities, gait disturbances, and seizures are uncommon until the late phases of the disease (Cummings, 2004). In a typical case, onset of AD is slow. Some individuals with incipient AD are aware of their declining abilities, but most patients with evolving AD never acknowledge that they have memory dysfunction. Eventually, recognition may occur because of an apparent sudden crisis, such as getting lost, an accidental fall, or discovery by neighbors or relatives of an unsafe, messy home environment, or acute confusion (delirium) during illness, after surgery or hospitalization, or environmental stress (Desai and Grossberg, 2005). Careful questioning will usually reveal that cognitive impairment and dysfunction have been

Table 1.3 Diagnosis of dementia, probable AD and MCI

Dementia
Loss of memory and one or more other cognitive abilities (aphasia, apraxia, agnosia, or disturbance in executive functioning)
Substantial impairment in social or occupational functioning (decline from a previous level of functioning)
Deficits that do not occur exclusively during the course of delirium
Probable Alzheimer's disease
Typical history of Alzheimer's disease
Insidious onset of symptoms
Gradual progression of symptoms
Cognitive loss documented by neuropsychological tests
No physical signs or neuroimaging or laboratory evidence of other diseases that could cause dementia (such as strokes, Parkinson's disease, subdural hematomas, or tumors)
Mild cognitive impairment
Report (by the patient or an informant) of memory loss
Abnormal memory performance for age (score >1.5 SD below mean for age)
Normal general cognition
Normal activities of daily living
Criteria for dementia not met

Adapted from Kawas, 2003

present for several years before the acute crisis. A decline in calculation abilities is one of

the hallmark cognitive features of AD. Other clinical presentations of AD are psychosis, depression, and agitation/behavioral disturbances.

The clinical phenotype of senile dementia can also be caused by a variety of other diseases, including dementia with Lewy bodies, frontotemporal dementia, Pick's disease, vascular dementia, and so on. Diagnosis of AD can be made only after these disorders have been excluded.

Cognitive tests

The diagnostic utility of memory tests in AD varies according to severity of cognitive impairment. In the preclinical phase, tests of verbal learning and immediate visual recall have the greatest accuracy in predicting the progression to AD (Albert, 1996). For established AD, tests of delayed recall are most sensitive for early AD but less useful for tracking disease progression because performance on these tests often declines rapidly to a plateau. Recognition memory tests, including verbal and visual subtests, are less sensitive than recall tasks for detecting early AD but are more useful for staging disease severity (Albert, 1996).

Brain imaging

Brain structural imaging, including computed tomography and magnetic resonance imaging, is particularly helpful and routinely used in the diagnosis of AD, to exclude alternative causes of dementia and to provide objective measures of preclinical disease and, when measured serially, the rate of change.

Understanding the pattern of pathological changes in AD has prompted the assessment of regional brain volume instead of whole-brain volume. Generalized cortical atrophy with a thinning of medial temporal lobe structures is characteristic of AD, though not pathognomonic of the disease because of the overlap with “normal” aging. Of the many quantitative techniques, volumetric analysis has been shown to have greater diagnostic specificity and sensitivity than linear measurements of atrophy. Volumetric changes on magnetic resonance imaging are consistent with the patterns of neuropathological progression in AD, and the severity of volume loss is correlated with disease severity (de Leon et al, 1997). AD is associated with an increased rate of hippocampal atrophy (Jack et al, 1998), which is prominent even at the presymptomatic stage (Convit et al, 1997). A substantial number of studies have shown that MRI measurements of hippocampal atrophy can distinguish AD from cognitively normal elderly people with 80–90% accuracy (e.g. Blennow et al, 2007). Substantial neuronal loss has occurred by the time atrophy is detectable by MRI (Killiany et al, 2002). It must be mentioned that the absence of cortical

atrophy or medial temporal lobe changes is not sufficient to exclude a diagnosis of AD. In addition, the fact that there is an overlap between AD patients and healthy aged controls and that structural changes at visual inspection are not evident until late in the course of the disease, has prompted the development and refinement of serial MRI coregistration techniques. These techniques are capable of revealing and monitoring the progressive changes of brain volumes in an individual. AD cases show an average 2% atrophy rate, in contrast to 0.25–0.5% in control subjects (O'Brien et al, 2001).

In recent years increasing attention has been devoted to the utility of positron emission tomography (PET), single photon emission computed tomography (SPECT) and functional magnetic resonance imaging (fMRI) in AD diagnosis, because they can detect the subtle pathophysiologic changes of surviving neurons before structural changes are present. (Silverman and Phelps, 2001). PET demonstrates bilateral temporoparietal hypometabolism and hypoperfusion in AD, which is correlated with hippocampal atrophy and the severity of dementia. In more advanced AD, hypoperfusion changes are more widespread and additional reductions in cerebral blood flow are seen in frontal lobes (Jagust, 1998). SPECT studies evaluating regional cerebral blood flow have shown a similar pattern as the one described for PET studies, with relative regional cerebral blood flow paucity in the temporoparietal regions (Camargo, 2001). Novel SPECT cholinergic markers, including muscarinic and nicotinic receptors and cholinesterase activity, have also attracted researchers (O'Brien et al, 2007).

Other tests

Investigators have begun to assess the diagnostic value of determining the levels of β -amyloid protein ($A\beta$) peptides (soluble monomeric species decreased, multimeric species possibly increased) species and of tau (increased) in the cerebrospinal fluid and of establishing apoE genotypes. Currently, however, these assessments are not appropriate for routine diagnostic evaluations (Trojanowski and Growdon, 1998).

MCI

Recognition that MCI may represent a transition state between normal cognitive decline due to ageing and dementia offers possibilities for early diagnosis of AD. Without exhaustive questioning, MCI is often difficult to detect clinically. Patients with MCI should undergo the same laboratory studies as other patients with dementia (Table 1.3). It needs to be noted that MCI is heterogeneous and amnesic MCI subtype may not encompass all of the prodromal states of dementia.

The best predictors of conversion from MCI to AD are functioning in everyday

situations requiring judgment and problem-solving, presence of depression, and hippocampal atrophy (Knopman et al, 2003; Desai and Grossberg, 2005). Prospective studies of people with amnesic MCI have shown that episodic memory (such as delayed recall of word lists and paired-associates learning), semantic memory, attention processing, and mental speed can consistently predict which patients will develop dementia (Chong and Sahadevan, 2005). Similarly, in a retrospective study of people with MCI who had developed AD, verbal and visual memory, associative learning, vocabulary, executive function and other verbal tests of general intelligence were impaired at baseline (Guarch et al, 2004). Longitudinal studies have shown that decreased entorhinal cortex (deToledo-Morrell et al, 2004) and hippocampal volumes (Visser et al, 2002) can predict which patients with MCI will develop AD. Whole-brain, hippocampal, and entorhinal-cortex atrophy assessed by MRI are more common among patients with amnesic MCI who go on to develop AD than among non-converters (Jack et al, 2003; Apostolova et al, 2006).

5 Treatment of Alzheimer's disease

There are no ways to cure AD at present. Current treatments focus on early application of cholinesterase inhibitors and/or NMDA receptor-targeted therapy and treating behavioural and psychological symptoms of AD (Desai and Grossberg, 2005).

Cholinesterase inhibitors (ChEIs) are the first pharmacologic treatments for AD and are being prescribed for more and more people with mild to moderate AD, helping reduce behavioural disturbances, maintain cognitive abilities and delay first dementia-related entry into a nursing home (Doody et al, 2001). However the benefit is rapidly lost after discontinuation, suggesting that the primary benefit is symptomatic rather than neuroprotective. Another limitation is that efficacy may not be sustained in the long-term because of the development of drug tolerance and disease progression (Doody et al, 2001).

Memantine is a non-competitive, moderate-affinity, phencyclidine-site, NMDA receptor antagonist that protects neurons from glutamate-mediated excitotoxicity without preventing physiological activation of the NMDA receptor (Parsons et al, 1999). It has been shown that A β enhances the toxicity of glutamate, while activation of NMDA receptors appears to enhance production of pathologic forms of tau and A β (Mattson et al, 1997). The approval of memantine's application in AD was the result of two randomized placebo-controlled clinical studies, where memantine treatment significantly improved symptoms and slowed the rate of cognitive and functional decline in moderate and severe AD (Winblad and Poritis, 1999; Reisberg et al, 2003).

Potential therapies under investigation target multiple aspects of the disease cascade, including aberrant inflammation, neurotrophic function, and processing of beta amyloid and tau proteins. These novel approaches hold promise for disease modification but are as yet unproven, and are outside the scope of this thesis.

Chapter 2 Alzheimer's Disease: Neuropathology

The heterogeneous clinical phenotype of AD probably results from the various molecular and cellular changes in this disorder. AD has a well-defined neuropathologic profile characterized by a surfeit of extracellular amyloid deposits and accretion of intracellular neurofibrillary tangles (NFTs), together with a degeneration of neurons and synapses (Selkoe, 2001). This chapter discusses in detail the neuropathology of AD, both in a macroscopic view – the vulnerable neural systems in AD, and in a microscopic view – the molecular and cellular changes in AD.

1 Macroscopic pathology and regional hierarchy

On macroscopic examination, a typical AD brain is severely atrophic, shown by widening of the sulci and ventricular enlargement (Figure 2.1A). This is more obvious in early-onset FAD cases. Despite generalized atrophy of the cerebral cortex, not all the brain regions are equally implicated. AD selectively affects brain regions critical for cognition and memory. Closer inspection of the mediotemporal lobes reveals a greater degree of atrophy in the hippocampus, parahippocampal region, and amygdala. Indeed, there appears to be a temporal and regional hierarchy with respect to neurodegeneration, with both pathological (Braak and Braak, 1991) and brain imaging (Smith and Jobst, 1996; Scahill et al, 2002) studies indicating that medial temporal lobe regions are affected early and relatively rapidly in the disease process followed by the spread of gradual degeneration to other neocortical areas. The pathological changes in AD are thought to start from the entorhinal cortex, progress to the hippocampus, and involve the neocortex at later stages (Braak and Braak, 1991). In the neocortical regions, higher-order association cortex is the principal site of brain pathology with evolving disease whereas primary sensory and motor regions show relatively little pathology. In contrast, regions of the brain such as the striatum and cerebellum may show some degree of plaque formation but do not develop significant neuronal pathology. The spread of pathology throughout these circuits reflects a general staging of the disease, which is consistent with clinical findings, from an early preclinical phase of deficits in memory formation to later stages with gradual deterioration of other cognitive abilities.

Figure 2.1

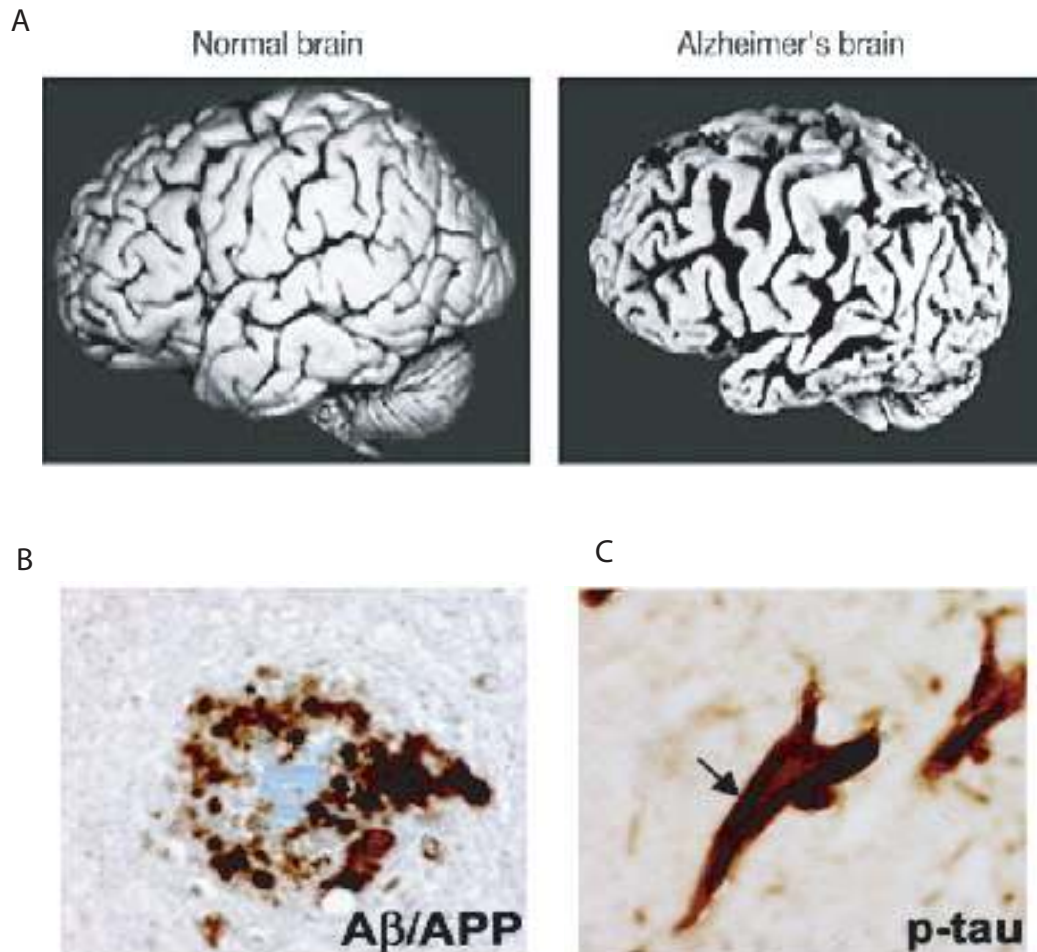


Figure 2.1 Pathological changes of Alzheimer's disease. (A) Compared with the brain of a healthy person, the brain of an Alzheimer's disease patient exhibits marked shrinkage of gyri in the temporal lobe (lower part of the brain) and frontal lobes (left part of the brain), both of which are involved in learning and memory. (B) Amyloid plaques detected with antibodies against A β (blue) and APP (brown). (C) Phospho- γ -immunopositive neurofibrillary tangles.

Adapted from Mattson, 2004 (A) and Jin et al, 2004 (B) and (C)

2 Amyloid pathology

One of the two defining pathological changes of AD is the amyloid plaque. Amyloid plaques contain extracellular deposits of toxic amyloid β -protein ($A\beta$) that are produced by aberrant processing of APP. They are complex lesions that vary in size, ranging from 10 to 120 μm , best visualized with silver stains or immunohistochemical techniques using an antibody to $A\beta$ (Figure 2.1B).

2.1 Plaque types

In AD brain tissue, amyloid plaques can be divided into two main types according to their maturation: neuritic plaques and diffuse plaques (Selkoe, 2001).

Neuritic plaques, generally found in the limbic and association cortices, are microscopic foci of extracellular amyloid deposition with dystrophic neurites within and immediately surrounding this amyloid deposit. Microglia are usually seen within and adjacent to the central amyloid core of the neuritic plaque, whereas astrocytes often ring the outside of the plaque, with some of their processes extending centripetally toward the amyloid core. Neuritic plaques contain fibrillar $A\beta$ and stain with Congo red.

Many of the plaques found in limbic and association cortices, and virtually all of those in brain regions not clearly implicated in the typical symptomatology of AD (e.g., thalamus, caudate, putamen, cerebellum), lack the compacted, fibrillar appearance of the classical neuritic plaques, but show relatively light, amorphous $A\beta$ immunoreactivity that occurred in a finely granular pattern. These lesions have thus been referred to as diffuse plaques or preamyloid deposits. Diffuse plaques are composed of homogeneous deposits of non-fibrillary material and contain only scant numbers of amyloid fibrils and do not stain with Congo red.

Later it was determined that the $A\beta$ peptides deposited in Alzheimer brain principally ended at either $A\beta 40$ or $A\beta 42$. Diffuse plaques are primarily composed of $A\beta 42$, with little or no $A\beta 40$ immunoreactivity. In contrast, while much of the fibrillar $A\beta$ found in the neuritic plaques is $A\beta 42$, $A\beta 40$, which is normally more abundantly produced by cells than $A\beta 42$, is usually colocalized in the plaque (Iwatsubo et al, 1994; Selkoe, 2001).

The earliest structural pathological changes in AD are diffuse $A\beta$ deposits. They are also observed in normal aging individuals, but at a lower density (Perry et al, 1978). Furthermore, the detection of diffuse plaques in regions that also contained many neuritic plaques (limbic and association cortices) led to the hypothesis that diffuse plaques represent precursor lesions to neuritic plaques. This notion was later supported by studies of transgenic mice expressing mutant human APP. These mice usually showed diffuse

deposits before developing fibrillar, thioflavin S-positive, and Congo red-positive neuritic/glial plaques. The time that it takes to develop a plaque is unknown, but these lesions probably evolve very gradually over a substantial period of time. While senile plaques are associated with degeneration of neurites and neuronal cell bodies, the diffuse plaques can also induce morphological degeneration and kill neurons in the absence of fibrillar A β (Lambert et al, 1998).

2.2 Genetics and molecular biology of amyloid pathology

All known gene mutations leading to FAD influence APP processing and the production of A β . The first FAD-associated genetic mutations were found in APP gene (Chartier Harlin et al, 1991), whereas the majority (30-50%) of FAD cases are caused by mutations of the PS gene. The apoE4 allele of the apoE gene represents the most prominent genetic risk factor for the sporadic late-onset cases of AD, and also influences A β metabolism.

APP gene

A β -related peptides consist of 39–43 amino acids derived from the amyloid β -protein precursor (APP). The APP gene is localized to chromosome 21 (21q21.2–3) and encodes APP protein which is a single membrane-spanning protein possessing a large extracellular amino-terminal domain and a small intracellular cytoplasmic domain. There are three main isoforms of APP: APP770, APP751 and APP695, arising from the alternative splicing of its pre-mRNA. The APP splice forms containing 751 or 770 amino acids are widely expressed in nonneuronal cells throughout the body and also occur in neurons. However, neurons express even higher levels of the 695-residue isoform, which occurs at very low abundance in nonneuronal cells (Selkoe, 2001). The ubiquitous expression of APP gene throughout the body suggests that APP must serve critically important functions. A general observation regarding APP function is that it acts at the cellular level in diverse processes such as axonal transport, cell adhesion, cholesterol metabolism and gene transcription (De Strooper and Annaert, 2000). However, APP knockout mice are viable and often show near normal phenotypes except for decreased locomotor activity and forelimb grip strength (Zheng et al, 1995). The absence of substantial phenotypes in these mice may result from functional redundancy provided by homologous amyloid precursor-like proteins (APLP1 and APLP2), which function in a similar manner to APP in many biochemical pathways (Heber et al, 2000).

A β is derived from APP by sequential proteolytic cleavages (Figure 2.2; Selkoe, 2001). The APP is processed by three proteases, which, starting from the amino terminus of the

Figure 2.2

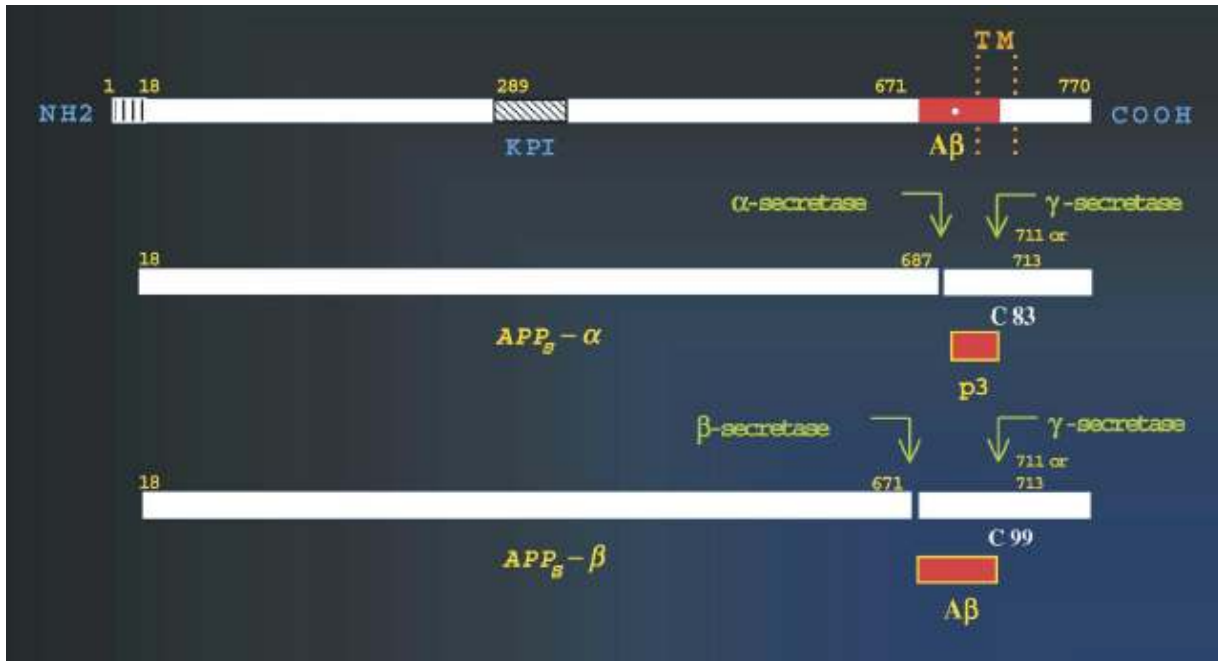


Figure 2.2 Schematic diagrams of the β -amyloid precursor protein (APP) and its principal metabolic derivatives. Top diagram depicts the largest of the known APP alternate splice forms, comprising 770 amino acids. Regions of interest are indicated at their correct relative positions. A 17-residue signal peptide occurs at the NH2 terminus (box with vertical lines). Two alternatively spliced exons of 56 and 19 amino acids are inserted at residue 289; the first contains a serine protease inhibitor domain of the Kunitz type (KPI). A single membrane-spanning domain (TM) at amino acids 700-723 is indicated by the vertical dotted lines. The amyloid β -protein ($A\beta$) fragment includes 28 residues just outside the membrane plus the first 12-14 residues of the transmembrane domain. In the middle diagram, the arrow indicates the site (after residue 687; same site as the white dot in the $A\beta$ region of APP in the upper diagram) of a constitutive proteolytic cleavage made by protease(s) designated α -secretase that enables secretion of the large, soluble ectodomain of APP ($APP_s-\alpha$) into the medium and retention of the 83-residue COOH-terminal fragment in the membrane. The C83 fragment can undergo cleavage by a protease(s) called γ -secretase at residue 711 or residue 713 to release the p3 peptides. The bottom diagram depicts the alternative proteolytic cleavage after residue 671 by a protease(s) called β -secretase that results in the secretion of the slightly truncated $APP_s-\beta$ molecule and the retention of a 99-residue COOH-terminal fragment. The C99 fragment can also undergo cleavage by γ -secretase to release the $A\beta$ peptides.

Picture and text adapted from Selkoe, 2001

protein, are known as the β , α , and γ secretases. The β -cleavage site occurs between residues 596 and 597 of APP695, which marks the N-terminus of the A β peptide, whereas the α -cleavage site lies between lys613 and leu614 (of the 695 isoform) within the critical A β peptide region and therefore precludes the release of the plaque-forming A β fragment. The α and β cleavages seem to be mutually exclusive events and each liberate a large extracellular domain of the protein, differing in size by only 17 amino acids at the carboxy terminus. The remaining portion of APP is tethered to the membrane and is referred to as the carboxy-terminal fragment (CTF), C99 for the β - and C83 for the α -generated product.

The transmembrane cleavage event catalyzed by γ -secretase occurs subsequent to α or β cleavage at several sites in close proximity within the CTF sequence. After α cleavage, C83 can be processed by γ -secretase to generate a shortened A β -like fragment termed p3, plus the same C-terminal fragments. After β cleavage, C99 may be processed by γ -secretase and, depending on the site of γ -secretase cleavage, produces “short” (A β 1-40) or “long” (A β 1-42, 43) A β fragment. Both forms assemble amyloid fibrils via analogous steps and elongate at comparable rates, but A β 1-42 is populated more rapidly and to a greater extent than A β 1-40 (Harper et al, 1999). The majority (90%) of the secreted soluble A β fragments are A β 1-40. A shift to a higher proportion of the longer isoform may be crucial to the earliest stages of fibril deposition into plaques, because this form is far more prone to oligomerization and fibril formation than is the more abundantly produced A β 1-40 (Bitan et al, 2003). Discrete intermediate A β species, designated protofibrillar A β , are formed before A β fibrils appear. Although protofibrillar A β appears transiently, its potential neurotoxic effects in vitro has led to the hypothesis that this form of A β , rather than fibrillar A β , is pathogenically important (Walsh et al, 2002).

Initially it was believed that α -cleavage is the physiological one (Esch et al, 1990; Sisodia et al, 1990), and prevents amyloidogenesis in AD. In reality this is an oversimplification, because only part of the total pool of APP is cleaved by α -secretase in most cell types. In addition, β -secretase processing of APP also occurs under physiological conditions, though the levels and activity of β -secretases are increased in AD (Fukumoto et al, 2002). This indicates that all fragments of APP, including the A β peptide, are part of normal physiology (Haass et al, 1992; Seubert et al, 1992).

More than 20 pathological mutations have been identified in the APP gene, all of which cluster at or near the proteolytic cleavage sites and increase the production of A β peptide or the proportion of the more amyloidogenic A β 42. The fact that, despite substantial investigation, no other mutations in the large APP protein that cause AD have

been discovered strongly suggests that these missense mutations lead to AD by altering proteolytic processing at the three secretase sites in subtly different ways.

Presenilin genes

Establishment of a linkage of some of FAD cases to chromosome 14 led to the identification of a novel FAD-related gene on this chromosome, which came to be known as presenilin-1 (PS-1) (Sherrington et al, 1995). Shortly thereafter, a homologous gene was discovered on chromosome 1, which was designated presenilin-2 (PS-2) (Levy-Lahad et al, 1995).

The presenilin proteins PS-1 and PS-2 are polytopic integral transmembrane domain proteins that express throughout most peripheral tissues and all brain regions, primarily in neurons. The exact functions of these proteins remain unclear, but may include regulation of β -catenin stability and trafficking of membrane proteins. There is also substantial evidence that PS proteins are associated with the γ -secretase activity that generates the A β peptide (De Strooper et al, 1999). In PS-1/PS-2 double knock-out cells, A β secretion into the medium is completely inhibited. Mice with a targeted disruption of the PS-1 gene have also been generated (Shen et al, 1997; Wong et al, 1997). Homozygous mutant mice failed to survive the early postnatal period. The most striking phenotype observed in PS-1 knockout embryos was a severe perturbation in the development of the axial skeleton, which may be related to abnormal somite patterns. In addition, PS-1 knockout embryos exhibited intraparenchymal hemorrhages after day 11 of gestation and massive neuronal loss in specific subregions after day 16.5. These observations have been interpreted to indicate that PS-1 is required for normal neurogenesis and neuronal survival (Shen et al, 1997). Removal of PS-2 gene is not as harmful as PS-1. PS-2 knockout mice are viable but develop pulmonary fibrosis and hemorrhage with age (Herreman et al, 1999).

The majority of FAD cases are linked to mutations within the PS genes. More than 150 mutations have been reported in the PS-1 gene and 20 in the PS-2 gene that subsequently result in AD. The AD-causing missense mutations in both PS genes selectively increase the production of the 42-residue form of A β peptide that is particularly prone to precipitation and aggregation. This is because PS is the catalytic subunit of γ -secretase (De Strooper et al, 1999). Its mutations alter the conformation of PS, enhancing coordination between the 2 catalytic aspartates and the A β 42 peptide bond in the transmembrane domain of APP.

Apolipoprotein E

ApoE is the most important genetic risk factor for late onset AD cases. ApoE plays a role in A β deposition because a lack of apoE dramatically reduces A β deposition in a

transgenic model (Bales et al, 1997; Holtzman et al, 1999). The human apoE gene is located in chromosome 19 and encodes a 34 kDa lipid transport protein. ApoE is normally present in oligodendroglia, astrocytes and microglia. As a lipid carrier protein involved in the transport of cholesterol and phospholipids, apoE is believed to play an important role in synaptic plasticity and tissue repair mechanisms, including control of neurite outgrowth and branching, microtubule depolymerization, adhesion to basal membrane components, immunomodulation and potentiation of the activity of ciliary neurotrophic factor (Poirier, 1994; Weisgraber et al, 1994; Masliah et al, 1995).

ApoE is polymorphic with three alleles, $\epsilon 2$, $\epsilon 3$ and $\epsilon 4$, encoding protein isoforms apoE2, E3 and E4, respectively. In many assays, E2 and E3 produce similar effects that vary only in amplitude whereas the presence of E4 often leads to a different outcome. For example, in primary neuronal cultures, the presence of E2 or E3 enhances neurite outgrowth and branching whereas E4 inhibits these processes (Nathan et al, 1994; Weisgraber et al, 1994). The presence of $\epsilon 4$ allele is more frequent in subjects with AD compared with the general population whereas inheritance of the $\epsilon 2$ allele may confer protection against AD (Corder et al, 1993; Corder et al, 1994). It should be emphasized that apoE4 is a risk factor for, not an invariant cause of, AD. Some humans homozygous for the $\epsilon 4$ isoform still show no Alzheimer symptoms in their ninth decade of life and beyond. Conversely, a great many humans develop AD without harboring $\epsilon 4$ alleles.

Although the association of apoE with AD is well characterized, its role in the pathogenesis is still unclear. ApoE proteins have been shown to be components of AD neurofibrillary tangles and to bind to the beta-amyloid peptides that are involved in fibril formation (Haas et al, 1997). One view is that apoE, especially the E4 isoform, acts as a chaperone protein for A β and promotes the development of the insoluble fibrils in plaques (Wisniewski and Frangione, 1992; Irizarry et al, 2000). An alternative view is centred on the role of apoE in the remodelling and sprouting of axons and synaptic terminals following neural damage (Poirier, 1994). In this regard, inheritance of a particular apoE genotype may influence how the nervous system responds to injury. $\epsilon 4$ allele inhibits neurite growth and dendritic plasticity, whereas the $\epsilon 3$ allele promotes these processes (Nathan et al, 1994; Weisgraber et al, 1994; Poirier, 1994).

2.3 Neurotoxicity of A β

The gradual accumulation of A β 42 leads to its aggregation into oligomers, polymers and fibrils. For a long time it has been assumed that only “aggregated” form of A β is neurotoxic (Lorenzo et al, 1994; Geula et al, 1998; Lambert et al, 1998; McKee et al, 1998).

However, more recent work emphasizes the importance of A β in the soluble form. A species of diffusible soluble A β oligomers has been shown to be primarily responsible for synaptic dysfunction and behavioural impairments (Westerman et al, 2002; Walsh et al, 2002; Cleary et al, 2005; Lesne et al, 2006).

How the soluble oligomers of A β induce neurotoxicity remains unclear but a general theme has emerged whereby the interaction of A β with lipid membranes is a necessary step in neurotoxicity (Masters et al, 2006). These interactions cause changes in membrane fluidity (Eckert et al, 2005), disrupted calcium homeostasis (Kawahara, 2004), lipid peroxidation via membrane-associated free radical formation (Butterfield and Boyd-Kimball, 2004) and cholesterol oxidation (Puglielli et al, 2005). All of these events converge into similar pathways of synaptic disruption and neuronal necrosis or apoptosis, leading to progressive loss of specific neuronal cell populations (Vickers et al, 2000; Hardy and Selkoe, 2002; Mattson, 2004; Masters et al, 2006).

Functional domains of A β involved in fibrillogenesis and neurotoxicity

Studies on A β fragments have suggested several functional domains in the A β sequences (Tran et al, 2002; Figure 2.3). The peptide fragment A β 25-35 is responsible for the neurotoxic effect of A β (Harkany et al, 1998; Tran et al, 2002). This fragment was reported to cause dendritic and axonal retraction followed by neuronal death in differentiated neurons and mature neurons in vitro (Yankner et al, 1990). For learning and memory, the A β 18-20 sequence is important for the amnesic effect (Flood et al, 1991). The peptide fragment A β 16-20 serves as a binding sequence during A β polymerization and fibril formation (Tjernberg et al, 1996). A β 13-16 is necessary for an initial interaction of microglia with plaques through a cell-surface-binding site involving heparan sulfate (Giulian et al, 1996). Using an analogue of A β 31-34, several investigators demonstrated that this putative antagonist prevented an A β 1-42-induced elevation of intracellular Ca²⁺ and extracellular aspartate and glutamate levels in rat astrocytes. Thus, the sequence of A β 31-34 is considered a putative domain involved in cellular A β recognition and cell surface binding (Laskay et al, 1997).

Oxidative stress

A β is capable of generating free radicals and reactive oxygen species (Hensley et al, 1994), which in turn initiate and promote neurodegeneration in AD. Free-radical-mediated molecular damage is particularly prominent in the environment of plaques and in NFT-bearing neurons (Mattson, 2004). The aggregation of A β can attract inflammatory mediators which, in turn, stimulate nitric oxide (NO) production and expression of the

Figure 2.3

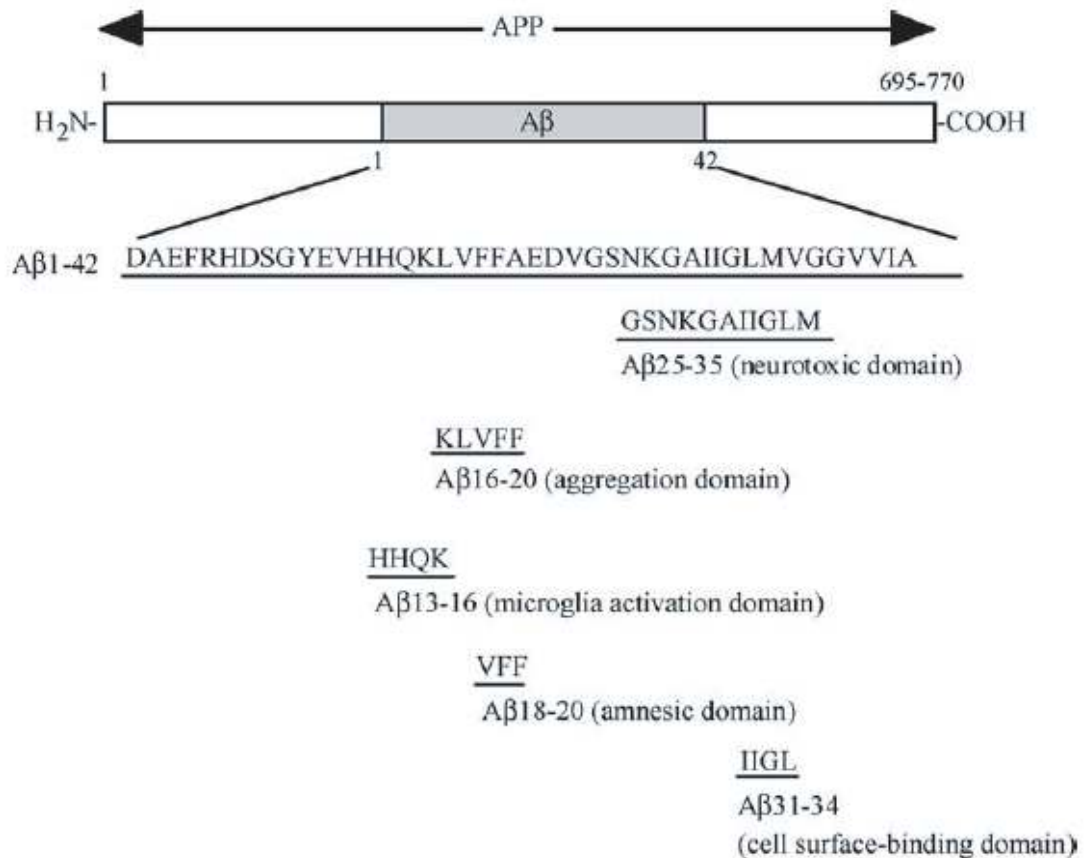


Figure 2.3 Structure of Aβ and functional domains involved in fibrillogenesis and cytotoxicity. Processing of APP precludes the formation of Aβ. Two major species of full-length Aβ defined by their C-terminus lengths are Aβ1-42 and Aβ1-40. Several functional domains in the Aβ sequence are proposed, including the neurotoxic, aggregation, microglial activation, amnesic, and cell-surface binding domains.

Adapted from Tran et al, 2002

high-output isoform, inducible nitric oxide synthase (iNOS) (Yates et al, 2000). This A β -induced iNOS expression can result in an overproduction of NO, which may react with O $_2^-$ to yield highly reactive peroxynitrite and may, therefore, increase the overall radical burden in A β -loaded brain regions (Tran et al, 2002). By an oxidative-stress-mediated mechanism, A β impairs the function of membrane ion and glutamate transporters, compromises mitochondrial function, and render neurons vulnerable to excitotoxicity and apoptosis.

It needs to be mentioned that oxidative stress can also induce amyloidogenic processing of APP and accumulation of potentially neurotoxic forms of A β (Gabuzda et al, 1994). This could contribute to increased amyloid production in late-onset forms of AD, because oxidative stress and metabolic impairment increase with advancing age. Thus, a vicious circle exists where oxidative stress and A β promote each other in the course of AD.

Calcium dysregulation and excitotoxicity

In addition, A β may cause excitotoxicity by inducing a harmful elevation of intracellular calcium levels (Mattson et al, 1992). The calcium plays crucial roles in a variety of fundamental biological processes. The impairment of neurons to regulate calcium homeostasis is an aspect of AD pathogenesis that appears to be intimately involved in the dysfunction and death of neurons (Mattson, 2004). A β can promote Ca $^{2+}$ influx by forming channels in membranes or by activating cell surface receptors coupled to calcium influx (Mattson and Chan, 2003). In natural cell membranes of hypothalamic neurons, soluble A β 40 forms calcium-selective channels across excised membrane patches (Kawahara et al, 1997). Although the permeability for Ca $^{2+}$ is selective to the channels formed by A β , the permeability of other monovalent cations such as K $^+$, Na $^+$, and Cs $^+$ to these channels suggests that these A β -formed channels are not classical calcium channels. In addition, A β -induced oxidative stress may impairs membrane calcium pumps and enhances calcium influx through voltage-dependent channels and ionotropic glutamate receptors. Efforts to stabilize intracellular Ca $^{2+}$ homeostasis provide a way to protect against A β neurotoxicity, and may represent therapeutic approaches for AD (Tran et al, 2002).

Glial cell changes and inflammation

A β -induced alterations of glial cells (astrocytes, oligodendrocytes and microglia) in the brain have been documented in studies of AD patients, animal models and cell cultures (McGeer and McGeer, 2002). Abnormalities in astrocytes include impaired glutamate transport and perturbed calcium regulation. Astrocytes, together with microglial cells, are

also involved in the chronic inflammatory responses in AD through the up-regulated expression of phospholipase A2, leading to increased arachidonic acid/prostaglandin inflammatory pathway activity and production of a variety of potentially neurotoxic compounds, including superoxides, glutamate and NO (Brown and Bal-Price 2003). Blocking A β oligomers/fibrils formation prevents this toxicity (Meda et al, 1995).

Similarly, a range of inflammation-related markers has been reported to be elevated in AD (Vickers et al, 2000). Inflammatory changes may promote plaque formation by increasing APP expression or enabling the transition from diffuse to neuritic forms of the plaque (Mrak et al, 1995), whereas stimulation of plaque formation would result in a further activation of microglia and astrocytes, resulting in a 'vicious cycle' of increased inflammatory involvement and β -amyloid plaque formation (Vickers et al, 2000).

It must be noted that whereas many of the changes in glial cells in AD promote neuronal degeneration, some of them may actually represent adaptive responses aimed at promoting neuronal survival. For example, microglia may act to remove A β , a potentially beneficial action (Jantzen et al, 2002). Moreover, astrocytes associated with A β deposits may compensatively increase the production of neurotrophic factors so as to counteract the neurodegenerative process in AD (Cummings et al, 1993).

Apoptosis

It has been suggested that A β may cause DNA damage and/or signal apoptotic pathways (LaFerla et al, 1995). Pro-apoptotic proteins are associated with A β deposits in AD brains and exposure of neurons to A β induced an apoptotic cascade that include a number of well-known apoptotic proteins (Mattson, 2000). Importantly, apoptotic markers have been shown to be present in a larger proportion of neurons than those affected by NFTs, suggesting the presence of alternate pathways that lead to neurodegeneration (Su et al, 1997).

Metal metabolism

Several lines of evidence point to the participation of transition metals in A β neurotoxicity (Bush, 2003). Very high concentrations of Cu, Zn and Fe have been found in plaques of AD brains (Lovell et al, 1998). This would lead to high concentrations of soluble metallated A β , thereby promoting its toxicity (Lee et al, 2002). The high Zn concentrations also promote the aggregation of the Cu/Fe-metallated A β , creating a reservoir of potentially toxic A β that is in equilibrium with the soluble pool. The large polymeric deposits of misfolded proteins not only represent the end result of the aggregation process but may act as inactive reservoirs in equilibrium with the small

diffusible oligomeric toxic species responsible for the neurodegenerative pathology (Masters et al, 2006). Genetic ablation of the zinc transporter 3 protein, required for zinc transport into synaptic vesicles reduced plaque formation in tg2576 transgenic mice (Lee et al, 2002).

Issues to be noticed

There are some issues to be considered regarding the relevancy of experimental A β toxicity models. One important point is that some assays rely on an acute mechanism of neuronal damage being caused by A β whereas the degeneration of neurons in AD is likely to be a gradual event, perhaps occurring over years or decades. The case for true AD-like neuronal degeneration in experimental models, in which β -amyloid peptides are injected into the brain (or cerebral ventricles), is much less convincing, since the concentration of A β required to kill cells is often much higher than presumed physiological levels (Neve and Robakis, 1998). Neurons may take a long time to degenerate and eventually die. Indeed, neuronal loss is not obvious in a number of transgenic models of AD overexpressing mutated forms of APP, which better mimic the real condition in AD brains (Games et al, 1995; Irizarry et al, 1997; Sturchler-Pierrat et al, 1997; Urbanc et al, 2002). Similarly, the β -amyloid plaques that occur during the preclinical phase of the disease are also not associated with profound neuronal pathology (Vickers et al, 1996).

2.4 Sequence of amyloid deposition in medial temporal lobe

The evolution of A β -deposits in the human medial temporal lobe follows a distinct sequence. In contrast to the high degree of inter-individual variability in the number of plaques, the successive involvement of anatomically related regions exhibiting distinct types of A β -deposits shows no greater degree of inter-individual variability in a given phase. In a given phase A β -deposits always appear in distinct regions and all of the regions affected in an earlier phase always exhibit A β . Four phases of A β -deposition can be distinguished (Thal et al, 2000). In brief, the pathology progresses as follows: the basal temporal neocortex \rightarrow entorhinal cortex, CA1 and subiculum \rightarrow molecular layer of fascia dentate \rightarrow CA4. The mechanisms responsible for this progression remain unclear.

3 Neurofibrillary tangles (NFTs)

Profound cytoskeletal changes occur in neurons and their processes in AD. Abundant neurofibrillary tangles (in cell bodies and apical dendrites, Figure 2.1C), neuropil threads (NTs, in distal dendrites) and plaque neurites constitute the neurofibrillary pathology of AD. They form in the nerve cells that undergo degeneration in the disease where their regional

distribution correlates with the degree of dementia. Ultrastructurally, each lesion contains paired helical filaments (PHFs) as its major fibrous component and straight filaments as its minor fibrous component (Kidd, 1963). PHFs are composed of two strands of filament twisted around one another with a periodicity of 80 nm whereas straight filaments lack this helical periodicity. Both PHFs and straight filaments are composed predominantly of the microtubule-associated protein tau in a hyperphosphorylated state (Grundke-Iqbal et al, 1986).

NFTs are usually intracellular fibrillar structures commonly flame-shaped in appearance and occupy the cell body and proximal portion of the apical dendrite of the affected neuron. Since the abnormal filaments are highly insoluble, NFTs remain in the extracellular space following the complete degeneration of the affected neuron and become extracellular 'ghost' tangles.

3.1 Molecular biology

NFTs are composed of tau, which is a microtubule-associated protein abundant in both central and peripheral nervous systems. In the brain it is found predominantly in nerve cells, where it is concentrated in nerve cell axons. This contrasts with the distribution of the neurofibrillary lesions in AD that are found in nerve cell bodies, axons, and dendrites. Tau protein appears as a series of polypeptides of differing lengths on electrophoresis gels, which is a characteristic phenomenon of alternative RNA splicing and/or various phosphorylation levels.

The gene encoding tau, consisting of 16 exons, is located on chromosome 17 (Figure 2.4; Neve et al, 1986; Lee et al, 2001). The adult brain expresses six isoforms of tau, which derive from alternative mRNA splicing of exons 2, 3 and 10 and differ by the presence of three (τ 3L, τ 3S or τ 3) or four (τ 4L, τ 4S or τ 4) tubulin binding domains (repeats) of 31 or 33 amino acids in the C-terminal portion, and none (τ 3, τ 4), one (τ 3S, τ 4S) or two (τ 3L, τ 4L) inserts in the N-terminal region (Figure 2.4; Goedert et al, 1989).

3.2 Physiology

Neuronal morphology and structural integrity are maintained largely by the cytoskeleton, which is partially composed of microtubules. The assembly and stability of microtubules, in turn, are maintained by microtubule-associated proteins (MAPs). Tau, MAP1 (A/B) and MAP2 are the major microtubule-associated proteins of a normal mature neuron. These three MAPs perform similar functions, probably due to the essential requirement of microtubules for axoplasmic flow, which, in turn, is critical to neuronal activity. Physiologically, tau binds to and stabilizes microtubules, polymerizes actin, and

Figure 2.4

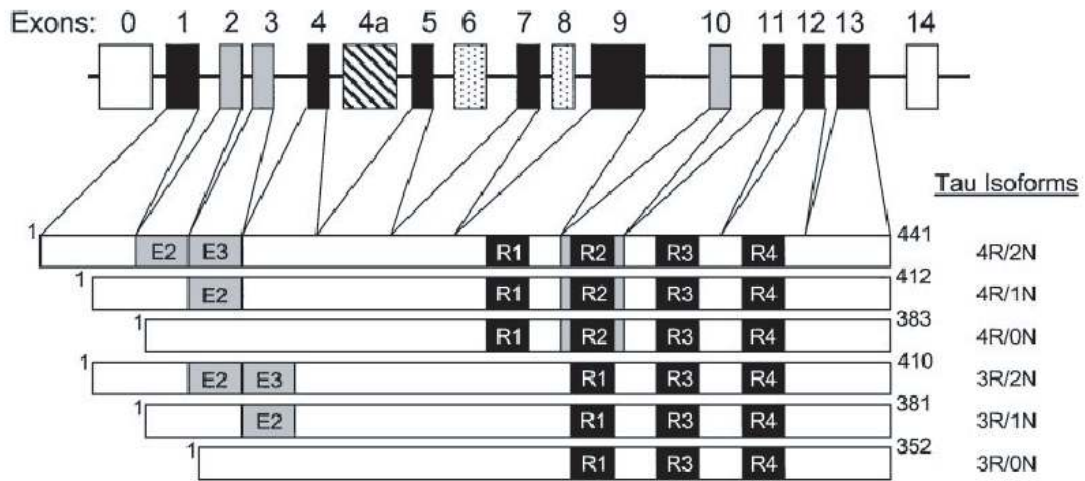


Figure 2.4 Schematic representation of the human tau gene and the six central nervous system tau isoforms generated by alternative mRNA splicing. The human tau gene contains 16 exons, including exon (E)0, which is part of the promoter. Alternative splicing of E2, E3, and E10 (gray boxes) produces the six tau isoforms. E6 and E8 (stippled boxes) are not transcribed in the human CNS. E4a (striped box), which is also not transcribed in the human CNS, is expressed in the peripheral nervous system, leading to the larger tau isoforms, termed big tau (see text). The black bars depict the 18–amino acid microtubule binding repeats and are designated R1 to R4. The relative sizes of the exons and introns are not drawn to scale.

Adapted from Lee et al, 2001

participates in intracellular trafficking (Buee et al, 2000; Lee et al, 2001). However, tau is not necessary for normal cell function. A tau-deficient mouse produced by gene targeting is viable and phenotypically similar to tau-containing mice, except for a reduction in the number and density of axons in parallel fibers from the cerebellum (Harada et al, 1994). This is largely due to compensation effects from another two MAPs.

The microtubule binding domains of tau are localized to the C-terminal half of the molecule within the three or four microtubule binding motifs. It has been suggested that the four-repeat forms are more efficient at promoting microtubule assembly, have a greater microtubule binding affinity, and favour fibril formation, compared with three-repeat forms (Heutink, 2000; Lee et al, 2001). The binding of tau to microtubules is a complex process and highly regulated by phosphorylation (Lindwall and Cole, 1984). A variety of protein kinases and phosphatases have been implicated in the regulation of tau phosphorylation (Bree et al, 2000). There are 79 potential serine (Ser) and threonine (Thr) phosphate acceptor residues in the longest tau isoform, and phosphorylation at ~30 of these sites has been reported in normal tau proteins (Buee et al, 2000). The tau phosphorylation sites are clustered in regions flanking the microtubule binding repeats. Normal tau contains 2–3 mol phosphate/mol of the protein (Kopke et al, 1993), the level of phosphorylation for its optimal activity. Hypophosphorylated tau binds with high affinity to microtubules, whereas hyperphosphorylated tau, similar to that present in AD, depresses its microtubule assembly activity and its binding to microtubules (Garcia de Ancos et al, 1993).

Tau phosphorylation is regulated by the balance between multiple kinases (eg, glycogen synthase kinase 3 β and cyclin-dependent kinase 5) and phosphatases (eg, protein phosphatase-1 and protein phosphatase-2A) (Lee et al, 2001).

3.3 Tau pathology

Tau pathology is not specific to AD and is also seen in a variety of other neurodegenerative disorders, such as frontotemporal dementia, subacute sclerosing panencephalitis etc. The discovery of mutations in tau gene and their cosegregation with the disease in the inherited frontotemporal dementia with Parkinsonism linked to chromosome-17 (FTDP-17) has established that abnormalities in tau protein as a primary event can lead to neurodegeneration and dementia (Hutton et al, 1998).

In AD brains the levels of tau, but not its mRNA, are four- to eightfold increased as compared to age-matched control brains and this increase is in the form of the abnormally hyperphosphorylated tau (Khatoon et al, 1992). Furthermore, tau in healthy adult brain is located in axons, but in AD and other tauopathies, it is found in cell bodies and dendrites as

well as axons. The abnormally hyperphosphorylated tau is found in AD brain in two subcellular pools: as polymerized into NFTs of PHF mixed with straight filaments; and as non-fibrillized form in the cytosol (Iqbal et al, 1986; Köpke et al, 1993). The tau polymerized into neurofibrillary tangles is inert, whereas the cytosolic abnormally hyperphosphorylated tau does not interact with tubulin/microtubules but instead sequesters normal tau, MAP1 and MAP2, causing inhibition and disassembly of microtubules (del C Alonso et al, 1996). As a consequence, AD is associated with a loss of microtubule-binding capacity and a consequent accumulation in neuronal bodies, which further interfere with cytoskeletal integrity, intracellular transport, cellular geometry, and neuronal viability (Lee et al, 2001). The mechanisms underlying PHF formation in neurons are still unclear. But it is possible that hyperphosphorylation disengages tau from microtubules, thereby increasing the pool of unbound tau. Unbound tau may be more resistant to degradation and more prone to aggregate. After aggregation, PHF-tau undergoes posttranslational modifications, including ubiquitination (Mori et al, 1987), glycation (Yan et al, 1994) and oxidation (Schweers et al, 1995).

3.4 Neurotoxicity of NFTs

PHF-tau may cause neurotoxicity in a loss of function and/or a gain of toxicity manner. Since phosphorylated tau inhibits microtubule assembly and causes the disassembly of microtubules (Alonso et al, 2001), PHF-tau compromises microtubule stability and function, resulting in a loss or decline in axonal or dendritic transport in disease (Salehi et al, 2003). Furthermore, PHF-tau disrupts intracellular compartments that are essential for normal metabolism. Cell culture studies show that the overexpression of tau causes a change in cell shape, retards cell growth and dramatically alters the distribution of various organelles transported by microtubule-dependent motor proteins (Stamer et al, 2002). Moreover, transgenic mice that overexpress the four-repeat human tau protein isoform specifically in neurons develop axonal degeneration in the brain and spinal cord and have notable axonal dilations due to the accumulation of neurofilaments, mitochondria and other vesicular structures (Spittaels et al, 1999).

3.5 Braak staging

The vulnerability to NFT formation is highest in the limbic areas of the medial temporal lobe. This vulnerability appears to become transferred along monosynaptic connections from limbic to paralimbic areas, then from paralimbic areas to association neocortex, and only terminally from association neocortex to primary sensory–motor areas. The putative role of axonal connections is highlighted by the fact that subcortical nuclei

monosynaptically interconnected with the cerebral cortex (such as the hypothalamus, brainstem raphe, and nucleus locus coeruleus) develop numerous NFT, whereas those with no cortical connections (such as the globus pallidus) do not. The interval between the initial appearance of the NFT in limbic areas and their widespread neocortical distribution in the terminal stages of the dementia may be as long as 50 years (Braak and Braak 1996).

A hierarchical staging system for the neuropathological changes in AD has been elaborated based on the distribution of NFTs in the cerebral cortex (Braak and Braak, 1994). In stages I and II (the transentorhinal stages), NFTs and NTs are restricted to the entorhinal cortex and the CA fields of hippocampus. Stages III and IV (the limbic stages) are characterized by moderate numbers of NFTs and NTs in the hippocampus, basolateral amygdala, and limbic nuclei of the thalamus. In stages V and VI (the isocortical stages) all hippocampal subfields and isocortical association areas are severely affected. The principal consequence of these lesions is a diminution of synaptic inputs in these regions of the brain (DeKosky and Scheff, 1990; Terry et al 1991).

4 Neuronal and synaptic degeneration

Although plaques and NFTs are hallmark changes, they are not the only significant pathological changes in the AD brain, additional structural changes occur in the AD brain, including inflammation and oxidative stress. The combined consequence of these changes is severe neuronal and synaptic degeneration. Many researchers have sought to establish correlations between cognitive impairments and pathological changes in AD. In general, the number of NFTs correlates better with severity of dementia than the number of amyloid plaques, but the best statistical correlates with degree of dementia are measures of synaptic density (DeKosky and Scheff, 1990; Terry et al, 1991). Quantification of synaptic markers has documented significant decreases in synaptic density in the association cortices and hippocampus of AD brain, which appear to precede neuronal degeneration (Davies et al, 1987; DeKosky and Scheff, 1990; Terry et al, 1991; Masliah et al, 2001). Furthermore, studies from both AD patients and animal models indicate that the decrease in synapse number and density is disproportionate to the loss of neuronal cell bodies, suggesting that synaptic dysfunction is compromised prior to the physical degeneration of the synapses (Davies et al, 1987; DeKosky and Scheff, 1990; Westphalen et al, 2003). Extensive neuronal loss in circuits critical for learning and memory characterizes AD. Neurons that remain also undergo extensive morphological changes: dystrophic neurites form, dendritic and axonal trees are remodeled, and synapse and dendritic spine densities change (Spire

and Hyman, 2004). Such changes, both functional and structural, disrupt neuronal circuitry and represent early and pivotal events in the emergence and progressive development of dementia.

This loss of populations of neurons would have a variable time course between individuals, but is likely to take months to years for individual nerve cells to degenerate. Importantly, neuronal degeneration is highly selective for certain brain regions and types of nerve cells. Neocortical interneurons, many of which would be GABAergic and inhibitory, are relatively unaffected, whereas a subset of pyramidal cells in layers II, III and V in the entorhinal cortex, hippocampus, frontal, parietal and temporal cortices, that use excitatory amino acids as transmitters, show a high degree of vulnerability (Vickers et al, 2000). Neuronal loss in AD brains is associated with the presence of amyloid plaques and NFTs (Khachaturian, 1985). In general, AD essentially attacks the integrity of cortical circuitry, affecting both intracortical pathways as well as neurons in other regions of the brain that project to the cerebral cortex (Vickers et al, 2000). In this respect, there are numerous subcortical pathways affected, involving catecholaminergic, serotonergic and cholinergic transmission. Neurons in layer II of the entorhinal cortex and hippocampal CA1 neurons are particularly vulnerable. The pattern of neuronal loss in AD overlaps with, but is not identical to, that of normal ageing -- suggesting that AD pathogenesis is not simply an acceleration of normal brain ageing (Mattson, 2004).

Among various neurotransmitter systems affected, one neural system that needs special attention is the cholinergic system. The central cholinergic transmitter system is involved in learning and memory. Cholinergic afferents innervate all cortical regions, with particular projections to the limbic and paralimbic cortices (Mesulam et al, 1983). Most of the cholinergic afferents to the hippocampal formation arise from the medial septal nucleus and the nucleus of the diagonal band, with a smaller portion from the basalis of Meynert. By contrast, the cerebral cortex receives its major cholinergic input from the basalis of Meynert, with additional projections from the pedunculo-pontine and lateral dorsal nuclei.

Basal forebrain cholinergic neurons are vulnerable in AD (Geula, 1998). Amyloid plaques and NFTs are observed in the basalis of Meynert, the nucleus of the diagonal band, and the medial septal nuclei, with marked neuronal loss is in the first two regions and selective loss of nicotinic receptor subtypes (Whitehouse et al, 1981; Doody et al, 2004). The severity of the neuropathological changes in the basalis of Meynert has been found to correlate with clinical disease severity (Davies and Maloney, 1976). These findings underpin the “cholinergic hypothesis of AD”, which proposes that the cognitive

dysfunction in AD at least partly attribute to impairment of cholinergic neurotransmission (Coyle et al, 1983). A β may induce cholinergic dysfunction via three mechanisms: impairing Ach synthesis and release; functioning as an antagonist of the nAChR system; and oxidative stress (Tran et al, 2002). However, the cholinergic system is not selectively affected in AD, and there is relative preservation of the cholinergic neurons of the nucleus basalis in MCI and early AD (Gilmor et al, 1999), casting doubt onto the hypothesis. Currently the cholinergic deficiency in AD is considered to be a secondary event (Albers and Beal, 2000). But cholinesterase inhibitors remain important pharmacologic treatments for mild to moderate AD.

Chapter 3 *Alzheimer's Disease: Pathogenesis*

Understanding of the etiology and mechanisms of cognitive impairment in AD is pivotal for the development of effective treatments that prevent or ameliorate symptoms of the disease. AD clearly has a complex etiology, existing in early-onset familial forms in addition to manifestation in a common sporadic form. This chapter focuses on the pathogenetic theories and current animal models of AD.

1 Alzheimer's disease at the molecular level: A β hypothesis

As discussed in the previous chapter, amyloid plaques and NFTs are two hallmark pathological changes in AD. The relative importance of these two changes in the development of AD has been intensely debated, with proponents of two camps dubbed as 'baptists' and 'tauists', respectively.

1.1 Amyloid hypothesis

The A β hypothesis of AD stipulates that altered amyloid processing is the key pathogenic factor in disease progression, and that the rest of the disease results from amyloid-induced changes. According to the hypothesis first formulated over 10 years ago, overproduction and aggregation of A β in amyloid plaques trigger neurotoxic events, leading to progressive neurodegeneration underlying the typical cognitive decline associated with AD (Hardy and Allsop, 1991; Hardy and Higgins, 1992). Later, however, attention is turning away from the deposits of extracellular insoluble A β in plaques to soluble, oligomeric and intracellular A β peptides. These smaller aggregates exert a number of physiological effects that are sufficient to induce synaptic dysfunction and to account for much of the symptomatology in AD animal models and patients (Walsh et al, 2002; Cleary et al, 2005; Lesne et al, 2006). Furthermore, APP itself, its other fragments, and A β , are all physiologically involved in modulating neuronal and synaptic functions (Turner et al, 2003). These findings led to a modified A β hypothesis (Figure 3.1; Hardy and Selkoe, 2002), which shifts its emphasis away from the dense core amyloid to smaller, pre-fibrillar aggregates and the functional balance between different APP fragments, as major contributors to neural and cognitive dysfunction in AD.

Supporting evidence

There is a wealth of evidence to support this hypothesis.

Figure 3.1

Amyloid cascade hypothesis

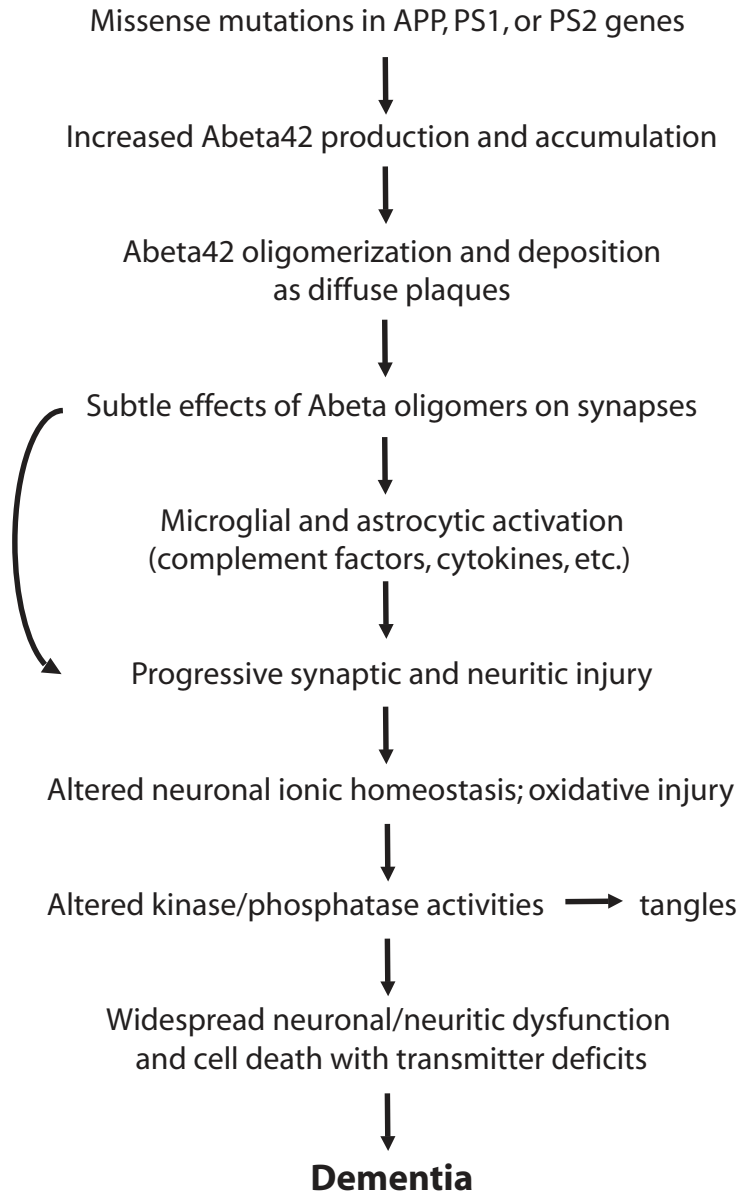


Figure 3.1 The sequence of pathogenic events leading to AD proposed by the amyloid cascade hypothesis. The curved violet arrow indicates that A β oligomers may directly injure the synapses and neurites of brain neurons, in addition to activating microglia and astrocytes.

Adapted from Hardy and Selkoe, 2002

1. The strongest evidence comes from molecular genetic studies showing that all FAD-causing mutations, either to APP itself or to presenilins, affect the processing of A β from APP and lead to proportionally greater production of the more neurotoxic “long” form of A β (Chapman et al, 2001; Selkoe, 2001).

2. The ϵ 4 allele of apoE, a major risk factor for sporadic AD, promotes the precipitation of A β into insoluble plaques. Crossing APP transgenic mice with apoE-deficient mice markedly reduces cerebral A β deposition in the offspring (Bales et al, 1997), suggesting that the pathogenic role of genetic variability at the human apoE locus is very likely to involve A β metabolism.

3. Patients of trisomy 21 (Down's syndrome), who possess an additional copy of the APP gene, invariably show an early emergence of AD-like neuropathology (Wisniewski et al, 1985).

4. A β is neurotoxic both in vitro and in vivo.

5. Transgenic mice overexpressing AD-causing mutants of APP and PS show AD-like pathological, electrophysiological and behavioural changes (see below).

6. In patients with PS-1 mutations or individuals with Down's syndrome who died prematurely from other diseases, the presence of A β precedes the appearance of NFTs (Lippa et al, 1998). Additionally, APP transgene accelerates NFT formation in APP/tau double transgenic mice (as compared with mice overexpressing tau alone) whereas NFTs do not influence the structure and number of amyloid plaques (Lewis et al, 2001). Similarly, injection of synthetic A β 42 into somatosensory cortex and contralateral hippocampus of P301L mice caused a 5-fold increase in NFT numbers in the amygdala (Gotz et al, 2001). Results of these studies strongly suggest that A β accumulation precedes and accelerates the development of neurofibrillary lesions, although the exact mechanism of this interaction is unknown.

7. A β immunization in multiple lines of APP transgenic mice reduces cerebral A β levels and plaque burden and/or alleviate memory impairment (Schenk et al, 1999; Bard et al, 2000; Chen et al, 2000; Morgan et al, 2000; Dodart et al, 2002).

Refuting evidence

Despite these supporting findings, there are also observations arguing against the A β hypothesis. The strongest challenge is that a specific neurotoxic species of A β and the nature of its effects on synaptic function in vivo remain ill-defined. However, several lines of evidence have converged to demonstrate that soluble oligomers of A β , but not monomers or insoluble amyloid fibrils, are toxic to cultured neurons and responsible for

synaptic dysfunction in the brains of AD patients and animal models. This will be discussed in detail in a later section.

Another frequently voiced objection is that spatial and temporal patterns of amyloid deposition do not correlate very well with the clinical degree of dementia in AD (Arriagada et al, 1992; Braak and Braak, 1998). Some humans without symptoms of AD have many cortical A β deposits (Neve et al, 1998). However, the extent of β -amyloid deposition in “healthy” elderly is less than in AD and these plaques are almost exclusively diffuse forms of amyloid plaques that are indicative of an early stage of the disease (Troncoso et al, 1998). More importantly, a study by Morris et al (1996) demonstrated that aged non-demented individuals who demonstrate neocortical β -amyloid deposits do show specific minor cognitive impairments that indicate that they were in an early stage of AD. Moreover, the degree of dementia in AD correlates much better with A β assayed biochemically than with histologically determined plaque counts, and the concentration of soluble A β species (which are invisible to immunohistochemistry) appears to correlate with cognitive impairment (Lue et al, 1999; McLean et al, 1999; Wang et al, 1999; Naslund et al, 2000). The aggregated plaques may be either a reservoir for the soluble oligomers or the sequestered pool of soluble and precipitated A β representing the end stage or final product of the A β cascade.

Finally, whereas transgenic mouse models bearing the FAD mutations show an increase in A β 42 levels and subsequent plaque pathology, most of these models do not show significant neuronal loss or tangle formation as predicted by the amyloid cascade hypothesis. The reasons for this lack of neuronal loss and tau pathology are unclear but could result from the difference in tau sequence between mouse and human, species differences in neuronal vulnerability, the relatively short duration of exposure to A β and the lack of certain inflammatory mediators necessary for a full complement inflammatory response.

Overall, the amyloid cascade hypothesis comprehensively articulates the current available knowledge regarding the cellular and behavioural alternations in AD and has had few compelling challenges. Nonetheless, there remain areas of doubt that A β by itself is both necessary and sufficient for AD. Its most robust test will come with the ‘amyloid vaccine’. Both passive and active immunization against A β has been shown to arrest, and even reverse, plaque pathology and/or behavioural phenotypes in the transgenic animals (Schenk et al, 1999; Bard et al, 2000; Chen et al, 2000; Morgan et al, 2000; Dodart et al, 2002). However, studies on AD patients are lacking and if the same result is obtained in

human studies the A β hypothesis is all but proven. On the other side of the coin, if clearing amyloid neither reverses dementia nor affects tangles then the hypothesis needs considerable revision.

1.2 Tau and tangle hypothesis

Despite the great persuasiveness of the amyloid hypothesis, there is a persistent group of researchers arguing that tau and tangles are central to AD. The tau and tangle hypothesis argues that in AD the normal role of tau in stabilizing microtubules is impaired and, induces other pathological changes.

Supporting evidence

Support for tauists mainly comes from the fact that cognitive decline in the elderly has a good correlation with tangle formation but correlates poorly with amyloid burden (Nagy et al, 1995; Cummings et al, 1996) and that the process of tangle formation starts with increased phosphorylation and then aggregation of tau in those areas known to be critical for memory (Braak et al, 1994; Braak and Braak, 1998).

Refuting evidence

However, there is no known genetic linkage between AD and tau-processing abnormalities that might lead to NFT formation. Indeed, tau gene mutations have been reported to associate with the development of frontotemporal dementia with parkinsonism (Heutink, 2000). Furthermore, the fact that NFTs composed of altered, aggregated tau proteins occur in disorders (e.g., subacute sclerosing panencephalitis, Kuf's disease, progressive supranuclear palsy, etc.) in the absence of A β deposition suggests that tangles are not sufficient to induce amyloid plaques characteristic of AD, and may arise secondarily during the course of a variety of etiologically distinct neuronal insults, including the gradual accumulation of A β and A β -associated molecules.

Whatever the mechanism, the fact that mutations in tau give rise to tau-inclusion tangles but not plaques and yet mutations in APP or in the probable APP-proteases (PS-1 and -2) give rise to both plaques and tangles almost proves that amyloid pathology occurs upstream of tau pathology. Two double-pathology models have also been created that come tantalizingly close to harmonizing the tauist and baptist camps. A transgenic mouse overexpressing both mutant APP and mutant tau has both plaques and tangles (Lewis et al, 2001). Interestingly, the double mutant has more tangles than tau-mutant mice alone and tangles appear in areas of the brain that are unaffected in single mutant-tau transgenic mice. In a parallel experiment another group showed that injection of amyloid into the cerebrum of mutant tau transgenic mice exacerbated tangle pathology in another brain region from

where neurons project to the injection site (Gotz et al, 2001). These models together show that tangles and plaques are related pathogenetically.

1.3 AD as a neuroplasticity failure: A unifying theory for AD?

Amyloid hypothesis is the dominant etiologic paradigm at present. However, one important limitation of both hypotheses is the assumption that one of the pathological hallmarks directly induces the other, even though the development of both pathologies is temporally and spatially separated in AD (Thal et al, 2000). Indeed, amyloid plaques and NFTs can occur independently of each other. Tangles composed of tau aggregates that are biochemically similar to or, in some cases, indistinguishable from those in AD have been described in more than a dozen less common neurodegenerative diseases (Heutink, 2000), in almost all of which one finds no A β deposits and neuritic plaques. Conversely, A β deposits can be seen in the brains of cognitively normal-aged humans in the virtual absence of tangles. An alternative proposal would be that both pathologies, instead of developing interdependently, might be prompted by the same mechanism.

AD as a neuroplasticity failure

AD selectively attacks those neural systems that play a crucial role in “higher cognitive functions” such as hippocampus, neocortical association areas, and the cholinergic basal forebrain neurons. A common feature of these brain regions is that they retain a high degree of structural plasticity throughout life (Arendt et al, 2001). Neuroplasticity is a life-long process that mediates the structural and functional reaction of dendrites, axons, and synapses to experience, attrition, and injury. The manifestations of neuroplasticity in the adult brain include alterations of dendritic ramifications, synaptic remodeling, long-term potentiation (LTP), axonal sprouting, neurite extension, synaptogenesis, and neurogenesis. Multiple lines of evidence indicate that the potential for neuroplasticity is distributed unevenly in the adult brain so that it is higher in the limbic system than in other parts of the cerebral cortex (Arendt et al, 2001). The fact that the spatial and temporal distribution of AD pathology follows the uneven pattern of neuroplasticity in the adult brain suggests that a breakdown of mechanisms regulating neuroplasticity is likely to be central in the pathogenesis of this disorder.

Mesulam (1999) proposed that AD represents a form of neuroplasticity failure. Neuroplasticity depends on the strengthening of existing synapses, formation of new synapses, and destabilization of previously established synapses. With the increasing complexity of brain structures in evolution, these dynamic processes may become more and more important. Meanwhile, the delicate balance between stabilization and

destabilization might also provide the basis for an increasing rate of failure (Arendt, 2004). The core idea of the hypothesis is that, AD-promoting factors create a setting where neurons must work harder to keep up with the increasingly more burdensome plasticity needs. Over many years, such compensatory processes would result in chronically high and eventually unsustainable levels of plasticity-related cellular activity and finally neurodegeneration and neuronal death. In other words, neuronal modifications that are supposed to facilitate neuroplasticity may also have detrimental effects that lead to neurodegeneration.

Support for this hypothesis comes from several pieces of evidence.

First, all AD-related risk factors potentially perturb neuroplasticity. Age, the single most important risk factor for AD, and all known genetic linkages of AD are associated with a decrease in the biological capacity for plasticity (previous chapter). Under these conditions, a chronically high demand for plasticity-related activity would lead to prolonged elevation of plasticity related cellular activities. In vivo and in vitro experiments show that high levels of neuroplasticity tend to be associated with increased expression and phosphorylation of tau (Black et al, 1996; Lovestone and Reynold, 1997). Similarly, animal experiments show that injury- and denervation-induced neuroplasticity can also lead to an upregulation of APP (Wallace et al, 1993; Beeson et al, 1994; Turner et al, 2003). In this way, the two principal pathological changes of AD become linked up by a single mechanism – neuroplasticity failure. These changes should initially appear within limbic-paralimbic neurons because these neurons have the highest baseline levels of plasticity and would thus have the highest exposure to compensatory upregulations of plasticity-related cellular activity. The resultant cytoskeletal dysfunction and neuronal degeneration in these regions would promote both “horizontal (within the tightly interconnected components of limbic-paralimbic cortices)” and “vertical (to the reciprocally interconnected association cortices)” expansion of the disease (Mesulam, 1999). Therefore, genetic mutations or risk factors do not cause AD – they simply operate through a common downstream mechanism and accelerate the temporal course of events that lead to plasticity failure. This helps to explain how the numerous genotypes of AD cause an identical clinical and neuropathological disease phenotype. In addition, the advanced cognitive and mnemonic activities of the human brain impose a very high plasticity burden, which helps explain human’s unique susceptibility to AD.

Additionally, evidence also exists that A β and NFTs, instead of “causing” AD, may actually represent protective or compensatory responses to cellular stress. Oxidative

damage is one of the earliest events in AD and activates several kinases, including glycogen synthase kinase-3 and mitogen-activated protein kinases, which are activated in AD and are capable of phosphorylating tau and neurofilaments (Reynolds et al, 2000). The regulation of tau phosphorylation in the adult mammalian brain is a naturally occurring process associated with neuroprotective mechanisms. The AD hyperphosphorylated-tau readily self-assembles into tangles in vitro under physiological conditions of protein concentration, pH, ionic strength and reducing conditions (del C. Alonso et al, 2001). Embryonic neurons that survive after treatment with oxidants have more phospho-tau immunoreactivity relative to those that die (Ekinici and Shea, 2000). In addition, cellular antioxidant induction and tau expression are opposing, suggesting that the reduced oxidative damage in neurons showing tau accumulation might be a direct consequence of an antioxidant function of phosphorylated tau (Wataya et al, 2002). Thus, the presence of NFTs may protect crucial cellular components from attack by reactive oxygen species. The fact that the tangle-bearing neurons seem to survive for many years (Morsch et al, 1999) is consistent with such a self-defense role of the formation of tangles. As for amyloid pathology, it has been reported that in AD cellular stress precedes increases in A β (Nunomura et al, 2001) and that a reduction in stress consequentially leads to lowered levels of A β (Yan et al, 1996; Lim et al, 2001). More interestingly, stress-induced increases in A β are associated with a decrease in stress (Nunomura et al, 2001), suggesting that the upregulation of A β could play a protective function.

In this way, the dilemma of amyloids and tau in AD pathogenesis can be solved by assuming that these two markers of AD are independent manifestations of a common underlying phenomenon -- a prolonged perturbation of neuroplasticity and the resulting compensatory response. An important inference of this hypothesis is that, treatment for AD may not be entirely successful unless the underlying plasticity failure is also addressed. One of the most important goals in AD research will be to understand the processes that influence plasticity in the adult human brain and to determine whether their vulnerability to aging and to the other AD-causing factors can be modified.

2 Alzheimer's disease at the synaptic level: AD as a synaptic failure

Synaptic loss in the hippocampus and neocortex is the major structural correlate of cognitive dysfunction in AD. (Davies et al, 1987; DeKosky et al, 1996). The degree of cognitive decline in patients with AD has been correlated with changes in the presynaptic vesicle protein synaptophysin in the hippocampus and association cortices (Terry et al,

1991; Dickson et al, 1995; Sze et al, 1997). Even at the end of the disease, quantitative correlations of postmortem cytopathology with premortem cognitive deficits indicate that synapse loss is more robustly correlated than are numbers of plaques or tangles, degree of neuronal perikaryal loss, or extent of cortical gliosis (Terry et al, 1991).

2.1 Synapses as the initial target in AD

Many biochemical and morphological studies suggest that early AD represents an attack on synapses (Small et al, 2001). Synaptic pathology is reflected by a loss of all major components of small synaptic vesicles and most peptides, stored in large dense core vesicles accompanied by extensive pathological changes of the synapse (Lassmann et al, 1993). Although deficits in numerous neurotransmitters (including corticotropin-releasing factor, somatostatin, GABA, and serotonin) accrue as the disease progresses, the early symptoms appear to correlate with dysfunction of cholinergic and glutamatergic synapses. This is consistent with the facts that the defining lesions of AD – the A β -containing neuritic plaques and the tau-containing NFTs – first attack septal-hippocampal and basal forebrain-neocortical pathways (Whitehouse et al, 1982). A quantitative morphometric study of temporal and frontal cortical biopsies performed within an average of 2 to 4 years of the onset of clinical AD revealed a 25 to 35% decrease in the numerical density of synapses in biopsied AD cortex, and a ~15 to 35% decrease in the number of synapses per cortical neuron (Davies et al, 1987). Synaptophysin immunoreactivity has also been reported to be decreased ~25% in the cortex of patients with MCI or very mild AD (Masliah et al, 2001). In some APP transgenic mouse lines, a decrease in the numbers of synaptophysin-positive presynaptic terminals and MAP2-positive neurons is the first histological change and appears at an age prior to A β plaque formation (Hsia et al, 1999).

It must be emphasized that AD is a chronically progressing disorder preceded by a clinically silent period of several years or even decades. Similarly, synaptic degeneration is a slow process progressing from an initial reversible stage to later stages irreversibly associated with marked synaptic loss. While massive loss of neurons and synapses can undoubtedly produce behavioural deficits, structural changes (loss of neurons and synapses) are not key factors for the physiological or behavioural deficits (Small et al, 2001), as these deficits can occur in the absence of cell death in AD models (Hsiao et al, 1996). In contrast, dysfunction of existing neurons and synapses may be sufficient to explain the behavioural deficits in AD.

Support for this notion comes from a large number of electrophysiological studies. Exogenously applied A β inhibits hippocampal LTP both in vitro and in vivo (for reviews,

see Selkoe, 2002; Rowan et al, 2003; Turner et al, 2003). Moreover, disrupted basal synaptic transmission and/or plasticity have been demonstrated in each of the APP-overexpressing mouse models that have been thoroughly tested (eg, Chapman et al, 1999; Larson et al, 1999; Janus et al, 2000a; Fitzjohn et al, 2001). In many cases, this synaptic dysfunction develops before plaque formation thus may result from soluble species of A β , especially soluble oligomers (Walsh et al, 2002), instead of amyloid deposits. Thus, AD has been proposed to be an A β -induced synaptic failure and such effects could contribute to the cognitive dysfunction (Selkoe, 2002).

2.2 A β -induced synaptic dysfunction

The concept that progressive accumulation of A β in brain regions important for memory and cognition initiates AD is currently the leading theory of causation. Several electrophysiological studies of young mice transgenic for human APP with AD-causing mutations have revealed significant deficits in basal synaptic transmission and/or LTP in the hippocampus, well before the development of microscopically detectable A β deposits (next section). Although the use of different electrophysiological protocols and mouse lines has led to some variability, all these studies support the concept that mutant APP transgenic mice undergo synaptic dysfunction. However, the presence of a mixture of A β forms (monomers, soluble oligomers, insoluble oligomers, and some insoluble amyloid fibrils) that are likely to exist in dynamic equilibrium in the brain of AD patients and animals has made it difficult to ascribe the specific synaptotoxic A β species.

Recently, there is evidence that soluble oligomers of A β , but not monomers or insoluble amyloid fibrils, are both necessary and sufficient for synaptic dysfunction in the brains of AD models (Walsh et al, 2002; Cleary et al, 2005; Lesne et al, 2006). Impaired LTP has been observed in APP-V717 (PD-APP) mutant transgenic mice before the development of A β deposits (Larson et al, 1999). In certain cultured cell lines expressing mutant human APP, natural oligomers of human A β are formed soon after generation of the peptide within intracellular vesicles and are later secreted from the cell at low nanomolar levels (Walsh et al, 2002). Intracerebroventricular microinjection of cell medium containing these oligomers in the absence of monomers and amyloid fibrils potently inhibited hippocampal LTP in adult rats. Pretreatment of the medium with a protease that selectively degrades A β monomers but not oligomers failed to prevent the LTP inhibition. Conversely, treatment of the cells with an inhibitor of β -secretase (one of the two proteases that generate A β from APP) markedly decreased oligomer formation at doses that still allowed appreciable monomer production, and such medium no longer

disrupted LTP (Walsh et al, 2002). Such findings indicate that neurodegeneration in AD may arise from injury caused by small, diffusible oligomeric assemblies of A β , whereas large polymeric aggregates (amyloid plaques) represent inactive reservoirs of species that are in equilibrium with the smaller, putatively neurotoxic assemblies (Selkoe, 2002). This animal work fits nicely with growing evidence that memory and cognitive deficits in MCI and AD patients correlate far better with cortical A β levels than with plaque numbers (Naslund et al, 2000) and correlate best with the soluble pool of cortical A β , which includes soluble oligomers (Kuo et al, 1996; Lue et al, 1999; McLean et al, 1999). Even in very mildly impaired patients, soluble A β levels in the cortex show a significant correlation with degree of synaptic loss (Lue et al, 1999).

3 Animal models of Alzheimer's disease

3.1 Criteria for a successful AD model

Animal models of AD greatly improve our understanding of the disease. However, it is important that the created animal models mimic AD both pathologically and behaviourally. Janus et al proposed 5 expectations for a credible rodent model of AD (Janus et al, 2000a).

1. Mice should exhibit progressive neuropathology culminating in at least one or more, of the accepted pathologic hallmarks of AD.

2. Mice should exhibit robust cognitive deficits evident in different behavioural paradigms targeting the same memory system. Ideally, behavioural paradigms employed to test mice should address neuroanatomic structures that are affected in AD (e.g. the hippocampus).

3. In the case of experiments employing FAD mutations, phenotypic changes should be correlated with the presence of the FAD mutations, and should be absent or less overt in age-matched mice expressing wt gene alleles expressed at equal (or greater) steady-state levels.

4. Key facets of the phenotype as per 1–3 should be confirmed in independent tg lines harboring the same construct, to exclude the contribution of insertional mutations.

5. Key facets of the phenotype as per 1–3 should have been confirmed in several laboratories.

Despite intensive efforts, a fully authentic mouse model has not been created. Early models focused on reproducing specific aspects of neuropathology. In the past decade, several strains of transgenic mice overexpressing FAD-related mutated genes have been created. These animals show a variety of phenotypes that are similar to AD but none of

them represent a full scene of AD. However, studies of these mice have begun to define the character and spatial/temporal evolution of cellular/biochemical abnormalities in brain, to delineate the pathogenesis that causes these lesions, to identify a variety of new therapeutic targets, and to evaluate potential therapeutic intervention of AD.

3.2 Early models

Before the identification of AD-related gene mutations, animal models focused on reproducing more specific aspects of neuropathology. For example the hypothesis that a cholinergic deficit is responsible for the cognitive impairments seen in AD led to the models with destroyed forebrain cholinergic nuclei, initially by electrolytic lesions, then by excitotoxins and finally by the selective immunotoxin 192 IgG-saporin. Another commonly used model is aging animals. Although informative, these models are too restricted and could not recapitulate the overall neuropathological picture of AD. In the past decade, with the identification of several genes directly linked to AD, it has been possible to develop transgenic mouse models to test many of the observations and hypotheses established from *in vitro* experiments and to evaluate therapeutic concepts.

3.3 APP transgenics

Transgenic mice are created by expressing variants of APP, PS-1, PS-2, apoE, or tau genes. Mice that overexpress mutated forms of APP gene were the first transgenic models of AD developed. Now there are over a dozen APP models. As a family, these mice develop a wide variety of behavioural, biochemical, pathological, and physiological traits simulating AD, although not all traits have been represented within an individual mouse line. One of these models, the tg2576 line, is used in the current study and its features will be discussed in detail in a separate section.

The earliest transgenic models of AD overexpressed full-length (Lamb et al, 1993; Pearson et al, 1993) or C-terminal fragments (Kammesheidt et al, 1992) of wild-type APP (Mucke et al, 2000). However, even high-level expression of wild-type human APP was insufficient to produce significant plaque deposition (Mucke et al, 2000) until very high A β concentrations had been achieved (Czech et al, 1997; Lamb et al, 1999). Thus the key factor in producing APP transgenic mice that do form plaques appears to be a high concentration of A β , which can be obtained by the use of a strong brain specific promoter driving high levels of protein expression or inserting an APP gene with an FAD mutation. Another crucial factor for plaque formation is age; all the APP-overexpressing mouse lines that develop AD pathology are apparently normal at birth, and begin to develop histopathology by approximately the middle of the normal mouse lifespan.

Neuropathology

There is some consistency among APP transgenic mouse lines in terms of histopathological profiles. Three different promoters (PDGF, PrP and Thy-1) and at least three mutations [V717I (Moechars et al, 1999), V717F (the PDAPP line, Games et al, 1995) and K670N/M671L (Hsiao et al, 1996)] all produce age-dependent increases in A β concentration and, eventually, deposition into dense-core amyloid plaques with reactive astrocytes, dystrophic neuritis and gliosis. Diffuse plaques also exist. The plaque distribution is not uniform throughout the brain, but has a similar regional distribution of plaques which mimics that in AD patients. There are differences, however, that could prove to be significant regarding the nature of the deposits. For example, mice expressing the V717F mutation produce vast amounts of A β 42 but little A β 40 and a greater proportion of diffuse plaques (Games et al, 1995), whereas K670N/M671L mutants demonstrated elevated concentrations of both types of A β and most of the amyloid load consists of dense cored plaques with relatively few diffuse deposits (Hsiao et al, 1996; Sturchler-Pierrat et al, 1997).

However, none of these models demonstrate all the pathological changes seen in human AD. Overt NFTs were absent in all these models, though hyperphosphorylated forms of tau are detectable. Another important difference regards the loss of neurons and synapses. A severe loss of neurons and synapses characterizes human AD, even in its early stages, whereas it is not prominent, if not absent, in APP transgenics (Games et al, 1995; Irizarry et al, 1997; Sturchler-Pierrat et al, 1997; Urbanc et al, 2002; but see Calhoun et al, 1998). In contrast, synaptic degeneration and dysfunction has been reported in nearly all the models studied, despite marked variability among them. Structural changes include reduced synaptophysin immunoreactivity, altered neurite trajectory, dendritic spine loss and thinning of dendrites (Games et al, 1995; Hsia et al, 1999; Mucke et al, 2000; Tsai et al, 2004; Spires et al, 2005; Jacobsen et al, 2006). However, in some models, these structural changes only occur at very late stage (>20 months), suggesting that synaptic dysfunction may underlie the cognitive deficits seen in younger subjects (>12 months) (Spires et al, 2005). Consistent with this idea, some forms of synaptic dysfunction, either basal synaptic transmission, or synaptic plasticity, or both, have been demonstrated in each of the APP-overexpressing lines that have been thoroughly tested (Chapman et al, 1999; Fitzjohn et al, 2001). It is currently uncertain how the synaptic changes observed in transgenic mice relates to the process of neurodegeneration in human AD, and whether it could be a precursor of neuronal cell death given longer time periods than are possible in a

mouse. However, it is clear, from the absence of neuronal loss in some models, that physiological A β accumulation does not cause rapid neuronal cell death in vivo.

Behaviour

It is difficult to compare the behavioural phenotype of a rodent model of AD with human patients, because of the significant differences in human and rodent cognition. Indeed, many of the diagnostic features of AD, such as language disturbance and loss of judgement, are impossible to examine in rodents. However, hippocampal dysfunction is the earliest and most prominent feature of AD, so behavioural testing of mouse AD models has focused on spatial learning and memory.

Before testing learning and memory, some measure of general activity and sensorimotor ability needs to be performed so as to exclude potential gross impairments of neurological function in these animals. No severe impairments in these domains have been reported, although some transgenic lines show hyperactivity, anxiety and neophobia (Hsiao et al, 1995; Moechars et al, 1999).

Multiple lines of APP transgenics have been shown to develop deficits in spatial learning and memory in a variety of behavioural test paradigms, though the onset time differs across models and tasks (Moran et al, 1995; Hsiao et al, 1996; Chapman et al, 1999; King et al, 1999; Moechars et al, 1999; Chen et al, 2000; Janus et al, 2000c; Morgan et al, 2000; Jacobsen et al, 2006). One important issue in modelling the cognitive deficits of AD is the age dependence of the behavioural impairments. To date, each of the mutations that cause behaviour deficits produce a component that is essentially age-independent, and another that is age-dependent. Interestingly, although the onset of amyloid deposition coincides with significant increases in the severity of some behavioural deficits, others do not appear to require the formation of amyloid plaques, indicating the possible involvement of soluble forms of A β in the development of cognitive decline (Moran et al, 1995; Chapman et al, 1999; King et al, 1999; Lamb et al, 1999; Westerman et al, 2002; Walsh et al, 2002a).

3.4 PS transgenics

The majority of FAD cases are caused by mutations in PS genes. These mutations, both PS-1 and PS-2, have also been used to generate animal models (Duff et al, 1996; Borchelt et al, 1996; Borchelt et al, 1997; Herreman et al, 1999). These models only show increased production of A β 42 without formation of neuritic plaques or neuronal loss (Borchelt et al, 1996; Duff et al, 1996; Borchelt et al, 1997; Citron et al, 1997). Similarly, they do not

display significant sensorimotor- or age-dependent cognitive impairment (Janus et al, 2000b).

PS-1 null mice fail to survive but this lethality can be rescued by crossing these mice to human PS-1 transgenic lines (Davis et al, 1998). Interestingly, not only transgenic lines expressing the wild-type, but also the mutant PS-1 gene carrying the human PS-1 (A246E) mutation can rescue the severe phenotype of the knockout mouse to similar degrees. Moreover, the level of A β 1-42 and the ratio of A β 1-42/40 are significantly increased in the human mutant PS-1 rescued PS-1 knockout mice than in the mutant PS-1 transgenics alone (De Strooper et al, 1998). These findings indicate that the mutation of the PS-1 gene does not lead to a loss of function during mouse development. Rather, it comprises a gain of function, which can be suppressed by competition of normal functioning murine PS-1 alleles, which are present in the human mutant PS-1 transgenic, but are absent in the PS-1 rescued mice.

When mutant PS1-overexpressing mice are crossed with the APP transgenics, the A β 42/A β 40 ratio in the brain is greatly enhanced and amyloid deposition is accelerated (Borchelt et al, 1996; Borchelt et al, 1997; Citron et al, 1997; Holcomb et al, 1998). Large numbers of amyloid deposits in the cerebral cortex and hippocampus were found already starting at 6 months of age in the double transgenics in comparison to 9–12 months in the single APP695(K670N+M671L) mutant APP line alone. However, stereological investigation of the hippocampus and frontal cortex of these mice indicate that there is still no dramatic cell loss despite the increased severity of the model (Takeuchi et al, 2000). PS-1–APP double transgenics do show behavioural impairments, but the severity is not significantly greater than that seen in the APP single transgenics (Holcomb et al, 1998; Holcomb et al, 1999).

Evidence that PS-1 coexpression with APP accelerates plaque deposition and that PS-1 expression alone is not sufficient to induce amyloid pathology supports the role for PS-1 as a modifying gene (Spires and Hyman, 2005). Moreover, FAD-associated mutations of presenilins cause an increased probability of producing the highly fibrillogenic 42 amino acid version of A β instead of the less harmful 40-amino acid peptide.

3.5 ApoE transgenics

ApoE knockout mice have decreased synaptophysin and microtubule-associated protein 2 staining, supporting a role for apoE in the maintenance of synapses and dendrites (Masliah et al, 1995). Evidence also exists for the involvement of apoE in A β fibrillization and clearance (Irizarry et al, 2000).

Recently, the effects of apoE on amyloid deposition have been tested by mating apoE knockout mice with APP transgenics (PDAPP or tg2576 mice). These mice show only sparse, diffuse plaque deposition and almost no dense, thioflavine S (thioS)-positive plaques or neuritic degeneration normally associated with these dense plaques (Bales et al, 1997; Bales et al, 1999; Irizarry et al, 2000; Holtzman et al, 2000a; Holtzman et al, 2000b). There was no obvious influence of the apoE^{-/-} state on APP mRNA or protein or on levels of A β , suggesting that neither reduced expression of the transgene nor the processing of APP to A β accounts for the absence of A β deposits in these mice.

It was hypothesized that apoE isoforms may differentially promote the aggregation, fibril formation, or influence the clearance of A β . In APP-expressing mice on an apoE null background, overexpression of apoE3 or apoE4 “rescues” A β deposition in dense, neuritic plaques that are absent in apoE knockout mice expressing APP (Holtzman et al, 2000a). ApoE4 expression induced a 10-fold higher density of neuritic plaques than expression of apoE3. In contrast, overexpressing apoE2 in both PDAPP and tg2576 mice ameliorates amyloid-induced dendritic spine loss, providing a mechanism by which the apoE2 allele might exert its protective effect in AD (Lanz et al, 2003).

3.6 Tau transgenics

The above models mainly target amyloid pathology and none of them show NFTs. To model the NFT pathology, tau transgenic mice have also been developed. Early transgenic tau models expressing wild-type human tau resulted in hyperphosphorylation of tau and somatodendritic localization without formation of NFTs (Gotz et al, 1995; Probst et al, 2000; Ishihara et al, 2001). Thus overexpression of human wild-type tau is insufficient to induce NFT formation, but the somatodendritic localization of tau in neurons does resemble the “pre-tangle” state observed in AD.

Later, many groups turned to the newly discovered pathogenic tau mutations for producing animal models. Expression of the FTDP-17-associated mutation P301L in the shortest four repeat tau isoform caused massive NFT formation in the spinal cord, brainstem, cerebellum, diencephalon, and basal telencephalon. These mice also show significant neuronal loss in affected brain regions and spinal cord (Lewis et al, 2000; Gotz et al, 2001; Santa-Cruz et al, 2005; Ramsden et al, 2005). Furthermore, the progression of neurofibrillary pathology and neuronal loss is also correlated with the progression of deficits in spatial reference memory as assessed by the Morris water maze, indicating a detrimental effect of NFTs on memory (Arendash et al, 2004; Santa-Cruz et al, 2005).

One important finding from these animals is that the formation of NFT lesions could be dissociated from tau-induced neuronal loss and memory decline (Santa-Cruz et al, 2005; Ramsden et al, 2005). In the inducible rTg4510 model, onset of memory deficits (2.5–4 months) precedes the development of significant NFT pathology or neuronal loss (5.5 months). Furthermore, suppressing inducible transgene expression after initial NFT formation has occurred (>4 months) did not halt the continuing increase in NFT numbers but halted the loss of neurons and enabled at least partial recovery of spatial reference memory function (Santa-Cruz et al, 2005). These findings suggested that NFT formation is not directly responsible for neurodegeneration and memory loss in rTg4510 and a toxic intermediate tau species is more likely to underlie these processes. This is similar to the finding that soluble oligomeric A β species, rather than insoluble amyloid, drives memory loss in APP mice and implies that reversible neuronal dysfunction, as opposed to structural neurodegeneration, explains much of the early memory loss in AD.

3.7 Multiple-transgenic models

With the availability of both amyloid and tau transgenic models, intense interest became focused on how A β would affect NFT formation. Crossing tg2576 mice that produce A β with JNPL3 tau mice (P301L mutation) resulted in a double-transgenic strain that developed both plaques and tangles. Whereas the presence of tau did not affect amyloid pathology, the presence of amyloid greatly enhanced NFT formation in the amygdala, entorhinal cortex, and olfactory bulb of double transgenic mice (Lewis et al, 2001). Importantly, the enhanced NFT pathology in the TAPP mice was also associated with evidence of neuronal loss in the entorhinal cortex. Similarly, injection of synthetic A β 42 into somatosensory cortex and contralateral hippocampus of P301L mice caused a fivefold increase in NFT numbers in the amygdala (Gotz et al, 2001). Results of these studies strongly suggested that A β accumulation accelerated the development of neurofibrillary lesions, although the exact mechanism of this interaction was unknown.

Oddo et al (2003a) developed a 3xTg-AD mice harboring mutations of APP (Swedish), PS1 (M146V), and tau (P301L). This model accumulates intraneuronal A β , and subsequently forms amyloid plaques and MAPT lesions in an age-dependent fashion: plaques first develop in the neocortex (around 3 months of age) and spread to the hippocampus by 6 months; tangles develop after amyloid pathology, appearing first in the hippocampus (at 12 months) and spreading to the cortex (Oddo et al, 2003b). The appearance of amyloid pathology before tau pathology despite similar levels of expression also supports the amyloid cascade hypothesis of AD pathogenesis. 3xTg mice also exhibit

age-dependent synaptic dysfunction, including LTP deficits that surprisingly precede plaque and tangle formation. (Oddo et al, 2003a) Instead, LTP deficits and memory deficits correlate with the accumulation of intraneuronal A β (Oddo et al, 2003b; Billings et al, 2005).

In subsequent studies, treatment of 3xTg mice with anti-A β antibodies, or antibodies specific for oligomeric forms of A β led to the rapid clearance of accumulated A β deposition and early tau lesions in the cell body (Oddo et al, 2004; Oddo et al, 2005). The removal of both A β and tau lesions proceeded in a hierarchical, time-dependent manner, with clearance of accumulated A β occurring before a reduction in the tau pathology. Furthermore, after clearance of the injected A β antibody, A β pathology re-emerged before the appearance of tau lesions. However, later stage hyperphosphorylated tau lesions were resistant to clearance by A β immunotherapy (Oddo et al, 2004). This mimics the findings of Santa-Cruz et al (2005) that NFTs are stable and resistant to transgene suppression.

3.8 Summary

Studies in mice expressing APP, PS and tau transgenes have provided clear evidence of A β and tau interaction in the pathogenesis of AD. Moreover, the results clearly support a central role of A β in these processes. However, it seems likely that A β accumulation does not lead directly to neuronal cell death in AD but promotes the accumulation of toxic tau species or other secondary pathological events. The close link between NFT formation and neuronal loss observed in multiple tau transgenic lines strongly suggests that in AD the development of pathological tau species is a major pathway to neurodegeneration, which, by extension, accounts for most of the clinical syndrome.

3.9 Tg2576 model

The tg2576 mice line was one of the first successful mouse models of AD. It was developed by insertion of the human APP₆₉₅ construct with the double ‘Swedish’ mutation (Lys670→Asn, Met671→Leu) and hamster prion protein cosmid vector into a C57B6/J*SJL host (Hsiao et al, 1996).

Neuropathology

The mutant gene has been shown to induce a 5-fold overexpression of APP₆₉₅ in the brains of mice over 2 months of age, and consequently, elevated brain levels of A β (Hsiao et al, 1996). It is important to note that different forms of A β are present in varying amounts throughout the lifetime of these mice. Detergent-soluble A β is present since birth, whereas insoluble A β becomes elevated by 6–8 months of age and A β -containing neuritic

plaques begin to form in the neocortex and hippocampus by 10–16 months (Hsiao et al, 1996; Irizarry et al, 1997; Kawarabayashi et al, 2001; Jacobsen et al, 2006).

In addition to amyloid pathology, tg2576 mice also show activated microglia, reactive astrocytes and neuritic dystrophy, which mimic what is observed in AD (Frautschy et al, 1998). The presence of hyperphosphorylated tau has also been reported in dystrophic neuritis close to neuritic plaques yet no NFTs are observed (Tomidokoro et al, 2001). Another important difference of these mice from AD patients is, despite similar A β burdens as those in AD, there is minimal or no neuronal loss within the hippocampus and cortex of aged tg2576 mice (Irizarry et al, 1997; Dong et al, 2007).

Behaviour

Sensorimotor functions have been intensively studied in tg2576 mice. Shorter latencies to fall from a beam and increased activity in the open-field and Y-maze have been reported for young but not aged transgenic mice (Hsiao et al, 1995; King et al, 1999). These altered exploratory behaviours could result from emotional or motor changes in young transgenics.

Learning and memory abilities of these mice have also been measured using a number of behavioural tasks. In the conventional Morris water maze task, aged transgenic mice showed significantly longer escape latencies to the hidden platform and spent less time in the target quadrant during probe trials, when compared with age-matched controls (Hsiao et al, 1996; Morgan et al, 2000; Westerman et al, 2002). As for spatial working memory, Chapman et al (1999), Corcoran et al (2002) and Barnes et al (2004) all reported an age-dependent (>10 months) performance deficit of tg2576 mice in the forced T-maze alternation task. Similar results have been reported in the spontaneous alternation task in Y-maze and radial maze (Hsiao et al, 1996; Morgan et al, 2000).

Thus, it is clear that transgenic mice develop memory impairments with age. However, reported onset of these deficits ranges from 3 months to 15 months in different studies (Hsiao et al, 1996; Holcomb et al, 1999; King et al, 1999; Morgan et al, 2000; Westerman et al, 2002; Jacobsen et al, 2006). Possible explanations for these discrepancies may include different genetic background of the mice, different training protocols or the varied sensitivity to hippocampal damages of each task. Another potential reason may lie in the age-independent performance deficits related to both mutant and wild-type human APP overexpression, reported by Westerman et al (2002) after studying transgenic mice overexpressing comparable levels of wild-type and mutant human APP₆₉₅. When mice with these deficits not specifically related to the expression of mutant APP₆₉₅ were excluded

from the analysis, spatial memory loss was first detected at ~6 months, which coincided with the appearance of detergent-insoluble A β aggregates.

Synaptic function

Massive neuronal loss can undoubtedly cause behavioural deficits. However, as discussed earlier, neuronal loss is not apparent in tg2576 mice, suggesting that neuronal and synaptic dysfunction may account for the cognitive deficits in these mice when they get aged. Evidence for progressive synaptic degeneration and dysfunction has been reported in tg2576 mice. No evidence of synaptic degeneration has been reported in this line before robust amyloid plaque deposition (<12 months) (Irizarry et al, 1997), However, recent studies have shown that in older tg2576 mice (21–25 months) synaptophysin immunoreactivity is reduced only around dense cored amyloid plaques and this is associated with electrophysiological changes indicating abnormalities in synaptic function (Spires et al, 2005). In addition, 3D multi-photon microscopy has been used to demonstrate altered neurite trajectory, dendritic spine loss and thinning of dendrites in a zone around the dense cored plaques in tg2576 (Spires et al, 2005). These findings further support the view that AD represents a synaptic failure.

Three groups have measured synaptic transmission and/or plasticity in tg2576 mice. However, these three studies produced different results. Chapman et al (1999) did not detect changes in basal synaptic transmission in young (2 to 8 months) or aged (15 to 17 month) mice, but demonstrated severely impaired LTP deficits in dentate gyrus *in vitro* and *in vivo* and, to a much lesser extent in CA1 *in vitro*, by the latter age. Furthermore, there was a positive correlation between the magnitude of LTP recorded *in vitro* in CA1 and dentate gyrus and the performance of the same mice on the forced T-maze alternation task.

In contrast, Fitzjohn et al (2001) reported decreased baseline synaptic transmission but normal LTP *in vitro* at ages 12 and 18 months. One possible explanation for the difference between the *in vitro* LTP results in these two studies could be the application of the neuroprotective kynurenate (glutamate receptor antagonists) during the preparation of slices by Chapman et al., suggesting increased vulnerability of tg2576 hippocampal slices to NMDA-mediated excitotoxic damage with age. However, this would not explain the diminution of LTP recorded *in vivo* in the dentate gyrus of 13 to 15-month-old tg2576 mice (Chapman et al. 1999). There are several possible reasons for the disparity between *in vitro* and *in vivo* LTP results in tg2576, including the presence of a diffusible factor disrupting cognition that might be diluted in slice preparations, the involvement of modulating

afferent pathways to the hippocampus that would be severed in slice preparations, or the contributions of synaptically mediated cerebrovascular responses that would make no contributions in vitro. (Ashe, 2001)

More recently, Jacobsen et al (2006) reported impaired basal synaptic transmission and LTP of tg2576 mice at 5 months of age, before the measurable rise of insoluble A β levels at 6 months. This synaptic dysfunction coincided with decreased dendritic spine density and impaired performance in contextual fear conditioning.

It is difficult to fully reconcile the discrepancies of these three studies. But all of them have confirmed synaptic dysfunction in this model.

Nature of cognitive impairment in tg2576 mice

Cognitive impairments have been repeatedly reported in aged tg2576 mice. It is important to identify the nature of these deficits. One commonly used task is the T-maze alternation task. Several groups have reported performance deficits of tg2576 mice in this task (Chapman et al, 1999; Corcoran et al, 2002). Theoretically, such deficits may result from either a more rapid decay of trial-specific information or an impairment of encoding extramaze (spatial) cues necessary for successful navigation. Both types of deficits are present in AD patients (Foldi et al, 2003; Monacelli et al, 2003). Recently, Barnes et al (2004) examined this issue in their study. They found that introducing an interval delay had similar effects on the performance of transgenic and wild-type animals. Thus adult tg2576 mice were no more sensitive than control mice to temporal delays or interference between trials on this task. In contrast, when the T-maze was rotated 180° between sample and test trials, control mice, but not tg2576 mice, were able to use extramaze cues to encode the goal location. Tg2576 mice were, however, capable of using intramaze and extramaze cues to acquire a visual discrimination task and a context discrimination task at the same rate as control mice. These results suggested that aged tg2576 mice were able to process at least some features of the environmental cues but failed to use this information to solve the T-maze task.

Two later studies by the same group (Hale and Good, 2005; Good and Hale, 2007) examined the effect of an APP mutation on object recognition memory processes in tg2576 mice. They reported normal object novelty detection and relative familiarity judgments in aged tg2576 mice. In contrast, tg2576 mice showed impaired visuospatial recognition memory, indicated by their failure to investigate objects that had changed their relative spatial positions. These results suggest that the formation of representations involving a

combination of object identity and spatial information are particularly sensitive to amyloid pathology in adult APP^{swe} mutant mice.

Molecular correlates of cognitive deficits in tg2576 mice

Tg2576 mice have been shown to develop cognitive deficits in a variety of behavioural test paradigms. Determining the onset and progression of memory deficits, as well as their molecular correlates, in these animals helps understand the pathogenesis of AD and develop effective treatments. However, this is a potentially difficult task, not only because of the subtlety of initial memory deficits and lack of sufficiently sensitive behavioural tasks, but also because various forms of A β , characterized by their aggregation states, are present in the brains of transgenic mice at different ages (Kawarabayashi et al. 2001). Multiple studies in APP transgenic mice have shown that memory loss and A β are correlated (Hsiao et al, 1996; Chen et al, 2000; Janus et al, 2000c; Morgan et al, 2000; Gordon et al, 2001; Dodart et al, 2002). However there is no consensus on which form of A β is primarily responsible (Ashe, 2001).

The first study on this topic in tg2576 mice assigned the onset of deficits to ~6 months, coinciding with the appearance of detergent-insoluble A β aggregates but prior to mature plaque deposition (Westerman et al, 2002). The simplest interpretation for these observations is that memory loss in tg2576 mice is associated with insoluble A β aggregates. Genetically accelerating the formation of detergent-insoluble A β aggregates resulted in an earlier onset of memory loss in these mice (Westerman et al, 2002), further corroborating this association. However, further research argued against such a simple relationship (Westerman et al, 2002). Analysis of tg2576 mice over a broader age range (4–22 months) failed to show any overall correlation between memory function and levels of insoluble A β , although a correlation was observed when the mice were stratified into several age groups. These observations suggest that the insoluble A β may be a surrogate measure for one or more small soluble A β assemblies that cause memory dysfunction in tg2576, explaining why some cognitively intact aged tg2576 mice have very high levels of detergent-insoluble A β . A role for soluble A β in memory dysfunction has been supported by both clinical and experimental studies. Elimination of A β in APP and PS/APP mouse models with either active or passive immunization with anti-A β antibodies could fully reverse memory deficits in these mice, but resulted in minimal clearance of pre-existing amyloid plaque pathology or total A β load (Janus et al, 2000c; Morgan et al, 2000; Kotilinek et al, 2002). Additionally, several studies have focused on identifying such small A β assemblies. Walsh et al (2002) identified a population of stable A β oligomers,

predominantly trimers, in the media of APP-transfected cells that produced deficits in LTP in rats in vivo, and further studies showed that the same type of oligomers were sufficient to induce memory deficits when injected into the brains of rats (Cleary et al, 2005). The memory deficits induced by A β oligomers were dissociated from any signs of neurodegeneration in the treated rats, indicating that memory loss is probably caused by reversible neuronal dysfunction. Thus, early cognitive decline in pre-clinical AD might also reflect the impact of small oligomeric A β aggregates on neuronal function and might therefore be reversible. Recently a study by Lesne et al (2005) identified one specific 56-kDa oligomeric A β species that consistently correlates with memory function in tg2576 and also disrupts cognitive function when administered to rats.

The proposed role of soluble A β also explains a puzzling inconsistency in the relationship between amyloid load and memory in AD (Ashe, 2001). Several early reports showed little or no correlation between amyloid load and dementia (Terry et al, 1991; Arriagada et al, 1992; Berg et al, 1993). More recently, when more sensitive antibody-based methods were used to measure A β deposits and subject pools that represented a broad range of cognitive impairment were examined, highly significant and robust correlations were found (Cummings et al, 1996; Bartoo et al, 1997; Naslund et al, 2000). Despite these methodological improvements, it has remained difficult to explain why some individuals with high plaque loads are cognitively normal. One possibility is that small A β assemblies formed during the conversion of detergent-soluble to -insoluble A β are primarily responsible for the cognitive decline. Certain individuals with low levels of these A β assemblies could be cognitively intact but would nevertheless accumulate large amounts of deposits or A β_{insol} over time.

Chapter 4 *Anatomy of the Hippocampus*

Given the early implication of the hippocampus and related structures in AD, it is crucial to understand the anatomy, physiology and function of this brain. The following chapters focus on these areas.

1 Nomenclature

There is some confusion in the literature regarding what is meant by “the hippocampus” or “the hippocampal formation”. In this thesis I will follow the nomenclature proposed by Scharfman et al (2000) as below.

The *hippocampus* is defined as the dentate gyrus (DG) and the cornu ammonis (CA) fields (CA1, CA2, CA3).

The *hippocampal formation* includes the hippocampus and the subiculum.

The *parahippocampal region* comprises the pre- and parasubiculum, the entorhinal, the perirhinal and the postrhinal (in nonprimate mammals) or parahippocampal (in primates including human) cortex.

This segregation is based on connectivity and laminar organization. The hippocampal formation shows a continuous trilaminar organization, which defines the allocortex. This is different from the neocortex, which comprises six layers. The parahippocampal region can be thought of as the transition from allocortical to neocortical organisation. As for general connectivity, hippocampal formation connectivity is much simpler, and more serial compared with that of neocortex: the connections between different hippocampal formation fields are overwhelmingly in one direction only, lacking much of the strong, reciprocal innervation between different areas. In contrast, the parahippocampal region shows a more “neocortical” pattern of connectivity, including at least two sets of reciprocal connections, one between peri- and postrhinal cortices and entorhinal cortex, the other between pre- and parasubiculum and entorhinal cortex.

2 Gross morphology

In rodents, the hippocampal formation can be revealed by the removal of occipital and

temporal neocortex (Figure 4.1). It is an elongated structure with the long axis extending in a C-shaped manner from the septal nuclei of the basal forebrain rostrally and bending caudo-ventrally into a cashew-nut shape to the temporal lobe. This axis is referred to as septo-temporal (or dorso-ventral) axis, and orthogonal to this, running medial to lateral, is the transverse axis. These two axes represent important landmarks in the structural connectivity of the hippocampal formation. It is arbitrarily divided into a dorsal portion (located just behind the septum), a posterior portion (located at the commencement of the structure's ventrolateral curvature) and a ventral portion (located within the temporal portion of the brain).

The horizontal section in Figure 4.2, through the hippocampal formation and the parahippocampal region, reveals the shape of its basic components. This section illustrates the trilaminar organization of the structures that make up the hippocampal formation and shows the emergence of new layers at the border between subiculum and presubiculum. The darkly stained material that defines two interlocked, C-shaped structures represents the granule cell layer of the DG, and the pyramidal layers of fields CA1-3. Below lies the polymorph layer or stratum oriens, whilst layer I is continuous with the molecular layer of isocortex and contains the well aligned dendrites of the principal cells. It also shows how the deep layers of pre- and parasubiculum appear to be a continuation of the pyramidal cell layer of the subiculum. The border between the subiculum and the presubiculum is characterized by a sudden emergence of a more superficially positioned cortical sheet. This increase in number of layers indicates the border of the hippocampal formation with the parahippocampal region. This sheet consists of the superficial layers of pre- and parasubiculum, which are strictly separated from their deep layers by a cell-free zone, the lamina dissecans. At the border between the entorhinal and perirhinal or postrhinal/parahippocampal cortex, the lamina dissecans disappears, giving way to a more homogeneously layered cortex that resembles the six-layered neocortex, with the only difference being that peri- and postrhinal cortices lack a marked granular cell layer IV.

The major fibre bundle associated with the hippocampus is the fimbria-fornix. The deep surfaces of the hippocampal formation are covered in a thin sheet of efferent and afferent fibres, the alveus. These fibres collect in a bundle along the lateral edge of the hippocampal formation, named the fimbria. At the septal extreme of the hippocampal formation, these fibres descend into the basal forebrain, at which point the bundles are referred to as the fornix (Figure 4.1). The fornix then splits into several components, innervating different regions including medial and lateral septal nuclei, anterior and

Figure 4.1

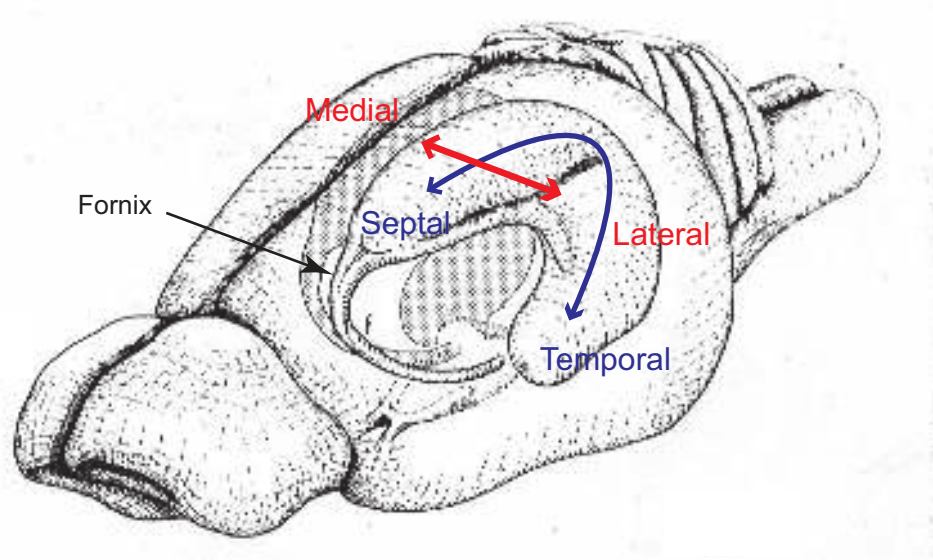


Figure 4.1 Three-dimensional drawings indicating the position of the hippocampal formation in the rodent brain, seen from a lateral frontal view. The septo-temporal axis (blue) runs between the poles of the hippocampal formation marked septal and temporal. The transverse axis (red) runs medial to lateral.

Adapted from Amaral and Witter, 1995

Figure 4.2

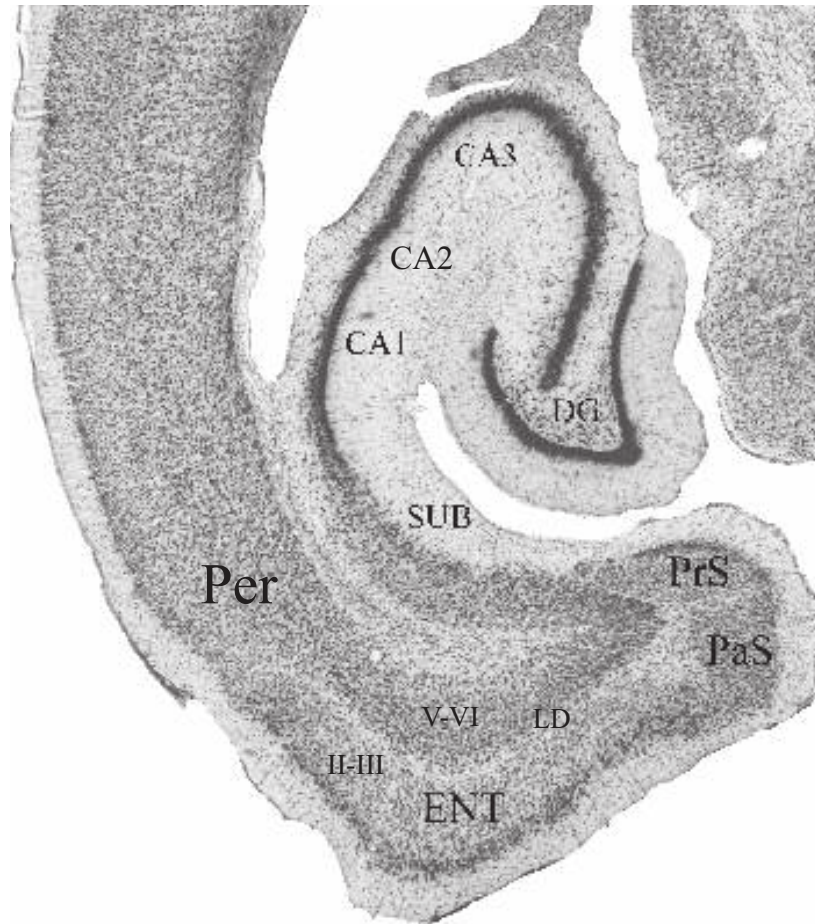


Figure 4.2 Horizontal section through hippocampal formation and parahippocampal region of the rodent brain. The trilaminar structures that compose the hippocampal formation are the Dentate Gyrus (DG), CA1-3 pyramidal cell layers, and the subiculum (SUB). The parahippocampal region is composed of the Presubiculum (PrS), Parasubiculum (PaS), Entorhinal cortex (ENT) and Perirhinal cortex (Per). The transition to the parahippocampal region is marked by an abrupt increase in the number of layers. The newly appearing layers form the superficial parts of these structures (II-III), while the deep layers (V-VI) are a continuation of the Subiculum. The transition between the Entorhinal and Perirhinal layers is marked by the disappearance of the cell-free zone, lamina dissecans (LD).

Adapted from Witter et al, 2000

posterior hypothalamic areas and anterior thalamic nuclei (Gloor, 1997). It should be emphasized that fornix fibres are both afferent and efferent to the hippocampal formation, and therefore represent subcortical input to, and output from, the hippocampal formation.

3 General connectivity of the hippocampal formation

Figure 4.3 illustrates the major connections of the parahippocampal-hippocampal regions. While connections between other parts of the hippocampal formation and various association areas of the neocortex are generally reciprocal, within the hippocampus they are largely unidirectional.

3.1 Afferents to the hippocampal formation

Cortical afferents

The entorhinal cortex plays a critical role in the hippocampal-parahippocampal network, in that it forms the gateway connecting the neocortex and the hippocampal formation. The major input to the hippocampal formation comes from the superficial layers of the entorhinal cortex, through the perforant pathway, whose organization will be discussed in detail in a later section.

Sub-cortical afferents

Some subcortical afferents directly innervate all fields of the hippocampal formation, while others are channelled through pre- and parasubicular cortices, via the entorhinal cortex.

Four major subcortical inputs to the hippocampal formation, not including thalamus, come from the medial septum, supramammillary hypothalamus, brain stem, and amygdala. The first three may be related to motor and arousal information. One of the most important inputs to the hippocampal formation, which may act as a pacemaker in the generation of the theta oscillation, is from the medial septum and the vertical limb of the Diagonal Band of Broca. Septal fibers terminate in all areas of the hippocampal formation, and are particularly prominent in the dentate gyrus (Amaral and Witter, 1995). The termination patterns of the supramammillary nuclei are much more specific, and target the dentate gyrus and CA2 in particular (Amaral and Witter, 1995). Monoaminergic input from the brain stem is primarily restricted to noradrenergic and serotonergic input. Noradrenergic input is quite dense to all regions of hippocampus proper and subiculum, while serotonergic input is densest to lateral entorhinal, dentate gyrus, and layer I of pre and parasubiculum (Amaral and Witter, 1995). Basolateral amygdalar input to hippocampus is largely restricted to CA1 and to its temporal third (Amaral and Witter, 1995). Finally, it

Figure 4.3

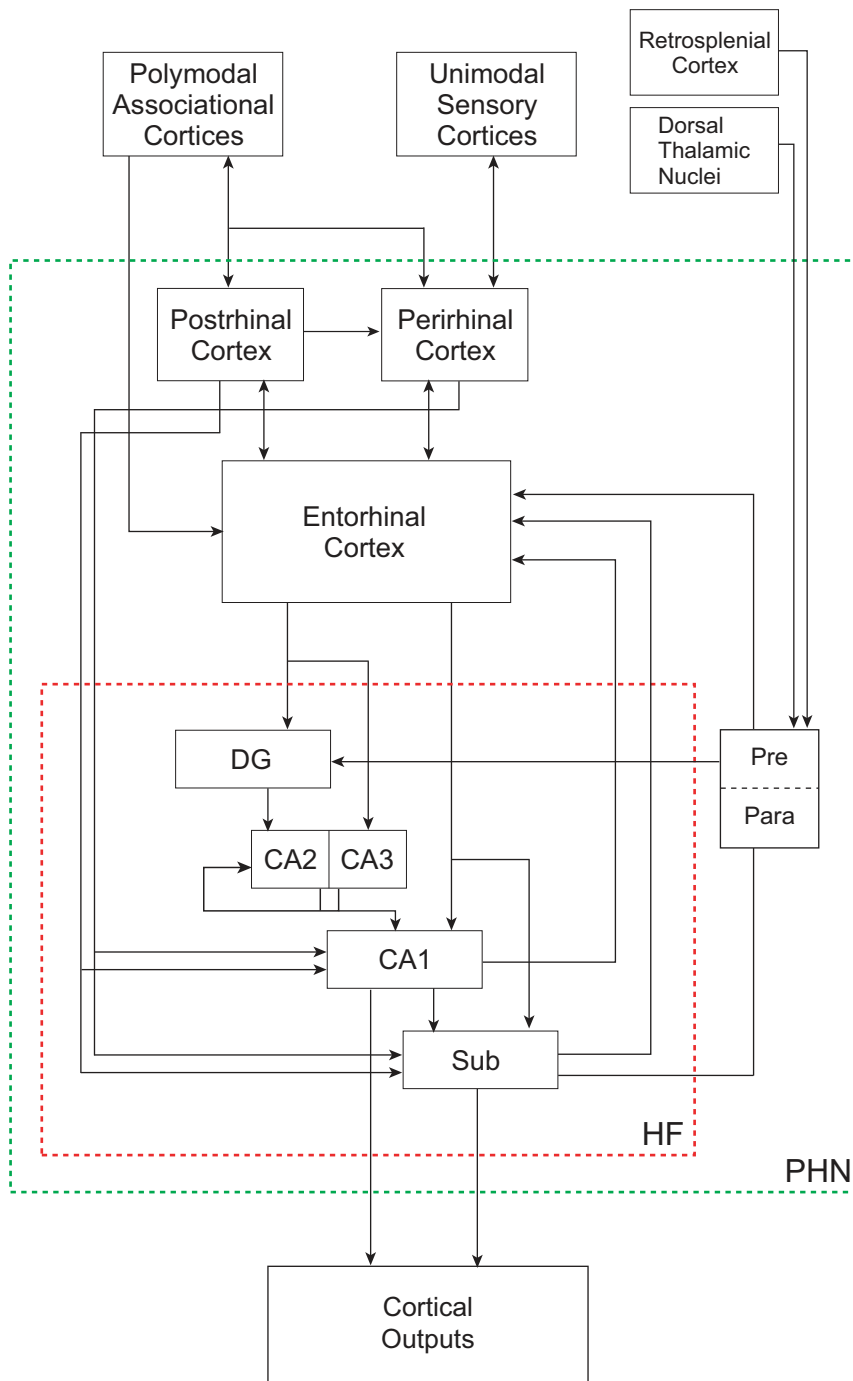


Figure 4.3 Diagram showing the internal connections of the hippocampal-parahippocampal regions, plus cortical input and output to these regions. The dashed boxes demarcate the hippocampal formation (HF, red box) and the parahippocampal network (PHN, green box). For details of cortical outputs of subiculum and CA1, see main text.

Adapted from Wills, 2005

should be added that CA1 receives a direct and massive input from the nucleus reuniens of the thalamus (Wouterlood et al, 1990), largely directed at the stratum lacunosum-moleculare and to CA1's middle septo-temporal levels.

An important sub-cortical input enters the hippocampal-parahippocampal network via the pre- and parasubiculum. These are afferents from dorsal thalamic nuclei, specifically the antero-dorsal, antero-ventral and latero-dorsal, which terminate in layers I, III and V of pre- and parasubiculum (van Groen and Wyss, 1990a; 1990b; 1995). The functional significance of this projection is that cells encoding the allocentric head direction of the animal have been found in dorsal thalamic nuclei (Mizumori and Williams, 1993). This input reaches the hippocampal formation mainly via the entorhinal cortex. Both pre- and parasubiculum send efferents to entorhinal cortex, presubicular efferents terminating in medial entorhinal area layer III, and parasubicular efferents terminating in layer II of medial and lateral entorhinal areas (Swanson and Cowan, 1977; Kohler, 1985; van Groen and Wyss, 1990a). There is also, however, a direct projection from parasubiculum to the dentate gyrus (Kohler, 1985).

3.2 Intrinsic connectivity of the hippocampal formation

Within the hippocampal formation, the major connections include:

- (1) mossy fibres from dentate gyrus to CA3;
- (2) CA3/CA2 to CA3/CA2 recurrent collaterals;

Strong associational/commissural projections to itself characterize the CA3 hippocampal field (MacVicar and Dudek 1980). The recurrent collaterals of CA3 pyramidal cells are highly divergently distributed along the hippocampal septo-temporal axis (Ishizuka et al, 1990; Li et al, 1994). In the rat (but not in primates), there is also a similarly widespread commissural projection to the contra-lateral CA3 (Blackstad, 1956). This organisation means that the CA3 system can be viewed functionally as a single network, within which each CA3 pyramidal cell can transmit information to every other pyramidal cell within a small number of synaptic steps. Such a CA3 recurrent network has been suggested to provide a mechanism for maintaining coherent information for short-term duration and to serve as a temporary storage site for short-term, episodic or working memories by reverberating activity in the recurrent collateral connections (Wiebe et al. 1997) and for pattern completion (Nakazawa et al, 2002).

- (3) Schaffer collaterals from CA3/CA2 to CA1;
- (4) projections from CA1 to subiculum.

However, as we shall see later, after discussing the organization of the perforant

pathway and the layout of the hippocampal intrinsic connections, the classical view of the information flow through the tri-synaptic loop is an oversimplification, and that the hippocampal connectivity should be viewed more as a series of parallel circuits.

3.3 Efferents from the hippocampal formation

CA1 and the subiculum are the only fields of the hippocampal formation that give rise to cortical efferents. The subiculum projects to a number of cortical and subcortical regions, and, in the parahippocampal region, to the deep layers of entorhinal cortex, perirhinal cortex, pre- and parasubiculum. The CA1 field also projects to deep layers of entorhinal cortex, perirhinal cortex, and, though not as strongly as the subiculum, to a number of cortical and subcortical regions. In this way, information processed within the hippocampal formation is relayed to the deep layers of entorhinal cortex. From there, it can be either fed back to the hippocampus, by way of the associational connections from deep to superficial layers of entorhinal cortex, or channelled to the cortical mantle, directly (from a very restricted area of entorhinal cortex) or through peri- and postrhinal cortices.

4 The entorhinal cortex

The entorhinal cortex (EC) plays a pivotal role in the function of the hippocampal formation. It is not only the main entrance for much of the cortical inputs, but also the main conduit for processed information to be relayed back to the neocortex. That means, the EC is both the beginning and the end point of an extensive loop of information processing within the hippocampal formation.

The EC can be divided into the lateral entorhinal area (LEA) and the medial entorhinal area (MEA) (Figure 4.4). These are cyto-architecturally defined regions of the entorhinal cortex. Briefly, LEA has a more clearly demarcated layer II, as compared to MEA, and LEA layer II cells are smaller, more densely packed and clustered into islands (Amaral and Witter, 1995). They are also differentiated in terms of their inputs and outputs, and are therefore relevant to the discussion below.

4.1 Afferents to the entorhinal cortex

The EC channels cortical inputs from the perirhinal and postrhinal cortices, and gives rise to most of the cortical input into the hippocampal formation via the perforant pathway. The origin of EC afferents is therefore crucial for understanding the nature of input to the hippocampal formation.

Afferents through peri- and postrhinal cortex

Much of the entorhinal input to hippocampus comes via the postrhinal and perirhinal

Figure 4.4

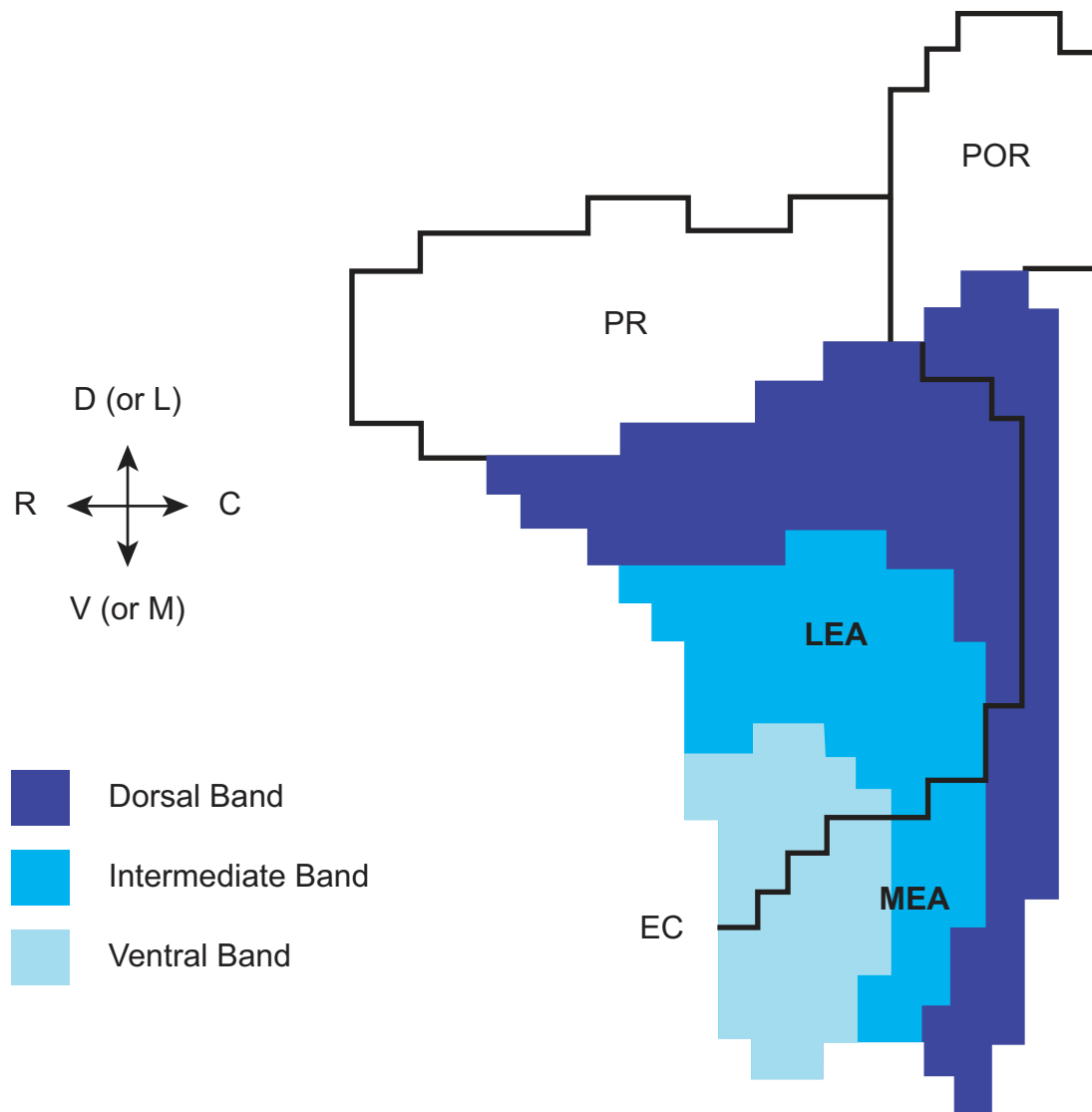


Figure 4.4 Diagram showing an unfolded map of the perirhinal (PR), postrhinal (POR) and entorhinal (EC, coloured) cortices. Black line within EC demarcates lateral entorhinal area (LEA) and medial entorhinal area (MEA). Shades of blue show the approximate location of the dorsal, intermediate and ventral dentate-projecting bands in the entorhinal cortex. Note that each band includes portions of both LEA and MEA.

Adapted from Burwell & Amaral (1998a).

cortices. The peri- and postrhinal cortices, in turn, receive most of their inputs from uni-modal and poly-modal neocortical association areas, suggesting that the hippocampus receives abstracted, highly-processed information from neocortex, but there is also some specialisation of inputs associated with each cortical area. Of the uni-modal input, particularly prominent efferents are olfactory (originating in piriform cortex) and visual. Visuo-spatial input, originating in the retrosplenial, cingulate, and posterior parietal cortices, also forms a robust projection (Burwell and Amaral, 1998a). There is some differentiation between peri- and postrhinal cortices: postrhinal cortex receives stronger visual and visuo-spatial inputs, whereas perirhinal cortex receives a stronger projection from olfactory areas.

However, the bulk of cortical input to rhinal cortices comes from polymodal associational cortices, in particular from ventral temporal areas. Perirhinal cortex receives from the whole extent of ventral temporal areas, while post rhinal cortex mainly from its most caudal aspect, which in turn receives visuo-spatial inputs.

Projections from the peri- and postrhinal cortices differentiate between MEA and LEA, with the postrhinal cortex sending more efferents to the MEA, and the perirhinal cortex preferentially projecting to the LEA (Burwell and Amaral, 1998b).

Direct afferents to entorhinal cortex

The EC also receives direct neocortical inputs. Similarly to peri- and postrhinal afferents, most projections are from uni-modal or poly-modal association areas. A particularly dense projection is from the piriform cortex (Burwell and Amaral, 1998a). Interestingly, there is a differentiation between inputs to MEA and LEA, with the MEA, similar to postrhinal cortex, receiving a higher proportion of cortical afferents from visual or visuo-spatial areas (Burwell and Amaral, 1998a) whereas the LEA, like perirhinal cortex, receiving predominantly olfactory information.

Thus, the pattern of differentiation between MEA and LEA is similar both for direct neocortical inputs to entorhinal cortex, and inputs via the peri- and postrhinal cortices. This strengthens the idea that there are two parallel streams of information coming from the peri- and postrhinal cortices, carrying distinct, highly-processed information to fields CA1 and subiculum in the hippocampal formation, by way of the medial and lateral components of the perforant pathway.

Afferents from pre- and parasubiculum

Another source of cortical inputs to the hippocampal formation via the entorhinal cortex is represented by the pre- and parasubiculum. Presubiculum receives strong

projections from visuo-spatial neocortical areas, including retrosplenial cortex (Swanson and Cowan, 1977; van Groen and Wyss, 1990a; 1990b), visual area 18b (Vogt and Miller, 1983) and cingulate cortex (van Groen and Wyss, 1990b). Cortical inputs to the parasubiculum are similar, but lighter than those to presubiculum and include occipital visual areas and retrosplenial cortex (Van Groen and Wyss, 1990a, Vogt and Miller, 1983). In addition, both pre- and parasubiculum receive a substantial input from the subiculum. Pre- and parasubicular intrahippocampal connectivity suggest that they lie at the crossroad between output and input, given that they receive strongly from the subiculum and project heavily to the entorhinal cortex and, in the case of parasubiculum directly to the dentate gyrus. This functional loop might be important in re-channelling into the hippocampus information already processed by the hippocampus itself.

4.2 Perforant pathway: entorhinal efferents to the hippocampal formation

Because the EC is the major relay for incoming sensory information in the hippocampal formation, the topographical organisation of the perforant pathway connections has strong functional implications for the kind of processing that will take place in the hippocampus. All fields of the hippocampal formation have a direct input from the perforant pathway. The organisation of the pathway is somewhat complex, however, it being possible to classify the projections according to three orthogonal divisions.

Layer II projections vs layer III projections

The EC gives rise to the “perforant pathway” to the hippocampal formation, which can be subdivided into two streams:

(1) Fibres originating in layer II of the EC project to the dentate gyrus, CA3 and CA2. The projections to the dentate gyrus terminate in the most superficial two-thirds of the dentate molecular layer, mainly on granule cell dendrites, but also on some GABA-ergic cells (Nafstad, 1967). Entorhinal layer II efferents to CA3/CA2 terminate in stratum lacunosum-moleculare (Nafstad, 1967; Steward and Scoville, 1976; Witter, 1993).

(2) Projections originating in entorhinal layer III, by contrast, project to CA1 and the subiculum. As in CA3/CA2, projections to CA1 terminate in stratum lacunosum-moleculare (Steward and Scoville, 1976). Projections to the subiculum terminate in the molecular layer (Witter, 1993).

MEA projections vs LEA projections

A further level of complexity is added by the fact that the perforant pathway comprises two components, orthogonal to the ones just described, which originate from two cytoarchitectonically defined subdivisions of the entorhinal cortex. These subdivisions

have been referred to as the lateral and medial entorhinal areas (LEA and MEA; Figure 4.4). The lateral and medial perforant pathways carry different information and show distinct terminal distributions in their respective hippocampal targets, depending on whether they originate in layer II or III.

Projections from cells in layer II discriminate along the radial axis of DG and CA3. LEA originating fibres terminate in the outer one-third of the molecular layer/stratum lacunocum-molecolare of the DG and CA3, while MEA originating fibres terminate in the middle one-third of these layers. However, they do not discriminate between transverse levels. In the dentate and CA3/CA2, therefore, the same cells are likely to be influenced by both MEA and LEA inputs (McNaughton and Barnes, 1977).

By contrast, MEA and LEA components of the layer III perforant path show a non-overlapping terminal distribution in CA1 and the subiculum along the transverse axis. Projections from the MEA terminate in the proximal part of CA1 (that closest to CA3) and the distal part of the subiculum (that closest to the presubiculum), whereas fibres originating in the LEA terminate in the distal part of CA1 and the proximal part of the subiculum (Witter et al, 2000).

This organisation is likely to have functional value, because inputs channeled through the medial and lateral portions of the entorhinal cortex are qualitatively different. Both the LEA and MEA receive strong inputs from the perirhinal and postrhinal cortices. While the LEA receives a stronger input from perirhinal cortex and anterior associational regions such as the medial and orbital frontal cortex, the MEA receives a more robust projection from the postrhinal cortex, and posterior associational regions like retrosplenial cortex (Burwell and Amaral, 1998b). It must be stressed that layer III neurons of MEA are also heavily influenced by presubicular input (Caballero-Bleda and Witter, 1993), which conveys more visuo-spatial information from retrosplenial and visual cortices, and dorsal thalamic inputs (head-direction cells which code for the allocentric direction of the animal's head have been found in the presubiculum of the rat; Taube et al, 1990). If we add that projections from CA1 to the subiculum and from CA1 and subiculum back to entorhinal cortex preserve this columnar organisation, we realise that two parallel streams of information can be recognised within the hippocampal formation, one originating from the MEA and most likely involved in coding of spatial orientation, the other from LEA, processing object-context relations (Witter et al, 2000; Bannerman et al, 2002; Moser et al, 2003). These parallel streams remain segregated at the CA1 and subicular level, while they are integrated at the dentate and CA3 levels.

Projections to septal vs temporal hippocampal formation

The EC can be further subdivided into three longitudinal strips, based on where perforant path fibres terminate along the septo-temporal axis of the hippocampal formation (Witter et al, 1989; Fyhn et al, 2004). Each of these strips contains portions of both MEA and LEA (Figure 4.4).

The most lateral and caudomedial portions of the EC, which receive major inputs from the adjacent perirhinal and postrhinal cortices, project predominantly to the septal third of the hippocampal formation. These include prominent olfactory, auditory, visual and visuo-spatial inputs. The most rostromedial portions of the EC, which receive prominent inputs from the limbic and periamygdaloid cortices, project more temporally in the hippocampal formation. The intermediate zone of the EC projects to the intermediate parts of the hippocampal fields. It is also interesting that the organisation of the entorhinal intrinsic connectivity preserves the segregation of the three longitudinal strips (Witter, 1989). Within the hippocampal formation, the projections from DG to CA3, CA1 to subiculum and subiculum back to entorhinal cortex, show a similar topographical organisation into three septo-temporal strips (Amaral and Witter, 1995). However, the CA3-CA3 auto-associative connections and the Schaffer collaterals show a highly divergent organisation, not preserving the septo-temporal organisation outlined above (Li et al, 1994).

The pattern of connectivity just described, suggests that septal levels (dorsal) of the hippocampal formation most likely process sensory-related information whereas the temporal hippocampus is more involved in visceral-related processing (Witter et al, 2000). Both behavioural and electrophysiological studies are beginning to demonstrate functional differences along the septotemporal hippocampal axis. Behaviourally, it has been shown that rats with lesions restricted to the septal extent of the hippocampus exhibit longer escape latencies in the Morris water maze task than rats with lesions restricted to the temporal portion of the hippocampus (Moser et al, 1993). A more recent paper (Bannerman et al, 2002) showed that ventral hippocampal lesions had no effect on T-maze alternation and on a working memory version of the water maze, even when task difficulty was increased by introducing delays. Moreover, place cells recorded from dorsal hippocampus show place fields with higher spatial selectivity than place cells recorded from more temporal portions of the hippocampus (Jung et al, 1994).

4.3 Entorhinal efferents to other regions

Entorhinal efferents: cortical

Deep entorhinal layers receive input from CA1 and subiculum; accordingly neurons in

these entorhinal layers may be very important in acting as the hippocampal conduits to the rest of the cortex. Most of the hippocampal-cortical connections in the rat are mediated by way of entorhinal-perirhinal-cortical connections, while only a very restricted part of the entorhinal cortex sends projections that can reach widespread portions of the neocortex (Insausti et al, 1997).

Entorhinal efferents: subcortical

The entorhinal subcortical efferents overlap those described for the subiculum and CA1. Like these two regions, but not the para- and presubiculum, entorhinal cortex projects to the septum, primarily to lateral septum. Entorhinal cortex also projects widely to the amygdala, especially to the basal nucleus. Other important efferents are those to the nucleus accumbens and olfactory tubercle. There have been no reports of entorhinal projections to the thalamus or brain stem (Amaral and Witter, 1995).

5 Summary

The unique anatomy of the hippocampal formation implies that its function is likely to be quite different from that of other cortical areas. For example, the neo-cortical inputs to the hippocampal formation originate in a variety of sources. Thus the hippocampal formation is one of the few brain regions that receive highly processed, multimodal sensory information. Within the hippocampal formation, its anatomical organisation allows for both serial and parallel information processing, which is ideally suited for mixing or comparing information. The output of the hippocampus is directed at many different areas, cortical and sub-cortical. Its ability to integrate, process and distribute information suggests that the hippocampal formation is most likely involved in general, abstract functions, for example memory or spatial cognition. Moreover, as discussed in chapters 1-3, the hippocampus is one of most vulnerable brain regions in AD. In this way research in this structure would undoubtedly provide crucial information about the pathogenesis of this disorder, especially at its earliest stage.

Chapter 5 *Physiology of the Hippocampus*

This chapter focuses on the physiological properties of the hippocampal network. The first part deals with the characteristics, the hypothesized generation mechanisms, and the behavioural and functional significance of the global electroencephalograph (EEG) states in the hippocampal system. The next sections look at two important aspects of the hippocampal network: long-term potentiation (LTP), as a form of synaptic plasticity, and hippocampal place cells, as its basic units. Finally, physiological changes of place cells, LTP and spatial behaviour in aging are briefly reviewed.

1 Electroencephalograph

Various EEG states have been recognized in the hippocampal formation. Four rhythmical patterns include theta (5-12 Hz), beta (12-30 Hz), gamma (30-100 Hz) and ripple (100-200 Hz) waves. Nonrhythmical patterns include large irregular amplitude activity (LIA) and small irregular amplitude activity (SIA). These oscillations are the product of synchronous activity within the hippocampal network, reflecting different functional states of the system, each correlating with a specific set of behaviours. Theta oscillations occur in the rodent during voluntary motion, exploratory activity, alert attention and rapid eye movement sleep. In contrast, during immobility, consummatory behaviours, and slow-wave sleep, the hippocampus enters LIA and ripples (Vanderwolf, 1969). The SIA state occurs during behavioural transitions, often when the animal awakens from rapid-eye-movement sleep or when it abruptly stops running. Little is known about the exact behavioural correlates of beta and gamma, but they may be associated with olfactory stimuli.

1.1 Theta

Basic properties

Theta activity consists of large amplitude (1-2 mV) oscillations in the frequency range of 5-12 Hz, which is the largest synchronous signal that can be recorded in the normal EEG of the mammalian brain. Theta has been observed in various animals, including rats, cats and dogs, and recently, in humans (Tesche, 1997; Kahana et al, 1999), and in various brain areas, including the hippocampal formation (Buzsaki et al, 1983), the entorhinal cortex (Mitchell and Ranck, 1980), and numerous cortical structures (Steriade, 2000).

Two components of theta

Theta can be classified into two components on the basis of behavioural correlates and pharmacology. The atropine-resistant component (t-theta) occurs during a class of “translational” movements that change the spatial relation between the animal’s head and the environment, such as running, turning, rearing, jumping, climbing, struggling when held, swimming, head movements, postural changes, manipulation (such as pressing a lever in a Skinner box), and digging in sawdust. The correlates of the atropine-sensitive theta (a-theta) are less well defined and may be related to arousal, attention and intention of movement.

Mechanisms of theta generation

The mechanisms of theta generation in the hippocampal-parahippocampal region appear to depend on three factors.

(1) External control circuits in the medial septum/diagonal band of Broca complex (MS/DBB) and other areas: Reversible or irreversible lesions of this region permanently abolish theta rhythm both in hippocampal and parahippocampal areas (Mizumori et al, 1990; Lawson and Bland, 1993). The majority of MS/DBB cells fire rhythmically at theta frequency and continue to do so even after deafferentation (Vinogradova, 1995). This septal population of bursting neurons likely includes both cholinergic and GABAergic neurons and both are important for theta generation in hippocampal and parahippocampal areas (Lee et al, 1994).

(2) Network properties within the hippocampal formation: The idea results from current density analysis of electrical activity in the hippocampus. A study by Buzsaki et al (1986), looking at current sink positions along the radial axis in CA1, proposed that hippocampal theta rhythm is the result of two inputs: one from the medial septum, and one from a theta generator in the entorhinal cortex. A later study by Brankack et al (1993) proposed that, in addition to the excitatory inputs from the entorhinal cortex, the excitatory connections between cells in the ‘tri-synaptic loop’ (the dentate gyrus to CA3 to CA1 pathway) also provide rhythmic inputs.

(3) Cells’ intrinsic oscillatory properties: In vitro work on hippocampal slices suggests that application of carbachol (a cholinergic agonist) to hippocampal slices induces membrane potential oscillations at theta frequencies in pyramidal cells in region CA1 (Bland et al, 1988) and CA3 (MacVicar and Tse, 1989). However, how this relates to the situation in the intact animal remains unclear.

Hippocampal cell firing and theta

The firing of cells throughout the hippocampal-parahippocampal formation is

modulated by the theta rhythm. In the hippocampus proper Ranck (1973) subdivided extracellularly recorded neurons into two broad classes: complex spike cells and theta cells.

Complex spike cells are mainly hippocampal pyramidal neurons. These cells fire at low rates (generally around 2 Hz and not greater than 30 Hz), have long duration action potentials (0.4-1.2 ms), and show complex spikes (bursts of two to ten spikes of decreasing amplitude and an interspike interval between 2 to 6 ms). An interesting phenomenon of complex spike cells is that they fire at progressively earlier phases of the concurrent locally recorded theta wave, as the animal crosses the place field of the cell (O'Keefe and Recce, 1993). O'Keefe and Recce (1993) proposed that this "phase-precession" phenomenon was generated by two oscillators of slightly different periods converging onto the hippocampus and that hippocampal pyramidal neurons (place cells) might encode information using both a rate (rate of firing) and a temporal (phase of theta oscillation at which firing occurs) code. This will be discussed in detail in the next section.

In contrast to pyramidal neurons, some hippocampal interneurons increase their firing rates in the presence of theta rhythm and thus have been termed "theta" cells. Theta cells fire at high rates (10 Hz to > 30 Hz), have short action potentials (<0.35 ms), and fire single spikes in bursts, preferentially at the negative peak of the extra-cellularly recorded CA1 theta wave (Fox et al, 1986; Csicsvari, 1999).

Functional significance

Three possible functions can be attributed to theta. Firstly, it acts as a synchronizing mechanism that locks the entire hippocampal formation into a global processing mode. Simultaneous recordings of EEG from different hippocampal subregions have shown that theta activity at comparable locations is in synchrony and coherent across large areas of the hippocampal formation (Buzsaki et al, 1983). Thus two cells, even if far apart in the hippocampus, have systematic temporal relations to each other if their firing patterns are related to the local theta cycle. However, recent research suggests that there is a systematic phase shift in theta across the dorsal-ventral axis of the hippocampus (Siapas et al, unpublished data).

Secondly, theta oscillation has been implicated in gating or facilitating synaptic modifications, which underlie LTP. It has been shown that theta-pattern stimulation induces LTP in vitro (Rose and Dunwiddie, 1986). This stimulation protocol has been referred to as primed burst stimulation and consists of a single priming pulse, followed 140-170 ms later by a high-frequency (100 Hz) burst of 2 to 10 pulses. It has also been reported that LTP is preferentially induced by burst stimulation on the positive phase of the theta rhythm in

urethane-anaesthetised rats (Pavlides et al, 1988).

Finally, the phase precession phenomenon allows for accurate location of one's position within a place field. During theta activity, all active hippocampal cells fire in bursts with the same frequency as or close to that of the theta rhythm. Theta cells do so whenever there is theta activity in hippocampal EEG, and they always maintain a fixed correlation to a particular phase of the sinusoidal EEG wave. In contrast, the phase correlation of complex spike cells does not remain constant, but change in a systematic way. These cells always began to fire on the same phase of theta, but the bursts of spikes occur at an earlier phase of each successive cycle. This phenomenon is named phase precession and allows for accurate location of position within a place field (section 3.3 of this chapter). Jensen and Lisman (2000) have elegantly shown that use of the phase precession data allows an increase in the accuracy of positional localization by around 43% if there is no error in estimation of an animal's position by monitoring its head position. In the more likely case that this estimate is somewhat inaccurate the value of phase coding increases further.

1.2 Gamma

Gamma oscillatory activity was first reported, in the rat hippocampus, in the hilar region of the dentate gyrus (Bragin et al, 1995), and has been later described in the superficial layers of entorhinal cortex (Chrobak and Buzsaki, 1998). Like theta, gamma oscillations show a high degree of coherence along the longitudinal axis of the hippocampal formation, along distances of up to several mms, but their average coherence decreases rapidly along the CA3 to CA1 direction.

Gamma oscillations occur during theta-associated behaviours (exploration, sniffing, rapid-eye-movement sleep), and are nested to the concurrent theta waves, their frequencies span from 40-100 Hz and there is a high correlation between theta and gamma frequency shift. This suggests that similar, still obscure, mechanisms modulate the frequency of both gamma and theta rhythms. Unlike theta, gamma oscillations persist after removal of all subcortical inputs to the hippocampal formation (Buzsaki et al, 1987), demonstrating that they are generated within the hippocampal circuits. A definite source of gamma rhythm is the entorhinal cortex, but after bilateral removal of this input gamma activity is still present in the hippocampus and is most likely sustained by the CA3 region (Bragin et al., 1995).

The function of gamma oscillations might be synchronising the activity among different subset of neurons within very short time windows (10-25 ms), complementing the role that theta waves have over longer time scales.

1.3 Large irregular activity (LIA) and ripples

LIA consists of large irregular, slow waves (slower than theta), which, in the deep layers of entorhinal cortex and in stratum radiatum of CA1 (just below the pyramidal layer), translates into a large amplitude (1-3 mV), aperiodic field potential also called the sharp wave (SPW; Buzsaki et al, 1983). They are thought to arise from a burst of activity in CA3, that produces a large depolarization in the Schaffer collaterals' targets, the dendrites of CA1 neurons and interneurons. Sharp waves coincide with a high frequency (140-200 Hz) oscillatory activity in the somatic layers of these structures, generally referred to as "ripples", which result from synchronous depolarization of CA1 neurons and inhibitory interneurons (O'Keefe and Nadel, 1978). LIA occurs when there are no changes of the animal's location in the environment: most frequently during slow-wave sleep and quiet sitting, less frequently during eating, drinking and grooming. Thus, LIA may represent a resting state of the hippocampal formation and is often inhibited by arousing stimuli.

Both interneuronal and pyramidal firing is phase locked to the rhythmic ripple oscillation. Pyramidal cells tend to fire a single action potential per ripple, phase locked to the troughs of the ripple oscillation. Interneurons generally fire several spikes during one ripple event with different populations showing different relationships to ripple events. The mechanism by which this highly coherent discharge of pyramidal neurons is brought about along the whole extension of the dorsal CA1 region is still unknown, but may be achieved through axo-axonic gap junctions (Draghun et al, 1998).

As for the functional role that ripples might have, Buzsaki (1989) has suggested a model of memory trace formation in the entorhinal-hippocampal region, in which the LIA, during rest or slow-wave sleep, plays an important role in potentiating memory traces of information acquired during exploration (the theta state).

2 Long-term potentiation (LTP) and synaptic plasticity

How the nervous system acquires, stores, and retrieves information derived from the external world has long been an attractive question in neuroscience. With the establishment of the neuron doctrine by Cajal, it has been proposed that modifications between neurons, or neuroplasticity, might underlie developmental, regenerative, as well as cognitive processes (Cajal et al, 1966). The manifestations of neuroplasticity in the adult CNS include alterations of dendritic ramifications, synaptic remodeling, long-term potentiation (LTP), axonal sprouting, neurite extension, synaptogenesis, and neurogenesis (Mesulam, 1999). Neuroplasticity is a life-long process that mediates the structural and functional

reaction of neurons and synapses to experience and injury. Experience-induced modifications of synaptic strengths, for example, enable the accumulation of knowledge that is unique to each individual and provide the critical substrates for adaptation and individuation (Mesulam, 1999).

The idea that memories are stored as changes in synaptic strength was first formalized by Hebb (1949), who suggested that coincident activity in two connected neurons leads to strengthening of their connection, thus providing a mechanism for the association of activity patterns.

The discovery of LTP (Bliss and Lomo, 1973) provided the first experimental support for Hebb's theory on the neural representation of memory. LTP is defined as a long-lasting increase of synaptic efficacy following a brief high-frequency stimulation. Since its first discovery in the rabbit dentate gyrus, LTP has been observed in many pathways of the brain. Within the hippocampus, LTP has been observed in all the three major excitatory synapses: the perforant pathway from the entorhinal cortex to the dentate gyrus (dentate LTP); the mossy fibers from the dentate to the CA3 (CA3 LTP); and the Schaffer collateral pathway from the CA3 to the CA1 (CA1 LTP) (for a good review, see Chen and Tonegawa, 1997). Now there is overwhelming evidence that LTP is engaged in spatial learning and memory (see below).

It must be made clear that LTP is not the only form of synaptic plasticity in the hippocampus and that Hebb's rule only applies to some forms of such plasticity. Various forms of synaptic potentiation exist with different temporal duration and induction and expression mechanisms (Bliss and Collingridge, 1993). Post-tetanic potentiation (PTP) refers to synaptic enhancement that decays within minutes. Short-term potentiation (STP) presumably lasts for 10-20 min. LTP can last at least 1 hour.

2.1 Properties

LTP can be either associative (Hebbian) or non-associative. CA1 LTP is Hebbian. It can be studied in hippocampal brain slices by intracellular or extracellular recording from CA1 neurons whilst electrically stimulating a bundle of Schaffer collaterals. In response to a brief tetanic burst of high frequency stimulation (typically 100 Hz for 0.5 s), subsequent low frequency pulses can elicit a larger excitatory postsynaptic potential (EPSP), which is LTP.

LTP has several features that make it suitable as a cellular substrate for learning and memory. The 3 most important features include its input specificity, cooperativity and associativity.

Input specificity

LTP is input-specific, which means that when generated at one set of synapses by repetitive activation, delivery of low frequency stimuli via a different untetanized afferents does not elicit LTP on the same cell. This property is advantageous because it greatly increases the storage capacity of individual neurons.

Cooperativity

The probability of producing LTP increases with the number of afferent fibers tetanically stimulated. While weak high frequency stimuli often fail to generate LTP because they excite only a few afferents, strong tetanic stimuli are successful because they recruit many afferent.

Associativity

LTP needs paired activity of multiple neurons. The majority of synapses that support LTP require NMDA receptors, which are thought to be a molecular coincidence detector because its activation requires simultaneous presynaptic activity (glutamate release) and postsynaptic depolarization. Depolarization is usually accomplished experimentally by repetitive tetanic stimulation of synapses or by directly depolarizing the cell while continuing low-frequency synaptic activation (a "pairing protocol"). It has been shown that synaptic activity in a "weak" afferent pathway that is unable to induce LTP due to insufficient postsynaptic depolarization can be made to do so if paired with stimulation of a "strong" pathway, which provides postsynaptic depolarization strong enough to activate NMDA receptors.

This property may be relevant (a) to associative or relational features of learning and memory (because associative induction implies the capacity to relate two arbitrary patterns of pre- and postsynaptic neural activity); (b) to storage capacity (because a synapse-specific mechanism endows greater storage capacity than would changes in cell excitability); and (c) to the permanence of memory (because the synaptic enhancement must last as long as the memory).

2.2 Molecular mechanisms

The above various properties of LTP arise from a complicated interplay of biological processes at the molecular and cellular levels. In recent years, there have been considerable advances in our understanding of these mechanisms. For convenience, we distinguish between LTP induction, which includes the events that trigger changes of synaptic weight without affecting them, and LTP expression, which comprises all the mechanisms that directly enhance synaptic efficacy.

It must be made clear that several types of LTP with varied mechanisms exist in the brain. Types of LTP can be distinguished whether they are associative, or more specifically, whether they depend on NMDA receptors (Bliss and Collingridge, 1993). Within the hippocampus, CA3 LTP is nonassociative, independent of NMDA receptors and thought to be initiated presynaptically (Zalutsky and Nicoll, 1990). In contrast, dentate and CA1 LTP are associative and are initiated postsynaptically by the activation of NMDA receptors. Even these two sets of synapses have different signalling mechanisms. For instance, CaMKII signalling is required at the latter but not the former (Zhang et al, 2005).

Thus, there is no generalized picture for the molecular mechanisms supporting LTP induction and expression. The following discussion will mainly focus on CA1 LTP, which has been shown to play an important role in spatial and contextual learning and whose physiology has been well studied (Chen and Tonegawa, 1997).

Induction

In general, LTP induction in the hippocampus is accomplished by applying brief trains of rhythmic high-frequency stimulation to excitatory axons that project to hippocampal neurons.

The triggering of LTP requires synaptic activation of postsynaptic NMDA receptors (Morris et al, 1986). Under normal conditions, hippocampal synaptic responses elicited by low-frequency stimulation are mediated primarily by the interaction of glutamate with AMPA receptors. Because the amount of glutamate released is low, few AMPA receptors are activated and the resulting EPSP is too small to open NMDA receptors. However, during high-frequency stimulation of excitatory afferents, strong postsynaptic depolarization coupled with presynaptic glutamate release results in the activation of NMDA receptors by releasing the voltage-dependent Mg^{2+} blockade of their associated ionic channels (Mayer et al, 1984). The combination of depolarization and binding of glutamate to synaptic NMDA receptors leads to the opening of these heteromeric voltage- and ligandgated ion channels and Ca^{2+} influx into postsynaptic structures (MacDermott et al, 1986). Calcium influx triggers a series of enzymatic cascades and immediate early genes encoding transcriptional factors that lead to a persistent modification of synaptic efficacy. The nature of these cascades is still under investigation, but involves protein kinases (such as protein kinase C and calcium-calmodulin kinase II), proteases (calpain) and phospholipases (phospholipase A_2) (Maren and Baudry, 1995; Lynch, 2004).

Expression

Once induced, LTP is expressed as a persistent and synapse-specific increase in the

amplitude of synaptic responses elicited by low-frequency stimulation of the excitatory afferents. It is generally accepted that both pre- and postsynaptic mechanisms are involved.

Postsynaptically, LTP causes the phosphorylation of AMPA receptors (Barria et al., 1997) and an increase in their single-channel conductance (Benke et al, 1998). But an increased expression of AMPA receptors is likely to be the primary requirement for the expression of LTP, which occur at both synapses that already contain functional AMPA receptors and those that do not express these receptors. AMPA receptor expression on cells is a dynamic process and is modulated by NMDA receptor activation. Incoming Ca^{2+} ions bind to calmodulin, which in turn activates Ca^{2+} /calmodulin-dependent protein kinase II (CaMKII), the beginning of a biochemical cascade that results in the net addition of AMPA receptors to the synapse. With more postsynaptic AMPA receptors available, subsequent presynaptic release of glutamate elicits a larger depolarization (Lynch, 2004).

In addition to the postsynaptic changes, LTP also involves an increase in the probability of presynaptic glutamate release. Quantal analysis in CA1 has demonstrated increases in quantal size, potentially reflecting increased probability of quantal release or changes in the number of release sites (Oliet et al, 1996). A further possibility is that glutamate release can be regulated by the time of vesicular fusion or the size of the fusion pore. Indeed, studies using a fluorescent marker of presynaptic activity, FM 1-43, have shown LTP in acute hippocampal slices to be associated with elevated glutamate release at single synapses (Zakharenko et al, 2001).

The evidence for postsynaptic induction mechanisms yet presynaptic expression mechanisms suggests communication between the two sides of the synapse. Postsynaptic activation of enzymatic cascades produces the synthesis and release of retrograde messengers which diffuse to the presynaptic terminals and interact with presynaptic enzymatic cascades to produce changes in transmitter release. Candidate retrograde messengers include nitric oxide, carbon monoxide and arachidonic acid, although other means of communication are possible, for example, through adhesion molecules binding the presynaptic and postsynaptic elements.

Late phase LTP

Similar to memory storage, LTP has both short- and long-term phases. One single stimulus train produces a short-term early phase of LTP that lasts 1-3 hours. Four or more stimulus trains induce a more persistent late phase of LTP that lasts for more than 24 hours. Persistent activity of synaptic kinases and posttranslational modifications are not sufficient to maintain LTP over the long term. Instead, this phase of LTP requires new gene

transcription, protein synthesis and formation of new synaptic connections (Nguyen et al, 1994). The cAMP-responsive element-binding protein (CREB) is a candidate that mediates late-LTP. CREB is a constitutively expressed transcription factor that, once phosphorylated, forms homodimers or heterodimers with other members of the B-Zip family of transcription factors (Herdegen and Leah 1998). This transcription factor binds to genes with the cAMP-responsive element (CRE) sequence, thereby stimulating the expression of these genes and leading to the generation of new dendritic spines, the primary targets of excitatory synaptic inputs associated with long-term morphological modifications during LTP (Murphy and Segal, 1997).

2.3 Functional considerations

The hippocampus is a major component in the medial temporal lobe, which plays an important role in declarative memory. Lesion studies in rodents confirmed that two forms of declarative memory, spatial and contextual fear memory, depend on the intact hippocampus (Morris et al, 1982; Philips and LeDoux, 1992). There are many parallels between LTP and memory. For example, both can be induced rapidly and once induced, persist for various periods of time. Both require CREB activation and new protein synthesis in their late phase. Optimal LTP induction conditions mimic naturally occurring theta rhythm that dominates the hippocampal EEG during information-gathering behaviours (section 1 of this chapter). There is also extensive evidence that LTP formation can be disrupted by a variety of manipulations such as hypoxia, seizure activity, cooling shocks and so on, prior to its stabilization, mimicking the consolidation period frequently observed in behavioural studies of learning and memory. Molecularly, LTP and memory are supported by similar mechanisms. Blocking NMDA receptors with APV prevents induction of LTP and impairs some types of learning. Similarly, gene mutations of the NMDA-receptor or of enzymes downstream of those receptor channels prevent LTP in CA1, prevent the formation of normal or stable place fields, and impair spatial learning. These mutations include NMDA receptor knockouts (McHugh et al, 1996), NMDA receptor knockouts only in area CA1 (Tsien et al, 1996 a,b) and CamKII mutations (Rotenberg et al, 1996; Cho et al, 1998; Giese et al, 1998).

If LTP is the neural basis of learning and memory, it (or other activity-dependent synaptic plasticity) should be induced at coactive synapses during memory formation. Later reactivation of these altered connections causes patterns of cell firing that collectively constitute the experience of memory for these events or the expression of learned changes in behaviour triggered by them. This is the core of the synaptic plasticity and memory

hypothesis (Morris et al, 2003).

However, it is not easy to verify or refute this hypothesis experimentally. Martin et al (2000) proposed four criteria for a rigorous test of the hypothesis: detectability (in association with the formation of memory, LTP must occur at certain synapses in one or more brain areas and should be detectable), anterograde alteration (blocking the mechanisms that induce or express changes in synaptic weights should have the anterograde effect of impairing new learning), retrograde alteration (altering the pattern of synaptic weights after learning should affect an animal's memory of past experience), and mimicry (artificial induction of LTP should give rise to a memory for a stimulus or event that did not actually occur). A detailed review on these topics is outside the scope of this thesis but can be found elsewhere (eg, Martin et al, 2000; Lynch, 2004). In brief, the first three criteria have been successfully fulfilled, but no studies have adequately met the mimicry criterion. In addition, LTP at different regions contributes differently to learning and memory. Most of the results from transgenic mice support the view that CA1 LTP is the primary synaptic mechanism for hippocampal learning, whereas dentate and CA3 LTP seems to be dispensable for these forms of learning (Chen and Tonegawa, 1997). Overall, LTP is necessary at least for some forms of spatial learning and memory. However, there is no evidence by now that LTP by itself is sufficient for spatial cognition.

3 Hippocampal place cells

In 1971, O'Keefe and Dostrovsky made the remarkable discovery that hippocampal pyramidal neurons in rats increased their firing rates over limited portions of an environment through which the animal passed (the "place field" of the cell) (O'Keefe and Dostrovsky, 1971). This obvious correlation leads these cells to be named "place cells".

Anatomically, place cells are pyramidal cells of the hippocampus and have been shown to be the complex spike cells (Muller et al, 1987). They are found in both the CA3 and CA1 regions of the hippocampus. Neurons with properties similar to place cells are found in other parts of the hippocampal formation (see below), but here the term "place cell" is reserved for the principal cells of CA3 and CA1.

3.1 Place field properties

A place field is cell specific and refers to the portion of any given environment within which a place cell fires at a high rate. Peak firing rates reported are very variable, ranging from 2-20 Hz. This is due to the intrinsic properties of place cells (some fire at higher rates than others) but also to the varied choice of methods and parameters used to display place

fields (smoothing, bin size etc.). Outside the place field, the discharge rate drops dramatically to virtually 0 Hz in well isolated cells.

Basic properties

It is possible, though not common, that a single place cell has more than one place field in an environment (Muller et al, 1987). A typical place field is continuous and compact, showing a single peak that falls off smoothly in all directions, approaching a 2-dimensional Gaussian distribution (Muller et al, 1987; O'Keefe and Burgess, 1996). However, not all place fields can be approximated to a two dimensional Gaussian, but can be influenced by the shape of the enclosure the animal is in. For instance, it is common to observe crescent shaped fields at the edge of cylindrical environments as well as elongated fields (linear) that "hug" the walls of rectangular or square walled enclosures (Muller et al, 1987; O'Keefe and Burgess, 1996).

The size of place fields vary considerably, due to the threshold used for separating signal from noise and the recording site. With a 1 Hz threshold as the minimum firing rate within a place field, field sizes ranged from 4% to 62% of the surface area in a cylinder of 76 cm radius (Muller et al, 1987). Within the hippocampus, the size of place fields expands as the electrodes are moved to more ventral portions (Jung et al, 1994).

Spatial distribution

The field centers are fairly evenly distributed over the surface of the cylinder and similar apparatuses, although there may be some tendency for fields to be more common near walls and proximal to prominent stimuli on the apparatus wall (Hetherington and Shapiro, 1997).

A very important question is if there is an anatomical-topographic relationship between place cells and place fields in the hippocampus. The vast majority of studies have not found any such relationship (O'Keefe and Nadel, 1978; Muller and Kubie, 1987). Neighbouring cells are as likely to code for distant regions of an environment as they are to code for nearby regions. Thus, there are no systematic relationship between the anatomical location of cells within the hippocampus and the places that they represent in an environment.

Field directionality

In open apparatuses like the cylinder, place cell firing depends only on the position of the animal's head and is independent of the head orientation. However, when the animal traverses repeated paths in a stereotyped manner, by environmental restrictions or reward-shaping or both, place fields can be directionally selective. For instance, where the rat

repeatedly runs back and forth between the rewarded ends of a linear track, the majority of place cells fire only when the animal runs either on the outward or the inward journey, showing marked directionality (O'Keefe and Recce, 1993; Huxter et al, 2002).

Markus et al (1995) investigated this question in two experiments. First they recorded place cells in two differently shaped environments: a cylindrical open field where place fields were omnidirectional, and a plus maze, where the rat trajectory is constrained by the shape of the environment and place fields were very directional. Next, place cells were first recorded while the rats were sampling the environment uniformly, chasing randomly scattered food, then recorded when the rats were trained to run stereotypically between four locations in the cylinder, where the food was sequentially placed. The proportion of directional fields grew from less than 20% to nearly 40%. This study suggests that the directionality seen in place fields is primarily attributable to the rat's restricted behaviour and, only secondarily, to environmental constraints.

3.2 Determinants of place fields

Why do place cells fire in particular locations in an environment? One possibility is that each cell has its simple sensorimotor or behavioural firing correlates: it responds to a simple sensory characteristic of the environment, such as a localized odour, or fires only when the subject is doing a peculiar behaviour, such as eating. The nondirectional mode of place cell activity rules out this possibility, as such particular scenes change radically as a function of the heading direction. Other evidence also supports this view. For example, place fields are frequently maintained even if significant landmarks are removed or recording is performed in the dark (O'Keefe and Dostrovsky, 1971; O'Keefe, 1976; O'Keefe and Speakman, 1987; Quirk et al, 1990). Indeed, the primary correlate of place cells is the animal's location in an environment, and it seems that place cells constitute an abstract, high-level cognitive representation of the environment, which is triggered by a combination of stimulus features, so that most cells fire in only one place because the combination occurs at that place in the environment. The sensory input necessary for this cognitive map can be obtained from both external (visual, somatosensory, auditory, olfactory) and internal (self-motion, proprioceptive, vestibular) systems.

External and internal cues

External or exteroceptive cues convey information about the proximal and distal features and landmarks in the environment. Internal or interoceptive information is derived from idiothetic or path integration system based on the animal's own movement. It has been suggested that distal, proximal, and idiothetic cues interact to determine place-cell

activity (O'Keefe, 1976; O'Keefe and Nadel, 1978; Best et al, 2002).

Place cells use information from different sensory modalities to identify landmarks. Of the various types of external information, stable distal visual cues, when present, exert a particularly powerful influence over place cell activity (O'Keefe and Conway, 1987; Muller and Kubie, 1987; Jeffery, 1997; Jeffery and O'Keefe, 1999). It has been repeatedly shown that place fields' angular location may almost be totally controlled by salient distant landmarks, for instance, a single large white card either on the wall of the recording environment (O'Keefe and Conway, 1987; Muller and Kubie, 1987; Jeffery, 1997; Jeffery and O'Keefe, 1999). In such circumstances, when the distal cues are rotated by a given amount, place fields can be observed to rotate both in unison and by a similar amount to the controlled cues. Furthermore, simultaneously recorded place cells rotate in unison (O'Keefe and Conway, 1978; O'Keefe and Speakman, 1987; Muller et al, 1987; Jeffery et al, 1997; Jeffery and O'Keefe, 1999). This is a well-established finding, and it is true of all the situations where the cues are not in conflict with the other sensory systems (vestibular, motor feed-back, olfactory etc).

Though important, visual cues are neither necessary nor sufficient for place field formation and maintenance. Place fields stay unchanged in darkness (O'Keefe and Dostrovsky, 1971; O'Keefe, 1976; Quirk et al, 1990) or when the experimentally controlled visual cues are removed (Muller and Kubie, 1987; O'Keefe and Speakman, 1987). Thus, non-visual information is capable of maintaining place fields when visual sensory input is removed. Research suggests that, under these circumstances, the fields are maintained by olfactory and tactile cues on the floor and walls of the enclosure, and by internal idiothetic cues, in particular vestibular and proprioceptive ones, providing information about the animal's own movement. Save et al (1998) examined place cell activity in animals that were blinded shortly after birth. Place cells were recorded in a cylindrical arena containing three objects (a wooden cone, a plastic cylinder, and a bottle of wine) near the wall of the apparatus. Both blind animals and sighted controls showed stable place fields. The difference was, while place field appeared during the subject's first pass through the field in sighted animals, blind rats did not show place field until at least one of the three objects was approached and explored. Hill and Best (1981) also found reliable place fields in rats that were both blind and deafened. After maze rotation, the place fields of the majority of the cells rotated with the physical apparatus. Because these subjects were receiving no visual or auditory information, it appears that they were relying on idiothetic information to track changes in location in an internal map of the environment.

What will happen if external and internal cues are in conflict? Jeffery and O'Keefe (1999) showed that distal visual cues could lose their control over place cell activity when the animal learned that the cues were unstable. They recorded place cells as rats explored a platform located in a curtained enclosure containing a single, prominent visual cue. After the locations of place fields were determined, the cue was moved to a new location. When rats were not able to see the experimenter move the cue, the place fields rotated with the cue. However, when rats were able to see the experimenter move the cue, place fields did not reliably rotate with the cue. Similar findings have been reported in other studies (e.g. Knierim et al 1995). Such evidence suggests that, external and internal cues compete to control the location of place fields.

Shape of immediate environments

Another important determinant of place fields is the distance of the animal to some of the walls of the immediate environment. O'Keefe and Burgess (1996) recorded place cells in four rectangular boxes that varied in the length of one or both dimensions. Interestingly, the location of place fields seemed to follow a subset of the box walls, and each individual cell is governed by its own particular combination of two or more walls in orthogonal directions, firing maximally when the subject is at the optimal distance from that particular subset of walls. For some cells, moving a wall moved the field without changing its shape, while for others, increasing the distance between two opposite walls shifted the field center so that it maintained the correct distance to both walls. This distorted the field shape, causing it to expand along that dimension, and it sometimes resulted in double peaks or the splitting of the field into two. This distance might be estimated either visually or by path integration. The influence of immediate environments on place cell firing was further demonstrated in Lever et al (2002). In this study, when rats were exposed to two geometrically different environments (cylindrical and square walled enclosures), the locations of place fields in these two environments were similar at the beginning, and diverged gradually and incrementally with more experience.

Non-spatial correlates

According to the cognitive map theory, the fundamental firing correlate of place cells is the location of the subject. But there are other correlates such as the existence or absence of objects in places (O'Keefe and Nadel, 1978) and the future action of the subject (Markus et al, 1995; Wood et al, 2000). In one study (Wood et al, 2000), rats performed a spatial alternation task in a T-maze. Each trial began with the rat at the base of the stem of the T and commenced, when the rat traversed the stem and then selected either the left- or the

right-choice arm. Rewards were available at the end of each arm according to an alternation sequence, requiring the rat to distinguish between their left- and right-turn experiences and to use their memory for the most recent previous experience to guide the current choice. Individual hippocampal cells fired as the rats passed through a sequence of locations within the maze during each trial. The key observation in this experiment was that the firing patterns of many of the cells depended on whether the rat was in the midst of a left- or right-turn episode. Thus, these cells fired selectively on either a left- or a right-turn trial, even when the rat was in the same segment of the stem of the T and running similarly on both types of trials.

3.3 The phase shift phenomenon

O'Keefe and Recce (1993) observed an interesting relationship between the theta wave of the EEG and place cell firing in rats running on a linear track. They found that as the rat traversed the place field of any given place cell, the cell fired at progressively earlier phases of the local EEG theta oscillation. They were recording from CA1 pyramidal cells. Since then the phase shift phenomenon has been observed in DG/CA3 cells, although the phenomenon is less pronounced (the precession occurs over more restricted phase ranges per any given run within the field; Skaggs et al, 1996; Huxter et al, 2002). Recently, phase precession has also been demonstrated in two-dimensional open fields (Huxter et al, 2008).

O'Keefe and Recce (1993) proposed that the phase precession observed in CA1 was generated by two oscillators of slightly different periods converging onto the hippocampus. One of these oscillators was the theta wave, whereas the other has still to be identified. They also proposed a functional role for phase precession, postulating that it could represent a temporal code which improves the ability of the hippocampus to represent space. Given that place fields on a linear track can be modeled in terms of symmetric Gaussians, firing rates do not distinguish between various portions of the edges of a field. Thus phase coding could in principle disambiguate the entry and exit portions of a place field. Jensen and Lisman (2000) showed that the position reconstruction accuracy of an ensemble of place cells recorded on a linear track can be improved by more than 43% using phase data and rate data as compared to using rate data alone. These findings strengthen the hypothesis that phase contributes to hippocampal spatial representation.

However, if this claim is correct, one should expect that temporal and rate codes should be independent of each other. Two studies failed to such a functional independence. Harris et al (2002) claimed that the theta phase of pyramidal cell firing is best predicted by the instantaneous firing rate of the cell, and therefore proposed that phase shift results from

the dynamics of the spike train. Mehta et al (2002) reported that, during initial runs through the place field, place firing became more asymmetric, and phase shift strengthened (Mehta et al, 2000). However, when Huxter et al (2003) looked at phase shift on sub-sections of the place field, and on individual runs through the place field, they found that the strongest correlation of phase shift was still the animal's position. They therefore maintained that place cells could represent position with independent rate and time codes.

3.4 Formation, stability and plasticity of place fields

It is important to understand how place fields are formed, developed and maintained in an environment. Some models of the hippocampal function (Wilson and McNaughton, 1993; McNaughton et al, 1996) suggest that path integration is the initial metric of the hippocampal map, and cues and landmarks are bound to the map secondarily. Indeed, the firing patterns of place cells remain stable in a familiar environment for extended periods and new map representations emerge over time in a novel environment (Wilson and McNaughton, 1993; Lever et al, 2002). A candidate mechanism responsible for such stability and respective change of neuronal firing patterns is a use-dependent synaptic plasticity, such as LTP.

Emergence of place fields in novel environments

Place fields develop rapidly when the subject is exposed to a novel environment. However, there is no general consensus about how place fields emerge in new environments.

Hill (1978) reported that initial fields (the first time an animal passes through the field) are indistinguishable from fields recorded later on, when the animal is familiar with the environment. This is in contrast with a study by Wilson and McNaughton (1993), which showed that place fields took 6 to 10 minutes to be established, and that these initial fields were not as stable as those in a familiar environment. Frank et al (2004) reported that about one-quarter of the new fields in a novel environment developed rapidly within the first 2 min and that most fields stabilized after 5-6 min of experience.

More recently, one study clarified the importance of CA3 sub-field in the formation of place fields in a novel environment. This study was performed in a knockout mouse (CA3-NR1 KO) in which the deletion of the NR1 gene is restricted to the CA3 pyramidal cells of an adult mouse (Nakazawa et al, 2003). It was reported that the specificity of spatial tuning in the CA1 place cells of mutants was reduced during the first 15 min of exploration in the novel space compared with the same period in the familiar space. By contrast, no space shift-associated change of spatial tuning was observed when the mutant mice were returned

1 day later to the pair of spaces experienced on the previous day. Behaviourally, these mice were impaired in the spatial delayed-matching-to-place task when the platform was placed in a novel location, but were normal when the platform location employed a few days earlier was reused. This behavioural deficit was highly specific in that the mutants were normal in the acquisition of spatial reference memory as tested by the standard version of the Morris watermaze task. These results suggest that CA3 NRs, most probably those in the recurrent network, play a crucial role in rapid hippocampal encoding of a novel encounter and in one trial- or one experience-based rapid learning.

Stability of place fields

Place cell recordings are usually made in a continuous "session" that starts when an animal is put in an apparatus and ends when it is removed. There is a general consensus that, once place fields are established and sensory stimuli are kept constant, they are stable both during sessions that last for minutes and across sessions separated by hours, weeks, or months. If sessions are run in two or more apparatuses over extended times, fields are stable in each (Muller et al, 1987; Muller and Kubie, 1987). The long-term stability of firing fields implies that the representation is recalled and not created de novo each time the rat enters a familiar environment. The stability of different fields in different familiar environments implies that many representations can be stored without interference and represents a form of memory.

Place cell plasticity

The hippocampus is a brain region with high capacity for plasticity. There is increasing evidence of experience-dependent change in hippocampal cells that might suggest a learning process (Bostock et al, 1991; Mehta et al, 1997; 2000; Lever et al, 2002).

Place fields develop asymmetries (an elongation of the field in the direction from which the animal is moving) and increase in size over a series of trials in which the animal travels repeatedly along a given path in the same direction (such as when rats run along linear tracks) (Mehta et al, 1997). However, place fields return to their original shapes by the next day (Mehta et al, 2000), indicating that the change is only temporal. An NMDA-function antagonist blocked these experience-dependent changes (Ekstrom et al, 2001), suggesting that this phenomenon might involve an asymmetrically associative LTP-like process, and that such plasticity mechanisms might be engaged in spatial behaviours of freely behaving animals.

Another type of plasticity takes the form of a long-term incremental change in the place fields and may underlie long-term incidental spatial learning and memory. In the

Lever et al. (2002) study, rats were exposed to two geometrically different environments (cylindrical and square walled enclosures) and place cell representations were shown to diverge gradually and incrementally. The divergence occurred in a totally incidental manner, in the absence of explicit reward. The changes were long-lasting, persisting for at least one month, in the absence of exposure to the environments during the delay period. Moreover the “cylinder” and “square” representations were shown to generalize across two environments of similar shape but different material, implying that the hippocampus had encoded the geometric features of the environments it was exposed to. These results have not only confirmed the view that the distance from two or more walls in particular directions is one of the major determinants of the location and shape of place fields (O’Keefe and Burgess, 1996), but also suggested that place cells might underlie the long-term incidental learning in the hippocampus.

3.5 Remapping

One important feature of the place cell is that, a dramatic change of sensory cues or moving the animal to a new environment result in a reconfiguration of place fields. This phenomenon is named ‘remapping’. In remapping, place fields can change in magnitude, shift in position, appear or disappear. The last two phenomena are worth particular attention. A robust place cell in one environment may stop firing in another. This suggests that every “sufficiently different” environment is represented by an independent subset of place cells chosen, with replacement, from the pyramidal cell population.

The remapping phenomenon suggests that the hippocampus learns and holds distinct maps for distinct contexts, with each specific map being reactivated as the subject switches between the different contexts. There is currently no consensus for predicting which kind of environmental changes will produce remapping. This is not surprising as some subjects may judge two environments to be quite similar while others judge the environments to be quite different. This issue is further complicated with the discovery of partial remapping.

Complete remapping vs partial remapping

A sufficiently large environmental manipulation may cause an ‘all-or-none’ switch in the responses of place cells, with almost all cells in one environment being either silent or having unrelated fields in a second environment. Such a dramatic change of spatial representation is called “complete remapping”. One example of complete remapping was given by Muller and Kubie (1987), who trained rats to forage for food pellets scattered randomly over the floor of either a circular or a rectangular box in the same curtain-enclosed environment. Under these conditions, place cells fields in one box did not predict

fields in the other (Muller and Kubie, 1987). Of a sample of 22 CA1 place cells, 12 had fields in only one environment and 9 had fields in both that could not be related by any ‘simple’ (rotational or radial) transformation. Later, however, this “complete” model of remapping was challenged by several other studies.

Skaggs and McNaughton (1998) recorded CA1 place cells in two visually identical environments connected by a corridor, through which the rat walked between them. When place fields in the two boxes were compared, cells were fairly evenly split into those which had similar fields, and those whose fields were completely different. There was, therefore, ‘partial’ remapping between environments. Another study investigated systematically changing stimuli across modalities: the colour (black/white) and odour (vanilla/lemon) of the recording environment (Anderson and Jeffery, 2003; Jeffery and Anderson, 2003). Place cells were found to have heterogeneous responses, with some remapping in response only to colour changes, some only to odour changes, and some to both. There did not appear to be any relationship between the responses of individual cells: remapping appeared to occur on a cell-by-cell basis. Taken together, in partial remapping, significant proportions of simultaneously recorded place cells are both remapped and non-remapped.

The reason why some manipulations produce complete remapping whereas others produce partial remapping is unclear. It seems which response is seen depends on the exact nature of the manipulation, and on the individual animal being studied.

Global remapping vs rate remapping

In addition to complete and partial remapping, Leutgeb et al (2005) identified another form of remapping – rate remapping, which may account for encoding different episodes occurred in the same place.

In that study, place cells were recorded under two different conditions: (1) two recording chambers with different shapes but always placed in the same location of the same room; and (2) the same recording chamber located in two different rooms. In the second condition, both the spatial locations of activated place cells and their discharge rates were statistically independent, representing a complete (or “global”, applied by the authors) remapping, as expected from previous studies. In contrast, in the first condition, virtually all the activated hippocampal neurons remained anchored to the same Cartesian coordinates, but the firing rate of the individual neurons varied more than an order of magnitude in the different chambers (rate remapping). A further study from the same group reported that hippocampal rate remapping was associated with stable grid cell firing patterns whereas as global remapping was accompanied by a coordinate shift in the firing

vertices of grid cells (Fyhn et al, 2007). Based on these findings, the authors suggested a dual coding scheme of spatial vs episodic information: global remapping permits the distinction between similar experiences that occur in different places, whereas rate remapping permits the generation of representations of unique episodes of experience independent of the ensemble-coded place.

However, some studies with similar aims have generated different results. For example, Wills et al (2005) reported abrupt and simultaneous switch of place cell ensembles (global remapping, according to Leutgeb et al) when rats explored either square or circular chambers at the same location of the room. One possible reason for such discrepancies is the subjects' varied familiarity with the testing conditions in different studies. One subject's "similar environments" may be judged to be significantly "different" by another with more experience. Indeed, Lever et al (2002) has shown that with more experience, place cells slowly and incrementally discriminate two similar environments.

3.6 Place cells as a coherent representation of space

An important component of the cognitive map theory (O'Keefe and Nadel, 1978) is that the hippocampus holds a holistic map-like representation of space. If that were the case, place cells should show a tendency to stably retain their field positions relative to each other, despite perturbations to the environment. In such a model, changes in the environment will not cause the positional firing patterns of individual cells to change independently of each other unless the change is big enough. At this point, the positional firing patterns of all cells and their relationships to each other in two-dimensional space will change all at once, as seen in complete remapping. The term 'coherent' will be used to describe place cells displaying these properties.

Pattern completion

Much evidence supports such a model. In a study by O'Keefe and Conway (1978), place cells were recorded on a 3-arm maze inside a curtained environment. Apart from the maze itself, the only cues inside the curtained environment were four prominent, distinct objects placed near the curtains. When these objects were rotated to a new position, all the fields rotate simultaneously, as if all the cells were bound to each other. Furthermore, eight cells were then tested with removal of one or two of the cue objects. All cells maintained the same fields despite removal of at least one object, and for most cells two objects.

The conclusion from this study is that the cognitive map is not a collection of independent place cells but is instead a stable unit, which responds to a degraded input pattern with the entire previously stored output pattern. Specifically, normal place fields

from all place cells can be elicited when the animal is exposed to a familiar environment but with some usual cues missing. This phenomenon is termed “pattern completion” and is in contrast to “pattern separation”, the ability to make the stored representations of two similar input patterns more dissimilar in order to decrease the probability of errors in recall, which will be discussed later (Marr, 1971; O’Reilly and McClelland, 1994; Guzowski et al, 2004).

The CA3 sub-field is known to have a robust recurrent network with pyramidal cells receiving synaptic contacts from 2% of other CA3 pyramidal cells (MacVicar and Dudek 1980) and is often proposed as an anatomical substrate of place cell pattern completion. Their extensive network of recurrent collaterals could act as an auto-associative network, allowing any cell missing its own particular input for that environment to be fired by others active in CA3. Direct support for this proposal has come from a mutant mouse that selectively lacked the NR1 subunit of the NMDA receptor in the CA3 area of the hippocampus (Nakazawa et al, 2002). The removal of the NMDA receptors prevents the selective strengthening of synaptic connections needed to create an auto-associative network specific for an environment. CA1 pyramidal cells were recorded while the mice were foraging in a cylindrical environment, placed within a curtained enclosure with four prominent visual cues. No difference was found in mean firing rate, spike width and place field size between control and knock-out (KO) place cells. Thus, spatial information within CA1 is relatively preserved despite the loss of CA3 NRs. The main finding of Nakazawa et al (2002) is that when place cells were recorded in trials where three of the four visual cues were removed, the firing rate, burst frequency and place field size of CA1 place cells in KO animals were dramatically reduced, even though the spatial selectivity of the cells remained intact. This was in contrast to the control mice, which showed no significant changes in place field properties associated with the environmental change. Behaviourally, KO animals were normal in the acquisition and retrieval of spatial memory tested in the conventional version of the Morris watermaze with four visual cues surrounding the pool. However, when a probe trial was run with only one of the four visual cues present, control animals’ performance remained intact, while KO animals exhibited a clear deficit of memory retrieval. KO animals still showed a selective search strategy in the correct quadrant, but the time spent in this quadrant was significantly shorter when compared to probe trials with the four visual cues present.

Thus, electrophysiological results are compatible with the behavioural results, suggesting that lack of CA3 drive impairs pattern completion ability and makes it more

difficult for mutants to retrieve old spatial memories when only partial cues are available. The fact that in the single cue condition the spatial selectivity (both at the behavioural and place cell level) is reduced but not completely abolished prompted the authors to suggest that this might be sustained by dentate to CA3 NMDA-independent plasticity and by the direct entorhinal to CA1 projections.

Pattern separation

In addition to pattern completion, CA3 is also involved in pattern separation. Indeed, computational models based on hippocampal connectivity have proposed that CA3 is uniquely positioned as an autoassociative memory network, capable of performing the competing functions of pattern completion and pattern separation (Marr, 1971; O'Reilly and McClelland, 1994; Guzowski et al, 2004).

Leutgeb et al (2004a) simultaneously recorded CA1 and CA3 place cells whilst animals were in enclosures with varying geometric similarity in three similar but distinguishable rooms. In these rooms, the overlap between active CA1 cell populations was much higher than that between CA3 populations. Thus, the CA1 representations shared more cells firing in different environments, whereas the CA3 representations were based on more distinct active subsets of cells. These results show that overlapping input patterns are orthogonalized in ensembles of CA3 neurons whereas CA1 neurons remain more faithful to their inputs. Further research from the same group suggested that this pattern separation function of the CA3 area was triggered by direct projections from entorhinal grid cells to the CA3 (Leutgeb et al, 2007).

It is interesting to compare this study, which shows pattern separation in CA3, with the Lee findings (2004), which shows pattern completion in the same region. The Leutgeb study recorded place cells in different rooms while the Lee study did in the same room but with altered cues. Thus, a competition exists in CA3 between pattern completion and pattern separation processes. When the environmental change is minor, the CA3 subregion employs autoassociation via the recurrent collaterals to make the representations more similar thus pattern completion predominates, whereas, without this extensive recurrent system, CA1 shows less overlap in its spatial representations (Lee et al, 2004). When the sensory inputs are changed drastically, CA3 cells produce very different spatial firing patterns (pattern separation), whereas CA1 cells maintain more similar place fields (Leutgeb et al, 2004).

One recent study has directly confirmed this hypothesis. Vazdarjanova and Guzowski (2004), using time-dependent RNA imaging of immediate-early gene (IEG) activity as a

measure of ensemble activity patterns, examined the responses of CA3 and CA1 ensembles in rats exposed sequentially to various environmental manipulations. When the animal was introduced to two similar environments in which either the proximal or distal cues were altered, ensembles in CA3 demonstrated greater overlap between contexts than in CA1, as in the Lee et al (2004) paper. In contrast, when the rats were exposed to two dissimilar environments in which both proximal and distal cues were varied, the overlap of CA3 ensembles active in each exposure was at chance levels, whereas that of CA1 ensembles was higher, as in the Leutgeb et al. (2004) paper. The obvious caveat to this result, however, is that assaying cell firing through gene activity cannot show the spatial nature of cell firing. If CA1 cells remapped by shifting field position, the techniques used in this study would not detect this.

Angular cue dissociation

Another way to investigate the coherent nature of the cognitive map is to put external cues in conflict with each other, for example by rotating distal cues in a different direction to the local (i.e. intra-maze) cues, and study the response of place cells. A coherent representation would be expected to rotate as a whole, following only one set of cues and ignoring others. If, on the other hand, different cells rotated with different cues, it would suggest that cells responding independently to individual stimuli.

The first study to address this issue found a complex set of results (Shapiro et al, 1997; Tanila et al, 1997). Rats were trained to run on a four-arm plus maze, where the spatial reference was defined by both intra-maze (distinct materials on each arm of the maze) and extra-maze (four prominent objects surrounding the maze) cues. On some trials, these two sets of cues were rotated 90° in opposite directions. The fields of some cells rotated with the distal cues, while many other fields rotated with the proximal cues. In another condition, the relative positions of distal and proximal cues were scrambled. As with the previous result, some fields moved with particular proximal stimuli and some with particular distal stimuli, and some cells fired maximally only when the proximal and distal cues were oriented in a particular way relative to one another. These findings argue against a coherent representation, suggesting, rather, that different cells are ‘tuned’ to different sets of cues. Brown and Skaggs (2002) repeated the experiment, recording 4-70 cells simultaneously. They found that, initially, cells rotated coherently, all following the intra-maze cues. After repeated manipulations, the likelihood of cells remapping increased. When the effects of remapping were controlled for, the incidence of cells simultaneously following both cue sets was no more than expected by chance. Knierim and McNaughton (2001) came to a

similar conclusion, although using a very different experimental protocol (3-dimensional rotation of the recording apparatus). In this case, no more cells were bound to the local cues than would be expected by chance.

Later, Knierim et al (2002) substituted a circular track for the plus-maze as the recording environment. This study claimed to demonstrate clear split control of place fields. This certainly appears true of some of the animals in the study: some fields rotate with local cues, some with distal, and some develop double fields, as if rotating with both cue sets. The field rotation data, grouped over all animals, shows a corresponding bi-modal distribution. However, in most cases, the field rotation data for individual animals does not show this. Only 2/8 animals show an obvious bi-modal distribution, in the others, fields seem to rotate overwhelmingly with either one cue set or the other. (The study does not provide formal analysis of this data). This study therefore showed that split control over place fields is possible, but not necessarily that it is the normal response to cue dissociation.

It remains unclear what kind of mechanism drives place cells to respond to local or distal cues. Since different place cells receive different sensory inputs about the environment, there might be a competition between this information so that the more powerful set controls the activity of the cell (Shapiro et al, 1997).

All the above studies were conducted in CA1 cells. Lee et al (2004) repeated the experimental protocol of Knierim (2002), but simultaneously recorded CA1 and CA3 place cells. In probe tests, the cues on the track and those on the walls were rotated counterclockwise and clockwise respectively so as to cause a mismatch between the sensory input provided by the proximal (track) and distal (wall) cues. When the magnitude of the mismatch was small (45°), both CA1 and CA3 ensembles output coherent representations that were similar to those of the original, familiar cue configuration. When the mismatch amounts were larger than 45° , however, the CA3 and CA1 cells reacted differently. The CA3 representation was still coherent, in that approximately half of the place fields rotated with the proximal cues and only a minority remapped or displayed ambiguous behavior. In contrast, the CA1 representation lost its coherence, as most place fields remapped or displayed ambiguous behavior. The finding of increased coherence in CA3 place fields is interesting, given the common idea that CA3, with its extensive recurrent collaterals, may be able to act as an auto-associative network (chapter 4). An alternative explanation, however, may simply be that CA3 receives different inputs to CA1, and that these are more controlled by the local cues.

All studies described so far have used angular dissociation of local and distal cues as

their primary experimental technique. Local and distal cues exert control over place fields in different ways, however, and their effects may interact in non-obvious ways, making prediction or modelling difficult. Fenton et al (2000a; 2000b) also investigated angular cue dissociation, but with two similar distal cues (a white card and a black card on a circular environment wall). The amount of angular dissociation was also smaller: 25° together or apart, compared to 90° or 135° in other studies. Under these conditions, place fields neither followed different cues independently, nor remapped. Rather, place representation was topologically distorted, such that fields near each cue moved rotationally with the cue, while those away from the cues moved radially towards or away from the centre. This experiment does show a type of coherent representation, therefore, though a flexible and deformable one, rather than a rigid one.

In conclusion, if there is a coherent place representation in the hippocampus, it is clear that it can be ‘broken up’ by a severe manipulation such as placing local and distal cues in conflict. All studies using this protocol found that some cells rotate with the cues and others remap. However, this does not disprove the existence of a coherent representation under more normal conditions. Subjecting animals to a lesser cue manipulation, as in Fenton et al (2000a, b), shows a place representation that is coherent, though deformable rather than rigid.

Attractor dynamics of the hippocampal network

An attractor network has one or several preferred positions or volumes in state space such that when the system is started from any other location, it will evolve until it arrives at one of the attractors and will then stay there in the absence of new inputs (Marr, 1971; Hopfield, 1982; Amit, 1992). In such a network, stored memories can be recalled from degraded versions of the original input stimuli (pattern completion), while at the same time the retrieved pattern stays separate from other, more different memories in the same network (pattern separation). The fact that place cells show both phenomena (previous sections) suggests that the hippocampal network may have attractor properties.

Strong support for this hypothesis comes from Wills et al (2005). In their study, rats were first extensively exposed to two very different environments (a wooden circle and a square made of morph material). Such exposures resulted in rapid occurrence of remapping, which persisted when rats were exposed to circular and square boxes both made of morph material. Then Wills et al probed rats to a series of morph boxes, whose shapes took a variety of configurations from more circular to more square-like, in a pseudo-random order across successive recording sessions. Interestingly, morphing the recording environment

from one familiar shape to another led to an abrupt transition in the hippocampal network state near the middle of their sequence when the intermediate shapes were presented. Not only was the switch point similar for all rats, it was also the same for all simultaneously recorded cells within a given rat.

These results suggest that place cells act in a unitary coherent ensemble fashion, with both pattern separation (which permits abrupt switch from circle to square pattern despite a small change in the geometry of the boxes) and pattern completion (which allows some type of cooperation among different place cells) at work.

3.7 Place cells and synaptic plasticity

Place cells are not isolated neurons but receive lots of synaptic input. It has been shown that NMDA receptor-dependent synaptic plasticity is not necessary for forming place fields, nor for maintaining them between consecutive test sessions in the same environment on the same day. However, longer-term stabilization of the place fields is seriously dependent on this pathway. Kentros et al (1998) found that NMDA receptor blockade by competitive NMDA receptor antagonist CPP neither affected the already established place fields in a familiar spatial environment before drug administration, nor prevented new place fields emergence (remapping) in a novel environment. The representation of the novel environment was stable when re-tested 1 hour later, but remapped again when tested the next day: different from that of the first day of exposure. Thus, NMDA receptor pathway is important for long-term stability of new, remapped place fields. Further research (Shapiro and Eichenbaum 1999) reveals that NMDA receptor blockade prevents even the short-term stabilization of place fields. As before, when drug-treated animals were placed in a novel environment, new place fields were formed. However, when the room lights were turned off for 5 min and then turned back on, place fields in drug-treated animals moved to new locations. These results are consistent with behavioural data that NMDA receptors, although required for some component of spatial learning, may not be required for the generation of the spatial representation of a specific environment (Bannerman et al, 1995).

Interestingly, deleting NMDAR1 subunits specifically in CA1 or the CA3 regions resulted in different alterations in the properties of place fields. The place fields of the NMDAR1-CA1 mutants were roughly one-third larger than fields in wild controls, and place cells with overlapping fields showed lower levels of coordinated firing. Not surprisingly, these mice exhibited impaired spatial learning (McHugh et al, 1996). In contrast, deletion of the NMDAR1 in the CA3 region resulted in a higher sensitivity to

partial removal of spatial cues, suggesting that NMDAR1-synaptic plasticity in CA3 networks is critical for pattern completion of spatial information (Nakazawa et al., 2002).

3.8 Place cells and spatial behaviour

If the cognitive map theory is correct, changes in that map should correlate with simultaneous changes in the animal's spatial behaviour. Several lines of transgenic mice without stable place fields are also impaired in spatial learning (McHugh et al, 1996; Tsien et al, 1996 a,b; Rotenberg et al, 1996; Cho et al, 1998; Giese et al, 1998).

An alternative way to test this theory is to examine place cell activities in normal animals performing behavioural tasks. O'Keefe and Speakman (1987) trained rats to perform a spatial memory task which required them to choose the correct arm of a 4 arm plus-shaped maze identified by distal cues to obtain reward. Removal of distal cues from the testing environment (with the rat absent) caused an increase in behavioural errors, as well as a change in the place-cell firing patterns relative to the trials performed with distal cues present. When the same data were realigned according to the rat's choice of goal arm (rather than the actual goal arm), the place fields returned to the same configurations obtained in the cues-present trials. In other words, the rat's choice of goal arm corresponded with the information contained in the rat's hippocampal map. In another study (Lenck-Santini et al, 2001), rats were trained in a continuous spatial alternation task in which they had to alternate between the two arms of a Y-maze to get a food reward in the third (goal) arm. By manipulating the information available to the animals, the researchers induced the cells to establish their fields in locations that were out of register relative to their standard position, thus making them inconsistent with the learned spatial task. When this happened, the rats' performance in the alternation task was markedly decreased. In addition, the nature of the behavioral errors during inconsistent field placements also changed dramatically in a way that was highly indicative of the rats' spatial disorientation.

Jeffery et al (2003) took a different approach with different results. They trained rats to go to a particular corner of a square-walled box (box A) after the occurrence of a tone. This task was shown to be hippocampal dependent. They recorded place cells from these rats in box A. When the animals were introduced to the same shaped box with different walls (box B), the animals' place cells remapped. However, the animals' corner choice performance in box B was significantly impaired relative to their performance in box A, but was much better than chance (about 63%, chance being 25%). These results appear to show some dissociation between place cell firing and behaviour.

In summary, although some studies confirmed a functional association between the firing patterns of place cells and the spatial behavior of the animal, current indications suggest some caution in attributing causal relationships between them.

3.9 Head-direction cells and grid cells

In addition to place cells, another two types of cells: head-direction cells (HDCs) and grid cells, have been discovered, which are equally important for spatial behaviour.

Head-direction cells

HDCs fire maximally when the animal points its head in a given compass direction, regardless of its position or behaviour in the environment, therefore signal the allocentric direction of the head of the animal in the horizontal plane. These cells have been found in a variety of strongly interconnected brain areas, including the presubiculum (Taube et al, 1990), lateral mammillary nuclei (Blair et al, 1998; Stackman and Taube, 1998), anterodorsal thalamic nuclei (AND; Blair and Sharp, 1995) and latero-dorsal thalamic nuclei (LDN; Mizumori and Williams, 1993).

Several studies have recorded the activity of HDCs before and after landmark manipulations, in an attempt to define to which environmental features the directional system can be tuned. When distal visual cues are rotated the preferred direction of the HDCs recorded, like place cells, shifts a near-equal amount as the cues. This has been found for all types of HDCs recorded so far, even if the amount of under-rotation varies across brain region (Taube, 1998). These results indicate that HDCs can be controlled by prominent visual stimulus. In the absence of visual stimuli, the head-direction signal is disrupted, but not suppressed (Goodridge et al, 1998).

So the firing of both place cells and HDCs may be controlled by distal landmarks when present. When they are simultaneously recorded in this type of cue-control experiment, their signals are strongly coupled (Muller et al, 1987; Knierim et al, 1995; 1998). However, this correlation by itself does not tell us if one system controls the other and to which extent they are operating in parallel. Current indications are that the head direction system controls the hippocampal place system, rather than *visa versa*. Dorsal presubicular and ADN lesions degraded the spatial selectivity of hippocampal place cells, and also made their signal more directional (Calton et al, 2003). Mizumori et al (1994) reversibly inactivated the LDN, showing a disruption of both hippocampal place fields and behavioural accuracy on a spatial memory task (radial arm maze). However, the converse is not true. In animals with complete dorsal hippocampal lesions it is still possible to record HDCs from the presubiculum and ADN (Golob and Taube, 1997, 1999). The properties of

these HDCs are very similar to those recorded from control animals. Thus, the place system relies on the HD system to provide it with the direction information for a fixed framework.

Grid cells

Another important but only recently discovered type of space-related cell is the grid cell in the medial entorhinal cortex (Hafting et al, 2005). The entorhinal cortex represents the major input structure to the hippocampal formation, therefore the electrophysiological studies conducted on this area are of crucial importance for understanding where and how spatially selective signals emerge in the hippocampus.

Early studies reported much lower spatial specificity and selectivity of neurons in some areas of the entorhinal cortex compared with that of hippocampal place cells. They are also less sensitive to enclosure changes (Quirk et al, 1992; Frank et al, 2000, 2001). Now, however, it has become clear that the spatial information carried by neural activity in the entorhinal cortex has been underestimated, because the entorhinal cell population was undersampled and large variations exist among different parts of this region.

Spatial representations in the entorhinal cortex follow a two-dimensional anatomical topography (Leutgeb et al, 2005). First, the spatial content varies between the lateral and the medial entorhinal cortex (Hargreaves et al, 2005). Neurons have stable and sharp spatial modulation in the medial subdivision, but not in the lateral subdivision. Second, in the medial entorhinal cortex, firing fields become gradually sharper and more concentrated along a ventromedial-to-dorsolateral axis (Fyhn et al, 2004). In the ventromedial-to-intermediate bands, where the early recordings were made (Barnes, 1990; Quirk et al, 1992; Frank et al, 2000), firing fields are broad and dispersed. Towards the dorsolateral end, however, firing fields get smaller and more confined. A recent study has shown a strong spatially selective signal in the superficial layers of the dorsolateral band of the entorhinal cortex (Fyhn et al, 2004), which projects to more dorsal areas of the hippocampal formation. These cells' firing maps form a regular grid-like pattern of locations across all environments the animal enters. Each grid cell fires in several locations in each environment, with the locations forming a regular pattern as though they were nodes on a triangular grid. Different cells recorded at the same location have the same grid spacing and orientation relative to the environment but differ in the location of the nodes such that the firing peaks of one cell are slightly shifted from those of its neighbours in a random manner (Hafting et al, 2005). The overall set of fields of several such cells together spans the whole surface of the environment. For each cell, the size of the grid appears to be

independent of the size or shape of the environment.

The firing of grid cells can express information about position (grid vertices), direction (grid orientation) and distance (number of grid cycles) (Hafting et al, 2005). The grid-like firing persists after the removal or displacement of extrinsic sensory input, suggesting that path integration is a primary mechanism for generating such a metric representation of the environment. In addition, the orientation of the grid is dependent on the location of a polarizing visual cue in much the same way as head direction cells and place cells. More importantly, it has been shown that the localized firing of these neurons can be maintained without a functional hippocampus. Thus, the key features of the map are probably not mere reflections of hippocampal output. Instead, the position vector may be computed, at least partly, in the medial entorhinal cortex itself. Support for this proposal comes from the finding that layers III and V of the entorhinal cortex contain grid cells, head-direction cells and conjunctive grid x head-direction cells (Sargolini et al, 2006). These layers are major target areas of projections from the presubiculum, which probably convey signals from the head-direction cells in this area (Taube et al, 1990). All three cell types are responsive to speed of movement of the animal (Sargolini et al, 2006). Thus, the medial entorhinal cortex contains the translational and directional input necessary for computing a continuously updated metric representation of location.

The exact relationships between entorhinal grid cells and hippocampal place cells require further research. According to some models, the amazingly regular firing pattern of grid cells suggests that they may be able to calculate spatial information and act as the primary site of path integration, whereas the hippocampus serves to encode episodic information (Leutgeb et al, 2005; Fyhn et al, 2007). Moreover, because the multiple fields of grids cells make them inappropriate to associate the sensory information to a specific location, the hippocampus might also serve as the read-out structure that provides feedback information to grids cells to maintain their association to sensory inputs from the environment, and to connect grids with different orientations and scales together (O'Keefe and Burgess, 2005).

4 Hippocampal physiology in aging

Aging is accompanied by a decline in cognitive abilities, particularly in spatial learning and memory. When young and old rats are compared on spatial navigational tasks, such as the Morris water maze task, the clear and consistent finding between laboratories is that old rats do not remember the spatial location of the escape platform as well as do young rats

(Barnes et al, 1997). Aging also causes deterioration of hippocampal circuitry and plasticity, which may explain the cognitive impairment seen in the same subjects.

4.1 Changes of hippocampal anatomy in aging

In the past it was considered that substantial neuronal loss might be associated with aging and thus responsible for the cognitive decline. Though early studies noted a loss of hippocampal principal cells with age (Coleman and Flood, 1987), careful anatomical studies have shown that there is no loss of neurons in the primary CA fields of the hippocampus, nor do granule cell numbers change during normal aging in humans (West et al, 1994), rats (Rapp and Gallagher, 1996; Rasmussen et al, 1996), or mice (Calhoun et al, 1998). Thus, widespread hippocampal cell loss is not required for the appearance of age-related cognitive deficits.

As for synapses, there is no evidence of gross synaptic changes in aging. Instead, synaptic loss is highly region-specific and mainly affects cortical inputs to the hippocampus. Geinisman et al (1992) used an unbiased stereological method to count synapses in memory-impaired aged rats and reported a decrease in the number of axospinous synapses in the mid-molecular layer of the dentate gyrus. More recent work examined synapse-associated protein levels in the hippocampus (Smith et al, 2000) and found significant losses of synaptophysin in aged, memory-impaired rats, but only in stratum lacunosum moleculare of CA3. Moreover, when all aged rats, regardless of spatial learning ability, were considered together, synaptophysin levels (in CA3 lacunosum moleculare and the outer and middle portions of the molecular layer of the dentate gyrus) were significantly correlated with performance in the Morris water maze. Similar results have been reported in humans (Scheff et al, 1996).

4.2 Changes of hippocampal synaptic transmission in aging

Electrophysiological data support the anatomical observation that there is a reduction in synapse number in the dentate gyrus of aged animals. In the aged rat, the field EPSP recorded in the dentate gyrus is reduced (Barnes, 1979; Barnes and McNaughton, 1980). There is general agreement that synaptic plasticity is also impaired in aging. However, the changes are complex, protocol-dependent and region-specific (reviewed by Burke and Barnes, 2006). Aged rats have deficits in both LTP induction and maintenance, which may contribute to the observed cognitive deficits.

Aged animals appear to have intact hippocampal LTP induction when robust, high-intensity stimulation protocols are used. However, when peri-threshold stimulation parameters are used, LTP induction deficits can be observed in both the dentate gyrus and

CA1, but in different manners. In the dentate gyrus, larger amplitude current injection is required to elicit LTP at the perforant path–granule cell synapse of aged rats compared with young rats, indicating an increased threshold for LTP induction in these synapses (Barnes et al, 2000). Aged neurons in area CA1 do not have an increased threshold for LTP, but when peri-threshold stimulation parameters are used, the level of LTP induction in aged rats is less than in young rats (Rosenzweig et al, 1997). In addition, at CA1 synapses, there is also a reduction in the magnitude of LTP, which may be explained by less depolarization during induction and consequently less activation of NMDA receptors (Rosenzweig et al, 1997).

As for LTP maintenance, there appears to be no difference in LTP decay rates over the course of 1 h in aged and adult rats, assuming high-intensity stimulation parameters are used so that induction is equivalent across age groups. Over a much longer time scale, however, age-related maintenance deficits appear in both dentate gyrus (Barnes, 1979; Barnes and McNaughton, 1980) and CA3 (Dieguez and Barea-Rodriguez, 2004).

4.3 Place cell physiology in aging

Much work has been done in place cell physiology in aged rats, with different studies focusing on different aspects. Consistent with known age-related changes in hippocampal plasticity, there is a loss of rapid experience-dependent place field expansion in aged hippocampal CA1 paramidal cells (Shen et al, 1997). The lack of field broadening in old rats might therefore be expected to lead to a failure to link together the spatial components of a route. Deficits in this form of plasticity may contribute to the differences between age groups in the stability of map formation.

As for spatial encoding, different studies have generated divergent views on the nature of age-related changes in spatial representation in the rat. In the first study, it was reported that the older animals had larger firing fields (Barnes et al, 1983). Subsequent studies, however, have not been able to replicate this change but have found that the field sizes of the aged animals were comparable or even more compact than those of the adult controls (e.g. Mizumori et al, 1996; Tanila et al, 1997). In contrast, all these studies report age-related changes when hippocampal network (composed of place cells) properties are examined.

For example, Wilson et al (2003) and Oler and Markus (1995) described place cells in aged rats as "rigid," in that they maintain the same spatial representation abnormally across multiple environments that share some cues. Barnes et al (1997) characterized place cells in aged rats as "multi-stable," that is, unpredictably switching among multiple spatial firing

patterns on repeated explorations of the same environment. Alternatively, Rosenzweig et al (2003) have characterized place cells in aged rats as delayed in anchoring spatial representations to salient external cues.

Though contradictory at the first glance, all these descriptions can be explained by weakened control of external visual cues over hippocampal spatial representations in aged rats. Place fields in these rats may present as overly stable, unstable, or delayed in stabilizing to external stimuli under different experimental protocols. In a recent study, Wilson et al (2004) recorded place cell activity from aged and young rats as they repeatedly explored one familiar and one novel environment that differed in both geometry and visual landmarks. To test whether the place fields in the new environment were anchored to external visual cues, the authors occasionally rotated the arena frame and observed a course of place cell firing patterns that sequentially reflected each of the previous descriptions of age-associated abnormalities in spatial representation. They showed that while hippocampal neurons of young rats rapidly remapped in a novel environment, hippocampal spatial representations in aged rats abnormally maintained the spatial representation in the familiar environment, as reported by Tanila et al (1997), Oler and Markus (2000), and Wilson et al (2003). However, the rigidity between two distinct environments is temporary. Also, consistent with Rosenzweig et al (2003), place fields of aged rats were not initially controlled by the external cues. With additional experience, place fields of aged rats did rotate with the landmarks, demonstrating that the hippocampal spatial representations in aged rats were eventually bound to external cues. However, even after numerous repeated exposures, the hippocampal spatial representations of aged memory-impaired rats rotated with the landmarks on some but not all occasions and differed across experiences within the same environment, reflecting the characteristics of "multi-stability" described by Barnes et al (1997).

Most of the studies mentioned above did not discriminate between CA3 and CA1 place cells. As discussed earlier, CA1 and CA3 place cells show distinct characteristics of information coding (Guzowski et al, 2004). This raises the question if aging specifically influences a particular subregion of the hippocampus. To answer this question, Wilson et al (2005) compared the spatial firing patterns of CA1 and CA3 neurons in aged memory-impaired rats with those of young rats as they explored familiar and novel environments. They found that CA1 place cells in aged and young rats had similar firing characteristics in the familiar and novel environments. In contrast, aged CA3 place cells had higher firing rates and larger field size in general, and failed to change their firing rates and place fields

as much as CA3 cells of young rats when the rats were introduced to a novel environment. Thus, aged CA3 cells failed to distinguish change when new information was provided, which may underlie the age-associated behavioral impairment observed in spatial learning. The dissociation between CA3 and CA1 responses strongly implies that the aging effects on hippocampal physiology are not secondary to any more general deficit, such as sensory or perceptual impairments. Instead, they may stem from intrinsic properties of hippocampal subregions. Whether similar subregional specificity is also present in age-related diseases, such as Alzheimer's disease, remains unclear.

There is only one study investigating place cell physiology in aged mice. Unlike data from rats, Yan et al (2003) reported reduced spatial tuning and stability of hippocampal place cells in aged mice, which mainly resulted from significantly higher firing of place cells out of their place fields.

Chapter 6 *Theories of Hippocampal Function*

“What does the hippocampus do?” has been a highly debated question in neuroscience. It is generally accepted that the hippocampus is important for spatial cognition in animals. Similarly, there is little doubt that the hippocampus is crucial for memory in humans. But what is the exact role of the hippocampus in these cognitive processes remains controversial. In this chapter, I will discuss some of the most influential theories on hippocampal function.

1 The cognitive map theory

As the first of several spatial theories of hippocampal function, the cognitive map theory stemmed from the discovery of place cells by O’Keefe and Dostrovsky (1971). In this theory, O’Keefe and Nadel (1978) proposed that the hippocampus acted as a neural system that organized the encoding and representation of perceived stimuli with respect to an allocentric framework of absolute, unitary space, or a cognitive map. This framework is thought of as innate, and the underlying geometry of the system is Euclidean.

O’Keefe and Nadel spelt out the differences between two major systems they believed could support spatial information and processing: a taxon and a locale system. Only the latter was instantiated by and depended upon the proper functioning of the hippocampus. The locale system automatically produces a map of an environment as soon as it is explored, and stores it indefinitely. The creation of the map is all-or-none, meaning that once a map for an environment exists, it is not modified. (References to changes in the environment could be added, but these do not affect the spatial structure of the map). The locale can ‘act at a distance’ allowing animals to move with reference to places they cannot perceive, for instance, taking shortcuts. By contrast, the non-hippocampal taxon navigation system is comprised of guidance and orientation strategies, and learns, incrementally, only those particular routes relevant to a given behavioural task.

According to the theory, the hippocampus of rodents and other animals generates cognitive maps of all environments the organism encounters during its life. The map of each environment is composed of a set of place representations (entailed by the place fields of hippocampal pyramidal cells) connected together by rules that represent the distance and directions among them. Such maps are stored in the hippocampus and are used for spatial navigation. Moving from the animal sphere, O’Keefe and Nadel proposed that in humans

the hippocampus has come to provide the basis of episodic memory. A possible link between topographic and episodic memory is the existence of an allocentric cognitive map with the addition of verbal and temporal inputs. O'Keefe and Nadel have postulated that while the right hippocampus maintains a primarily spatial function, the left hippocampus might incorporate a temporal sense and linguistic functions that together provide the basis for episodic memory and narratives.

The theory was supported by features of hippocampal anatomy, correlates of the hippocampal EEG, behavioural studies of hippocampal lesioned animals and, primarily, by spatial correlates of single unit firing in the hippocampus. These cells were proposed to form the neural substrate of the cognitive map. Though the place cell literature described in a previous section seems to broadly support the cognitive map theory, some of the properties of the place cell system cannot be adequately explained on the basis of this theory alone. For example, the results of Lever et al (2002) cannot be easily accommodated by the cognitive map theory. This study showed that the changes that occurred in the hippocampal place representations of two geometrically distinct environments were incremental, not all or none and thus were unlike those predicted for the locale system by O'Keefe and Nadel. Moreover, the changes were observed at different times in different place cells, showing that the hippocampal cognitive map is not as unitary as originally thought (and see also the results by O'Keefe and Burgess, 1996). Another set of difficulties for the theory is that subjects, both rodents and humans, with hippocampal damage can sometimes acquire allocentric spatial information, though at a slower rate (Wise and Murray, 1999; Gaffan et al, 2000). However, this is not fatal to the theory because recent studies have established that effective spatial navigation can be achieved in many other ways (see below).

2 The declarative theory

This theory emphasizes the distinction between declarative memory and procedural memory (Cohen and Squire, 1980). The declarative theory claims that the primary function of the hippocampal formation is memory. Moreover, the hippocampal formation selectively mediates declarative memory and shows rapidity, flexibility, richness, and conscious accessibility. This account was based more on the human and clinical literature on amnesia, but virtually all hippocampal theorists predict continuity between animals and humans.

One successful feature of this theory is its prediction about consolidation. Squire's theory, based on the clinical literature on retrograde amnesia, has emphasized the idea that

hippocampal storage is time-limited and that neocortical areas gradually acquire memory traces stored in hippocampus. In essence, memories that used to depend on the hippocampal formation can become neocortical-dependent and hippocampal-independent. Two one-subject studies have shown intact remote spatial memory in patients with profound hippocampal damage (Teng and Squire, 1999; Rosenbaum et al, 2000). These two papers undermine the view that the hippocampus is required at all times to store and retrieve spatial memories. The current view is that the hippocampus initially binds together the distributed sites in neocortex that together represent the memory of a whole event (Zola-Morgan and Squire, 1990). This low-capacity, fast system permits the acquisition and storage of representations involving arbitrarily different elements, and for a period it provides a basis for retrieving the full representation, even when a partial cue is presented. As time passes, the burden of long-term memory storage is assumed fully by neocortex (however, see next section for slightly different views).

As mentioned earlier, declarative memory can be sub-divided into episodic and semantic components. The declarative theory predicts that the medial temporal lobes are involved in both types of memory and that the extent of the memory impairment varies directly with the amount of tissue damaged. However, while it is generally accepted that episodic memory is severely impaired in amnesic patients and is dependent on the hippocampus in humans, the relationship between semantic memory and this brain region remains debated. Amnesic patients do have difficulty acquiring semantic knowledge but they can typically succeed to some extent after much repetition (Tulving et al, 1991). There has been considerable recent interest in the idea that the hippocampal formation is essential for episodic rather than all forms of declarative memory in humans. Recent work on three patients who sustained bilateral damage limited to the hippocampus early in life (Vargha-Khadem et al. 1997) suggests that such damage results in memory deficits for episodes and events but not for semantic or other factual material.

In the animal literature, the most used task to test declarative memory is the non-match to sample task with various delays. Other recognition memory tasks are also used. The vast majority of studies using restricted ibotenic acid lesions of hippocampus have failed to support a role for the hippocampus in non-match to sample tasks and recognition memory tasks (e.g. Jarrard, 1993; Mumby et al, 1996). Furthermore, Mumby et al, (1996) helped to explain previous recognition deficits associated with ischaemic hippocampal lesions (Zola-Morgan and Squire, 1986; Zola-Morgan et al, 1992) by showing that ischaemic-induced hippocampal rats showed recognition memory deficits but when their hippocampi were

removed after ischaemia, their performance was unimpaired.

3 Multiple trace theory

In contrast to the declarative theory, Nadel and Moscovitch (1997) drew evidence from the animal literature, amnesic patients studies and neuroimaging studies in healthy subjects to support the opposite view that certain types of memories are encoded and permanently stored within the hippocampus.

According to this theory, the hippocampus automatically encodes memories, acting as an index to the neocortex that represents the sensory cues and other features of the attended event (Teyler and DiScenna, 1986). Thus, in order to reactivate a memory one requires an intact hippocampus. When a memory is replayed, in a different temporo-spatial and neural context a new trace is laid down in the hippocampus. This means that over time, some memories get strengthened through rehearsal and are represented by several traces in the hippocampus, while some others are lost. Thus, the evidence of a graded retrograde amnesia that the consolidation theory holds onto to demonstrate that memories, once “mature”, are shuttled to the cortex, could be reinterpreted on the basis that partial hippocampal lesions could selectively spare well-rehearsed memories as opposed to more recent memories which are encoded in fewer hippocampal traces.

Another important feature of the multiple trace theory is that the hippocampus is thought to be involved in the processing of only a subset of mnemonic material. For Nadel and Moscovitch the hippocampus is the seat of episodic memories and in particular of autobiographical episodic material, whereas more semantic materials, like factual knowledge about the world and the self might become, over time, hippocampally-independent.

In support of Nadel and Moscovitch’s views, several neuroimaging studies in healthy subjects have showed that activation of the hippocampus is as robust when remote memories are being retrieved as when recent memories are retrieved (Ryan et al 2001; Maguire, 2001). These findings speak in favour of a permanent hippocampal role in memory retrieval. Studies on amnesic patients and animals also support this theory, given that the retrograde amnesia for autobiographical events (or spatial tasks in animals), when tested, extends over very long periods of the subjects’ life (30 years or over), and it shows, in general, a rather flat gradient (the deficit being similar in intensity at all time periods considered), while semantic information (or non-spatial tasks in animals) is generally more mildly affected and shows a more graded retrograde amnesia (Salmon et al, 1985; Zola-

Morgan and Squire, 1990; Clark et al, 2005).

Chapter 7 Aims and Design of the Present Study

After a detailed review of the background information, this chapter outlines the aims, design and predictions of results of the present study.

1 Rationale and aims of the present study

In summary, the unique anatomical and physiological position of the hippocampus in the brain endows this brain region with higher susceptibility to diseases characterized of neuroplasticity failure and cognitive impairments, such as AD. Accumulating evidence suggests that AD represents an A β -induced synaptic failure. Yet how these molecular and cellular changes induce cognitive impairments at the behavioural level remains largely unknown. The hippocampal place cell is a well understood model for studying spatial cognition in animals. It has been repeatedly shown that pharmacological and genetical manipulations that disrupt hippocampal neuroplasticity are always accompanied by place cell and behavioural deficits (chapter 5). However, there have been no reports of place cell physiology in AD.

Based on the evidence presented in previous chapters, I hypothesize that place cells in aged transgenic mice of AD could not encode and process spatial information as well as those in control animals. In other words, these animals showed degraded neuronal representations of the environment. Such deficits may result from synaptic dysfunction caused by soluble A β or amyloid plaques in the brains of the subjects. As a result, a normal “cognitive map” cannot be formed in these animals, which underlies the neural basis of spatial cognition deficits in this disorder. In this way, the hippocampal network deficit provides the linkage between molecular and behavioural changes in AD.

The aim of the present project is to test this hypothesis.

2 Design of the present study

2.1 General design

To test this hypothesis, I train young and aged AD mice and their littermate controls in the forced T-maze alternation task, to examine their spatial working memory ability. After they acquired the task, these subjects are implanted for place cell recording. Once place cell activities are detected, behavioural training resumes with various delays to provide a

better test for their memory. Meanwhile, place cells are recorded in the same subjects, to examine their place cell representation of the space. During this period, a simple environmental manipulation – a 180° maze rotation – is introduced between sample and test trials of the task, to examine place cells' physiological as well as the animals' behavioural responses to this change. Finally, after all experiments are finished, the animals are sacrificed to examine the pathology of their brains. When all these data are obtained, statistical analysis is performed among behaviour (spatial memory task performance), physiology (place cell ability to encode spatial information), and pathology (cortical and hippocampal load of amyloid plaques or soluble A β) for possible correlations.

2.2 Choice of subjects

The tg2576 model is selected to be the subject of the present study, because it is one of the first successful models of AD and has been well characterized behaviourally, physiologically and pathologically in the past ten years. These animals develop age-dependent amyloid pathology, synaptic dysfunction and cognitive impairments (chapter 3). One disadvantage of this model is that these animals do not show all the pathological changes in AD, eg, they do not have NFTs or neuronal death in their brains (chapter 3). However, this drawback makes it easier to analyze the molecular correlates, if any, of the potential physiological changes in these subjects. By the time this thesis is submitted, several double or triple models of AD have been developed, which show NFTs and neuronal death as in AD patients. These models may serve as appropriate subjects for further research in this area.

In the present study, two age ranges of the animals are selected: 3-5 months and >13 months. It may be argued that investigation on middle-aged animals provides more important information about the pathogenesis of AD, especially at its earliest stages. However, since no place cell recording has ever been performed in AD models, I am not sure whether there are any changes in place cell properties in these animals. For this reason, more aged animals that show more severe pathological and behavioural deficits are selected for the present study.

2.3 Choice of behavioural task

The forced T-maze alternation task is selected for the present study because it has several advantages over other tasks. Firstly, this task has been shown to be very sensitive to hippocampal damages in the rodent (Burton et al, 2000). At the same time, performance deficits of aged tg2576 mice in this task have already been reported by several groups (Chapman et al, 1999; Corcoran et al, 2002; Barnes et al, 2004). Secondly, delays, long or

short, can be easily introduced into the task to increase its difficulty, enabling a better evaluation of memory in these animals. Thirdly, one advantage of spatial working memory tasks over reference tasks is that the same task can be used over weeks or months, to monitor the longitudinal changes of cognitive abilities in the same subject. Finally, this dry-land task enables simultaneous place cell recording.

2.4 Procedures considered but not performed

Given that this is the first attempt to study place cell physiology in a transgenic model of AD, a number of other potentially interesting experiments have been considered. However, these procedures have not been carried out, due to (1) shortness of space and manpower: all the experiments have to be performed in the same environment in the same room, whereas training and recording trials with long delays are extremely time-consuming; and (2) technical difficulties: long-term place cell recording in mice is quite difficult compared with rats. According to experience in our laboratory, mouse place cells tend to decrease or even stop firing after 4-5 hours' continuous recording within a day. Moreover, it is very difficult to perform long-term place cell recording from one mouse for more than 5-6 days, because of tetrode movements or cell death. Even across days, the clusters might change dramatically. These problems could result from the relatively unstable construction of mouse microdrives and the small size of mouse brains.

More training in longer-delay trials

It has been suggested that performance in long-delay trials of spatial working memory tasks provides more specific evaluation of hippocampal function than short-delay trials (Lee and Kesner, 2003). However, as mentioned earlier, training of long-delay trials was extremely time-consuming and such a design was not practicable.

Recording in the T-maze

Wood et al (2000) suggests that rat place cells may remap depending on the subject's future actions even at the same spatial location. An intriguing inference from this study is that by recording place cells at the stem arm of the T-maze, one may be able to predict the subject's intended arm choice. However, such a proposal could not be tested, because single runs in the T-maze would not provide enough sampling for clear identification of place fields.

Other environmental manipulations

Environmental manipulations are the best way to study place cell activities in animals. In the present study, a simple maze rotation has been applied. Many other procedures, such as those discussed in section 3.6, chapter 5, would undoubtedly provide more information

about hippocampal network dynamics in transgenic animals. However, for time and technical difficulties, these interesting manipulations could not be performed in the present study, but represent intriguing ideas for future work.

Long-term place cell stability

It has been shown that aging affects long-term stability of place fields, eg over days (Yan et al, 2003). Such analysis was not performed due to technical difficulties (unstable clusters in mice across days).

3 Predictions of results

If the above hypothesis were correct, the following results would be expected.

Behaviourally, transgenic animals should show an age-dependent impairment in the T-maze alternation task, as repeatedly shown from previous studies (Chapman et al, 1999; Corcoran et al, 2002; Barnes et al, 2004). In addition, longer delays introduced between sample and test trials should equally affect performance of transgenic and control animals (Barnes et al, 2004).

Pathologically, transgenic animals should develop age-dependent changes in their brains, especially in the hippocampus, including amyloid plaques and soluble A β , as repeated shown from previous studies (Hsiao et al, 1996; Irizarry et al, 1997; Chapman et al, 1999; Corcoran et al, 2002; Westerman et al, 2002).

Physiologically, place cells in aged but not young transgenic animals should show deficits in encoding spatial information about the environment, as the result of severe brain pathology and synaptic dysfunction. For example, field sizes of these cells should be bigger than those of control animals, whereas spatial information content lower, suggesting degraded place cell representation of space in these animals. When the 180° maze rotation was introduced, place cells in transgenic mice might also respond differently compared with controls. External cues might exert weakened control over place cells in aged transgenic animals, as inferred from previous studies on aged rats, though the exact change would be difficult to predict. But the behavioural response should be consistent with the physiological response, which could be deducted from the animal's performance change in the task.

Finally, when behavioural, physiological and pathological data were combined, correlations were expected between each pair of them.

Methods

Chapter 8 Materials and Methods

This chapter describes the materials and experiment procedures in the present study. The experimenter was blind to all experiment procedures, including animal training, surgery, place cell recording, cluster cutting, quantitative analysis of place cell properties, and histology.

1 Subjects

The subjects studied were APP₆₉₅SWE transgenic (tg2576) mice in a SJL background backcrossed to C57/BL6, obtained from Merck Sharp and Dohme (Harlow). The founder of the tg2576 line was a C57Bl/6jxSJL F3, which was subsequently crossed twice into C57Bl/6j. Subsequent generations were then crossed to C57Bl/6jxSJL F1. Thus, in all cases, the average contribution of C57Bl/6j ranged from 59% to 88%, with the remainder from SJL (Chapman et al, 1999). On each individual test, transgenic mice were always compared to age- and background-matched littermate controls. Four groups were included: young (3-5 months) control and transgenic groups, and aged (15-17 months) control and transgenic groups.

All subjects were singly housed in Perspex cages, and maintained on a 12/12 hour light/dark schedule, with lights off at 6 pm. Mice were weighed daily, and maintained at about 90% and no lower than 85% of their free feeding weight during training and recording. All experiments were carried out blind to genotype and in accordance with the UK Animals (Scientific Procedures) Act 1986.

2 Apparatus and laboratory layout

The start box (30 * 30 * 30 cm) with a removable door (6 cm wide) in one side of its walls, and two identical T-mazes (length of stem and choice arms = 45 and 35 cm respectively, width = 7 cm, height = 10 cm) were all built from wood and painted grey.

All the experiments were conducted in the same environment enclosed by black curtains (diameter = 2 m) with 4 distinct cardboards as distal visual cues, as shown in Figure 8.1.

Figure 8.1

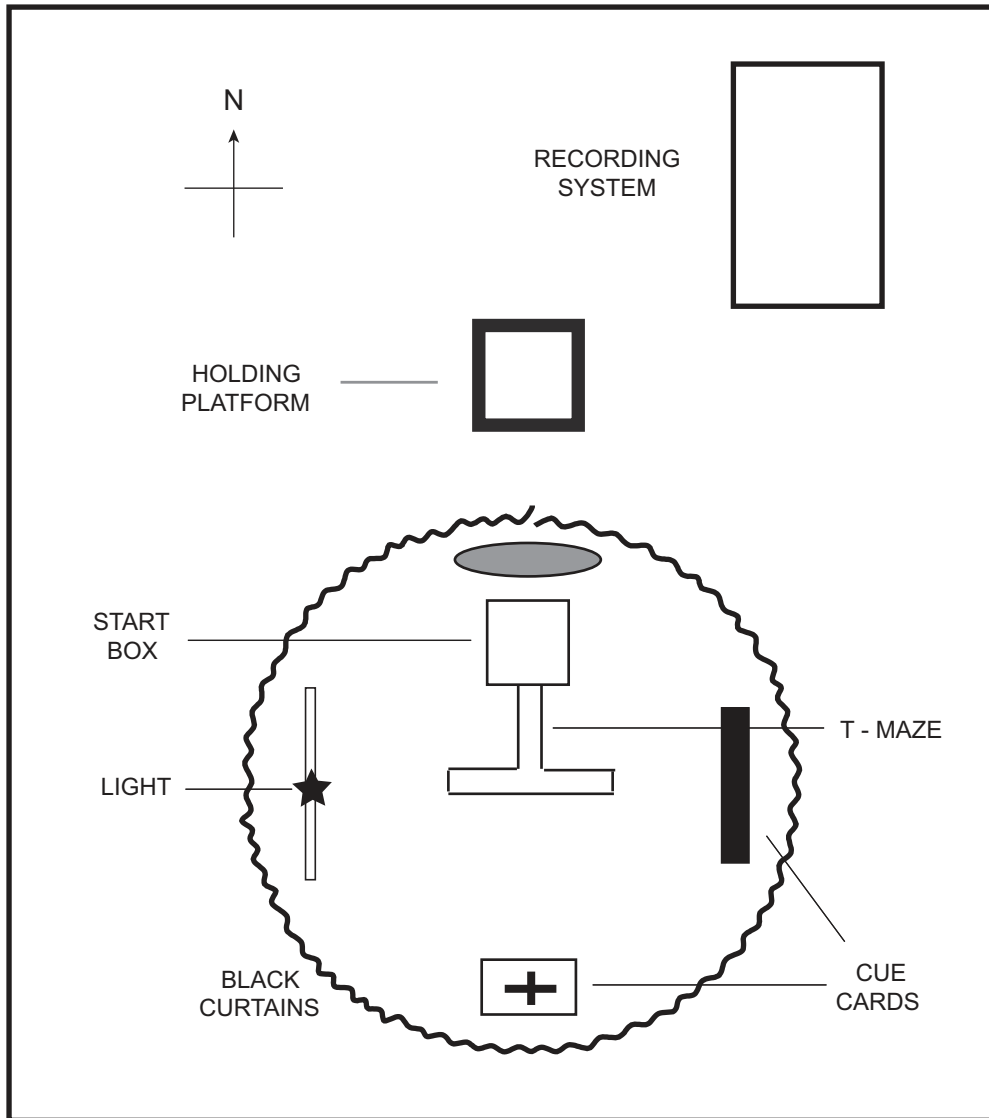


Figure 8.1 Plan view of laboratory, showing general setup of the recording environment. All training and recording sessions take place within the curtained environment with four distinct cue cards. At other times, the mouse is placed on the holding platform. A light is hung above the cue card in the west side to illuminate the environment.

3 Experiment 1: T-maze alternation task acquisition

A total of 62 animals were trained on the T-maze forced alternation task. Animals were assigned to 4 groups on the basis of age and genotype: young control (n = 15; mean age = 2.5 months, range 1.9-3.2 months), young tg2576 (n = 16; mean age = 2.7 months, range 1.9-3.2 months), aged control (n = 13; mean age = 15.4 months, range 13.4-16.4 months) and aged tg2576 (n = 18; mean age = 15.4 months, range 13.5-16.8 months).

For behavioural testing, mice ran from a start box for reward (condensed milk) on a forced alternation task in a T-maze as previously described (Chapman et al, 1999; Corcoran et al, 2002; Figure 8.2). After 5 days of habituation to the apparatus (for each day, 2 min in the starting box, 3 min in the maze and another 5 min in the whole apparatus) and food, each mouse was given 6 pairs of training trials per day for 12 days. On the first (sample) trial of each pair, the animal was forced to choose one of the choice arms of the T-maze (the other was blocked by a removable door) and received food reward at the end. 10-15 seconds after consuming the reward, the mouse was returned by the experimenter to the start box and confined there for 15 seconds, while the T-maze was replaced with another identical one to eliminate intramaze cues. The animal was then given a free choice between both T arms and only rewarded for choosing the 'novel' arm not entered on the first trial. The choice was made when the whole body of the mouse passed 1/3 of the length of either choice arm. The location of the sample arm (left or right) was varied pseudorandomly across trials so that mice received equal numbers of left and right presentations on each day, but no more than two consecutive trials with the same sample location. Mice were run in squads of 5-7 to minimize variation in intertrial interval, which was about 12 minutes for all animals.

4 Microdrive construction, surgery and screening

2-3 days after task acquisition, a subset of animals (due to environmental and manpower restrictions) underwent implantation of microelectrodes for chronic hippocampal recording (young control, n=6; young tg2576, n=7; aged control, n=10; aged tg2576, n=15). Dr Francesca Cacucci helped perform about half of the surgery and screening work.

Figure 8.2

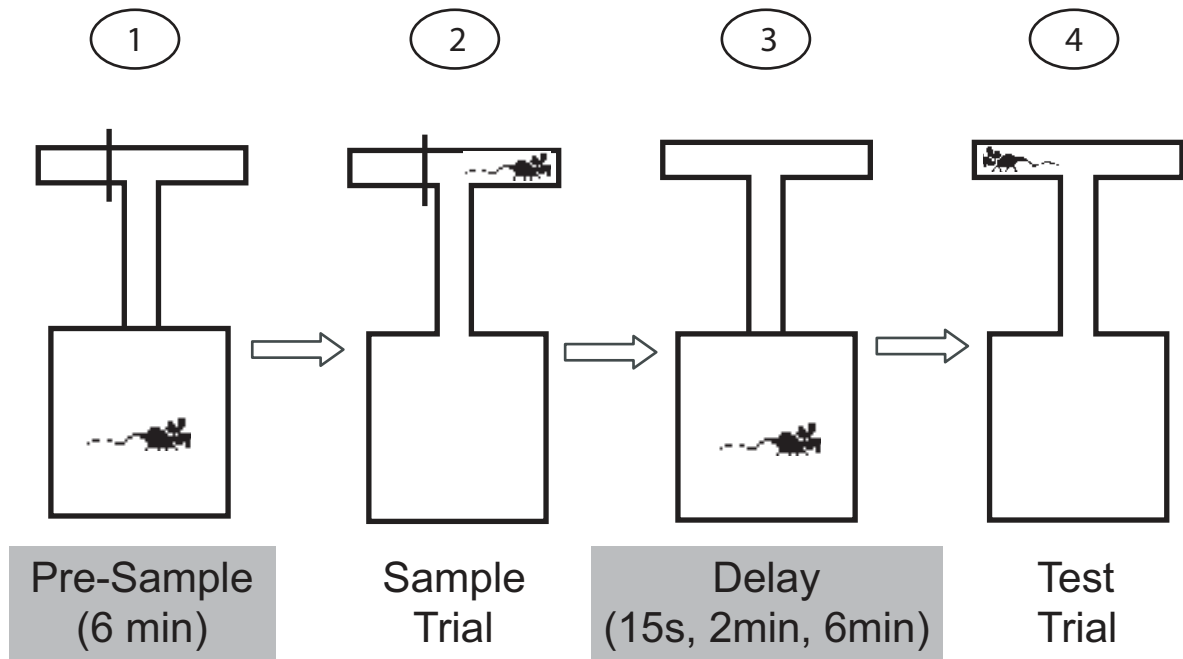


Figure 8.2 The T-maze alternation task. On the first trial (sample trial, phase 2), a mouse is forced to choose one of the choice arms of the T-maze and receives food reward at the end. After consuming the reward, the mouse is returned by the experimenter to the start box. After a delay (phase 3), the animal is then given a free choice (test trial, phase 4) between both T arms and only rewarded for choosing the ‘novel’ arm not entered on the first trial. Place cell recording takes place in phases 1 and 3.

4.1 Electrodes and microdrives

The electrodes were made from microwire (90% platinum, 10% iridium, HM-L insulated), 17 μ m in diameter (California Fine Wire). All electrodes used were tetrodes, constructed by twisting four individual pieces of wire together.

Electrodes were loaded into a moveable 16-channel microdrive (4 tetrodes). Figure 8.3 shows a diagram of the standard microdrive. Part of the microdrive, consisting of the two metal posts, is stationary with respect to the brain of the animal. This structure is attached to the head of the animal using dental cement. One of the posts (right hand side in Figure 8.3) carries a fine-threaded screw. Fitted over this screw is a nut (consisting of heat shrink plastic), attached to which, via dental cement, is the electrode cannula. When the screw turner (right top, Figure 8.3) is used to rotate the threaded post, the portion of the microdrive attached to the nut, including the electrodes, moves up or down. One 360° turn moves the electrodes 250 μ m.

The ends of the tetrode wires were stripped of insulation (exposure to naked flame), wrapped around microdrive wires, and the junction sealed with conductive silver paint.

4.2 Surgery

In surgery, a mouse was first anaesthetised as follows: oxygen at 3 litres per minute, with isoflurane at 3% of the gas volume. The animal's head was then shaved using electric clippers. Before the animal was fitted with the ear bars, nitrous oxide was introduced at 3 litres per minute, and the oxygen flow was reduced to 1.5 litres per minute. At this point, analgesic [Buprenorphine (Vetergesic), 45 μ g, i.m.] and prophylactic antibiotic [Enrofloxacin (Baytril), 2.5mg, s.c.] were administered. The percentage of isoflurane was gradually decreased throughout surgery, stabilising at 0.5 to 1.2 %. Once the animal was stably anaesthetised, it was fitted with ear bars and mounted in the stereotaxic frame.

The skull was exposed and the mouse's head adjusted so that the upper surface of the skull was parallel to the base plate of the stereotaxic frame. Six holes for screws were drilled using a 0.9 mm burr drill. Two holes were drilled over the occipital, two over the frontal bone, and two over the parietal bone contra-laterally to the implant side. Stainless steel screws were screwed into each hole for structural support. One screw served as the ground attachment for unit recording.

A hole 1.5 mm in diameter was made over the left hemisphere (2.0 mm posterior and 2.0 mm lateral to bregma, targeting the CA1 area). The microdrive-tetrode assembly was then stereotaxically positioned with the electrode tip at the target coordinates, and then lowered so that the electrodes were placed at the initial depth below the pial surface of the

Figure 8.3

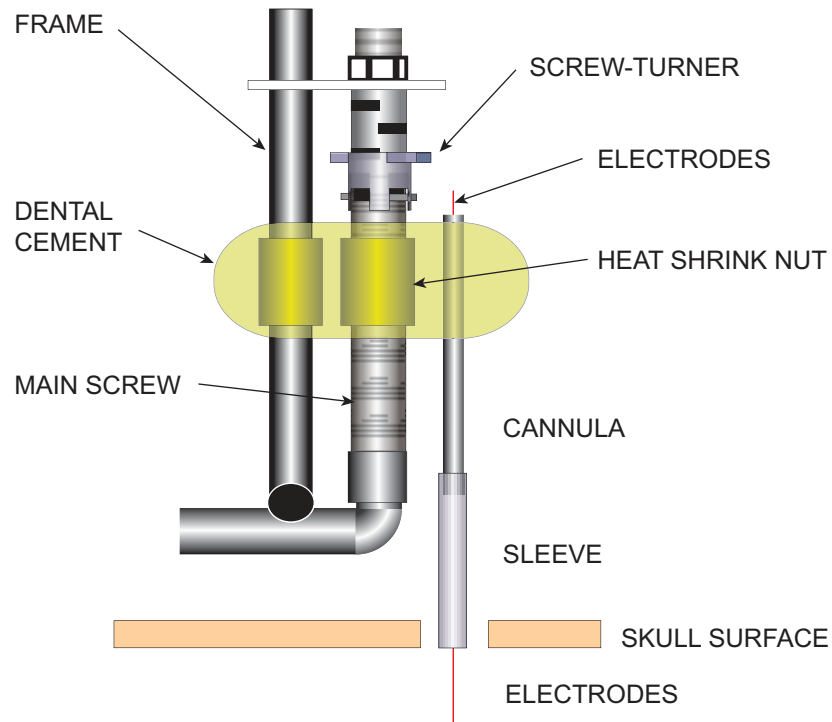


Figure 8.3 Microdrive construction. Diagram showing the main features of the microdrive. Microdrive is fixed with respect to the skull surface, using dental cement (not shown). Turning the screw-turner causes the cannula to move up and down relative to the main screw. The cannula was loaded with up to four tetrodes. 0.5 mm solid-core steel wire, mounted in dental cement, carried the signal between the tetrodes and the headstage connection (not shown).

Original version of this drawing by John Huxter.

brain (0.4-0.6 mm) . The sleeve around the electrode cannula was then lowered so that its lower surface rested on the brain surface. The exposed brain around the sleeve was covered with sterile vaseline. The tetrode-microdrive assembly was then fixed to the skull by applying dental cement around the sleeve, the feet of the microdrive, the screws and the skull.

4.3 Screening

5-7 days after surgery, screening for cell activity began. Screening took place while the mouse was on the holding platform (Figure 8.1). The main physiological marker used for the hippocampus was the high-frequency “ripples” state, seen when the animal is still and at low arousal levels (see chapter 5). The electrodes were slowly moved down towards CA in multiples of 62.5 μm steps until signs of low amplitude ripples were seen. When ripples were seen, the electrodes were moved down in 32 μm steps, until complex spike activity was detected.

5 Experiment 2: Delayed T-maze alternation task and place cell recording

Given environmental, time and manpower restrictions of the laboratory, no more than two animals were trained and recorded simultaneously at this stage. In total, 42 subjects have been included in this phase of experiment (9 young control, 6 young tg2576, 13 aged control and 14 aged tg2576 animals).

5.1 Standard trials and recording

Once place cell activity was detected, the mouse was further trained in the T-maze alternation task. On each training day, there were 4 pairs of trials with 15 sec delay (identical to the task acquisition phase), followed by 6 pairs of longer-delay trials (2min for the first 2 days and 6min for the next 3 days). Place cell activities were recorded during these longer-delay trials, prior to the sample trials (6 min) while animals navigated in the start box. Small chocolate flakes were used to encourage exploration of the box when necessary.

5.2 Probe trials and recording

In the next 3 days, animals were trained in both standard trials as above, and probe trials when the start box and the T-maze were rotated 180° between sample and test trials (Figure 8.4), to examine the hippocampal network dynamics in response to environmental changes. The animal first received the sample trial and returned to the start box as normal. Then the whole apparatus (the start box and the T-maze) was rotated manually 180° on a

Figure 8.4

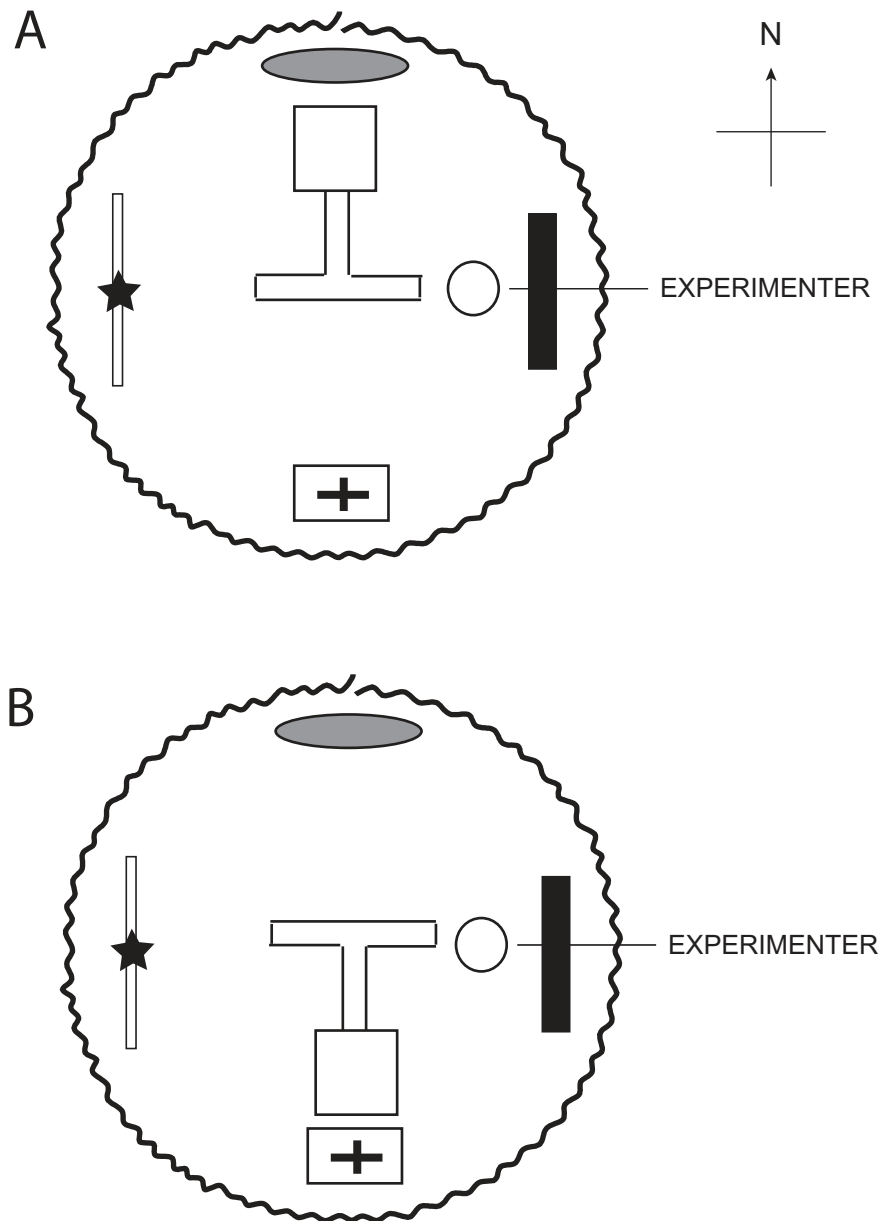


Figure 8.4 Plan view of the experimental environment in probe trials. In standard trials, the start box and the T-maze remain fixed through the whole trial (A). In probe trials, the start box and the T-maze are rotated 180 degree after the sample run, whereas the distal cue cards remain unchanged (B). The experimenter always sits in the east side of the environment.

swivel platform. The whole rotation procedure took around 3 seconds (the animal was able to perceive this manipulation). Then came the delay period of 2 min and the test trial. Place cell recording was performed both before sample trials (6min) and before test trials during the delay period (2min). Within these 3 days, each animal received 10 trials under standard condition and 10 with maze rotation. These two types of trials were pseudorandomly mixed up so that no more than 2 consecutive trials were the same type. In probe trials, the opposite arm in the environment (that was, the same arm in the maze) was taken as the correct choice.

5.3 Distal cue rotation recording

By the end of all training and recording procedures, a small proportion of subjects were tested for their visual ability (young control: n=3; young tg2576: n=1; aged control: n=4; aged tg2576: n=3). Each animal received three 10 min recording session in a white cylinder (d = 50cm; h = 50cm) with black floor. In the first and last sessions, the distal cues in the curtain-enclosed environment remained the same as before. In the second session, however, these cues were rotated 180° consistently. These sessions were separated by 20 min intervals, when the floor was cleaned with soap to eliminate local odour cues.

5.4 Unit recording and data acquisition

Unit recording apparatus

During recording sessions, each mouse was connected to the recording equipment via a headstage amplifier fitted onto the plug of the microdrive. These amplifiers and cables were light in weight and animals could move around freely with them without showing any aversive activities. These headstage amplifiers were unity-gain buffers, which served to isolate the electrodes from the wires carrying their signals to the recording system. The implanted electrodes were AC-coupled to these amplifiers. Lightweight hearing-aid wires 2 to 3 metres in length connected the headstage to a preamplifier (gain 1000).

Signals were amplified (15-50 thousand times) and bandpass filtered (500 Hz-7 kHz). The signals were recorded differentially, meaning that a reference signal (a channel on a different tetrode) was subtracted from the target signal. This technique is designed to remove noise and artifact from the signal. Each channel was continuously monitored at 20- μ s intervals and potentials were captured using 50 time sample points (200 μ s pre-threshold and 800 μ s post-threshold) whenever the signal from any of the pre-specified recording channels exceeded a given threshold set by the experimenter.

Two infrared light-emitting diodes (LEDs) were attached to the mouse, in order to track head position and orientation with a video camera (position sampling rate 46.875 Hz).

The two LEDs were separated by 4 cm and identified on the basis of their differential brightness. All unit recording apparatus was custom built (O'Keefe lab, Axona).

Cluster cutting

Isolation of single units from multi-unit data (cluster cutting) is based on the premise that an action potential from an individual cell will be recorded on more than one wire of the tetrode. As the amplitude of the signal decreases in proportion with $1/(\text{distance from neuron})^2$, the amplitudes of the action potential on the four wires of the tetrode depend on their position with respect to the cell. A set of action potentials with the same set of amplitudes is assumed to originate from one neuron.

For all the data used in this thesis cluster cutting was performed manually, blind to genotype, using custom software (TINT, Neil Burgess). A series of scatter plots are created, plotting peak-to-trough amplitudes for all action potentials. The series contains all possible combinations of comparison. (Six scatter plots for four amplitude values). A set of action potentials that share the same amplitude profile should form a coherent "cluster" on every one of the scatter plots. In order to reduce error, only clusters well separated from other action potentials in 4-D amplitude space are considered. Although cluster cutting is conducted mainly on the basis of Peak-to-Trough amplitude, other parameters used include voltage at an arbitrary time, time-of-peak and time-of-trough.

5.5 Quantitative analysis of place fields

Cell inclusion criteria

Only CA1 place cells (confirmed by histology) were included in the present study. Cells were included in the analysis if they had: a) spike width $> 300 \mu\text{s}$ and b) peak firing rate $> 1 \text{ Hz}$ (Cacucci et al, 2007).

Each subject received place cell recording for a maximum of 8 days. On each day, each place cell was included for property analysis in the last trial that it reached the above criteria. Some cells fired in more than 1 day during recording. These cells were double counted across days in the analysis.

Derivation of place fields

There is no standard protocol used to construct and present place fields. The procedures used in this thesis and described below follow, very closely, the protocols used recently in the O'Keefe laboratory (O'Keefe and Burgess, 1996; Lever et al, 2002). The software used is the previously mentioned TINT program (written by Neil Burgess).

Within the viewing area of the camera, it is possible to create an experimenter-defined co-ordinate frame, which was always set to 500 x 500 pixels. The environment was always

placed in a standard position with respect to this frame. One pixel in the firing rate maps therefore always represented 2.5 mm² of space on the floor of the recording environment.

In order to construct firing rate maps, the raw pixel data were first binned. For a given bin, the number of spikes of each cell in that bin is divided by the rat's dwell time in that bin, to give a firing rate for each cell in that bin. However, following previous work (O'Keefe and Burgess, 1996), rather than display the data bin-by-bin, a box-car averaging, or 'smoothing', is applied to the map. This is in order to compensate for the poor sampling of places that sometimes occurs during recording. When smoothing is applied, the rate of a particular bin is derived from the total number of spikes fired in that bin and surrounding bins, divided by the total dwell time in that bin and the same surrounding bins. The number of surrounding bins used is determined by the 'level' of smoothing. The level used in this thesis is 5 in the TINT program. Smoothing level 5 means that for each bin, a larger square with 5-bin-long sides, whose center is the current bin, is used to smooth the rate of that bin. This smoothing is applied to every bin in the environment, ie. the larger, 25-bin-in-area, smoothing squares are overlapping. Only visited bins are considered during smoothing, therefore at the edge of the environment, less than 25 bins would be used.

The firing rate in each bin was mapped as a colour or grey-scale plot. The 5 colours or shades were autoscaled so that each represented 20% of the peak rate. In descending order, bins with the highest rates were shown in red, then yellow, then green, then light blue, then dark blue for the lowest rates. A white bin represented an area that is unvisited. Place field size represented the percentage of sampled environment in which the cell's firing rate was greater than the overall mean firing rate during that trial.

Autocorrelograms

The auto-correlation function of a spike train gives the frequency of occurrence of a pair of spikes separated by a time τ .

$$\phi(\tau) = \lim_{T \rightarrow \infty} \frac{1}{2T} \int_{-T}^T f(t) \cdot f(t + \tau) dt.$$

The autocorrelogram is a histogram formed by dividing the range of τ into equal bins centred on τ_i and counting the number of spikes whose temporal separation falls into each bin, and it can be thought of as an approximation of $\phi(\tau)$. The autocorrelogram is then normalised by the number of pairs of spikes observed during the trial to give the probability density function for the time between the spikes.

Waveform analysis

During recording, whenever the voltage exceeds an experimenter-set threshold value, a 1-ms long signal trace is recorded. This 1-ms long trace encompasses 200 μ s before and

800 μ s after the time the threshold was crossed. For each cell isolated (see cluster cutting) a representative waveform is constructed by averaging the traces of all the spikes collected for that given cell. For this averaged waveform, two parameters are measured: (1) spike width: measured as the time interval between the peak and trough of the 1ms-long waveform; and (2) spike amplitude: measured as the voltage difference between the peak and trough of the waveform.

Information measures

Spatial information was a measure of the extent to which the cell's firing could be used to predict the position of the animal (Skaggs et al., 1993). It was computed using the spatial information measure introduced by Skaggs et al (1993). They measured the amount of spatial information carried by each spike the cell fired, expressing it in bits per spike.

Briefly the estimate of the rate of information $I(R|X)$ between firing rate R and location X proposed by Skaggs et al (1993) is:

$$I(R|X) \approx \sum_i p(\bar{x}_i) f(\bar{x}_i) \log_2 \left(\frac{f(\bar{x}_i)}{F} \right)$$

where $p(\bar{x}_i)$ is the probability for the rat being at location \bar{x}_i , $f(\bar{x}_i)$ is the firing rate observed at \bar{x}_i , and F is the overall firing rate of the cell.

There is an intrinsic problem with the estimation of the spatial information carried by each spike the cell fires: the data acquired is strongly under-sampled, and we generally can only produce an estimate of the firing rate of the cell in any given bin (estimated here as $f(\bar{x}_i)$, the mean firing rate observed at bin \bar{x}_i). There is not enough data to properly estimate the reliability of the observed $f(\bar{x}_i)$. The Burgess information measure is an attempt at tackling this problem, by assuming that the probability distribution of firing rate in bin \bar{x}_i follows a Poisson distribution with its mean equal to the observed rate $f(\bar{x}_i)$.

Field stability and correlation analysis

To study place field stability between pre-sample and delay recording sessions in standard probe trials, a smoothed locational firing rate map was generated for each session. Maps from these two sessions were compared using a bin-by-bin correlation. Bins in which the rate was zero in both maps were discarded, to avoid artifactually inflating the correlation values resulting from many zero-zero bin correlations. The resulting correlation coefficient (Pearson's r) served as a measure of the similarity of the maps.

To examine place cells' responses in rotation trials, two methods had been tried. First, for each pair of recording sessions, bin-by-bin correlations were obtained between the pre-

sample map and the delay map unchanged (0°) or 180° rotated. If the place field followed local cues (the box), the 180° rotated map should show a high correlation with the pre-sample map. If the field followed distal cues, a high correlation was expected for the 0° map. However, since this method did not work well (for details see next chapter), a second method was used. For each pair of recording sessions, the rate map of the delay session was rotated every 5° to find the angle that showed the highest bin-to-bin correlation with that of the pre-sample session. Under standard condition, this angle would be close to 0° , given that place fields remained stable in the same environment. In rotation trials, however, the angle would be close to 0° if the map fixed to the environment, close to 180° if the map fixed to the start box, and random (0 - 180°) if all cells remapped.

6 Histology

After the completion of all experiments, each mouse was killed with an overdose of sodium pentobarbital (Euthatal; 50 mg). The brain was extracted and hemisected sagittally. The implanted hemisphere (left) was stored in 4% paraformaldehyde (PFA) with 0.1 M phosphate-buffered saline (PBS, pH 7.6) at 4°C , whereas the other hemisphere (right) was quickly frozen in dry ice and stored at -80°C .

The following histological procedures were performed by our cooperators from Merck Sharp and Dohme (Harlow). Some analysis was performed with the aid of Dr Francesca Cacucci.

6.1 Measuring amyloid plaque deposition

The implanted hemi-brains were embedded in paraffin, sliced coronally on a microtome at $7\mu\text{m}$, and mounted on gelatin-coated slides. Some of the slides were stained with cresyl violet, for tetrode localisation, whereas the others were used to measure amyloid plaque deposition.

Plaques were identified with Congo red staining. For each hemi-brain, amyloid plaque quantification was assessed using a total of 10 coronal sections through the hippocampus (sections were spaced $280\mu\text{m}$ apart). Brain sections were routinely deparaffinized, incubated in alkaline sodium chloride solution (2.5 mM NaOH in 80% ethanol) for 20 min and then stained with alkaline Congo red solution (0.2% in 80% ethanol saturated with sodium chloride; Sigma), washed three times in absolute ethanol and coverslipped with DPX. Congo red positive $\text{A}\beta$ plaques were visualised with a Leica DMLB microscope (with a total x100 magnification factor), and images captured using a DC300F Leica camera. Quantitative image analysis was performed using Image J as the image analysis

system. The software uses red, green and blue (RGB) levels to segment objects in the image field. Segmentation thresholds were established using a series of standard slides that have extremes of intensity and remained constant throughout the analysis. Hippocampal and cortical measurements were performed by manually circumscribing the areas of interest. Hippocampal measurements refer to an area encompassing all hippocampal fields (CA1, CA3 and dentate gyrus), while cortical measurements refer to the neocortical areas overlying the hippocampus, including the retrosplenial cortex, the frontal cortex, the hindlimb area of cortex, the parietal cortex, the temporal cortex and the occipital cortex.

6.2 β -amyloid concentration assessment

Hemibrains contralateral to electrode implantation were immediately frozen on dry ice and stored at -80°C until use. The concentrations of soluble and total amyloid in the brain were assessed using a standard sandwich enzyme-linked immunosorbent assay (ELISA).

Soluble β -amyloid concentration

The frozen hemibrains were homogenized in 10 volumes (wt/v) of 0.2% DEA containing 50 mM NaCl, pH 10, and protease inhibitors (Complete; Roche Diagnostics, Mannheim, Germany) and then centrifuged at $355,000g$ at 4°C for 30 min (Optima MAX ultracentrifuge; Beckman Coulter Fullerton, CA). The resulting supernatant was retained as the soluble fraction and neutralized by addition of 10% 0.5 M Tris-HCl, pH 6.8. Samples were frozen at -80°C awaiting analysis by immunoassay.

Total β -amyloid concentration

The protocol used for extraction of total amyloid from the brain sections is based on the GnHCl extraction method described previously (based on Johnson-Wood et al, 1997). The hemibrains were homogenized in 10 volumes of 5 M GnHCl, 50 mM HEPES, pH 7.3, 5 mM EDTA plus 1X EDTA-free protease inhibitor cocktail (Complete). After mixing at room temperature for 3 h, the homogenate was diluted 10-fold into ice-cold 25 mM HEPES, pH 7.3, 1 mM EDTA, 0.1% bovine serum albumin plus 1X protease inhibitor cocktail and centrifuged at $16,000g$ for 20 min at 4°C . Aliquots of supernatant were stored at -80°C to prevent degradation.

Immunoassay analysis

The biotinylated antibody 4G8 was used in combination with the monoclonal antibodies G2-10 or G2-11 for detection of A $_{\beta}$ (40) or A $_{\beta}$ (42), respectively. These species reflect subpopulations of peptides with heterogeneous N termini encompassing at least the 4G8 epitope at residues 17 to 24. Analysis of the samples was performed using the

SECTOR Imager 6000 (Meso Scale Discovery, Gaithersburg, MD), as described previously (Best et al, 2005).

(* According to the discussion in chapter 3, hippocampal soluble A β , especially A β oligomers, is the most promising candidate for the physiological changes in the tg2576 mice. But this analysis was not performed because (1) the importance of soluble A β had not been fully recognized by the time the present project started; and (2) Merck Sharp & Dohme, who helped perform histology, experienced some difficulty in isolating the hippocampus from the first group of brain samples.)

7 Data analysis

All statistical analysis was performed using SPSS version 11.5.

Results

Chapter 9 Results

This chapter shows the findings from the experiments described in the previous chapter.

1 General characterization

Transgenic mice were comparable with their littermate controls regarding general appearance, body weight and survival rate (data not shown). However, it was noticed that tg2576 mice showed greater homecage activities than wild-type mice, especially in aged animals. This was consistent with earlier report that tg2576 mice exhibited disinhibitory tendencies, which possibly resulted from the disruption of normal cholinergic function and plaque loads (Ognibene et al, 2005).

2 Behavioural testing

2.1 T-maze alternation task acquisition

A total of 62 subjects have been included in this experiment (15 young control, 16 young tg2576, 13 aged control and 18 aged tg2576 animals). After 12 days of training, every 3 days' data were grouped into 1 training block (4 training blocks all together; Figure 9.1A). A mixed design ANOVA revealed significant effects of training blocks [$F(3, 174) = 32.47, p < 0.001$], indicating overall improvement after training, genotype [$F(1, 58) = 16.26, p < 0.001$] and age [$F(1, 58) = 5.32, p = 0.025$], indicating performance impairments caused by the transgene and aging. There was also a significant effect of blocks by genotype by age [$F(3, 174) = 3.23, p = 0.024$]. Further analysis indicated a performance difference between aged tg and aged control animals [$F(1, 29) = 11.60, p = 0.002$], and significantly worse performance of aged tg animals on the third [$F(1, 29) = 8.37, p = 0.007$] and final [$F(1, 29) = 11.71, p = 0.002$] blocks, indicating that aged transgenic mice performed much worse than aged control animals after training. This performance deficit was underscored by the observation that only 39% of aged transgenic mice reached the learning criterion in 12 days of training (>80% alternation rate for 3 or more consecutive days), compared with more than 69% of the other three groups [$\chi^2(3) = 10.67, p = 0.014$, Figure 9.1B]. In addition, there was a performance difference between young tg and young

Figure 9.1

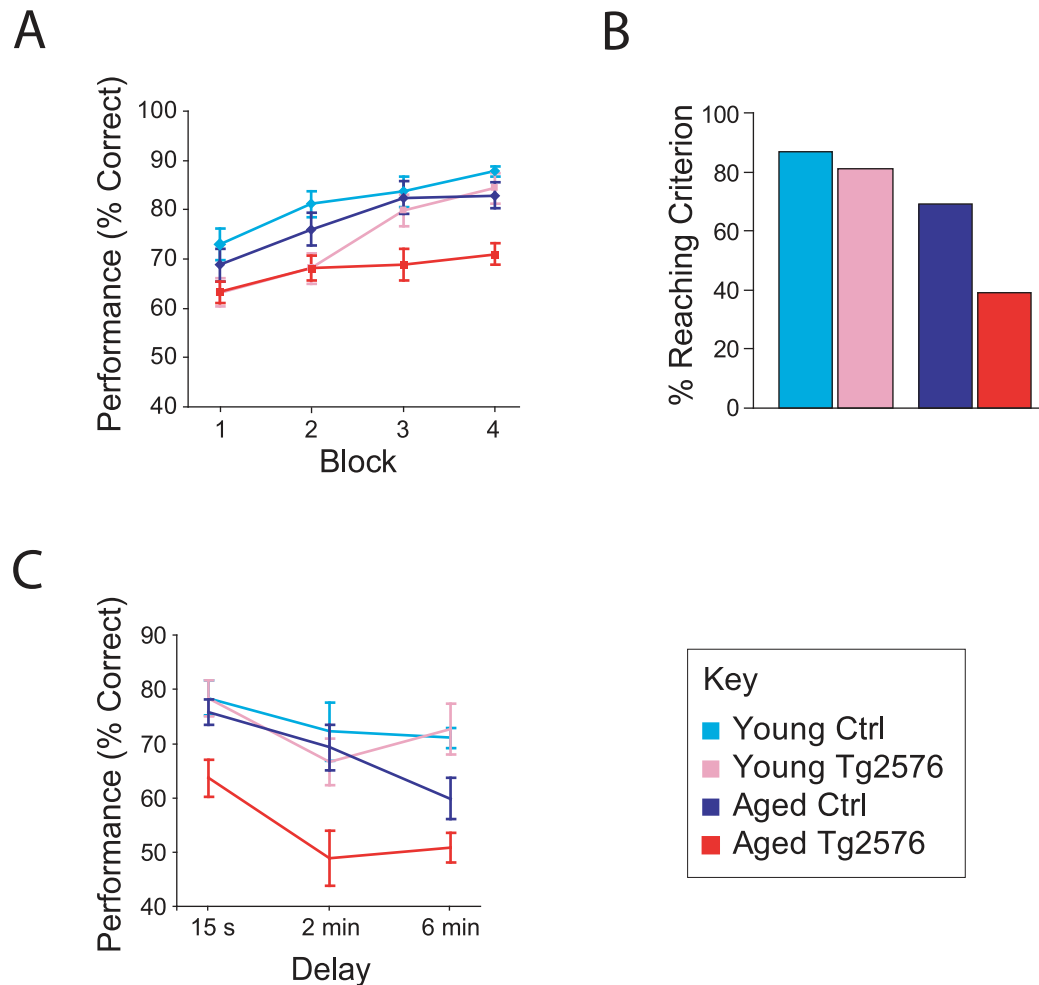


Figure 9.1 Performance in the T-maze alternation task. (A) Acquisition of the T-maze alternation task (15-secs delay version) was selectively impaired in aged transgenic animals [training blocks x age x genotype: $F(3,174) = 3.23$, $p = 0.024$]. Each block consisted of 3 training days (6 trials/day). (B) Only 37% of aged transgenic mice reached the learning criterion in 12 days of training (>80% alternation rate for 3 or more consecutive days), in contrast to > 70% of aged non-transgenic littermates and both transgenic and non-transgenic young mice [$\chi^2(3) = 10.67$, $p = 0.014$]. (C) Overall performance on the T-maze task decreased with increasing delay [effect of delay: $F(2,76) = 9.70$, $p = 0.003$]. Aged mice performed significantly worse than young mice upon introduction of delays but there was no additional deficit in the aged tg group [delay x genotype x age: $F(2,76) = 0.08$, $p = 0.782$]. Error bars showed S.E.M.s.

control animals as well [$F(1, 29) = 5.59, p = 0.025$]. However, this difference lied in the worse performance of young tg animals on the first [$F(1, 29) = 5.12, p = 0.031$] and second [$F(1, 29) = 9.95, p = 0.004$] but not the third [$F(1, 29) = 0.75, p = 0.393$] or fourth [$F(1, 29) = 1.02, p = 0.322$] blocks, indicating that young transgenic mice performed worse than control animals at the beginning, but equally well by the end of the training. Such an age-independent learning deficit has been reported earlier in tg2576 (Westerman et al, 2001) and other AD models (eg, Chen et al, 2000), and could result from the APP overexpression in these animals (Westerman et al, 2001).

2.2 Delayed T-maze alternation task

In total, 42 subjects have been included in this phase of experiment (9 young control, 6 young tg2576, 13 aged control and 14 aged tg2576 animals; the other animals from the previous phase of experiment were disgraded because of death or lack of manpower in training and recording). When longer delays were introduced, all 4 groups showed poorer performance with increasing delay time (Figure 9.1C). A mixed design ANOVA revealed significant main effects of delay [$F(2, 76) = 9.07, p < 0.001$], genotype [$F(1, 38) = 7.36, p = 0.010$], and age [$F(1, 38) = 18.02, p < 0.001$]. There was also a significant effect of age by genotype [$F(1, 38) = 5.08, p = 0.030$], suggesting that aged transgenic mice performed worse than other groups in the delayed alternation task. However, there were no effects of delay by genotype by age [$F(2,76) = 0.08, p = 0.93$], suggesting that introducing a delay equally affected these four groups. Thus, in further analysis, performance of all delays was pooled together for each group.

2.3 Probe trials

There were two broad strategies for the subject to solve the T-maze task, which could be easily differentiated by a 180° maze rotation between sample and test trials. If the animals were using the extramaze cues to guide their behaviour (extramaze strategy), they would go to the opposite location in the environment yet the same arm in the maze, whereas if they were using intramaze cues, body turn or path integration (all named intramaze strategy in contrast to the extramaze strategy), the same location in the environment yet the opposite arm in the maze (for more detailed discussion see the next chapter). Factors influencing the subject's choice of strategies would be discussed in the next chapter.

In total, 41 subjects have been included in this phase of experiment (9 young control, 6 young tg2576, 13 aged control and 13 aged tg2576 animals). Most tested subjects, regardless of genotype and age, have adopted the intramaze strategy. However, some

animals have performed in a random manner after maze rotation whereas a few others have clearly chosen the extramaze strategy. The adoption of various strategies by different subjects made it unreasonable to analyze these data at the group level. However, some details of these behavioural results will be discussed in the next section, together with electrophysiological data.

Overall, these behavioural results were comparable with earlier studies from other groups (Chapman et al, 1999; Corcoran et al, 2002; Barnes et al, 2004) and confirmed an age-dependent cognitive impairment in *tg2576* mice.

3 Place cell physiology

Only CA1 place cells were included, based on histological confirmation after all the experiment. A typical tetrode track was shown in Figure 9.2A.

Table 9.1 Summary of place cells included in the analysis in the present study

	Mouse code	Total No. of cells	Total No. of recording days	Averaged No. of cells/day
Young control	1	38	7	5.4
	2	22	6	3.7
	3	49	8	6.1
	4	12	5	2.4
Young <i>tg2576</i>	1	50	7	7.1
	2	61	7	8.7
	3	6	2	3
Aged control	1	83	8	10.4
	2	42	6	7
	3	37	4	9.3
	4	42	7	6
	5	36	4	9
Aged <i>tg2576</i>	1	55	5	11
	2	66	8	8.3
	3	37	6	6.2
	4	51	6	8.5
	5	47	7	6.7
	6	70	8	8.8
	7	10	5	2
	8	10	3	3.3
	9	27	5	5.4

Figure 9.2

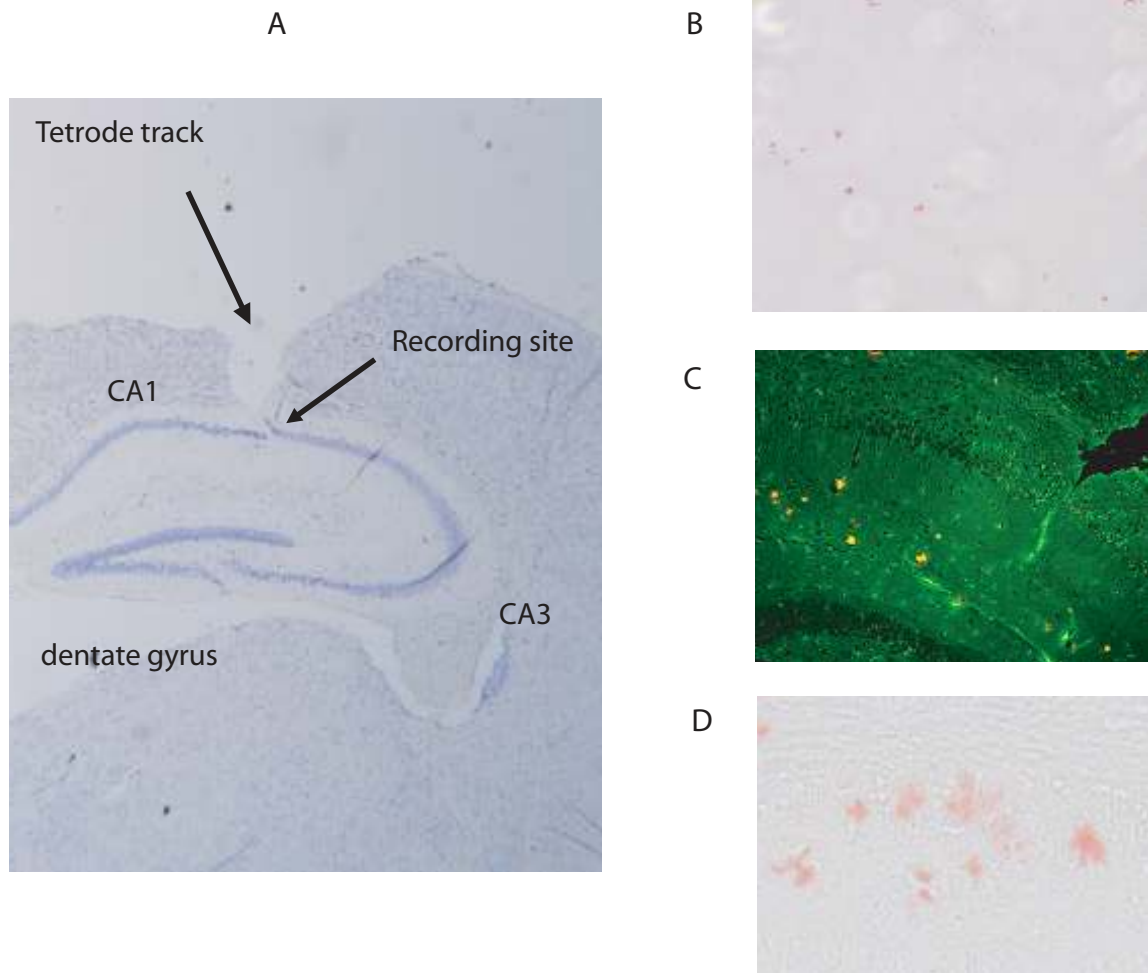


Figure 9.2 Histology. (A) A photograph showing the typical tetrode track in the hippocampus of an implanted mouse (cresyl violet staining). Only CA1 place cells were included in the present study. (B) Extensive amyloid plaques were detected in brains of aged tg2576 mice with Congo red staining. (C) Figure (B) viewed under blue fluorescent light. (D) A higher resolution photograph showing amyloid plaques.

3.1 Properties of single neurons

These property measures were obtained in the start box from standard trials.

Altogether, 121 place cells from young control mice ($n=4$), 115 from young *tg2576* mice ($n=3$), 240 from aged control mice ($n=5$) and 373 from aged *tg2576* mice ($n=9$) were included in the analysis of properties (Table 9.1). There were no differences across groups regarding the total number of cells [$F(3, 17) = 0.50$, $p = 0.685$] or the averaged number of cells recorded/day [$F(3, 17) = 1.83$, $p = 0.180$].

A first glance at field maps from different groups of animals suggested that some, but not all, aged transgenic animals showed larger and fuzzier place fields than animals of the other groups (Figure 9.3A and B). Such an impression was supported by statistical analysis. There were significant effects of age on place field size [$F(1, 17) = 15.06$, $p = 0.001$], spatial information content [$F(1, 17) = 17.30$, $p = 0.001$], and peak firing rate [$F(1, 17) = 6.80$, $p = 0.018$], but not on measures of basic neuronal properties, including mean firing rate [$F(1, 17) = 0.79$, $p = 0.385$], spike width [$F(1, 17) = 4.29$, $p = 0.054$] and spike amplitude [$F(1, 17) = 2.70$, $p = 0.119$], were comparable. However, when aged control animals were compared with young controls, no effects were seen in the above measures [place field size: $F(1, 7) = 3.11$, $p = 0.121$; spatial information content: $F(1, 7) = 3.04$, $p = 0.125$; peak firing rate: $F(1, 7) = 0.11$, $p = 0.756$; mean firing rate: $F(1, 7) = 0.08$, $p = 0.783$; spike width: $F(1, 7) = 2.77$, $p = 0.140$; spike amplitude: $F(1, 7) = 0.004$, $p = 0.951$], suggesting that place cells in young and aged control mice were indistinguishable.

Moreover, age and genotype interactions were found among groups regarding spatial information content [$F(1, 17) = 4.89$, $p = 0.041$, Figure 9.3C], place field size [$F(1, 17) = 6.10$, $p = 0.024$, Figure 9.3D], and peak firing rate [$F(1, 17) = 4.84$, $p = 0.042$, Figure 9.4B], while mean firing rate [$F(1, 17) = 0.28$, $p = 0.602$], spike width [$F(1, 17) = 0.33$, $p = 0.574$], and spike amplitude [$F(1, 17) = 2.49$, $p = 0.133$] did not differ amongst the groups (Figure 9.4A, C and D). A test of simple effects confirmed that place cell quality did not differ across genotypes in young animals [spatial information: $F(1,17) = 0.46$, $p = 0.50$; place field size: $F(1,17) = 0.33$, $p = 0.57$] but did significantly differ in aged animals [spatial information: $F(1,17) = 8.46$, $p = 0.01$; place field size: $F(1,17) = 12.22$, $p = 0.003$], suggesting that place cells in aged *tg2576* mice showed deficits in representing locations, indicated by larger place field size and lower spatial information content. Sample clusters, waveforms and autocorrelograms of place cells from an aged control mouse and an aged transgenic mouse were shown in Figure 9.5.

Figure 9.3

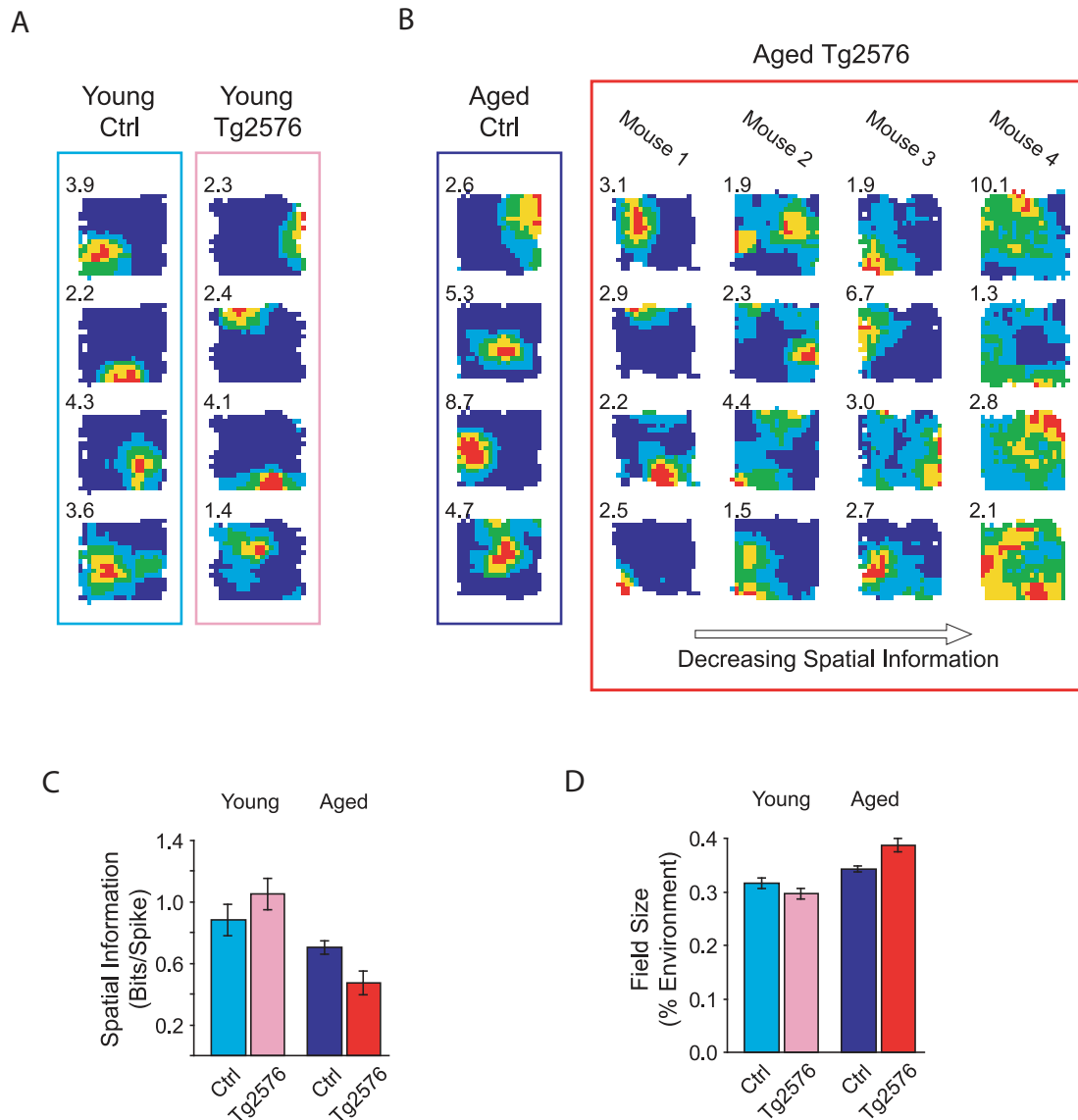


Figure 9.3 Degraded place fields in aged tg2576 mice. (A) Four representative place cells recorded from one young control mouse (left) and one young tg2576 mouse (right). (B) Four representative place cells recorded from one aged control mouse (left) and 4 different aged tg2576 mice (right 4 columns). Mouse 1 place cells showed the highest mean spatial information across aged Tg2576 mice, Mouse 4 the lowest, Mouse 2 and 3 reflected average values. (C) Place cells in the aged tg mice had lower spatial information content than aged controls and young animals [effect of age: $F(1,17) = 17.29$, $p = 0.001$; age x genotype: $F(1,17) = 4.89$, $p = 0.041$] (D) Place fields were markedly larger in aged transgenic animals [effect of age: $F(1,17) = 15.06$, $p = 0.001$; age x genotype: $F(1,17) = 6.10$, $p = 0.024$]. Numbers to the upper left of rate maps in (A) and (B) indicated peak firing rates.

Figure 9.4

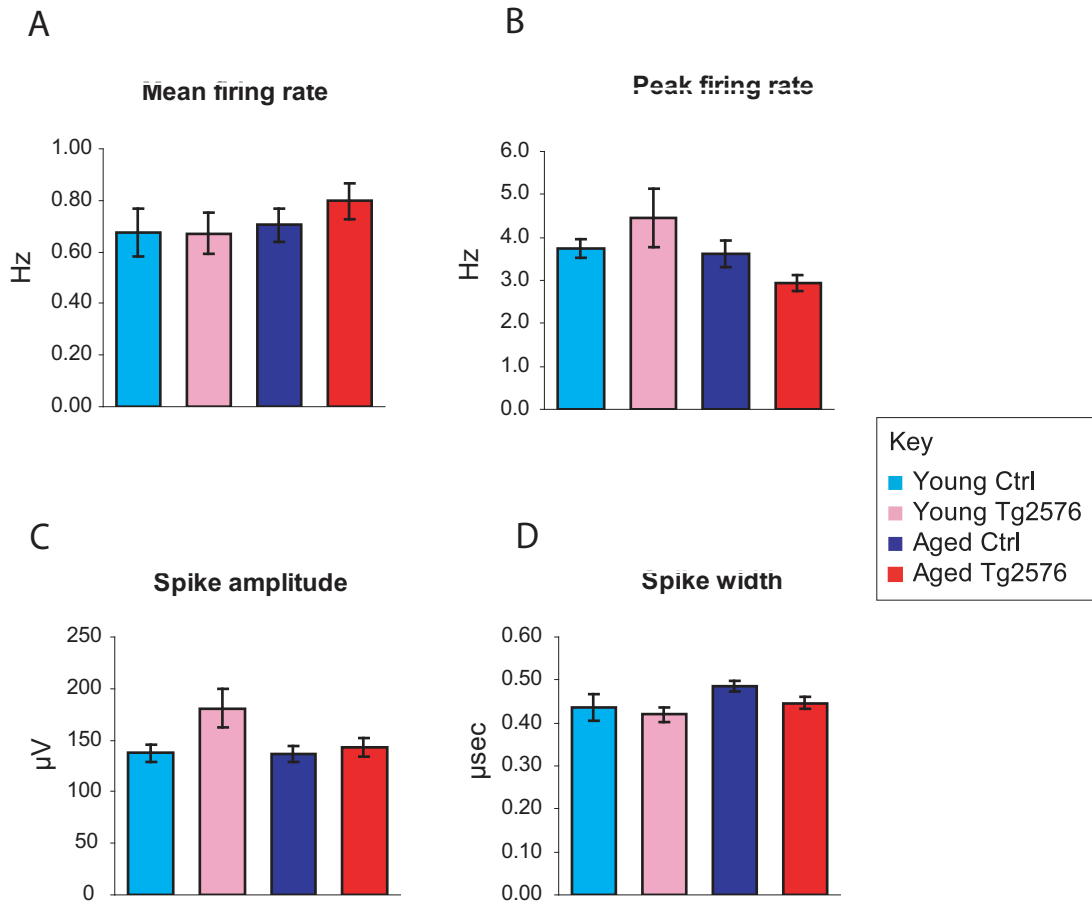


Figure 9.4 Basic properties of place cells in four groups of subjects. Aged transgenic mice showed lower peak firing rate than other groups [$F(1, 17)=4.84, p=0.042$, B], whereas mean firing rate [$F(1, 17)=0.28, p=0.602$, A], spike amplitude [$F(1, 17)=2.49, p=0.133$, C], and spike width [$F(1, 17)=0.33, p=0.574$, D] were not different across groups.

Figure 9.5

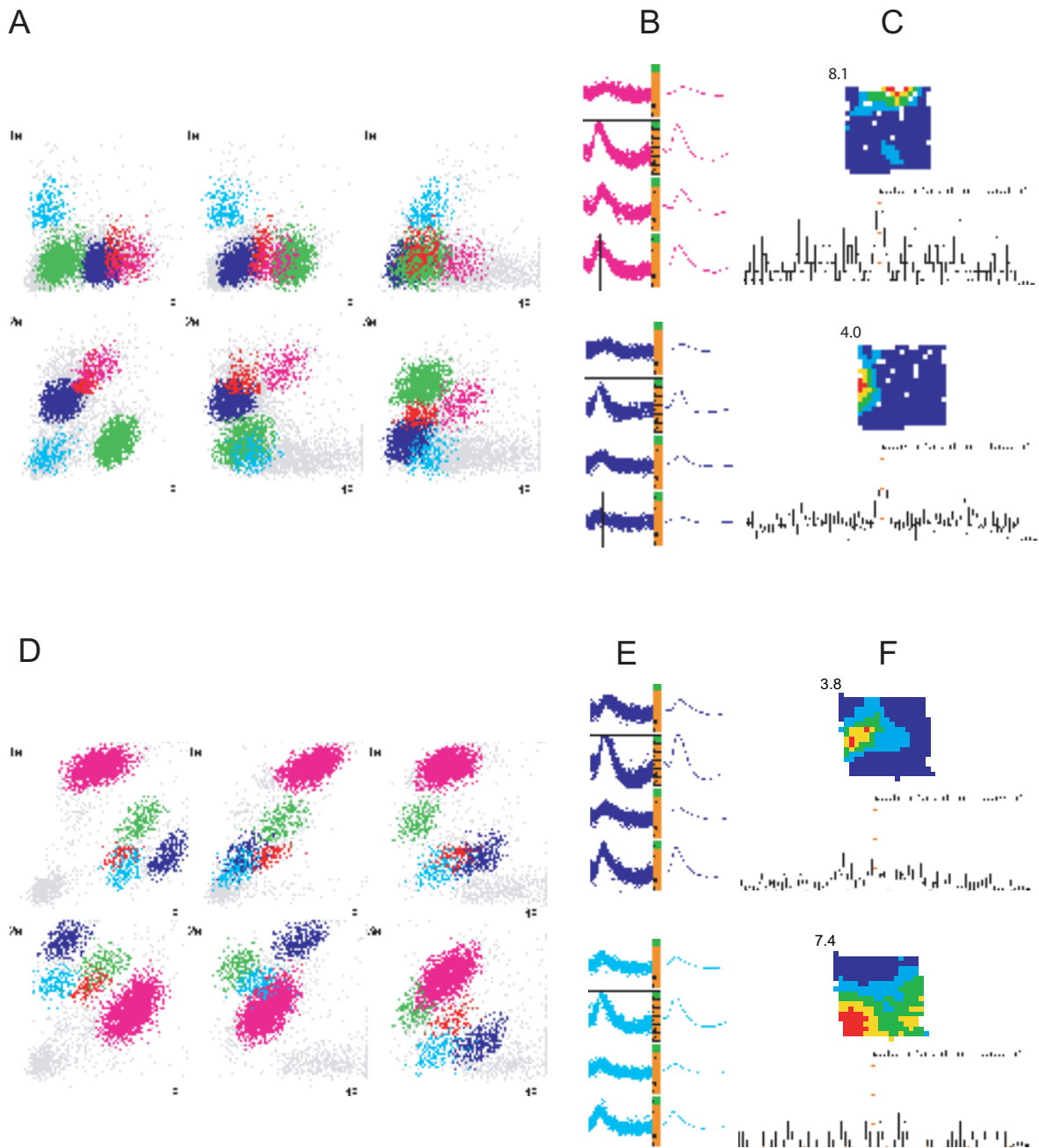


Figure 9.5 Sample clusters and waveforms of place cells. (A) An exemplar cluster plot from an aged control mouse. Action potentials were recorded extra-cellularly using tetrodes. Waveform parameters (here, peak-to-trough amplitude) for each recorded spike on each wire of the tetrode were then plotted against each other. Spikes that formed clearly isolated clusters were defined as being from different units (plotted in different colours). (B) Waveforms of two sample place cells from the trial in (A). Each waveform plot shows the waveforms on all four tetrode wires (top to bottom), for both the mean waveform (right-hand panel) and the overlaid plots of all recorded waveforms (left-hand panel). (C) Place fields and temporal autocorrelograms of 500ms for the cells in (A) and (B). (D) An exemplar cluster plot from an aged tg2576 mouse. (E) Waveforms of two sample place cells from the trial in (C). (F) Place fields and temporal autocorrelograms of 500ms for the cells in (D) and (E).

Part of this figure was constructed with the aid of Dr Tom Wills.

It was interesting and important to note, that the decrease in the accuracy of spatial representation was not uniform across the aged tg2576 mice (see Figure 9.2B). Some animals showed severely degraded place fields (mouse 4) while the place fields of others appeared normal (mouse 1), suggesting a large individual difference within the group.

Another interesting finding was a strong correlation between the place cell quality and the number of place cells detected in the aged tg2576 group. Given that different animals received place cell recording for different number of days, the average number of place cells recorded per day was calculated for each subject. There were strong correlations between the number of cells recorded and the average spatial information content ($r = 0.911$, $p = 0.001$; Figure 9.6A) and between the number of cells recorded and the average place field size ($r = -0.891$, $p = 0.001$; Figure 9.6B), suggesting that some aged tg2576 animals not only had poorer quality place cells, but also had fewer place cells to fire in an environment.

3.2 Place field stability

Place field stability was examined in standard trials where the start box remained at the same place between sample and delay sessions. All four groups showed a high bin-to-bin place field stability without age [$F(1, 15) = 1.67$, $p = 0.216$], genotype [$F(1, 15) = 0.26$, $p = 0.618$], or genotype by age [$F(1, 15) = 0.716$, $p = 0.411$] differences, suggesting that place cells from all groups of animals showed similar field stability in a highly familiar environment (Figure 9.7).

Longer-term (eg across-day) place cell stability analysis has not been performed because of technical difficulties.

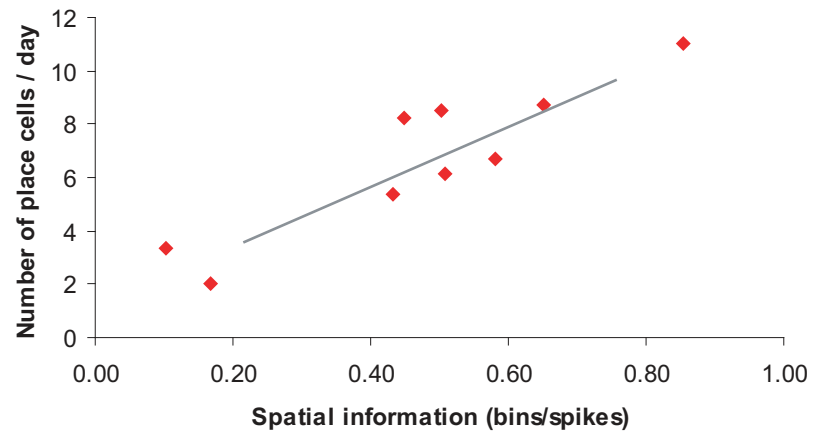
3.3 Network properties

The high place field stability allowed the examination of hippocampal network properties in probe trials, when the start box was rotated 180° between sample and test trials. Altogether, 51 place cells from young control mice ($n=4$), 57 from young tg2576 mice ($n=3$), 102 from aged control mice ($n=5$) and 184 from aged tg2576 mice ($n=7$, 2 had been eliminated from the previous phase because place cells could no longer be recorded from them at this stage) were included in the analysis of properties.

For each pair of recording sessions, bin-by-bin correlations were obtained between the pre-sample map and the delay map unchanged (0°) or 180° rotated. If the place field followed local cues (the box), the 180° rotated map should show a high correlation with the pre-sample map. If the field followed distal cues, a high correlation was expected for the 0° map. However, although place fields showed high correlations (in average >0.5) under

Figure 9.6

A



B

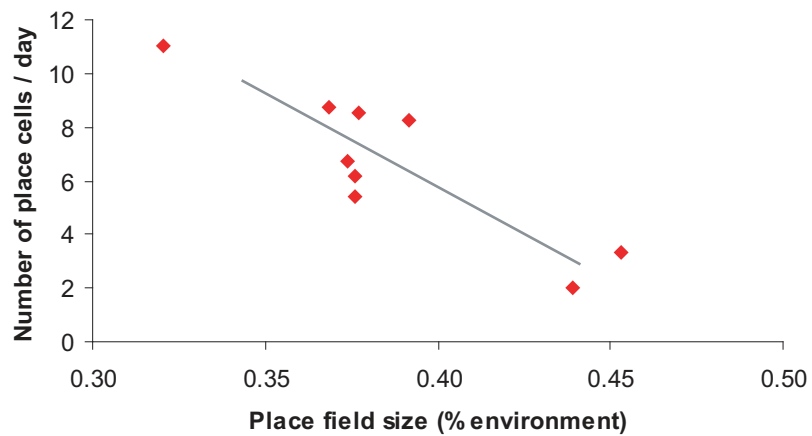


Figure 9.6 Correlations between place cell numbers and place cell quality in aged *tg2576* mice. The number of place cells recorded from each animals/day was strongly correlated with the spatial information content ($r=0.911$, $p=0.001$) (A) and place field size ($r=-0.891$, $p=0.001$) (B) of place cells from the same subject.

Figure 9.7

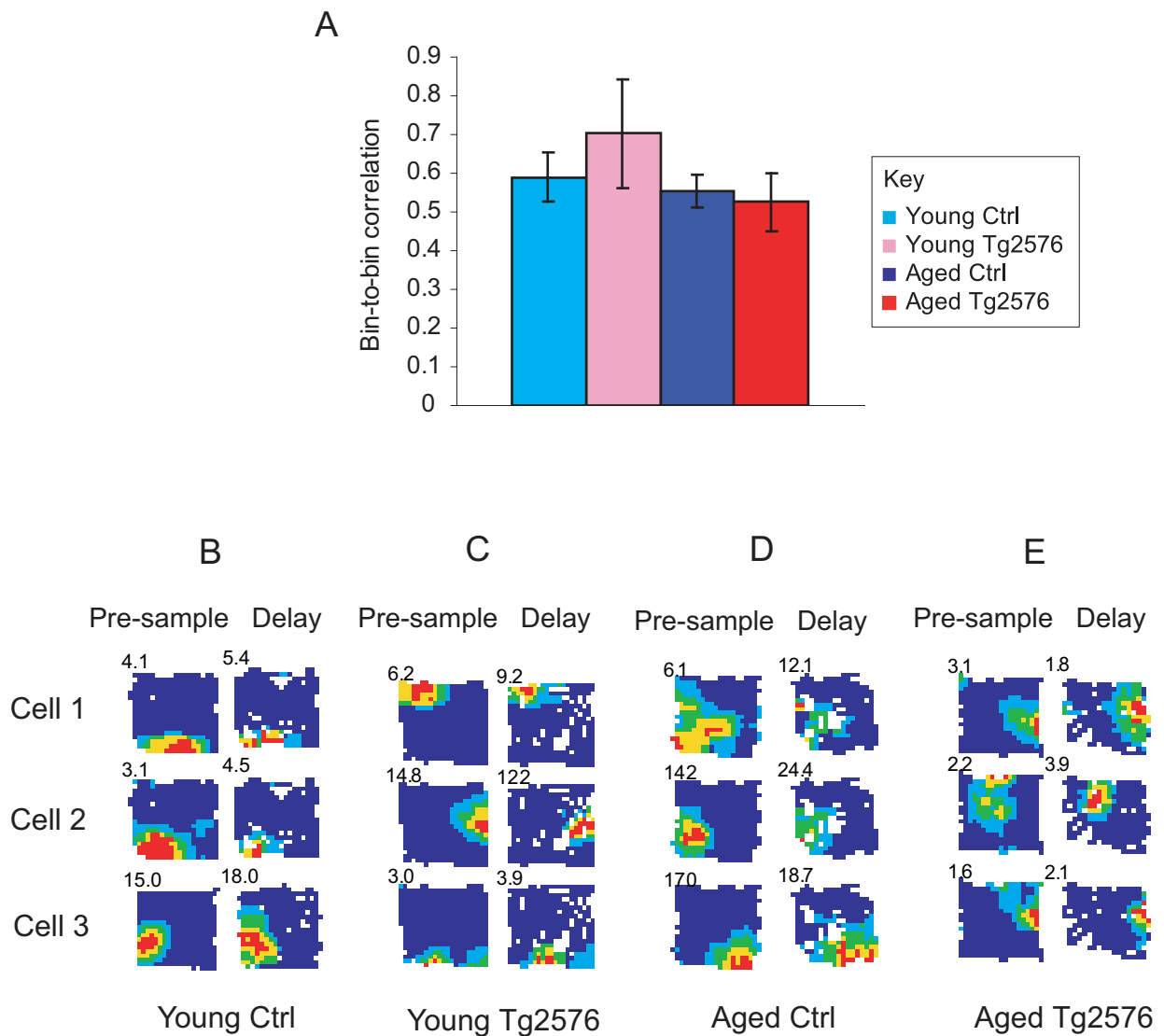


Figure 9.7 Place field stability in a familiar environment. (A) Place cells from four groups of subjects had similar field stability in a familiar environment, as measured by the bin-to-bin correlation between pre-sample (6 min) and delay (2 min) recording sessions. Effect of age: $F(1, 15)=1.67$, $p=0.216$; effect of group by age: $F(1, 15)=0.716$, $p=0.411$. (B), (C), (D) and (E) Sample place fields from animals of each group.

standard conditions when the start box remained at the same place (Figure 9.7), these values dropped to ~ 0.2 after either 0° or 180° map rotation. This low level correlation made it very difficult to draw convincing conclusions quantitatively about place cell responses to maze rotation, even if one could easily tell these cells' responses (following the box or the environment) by checking the field maps.

This technical failure could result from the following reasons: (1) The delay session was 2 min and in a novel location of the environment. Some animals were more cautious in exploration under this condition, making it difficult to get enough sampling for field analysis; (2) During the rotation process, the whole apparatus (the start box, its floor and the T-maze) was on a piece of wood which was a bit slippery, making it difficult to get accurate 180° rotations every time; (3) This procedure brought about a conflict between local and distal cues, making it unlikely that place cells responded purely to one set of cues with complete ignorance of the other; and (4) Some place fields were so small that even a tiny shift in the location would produce significantly low correlation values.

Since this method failed to provide convincing results, a second method was applied. For each pair of trials, the rate map of the delay session was rotated every 5° to find the angle with the highest bin-to-bin correlation with that of the sample session, and place cell responses to the 180° rotation of the apparatus between sample and test trials were classified into one of three categories:

(1) Box-centered: the location of the place field remained stable and always followed the box, defined by a $>135^\circ$ angle that showed the highest correlation;

(2) Environment-centered: the location of the place field remained stable and always followed the curtain-enclosed environment (distal cues), regardless of the movement of the maze, defined by a $<45^\circ$ angle that showed the highest correlation;

(3) Remapping: the location of the place field changed in a random manner, defined by a $>45^\circ$ yet $<135^\circ$ angle that showed highest correlation.

It must be noted that these definitions were arbitrary and not totally accurate. For example, remapping happened in a totally random manner so some remapping cells could be mis-categorized into box-centered or environment-centered groups.

Next, data were analysed in two ways: subject-based and cell-based, with behavioural data discussed as well.

Subject-based analysis

In this way, each group's response to maze rotation was analysed on the basis of individual subjects. For each subject, all place cells' field responses to maze rotation were

calculated and the subject's response to maze rotation was defined as box-centered if more than 50% cell fields followed the box, environment-centered if more than 50% cell fields followed the environment, and remapping if neither criteria applied.

(1) Young control: All 4 young control animals followed the box (Figure 9.9A, B). Sample place fields were shown in Figure 9.8A and B. Consistently, all of them used intramaze strategy to solve the task after maze rotation.

(2) Aged control: 3 out of 5 subjects in this group followed the box like young controls, and the other 2 followed the environment (Figure 9.8C and D, Figure 9.9A, B). Despite this dissociation, all subjects showed comparable performance between standard and rotation trials, and used box-centered strategy. However, it must be noted that the 2 environment-centered subjects performed more poorly than the other 3 (Figure 9.9A). These data were consistent with data from a rat study, which reported more powerful control of distal cues on place cells in aged cognitively impaired rats (Tanila et al, 1997).

(3) Young tg2576: Among the 3 subjects in this group, 1 mouse, like young wt, followed the box and used the same strategy in probe trials. However the other 2 subjects remapped and performed in a random manner after rotation (Figure 9.8E and F, Figure 9.9A, B). This result was particularly interesting, because one of them performed very well in standard trials, suggesting, though not proving, that place cell remapping might have impaired his performance in probe trials.

(4) Aged tg2576: 2 animals followed the environment and all the rest in this group remapped after maze rotation (Figure 9.8G and H, Figure 9.9A, B). Nearly all subjects performed randomly in probe trials, although some were not impaired in standard trials (Figure 9.9A).

(5) Statistical analysis

Statistics revealed a significant effect of groups [$\chi^2(6) = 15.39$, $p = 0.017$, Figure 9.8A], indicating different responses of the four groups to the maze rotation. Further analysis showed a difference between aged control and aged tg2576 [$\chi^2(2) = 7.89$, $p = 0.019$], and a nearly significant difference between young control and young tg2576 [$\chi^2(1) = 3.73$, $p = 0.053$]. These data suggest different hippocampal network dynamics between wild-type and transgenic animals in response to environmental manipulations, which could be present as early as 4-5 months of age. However, a clear conclusion could not be drawn given the relatively few number of subjects.

(6) Behaviour-physiology analysis

Figure 9.8

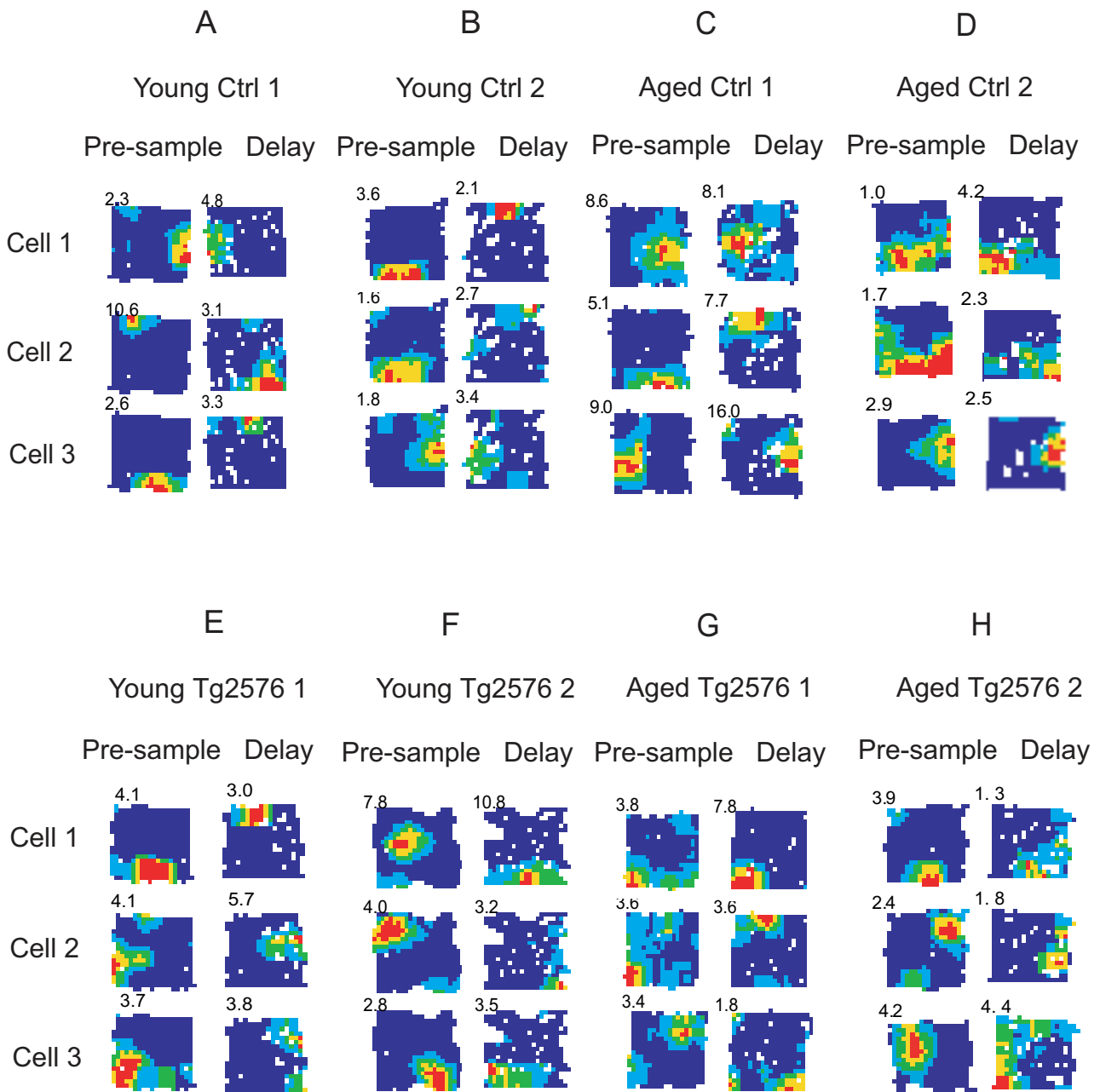


Figure 9.8 Sample place cell responses to maze rotation in probe trials. All young control animals followed the box (A) and (B). Some aged control animals followed the box (C) while others followed the environment (D). As for the young tg2576 group, some animals followed the box (E) whereas others remapped (F). Most aged tg2576 animals remapped (G) but two of them followed the environment (H). When the cell followed the box, its place field should be 180 degree opposite between pre-sample and delay sessions. When followed the environment, nearly identical. When remapped, the place field changed its location in a random way.

Figure 9.9

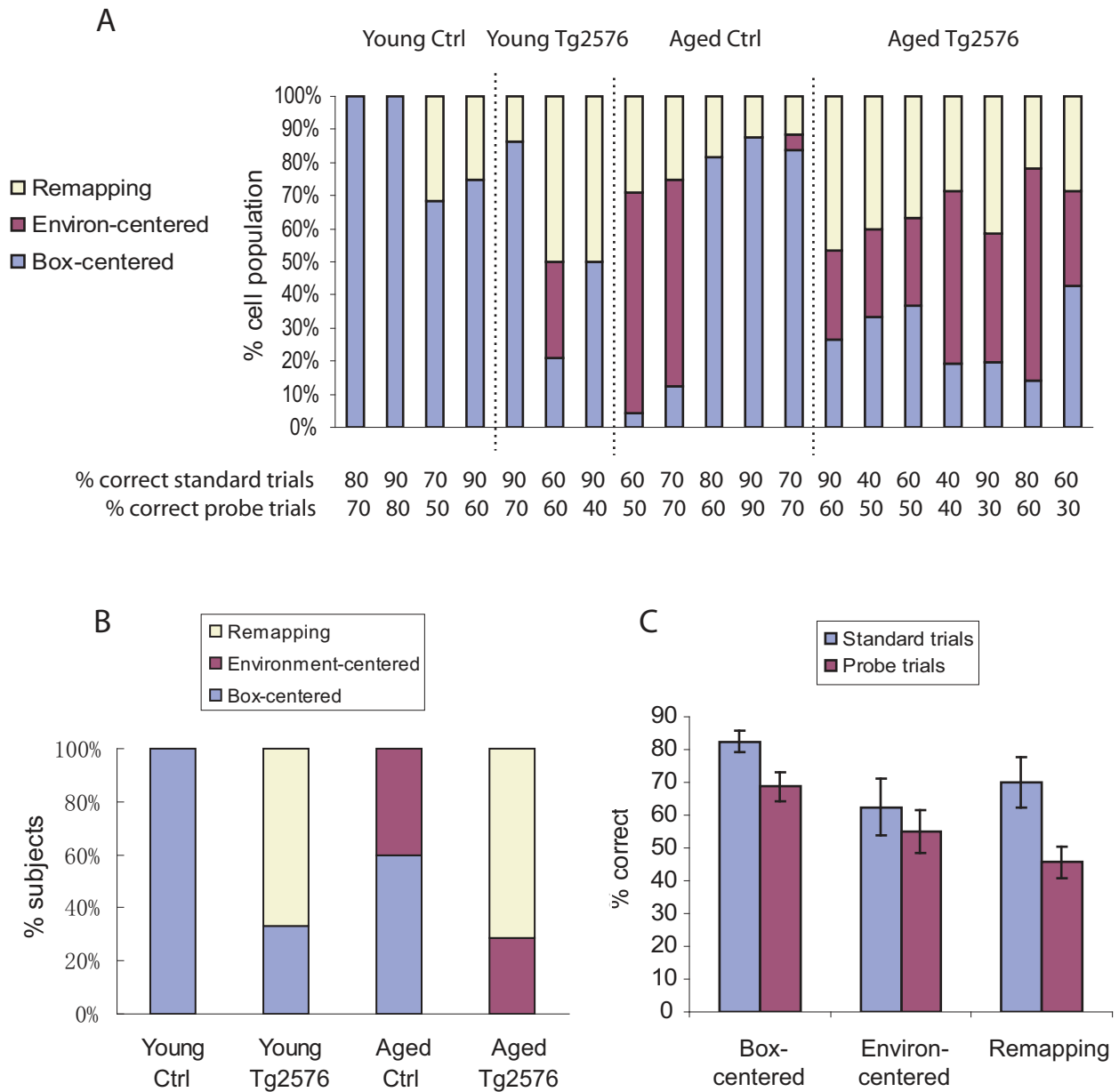


Figure 9.9 Subject-based analysis of electrophysiological and behavioural responses to maze rotation in probe trials. (A) Proportions of cell response to maze rotation in all subjects. All young control animals followed the box. Some young tg2576 animals followed the box whereas others remapped. As for the aged control group, some followed the box while some followed the environment. All but two aged tg animals remapped. Each subject's performance in the standard and probe trials was shown underneath its own column. (B) Different groups of animals responded to maze rotation in different ways [$\chi^2(6) = 15.39, p = 0.017$]. (C) Performance in standard and probe trials categorized by cell responses. The environment-centered and remapping groups were cognitively impaired compared with the box-centered group [$F(2, 16) = 5.84, p = 0.012$].

All subjects, regardless of their age and genotype, were divided into three groups based on their place cell responses to maze rotation, and their performance in standard and probe trials was compared (Figure 9.9C). Statistics indicated an effect of groups [$F(2, 16)=5.84$, $p=0.012$], indicating performance differences among groups, and an effect of training condition [$F(1, 16)=12.73$, $p=0.003$], suggesting that animals performed better in standard trials than in probe trials, but no effects of interactions [$F(2, 16)=1.28$, $p=0.304$]. Further analysis indicated a difference between box-centered and environment-centered groups [$F(1, 10)=6.12$, $p=0.033$], a difference between box-centered and remapping groups [$F(1, 13)=11.65$, $p=0.005$], but no differences between environment-centered and remapping groups [$F(1, 9)=0.011$, $p=0.909$], suggesting that environment-centered and remapping animals were cognitively impaired compared with box-centered animals.

Cell-based analysis

An alternative strategy to analyze these data was to pool all the cells of the same group of animals together. In this way, instead of getting a categorization for each individual subject, a categorization for the whole group was obtained.

(1) Young control: Consistent with subject-based analysis, fields of most cells from this group followed the box (Figure 9.10A).

(2) Aged control: Consistent with subject-based analysis, fields of most cells from this group followed the box (Figure 9.10A).

(3) Young tg2576: Inconsistent with subject-based analysis, fields of most cells from this group followed the box (Figure 9.10A). This was mainly because we had far more number of cells from the subject following the box than the other two remapping subjects.

(4) Aged tg2576: Fields of most cells either followed the environment or remapped whereas only a minority of cell fields followed the box (Figure 9.10A). This result was slightly different from the subject-based analysis but the consistent point was that place fields from this group did not tend to follow the box as control mice, young and aged.

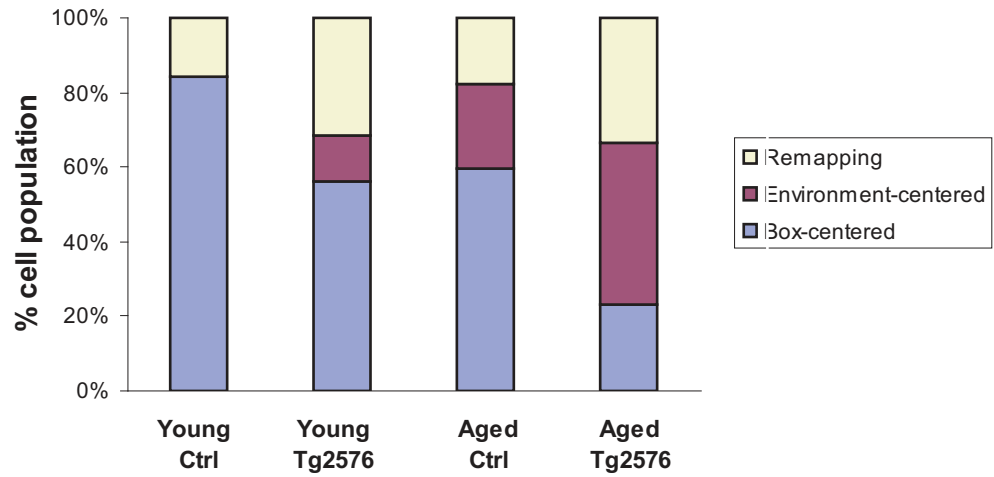
(5) Statistical analysis

Statistics revealed a significant effect of groups [$\chi^2(6) = 87.47$, $p < 0.001$], indicating different responses of the four groups to the maze rotation. Further analysis showed a difference between aged control and aged tg2576 [$\chi^2(2) = 37.65$, $p < 0.001$], and a significant difference between young control and young tg2576 [$\chi^2(2) = 12.16$, $p = 0.002$]. These statistical data were consistent with subject-based analysis and suggested different hippocampal network dynamics between wide-type and transgenic, even at a young age.

(6) Behaviour-physiology analysis

Figure 9.10

A



B

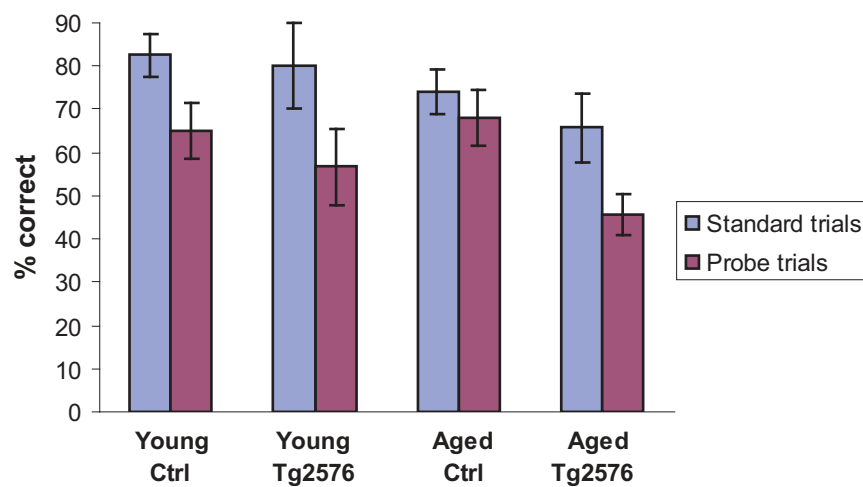


Figure 9.10 Cell-based analysis of electrophysiological and behavioural responses to maze rotation in probe trials. (A) Proportions of cell response to maze rotation in each group. Responses from transgenic groups differed from control groups at both young [$\chi^2(2) = 12.16, p = 0.002$] and aged [$\chi^2(2) = 37.65, p < 0.001$] stages. (B) Performance in standard and probe trials of the four groups. Maze rotation caused a significant performance drop for all groups [$F(1, 30) = 10.36, p = 0.003$], but not interactions were detected, suggesting that this manipulation affected four groups equally.

Statistics revealed a significant effect of maze rotation [$F(1, 30)=10.36, p = 0.003$], suggesting that this manipulation induced a performance drop (Figure 9.10B). However, this procedure affects different groups to the same level, since no interactions of rotation by age [$F(1, 30) = 0.51, p = 0.481$], or rotation by genotype [$F(1, 30) = 0.91, p = 0.347$], or rotation by age by genotype [$F(1, 30) = 0.16, p = 0.697$] were detected.

The small discrepancy between subject-based and cell-based analysis, especially in the young transgenic group, mainly resulted from the relatively small number of subjects and varied numbers of cells from each subject. However, given that this was only the pilot work in this field, further research needs to be performed to clarify this potential hippocampal network difference.

3.4 Distal cue rotation

An academically uninteresting explanation for the above behavioural and physiological changes in tg2576 mice was that these animals, instead of having spatial cognition deficits, experienced visual disabilities and could not see clearly. However, this possibility was unlikely, since this model had been well characterized previously and no such problems had been reported, even in experiments specifically designed to test their visual abilities (eg, Hsiao et al, 1996; Westerman et al, 2001; Barnes et al, 2004). In the present study, a small subset of subjects underwent a series of distal cue rotation recording sessions by the end of the whole experiment. Not all the subjects received this procedure either because place cells could no longer be recorded from them by this stage, or because their place fields were too disrupted or unstable to draw any clear conclusions. However, place cells from all tested animals consistently followed the distal cues in these sessions, further arguing against the possibility that these animals had visual problems. Sample place fields were shown in Figure 9.11.

4 Histology

All aged transgenic animals showed amyloid plaques in their neocortex and hippocampus (Figure 9.2B-D; Figure 9.12A-C). In contrast, none of the animals in the other 3 groups had plaques. Again, the pathological change in aged tg2576 mice was not uniform across subjects. Some animals showed large amounts of plaques (mouse 4) whereas some others had relatively few (mouse 1) (Figure 9.12A-C). Additionally, aged transgenics had elevated levels of soluble and total A β (Table 9.2).

Figure 9.11

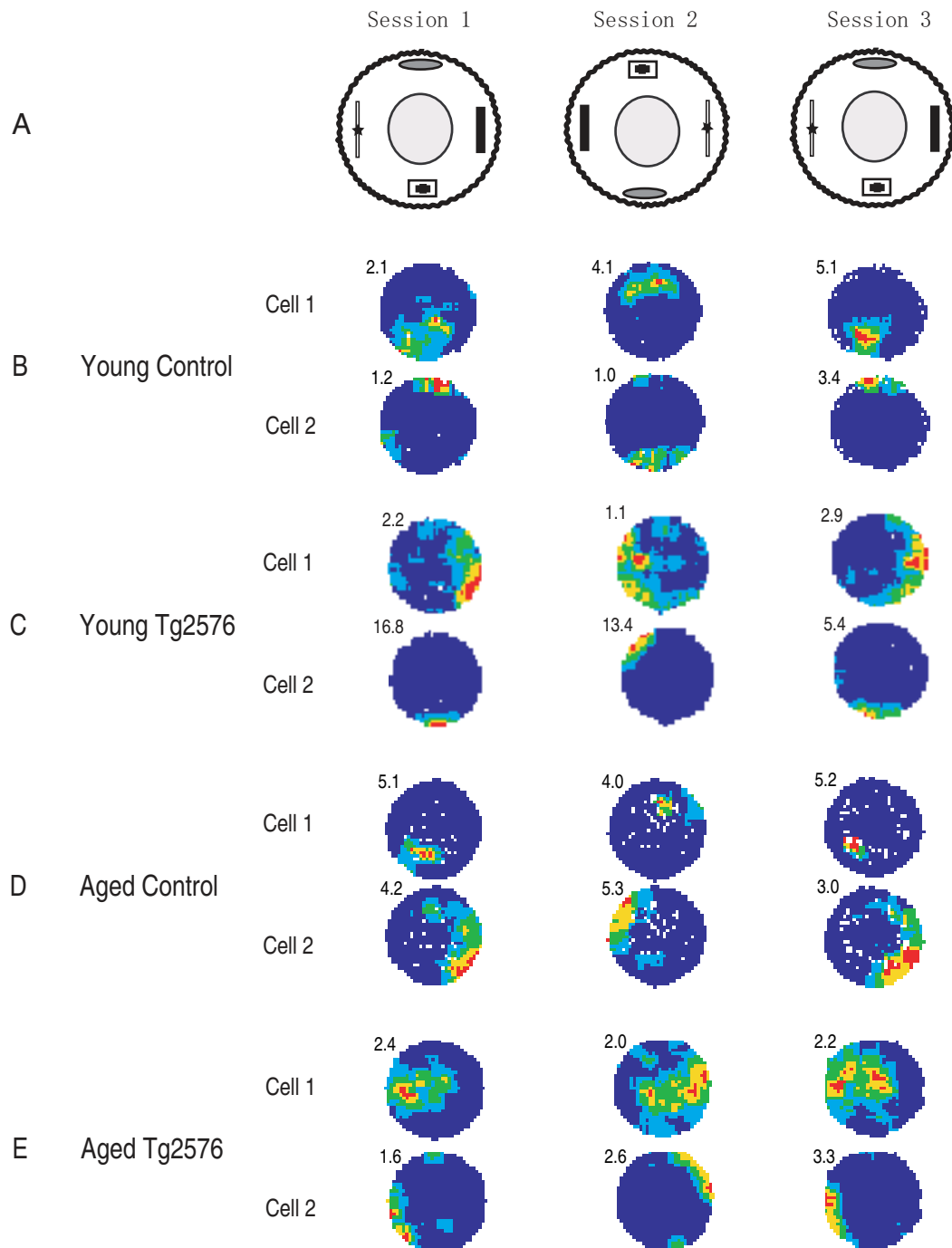


Figure 9.11 Distal cue rotation experiment. (A) Place cells from a subset of subjects were recorded in a cylinder for three sessions. In the first and third sessions, the distal cues remained the same as before (behavioural training and recording). In the second session, these cues were rotated 180 degree. Place cells recorded from all subjects followed the distal cues consistently during these sessions, indicating normal visual abilities of these animals. Two sample place fields from one mouse of each group were shown in (B) - (E).

Figure 9.12

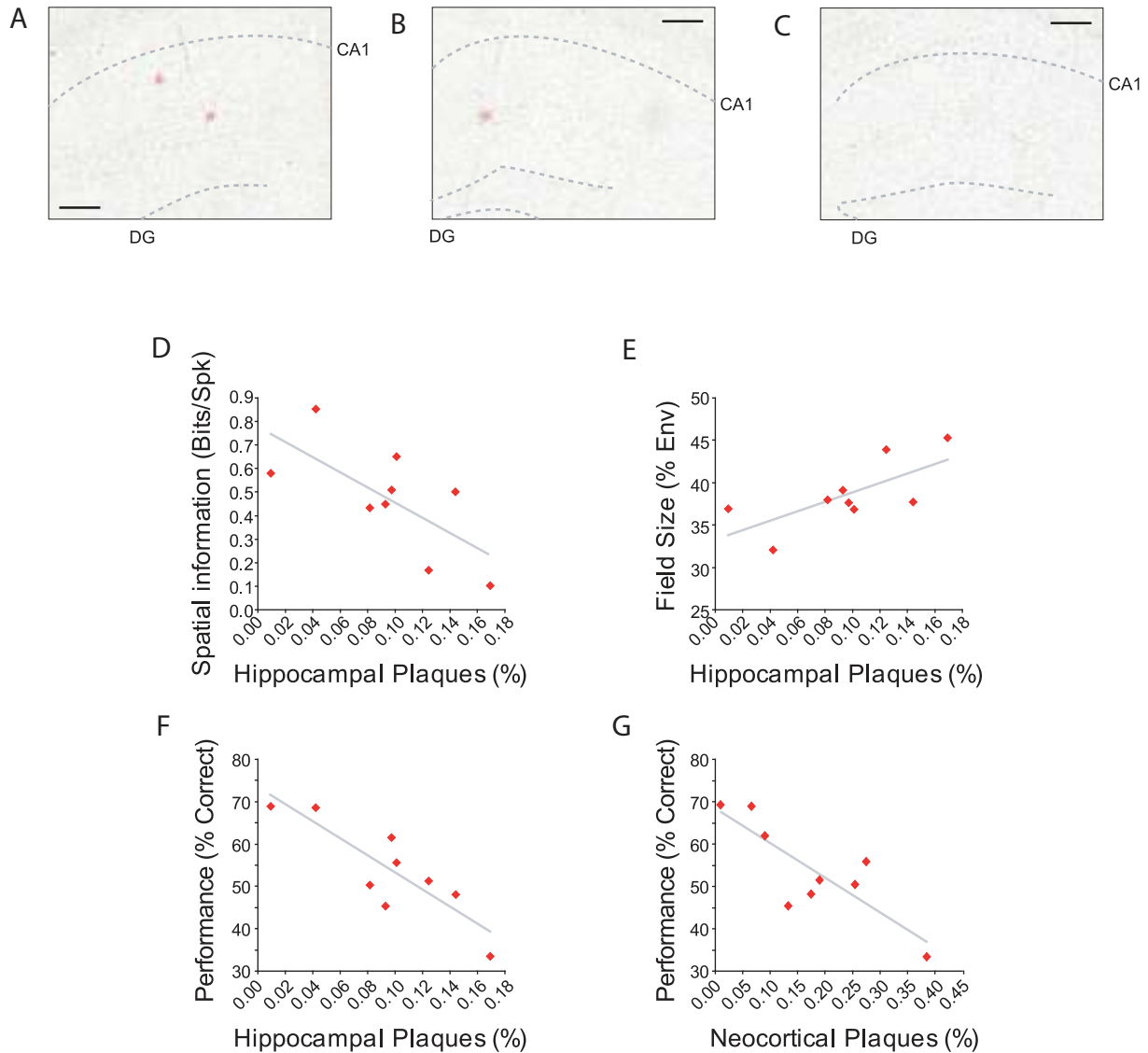


Figure 9.12 Percentage of hippocampal area occupied by amyloid plaques inversely correlated with place cell quality and behavioural performance. (A-C) Photomicrographs of 7- μ m coronal sections through the dorsal hippocampus of three aged Tg2576 mice showing highest (A), mean (B) and lowest (C) hippocampal levels of congophilic plaques deposition. (D) Place cell spatial information was negatively correlated with the percentage area of the hippocampus covered by congophilic plaques of aged Tg2576 mice ($r = -0.70$, $p = 0.036$). (E) Place field size was positively correlated with hippocampal amyloid plaques deposition ($r = 0.72$, $p = 0.029$). T-maze alternation rate was negatively correlated with the percentage area covered by congophilic plaques of the hippocampus ($r = -0.86$, $p = 0.003$) (F) and the overlying neocortex ($r = -0.83$, $p = 0.006$) (G). Scale bar in (A-C) = 100 μ m.

Table 9.2 Soluble and total A β amount of four groups (nM; mean \pm S.E.)

	Soluble A β_{1-40}	Soluble A β_{1-42}	Total A β_{1-40}	Total A β_{1-42}
Young control	0.64 \pm 0.10	0.37 \pm 0.20	2.14 \pm 0.21	2.83 \pm 0.87
Young tg2576	0.69 \pm 0.23	0.75 \pm 0.69	3.42 \pm 0.06	1.93 \pm 0.95
Aged control	0.61 \pm 0.16	0.23 \pm 0.08	1.59 \pm 0.14	2.04 \pm 0.09
Aged tg2576	292.33 \pm 67.66	50.22 \pm 14.95	982.20 \pm 126.86	300.05 \pm 78.23

5 Correlation

Since we had three measurements for each subject (behaviour, place cell physiology, and brain pathology), three sets of correlational analysis have been performed.

5.1 Behaviour and place cell physiology

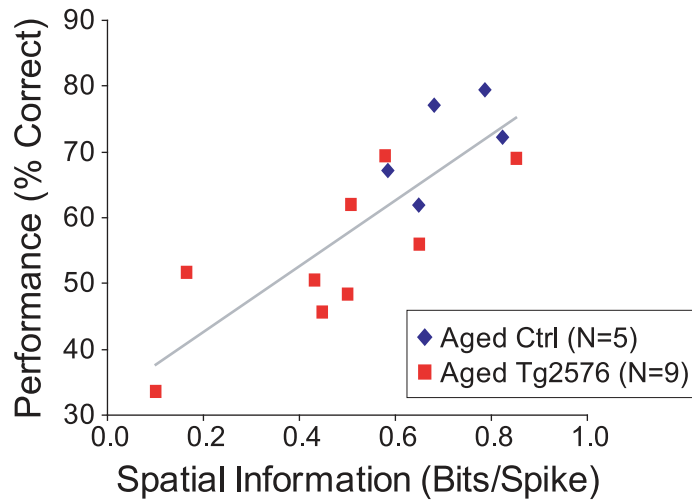
Performance on the T-maze alternation task is highly dependent on the hippocampus (Deacon et al, 2002): does it correspond to the amount of spatial information carried by the place cell population? The variability of both place cell firing quality (Figure 9.3) and behavioural performance (Figure 9.1) across the aged transgenic population allowed us to test this hypothesis. With place field size and spatial information content as indexes of the quality of place cell signalling in the aged (tg2576 and control) animals, there was a good correlation with behaviour averaged across all delays (Figure 9.13). This pattern of correlations did not change if the performance of three aged tg2576 animals performing at less than 50% correct is set to 50% (place field size: $r = 0.76$, $p = 0.001$; spatial information content: $r = 0.76$, $p = 0.002$).

5.2 Pathological correlates

Next, in order to identify the potential amyloid species responsible for the behavioural and physiological alterations observed in the tg2576 mice, dense core amyloid plaques in the hippocampus and overlying neocortex were stained with Congo red, and the concentration of soluble and total amyloid in the brain were assessed using a standard sandwich ELISA assay. There was a good correlation between congophilic (compact) amyloid plaque burden in the hippocampus (Figures 9.10A, B and C) and the spatial information content of hippocampal place cells (Figure 9.12D) and their place field size (Figure 9.10E). The plaque burden in the overlying neocortex did not correlate with hippocampal spatial representation quality (spatial information content, $r = -0.61$, $p = 0.084$; place field size: $r = 0.62$, $p = 0.076$; Figure 9.14) nor did the amount of soluble and total amyloid in the entire brain (Table 9.3). In addition, there was a strong correlation between each animal's overall performance on the spatial memory task and the burden of

Figure 9.13

A



B

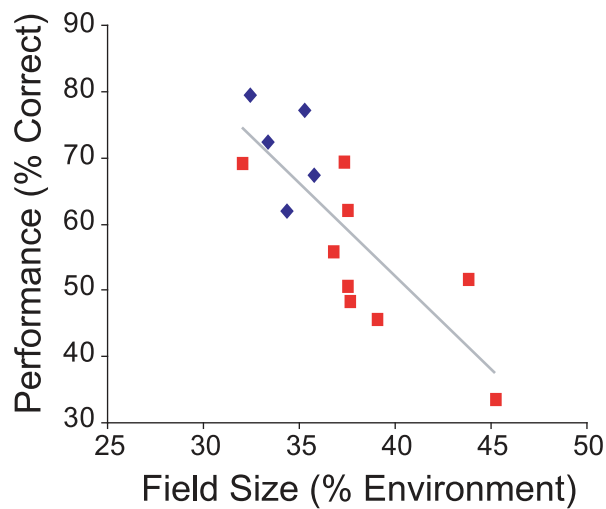


Figure 9.13 Correlations between T- maze performance and place cell quality in aged mice. (A) Mean spatial information content was positively correlated with alternation rate in aged tg animals (red squares) and aged control animals (blue diamonds) ($r = 0.83$, $p < 0.001$). (B) Mean place field size was inversely correlated with alternation rate ($r = 0.83$, $p < 0.001$). Alternation rates were means of three delays tested (15-secs, 2 min and 6 min).

Figure 9.14

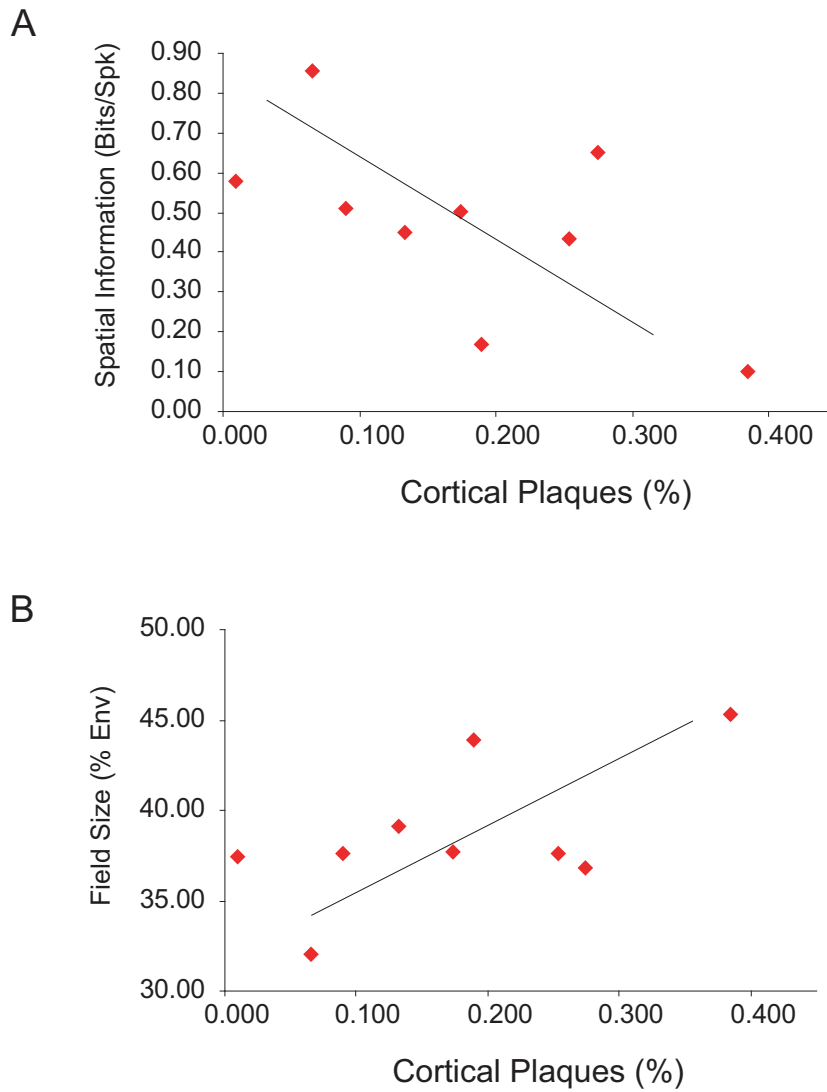


Figure 9.14 Percentage of cortical area occupied by amyloid plaques did not correlate with spatial information carried by place cells ($r = -0.61$, $p = 0.084$) (A) or mean place field size ($r = 0.62$, $p = 0.076$) (B).

plaques in hippocampus (Figure 9.12F) and in the neocortex (Figure 9.12G). Behavioural performance also correlated well with both soluble and total levels of A β -42 (Table 9.3).

Table 9.3 Correlations of brain A β load with place cells' properties and behaviour

	Soluble A β ₁₋₄₀	Soluble A β ₁₋₄₂	Total A β ₁₋₄₀	Total A β ₁₋₄₂
Spatial info	r=-0.47, p=0.20	r=-0.37, p=0.33	r=-0.43, p=0.25	r=-0.42, p=0.26
Field size	r=0.48, p=0.19	r=0.39, p=0.30	r=0.40, p=0.28	r=0.38, p=0.31
% Correct	r=-0.68, p=0.04*	r=-0.70, p=0.03*	r=-0.36, p=0.34	r=-0.70, p=0.04*

* p value significant at the 0.05 level.

Discussion

Chapter 10 Discussion

This chapter discusses possible explanations for the findings reported in the previous chapter, limitations of the present study, and potential future work.

1 Summary of main findings

1.1 Behaviour

The present study has confirmed previous findings that tg2576 mice showed an age-dependent impairment in spatial learning and memory.

1.2 Place cell physiology

Similar to spatial cognition, tg2576 mice showed degraded place cell representation of the environment in an age-dependent manner. Place cells in aged transgenic animals had reduced spatial information content and increased average field size, but their stability in a familiar environment and basic properties such as mean firing rate and spike measures were similar to controls.

At the network level, transgenic mice responded to environmental manipulation in a different way compared with control animals. Whereas place cells from all cognitively normal control animals followed the box after maze rotation, fewer cells from transgenic animals did in this way. Instead, many of them followed the environment or remapped. This difference could be present even at a young age when cognitive impairment was absent. However a clear conclusion could not be drawn because of the small number of subjects available.

1.3 Pathology

As described in previous studies, aged (>15 months) tg2576 mice but not young transgenics or control animals showed elevated levels of soluble and total A β and developed amyloid plaques.

1.4 Correlation

In aged transgenic animals, there was quite large inter-subject variability regarding both the performance in the T-maze alternation task and place cell quality. These two

measurements were strongly correlated with each other, and were associated with hippocampal plaque burden.

2 Cognitive impairment in tg2576 mice

2.1 The T-maze alternation task

The forced T-maze alternation task is frequently used to assess spatial working memory in animals. This is based on the fact that rodent species show a tendency to alternate their entries to left and right arm of a symmetrical T-maze. The rate of alternation depends on mouse strain, maze type, age of subjects, and the integrity of the septo-hippocampal system (Brito and Thomas, 1981).

An important issue is how the animal actually solves the task. These are at least four strategies available for the subject:

(1) The extramaze strategy (sometimes called place, spatial, or allocentric strategy in the literature): the subject uses spatial relationships between extramaze landmarks to identify its location within the environment in the sample trial, and avoids it in the free trial (“I was in the east arm in the sample trial, so should go to the west arm in the test trial.”) This strategy depends on the hippocampus (O’Keefe and Nadel, 1978);

(2) The intramaze map strategy: the subject uses the structure of the T-maze as the guidance cue to solve the task (“I went to the right arm in the sample trial, so I should go to the left one in the test trial.”). The hippocampus is also responsible for this strategy.

(3) The intramaze body-turn strategy (sometimes named the response strategy): the subject tracks his self-motion in the T-maze during the sample trial, and makes the alternative turn in the free trial (“I turned right in the sample trial, so should turn left in the test trial.”). It is not clear which brain regions primarily mediate this strategy, but the hippocampus (McNaughton et al, 1996) and the striatum (Packard et al, 1996; Chang and Gold, 2003) may be involved.

(4) The intramaze local cue strategy: the animal may use some uncontrolled local cues (for example, a small mark in one of the two maze arms, or olfactory cues in the maze) to identify his choice in the sample trial and turns in the other direction in the test trial. In this way this task becomes a recognition task that depends on peri- or post-rhinal cortex (Bussey et al, 1999). However, in the present study, this possibility has been eliminated, since the T-maze is always replaced with another identical one between sample and test trials.

There are several factors that influence the subject’s choice of strategies in the T-maze.

Indeed, animals may use a combination of these strategies in performing the task (Dudchenko, 2001). In general, animals trained in enriched environments are more likely to use the extramaze strategy than those trained in blank environments (Dudchenko, 2001). Additionally, animals tend to use the extramaze strategy in the early phase of training, but the intramaze strategy when they are over-trained (Packard et al, 1996; Chang and Gold, 2003).

Whichever strategy used, the animal must form a cognitive map that represents the environment, and retain information from a specific sample trial until the test trial, to successfully solve this task. Both procedures are sensitive to hippocampal damages (O'Keefe and Nadel, 1976; Aggleton et al, 1986; Deacon et al, 2002). However, there are some differences between these two situations. If the deficit results from an impairment of encoding spatial information, introduction of delays between sample and free trials will not influence the performance. In contrast, if the animal is unable to hold the trial-specific information, the impairment is only apparent when longer delays are introduced (Barnes et al, 2004). In this way, introduction of delays between sample and test trials can easily differentiate these two mechanisms.

2.2 Cognitive dysfunction of tg2576 mice

A number of studies have examined cognitive function of tg2576 mice, some of them using the forced T-maze alternation task. Despite some differences regarding the training procedure (trial numbers, learning criterion, introduction of delays and so on), these studies have reached very similar conclusions: aged (>10 months), but not young (3-5 months) transgenic mice show an impairment in acquiring this task. In a recent study, Barnes et al (2004) attributed this deficit to impaired spatial processing and encoding abilities in the aged transgenics, instead of an increased forgetting rate or interference. Additional support comes from two studies from the same group, showing that aged tg2576 mice are able to detect object novelty as well as control mice but show impaired conjunctive memory for specific object-location associations (Hale and Good, 2005; Good and Hale, 2007).

The current study has clearly confirmed these results. Performance of aged tg2576 animals was inferior to both young transgenic animals and aged control animals. Although the poor performance even at the shortest delay prevented an adequate assessment of the effects of retention interval and the conclusions from Barnes et al, 2004, we have directly examined the spatial encoding abilities of aged transgenics, by recording place cell activities in these animals.

Another finding from the present study is the performance impairment in young

tg2576 mice. These animals performed worse than controls when the training began. However, their performance became indistinguishable from controls in the end. Such an age-independent learning deficit has been reported in the same model in the conventional water maze task and could result from APP overexpression (Westerman et al, 2001).

3 Place cell physiology in AD: Neuronal properties

3.1 Place cell properties of tg2576 mice

The present study is the first attempt to examine place cell properties in a model of AD. It is interesting to compare current findings with those from studies on normal aging, given these two conditions, though different in nature, share some similarities both behaviourally and physiologically (chapters 1-3).

Although some early studies reported larger firing fields in aged rats (Barnes et al, 1983), later studies failed to replicate such a result. Individual place cells in aged rats seem to hold similar spatial information to young rats (Mizumori et al, 1996; Tanila et al, 1997). Only one study has examined place cell physiology in aged mice, which yielded very different results from rat studies. Yan et al (2003) reported lower spatial specificity and selectivity in of place cells in aged mice, which mainly resulted from higher firing rates outside the place field. The stability of place fields after a 24-h interval was also lower in old mice than in young mice.

Data from the present study were consistent with rat studies, and showed that place cells in aged transgenic animals show lower spatial content than those of their age-matched controls. This difference is unlikely to result from changes in the basic properties of hippocampal neurons in aged transgenic animals, since their wave amplitude, wave width and global mean firing rate are not different from other groups. In contrast, place cells in these animals show significant deficits in all spatial measures, including spatial information, field size and peak firing rate. Although place field stability in aged transgenic group was comparable with other groups, it must be noted that place fields in this group are significantly bigger and fuzzier than others, making it difficult to detect deficient stability with the bin-to-bin correlational analysis. Another intriguing finding was that aged transgenic animals with poor quality place cells also had fewer cells than those with high quality cells. Given that there is no neuronal death in the tg2576 model, this difference must result from cell dysfunction. That is, sensory or contextual information that drives place cell firing in normal animals can no longer do so in some aged tg2576 mice.

In this way, the ability of hippocampal place cells to code for locations in an environment is impaired in aged tg2576 animals. The demonstration of a deficit at the level of place cell representations complements previous findings of a deficit in hippocampal LTP and/or basic synaptic transmission in these animals (Chapman et al, 1999; Fitzjohn et al, 2001). According to the cognitive map theory (chapter 6), populations of place cells form a cognitive map that provides the necessary spatial information of the environment and enables successful navigation. In theory, a larger field size and/or a lower spatial information content decreases the cell's ability to encode and process locational information, whereas few place cells provide less locational information for the subject as a whole. However, the exact field size or amount of spatial information that support successful spatial navigation remains unclear. But consistent with this theory, breakdown of such a physiological system in aged tg2576 mice, indicated by the significantly lower spatial information and larger field size of their place cells, is accompanied by severe spatial cognitive deficits, as in some other transgenic lines (e.g. McHugh et al, 1996; Nakazawa et al, 2002; chapter 5). The present data have also directly confirmed conclusions from Barnes et al (2004), that impaired spatial processing and encoding abilities in aged tg2576 mice underlie their behavioural deficits in the T-maze, because these two measurements are correlated with each other (Figure 9.13).

3.2 Possible sources of place cell dysfunction

Given that tg2576 mice do not show neuronal loss despite immense pathology, it is rational to attribute the changes of place cell properties to the dysfunction of the hippocampal network, rather than a simple structural breakdown of it (neuronal death). Functionally, there are several possible sources of the place cell dysfunction.

The hippocampus

The most straightforward explanation is that mechanisms within the hippocampus attribute to this dysfunction. Anatomically, the hippocampus receives highly processed multimodal information from widespread cortical areas (chapter 4). Functionally, it is well accepted that the hippocampus is crucial for spatial cognition. Rodent hippocampal neurons encode the spatial location of the subject in an environment (chapter 5). The fact that place cell firing quality correlates better with hippocampal pathology, with only a non-significant trend towards correlation with neocortical plaques suggests that mechanisms within the hippocampus are the primary contributing factor to place cell dysfunction in the tg2576 model.

AD selectively implicates the hippocampus and related structures (chapter 2). The hippocampus of aged tg2576 mice are characterized by massive amyloid plaques and elevated levels of A β , both of which are neurotoxic and may induce neuronal dysfunction (chapters 2 and 3). The perforant path that projects from the entorhinal cortex to the dentate gyrus is among the most vulnerable pathways in the cortex with respect to both aging and AD pathology (Scheff et al, 1996). There are also reports of decreased spine density in the outer molecular layer of the dentate gyrus in tg2576 mice (Jacobsen et al, 2006; Dong et al, 2007). Anatomically, the entorhinal cortex provides most of the cortical input to the hippocampus. Damages to the perforant pathway and the dentate gyrus deprive the hippocampus of these inputs and result in an isolation of the hippocampus from the rest of the brain, which could explain the significantly smaller number of place cells in some aged tg2576 mice. Within the hippocampus, substantial synaptic loss and impaired synaptic plasticity have been repeatedly demonstrated in aged tg2576 mice (Chapman et al, 1999; Fitzjohn et al, 2001; Jacobsen et al, 2006; chapter 3). All these changes impact the hippocampus' ability to receive, integrate and process spatial information, and may result in disrupted place cell representations of the environment, as seen in the present study.

The entorhinal cortex

Similar to the hippocampus, the entorhinal cortex is early involved in AD (chapter 2). In AD patients, the entorhinal cortex shows even earlier pathological changes than the hippocampus (Thal et al, 2000; chapter 2). Yet no studies have examined this issue in tg2576 mice.

In recent years, especially with the discovery of grid cells (Hafting et al, 2005), the involvement of the entorhinal cortex in spatial cognition receives more and more attention. The hippocampus and entorhinal cortex are related structures located in the medial aspect of the temporal lobe. Anatomically, the entorhinal cortex provides most of cortical input to the hippocampus, and all three subregions of the hippocampus are directly innervated by the entorhinal cortex (chapter 4). Grid cells in this structure show strong spatial modulation (Hafting et al, 2005; Sargolini et al, 2006; chapter 5). According to some computational models (Burgess et al, 2007), the entorhinal cortex is the primary region of path integration and the firing of place cells is driven by a number of entorhinal grid cells (chapter 5). If this hypothesis were correct, functional changes of the entorhinal cortex would definitely induce hippocampal dysfunction, and one possible source of disrupted place cells in aged tg2576 mice could be disrupted grid cells in the same subjects. However, whether this is correct remains unclear until grid cell activities are recorded in these animals.

Association cortex

In addition to the hippocampus, neocortex, especially association cortex, also shows severe amyloid pathology in aged tg2576 mice (chapters 6 and 9). These areas received information from primary cortex and then relay it to the hippocampal formation. Damages to these regions deprived the hippocampus of sensory information about the environment, which is crucial for spatial cognition. However, the exact working mechanisms of association cortex remain poorly understood, making it difficult to judge their role in the place cell dysfunction seen in the present study.

The cholinergic system

Another possible source of place cell dysfunction is the basal forebrain cholinergic system, which is involved in the neural processing of learning and memory as well as attention (Geula, 1998). The cortical cholinergic system originates from a large population of neurons in the basal forebrain and innervates the entire cortical mantle. Among all brain areas, the hippocampus shows the highest density of cholinergic axons (chapter 2). Lesions or other manipulations of the cholinergic cells of the basal forebrain interfere with the cholinergic innervation of the cortex and impair learning, memory and attention in a number of animal species (for a review, see Geula, 1998). The cholinergic basis of this deficit was demonstrated through the reversibility of memory loss by cholinergic agonists.

The basal forebrain cholinergic system also appears to be vulnerable to the pathology of AD (Geula, 1998). The loss of cholinergic axons is not uniform, in that the areas with the greatest loss of cholinergic innervation are all in the temporal lobe, including the entorhinal cortex and the hippocampus (Geula and Mesulam, 1996). The frontal and parietal association areas, as well as the insula and temporal pole, show an intermediate magnitude of loss. Consistent with the loss of cortical cholinergic axons, the cholinergic neurons of the basal forebrain from which these axons emanate also display a major loss in AD (Lehericy et al, 1993). Deficits to this system have also been reported in the tg2576 model (Apelt et al, 2002; Luth et al, 2003; Klingner et al, 2003).

The head-direction system

This system provides head direction information to the hippocampus, which is important for spatial orientation of the subject in an environment, and its disruption severely influences place cell activities (chapter 5). In the present study, one interesting phenomenon that happened occasionally in some aged transgenic animals was that all place fields rotated 90° coherently across two standard trials (data not shown). This indicates that

aged transgenic animals may have an unstable head-direction system. However, physiology of head-direction cells in AD or aging remains unexamined.

The noradrenaline and olfactory system

The locus coeruleus (LC) is the main source of cortical noradrenaline (NA), and the LC/NA system is functionally involved in selective attention, arousal, learning, and adaptative behavioural responses (Berridge and Waterhouse, 2003). A dysfunction of the noradrenergic system has thus been proposed to be involved in the progression of dementia in AD (Marien et al, 2004). In rodents, LC/NA neurons provide strong projections to the olfactory bulb (McLean et al, 1989), and it is well known that rodents depend heavily on olfaction in their social behaviours. Consistent with this idea, neurodegeneration of the LC and olfactory deficiency have been recognized in AD patients (German et al, 1992; Murphy, 1999; Marien et al, 2004), as well as the tg2576 model (Guerin et al, 2007). The locus coeruleus degeneration in tg2576 mice happened between the age of 6.5 and 8 months, and was associated with olfactory memory impairments (Guerin et al, 2007). In the present study, local olfactory cues were removed by maze replacement during behavioural testing and by constant cleaning during recording, but an NA-system-induced place cell dysfunction cannot be ruled out.

The visual system

Finally, the explanation that all physiological deficits detected could simply result from disrupted basic sensory functions in aged transgenic animals, i.e., the animal could not see the distal cues as clearly as controls, is not plausible. It is well accepted that basic sensory (except olfactory) and motor functions of AD patients remain largely intact even at late stages of the disease (chapter 1). Additionally, the tg2576 model is one of the earliest and best characterized AD models and no studies have ever reported visual dysfunction of these animals (chapter 3). For example, these mice acquire an intramaze brightness discrimination task, a simple room discrimination task, and a contextual biconditional left-right discrimination task as well as control animals (Barnes et al, 2004). The distal cue rotation experiment in the present study also excludes such a possibility. However, it needs to be noted that deficits in the cholinergic system may affect visual attention, which is a potential mechanism for place cell activity changes (Wilson et al, 2004).

4 Place cell physiology in AD: Network properties

In the present study, the network properties of the animals have been examined by a simple maze rotation. Before discussing these data, I will restate results from studies

described in the “angular cue dissociation” part of chapter 5. Briefly, many of these studies report dissociations of place cells in response to environmental manipulations, in that some cells follow distal cues whereas others follow proximal cues. The maze rotation in the present study is similar to but also different from manipulations used in these studies. Similar, because in both cases there is a conflict between distal and proximal cues and hippocampal responses to such a conflict are examined. Different, because in the present study the animals are performing a spatial memory task whereas in those studies the animals are just performing free navigation.

Regardless of genotype and age, three different cell responses have been seen in the present study: following the box, following the environment, and remapping. Where does such a difference come from? It has been suggested that multiple input sets cooperate (or compete) to determine the activity of place cells (O’Keefe and Nadel, 1978; chapter 5). These functional input sets include external landmarks (including both proximal and distal cues) and internal or interceptive information derived from idiothetic or path integration system. Individual place cells have multiple firing correlates elicited by different sensory inputs or behavioural patterns (chapter 5). The various cell responses to maze rotation by different subjects seen in the present study suggest that varied sets of external or internal information dominate in each of them.

In control animals, young and aged, most cells followed the box, suggesting the dominance of proximal cues in these animals. Supposedly, these are “normal” animals but such a response seems to be inconsistent with angular cue dissociation studies (chapter 5; above). However, a careful examination of the training and recording protocol will reconcile this conflict. By the time these mice received rotation trials, they have been trained for long periods and are highly familiar with the environment and the task. Under such conditions, animals are more likely to use intramaze strategies to solve the task (Packard et al, 1996; Chang and Gold, 2003), which is consistent with the behavioural results in the present study (chapter 9). In this way, it is not surprising that place cells in these animals “ignore” the curtained environment and the idiothetic information, but only concentrate on proximal cues (the start box). Indeed a careful examination of Figure 9.10B indicates that the performance of both control groups and the young tg2576 group in probe trials seems better, though not significantly, than aged tg2576 group, whose cells mostly stick to distal cues.

In aged transgenic animals, most cells followed the distal cues. This result is comparable with Tanila et al (1997b)’s work on aged rats and it seems that the encoding

strategy of these animals relies on the most consistently available distal cues. There are several sources of the weakened control of proximal cues on place cells in these animals. First, these animals may have problems detecting changes in the local enclosure. For example, they have olfactory deficits. Aged (>6.5 months) tg2576 mice show locus coeruleus degeneration and olfactory memory impairments (Guerin et al, 2007), supporting this explanation. Second, these animals may selectively encode the most stable spatial cues (the distal cues), and ignore the rest. This could result from the specific pathological changes in the hippocampus of AD. The subregions that show the most severe damages in AD are the dentate gyrus and CA1, but not CA3 (chapter 1). Damages to the dentate gyrus reduce sensory input to the hippocampus about the environment, whereas intact CA3 can still perform pattern completion (chapter 5). In this way, these animals still hold a representation of the environment, but in a rigid manner that new changes cannot be incorporated. Reduced cholinergic innervation of the hippocampus in these animals may also contribute to this mechanism (Apelt et al, 2002; Luth et al, 2003; Klingner et al, 2003). There were also many cells that followed neither distal nor local cues. These cells could use idiothetic cues for their firing.

The situation in young tg2576 mice is difficult to discuss due to the small number of subjects in this group. Although the majority of cells from this group followed the box, the proportion was significantly less than the young control group. Most of the remaining cells remapped. Given that distal and proximal cues were in conflict, idiothetic cues could have overridden landmarks in these cells and remapping happens.

5 Molecular correlates of place cell dysfunction

Pathologically, AD brains are characterized by amyloid plaques and NFTs, plus neuronal and synaptic degeneration, inflammation and so on. All these changes are potentially neurotoxic and may induce physiological dysfunction. But in the tg2576 model, tau pathology and neuronal loss are not apparent, making it plausible to attribute these changes to amyloid pathology. However, A β deposits in the brains of AD patients and in animal models have a variety of morphologies, ranging from soluble A β oligomeric entities to insoluble fibrillar plaques, and their implication in AD has been heavily debated. The present study addressed this issue. The quality of place cell firing on the T-maze task was strongly correlated with the levels of congophilic plaque burden, but not soluble forms of A β . In particular, while behavioural impairments correlated with both cortical and hippocampal plaque levels, place cell firing quality correlated better with hippocampal

pathology, with only a non-significant trend towards correlation with neocortical plaques. This suggests that amyloid deposition in the hippocampus is crucial for hippocampal network dysfunction in the tg2576 model.

Although two previous studies on AD animal models reported correlations between amyloid plaque burden and cognitive decline (Chen et al, 2000; Gordon et al, 2001), most studies have failed to detect such a relationship (e.g. Terry et al, 1991; Arriagada et al, 1992; Braak et al, 1998). The number of fibrillar β -amyloid (A β) deposits in many areas of the cortex does not necessarily correlate with either the onset or the severity of cognitive decline (Arriagada et al, 1992; Terry et al, 1996). Additionally, functional deficits and synaptic loss have been observed even prior to fibrillar A β deposition (Hardy and Selkoe, 2002). This is frequently interpreted as evidence that amyloid plaques represent end-stage remains or by-products of the pathological processes in AD, and that more soluble forms of A β , instead of plaques, are the primary molecules responsible for the cognitive impairments (Hardy and Selkoe, 2002, Westerman et al, 2002). However, the present study, together with other recent studies (see below), suggests that compact amyloid plaques may be more actively involved in the physiological alterations in AD, which, in turn, result in the spatial cognitive deficits observed in tg2576 mice.

Plaque-related mechanisms

A β neurotoxicity has been demonstrated in cell culture where the extent of toxicity is clearly influenced by the aggregation state of A β , with fibrillar A β being far more toxic (chapter 2). There are several ways that plaques can cause physiological dysfunction.

Firstly, deposition of plaques results in synaptic loss. Abnormal neuronal morphology has been observed in several strains of transgenic mice that develop amyloid pathology, with the most pronounced changes near dense-cored amyloid plaques. Indeed, amyloid plaques are selectively associated with dystrophic neurites in these mice (Noda-Saita et al., 2004), and such deposits have been associated with local structural disruption of synapses and breakage of neuronal branches (Tsai et al, 2004). Specifically, in tg2576 mice synapse density is lower in areas surrounding compact plaques, providing evidence of a spatial relationship between synapse loss and β -amyloid plaques (Lanz et al, 2003; Tsai et al, 2004; Spires et al, 2005). Dong et al (2007), using electron microscopy, observed overall decreases of dendritic synapse density in the proximity of amyloid plaques in the dentate gyrus and the entorhinal cortex at 15-18 months of age in tg2576 mice. There was a correlation between lower synapse density and greater proximity to β -amyloid plaques. However, this study failed to detect overall changes in synapse density in the CA1 subfield,

where place cell activity was recorded in the present study. Another study, using intravital multiphoton microscopy, produced similar results. Spires et al (2005) revealed dramatic changes of dendritic trajectory and morphology near plaques, with a linear decrement in dendritic spine density in the immediate vicinity ($\sim 20 \mu\text{m}$) of plaques. In a later study from the same group (Spires-Jones et al, 2007), the structural plasticity of dendritic spines was examined. Tg2576 mice had normal spine density and plasticity before plaques appeared, but after amyloid pathology is established, spine elimination increased, specifically in the immediate vicinity of plaques. Spine formation was unchanged, resulting in spine loss. These data show a small population of rapidly changing spines in adult and even elderly mouse cortex; further, in the vicinity of amyloid plaques, spine stability is markedly impaired leading to loss of synaptic structural integrity. The change of synaptic structures in a pattern that centers on plaques implies a dramatic synaptotoxic effect of dense-cored plaques. Decreased spine density will likely contribute to altered neural system function and behavioural impairments observed in tg2576 mice.

The accumulation of fibrillar amyloid causes not only local structural disruption of synapses, but also neurite breakage, which might interfere with the integration and propagation of electric signals at the cellular level. Stern et al (2004) compared intracellular recordings from neocortical pyramidal neurons in tg2576 mice and showed that substantial accumulation of β -amyloid plaques was associated with defects in cortical synaptic integration. Spontaneous membrane potential dynamics were similar, suggesting that overall levels of synaptic innervation were intact and the deficits resulted from reduced synchrony of converging synaptic inputs. Jacobsen et al (2006) recently detected a relationship between decreased spine density in the outer molecular layer of the dentate gyrus and the declines in LTP and contextual fear conditioning.

Finally, dense-cored plaques may be selectively neurotoxic. Urbanc et al (2002), using triple immunostaining confocal microscopy, compared $A\beta$ deposition, $A\beta$ morphology, and neuronal architecture and found that the vast majority of $A\beta$ deposits do not alter the neuronal landscape whereas dramatic, focal neuronal toxicity and loss were associated only with thioflavin S-positive fibrillar $A\beta$ deposits. This may explain why neuronal loss is not apparent in mouse models of AD. ThioS cores occupy only 1~2% of the cortical surface and are distributed widely throughout cortical areas. Even if all neurons within ThioS cores were lost, this difference in total neuron numbers would be difficult to detect.

However, it remains difficult to determine whether these neuronal and synaptic changes in proximity to β -amyloid plaques are due to some type of plaque-related tissue disruption

or to increased concentrations of A β oligomers in the local area (next section). Plaques could be a source of specifically toxic species of oligomeric A β (Walsh et al, 2002), toxic plaque-associated free radicals (McLellan et al, 2003), or cytokines and other bioactive substances generated by the activated astrocytes and microglia associated with plaques (Nagele et al, 2004). It also has been hypothesized that A β may be toxic through an excitotoxic cascade (i.e. deregulation of calcium homeostasis inducing oxidative stress and abnormal increases in glutamate release), which is facilitated by the proximity of plaques (Mattson, 1997; McLellan et al, 2003).

Possible involvement of soluble A β and other molecules

Although structural changes are most pronounced near plaques, indicating focal toxicity, these dense plaques only occupy a small proportion of all the brain tissue, whereas the synaptotoxic effect and place cell dysfunction extend beyond the immediate vicinity of plaques. Additionally, loss of dendritic spines in the dentate gyrus of tg2576 mice could be detected in areas with little or no fibrillar A β deposition, or before plaque deposition (Holcomb et al, 1998; Jacobsen et al, 2006; Dong et al, 2007). In the present study, potential hippocampal dysfunction has also been detected at an age when no plaques are present. All this evidence implies the involvement of other molecules or a toxic environment in the pathogenesis of AD.

The identity of these synaptotoxic mediators beyond plaques remains uncertain. Recent studies have shown that soluble, low-molecular-weight (8-24 kDa) A β oligomers target synapses in cultured neurons (Lacor et al, 2004) and disrupt cognition when injected into the brain in vivo (Lambert et al, 1998; Walsh et al, 2002; Mattson, 2004; Cleary et al, 2005). These molecules are present at elevated levels of soluble A β species in the vicinity of plaques, disrupt synaptic function and impair hippocampal LTP (Larson et al, 1999; Moechars et al, 1999; Walsh et al, 2002; Cleary et al, 2005). Involvement of A β in synaptotoxic processes is further supported by a study showing that clearing A β using passive or active immunotherapy prevents synapse loss in transgenic mice (Buttini et al, 2005). Recently, Lesne et al (2006) have identified a 56-kDa soluble amyloid- β assembly (A β *56), which is responsible for cognitive deficits in middle-aged tg2576 mice when plaque formation is not very obvious. This molecule disrupts memory when administered to young rats.

The present results cannot rule out the possibility that place cell degradation and behavioural deficits in the tg2576 mice are caused by elevated soluble β -amyloid, and are just indirectly related to plaque deposition. Plaques could be a source of these toxic

oligomeric species of A β (Walsh et al, 2002). The hypothesis that soluble A β species are contributing to the behavioural impairments observed in the tg2576 mice is supported by the observation that soluble levels of A β -42 (as well as total A β -42) were correlated with aged tg2576 animals' performance on the T-maze task. Additionally, these molecules could be responsible for the hippocampal network dysfunction seen in young tg2576 animals. One way to test this hypothesis is to examine place cell physiology in middle-aged animals with the aid of A β antibodies.

APP transgene

The final possible source of physiological changes is the APP transgene expressed by the transgenic mice. APP itself, in addition to its product A β , is able to modulate neural activity (Arendt, 2004). The possible involvement of APP overexpression must be carefully considered in analysing physiological and behavioural changes of these mice, especially at the young age (<6months) when the level of A β is low. One way to solve this issue is to use transgenic mice overexpressing normal, instead of mutated form of, APP as a control group (Westerman et al, 2002).

6 Correlation between physiological and behavioural changes

The relationship between place cell activity and spatial behaviour of the subject has been a hot topic in hippocampal physiology. Whereas some studies have shown a strong correlation (O'Keefe and Speakman, 1987; Lenck-Santini et al, 2001), others do not (Jeffery et al, 2003) (see also chapter 5). Most of this work was done in normal animals and examined the relationship between place cell firing patterns and spatial choices in a multiple-choice task. Some groups have produced transgenic animals targeting molecules important for spatial learning, and reported disrupted place fields and impaired spatial cognition in behavioural tasks (e.g. Tsien et al, 1996; Nakazawa et al, 2002). One intriguing finding from the present study is that place cell dysfunction under pathological conditions is accompanied by impaired spatial cognition, supporting the view that place cells are part of a navigational system. This conclusion is strengthened by the strong correlation between the quality of place cell firing and performance levels in the T-maze alternation task. These findings have also directly confirmed the conclusion from Barnes et al (2004) that the behavioural deficit of aged tg2576 mice results primarily from the impairment of the hippocampus to generate an adequate representation of the environment within which the task is set.

7 Clinical significance

There is little doubt that the rodent hippocampus plays a vital role in spatial cognition. Similarly, it is widely accepted that this brain region is essential for episodic memory in humans. According to the cognitive map theory, the rodent hippocampus represents the environments and their contents, providing the neural basis for spatial memory and flexible navigation. When it comes to humans, this brain region has a broader function, based at least in part on lateralization of function. The right hippocampus still encodes spatial relationships, whereas the left has the altered function of storing relationships between linguistic entities and is more involved in episodic memory (O'Keefe and Nadel, 1978; Burgess et al, 2001).

An episodic memory deficit characterizes early AD and is possibly the most devastating symptom in this disorder. Although it is still controversial whether non-human species have episodic memory, several groups have identified episodic-like memory in rodents and shown that this type of memory is impaired in animals with hippocampal pathology (Chen et al, 2000; Dere et al, 2005; Good et al, 2007). Additionally, how place cell activities in rodents fit into such an episodic-like memory system has been proposed in several models (Ferbinteanu and Shapiro, 2003; Leutgeb et al, 2005; Knierim et al, 2006; Smith and Mizumori, 2006). Thirdly, place cell-like cell activities that signal spatial information have been detected in the human hippocampus (Ekstrom et al, 2003). And finally brain imaging studies have clearly demonstrated hippocampal hypometabolism and hypoperfusion in AD patients (chapter 1). All this evidence suggests that hippocampal network dysfunction seen in tg2576 mice may also be the neural substrate for cognitive impairments in AD patients and research on these animals helps clarify hippocampal dynamics in this disorder and possibly, evaluate potential treatment.

8 Limitations of the present study and future prospects

Despite the significance of these results, the present study bears undeniable limitations, which also direct future work.

Firstly, a general limitation of all animal studies on AD is that none of these models fully replicate the pathology and physiology of the human disease. The tg2576 model does not show neuronal loss and NTFs, which undoubtedly influence neuronal and synaptic function and contribute to behavioural impairment in humans. So results from the present study cannot reveal the full picture of the hippocampal network dysfunction in AD. In

recent years several double or triple transgenic models have been developed (chapter 3), whose pathology is much more similar to AD patients than the tg2576 model. Research on these models will better clarify the pathogenesis of AD at the hippocampal network level.

Secondly, correlation does not mean causation. The actual molecules responsible for physiological and behavioural changes remain unknown. A β has a variety of morphologies, ranging from soluble forms with little β -pleated sheet to dense core compact deposits. Only compact plaques and soluble A β have been measured in the present study. One way to prove the causative relationship is to ameliorate or postpone the development of pathological changes in this model and examine the resulting place cell physiology and behaviour. Strategies include active and passive vaccination, which has been shown to effectively reduce amyloid pathology and improve cognitive abilities (chapters 1-3), or other drugs under investigation.

Thirdly, the recording apparatus (the start box of the T-maze) in the present study is a highly familiar environment for the subjects. According to previous studies, effects of normal aging on place cell physiology are much more obvious in novel environments or after environmental manipulations. Similar procedures can be performed in Alzheimer mice to study changes of hippocampal network dynamics in this disorder. In the present study, a simple maze rotation has yielded some surprising results, which may be indicative of the pathogenesis of very early stages of AD.

Fourthly, only CA1 place cells have been examined. Within the hippocampus, CA1 and CA3 cells show different properties under some conditions, suggesting functional distinctions between these cells (chapter 5). Pathologically, different hippocampal subregions are not equally affected in AD (chapter 2). Simultaneous recording in multiple sites will help clarify such a pattern at the functional level. Additional interesting recording sites include the dentate gyrus and the entorhinal cortex.

Finally, only young (3-5 months) and very aged (14-18 months) animals have been included in the present study. Data from previous studies reveal that behavioural, physiological and pathological changes of these animals may start as early as 6 months (possibly even earlier with data from the present study). Work on these middle-aged animals may yield more significant information about hippocampal physiology in AD. Indeed some recording has been performed in such animals, though intriguing, these data are not enough to draw any clear conclusions.

9 Conclusions

In conclusion, the present study has revealed that tg2576 mice show an age-dependent deficit in their neuronal representations of the environment. The level of place cell degradation correlates the animal's poorer spatial memory and with hippocampal but not neocortical amyloid plaque burden.

AD is characterized by severe cognitive impairments at the behavioural level (chapter 1). Research in the past decades has suggested that AD represents an A β -induced synaptic failure at the molecular and cellular levels (chapter 2 and 3). The present study has complemented these findings and suggests that AD mice have degraded spatial representations at the hippocampal network level. The study of place cells in rodent models of AD may provide a powerful tool for identifying the pathogenesis and the respective contributions of hippocampal and neocortical pathology to the overall cognitive memory dysfunction in AD. Place cell recording may also serve as a powerful testing platform for therapeutic interventions.

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Appendix

List of Abbreviations

A β	β -amyloid
AD	Alzheimer's disease
ADN	anterodorsal thalamic nuclei
AMPA	α -amino-3-hydroxyl-5-methyl-4-isoxazole-propionate
ANOVA	analysis of variance
APLP	amyloid precursor-like protein
apoE	apolipoprotein E
APP	amyloid β -protein precursor
CA	cornu ammonis
CamKII	Ca ²⁺ /calmodulin-dependent protein kinase II
ChEI	cholinesterase inhibitor
CRE	cAMP-responsive element
CREB	cAMP-responsive element-binding protein
CTF	carboxy-terminal fragment
DG	dentate gyrus
DNA	deoxyribonucleic acid
DPX	deoxyribonucleic acid
EC	entorhinal cortex
EDTA	entorhinal cortex
EEG	electroencephalograph
ELISA	enzyme-linked immunosorbent assay
EPSP	excitatory postsynaptic potential
FAD	familial AD
FTDP-17	frontotemporal dementia with Parkinsonism linked to chromosome-17
GABA	gamma-aminobutyric acid
HDC	head-direction cell
IEG	immediate-early gene
iNOS	inducible nitric oxide synthase
KO	knock-out
LC	locus coeruleus
LDN	latero-dorsal thalamic nuclei

LEA	lateral entorhinal area
LED	light-emitting diode
LIA	large irregular activity
LTD	long-term depression
LTP	long-term potentiation
MAP	microtubule-associated protein
MCI	mild cognitive impairment
MEA	medial entorhinal area
MRI	magnetic resonance imaging
mRNA	messenger ribonucleic acid
MS/DBB	medial septum/diagonal band of Broca complex
NA	noradrenaline
NFT	neurofibrillary tangle
NMDA	N-methyl-D-aspartic acid
NO	nitric oxide
NT	neuropil thread
PET	positron emission tomography
PHF	paired helical filament
PKC	protein kinase C
PS	presenilin
PTP	post-tetanic potentiation
RNA	ribonucleic acid
SD	standard deviation
SE	standard error
SIA	small irregular activity
SPECT	single photon emission computed tomography
STP	short-term potentiation