Characterisation of visuospatial memory in the Tg2576 model of Alzheimer's disease

Gemma Hale

ProQuest LLC
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106-1346

DECLARATION

This work has not previously been accepted in substance for any degree and is not concurrently submitted in candidature for any degree.

Signed ..... Date 31st May 2018

STATEMENT 1

This thesis is being submitted in partial fulfilment of the requirements for the degree of PhD.

Signed ..... Date 31st May 2018

STATEMENT 2

This thesis is the result of my own independent work/investigation, except where otherwise stated. Other sources are acknowledged by explicit references.

Signed ..... Date 31st May 2018

STATEMENT 3

I hereby give consent for my thesis, if accepted, to be available for photocopying and for inter-library loan, and for the title and summary to be made available to outside organisations.

Signed ..... Date 31st May 2018

Acknowledgements

I would like to thank my supervisor, Professor Mark Good, for always making the time when I had a question and for helping me to stay motivated during those long hours in the lab.

I am also extremely grateful to Philip Barnes for passing on his wise words of wisdom and to Victoria Staal for all her help and patience. My thanks also goes to Andrew Nelson for keeping me sane in moments of mouse madness.

I also need to thank my family - Mum, Dad, Beth and Louise, for their continued support. Finally I would like to thank Daniel, he had to put up with a lot and for that I am extremely grateful.

This work was supported by an MRC research studentship.

Publications

Barnes, P., Hale, G., & Good, M. (2004). Intramaze and extramaze cue processing in adult APPSWE Tg2576 transgenic mice. *Behavioural Neuroscience 118(6),* 1184-1195.

Hale, G., & Good, M. (2005). Impaired visuospatial recognition memory but normal object novelty detection and relative familiarity judgments in adult mice expressing the APPswe Alzheimer's disease mutation. *Behavioral Neuroscience, 119(4),* 884-891.

Good, M.A., & Hale, G. (2007). The "Swedish" mutation of the amyloid precursor protein (APPswe) dissociates components of object-location memory in aged Tg2576 mice. *Behavioral Neuroscience, 121(6),* 1180-1191.

Good, M.A., Hale , G., & Staal, V. (2007). Impaired "episodic-like" object memory in adult APPswe transgenic mice. *Behavioral Neuroscience, 121(2),* 443-448.

Short Abstract

Previous behavioural characterization of the Tg2576 mouse has been limited to reference memory tasks in water or T /Y mazes. This thesis aimed to specify the underlying features of visuospatial memory in this model of Alzheimer's disease.

Chapter 2 presents a series of experiments that are consistent with the hypothesis that aged Tg2576 animals are impaired in forming allocentric representations of the environment. Experiments 1 and 2 demonstrated that aged transgenic mice were able to acquire a simple room discrimination and contextual conditional left-right discrimination in a T-maze. Experiment 3 showed that aged transgenic mice were able to learn a response strategy in a reference memory task in the plus maze. Tg2576 mice were however significantly impaired in the acquisition of the place version of the plus maze task and T-maze forced-choice alternation (Experiment 2a).

Using a spontaneous object recognition paradigm, Experiment 4 revealed that adult mutant mice were able to discriminate between familiar and novel objects with delays of up-to 24 hours and were also able to discriminate the relative familiarity of two objects. However, Tg2576 mice failed to investigate objects that had changed their relative spatial positions. Furthermore, Experiment 5, using an episodic-like memory task, demonstrated that Tg2576 mice were unable to integrate information concerning the temporal properties and spatial location of objects. This inability reflected a primary deficit in processing or memory for the spatial location of objects.

Chapter 4 examined the nature of the spatial representations that were impaired by the APPswe mutation in versions of a spontaneous object recognition paradigm. Experiment 6 evaluated Tg2576 mouse habituation. Experiment 8

demonstrated that Tg2576 performance was not facilitated by the presence of an intra maze cue. Experiments 7 and 9 revealed that Tg2576 mice were sensitive to changes in the spatial organisation of the objects when the objects were moved to locations previously unoccupied during the sample stage. This pattern of results suggests that Tg2576 mice manifest a specific deficit in the formation of object-location associations akin to the impairment observed in Alzheimer patients (e.g., Swainson et al., 2001).

Contents

Figure 4.3.1: Experiment 8 the mean contact time in seconds for transgenic (tg+) and wild type (tg-) mice with objects located in the same and different positions from the sample trial in the cue-card version of the maze.

Figure 4.3.2: Experiment 8 the mean contact time in seconds for transgenic (tg+) and wild type (tg-) mice with objects located in the same and different positions from the sample trial in the standard version of the task.

Figure 4.4.1: Summary of the behavioural procedures used for Experiments 9 phase 1 (absolute lateral task), Experiment 9 phase 2 (relative diagonal shift task) and Experiment 9 phase 3 (relative vertical shift task).

Figure 4.4.2: Experiment 9 the mean contact time in seconds for transgenic (tg+) and wild type (tg-) mice with objects located in the same and different positions from the sample phase. Objects have either moved to a novel location or changed their relative position and have therefore been repositioned in a familiar location.

Figure 4.4.3: Experiment 9 discrimination ratios for transgenic (tg+) and wild type (tg-) performance on each of the phases (objects either moved to a novel location or changed their relative position and have therefore been repositioned in a familiar location).

Table 1: Mean discrimination ratios for adult transgenic (tg+) and wild type (tg-) mice in 3 versions of the object recognition task in Experiment 4.

Table 2: Average amount of time (in seconds) wild type (tg-) mice spent in the arena during the exposure phases of Experiment 7.

General Introduction

The aim of this thesis is investigate visuospatial memory processes in the Tg2576 transgenic model of Alzheimer's disease (AD). Chapter 1 will present a brief overview of AD and current perspectives on transgenic models, with a focus on the Tg2576 animal. Previous research with Tg2576 mice has established an age-related impairment in spatial memory – characteristic of memory impairments in patients with AD. Parallels will be considered between spatial memory processes in the animal navigation literature and the nature of navigational deficits observed in AD patients.

I will first discuss the psychological and neuropathological features of AD followed by a short review of the transgenic literature. To gain a potential insight into the nature of the anatomical and psychological deficits observed in AD mice, a review of rodent navigation is presented. The contributions of the hippocampus, enthorinal cortex and parietal lobes to spatial memory are considered. Psychological and computational models of animal navigation are discussed with reference to the neural contributions these distinct anatomical regions may have in spatial memory. The final section considers spatial information processing in patients with AD.

1.1 Why Study Alzheimer's Diseases?

Thirty seven million people are estimated to have dementia, 18 million of these suffers have been diagnosed with AD (Vas et al., 2001, world health report). It is estimated that this neurodegenerative disease affects half of people over 85. With

an aging population this presents one of the greatest threats to our future health care systems and represents a considerable emotional and financial cost (Mount & Downton, 2006). At present it is estimated that dementia currently costs 55 billion euros in Europe per year (Andlin-Sobocki et al., 2005; Jonsson & Berr 2005). With an increasing aged population it is vital that we obtain a better understanding of the aetiology of AD.

1.2 Clinical Features

AD was first reported at the beginning of the twentieth century (Alzheimer, 1907). Over the past 100 years there has been many advances in the understanding of the neuropathological features of the disease. However, clinical symptoms are still fundamental to the diagnosis of AD (Bouchard & Rossor, 1996). The DSM IV (1994) criteria for probable AD can be summarised as follows: "impairment of memory, evidence of at least one of the following cognitive impairments: aphasia, apraxia, agnosia or disturbance of executive function, gradual onset and progressive decline; the deficits cause significant impairment of social and professional activities, represent a decline from a previous level of functioning, cannot be explained by any other neurological, psychiatric, systemic or substance-induced condition known to cause cognitive deficits and do not occur only in the context of delirium." (Bouchard & Rossor, 1996: p.42).

The progression of the disease can be categorised in stages. The most common symptom of early AD is disrupted memory function (Hart & Semple, 1990; Forstl & Kurz, 1999). Recent memories are the most vulnerable; whilst early memories remain relatively intact (Hart & Semple, 1990). Tests of declarative memory can reveal the

early stages of disease. Tests that index declarative memory in probable AD patients include the Wechsler Memory Scale-Revised (WMS-R), the logical memory subtest, and the Rey complex figure test (Welsh et al., 1992; Lehtovirta et al., 1996; Perry & Hodges, 2002). By the latter stages of the disease early episodic memories are lost (Forstl & Kurz, 1999).

Alzheimer patients also suffer with temporal and spatial disorientation, although there is disagreement within the literature as to which is more disrupted (Hart & Semple, 1990). Both aspects however have a grading effect. Spatial disorientation is initially worse in novel environments and progresses to a level of dysfunction in familiar locations (Cherrier et al., 2001). Temporal disorientation starts with a general forgetfulness regarding the date, and by the latter stages of the disease patients become unaware of the time of day (Smith, 1998). Further discussion of AD and spatial disorientation is presented at the end of Chapter1.

Early symptoms of AD also present themselves in the form of deficits in attention processes. Attention can be divided into three forms (Eysenck & Keane, 1995). Selective attention refers to processing one input among many possible sources of information. Divided attention requires processing all possible inputs. Sustained attention is the processing of one input for a prolonged amount time. Tests such as the Wisconsin Card Sorting task, the stroop test and trail-making test, demonstrate that AD manifests a global decline in selective, divided and sustained attention (Albert, 1996; Rizzo et al., 2000).

AD patients during the middle stages of the disease show marked deficits in working memory. Baddeley and Hitch (1974) define working memory as consisting of three components, the phonological loop which stores information as speech, visual and spatial information coded in the visuo-spatial sketch-pad, and the modality free

central executive involved in attention processes. By the middle stages of the disease patients demonstrate marked deficits in the Wechsler Adult Intelligence Scale-Revised (WAIS-R), (Rizzo et al., 2000), a clinical index of working memory. Patients also demonstrate deficits in implicit memory in the middle stages of the disease. Implicit memory differs from declarative memory in that it does not require conscious recall (Graf & Schacter, 1985). This form of memory is indexed in classical conditioning tasks such as the eye-blink reflex (Woodruff-Pak et al., 1996) and cued fear conditioning (Hamann et al., 2002).

During the final stages of the disease patients present symptoms of Apraxia (the loss of ability to regulate movement), Agnosia (an inability to recognise objects) and Aphasia (the inability to organise speech; Forstl & Kurz, 1999). Patients may even experience psychotic episodes and can present personality changes such as agitation, suspicion, apathy and irritability (see Hart & Semple, 1990; Forstl & Kurz, 1999).

1.3 Neuropathology

A brief description of the key neuropathological features of AD will now be presented. The two predominate features of AD are Amyloid Plaques and Neurofibrillary tangles (NFTs). Chapter 1 intentionally focuses upon Amyloid Plaques, as the Tg2576 mouse model evaluated in this thesis is a derivative of this pathology. It is however recognised that AD may be caused by a variety of neuropathological features including, inflammatory responses, oxidative stress, cholinergic, estrogen and cholesterol disruption, to name but a few (cf. Desai & Grossberg, 2005).

1.3.1 Amyloid plaques

Amyloid plaques consist of an A-beta core first identified by Masters et al. (1985). Two types of morphologically distinct forms of amyloid pathology have been identified (Dickson, 1997), dense cored plaques and diffuse plaques. Dense cored plaques, also known as senile or neuritic plaques, have an amyloid beta (Aβ) core and are distinguished by having degrading neuritic projections (Armstrong et al., 1986) and activated microglia and reactive astrocytes around the periphery (Selkoe, 1994). Neuritic plaques are specific to AD dementia and are required for a definitive diagnosis (see for example Braak & Braak, 1991). However it is important to note that senile plaques can also occur in the non-demented elderly (see Swerdlow, 2006). Senile plaques have been reported in the limbic system (dentate gyrus, CA1, CA3, CA4, enthorinal cortex, subiculum and amygdale), neocortex and several subcortical nuclei. Diffuse plaques do not have associated neuronal degeneration or reactive glia and occur in a variety of neurological diseases and are widely abundant in all cortical layers of the AD brain (Tomlinson et al., 1970: In Hart & Semple, 1990).

1.3.2 Amyloid Precursor Protein (APP)

Aβ is a 40-43 amino acid peptide which is produced from proteolytic cleavage from the amyloid precusor protein (APP). Three pathways, α, β and γ- secretase, are involved in the processing of APP. The gamma and beta pathways are both amyloidogenic, the alpha pathway is not. The α-pathway involves cleavage within the Aβ domain, precluding the formation of amyloid (Haass & Selkoe, 1993) and resulting in the production of a soluble amino terminal (N-terminal) fragment (sAPP

α) and an 83 amino acid, carboxyl-terminal fragment (C83). γ- secretase cleavage of the C83 fragment produces a P3 peptide found in diffuse plaques (Nunan & Small, 2000). Cleavage of APP by β-secretase takes place between amino acid residues 671 and 672 on the N-terminus of the Aß domain to produce a 99 amino acid, C-terminal fragment and another soluble protein (sAPPβ; Nunan & Small, 2000). γ- secretase cleavage of the C99 fragment at residues 711 and 713 produces $Aß_{1-40}$ and $Aß_{1-42}$ respectively (Selkoe, 2001). Probably the most influential theory of AD is the Amyloid Cascade Hypothesis (Selkoe, 1994). This theory proposes that the increase in the deposition of Aβ is the primary cause of the disease and its progression (including neurofibrillary tangle formation) and is the result of an imbalance between the production and clearance of Aβ. The amyloid cascade hypothesis has recently been revised, and the focus has been place upon the synaptotoxic properties of oligomeric Aβ as opposed to the fibrillar form found in neuritic plaques (see for example Hardy & Selkoe 2002; Tanzi 2005; Cole & Frautschy 2006).

1.3.3 Neurofibrillary tangles NFTs

Another type of lesion considered to be a cardinal feature of AD is neurofibillary tangles (NFTs). These lesions are composites of hyperphosphorylated tau protein and are found in the form of straight or paired helical filaments (Yen et al., 1987; Kosik et al., 1988). Hyperphosphorylation disrupts the role of the tau protein in cellular functions such as fast axonal transport (Goedert, 1993). Like diffuse plaques, NFTs can been found in the hippocampus and subiculum of the healthy aging brain (Hart & Semple, 1990). In the AD brain however, NFTs advance rapidly in these areas and are also found in the cortex (See Braak & Braak, 1991). A

major criticism of a 'Tauist' view of AD is that there is no clear genetic link between NFT's and the disease. However, NFTs correlate better with the severity index of AD than amyloid plaques (Arriagada et al., 1992).

1.3.4 Neuronal loss

The precise mechanisms of how amyloid plaques or NFTs contribute to neuronal death are unclear. Nevertheless in the AD brain, neuronal loss and cortical atrophy affects the neocortex and hippocampus formation (Hyman et al., 1984). The CA1 suffers extensive loss (Van Hoesen & Hyman, 1990) and this decrement correlates with the progression of disease severity (Bobinski et al., 1998). Braak and Braak (1991) argue that in the normal aging brain a loss is seen in the CA4 region, whilst AD patients demonstrate neuronal death in the CA1 region and the enthorinal cortex.

Neuronal loss has also been demonstrated in the Nucleus Basalis of Meynert (see Tagliavini & Pilleri, 1983), olfactory nerve and amygdala (Hart & Semple, 1990). More recently there is evidence to suggest that there is greater medial temporal cortex atrophy in AD cases caused by APP mutations, and greater frontotemporal atrophy in AD patients linked to the presenilin 1 (PS1) genetic mutation as compared to sporadic cases (Gregory et al., 2006).

1.4 Alzheimer Disease Aetiology

Ninety percent of all AD cases are sporadic; the remaining cases are believed to have a genetic cause (Vas et al., 2001). In general, it is accepted that both forms of

the disease, familial or sporadic, share the same neuropathological and clinical features (but see Blennow et al., 2006, for an alternative position).

Studies of Down Syndrome patients and AD related family studies provide strong evidence that there is a genetic link in the cause of AD in the form of plaque deposition (see, for example, Lemere et al., 1996; Mullan et al., 1992). A caveat to this suggestion is that there is a poor correlation between plaque deposition and behavioural deficits. This discrepancy however, may be resolved with the consideration of Aβ oligomers, which may play a role in the cognitive changes presented during the early stages of the disease (e.g., correlation between soluble Aß and memory impairment; Lesné et al., 2006).

After age, genetics is considered to be the second biggest risk factor in AD (Hock and Lamb, 2001). Five percent of cases are linked to an autosomal dominant trait associated with the mutation of three genes that code for APP on chromosome 21 (however see Swerdlow, 2006). The Swedish mutation expressed in Tg2576 model evaluated in this thesis, occurs on the N-terminal side of the Aβ sequence and the β-secretase site (Mullan et al., 1992). The APP670/671 mutation alters the proportion of α and β-secretase processing (Haass et al., 1995) which in turn increases Aβ production (Citron et al., 1992; Citron et al., 1994).

Missense mutations of the presilin 'modifier' genes PSI and PSII have also been implicated in AD. The presenlins act as catalysts for the γ-secretase pathway. PS1 found on chromosome 14, results in the most aggressive form of AD with an onset as early as 29 years (see Duff et al., 1996; Citron et al., 1997; Holcomb et al., 1998).

1.5 Transgenic Models

Animal models of AD can be considered useful as they can clarify the mechanisms of pathogenesis, serve as a basis for research into putative therapies and allow for a systematic evaluation of behavioural deficits. Past rodent models of AD have included lesion and pharmacological approaches as well as in-vivo infusion of A-β into rats' brains. More recently the use of transgenic animals, in particular mice, has become the predominate method of modelling this disease.

Transgenic animals have exogenous DNA integrated into their germline (Gordon & Ruddle, 1981). These models allow the pathology and behavioural impairments associated with the expression of the transgene to be evaluated in a longitudinal manner over the life of the animal (Janus & Westaway, 2001). The majority of transgenic animals express a form of the APP mutation. Other strains incorporate 'modifier' genes associated with an increased risk of developing AD such as PS1 and PS11 mutations. Tau models and APP/ Tau hybrid models have also recently been generated (for comprehensive reviews, see Higgins & Jacobsen, 2003; Hock & Lamb, 2001; Kobayashi & Chen; 2005 McGowan et al., 2006; Spires & Hyman, 2005).

Although an extensive review of the transgenic literature has not been presented in this introduction it is important to mention a major caveat with APP models of AD. The biggest criticism of APP models is that only the APP23 strain develops neuronal cell loss. This can be attributed to a number of potential reasons. For example, there are subtle biochemical differences between human and transgenic plaques. Human plaques are more robust and contain more proteins and lipids, and this may alter their toxicity. Furthermore the lifetime of plaques differ between

species. Finally, overexpression of the transgene in mice increases the production of soluble APP ectodomain, which may be a neuroprotectant (Mucke et al., 2004).

Caution should be taken when drawing comparisons across transgenic models. Each model differs in mutation, background strain and promoter variation, and use of cDNA. Methodological variations also emerge in the research conducted by different laboratories. Although a detailed analysis of all transgenic models to date, has not been provided in this introduction for the reader to draw their own comparisons, any discrepancies with regards to the findings reported in this thesis are highlighted in the appropriate chapters. The next section focuses on the critical experimental findings from the Tg2576 mouse model which is one of the more popular lines used in the research.

1.5.1 The Tg2576 Mouse Model

The Tg2576 mouse carries the ($HuAPP_{695}SWE$)2576 mutation in a hybrid background strain of C57Bl/6j with SJL and exhibits a 5 to 6 fold increase in APP expression compared to littermate controls (Hasio et al., 1996). These animals display the presence of soluble Aβ from birth and the deposition of diffuse and dense cored plaques from 6 to 8 months of age (Westerman et al., 2002; Kawarabayashi et al., 2001). Plaques are observed in the hippocampus, frontal, temporal, and entorhinal cortex and amygdala (Wen et al., 2002). Hyperphosphororylated tau in association with dystrophic neuritis and reactive astrocytes and activated microglial cells in the surrounding areas of amyloid plaques have also been reported in Tg2576 mice (Hsiao et al., 1996; Irizarry et al., 1997a; Frautschy et al., 1998; Tomidokoro et al., 2001; Wegiel et al., 2001).

A number of neurological screens have been conducted to test the motoric function of these animals. Holcomb et al. (1998) demonstrated that 3-month old mice were not impaired in the righting reflex, the preyer reflex, the coat hanger test and the forelimb place response test. Chapman et al. (1999) tested a smaller sample at 16 to 18 months of age and did not report any deficits in comparison to littermate controls in a neurological screen. King et al. (1999) reported a balance deficit at 3 months of age, however this impairment was not apparent in 9-month-old animals and is therefore indicative of a sampling error. King and Arendash (2002) also reported a deficit in the balance beam task at 14 months of age. This is, however, one of the more difficult neurological screen tasks. The same animals displayed normal motoric function in a watermaze task, suggesting that any age dependent deficit in motoric function may only be subtle.

The assessment of learning and memory in Tg2576 mice has been predominately based on spatial memory tests, such as the Morris watermaze, Y-maze alternation and Forced-Choice Alternation in the T-Maze. Results from watermaze tasks have generated rather mixed results (see Hasio et al., 1996; Westerman et al., 2002; and King et al., 1999; 2002). A careful analysis of the methodologies adopted in these studies suggests, however, that the discrepancies can be attributed to factors associated with the experimental design as different training procedures and scoring techniques were used. Furthermore, Whishaw et al. (1996) and Frick et al. (2000) have suggested that the watermaze task is not the most appropriate test to be used with mice due to their fear response and floating behaviour. Further discrepancies have been found with regards to the Y-maze alternation task. Hasio et al (1996) reported a deficit in 10-month old mice, in contrast King et al. (1999) and King and Arendash (2002) reported normal alternation in 9- and 14-month old Tg2576 mice.

Different scoring techniques were adopted and the size of the maze varied - all of which may have been factors that contributed to this discrepancy.

Greater consistency has been found in the results obtained form the T-maze Forced-Choice Alternation task (FCA). Chapman et al. (1999) reported a deficit in 10-month old Tg2576 mice although performance was comparable to wild type mice at 2-months of age. Furthermore, the deficit in the old Tg2576 mice correlated with an impairment in an in vivo measure of synaptic plasticity, long-term potentiation. Similarly, Corcoran et al. (2002) reported a deficit in FCA in aged animals at 14-16 months, but this impairment was absent in 2-4 month old mice. Alternative tasks that index spatial memory in Tg2576 mice include the Plus Maze Reference task (Middei et al., 2004) the Circular platform task (Pompl et al., 1999) and the detection of item displacement in object recognition (Ognibene et al., 2005; Meddi et al., 2005). The findings of these studies will be discussed in depth in the appropriate experimental chapters.

Cell recording studies have also provided evidence supporting a deficit in spatial memory in Tg2576 mice. Thus, Cacucci, Chapman and O'Keefe (Society for Neuroscience Abstracts, 2005) reported that aged Tg2576 mice (14 – 17 months) showed significantly lower spatial selectivity in place cell activation in familiar environments and delayed formation of compact and stable place fields in novel environments, relative to age matched wild type mice. Tg2576 mice demonstrating impaired spatial representation in hippocampal place cells also displayed poorer performance in a delayed T-maze alternation task. Place activation and T-maze performance was comparable to wild type mice in younger transgenic animals (3-5 months) and suggests that the impairments in place cell activity and spatial memory were related to the development of amyloid pathology.

1.6 Spatial Navigation

Given the extensive evidence of APP induced impairments in spatial memory, the next section will provide a brief literature review of theories of animal navigation and consider the nature of the strategies that animals may use to navigate. This may provide important theoretical insights into the nature of the anatomical and psychological deficits in AD mice. Specific consideration will be given to O'Keefe and Nadel's (1978) Cognitive Map theory and Poucet's (1993) topographic versus metric theory of navigation. These theories of navigation will then be discussed in the context of the spatial memory deficits displayed by patients with AD. This section will start with a discussion of the neurobiological and anatomical substrates of navigation.

1.6.1 Neurobiology

Hippocampal neurons fire in characteristic ways as the animal navigates around an environment. Some cells fire when the animal is in a specific location, and are referred to as 'place cells' (O'Keefe & Nadel 1978). Other cells in the subicular cortices appear to encode the position of the animals head, and are referred to as 'head direction' cells (Rank et al., as cited in Best et al., 2001). Cells in the entorhinal cortex, which provides a major projection pathway for sensory information into the hippocampus from the cortex, display a grid like pattern of firing (Hafting et al., 2005). These grid cells are thought to index the position, speed and direction of an animal's movement. When taken together, these electrophysioloical correlates of navigation suggest that the hippocampus and closely related cortices play an important role in memory processes supporting spatial navigation. The distinct firing

characteristics of cells in different regions of the temporal lobe reinforces the suggestion, that rodents may use multiple representational processes to encode information about the external and internal environments for the purposes of navigation.

1.6 Spatial Navigation: Neuroantamomy

This section considers the contribution of distinct anatomical regions to spatial memory and how this information might inform our understanding of the pathology in Tg2576 mice.

1.7.1 The role of the hippocampus in navigation: place memory

Place learning requires the use of distal cues to guide navigation. Animals navigate in accordance with the learned relationship between prominent landmarks. Historically, mazes have been used to interrogate the neurobiological mechanisms of learning in rodents (O'Keefe & Nadel, 1978). One of the most popular procedures has been the Morris watermaze task (Morris, 1981). The Morris water maze reference memory task requires the animal to navigate to a hidden platform submerged a few millimetres below the surface of a circular pool filled with opaque water. The animal is released from different start locations, but the platform and distal cues remain in a consistent relationship throughout training. Morris (1981) demonstrated that normal rats locate the platform more quickly with repeated exposure to the environment. In contrast rats with hippocampal lesions do not demonstrate this decrease in escape latency. Indeed, Morris et al. (1982) reported that lesioned rats either swam randomly

or demonstrated a thigmotaxic swimming strategy (i.e., the rats swam around the edge of the pool).

Rats with hippocampal lesions are also impaired on appetitive radial maze tasks (Olton, Becker & Handelman, 1979). During the working memory task all eight arms of the maze are baited with food and the rat can move freely around the maze to consume the rewards. It must however avoid arms that have previously been visited. Odour cues are controlled for, and distal visual cues are provided to guide navigation. Olton et al. (1979) found that hippocampal rats made more errors, visiting previously entered arms. Jarrard (1983) argued that this pattern of results reflected impaired working memory. The forced-choice alternation task in the T-Maze is also considered a spatial working memory task that is highly sensitive to rodent hippocampal dysfunction (e.g., Bannerman et al., 1999). Each trial consists of two phases. During the first phase the rat is forced to run down a particular arm, at the end of which is a food reward. During the subsequent test phase the rodent must enter the alternate arm to receive the food reward.

Although it is widely accepted that the hippocampus contributes to spatial memory, recent evidence has highlighted functional sub-division within this system. Potvin et al. (2006) argue that it is important to control for dentate gyrus and subiculum damage in hippocampal lesions. They raise the point that if the dentate gyrus is damaged then fewer inputs reach the hippocampus irrespective of the intended lesion site. They also found that whilst dorsal hippocampal damage led to a deficit in place learning in the eight arm radial maze, a deficit in a non-matching-to-place procedure in the T-Maze was only observed in hippocampal lesioned animals with substantial damage to the dorsal subiculum. This latter finding is consistent with the work of Jarrard (1989) who argued that hippocampal damage alone resulted in

more subtle effects on memory compared to when neighbouring structures were also damaged. In line with this argument, Allen et al. (2004) found that the extent of impairment in the intra maze version of the radial arm maze task was dependent on considerable subiculum damage in addition to an excitotoxic hippocampal lesion. In the distal version of the task the deficit was observed in hippocampal animals alone.

A controversy exists in the hippocampal literature with regard to the functional dissociation between dorsal and ventral areas. Dorsal lesions, but not ventral, have been demonstrated to disrupt spatial ability in the water maze (Bannerman et al., 1999, 2002) and T-maze (see Bannerman et al., 1999, 2002, 2003). The ventral hippocampus is purported to be involved with contextual fear conditioning (Rogers, Hunsaker & Kesner, 2006). However, it has been reported that ventral hippocampal excitotoxic lesions lead to spatial deficits in the water maze (see de Hoz et al., 2003; Ferbinteanu & Mcdonald, 2000; Ferbinteanu et al., 2003). This tendency to assign a spatial memory role to the dorsal area of the hippocampus is also seen in the place cell literature (Jung et al., 1994). Place cells have however been found in the ventral hippocampus (Poucet, 1994), but they are fewer in number and the place fields of these cells have a lower spatial specificity in comparison to dorsal place cells (Jung et al., 1994).

1.7.2 The role of the hippocampus in dishabituation of exploratory activity

One of the methods that has been used to investigate the representational structures underlying spatial memory is an object exploration paradigm that exploits the tendency of rats to explore novel or unexpected features in the environment. Thinus-Blanc et al. (1996) found that compared to controls, C57BL/6 mice with dorsal hippocampal lesions did not show increased exploration of objects (i.e., they

did not show dishabituation of exploratory activity) following the spatial re-arrangement of a familiar array of objects. The spatial manipulation incorporated in this task involved a change in the absolute location of an object and a consequent change to the geometric properties of the array, that is to say when the object was moved to a novel location previously unoccupied during the sample trial. It also incorporated a relative shift in object location, where two items were transposed (i.e., switched position), thus changing the spatial relationship between the objects. The fundamental importance of distinguishing between an absolute and a relative change to the topological rearrangement of familiar objects is highlighted in Chapter 4. These results support the view that the hippocampus contributes to the formation of a representation of the spatial layout of objects (or landmarks) in an arena. In contrast hippocampal lesions do not disrupt object novelty exploration in rats (cf. Mumby, 2001).

1.7.3 The neural basis of path integration: the role of the Hippocampus in Path Integration

Although there is wide ranging evidence supporting the role of the hippocampus in memory for spatial information, recent evidence suggests that the structure may contribute to multiple aspects of spatial information processing. In this section I will examine the neural contributions of distinct anatomical regions to navigation by path integration. In contrast to place learning where the relationship between numerous landmarks must be taken into account, route leaning, or vector formation, can be inferred from the relationship between the animal and a single cue. Hippocampal lesioned rats are able to navigate from fixed start locations to a goal.

They cannot, however, combine vector information to instruct navigation from novel start locations (Eichenbaum et al., 1990). This provides compelling evidence that hippocampal integrity is needed for place learning but not route learning, and consequently supports the fundamental premise of O'Keefe and Nadel's (1978) cognitive map theory. In contrast, Whishaw and Jarrard (1995) found that fimbria-fornix lesioned rats are able to demonstrate place learning. An explanation of this finding was provided by Jacobs and Schenk (2003) based on the principles offered by the cognitive mapping account. These authors argued that the lesioned rats in the Whishaw and Jarrard (1995) study had an intact representation of the proximal environment (what they refer to as a Sketch Map) and were thus able to navigate on the basis of local cues.

The ability of an animal to navigate back to its nest (homing behaviour) has been used to index path integration. Hippocampal or fornix lesions disrupt the homing behaviour of rats (Whishaw & Maaswinkle, 1998; Maaswinkle et al., 1999; Whishaw & Gorny, 1999) and mice (Gorny et al., 2002). Whishaw (1998) reasoned that the hippocampus had a role in calibrating spatial cues in a system based on path integration. Alyan and McNaughton (1999) countered this argument by demonstrating that under certain conditions hippocampal lesioned rats do not present a deficit in returning to nest/ start location using path integration.

1.7. 4 Parietal lobes

Although traditionally the hippocampus has been considered the major brain region supporting spatial information processing, other areas such as the parietal lobes

and the entorhinal cortex also contribute to this form of learning (e.g., Save & Poucet, 2000a; Jarrard et al., 1984).

Kolb (1990a) demonstrated that rats with parietal lesions showed poorer trajectory learning (the ability to learn to navigate from a fixed start location to a fixed goal location) than control animals, despite extensive training or pre-training. Save and Moghaddam (1996) trained parietal lesioned rats to navigate from a fixed start location in darkness, forcing their navigation to be based on self-motion cues. Post-surgery, trained rats were impaired in acquisition of this task. These studies suggest that the parietal cortex has a role in path integration. This idea also finds support from more recent experiments.

For example, Save, Guazzelli and Poucet (2001) used a homing task (again requiring the animal to return to a nest area), of varying complexity to index the effect of parietal and hippocampal lesions on path integration. Path integration was disrupted by parietal damage if the rats were required to make a complex journey. In contrast hippocampal lesioned rats were impaired on the basic elements of the task. The authors argued that this was indicative of the hippocampus being involved in general processes of spatial learning while the parietal cortex had a specific role in path integration.

Save and Poucet (2000a) found that parietal lesioned rats displayed an impairment in the water maze if they had to navigate using proximal cues, these animals could however navigate using distal landmarks. Hippocampal rats in contrast displayed a global deficit and could not navigate using either cue source. They argued that these results demonstrated that the parietal cortex was required in situations where visual and ideothetic cue associations were important, but was not needed for navigational processes that relied upon an allocentric representation of space.

This body of work by Save and colleagues suggests that rodent navigation comprises subsidiary functions, such as navigation based on the interrelation between proximal cues and ideothetic information. Furthermore, such subsystems complement a global spatial memory function based in the hippocampus (but see Rogers & Kesner, 2006). This idea is discussed in greater detail with reference to Save et al's (2005) elaboration on the parallel map theory (Jacobs & Schenck, 2003).

More recently Rogers and Kesner (2006) reported that the parietal cortex was required for subject based navigation (egocentric based learning), whilst the hippocampus was involved in allocentric processing. They used a modified Hebb-Williams maze to demonstrate that rats with dorsal hippocampal lesions were impaired in allocentric maze learning, while lesions to the parietal cortex impaired the acquisition of egocentric maze learning. During retention, the hippocampal lesions demonstrated transient impairments, whilst the parietal animals were significantly impaired on both forms of the task. Rogers and Kesner (2006) argued that their results suggest that the hippocampus and parietal lobes process information in parallel during the acquisition of spatial learning, but the long term retention of information requires the partial cortex. They go on to suggest that the hippocampus is required for retrieval of this information but does not necessarily act as the storage site.

In addition Save et al. (1992) demonstrated that rats with parietal lesions failed to show dishabituation of exploratory activity to the topographical re-arrangement of familiar objects. The test incorporated both an absolute change and a relative relocation of the objects. In summary, the aforementioned literature suggests that the parietal lobes serve a function in processing proximal environmental cues and path integration in rodents.

1.7.5 Entorhinal cortex

Early maze studies found that entorhinal cortex lesions resulted in deficits in the water maze (Schenk & Morris 1985), radial arm maze (Olton, Walker & Gage 1978; Jarrard et al., 1984), and the T-maze non-matching to place task with bilateral (Ramirez & Stein, 1984) and unilateral lesions (Karpiak, 1983; Reeves & Smith, 1987). However recent experiments that use refined chemical lesion techniques have found that enthorinal cortex lesions do not lead to deficits in the radial arm maze (Bouffard & Jarrard, 1998) or the reference task in the water maze (Hagan et al., 1992; Pouzet et al., 1999; Bannerman et al., 2001: Oswald et al., 2003). However, Steffenach et al. (2005) have demonstrated that dorsolateral lesions, but not ventromedial lesions, disrupt rat performance in the Morris water maze task, suggesting that the site of the enthorinal cortex lesion is important. The authors suggest that the dorsolateral band of the enthorinal cortex may play a role in spatial memory due to its connection with the dorsal hippocampus.

Rats with enthorinal cortex lesions exhibit deficits in an appetitive alternation task in the T-maze. Bannerman et al. (1999) argued that the enthorinal cortex plays a role in processing recent goal histories but is not critical to hippocampal function in the reference water maze task. Previous findings that implicate a role of the enthorinal cortex in place learning may have potentially been confounded due to the non-selectivity of the lesion techniques adopted in the earlier experiments. However with regards to the recent finding of grid cells (Hafting et al., 2005), the enthorinal cortex may have a potential role in path integration, although this has not been investigated using lesion approaches.

1.7.6 Frontal Cortex

Medial frontal cortex lesions in rats have been shown to impair delayed alternation performance in the radial arm maze, spatial navigation and spatial problem solving (see Kolb, 1984, 1990b). These lesioned animals do, however, show a normal tendency to explore and dishabituate to the topographical rearrangement of a familiar object array (Poucet, 1989). Poucet (1990) defines the frontal lobe impairment in terms of a working memory deficit, the inability to process spatial information needed to plan navigation. It represents a deficit in organising the correct spatial behavioural response, as opposed to a deficit in processing spatial information per se.

In contrast, more recent work using excitotoxic techniques has demonstrated that prelimbic cortex lesions in rats have a limited impact on the formation of spatial representations, the authors argued that the behavioural deficits were more likely to reflect a problem in attention and behavioural flexibility (Delatour & Gisquet-Verrier, 1996; Delatour & Gisquet-Verrier, 2000). Furthermore, rats with medial prefrontal cortex lesions were not impaired in an object location task (Ennaceur, Neave, & Aggleton, 1997). These studies suggest that although the work of Kolb implicated a role of the prefrontal cortex in spatial memory, this conclusion may have been wrongly drawn as a result of using none fibre sparing lesions.

1.8 Psychological theories of Spatial Navigation

This section will evaluate theories of animal navigation, and consider how 'normal' animals navigate. The influences of cognitive and ideothetic cues on the neural foundations of spatial navigation have been explored in the above sections.

Many theories provide an integrated account of how these strategies govern rodent navigation, but most postulate that either ideothetic or visual information dominates control of behaviour. The discussion of navigational theories will start with cognitive accounts that specify a primary role of visual information. In evaluating all of the accounts it is important to reflect on the evolutionary nature of the research. As our understanding of the neural underpinnings of navigation advances, theories evolve in line with these discoveries. It is easy to criticise early theories for their lack of comprehensiveness. This criticism should not obscure the fundamental importance of pioneering work encapsulated by O'Keefe and Nadel's (1978) cognitive map theory. The following section is not intended to be a comprehensive account of navigational theories or hippocampal function. The discussion has been tailored to focus on navigational strategies that may inform our understanding of navigational/spatial memory deficits displayed by patients with AD.

1.8.1 Cognitive accounts: Cognitive maps

The cognitive map theory (O'Keefe & Nadel, 1978) describes two systems of navigation. A rudimentary strategy based on route learning known as the taxon system and a mapping strategy based on place learning governed by the locale system. The taxon system is an inflexible form of learning based on goal driven routes, novel routes cannot be devised under this system. The taxon system is independent of the hippocampus, whereas the locale system is heavily dependent upon the integrity of the hippocampus and related cortices.

The basic premise of the locale system is that the hippocampus supports the formation of a cognitive map. The environment is represented by a number of

hippocampal place cells; each cell represents a specific location. Feedback from self-generated movement and head direction cues counteract egocentric changes to the angle and distances of objects relative to an animal as it explores an environment. The configurations of cells form an allocentric representation of space. As an animal moves around the environment the internal navigation system shifts the focus of excitement to different place representations. The defining feature of this strategy is that it allows the animal to map novel routes.

The locale system is also interested in novelty detection. Mismatch units fire in response to incongruent features of a previously habituated environment. The theory predicts that animals should be sensitive to topological re-arrangement of familiar objects, a premise evaluated in Chapters 3 and 4.

Support for the distinction between the locale and taxon system is found in the hippocampal lesion literature. Hippocampal lesions have been found to disrupt place learning but leave route leaning intact (Morris et al., 1986; O'Keefe & Conway, 1980; Packard & McGaugh, 1996). Manipulating place fields so that they fire incongruently with distal cues, effectively stimulating place cell instability, causes a similar impairment in place learning (Lenck-Santini et al., 2002). Taken together these findings suggest that the hippocampus is fundamental to place learning and that place cell congruency is an integral feature of a well functioning map. The study by Whishaw and Jarrard et al. (1995), however, demonstrates that place learning in hippocampal lesioned rats can occur under specific training conditions. This finding undermines the notion that hippocampal integrity is crucial to the mapping system as defined by O'Keefe and Nadel (1978). However, Jacobs and Schenk (2003) counter this argument by introducing the notion of parallel maps, as will be discussed shortly.

Compelling evidence that place cells form the basic units of an allocentric representation of space (that is a framework that encapsulates spatial relationships among landmarks independent of the subjects position), is provided by the numerous demonstrations that manipulation of visual cues can lead to changes in place field activity (e.g., O'Keefe & Conway, 1978). Furthermore, there are studies that demonstrate the coupling of place fields and navigational behaviour. O'Keefe and Speakman (1987) report that arm choice in a radial arm maze appetitive task, where reward was signalled by the position of distal cues, coincided with stable place representations when the distal cues were removed. During these memory trials incorrect arm entries corresponded with place fields, providing direct evidence that animals navigate in accordance with place field placement. In continuation of this argument, Lenck-Santini et al. (2001) demonstrated that changing the location of a distal cue after familiarization with task parameters based on the original location of that cue, caused erroneous field placement and this coincided with impaired spatial behaviour.

There is also molecular evidence that suggests that place cells underpin navigation. NMDA-receptors are involved in the mediation of LTP, the hypothetical neural basis of learning. NMDA receptor knockout mice show a navigational deficit in the water maze (Tsien et al., 1996; Wilson & Tonegawa, 1997). A counter to this argument was provided by Bannerman et al. (1995) who showed that spatial pre-training can attenuate the navigation deficits following NMDA receptor antagonism. However, Jacobs and Schenk (2003) suggest that the pre-training adopted in the Bannerman et al. (1995) study may have allowed CA1 independent residual learning to have taken place. A further discussion of this issue is produced in the evaluation of the cognitive map theory.

The Cognitive Map theory has also received criticism because its focus purely on a spatial role cannot explain certain associative leaning phenomena, such as overshadowing or blocking that have been demonstrated in the spatial domain (c.ref Cheng & Newcombe, 2005). Hippocampal rats also show impairments in spatially independent tasks such as negative patterning discrimination, acquisition and retention of transverse patterning discrimination and a configural task in the T-maze (Sutherland and Rudy, 1991). Assertions have emerged that suggest the hippocampus is involved in dealing with more general (relational) information of which spatial information is just one exampie (Eichenbaum et al., 1999; but see Kumaran & Maguire, 2005 for an alternative view). Indeed, place cells demonstrate tuning to several non-spatial properties of performance on a task (Wood et al., 1997). Recent studies have highlighted the important contribution of other neuroanatomical structures to both place cell functioning and normal navigation. The original cognitive mapping theory does not embrace the importance of other structures such as the entorhinal cortex or parietal lobe. The theory also does not provide a detailed account of how ideothetic cues contribute to hippocampal place cell functioning. Subsequent theories have therefore elaborated upon the concepts embodied by O'Keefe and Nadel's (1978) model.

1.8.2 Dual Network Hierarchical Model

Exploration is a key feature of cognitive mapping. Empirically, exploration of an environment has been demonstrated to be significant in the calculation of both ideothetic and allocentric navigation (Whishaw & Brooks, 1999). Poucet's (1993) Dual Network Model is a cognitive account of rodent navigation and is based on the

premise that specified areas of space receive heightened exploration. The dual network hierarchical model also contests that animals do not solely use metric information to compute spatial positions. Poucet (1993) argued that the properties of spatial information could be either metric or topological. In this model, topology represents the connectivity and overall arrangement of the environment, and the 'grain' of this representation is enhanced by metric (vector) information. Metric information is only available at key locations in space (location dependent reference frames).

Another key feature of this cognitive account is that egocentric representations are transformed to allocentric representation of space through a number of transitional steps. A local view is initially formed. This is a spatial percept interlinked through rotational movements at a given location and is based on exploration. Unlike McNaughton's accounts (McNaughton et al., 2006; see later discussion of path integration models), local views do not orientate navigation but are the building blocks of place representations. A place representation is the abstract product of amalgamated local views generated from a specific location. Place representations are, however, independent of location, and can be activated before the animal perceives a local view (unlike the path integration models e.g., McNaughton et al., 2006). This feature increases the flexibility of the model.

The next phase of the egocentric-allocentric transformation is the formation of local charts. These are constructed from a number of place representations that share common elements. They comprise both metric (vector) and topological information which are integrated through exploration. Long distance orientation relies on the spatial representation between charts. Poucet (1993) suggests that local charts are linked through the formation of 'location dependent reference frames'. These linking

points are generated through heightened exploration at that location. They provide the animal with a sense of orientation from which metric information can be used to generate novel vectors. To navigate, animals move from one chart to the next, a similar notion to cognitive mapping. The final stage is based on the controversial notion that after extensive exploration location independent reference frames, which are common to all places, are generated. Familiar environments are given an overall reference of direction from which common vectors are surmised. This idea affords the theory great flexibility as long distance orientation of the map becomes independent of the animals location.

Poucet (1993) argues that the metric information is computed in the parietal cortex while the hippocampus has a more extensive role. The hippocampus locates the current environment, stores local views, which in turn become place representations. Exploration leads to an increasing amount of information entering the hippocampus, and topological information based on relationships of proximity and connectivity is incorporated into the spatial representation via this structure.

Buzsáki (2005) touches upon the first premise of Poucet's (1993) account, the idea of 'privileged' exploration sites. This computation theory refers to landmark junctions. Areas of space, that through exploration, alter 1-dimensional place cells with unidirectional firing properties, to omnidirectional firing cells. This transformation forms the foundation of map-based navigation. The second concept of Poucet's (1993) model, the distinction between topological and metric information, is not elaborated upon in future work. A greater emphasis has been attributed to the role of the parietal cortex in processing egocentric metric based ideothetic information, and the potential function of the hippocampus in converting this to an allocentric representation (see Save & Poucet 2000b). Similar ideas of ego-to-allocentric

processing and the contribution of the parietal lobes and hippocampus are expressed by Burgess (2002, also see Rogers & Kesner, 2006).

In a recent paper Poucet and colleagues (c.ref Save et al., 2005) support the parallel map theory of spatial navigation (Jacob & Schenk, 2003) by providing empirical evidence for a dissociation between control/ use of distal and proximal cues. This cognitive account shares some similarities with Poucet's (1993) theory. It stipulates that the hippocampus and associated structures integrate different types of information. Furthermore, the parallel map theory incorporates a fine-grained map based on proximal cues and a coarse map based system based on principles that are not specific to any one environment. Both theories postulate that the fine-grained mapping system comprised of egocentric information in its basic form, is built up into an allocentric representation of space that is independent of heading.

1.8.3 Parallel Map Theory

Jacobs and Schenk's (2003) parallel map theory suggests that the hippocampus integrates two different types of map, the sketch and the bearing map. The sketch map chunks together disconnected local views to form an allocentric representation of space where heading is irrelevant. Specifically the sketch map processes proximal cues (objects) within an animal's environment and these 'positional cues' are incorporated into a topographical map. The sketch map is a fine grained representation of space. Explicit relationships between objects and locations are learnt, the information in the sketch map cannot be generalized across different environments. Novel route acquisition can only occur when this representation is integrated with the bearing map. The bearing map uses directional cues such as

stimulus gradients (odour or electromagnetic gradients), ideothetic information, the global geometry of the environment and distal landmarks, to form a coarse grained representation of space. The features incorporated in this map can be generalised from one environment to the next, it can therefore guide navigation across long distances.

The parallel map theory (Jacob & Schenk, 2003) suggests that the medial septum uses the theta rhythm to calibrate self-movement. This in turn provides trajectory information. The dentate gyrus receives a sensory input from the entorhinal cortex, this information, added to the trajectory, creates a one-dimensional map, and these vectors intercept to form the two-dimensional bearing map. CA3 hippocampal cells combine the information from the medial septum, enthorinal cortex and dentate gyrus to localise the current position of the animal on the bearing map. CA1 hippocampal cells create the sketch map and localise this representation on the bearing map. This integrated map projects to the subiculum, where reference memory representations of integrated maps are stored.

The authors argue that the parallel mapping system receives great support from extant neurophysiological and behavioural studies. The distinction between different types of maps, bearing (distal), sketch (proximal) and the integrated map, finds support in Gothard's (1996) work that showed that there are distinct classes of hippocampal place cells sensitive to cue distance. Furthermore, the distinctions made between the roles of the septum and CA3 cells versus CA1 cells, can explain the results of Mizumori et al. (1989) who found that reversible septal inactivation disrupted CA3 activity but not CA1 place cell activity. This study would suggest that CA1 cell activity (the sketch map) is independent of septal and CA3 cell activity (the bearing map). The redundant nature of these maps enables the theory to explain certain discrepancies in the navigation literature, as will be discussed shortly.

Jacobs and Schenk (2003) suggest that the bearings and sketch maps can act independently. Therefore if one system is damaged, a residual function remains in the other map. This idea of residual functioning can explain numerous findings in the navigation and place cell literature. For example, Jacobs and Schenk (2003) argue that damage to either map leads to different navigational strategies. A bearing map impediment forces the animal to navigating in local loops (tight circular paths), while a sketch map impairment results in the animal searching the environment in transects, large and angular search paths. Damage to both maps leads to navigation by global loops, this is arguably similar to the thigmotaxis observed in hippocampal rats in the water maze (Morris, 1982).

The notion of residual maps can also account for the finding that rats with fibria-fornix lesions can demonstrate place learning (Whishaw & Jarrard, 1995). Jacobs and Schenk (2003) argue that these animals had intact sketch maps and as of such, the training procedure adopted in Whishaw and Jarrad's (1995) experiment allowed the animals to navigate on the basis of local cues. Another discrepancy in the hippocampal lesion literature, the finding that hippocampal lesions do not always disrupt path integration (Alyan & McNaughton, 1999), can also be accounted for by the parallel map theory. Jacobs and Schenk (2003) suggest that while hippocampal lesions would disrupt the integration of ideothetic information into the two-dimensional bearing map, Alyan and McNaughton's (1999) experiment allowed animals to navigate on the basis of one-dimensional trajectories.

Further discrepancies in the literature can also be accounted for in terms of residual strategies. Jacobs and Schenk (2003) argue that Bannerman et al's (1995) dissociation between NMDA-mediated LTP and spatial learning can be explained by reasoning that because NMDA acts upon CA1 cells, a bearing map function would

have been spared by this manipulation. The animals could therefore navigate on the basis of a residual bearing map formed during the pre-training stage of this study. Interestingly the parallel map theory also offers an explanation for the place cell behaviour observed in distal and proximal cue competition studies. Cues in the centre of a cylindrical chamber would be processed by the sketch map, and the dynamics of this environment would prevent the formation of a bearing map. The sketch maps formed could not therefore be consolidated into an integrated map. Jacobs and Schenk (2003) suggest that under these conditions, sketch maps would form continuously, and proximal cues would consequently demonstrate a low resistance to cue rotation (see Cressant et al., 1997,1999).

Jacobs and Schenck (2003) acknowledge the dangers associated with only finding empirical support for their theory in the work of others. In particular they express concern about the lack of lesion specificity and the low level of cue control in the behavioural studies. Save et al. (2005) do, however, elaborate upon the parallel map theory on the basis of their own empirical research. They demonstrate that the parietal cortex is associated with processing proximal cues, and consequently argue that this region also contributes to the formation of a sketch map. Save et al. (2005) also acknowledge a caveat with this theory, the fact that the hippocampal- parietal pathway is undefined.

Alternative cognitive accounts of animal navigation that do not rely on the concepts of map based or route based learning shall now be discussed.

1.8.4 Geometric models

Geometric models contest that the shape of the environment guides navigation. These theories stem from 'rotational error' experiments. Cheng (1986) postulates that animals store the shape of the environment in a geometric module, and this metric frame can have landmark features 'glued' onto it to aid navigation. Gallistel (1990a) argues that animals use modular matching to guide navigation, the shape of a previous encountered environment is stored in memory and the current environment is compared to this geometric module. As previously discussed O'Keefe and Nadel's (1978) cognitive map theory can be criticised for not being able to account for overshadowing effects in the spatial domain, Cheng and Newcombe (2005) argue that no such criticism is available in terms of geometry. However, a fundamental criticism is provide by Pearce et al. (2004), who demonstrated in a set of experiments conducted with rats in the water maze, that properties of local geometric cues as opposed to global geometry are critical in guiding rat navigation.

1.8.5 Path integration models

The next section discusses theories that posit ideothetic information as the primary influence in navigation. The basic notion of a path integration model is encapsulated in McNaughton et al's (1996) model. An internal representation of space is physically constructed in terms of a co-ordinate matrix. The principal source of information is generated from self-motion cues and head direction feedback as the animal moves through space. Each environment is represented by a chart. Associations between map co-ordinates and external stimuli are learnt, and these are

used to correct for the accumulated drift error that would occur if navigation was guided by internal co-ordinates alone. McNaughton specifies that the process primarily involves the hippocampus.

Redish and Touretzky (1996, 1997) conceptualise an environment in terms of reference frames. Only one reference frame can be active at any one time. Each reference frame comprises a place code that represents where an animal is in the environment and is dependent on a local view, and ideothetic cues in the form of path integration and head direction. This information is combined in the hippocampus. Behaviour is also influenced by a goal subsystem in the nucleus accumbens. This system receives location information from the hippocampus via the fornix. The hippocampus is thought to hold multiple reference frames. A reference-frame selection subsystem allows the animal to navigate through different environments. Redish and Touretzky (1997) posit that the path integration system re-sets each time the animal enters a familiar environment. Similar theories have been conceptualised in attractor neural networks.

Attractor neural networks consider memories as discrete sets of stable activity states known as attractors. Navigation models require a slight adaptation to allow for the continuous and smooth transition between states, a necessary requirement to assimilate the information generated through exploration of an environment (see Tsodyks, 1999). Samsonovich and McNaughton (1997) suggest that the CA3 is the location of multiple charts, two-dimensional continuous stimulus-dependent attractors akin to reference frames. The model suggests that the activity of a hippocampal place cell is dependent on the activation of cells with adjacent receptive fields and a directional signal from a path integration system. A defining feature of this model is that the role of visual information is secondary to ideothetic information. However

once location co-ordinates and visual information have been associated, visual information can be used to re-set the path integration register.

Etienne and Jeffery (2004) dispute the fundamental claim made by path integration models that ideothetic cues contribute primarily to spatial navigation and visual cues act as a secondary, complementary source. They cite evidence for this in studies that show that visual information dominates control of place and head direction cells (but see Knierm et al., 1998, for the counter argument). They also dispute the premise of attractor models that cells act as unified populations, as previously discussed place cells can be classified into distinct categories (see Gothard 1996).

McNaughton et al's (1996) emphasis on the hippocampus being the site of path integration has also been criticised. As previously noted, Save et al. (2001) provide evidence that the parietal cortex may play a role in computing ideothetic based information. O'Keefe and Burgess (2005) argue that the combination of visual and metric ideothetic cues is less likely to take place in the CA3 area as proposed by McNaughton and more likely to be computed by enthorinal grid cells because of their ability to encapsulate location and time. In reconciliation with the discovery of grid cells, McNaughton et al. (2006) entertain the idea of a relationship between grid and place cells. They argue that the primitive nature of grid cell organisation provides a code that allows the hippocampus to have an orthogonal firing pattern. They argue that this conceptualisation is however, limited by the lack of knowledge known about the mapping of the enthorinal cortex.

1.8.6 Navigation: A prerequisite of episodic memory?

Although AD patients can show impairments in navigation and spatial orientation, the most well documented deficit in patients is impaired autobiographical or episodic memory. In this next section I will consider the components of this type of memory. Eichenbaum (1999) argues that place cells encode both spatial and non-spatial information in clusters that denote the animals experience. Spatial processing is considered the by-product of hippocampal function. In contrast O'Keefe and Nadel (1978) argue that spatial information is the pre-requisite of temporal processing. The precise nature of episodic memory is difficult to assess in animals as they do not have the ability to verbally recollect. However, Eacott and Norman (2004) designed a task ran in the E-Maze which used a 'which' element to denote a temporal sequence. Animals were trained to habituate to an object presented in a specific location within a specific context. The experiment worked on the notion that the animals should show a preference for novelty. The test manipulation required the animal to 'recollect' the spatial location, associated with the particular non-habituated object, from previous exposure. The relationship between spatial and episodic memory is addressed in greater depth in Chapter 3.

Samsonovich and Ascoli (2005) propose a neural network model for spatial navigation that is a prerequisite of an episodic pathfinder role. The model works on the premise that there are corresponding CA1 and CA3 units. During exploration an associative process takes place through the occurrence of a sharp wave (high frequency oscillation). When a sharp wave fires the system pauses and this arbitrary point is noted as a potential goal location. CA3 cells that were recently active are reactivated along with the CA1 cells that are signalling the current location. These

CA1 and CA3 cells become associated. This process continues until a goal is reached, and CA3 units acquire modulated and weighted connections to the CA1 representation of the goal location. The model uses an algorithm with phase precession to explore several local moves from any given location. The animal selects the move that produces the greatest excitation of place cells associated with the goal.

Banquet et al. (2005) also describe a model that proposes that a navigational function of the hippocampus is a composite of episodic memory. Object location is computed via multiple sensory inputs in the parahippocampal cortices, while enthorinal place cells encode spatial but context independent maps. Similarly to path integration models, local-view and movement information are integrated to compute the subject's current location. Transition cells (units that encode the transitions between events) in the CA3-CA1 hippocampal region encode context dependent maps and their intermediary properties allows learning of temporal spatial sequences.

1.9 Spatial Navigation in Alzheimer patients

Historically the hippocampus has been the focus of the navigational debate. The hippocampus is one of the primary targets of pathology during the early stages of the disease (Braak & Braak, 1995). An association between the human hippocampus and spatial memory is evident from both lesion and fMRI studies (e.g., Burgess et al., 2002). One might predict therefore that impairments in spatial information processing and memory would be a component of the AD amnesia syndrome. Anecdotally evidence from carers reveals that AD patients often become disoriented in unfamiliar environments, or forget where they have put things (Monacelli et al., 2003). In order to evaluate spatial memory processes that are disrupted in cases of AD I shall draw on

the distinction, derived from the animal navigation literature, between path integration and map-based navigation processes.

1.9.1 Problems in path integration

Optic flow (the percieved pattern of motion in a visual scene caused by the relative motion between an observer and objects) has been linked to path integration as a visual indication of movement but its role is much less clear than the influence of feed back from vestibular proprioceptive sources as demonstrated in the human (Kearns et al., 2002; Telford et al., 1995) and rodent literature (Sharp et al., 1995; Blair and Sharp, 1996). Optic flow does nevertheless contribute to path integration. An increasing body of evidence suggests that optic flow is disrupted in Alzheimer patients, in that they don't perceive movement as well as controls, and this impairment is coupled with spatial disorientation - demonstrated in a variety of navigation task including the ability to recall information about the layout of a route and landmarks (see Tetewsky & Duffy 1999; O.Brien et al., 2001; Mapstone et al., 2003).

1.9.2 Ideotheic preservation

The notions of route versus place learning was evaluated by Kalová et al. (2005) in a real version and computer based simulation of the Morris water maze task. The study found that patients with early stage Alzheimer's disease had preserved ideothetic navigation but were impaired in landmark navigation. They argued that the patients had a deficit in allocentric orientation. However, it is important to note that

both task conditions were artificial and this may have obscured a true deficit in ideothetic navigation. Alternatively, it could demonstrate that ideothetic learning is preserved during the early stages of the disease.

1.9.3 Problems with place information

Alzheimer's disease disrupts the ability of patients to use landmarks to guide navigation. Monacelli et al. (2003) found that all the Alzheimer patients in their study showed a tendency to 'get lost' in that they made spatial orientation errors. They argued that this impairment did not represent a global memory problem. The deficit could be characterized by an inability to link familiar scenes to an overall allocentic representation of space. Similarly, Kavic et al. (2006) reported that Alzheimer patients diagnosed at the mild stage of the disease were unimpaired in route learning, the ability to walk from one location to next, and could recognise familiar landmarks, but they could not integrate the two forms of information to guide navigation. This study is particularly interesting as it is consistent with the navigation strategies posited in O'Keefe and Nadel's (1978) cognitive map theory.

1.9.4 Topographical misperception

Monacelli et al. (2003) identified that Alzheimer patients did not take into account the 'architectural geometry', the angles of the walls and ceilings, when navigating around an environment. They agued that this topographical misperception could be attributed to optic flow deficits, and parietal or parietotemporal damage – as this has been linked to dysfunction in ability to link multisesory cues and self motion

in humans (Peuskens et al., 2002) and rats (Save et al., 2002). It is important to note however, that the elderly controls in this study also displayed a similar tendency although it was not as marked.

1.9.5 Allocentric disorientation

Burgess et al. (2006) embellishes upon the work of previous studies such as Monacelli et al. (2003) and Kavic et al. (2006) by demonstrating that the topographical disorientation observed in one Alzheimer patient represented a specific deficit in allocentric orientation. That is the ability to locate landmarks relative to the environment as opposed to ones perceived view (egocentric processing). The Patient could identify scenes as familiar or unfamiliar, and could detect changes in an object if it was viewed from a familiar viewpoint. The patient could not identify locations if viewed from a shifted viewpoint. The spatial perception and mnemonic skills of the patient were normal for their age group. Burgess et al. (2006) argued that this deficit was probably due to hippocampal and retrosplenial cortex damage, implicating the circuitry between the hippocampus and head direction system. In contrast to Monacelli et al. (2003) they argue that the patients preserved object perception and spatio-motor performance was indicative of parietal cortex integrity. Caution however must be taken when interpreting the results of this study as Burgess et al. (2006) only examined one patient, the robustness of this finding can theretofore not be determined.

1.9.6 Alzheimer Spatial Disorientation: Is it a global problem?

Topographical disorientation (orientation and navigational problems and an inability to become familiar with an environment) has been considered to be the consequence of general mental decline (Passini et al., 1995; Aquirre et al., 2003). Pai and Jacobs (2004) argue that this hypothesis is inaccurate, as spatial impairments are often observed during the earlier stages of the disease and these patients often demonstrate mnemonic functioning comparable to age matched controls (c.ref Monacelli et al., 2003; Burgess et al., 2006). Passini et al. (1995) argue that navigational problems stem from executive function impairment. However, there is little support in the Alzheimer literature that spatial disorientation observed during the early stages of the disease is the result of poor planning. Kavic and Duffy (2003) demonstrate a greater 'attentional blink' in Alzheimer patients, but this finding is more appropriate to perceptual (optic flow) impairment as opposed to a cognitive explanation of the deficit.

1.9.7 Object-Place learning

Tasks of visuospatial paired associate learning can discriminate between Alzhiemer-related cognitive deficits and those generated by depression (Swainson et al., 2001), frontotemporal dementia and questionable dementia (Lee et al., 2003) and may therefore provide an accurate preclinical marker for Alzheimer-related cognitive decline (Blackwell et al., 2004). Interestingly a specific deficit in acquiring object-place associations is also observed in people with left or right hemispheric lesions of the hippocampus (Stepankova et al., 2004).

The following chapters present evidence that the APP Tg2576 mouse model of Alzheimer's disease possess a circumscribed impairment in specific aspects of spatial memory. These results suggest that impairments in amyloid processing contribute to spatial memory deficits observed in patients with AD.

1.10 Section Summary and aims of thesis

This chapter has addressed the nature of the spatial memory deficits observed in AD patients. In an effort to characterise the nature of visuospatial impairments in transgenic mice, concepts (e.g., the formation of allocentric maps, topological representations, egocentric metric information, and path integration) have been borrowed from the rodent navigation literature. Furthermore, lesion data have been discussed in an attempt to establish the anatomical regions that may be disrupted by the transgene pathology; with deficits in spatial memory being linked to hippocampal, enthorinal cortex and parietal lobe impairments. It would seem that AD patients and transgenic mice have a difficulty in processing allocentric space. Chapter 1 confirms that this deficit is present in our cohorts of animals. This thesis aimed to further our knowledge of the visuospatial deficit in Tg2576 mice. Chapter 3 introduces the use of the spontaneous object recognition paradigm which allowed us to systematically manipulate the 'what' 'where' and 'when' components of episodic memory. This in turn led to the adoption of an episodic-like memory task, where the relationship between spatial and episodic memory was evaluated. Chapter 4 investigates the ability of the Tg2576 mice to process topological and metric information.

Place versus Response learning in the Tg2576 mouse

The present set of experiments examined spatial and non-spatial maze learning in adult Tg2576 mice. Transgenic mice were impaired in acquisition of a T-maze forced-choice alternation task but were able to acquire a simple room discrimination and contextual conditional left-right discrimination in a T-maze. A deficit in allocentric processing was demonstrated by Tg2576 performance in a reference memory task in a plus maze. Although Tg2576 mice were able to learn a response strategy at a faster rate than the controls, they were significantly impaired in acquisition of a place task. These results are consistent with the hypothesis that, although Tg2576 mice are able to process extramaze cues, they are impaired in forming allocentric representations of the environment. Alternative interpretations of the data are, however, discussed in the relevant discussion sections of this chapter.

2.1 Experiment 1: Simple room discrimination

2.1.1 Introduction

Previous work with Tg2576 mice has shown that these mutants display age-dependent deposition of amyloid plaques and impairments in learning on a range of spatial navigation tasks (e.g., the watermaze, T-maze and radial-arm maze, Hsiao et al., 1996; Holcomb et al., 1998; Chapman et al., 1999; Arendash et al., 2001; Barnes et al., 2004).

In order to establish that the spatial navigation impairments in Tg2576 mice do not reflect a theoretically uninteresting perceptual deficit, Experiment 1 used a simple discrimination paradigm to determine whether adult transgenic mice were able to associate different extramaze cues with the availability of reward.

2.1.2 Method

Subjects

Male Tg2576 mice expressing the 'Swedish' double mutation ($HuAPP_{695}SWE$; using a hamster prion protein promoter) in a hybrid strain of C57Bl/6j with SJL were used in experiment 1. All offspring were weaned and re-caged at approximately 5 weeks of age. The mice were housed in same-sex mixed genotype litter groups of 2 to 6 animals. On reaching sexual maturation some male mice demonstrated heightened levels of aggression. The aggressor was always removed and housed separately if the welfare of its littermates required it. Transgenic mice were compared to littermate controls to ensure that age and background strain were comparable. In fact, aggression tended to be more apparent in transgenic males. Animals housed in groups were preferably used over animals housed in isolation (see Dong et al., 2004 for discussion of the effects of isolation stress in Tg2576 mice). In the event that a transgenic animal that was housed in isolation was used, a wild type animal caged on its own would also be run in the behavioural cohort. Other details of mouse breeding and maintenance of the colony were similar to those described previously (Chapman et al., 1999).

All behavioural testing was conducted during the light phase of a 12 hour-light/dark cycle. Training sessions began at 09:00 each day, and the animals were

tested 7 days per week. Animals were placed on a food withdrawal schedule of 4g of food chow per day, sufficient to maintain their weight above 80% of baseline.

The animals were weighed regularly to ensure that the food restriction was not affecting their general health. The experiment was completed in full compliance with Home Office (UK) guidelines. These are the primary details of the strain of mice and general husbandry and testing procedures used throughout this thesis. Variation in factors such as the age or sex of the mice, food or water withdrawal details and any changes in testing schedules will be highlighted in the appropriate method sections.

Experiment 1 used 16 behaviourally naïve male animals (8 tg+ and 8 tg-) aged between 12-14 months of age. These animals were tested on an appetitive context discrimination procedure (Experiment 1), a conditional context left-right discrimination task (Experiment 2) and on a subsequent T-maze forced-choice acquisition task to confirm phenotype.

Apparatus

Open field

The base of the open field arena (82 cm x 82 cm) was constructed from laminated plywood. The arena floor was white and divided into16 equally sized squares by six bisecting lines scored into the wood to form a 4 x 4 arrangement. The walls measured 30 cm in height and were made of clear Perspex that was covered externally with white card. The apparatus was placed on a stand that elevated it to 30 cm from the floor of the room. The arena was situated in the centre of a quiet testing room, distal visual cues such as the position of the experimenter and arrangement of video and tracking equipment remained constant throughout testing. The floor and walls of the arena were wiped down with 70% alcohol between each trial.

Behavioural Training

Experiment 1: Simple room discrimination

In order to determine whether adult Tg2576 mice are able to process extramaze cues and associate these with the availability of reward Experiment 1 tested a naïve cohort of mutant and control mice on the acquisition of a simple context (room) discrimination using an open field. The mice received an initial non-reinforced 10-minute exposure to the apparatus in each context on Day 1. The order in which the mice were exposed to the apparatus in each context was counterbalanced and each mouse received one exposure session in the morning in one context and another session in the afternoon (approximately 4 hours later) in the remaining context. Half the Tg2576 and control mice would be exposed to room A in the morning and room B in the afternoon, the other half of the animals (4 tg+ and 4 tg-) would be exposed to room B in the morning and room A in the afternoon.

This procedure was carried out to evaluate locomotor activity in the two groups and to establish the levels of locomotor activity elicited by each context. The mice were then exposed to one room in the morning and a second distinctive room in the afternoon (approximately 4 hours later). In each room, the mice were placed into the centre of the open field and left for 5 minutes. After this period, food (10, 20 mg Noyes food pellets) was scattered across the maze floor in the room designated as the reinforced context. The mice remained in the open field for another 5 minutes and were then removed. In the second (non-reinforced) room, no food was presented at any point during the 10 minutes of exposure to the apparatus.

The order in which the mice were exposed to the two rooms within a day was counterbalanced between days. The assignment of the reinforced room was also counterbalanced between groups. Half of the animals (4 tg+ and 4 tg-) were rewarded in room A and the remaining animals (4 tg+ and 4 tg- mice) were rewarded in room B. For those animals rewarded in room A, half (2 tg+ and 2 tg-) were exposed to this reinforced context in the morning session of Day 1, followed by the non-reinforced context of room B in the afternoon session. On Day 2 these animals would be exposed to the non-reinforced context of room B in the morning and the reward context of room A in the afternoon. This would be reversed on Day 3 (reinforced context first followed by non-reinforced room in the afternoon) and alternated again on Day 4 (non-reinforced context followed by reward room A in the afternoon session). This pattern was used for those animals (2 tg+ and 2 tg-) initially rewarded in room B on the first test session (e.g., Day 1 reinforced room B followed by non-reinforced room A, Day 2 non-reinforced room A followed by reinforced room B, Day 3 reinforced room B followed by non-reinforced room A, Day 4 non-reinforced room A followed by reinforced room B). The reverse pattern (i.e Day 1 non-reinforced session in morning followed by reinforced session in afternoon, Day 2 reinforced session in morning followed by non-reinforced session in the afternoon, Day 3 non-reinforced session in morning followed by reinforced session in afternoon, Day 4 reinforced session in morning followed by non-reinforced session in afternoon) was applied to the remaining animals, half of which (2 tg+ and 2 tg-) were rewarded in room A and half (2 tg+ and 2 tg-) in room B.

The floor of the arena was marked by a series of 16 equally sized squares. The dependent measure was the number of times each animal entered into one or more of the 16 square areas on the floor of the arena. Entry into a square was considered to

have occurred when both the front and hind paws crossed into a square region. Data collection was carried out by an experimenter who sat in the room (out of sight of the animal) and observed the animal via a camera located on the ceiling above the maze.

2.1.3 Results

Experiment 1: Extramaze cue room discrimination.

Control mice showed significantly lower levels of crosses across the region subdivisions of the arena floor averaged across the two contexts (122 crosses, SEM ± 9.79) than Tg2576 mice (156.87 crosses, SEM± 29.37; t(14)=3.097, $p < 0.01$). In order to provide a meaningful comparison between the two groups, locomotor activity during the first 5 non-reinforced minutes of exposure to each context was expressed as a percentage of the level of activity observed in the habituation session in each context on Day 1. Figure 2.1.1 shows activity scores during the 4 days of training expressed as a percentage of baseline activity. Locomotor activity in the non-reinforced context declined systematically over the course of training in both Tg2576 mice and littermate control animals. Activity in the reinforced context was maintained at a higher level than in the non-reinforced context across the four days of training, $F(1,14)= 9.28$, $p < 0.01$. The levels of activity elicited by the two contexts did not differ between Tg2576 and control mice ($F<1$) and this did not vary across training session ($F<1$). The results suggest that transgenic mice were able to use cues provided by the extramaze environment to acquire a simple context discrimination.

A potential problem with these results has been acknowledged. Visual inspection of Figure 2.1.1 suggests that on Day 1 there is a significant difference

between room type (reinforced versus non-reinforced). This was confirmed by a one-way ANOVA $F(1,14) = 6.848$ $p<0.05$. It would appear that this is an anomaly with the transformed data because there was not a significant difference between rooms on Day 1 when the raw scores are analysed ($F<1$). It is important to note however that the animals showed increased activity in the rewarded context, and both types of mice displayed habituation in the non-reinforced room. The rooms were fully counterbalanced, so the initial difference in room preference cannot be attributed to adverse features in one room over another.

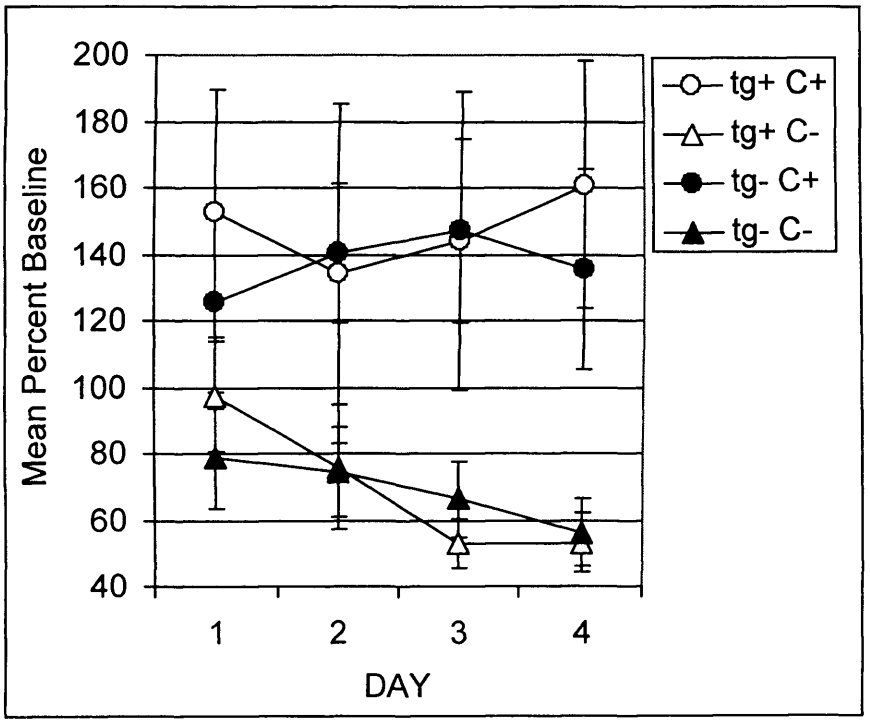


Figure 2.1.1 The mean level of locomotor activity during a five minute period of non reinforced exposure to an open field arena across four session of training. Locomotor activity is expressed as a percentage of baseline activity for transgenic (tg+) and wild type (tg-) mice, respectively. After 5 minutes of exposure to the apparatus food was delivered into the arena in one laboratory context (C+) but not in another visually distinctive context (C-). Error bars show the standard error of the mean.

2.1.4 Discussion

The results from Experiment 1 demonstrate that Tg2576 mice can distinguish between two visually distinct rooms. This would suggest that the mutant mice are sensitive to extramaze cues. Experiment 2 was designed to determine whether the Tg2576 mice could use extramaze cues to learn a conditional discrimination.

2.2 Experiment 2: Conditional left-right discrimination and Forced-Choice Alternation task in a T-maze.

2.2.1 Introduction

In general, Tg2576 deficits on spatial navigation tasks emerge at approximately 6-8 months of age when the production of insoluble forms of Aβ in cortical and hippocampal areas is increasing (Westerman et al., 2002; Kawarabayashi et al., 2001). Theoretical consideration of the impairment in Tg2576 mice has, therefore, understandably borrowed from theories of hippocampal function (e.g., King & Arendash, 2002).

Traditionally hippocampal function has been characterized as a cognitive map. O'Keefe and Nadel (1978) proposed that the hippocampus served as a neural correlate of allocentric positions in space, a process known as the Locale system. In contrast the hippocampally independent Taxon system allows rudimentary navigation on the basis of simple stimulus-response associations. This is a useful dichotomy to investigate spatial behaviour in Tg2576 mice.

The dual process concept of spatial navigation can be assessed in simple maze experiments such as the T-maze forced-choice alternation (FCA) task. The FCA task requires the animal to retain information from a sample trial and is very sensitive to hippocampal cell loss (Aggleton et al., 1986) and impairments in adult Tg2576 mice (Chapman et al., 1999; Corcoran et al., 2002). Historically, the strategies that animals use to guide performance on this T-maze task have been characterised as response (turning) or place-based strategies (Blodgett & McCutchan, 1947; Tolman, et al., 1946). The latter requires the ability to use extramaze cues to guide navigation (see Dudchenko, 2001 for a review).

Barnes et al. (2004) examined the strategy adopted by adult control and Tg2576 mice to perform the T-maze FCA task. To establish which strategy is used during acquisition a 180 degree probe can be used; as the rotation of the start arm at the choice phase of the tasks places extramaze cues into conflict with response-based performing. Using a 180 degree probe Barnes et al. (2004) found evidence that control mice spontaneously adopt a strategy based on the extramaze cues (place) during FCA, as their performance was not disrupted by the rotation of the start arm. In contrast, the mutant animals were found to perform at a level of chance - which is indicative of hippocampal damage in mice (see Passino et al., 2002; Middei et al., 2004b) and demonstrated that the processing of extramaze cues was compromised in adult APP_{SWE} mice. Control mice have also illustrated an automatic adoption of a place strategy to solve the plus maze task. Interestingly, Tg2576 mice were shown to unambiguously use a response based strategy to reach equivocal levels of acquisition in this reference memory task (Middei et al., 2004a).

Experiment 1 addressed a potential reason why Tg2576 mice failed to adopt place-based responding. If transgenic mice were unable to process cues distal to the

goal locations it would considerably limit the use of these cues to support navigation. Experiment 2 examined whether Tg2576 mice could use this contextual information to acquire a conditional left-right discrimination in a T-maze. If Tg2576 mice were unable to process extramaze cues then we would predict impairment in this task. The mice were then ran in a FCA task in the T-Maze (Experiment 2a) to confirm the Tg2576 phenotype of a deficit in this task.

2.2.2. Method

Subjects

Experiment 2 and 2a used the same 16 male animals (8 tg+ and 8 tg-) as described in Experiment 1. All mice were approximately 16 months of age at the start of Experiment 2a. Training on the conditional left-right discrimination and forced-choice alternation tasks required the animals to be water deprived for 22 hours prior to testing to motivate them to run for a 25 % sucrose reward in the T-maze. The animals were weighed regularly to ensure that the water restriction was not affecting their general health. The experiments were completed in full compliance with Home Office (UK) guidelines.

Apparatus

T–maze

The T-maze was constructed from three arms, each 9 cm wide (length of start arm: 52cm, length of each goal arm 26cm). The base of the T-maze was made from white wood laminate and the walls were made from clear Perspex and were 11 cm high. Three opaque Perspex guillotine doors operated by hand were used to block the

animal in the start box and goal arms. Mice ran for a 25% sucrose reward, 50μl of the solution in distilled water was placed in black plastic oblong drinking wells situated at the end of each goal arm. The T-maze was placed on a stand that elevated it 75cm from the floor and positioned in the centre of a quiet testing room. The position of the maze remained constant. Three different rooms were used for this experiment. All were of similar dimensions and had comparable lighting but differed in the type and arrangement of posters on the walls. These distal cues and others such as position of experimenter always remained the same throughout testing. The arms and walls of the maze were wiped down with 70% alcohol in distilled water between each trial to remove any odour cues.

Behavioural Training

Experiment 2: conditional room discrimination

Experiment 2 examined whether Tg2576 mice could use information provided by ambient extramaze cues to acquire a conditional contextual discrimination in which room cues identified which goal box in the T-maze contained food. Following acquisition we examined whether control and mutant mice used either an egocentric or allocentric strategy to perform the task by rotating the maze 180°.

In stage 1, the same cohort of mice used in the room discrimination in Experiment 1, was habituated to the T-maze and then trained to retrieve a sucrose reward from the goal boxes. Habituation lasted for four days. Each animal received 5 minutes of free exploration in the maze per day. 50μl drops of sucrose were placed in the drinking wells and along the length of the open start and goal arms. The number

of reward sites decreased with each day, such that by the final day of habituation sucrose was only placed in the drinking wells situated at the end of each goal arm. These were subsequently used as reward sites during acquisition of the task. During habituation, a measure was taken of the preference that each mouse showed for each goal box in either room (in terms of number of entries during a session). The mice were then trained against this bias in the second stage of the procedure. Thus, if a mouse showed a stronger preference to visit the left goal box in room A than in room B, the left goal box was assigned as the non-reinforced arm in Room A and the reinforced arm in Room B.

In stage 2 the mice received two sessions of training, each comprised 6 trials, with one session in the morning and the second in the afternoon (approximately 4 hours later). The order in which the rooms were presented each day was randomised so that on some sessions they were exposed to room A first and on other sessions room B first. Each mouse was assigned a correct goal box (left or right) for each context. If a mouse entered a correct goal box, (i.e. it ran to the end of the arm designated as their reward site in a particular room) it obtained access to a sucrose reward and was contained in the goal box for approximately 30 seconds. It was then removed from the apparatus and placed in a holding cage for the inter-trial interval (ITI). If the animal entered the incorrect goal box it was contained in the box for 30 seconds before being removed to the holding cage for the next trial. The ITI was approximately 10-12 minutes. Training continued for 14 days after which the mice received a series of probe trials (stage 3).

Stage 3: Conditional context discrimination – Probe trials

In stage 3, the mice received two days of testing to evaluate whether performance in each room was governed by a place or a response-based strategy. To minimize negative learning effects the animals received a single test session each day in either room A or room B. For example, on Day 15 a mouse could receive a single test session in the morning in room A and on Day 16 a single test session in Room B in the afternoon. The order in which the testing was carried out was counterbalanced within and between groups. A group of mice (2 tg+ and 2 tg-) would be exposed to room A in the morning of Day 15 followed by room B in the afternoon of the Day 16, a group (2 tg+ and 2 tg-) would be exposed to Room B in the morning of Day 15 followed by room A in the afternoon of Day 16, another group (2 tg+ and 2 tg-) would be exposed to room A in the afternoon of Day 15 followed by room B in the morning of Day 16, the remaining mice (2 tg+ and 2 tg-) would be exposed to room B in the afternoon of Day 15 followed by room A in the morning of Day 16.

In each test session, the mice received two normal training trials under the appropriate reinforcement contingency for that context. For the remaining 4 trials, the T-maze was rotated by 180° and the mouse was released from the new start location. Sucrose reward was available in the goal box location specified by the extramaze cues during training. If the mice used a strategy based on the location of the reward, then their level of performance should be above chance. In contrast, if the mice had adopted a turning response strategy (i.e., room A turn left; room B turn right) then their performance on the probe test would be severely disrupted.

Experiment 2a: Forced-choice alternation

In Experiment 2a, mice were trained on a T-maze FCA task to ensure that the procedures remained sensitive to deficits in Tg2576 mice. T-maze acquisition was conducted in a novel room that was of similar dimensions and illuminated in the same way as the rooms used in the conditional discrimination task. This room also had a unique arrangement of posters on the walls to act distal visual cues. In stage 1, the mice received one day of habituation to the T-maze and sucrose reward in the novel environment.

Acquisition consisted of six pairs of trials per day for seven days. The first part of each trial, the sample run, entailed a forced-choice entry into one of the two goal arms. On its release from the start box the mouse was allowed to freely enter the arm selected by the experimenter. A Perspex guillotine door blocked the remaining arm. Three forced choices were assigned to the left and three to the right in a pseudo-random order with no more than 2 consecutive trials having the same sample location in each session. Mice always received a sucrose reward in the drinking well situated at the end of the pre-selected goal arm. Another guillotine door was used to restrict the animal to the goal box for 15 seconds, where it was allowed to consume the sucrose reward. The animal was then removed from the T-maze and placed back in its holding cage. The guillotine doors were removed and the floor and walls of the maze were quickly but thoroughly wiped down with 70% alcohol in distilled water. Any sucrose residue was wiped away from the sample reward site and the drinking well in the alternate goal arm was baited with a 50µl drop of sucrose reward. The animal was then placed back into the start box and on its release had a free choice of goal arm to enter. To gain a reward during the free choice phase of the trial the animal had to

enter the previously unvisited arm. The animal was restricted into which ever arm (baited or non-baited) it initially entered and held there for 15 seconds or if necessary, until it had consumed the sucrose reward. It was then removed and placed back in its holding cage. The mice were tested in squads of 8 with an equal number of transgenic and wild type animals in each, the duration of the ITI was approximately 10-12 minutes.

2.2.3 Results

Aging Tg2576 mice have been shown to demonstrate deficits in spontaneous alternation (see Hasio et al., 1996; King & Arendash 2002, Lalonde et al., 2003). Alternation behaviour was investigated during the T-maze habituation sessions prior to stage 2, the conditional context discrimination task. The total number of arm entries and the total number of consecutive alternations made by each animal was recorded. The start arm was included in both of these observational measures. Figures 2.2.1 a and b show the average number of arm entries and consecutive alternations made by each animal across both rooms per day. The aged transgenic mice show a comparable rate of habituation to their littermate controls, in terms of both the number of arm entries (Figure 2.2.1.a) and the number of consecutive alternations (figure 2.2.1.b) made across the 4 sessions of habituation. An ANOVA conducted on the total arm entry data revealed a main effect of day $F(3,42)$ 46.34, $p<0.001$ but no main effect of genotype ($F<1$) and no significant interactions between these factors ($F<1$). Similarly a main effect of day $F(3,42)$ 36.01, $p< 0.001$ but no main effect of genotype ($F<1$) or day by genotype interaction ($F<1$) were found for the alternation data. Aged Tg2576 alternation behaviour was comparable to their littermate controls. Rewards were available during the habituation sessions, and this may call into dispute the

spontaneous nature of the animals' behaviour. However, all T-maze and plus maze work reported in this thesis was appetitive, and for this reason further analysis of transgenic alternation behaviour was not considered necessary.

a *b*

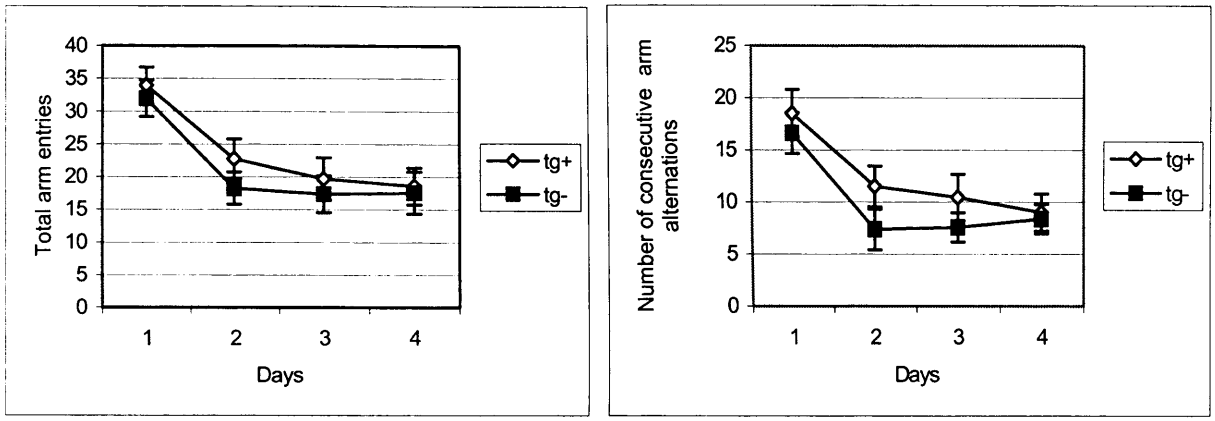


Figure 2.2.1(a) Mean number of arm entries made in a T-maze across 4 sessions of habituation in two visually distinct rooms. Error bars show the standard error of the mean. *(b)* Mean number of consecutive alternations made in a T-maze across 4 sessions of habituation in two visually distinct rooms. Error bars show the standard error of the mean.

After the four days of habituation animals were trained in the conditional context left-right discrimination. Figure 2.2.2 shows the mean percentage correct responses averaged across the two sessions of training each day and collapsed into two-day blocks. The mice found the conditional discrimination task challenging and their performance reached a stable but below asymptotic level of performance after approximately 14 days of training. Inspection of Figure 2.2.2 shows that both Tg2576 and control mice acquired the conditional discrimination at the same rate. This was

confirmed by an ANOVA which revealed a main effect of block, $F(6,84)=6.89$, $p < 0.001$, but no main effect of group ($F<1$) and no significant interaction between these factors, $F(6,84) = 1.18$, $p> 0.32$.

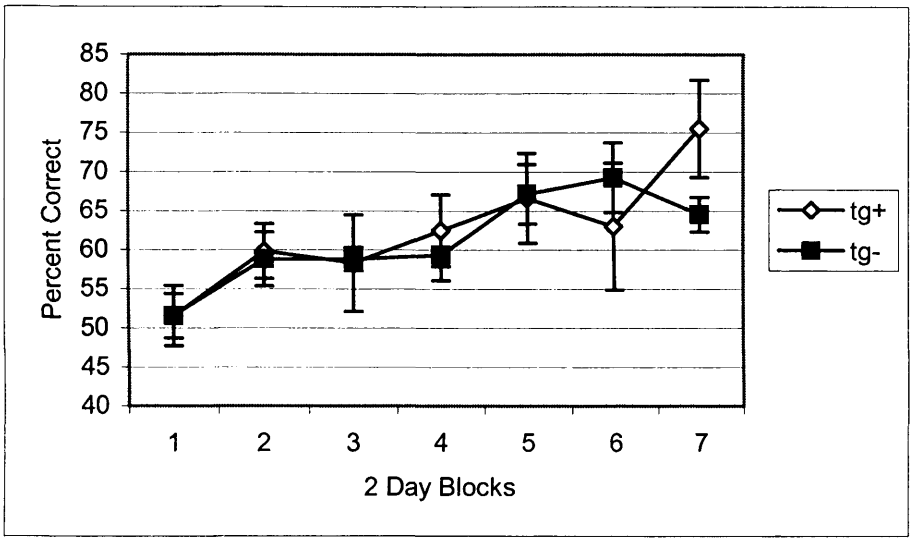


Figure 2.2.2 The mean percent correct choices during the acquisition of a conditional context left-right discrimination in a T-maze for transgenic (tg+) and wild type (tg-) mice. The mice were reinforced for entering one goal box in one context and the remaining goal box in a different context. Error bars show the standard error of the mean.

To investigate whether the mice were responding during each session on the basis of the current reinforcement history we examined trial 1 performance. If the mice adopted the latter strategy, rather than using the contextual cues to guide performance, accuracy should have been at chance on trial 1. The percentage of correct choices on trial 1, averaged across the final 6 days of training was 66.7% (SE= ±5.22) for control mice and 61.46% (SE = ±8.76) for transgenic mice. There was no significant difference between these means ($t<1$) and the overall level of performance

on trial 1 was significantly above chance ($t(15) = 2.83$, $p<0.01$). The results suggest Tg2576 and control mice were sensitive to the extramaze environmental cues and were able to use this information to guide performance on the T-maze task.

The performance of the Tg2576 and control mice on the probe test (stage 3) is shown in Figure 2.2.3. Relative to their performance on the last standard day of training, it is clear that the (rotation) probe severely disrupted performance for both Tg2576 and control mice. An ANOVA revealed a main effect of test (standard versus probe trial) $F(1,14)= 16.98$, $p < 0.01$, but no main effect of group ($F<1$) and no significant interaction between these factors, $F(1,14)= 3.78$, $p > 0.05$. The performance of both groups differed from chance on the last day of standard training (min $t(14) = 3.74$, $p <0.05$) but did not differ from chance on the probe trial (both t's < 1). Clearly, if control and mutant mice had been using an allocentric or place response, their performance would have been above chance. This pattern of performance may suggest that both groups of mice used a response-based strategy (i.e., room A, turn left; room B, turn right). This account also supports our interpretation of the trial 1 performance during training. If the mice had used contact with the within-session reinforcement contingencies to bias their behaviour, then, because reward was available during the probe test, performance should have been above chance. The probe trial data are somewhat inconclusive, if the animals were using a pure turning response than we would have expected their performance to be significantly below chance, the at chance performance may be indicative of general disruption caused by the novel start location.

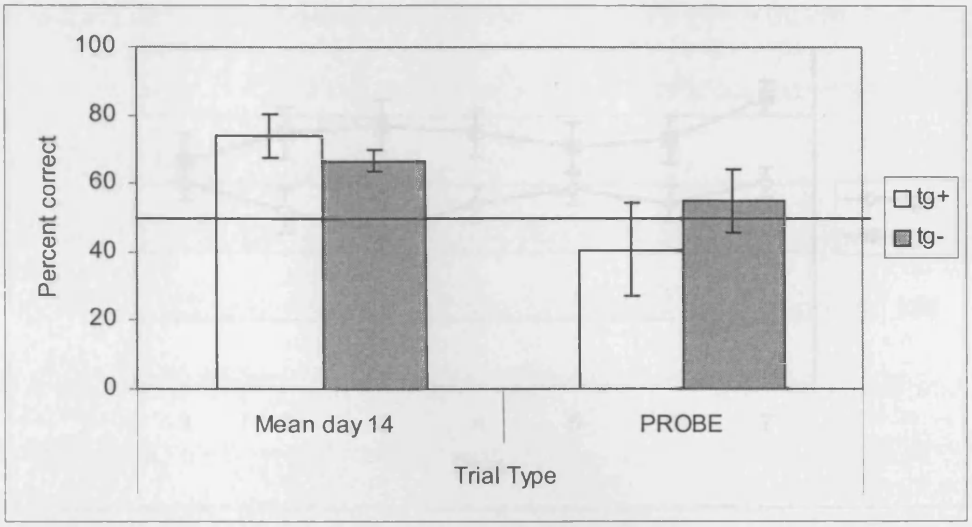


Figure 2.2.3 The mean percent correct choices of the last day of training on the conditional discrimination and during a probe trial in which the start location was rotated 180°. Error bars show the standard error of the mean. The single grid line represents chance at 50 %.

Figure 2.2.4 shows the mean percent correct responses during acquisition of the T-maze FCA task across the 7 sessions of training. Inspection of Figure 2.2.4 shows that there was a clear difference between the Tg2576 and control mice during acquisition and that mutant mice performed less accurately than control animals, $F(1,14)=26.75$, $p < 0.001$. These results confirm that the Tg2576 mice remained sensitive to deficits in T-maze performance despite normal acquisition of the conditional task in the same apparatus.

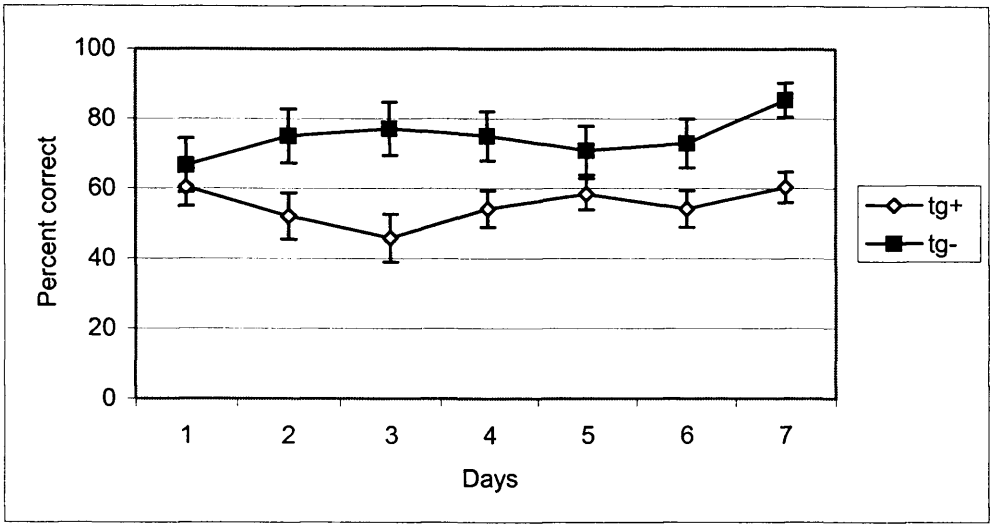


Figure 2.2.4 The mean percent correct choices of the same animals during acquisition of a T-maze FCA procedure. Error bars show the standard error of the mean.

2.2.4 Discussion

Acquisition of a T-maze forced-choice alternation task was impaired in the 16 month old Tg2576 mice. The present results are consistent with other published reports of deficits in spatial navigation in adult Tg2576 mice (Hsaio et al., 1996; King et al., 1999; Kotilinek et al., 2002; Westerman et al., 2002). The results also confirm earlier findings of impaired T-maze FCA alternation performance in these mutant mice (Chapman et al., 1999; see also Corcoran et al., 2002). Tg2576 mice were however able to associate different laboratory contexts with the availability of reward (Experiment 1). This suggests that Tg2576 mice were able to process at least some features of their extramaze environment. Furthermore, adult Tg2576 mice were able to use this contextual information to successfully guide their response on a T-maze left-right discrimination (Experiment 2). The implications of these results suggest that

failure to form and use an allocentric representation of the environment to guide navigation cannot be due to gross perceptual impairments or a generalised learning deficit in Tg2576 mice.

Mutant mice acquired the conditional task at the same rate as control mice. In addition, lateral shift probes ran in a T-maze provide evidence that control and transgenic mice are uniformly sensitive to exposure to novel positions within a well-habituated context (unreported data). These results suggest that Tg2576 mice can use distal visual cues to distinguish between different contexts and novel locations within the same environment, and this ability is comparable to controls. Any deficit in an allocentric use of spatial cues cannot therefore, be based on perceptual impairment. It is also important to note that the control mice required extensive training to learn the conditional discrimination task. The fact that Tg2576 mice learnt the task at an equivalent rate to the controls demonstrates that theses mutant animals can acquire difficult tasks, and suggests that deficits in spatial navigation cannot be attributed to task demands of the experiment per se.

The room discrimination and the conditional discrimination could, theoretically, be solved on the basis of non-visual, i.e., auditory or olfactory information, that was unique to each context. Evidence suggests, however, that the C57BL/6 strain of mouse (that forms part of the background strain for the Tg2576 mutation) place a strong reliance on the use of extramaze visual cues to solve other types of maze tasks, such as the radial maze (Roullet et al., 1993). It remains likely, therefore, that the stimuli used by the adult control mice were the distinctive visual features of the rooms.

Adult and aged Tg2576 mice have demonstrated comparable place performance to controls under the adverse conditions of the circular platform task

(Pompl et al., 1999; King & Arendash, 2001). This may suggest that when forced, Tg2576 mice can use extramaze cues to navigate efficiently around an environment. Pompl et al. (1999) however, also report a reversal deficit in Tg2576 performance on the circular platform task, and argue that the inability to re-calibrate the salience of the distal spatial cues was indicative of the mutant mice (unlike the controls) acquiring a "sub-optimal" place strategy.

The results from the conditional probe demonstrate that the transgenic mice did not adopt a place–based strategy, but in parallel with previous reports, the results were suggestive of the mutant animals adopting a response strategy to solve the spatial reference memory task (see Middei et al., 2004a). It should be noted however, that the Tg2576 probe behaviour although numerically below fifty percent, was not significantly below chance – which would have been indicative of a pure turning response. A difference in reward contingencies may account for the slight discrepancy between the two studies. Only one probe trial was used in the plus maze study, consequently there was no potential for within session learning. The availability of reward for responding to place in the conditional probe trials (8 in total) may have obscured our sensitivity to revealing systematic responses biases in mutant (and control) mice.

The fact that control animals adopted a similar response tendency in the conditional task is intriguing given the demonstration of place-based responding during FCA performance (Barnes et al., 2004). However, unlike the FCA acquisition that entailed six trials per day over 10 days, the conditional discrimination required 12 sessions per day (6 in each room) over a period of two weeks. This arguably amounts to extensive training, which Packard and McGaugh (1996) suggest encourages animals to adopt a stimulus-response type of response strategy. The length and

intensity of the training employed in the conditional task may have biased the performance of the control animals. The findings therefore suggest that under normal training conditions where either a place or a response strategy can be used to solve a discrimination, Tg2576 animals (unlike the controls) fail to adopt the first strategy (Barnes et al., 2004) and implement the latter (Middei et al., 2004a).

An alternative explanation that has not been addressed in this study is that animals use an inertial sense of direction to solve the FCA task (Douglas, 1966; Dudchenko & Davidson 2002). It has been suggested that the hippocampus integrates inertial information to form an on-line representation of the animal's location (McNaughton et al., 1996). The ability to use self-motion cues to guide behaviour is disrupted by hippocampal lesions in mice (Gorny et al., 2002). Experimental observations have noted that control animals tend to make more entries into the start arm during habituation and spend more time running up and down the length of the start arm during acquisition (this was an observation and has not been statistically verified). Such behaviours resemble the formation of a 'home-base' – the point of reference from which subsequent self movement is calibrated (Gorny et al., 2002). The lack of such responding in mutant mice may be indicative of a hippocampal-dependent path integration deficit. Further experiments are required to determine whether the APPswe mutation disrupts specific navigation strategies in these mice.

Further research is also needed to determine why Tg2576 mice can demonstrate effective response learning in spatial reference memory tasks (conditional, cross maze learning; see Experiment 3) but cannot translate this behaviour into FCA acquisition comparable to controls. A possible explanation may be reflected in the perseverative tendencies of aged Tg2576 mice (personal communications, Barnes, 2004). Unlike the conditional task reported in this study

and cross-maze learning, the location of the goal arm within a specific environment, does not remain constant over choice phases. The poor FCA performance demonstrated by Tg2576 mice in Experiment 2a might therefore represent an inability to modulate behavioural response. Evidence for an impaired ability to shift response in accordance with the availability of a reward can be found in the reversal deficit observed in Tg2576 mice during a simple visual discrimination (personal communications, Barnes 2004). Acquisition of this task was comparable to controls (see Experiment 3a, Barnes et al., 2004). Such results may be indicative of a deficit in response inhibition. An account of impaired response inhibition was used by Head et al. (1998) to explain reversal deficits in aged Beagles with amyloid-related pathology in the pre-frontal cortex. However, it is important to note that the perseverative tendencies of animals may not denote a deficit in itself, but represents a strategy to gain 50% reward (c.f Bannerman et al., 2001). Nevertheless, further experiments are needed to determine whether Tg2576 mice have a deficit in response inhibition. Such an experiment would need to be non-spatial and therefore hippocampal-independent, if it is to inform us about other neuro-anatomical regions (prefrontal cortex) that may be sensitive to amyloid pathology.

2.3. Experiment 3: Response and Place learning in a plus maze

2.3.1 Introduction

Experiments 1 and 2 demonstrate that Tg2576 mice are sensitive to extramaze cues and can use these cues to learn a conditional discrimination in a T-maze. Experiment 2a reaffirms the mutant's genotype of a deficit in the FCA T-maze task. Using the dichotomy of response versus place learning, I have alluded to the fact that Tg2576 mice have preserved response learning but despite a degree of sensitivity to distal visual cues, are unable to form an allocentric representation of space. Nevertheless, our experiments thus far, have not conclusively replicated previous reports that Tg2576 mice demonstrate response learning in a maze environment whilst control animals show a preference for place learning (see Middei et al., 2004a, 2006). Experiment 3 comprised a reference memory task in the plus maze and was conducted to evaluate this suggestion.

Middei et al. (2004a, 2006) reported that Tg2576 mice make significantly more correct choices during training on a plus maze reference task at 7, 14 and 15 months of age than their littermate controls. Middei et al. (2004a) acknowledge that the reference memory task may favour Tg2576 mice because they do not demonstrate a normal preference to alternate and would consequently acquire a simple turning response strategy more readily (King & Arendash, 2002, but see habituation results for Experiment 2). In line with this thinking, hippocampally compromised animals show augmented response learning (see Packard & McGaugh, 1996; Schroeder et al., 2002). Striatal circuitry has been implicated in rodent response based learning (see Packard, 1999). Importantly, Middei et al. (2004a) have shown that frontal-striatal

plasticity is unimpaired in 15-month-old Tg2576 mice. An experimental design that favoured animals that do not spontaneously alternate, coupled with a neurological augmentation of response learning, may account for why the transgenic mice outperformed the wild type animals on the training trials of the plus maze reference task.

Bizon et al. (2007) report that 15-month-old female Tg2576 mice demonstrate more established place learning than their littermate controls in a similar reference memory and 180 degree probe trial to the Middei et al. (2004a, 2006) studies. Bizon et al. (2006) also reported that the control animals failed to perform above chance on any of the training days. The latter finding is consistent with Middei et al. (2004a; 2006). However the claim that transgenic mice were demonstrating place learning is inconsistent with majority of published reports using this mutation.

Despite Bizon et al's (2007) claim that the Tg2576 mice were using a place strategy they did not conduct any statistical analysis against chance on the probe data. Closer inspection of the data reveals that only 54% of the Tg2576 animals adopted a place strategy over the two trials, suggesting that on average the transgenic mice were behaving at chance during the probes. It is therefore possible that the transgenic mice were adopting a response strategy during the training trials. Why these transgenic mice failed to demonstrate unequivocal response learning in the probe trial is undetermined. The important thing to consider however, is the fact that Tg2576 mice have not unequivocally demonstrated place learning in the T-maze or Plus maze and this deficit can be attributed to hippocampal dysfunction (O'Keefe & Nadel, 1978).

Stimuls-Response (S-R) behaviour is governed by the basial ganglia (for a review see Packard & Knowlton, 2002) and such processes are thought to simultaneously run in parallel with hippocampal based place learning (cf. Poldrack &

Packard 2003). It has been suggested that if hippocampal place learning is compromised then response-based learning can be augmented (see Packard & McGaugh, 1996, Schoroder et al., 2002). Experiment 3 assessed Tg2576 performance in a place and a response version of a reference memory task conducted in a Plus maze. Animals were trained to respond to a location that remained consistent across trials or to perform a specific turning response (always turn left or right at the choice point). It was predicted that Tg2576 mice might show facilitated response learning to the extent that performance was not compromised by the presence of allocentric cues that (in wild type mice) might compete for control of behaviour. Furthermore, based on a number of previous findings we predicted that the transgenic animals would show a deficit in place learning.

2.3.2 Method

Subjects

17 transgenic and 17 wild type mice were used in Experiment 3. All mice were male and 12 months old at the start of the experiment and were naive to T-maze and Plus maze training. 9 transgenic and 8 wild type mice were trained in a response protocol, and 8 transgenic and 9 wild type mice were trained in a place-based version of the task, both conducted in a Plus maze. Animals underwent water withdrawal following the same procedure as described in the method section of Experiment 2 and 2a.

Apparatus

Plus Maze

The plus maze was constructed from four arms each 40 cm long and 6.5 cm wide. The base of the maze was constructed of wood and painted white. The maze

had clear Perspex walls that were 13 cm high. Guillotine doors could be used to block off approximately 10cm of the end of each of the four arms, creating a start box and three goal locations. The doors could also be positioned at the entrance of each arm to prevent the animal from entering it. The guillotine doors were made from opaque Perspex. Each arm had a circular food well sunken into the floor approximately 2cm from the back wall. Mice ran for a 25% sucrose reward, 50µl of the solution in distilled water was placed in the food well at the end of the goal arms. The plus maze was stood on a stand, and elevated 75cm from the floor. It was positioned in the centre of a quiet testing room. Distal visual cues were provided in the form of posters on the walls, benching, ventilation vents and the experimenter. The location of these cues and the position of the experimenter remained constant throughout the testing.

Behavioural Training

All 34 animals underwent the same habituation procedure. On the first two days of habituation the animals were allowed to freely explore the maze for 5 minutes. The animals were introduced to the maze at the end of a particular arm, the location of this start arm was counterbalanced across animals and across days. On the second day of the maze habituation a guillotine door was introduced. The mouse was initially held in the start box for five seconds before being released into the maze. The arm entries made by the animals were recorded. If an animal displayed a preference for a particular arm (location), of for a specific turning response (left or right), it was trained against this bias during the subsequent test procedure.

Days three and four of the habituation process were conducted to equate the exposure each animal had to receiving a sucrose reward in all four potential goal sites

and after running in all three of the potential directions (straight on from start arm, or left and right at the choice point). During this phase of habituation two guillotine doors were used to block entry into two of the arms. To receive sucrose the mouse had to enter the free arm. Six of these trials were conducted per day. The location of the goal arm was counterbalanced so that each animal experienced a reward in each of the four potential locations three-times over the two-day period. The counterbalancing also ensured that the animals received reward for each of the response strategies (straight, left, right) four times over the two sessions. Latency scores were obtained for each animal, by the end of habituation all animals were running for reward within 20 seconds of being released from the start box.

Animals were randomly assigned to either the place or the response group. All animals received twelve trials per day. Once all animals had completed a trial the Plus maze was rotated to obscure any intra-maze cues that may have been present, and prevent their use on subsequent trials. Once each animal had completed a trial the maze floor and walls were quickly but thoroughly wiped down with 70% alcohol solution to remove any odour cues. During testing all arms were open, such that the animal had a choice of three arms to enter on its release from a pre-designated start arm.

Animals in the place group were designated a goal location (i.e., a North, South, East or West facing goal box; counterbalanced within and between groups). The goal location remained the same for each mouse across training and was never used as the start arm. Animals were enclosed into the first arm they entered following release from the pre-designated start arm. If a mouse entered its designated goal arm it was given 15 seconds to consume the sucrose reward, it was then removed and returned to its home cage. If an animal entered either of the two non-reinforced arms

on a particular trial, it was held in this arm for 15 seconds and then returned to its home cage for the ITI.

Start arm location differed over trials, each of the three non-goal locations were used. No more than 2 consecutive trials had the same start location. The counterbalancing meant that the animals in the place group had to utilize all three directional responses to gain a reward on four different trials during the training session. Start arm location was counterbalanced within and between groups and across days. For example, the north arm would be used as the reward location for a quarter of the transgenic and wildtype animals in the place group. Throughout the 12 trials conducted in a test day, these animals would be released from each of the other arms (East, South or West) on four occasions. This was conducted in a pseudorandom order. Animals were not started from the same arm on more than 2 consecutive trails.

Animals in the response group were reinforced for turning either left or right into a goal arm, irrespective of arm location. Turning response remained consistent for each animal across all the trials and training sessions, such that if a mouse was reinforced for turning left on the first trial of training, it was always reinforced for turning left throughout all of the sessions. Reinforcement of a turning response (left or right) was fully counterbalanced within and between groups. Animals were gaited into the first arm they entered on release from the start arm. If the animal made a correct turning response it had 15 seconds to consume the sucrose reward at the end of the designated goal arm, it was then removed and returned to its home cage. If the animal made the wrong choice it was gated into the non-reinforced arm for 15 seconds, it was then removed and replaced in its holding cage. Unlike the place group, response animals were started from all four arms during the training sessions. Goal location

was counterbalanced such that each arm was reinforced three times during a training session, but no more than 2 consecutive trials had the same goal location.

Animals were run in two squads of 8 and two squads of nine. Response mice were interspersed with place learners in each squad. Training was conducted over ten days, and there was an approximate ITI of 10-12 minutes between each of the 12 trials ran per day.

2.3.3 Results

Place learning

Figure 2.3.1 demonstrates that tg+ mice were impaired in acquisition of the place-learning task over the 10 training sessions. An ANOVA conducted on the data revealed a main effect of day $F(9,135) = 10.35$, $p < 0.001$ and a main effect of genotype $F(1,15)$ 12.06, $p<0.01$ and a significant day by genotype interaction $F(9,135)$ 3.041, $p <0.01$. Tests of simple main effects showed that transgenic animals were significantly impaired relative to litter mate controls from day three to day 10 of training, minimum $F(1,49) = 4.98$, $p< 0.05$.

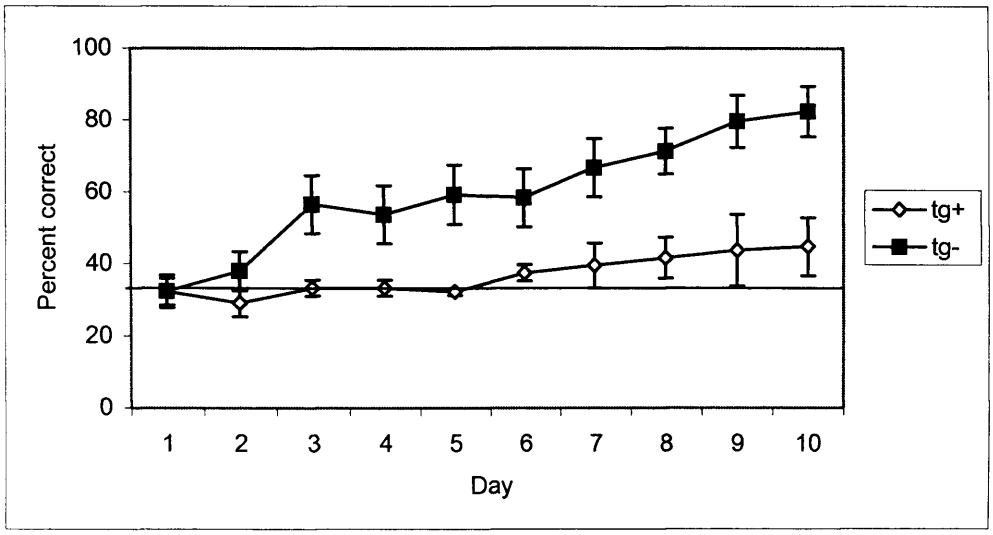


Figure 2.3.1 The mean percent correct choices of 12 month old animals during acquisition of a place learning task in the plus maze. Error bars show the standard error of the mean. The single grid line represents chance at 33.3 %.

Response learning.

In contrast to the impaired Tg2576 mouse performance on the place task, visual inspection of figure 2.3.2 clearly demonstrates that the transgenic animals in the response group acquired the response task at a faster rate than the wild type controls. This initial difference was not however significant. An ANOVA conducted on the data revealed a main effect day $F(9,135) = 10.013$, $p < 0.001$ but no main effect of genotype ($F < 1$) or group by day interaction $F(9,135) = 1.52$, $p > .48$.

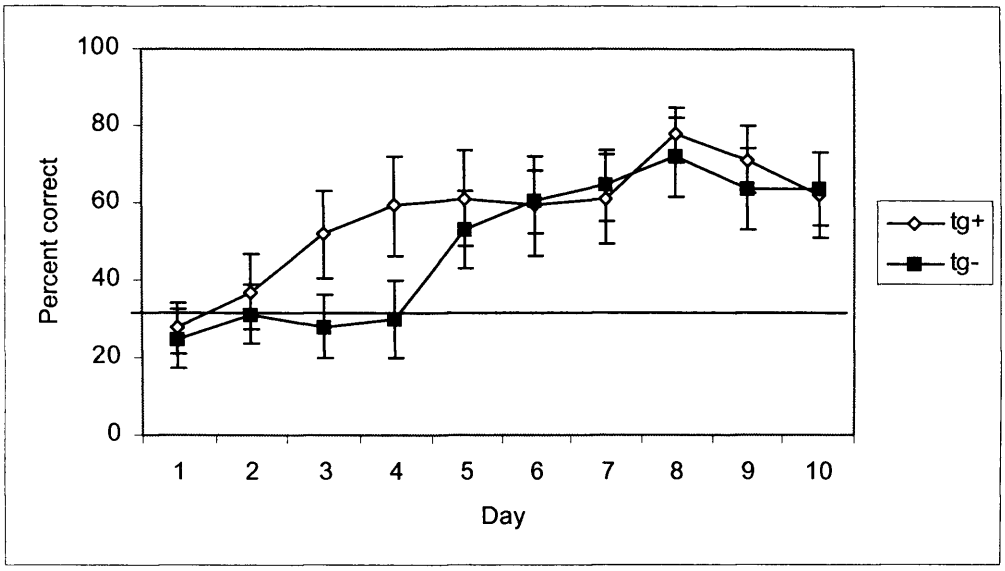


Figure 2.3.2 The mean percent correct choices of 12 month old animals during acquisition of a response learning task in the plus maze. Error bars show the standard error of the mean. The single grid line represents chance at 33.3 %.

2.3.4 Discussion

These results contribute to the growing body of literature that demonstrates a Tg2576 deficit in place learning but preserved response learning. Consistent with our prediction Tg2576 mice acquired the response task more rapidly on average that their littermate control mice – although this failed to reach conventional levels of statistical significance. In contrast, Tg2576 showed a marked impairment in learning the place version of the task.

The demonstration of impaired place but preserved response learning in Tg2576 mice is consistent with dual process theories of spatial navigation (e.g., O'Keefe & Nadel, 1978). The results from Chapter 3 coupled with this dissociation, complements evidence of impaired and spared learning in patients with AD. Typically

patients in the early stages of the disease are unimpaired on simple route learning, where S-R relationships can be utilized, but show an inability to process allocentric spatial information (e.g., Kavic et al., 2006). In addition, ideothetic processes are unimpaired in early stage Alzheimer patients (Kalová et al., 2005). It remains possible that the mice in the response task could have utilized an inertial sense of direction, and/or ideothetic information to solve the task (Dudchenko 2002). In order to evaluate the ability of mice to navigate on the basis of this type of information we would need to carry out a more formal test of path integration using procedures similar to those developed by Whishaw (e.g., Whishaw et al., 2001). However, the following chapters focus on the question of what elements of allocentric spatial memory are impaired by the mutation in the Tg2576 mice as this is relevant to understanding the pattern of deficits presented by these mice in the literature.

Tg2576 performance in a spontaneous object recognition paradigm

The experiments presented in Chapter 2 demonstrate that aged Tg2576 mice are impaired in navigational tasks that require place learning. However, symptoms of AD go beyond deficits in navigation. One of the earliest clinical symptoms is impairment in episodic memory; that is memory for the spatio-temporal context in which events occur. The precise pathological events that underpin these early cognitive deficits in AD remain unclear. A simplistic way to evaluate episodic memory is to break it down into three basic components of 'what' 'where' and 'when'. An object recognition paradigm lends itsself to evaluating these factors. Object identity / novelty represents the 'what' component, the temporal order in which the objects are presented constitutes the 'when' factor, and the location of the object the 'where' component. Experiment 4 adopted an object recognition paradigm to assess the ability of wild type and Tg2576 mice to independently process information pertaining to object identity, the temporal order of object presentation and memory for object location. The main aim of the Experiment 5 phase 2 was to replicate the pattern of results reported in the previous study by using a design that permitted a simultaneous assessment of the influences of spatial location and temporal order on exploratory activity elicited by objects.

3.1. Experiment 4: Spontaneous object recognition paradigm

3.1.1 Introduction

Recognition memory is disrupted in patients with AD and is closely associated with damage to medial temporal lobe structures in both humans and animals (Aggleton & Brown, 1999; Mumby, 2001). Patients with AD disease are particularly sensitive to visuospatial memory tasks, in which patients are asked to recall the spatial position of a target item (see Swainson et al., 2001; Lee et al., 2003). These findings suggest that visuospatial memory may be particularly sensitive to pathological processes involved in AD.

Recent work in rodents has highlighted the distinct roles played by temporal lobe structures in recognition memory. For example, the perirhinal cortex is critically involved in novelty detection (see Mumby, 2001; Winters et al., 2004) and the hippocampus in object-place memory. The frontal cortex is also thought to contribute to recognition memory processes, and in particular to the memory for the temporal sequence of events (Mitchell & Laiacona, 1998). These functional differences between neural systems provide a basis for understanding the effects of the APPswe mutation on recognition memory and the likely neural systems influenced by amyloid pathology

As discussed in Chapters 1 and 2 amyloid plaques are most pronounced in the hippocampus of adult Tg2576 mice (Lehman et al., 2003) but amyloid deposits are also present in the cortex, including, the frontal cortex (Arendash et al., 2001; Wilcock et al., 2003), and parahippocampal regions, such as the entorhinal cortex (cf. Frautschy et al., 1999). This raised the possibility that multiple components of object

recognition memory may be disrupted in adult Tg2576 mice. We, therefore, examined three characteristics of object recognition memory that are associated with the integrity of different neural systems; (1) object novelty detection; (2) object relative recency discrimination, and (3) object-location memory.

3.1.2 Method

Subjects

The spontaneous object exploration paradigm used a cohort of experimentally naïve, female and male Tg2576 mice (4 female, 6 male) and ten littermate controls of an equivalent sex ratio. All mice were 14 months of age at the start of Experiment 3. The same cohort was used on a subsequent FCA T-maze task with the exception of one female transgenic and one male wild type. Both mice were excluded on the grounds that they did not consistently consume the sucrose reward and thus reduced their contact with the appropriate reward contingencies. Breeding and maintenance information have previously been detailed in Chapter 2, section 2.1.2, page 58.

Apparatus

Open field

The base of the open- field arena (82 cm x 82 cm) was constructed from laminated plywood. The arena floor was white and divided into16 equally sized squares by six bisecting lines scored into the wood to form a 4 x 4 arrangement. The walls measured 30 cm in height and were made of clear Perspex that was covered externally with white card. The apparatus was placed on a stand that elevated it to 30 cm from the floor of the room. The arena was situated in the centre of a quiet testing

room, distal visual cues such as the position of the experimenter and arrangement of video and tracking equipment remained constant through out testing. The floor and walls of the arena were wiped down with 70% alcohol between each trail.

Objects

Objects were obtained from a variety of sources. They were constructed from materials that could not be easily gnawed by the mice and were non-porous (e.g., glass, glazed ceramic, metal). The shape and size of the objects was approximately matched and each item was free standing and weighted to withstand the investigative behaviour of mice. Objects were also of a substantial height/proportion to ensure that the animals could not climb on them. All objects were of minimal detail and were symmetrical on a horizontal plane. Examples of objects used include glass bottles, tin cans, ceramic ornaments and glassware. Objects were wiped down with 70% alcohol before they were placed into the maze to eliminate any possible odour cues.

Tracking equipment

A camera was suspended from the ceiling 90 cm above the centre point of the arena and was attached to a video recorder (Panasonic) and monitor and a computer. The movement of the animals in the maze was tracked using Etho Vision (Tracksys Ltd., Nottingham, England). Each object was assigned a zone and a keyboard button to identify it. Pressing the key signified the beginning or end of investigative behaviour. Object exploration was defined as the time spent attending to (actively sniffing or interacting with) the object at a distance no greater than 2 cm (Ennaceur & Delacour, 1988). Object exploration was not scored if the animal was in contact with

but not facing the object or if it sat on the object or used it as a prop to look around or above the item. EthoVision recorded the total exploration time for each target zone.

T-Maze

For details of the T-maze please refer to Chapter 2, section 2.2.2, page 66.

Behavioural Training

Experiment 4 : Phase 1 – Open field and object habituation; Phase 2 - Object novelty detection with 2 minute, 30 minute and 24 hour delays

A battery of non-spatial tests (Experiments 4 phase 2 and 3) were used to assess memory functioning independent of hippocampal pathology in the Tg2576 mice. Stage 1 of the novelty discrimination involved habituation to the open field. Animals were always transferred from a holding cage and placed in the centre of the arena. This start location remained the same for stages 1 – 3. Prior to the start of a trial, two identical objects were placed in the middle of the arena. The position of the target zones remained the same throughout stages 1 –3. An animal was allowed to explore the objects for 10 minutes and was then removed from the arena and placed back into its holding cage. The same procedure was repeated on the subsequent day with a novel pair of objects. The behaviour of the mice was scored in real time by the experimenter who sat in the corner of the room out of view of the animal. Etho Vision tracking software was used to manually score the exploratory behaviour. Object exploration was defined as the time spent by the animal in contact with the object (parameters detailed above) when its head was orientated towards the object.

For each of the phases of the object recognition task the same basic procedure was used. The animals were initially presented with two identical sample objects placed in the positions described above, and allowed to explore them for 10 minutes. The mouse was then removed from the arena and placed in its holding cage. Both objects were then removed from the arena, a version of the sample object and novel objects were wiped down with 70% alcohol and the objects replaced in the arena. The side on which the novel object was placed was counterbalanced between groups and within sessions. Therefore if the novel object was presented on the left on Day 1, the novel object would be located on the right on Day 2. After a predetermined delay period the mouse was placed back into the arena with the test set of objects for a further 10 minutes. For both the sample and test phase the animals exploratory behaviour was scored using the same criterion as described above. The animals received two days of testing for each trial type. Thus on the first two days of novelty testing the animal experienced 10 minute exploration of the sample objects with a two minute delay before commencing with the test phase. On the subsequent two days, a 30-minute delay was introduced between the sample phase and the test phase. To allow for efficient running the mice were ran in squads of two (i.e. two exposure phases were conducted followed by two test phases). For the 24 hour delay condition, the mice received exposure to a pair of identical objects on Day 1. On the following day and at the same time as their initial exposure, each mouse then received a test trial with a novel and familiar object. This procedure was then repeated with a new set of sample/novel objects the following day. The type of object used in the sample phase and as a novel cue, and the left right positioning of the novel item was counterbalanced across sex, genotype and day; such that if animals experienced the novel object on the left during the test phase on Day 1, then the novel object would be

positioned on the right during Day 2. The duration of contact made with each object was recorded during both the exposure and test phases.

Experiment 4 phase 3: Object relative recency discrimination.

The recency task involved two sample phases. The procedure for the first and second sample phase was identical to that described for the object novelty phase. Animals were initially allowed to investigate an identical set of objects for 10 minutes. The animal and the objects were then removed. The arena was cleaned and a new set of identical objects was place in the target zone. After an ITI of approximately 2 minutes the animal was placed back at the start point and allowed to explore the new set of objects for a further ten minutes whereupon the mouse and the objects were removed. The arena was thoroughly cleaned and one item from each of the object sets were wiped down with 70% alcohol and then replaced in middle of the maze. The mice were then introduced into the maze for the test phase which lasted for 10 minutes. The order of object pairs during the sample phases and the left/right positioning of the objects during the test phase were counterbalanced across sex, genotype and day. Therefore if the remote object was presented on the left on Day 1, the remote object would be located on the right on Day 2. The duration of contact made with each object was recorded during both the exposure and test phases.

Experiment 4 phase 4: Object-in place memory

Experiment 4 phase 4 evaluated whether the hippocampal-dependent deficits observed in Tg2576 spatial navigation could be generalized into an object-location impairment akin to visuo-spatial deficits observed in AD patients. Four different objects were place in the middle of the central four squares of the arena (approximately 10 cm apart). The mouse was placed into the arena at the mid point between the four objects. During the sample phase the animal was allowed to explore the objects for 10 minutes whereupon it was removed and placed in a holding cage. The arena and all the objects were thoroughly cleaned with 70% alcohol to remove all odour cues. The same four objects were then repositioned in the maze. Two objects were replaced in the same position that they occupied during the sample phase; the remaining two objects switched positions. The spatial shift took place on a diagonal plane such that the top left object during exposure would be placed in the bottom right position during test phase and vice versa. The direction of the diagonal switch top left – bottom right versus top right – bottom left, was counterbalanced across sex, genotype and day. Therefore if the switch was top left – bottom right on Day 1, it would be top right – bottom left on Day 2. The animal was then placed between the four objects and allowed to explore the new arrangement for 10 minutes. The duration of contact made with each object was recorded during both the exposure and test phases.

Mice were subsequently trained on T-maze forced-choice alternation task to ensure that the procedures remained sensitive to deficits in Tg2576 mice. The procedure was identical to that described in section 2.2.3 except that mice received 4 days of habituation to the T-maze and sucrose reward. The mice then received 5 days

of training, with 6 trials each session. A trial comprised of two runs in the maze as previously described. The experiment was carried out in a different room to that used during the object recognition experiments.

Interrater reliability.

An independent experimenter who was blind to the animal assignment and objects contingencies rescored 20% of all test phases from the original video footage. The rescored results significantly correlated with the original scores ($r = .82, p < .01$) indicating robust interrater reliability.

3.1.3. Results

Experiment 4 phase 1: Object exploration

Figure 3.1.1 shows the mean total amount of contact time with the objects on Day 1 and Day 2 of habituation. Inspection of this Figure suggests that Tg2576 and control mice explored a novel set objects at the same rate across the two days of habituation. This interpretation was confirmed by an ANOVA with group and days as factors and revealed a nonsignificant main effect of group ($F<1$), a main effect of day, $F(1,18)= 8.05, p < 0.05$ and a nonsignificant interaction between these factors ($F>1$). These results establish that there are no sampling biases between the two groups.

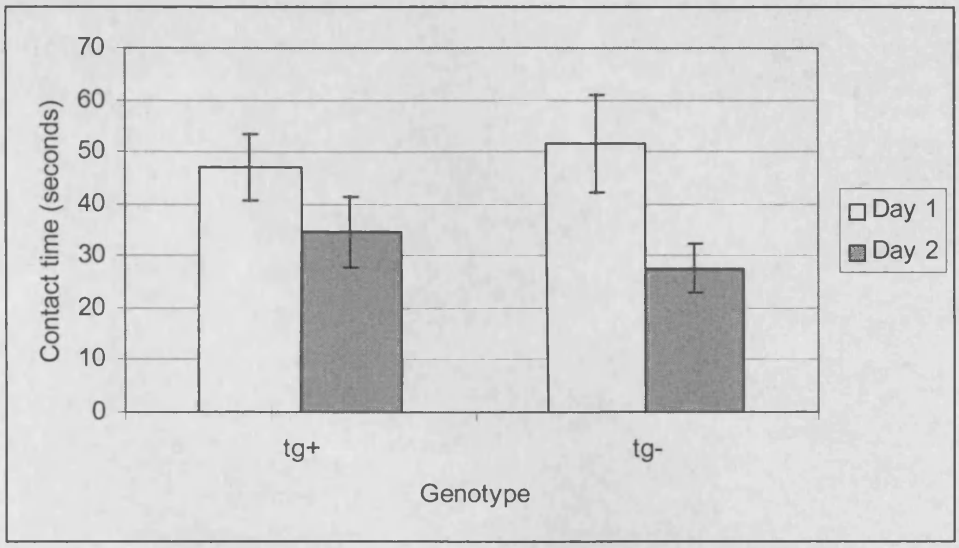


Figure 3.1.1 The mean contact time in seconds for transgenic (tg+) and wild type (tg-) mice with a pair of novel objects over 2 days of habituation. A novel set of objects was presented each day. Error bars show the standard error of the mean.

Experiment 4 phase 2: Object novelty detection with 2 minute, 30 minute and 24 hour delays

Figure 3.1.2 shows the mean contact time with the novel and familiar object across the three retention intervals (2 min, 30 min and 24 hrs). Inspection of this figure suggests that both Tg2576 and control mice showed a preference for exploring the novel object over the familiar object and this preference declined at comparable rates in the two groups as the retention interval increased. An ANOVA confirmed this interpretation and revealed a nonsignificant main effect of group, (F <1), a main effect of retention interval, $F(2,36)= 3.88$, $p < 0.05$, a main effect of Object (novel vs familiar) $F(1,18)=36.39$, $p < 0.001$, a significant interaction between retention interval and object, $F(2,36)= 3.80$, $p < 0.05$. No other main effects or interactions were significant (maximum $F(2,36)=1.15$, $p > 0.10$, group x retention interval x object

interaction). Test of simple main effects revealed a significant effect of retention interval on exploration of the novel object, $F(2,36)=5.03$, $p < 0.05$ and a nonsignificant effect of retention interval on exploration of the familiar object, ($F < 1$). These results show that there were no differences in object novelty detection between adult control and Tg2576 mice at each of the retention intervals. To ensure that we were sensitive to differences between groups, the data were converted into a discrimination ratio (of the form: time spent with novel object [A]/ time spent with both novel and familiar objects [A+B]) and are shown in Table 1. An ANOVA confirmed the absence of an impairment in Tg2576 mice and revealed no main effect of group, ($F<1$), nor delay $F(2,36=1.85$, $p >0.15$, nor interaction between these factors, $F(2,36)=1.80$, $p >0.1$. Both the control and Tg2576 mice differed from 0.5 (no discrimination) at each delay: 2-min delay, $t(9) = 3.35$ and 3.77, $ps < .05$, respectively; 30-min delay, $t(9)= 3.33$ and 6.10, $ps < .05$, respectively: 24- hr delay, $t(9) = 2.82$ and 2.83, $ps < .05$, respectively.

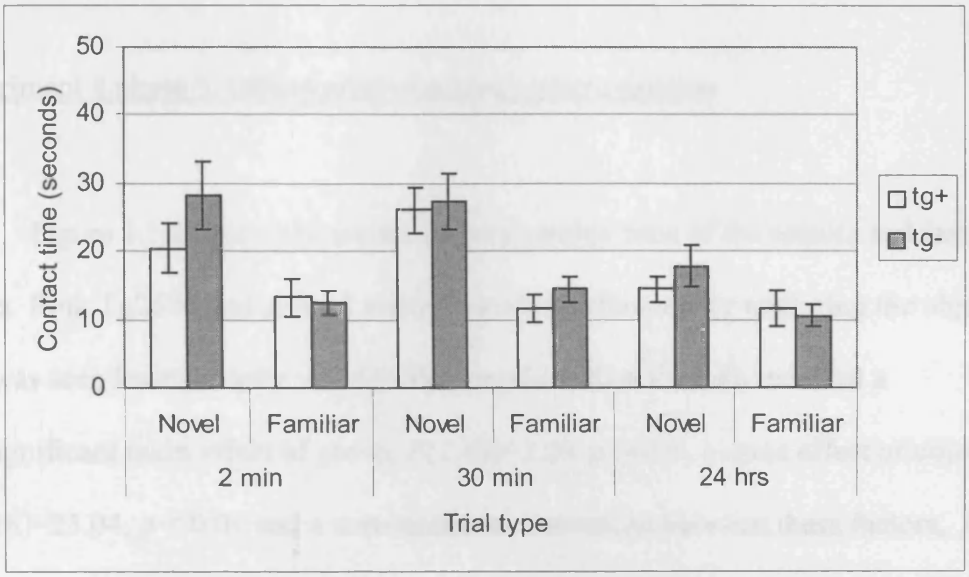


Figure 3.1.2 The mean contact time in seconds for transgenic (tg+) and wild type (tg-) mice with a novel versus a familiar object over successive delay intervals interpolated between the sample and test presentations. Error bars show the standard error of the mean.

Discrimination Ratio					
Novelty				Recency	Spatial Shift
Retention:	2 min	30 min	24 hr		
tg+	0.63	0.69	0.58	0.65	0.46
(SEM)	(0.03)	(0.03)	(0.03)	(0.03)	(0.03)
tg-	0.63	0.64	0.53	0.65	0.61
(SEM)	(0.04)	(0.04)	(0.05)	(0.02)	(0.03)

Table 1 Mean discrimination ratios for adult transgenic (tg+) and wild type (tg-) mice in 3 versions of the object recognition task. Standard error of the mean is shown in brackets.

Experiment 4 phase 3: Object relative recency discrimination

Figure 3.1.3 shows the mean total exploration time of the remote and familiar object. Both Tg2576 and control mice showed a preference for exploring the object that was seen least recently. An ANOVA conducted on the data revealed a nonsignificant main effect of group, $F(1,18)=3.20$, $p>0.05$, a main effect of object, $F(1,18)=23.04$, $p < 0.01$ and a nonsignificant interaction between these factors, $F(1, 18) = 0.84$, $p > .10$. Both the Tg2576 and control mice explored the objects during the test phase at a comparable rate and both groups showed a preference for exploring the object sampled least recently. A similar analysis of a discrimination ratio derived from these data also revealed no significant difference between the groups $t(18) = 0.73$, $p > .10$ (see Table 1), and both the ratios of control and Tg2576 mice differed from 0.5, $t(9) = 5.10$ and 6.08, $ps < .05$, respectively. Both the Tg2576 and control mice explored the objects at a comparable rate and both groups showed a preference for exploring the object sampled least recently.

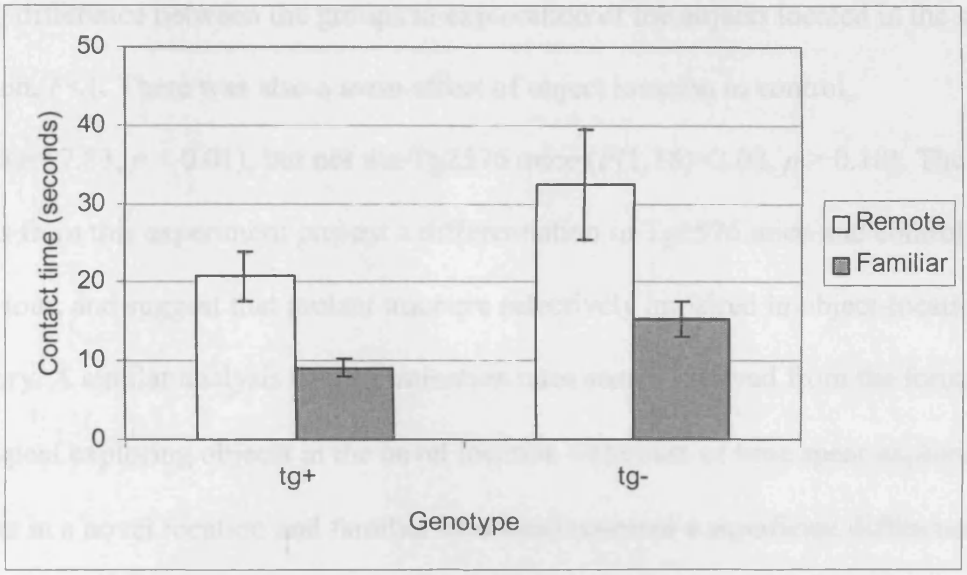


Figure 3.1.3 The mean contact time in seconds with objects presented either recently (Familiar) or more remotely (Remote) in time for transgenic (tg+) and wild type (tg-) mice. Error bars show the standard error of the mean.

Experiment 4 phase 4: Object location memory

Figure 3.1.4 shows the mean contact time with the objects in the same spatial location (Same location) and the objects moved to a different spatial location (Different location). Inspection of this figure suggests that the control mice showed a preference for exploring the objects located in a different spatial position. This effect was absent in Tg2576 mice. This interpretation was confirmed by an ANOVA which revealed a significant main effect of group, $F(1,18)=5.22$, $p < 0.05$, a nonsignificant effect of object location, $F(1,18)= 3.91$, $p > 0.05$, and a significant interaction between these factors, $F(1,18)=15.94$, $p < 0.001$. Subsequent tests of simple main effects showed that there was a significant difference between the groups in exploration of the objects positioned in a different location $F(1,25)=14.09$, $p < 0.01$,

but no difference between the groups in exploration of the objects located in the same position, $F<1$. There was also a main effect of object location in control, $F(1,18)=17.83, p < 0.01$), but not the Tg2576 mice ($F(1,18)=2.02, p > 0.10$). The results from this experiment present a differentiation in Tg2576 mice and control behaviour, and suggest that mutant mice are selectively impaired in object-location memory. A similar analysis of discrimination ratio scores (derived from the formula: time spent exploring objects in the novel location ÷ the sum of time spent exploring objects in a novel location and familiar locations) revealed a significant difference between groups $t(18)= 3.62, p < .05$. The performance of the wild type but not Tg2576 mice differed from 0.5, $t(9) = 4.24, p < .05$ and $t(9) = 1.12, p > .10$, respectively. Tg2576 mice explored the objects in a familiar location as often as control mice. Nevertheless Tg2576 mice failed to increase exploration of familiar objects that had been moved to a new spatial location.

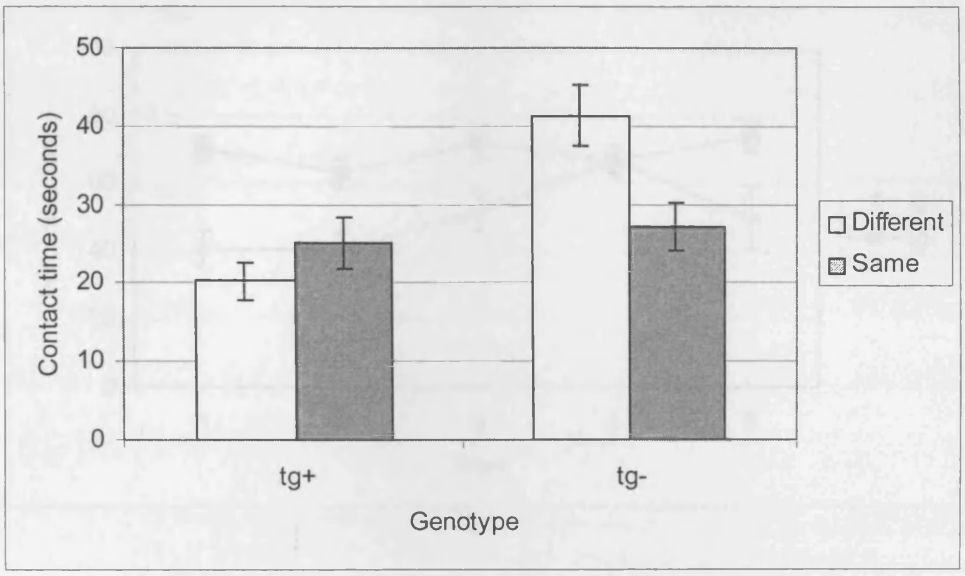


Figure 3.1.4 The mean contact time in seconds for transgenic (tg+) and wild type (tg-) mice with objects located in the same and different positions from the sample trial. Error bars show standard error of the mean.

Figure 3.1.5 shows the mean percent correct responses during acquisition of the T-maze FCA task across the 5 sessions of training. Inspection of Figure 3.1.5 shows that there was a clear difference between the Tg2576 and control mice during acquisition and that mutant mice performed less accurately than control animals. An ANOVA confirmed this interpretation and revealed a main effect of group $F(1,16) = 4.13$, $p < 0.001$. There was no significant main effect of day, $F(4,64) = 1.69$, $p > .10$, and a nonsignificant day by group interaction, $F(4,64) = 1.56$, $p > .10$. These results confirm that the Tg2576 mice remained sensitive to deficits in T-maze task.

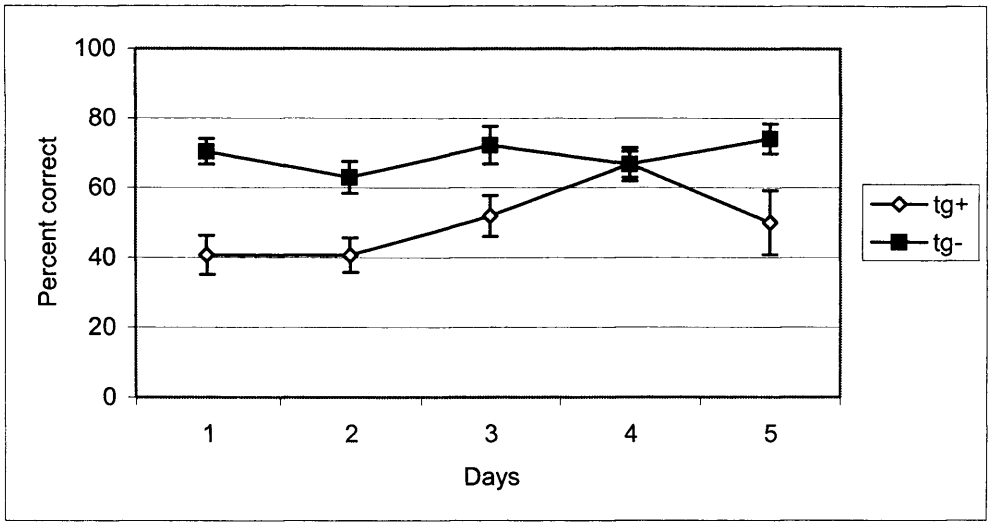


Figure 3.1.5 The mean percent correct choices averaged over 5 days of training on a T-maze forced-choice alternation task for transgenic (tg+) and wild type (tg-) mice. Error bars show the standard error of the mean.

3.1.4. Discussion

The results of the object recognition study show that adult mutant mice were able to discriminate between familiar and novel objects with delays of up-to 24 hours at a level comparable to that of control mice. Adult Tg2576 and control mice were also able to discriminate the relative familiarity of two objects. Both groups of mice showed a preference for investigating the least familiar of two recently presented objects. In contrast to their normal performance on object recognition and relative recency, Tg2576 mice failed to investigate objects that had changed their relative spatial positions. This latter result is indicative of hippocampal damage. However, the analogy between Tg2576 mice and rodents with hippocampal lesions breaks down in consideration of the relative recency results (cf. Marshall et al., 2004).

The pattern of results presented in this study demonstrates a specific deficit in processing spatial information to guide behaviour and has some similarities with the behavioural deficits displayed by AD patients. In particular the use of allocentric cues to navigate around an environment (Monacelli et al., 2003; see also Kavcic & Duffy, 2003), and the visuospatial paired associate learning impairment that appears highly specific and sensitive to early signs of AD cognitive dysfunction (see Swainson et al., 2001, Blackwell et al., 2004, see also Lee et al., 2003).

Tg2576 deficits on spatial navigation tasks generally emerge at 6-8 months of age when the production of insoluble forms of Aβ in cortical and hippocampal areas is increasing (Westerman et al., 2002; Kawarabayashi et al., 2001). The hippocampus is primarily but not exclusively targeted by amyloid deposition in both AD patients and Tg2576 mice (see Braak & Braak, 1995; Hsiao et al., 1996; Chapman et al., 1999; Kawarabayashi et al., 2001). The theoretical underpinnings of the behavioural deficits observed in the mutant mice have, therefore, understandably borrowed from theories of hippocampal function (e.g., King & Arendash, 2002).

Lesion studies with rats have shown that hippocampal cell loss produces a deficit in identifying novel configurations of familiar objects analogous to the deficit observed in AD patients visuospatial memory (Swainson et al., 2001). For example, Gilbert and Kesner (2002) reported that rats with hippocampal lesions were impaired in learning object-place and odour-place associations but were unimpaired in learning object-odour paired associations. Thus the hippocampus in rodents appears to be critical for paired associate learning when object and location associations are required (see also Parkinson et al., 1998; Mumby et al., 2002; Gilbert & Kesner, 2003). However, lesions to the pre-frontal cortex produce a similar deficit in object-place association learning in rats (Kesner & Ragozzino, 2003). This raises the

possibility that the spatial shift deficit demonstrated in this study might reflect more than amyloid related hippocampal pathology. However, the experimental design of this study did not permit the behavioural effects of any such pathology to be independently assessed.

Novelty detection was used in this study to index Tg2576 recognition memory. The results demonstrate that novelty detection was not differentially impaired in the transgenic mice with-up to a 24 hour retention interval. Such findings may initially seem to present a dissociation between APP_{SWE} mouse behaviour and AD related cognitive dysfunction and contrast previous demonstrations of hippocampal pathology in the Tg2576 mice. This discrepancy can however, be resolved with consideration of the dual process theory of recognition memory (Aggleton & Brown, 1999).

Brown and Aggleton (2001) argue that recognition memory can be viewed as a dual process, one that involves both a sense of familiarity and recollection - the process where contextual and temporal information is included in the retrieval of information. This distinction has received support from animal studies which have shown that the perirhinal cortex is involved in the detection of object novelty and the hippocampus in processing spatial and object-location associations (Winters et al., 2004).

The memory deficits observed in AD patients also lend themselves to the dual process distinction. Fox et al. (1998) argue that AD is initially characterized by deficits in recollection. Indeed, early stage AD patients have demonstrated selective recollective memory impairment with an intact sense of familiarity - which recordings of event-related potentials suggest to be a hippocampal-independent process (Tendolkar et al., 1999). Furthermore patients with AD show normal perceptual

priming (Willems et al., 2002) and are able to perform well on recognition memory tasks that do not rely on explicit recall of item information (Karlsson et al., 2003; Dalla Barba, 1997).

The fact that Tg2576 mice showed normal object novelty detection with delays up to 24 hours suggests that the amyloid deposition in mice may leave intact a hippocampal-independent familiarity-based recognition memory processes which seems to be preserved in AD patients. In addition these results indicate that the deficit in object-place memory was unlikely to be the result of some non-specific effect of the mutation on novelty detection *per se* or impaired memory for object information. The object exploration results also signify that such a deficit cannot be due to sampling biases.

A recency discrimination was included in the present study as a task that is sensitive to frontal cortex dysfunction (see Mitchell & Laiacona, 1998). Evaluation of pre-frontal cortex functioning in aged APP_{SWE} mice is important because like advance AD patients they display pervasive plaque pathology throughout many cortical structures (Hardy, 1997; Arendash et al., 2001; Wilcock et al., 2003). Secondly, AD patients demonstrate pre-frontal related behavioural deficits (see Nagahama et al., 2003; Albert, 1996).

The absence of a deficit in recency discrimination in Tg2576 mice suggests that frontal cortex function may remain relatively intact at this age. This conclusion however, illustrates a discrepancy between the mutant mice and AD patients. The ability to identify the temporal order of items presented in a list is impaired in patients with AD (Sullivan & Sagar, 1989; Becker et al., 1993; Storendt et al., 1998). It is of interest to note that Kesner et al. (2002) have showed that rats with hippocampal lesions were impaired relative to control rats in memory for the temporal sequence of

non-spatial odour cues. The hippocampus has also been theorized as playing an integral role in the sequential recall of events (see Fortin et al., 2002). Alternatively recency may be the effect of trace decay (see Wagner, 1981). On the basis of the spatial deficits that may reflect hippocampal damage in these mice, a recency deficit might have been predicted. For example, Marshall et al. (2004) demonstated that rats with excitotoxic hippocampal lesions were more likely to orient to a primed stimulus (one presented more recently) than an unprimed stimulus (one presented less recently). The Tg2576 mice did not behaviour like animals with hippocampal lesions, and showed a preference for the object presented least recently. There are at least two explanations for this null result: a) the hippocampal and/or pre-frontal based mechanisms required for recency discrimination remain insensitive to the plaque pathology in 14 month old Tg2576 mice or b) the task used in this study was not sensitive to a temporal sequencing deficit in the Tg2576 mice (see also Good et al., 2007b). It remains possible that a task that provides a greater challenge to non-spatial sequence memory may reveal impairments in adult Tg2576 mice. Further studies are needed to evaluate this proposal. As present, the ability to discriminate between two familiar items that differ in terms of relative recency is intact in aged Tg2576 mice.

The effects of other APP mutations on recognition memory have met with mixed results. Dodart et al. (1999) reported that 9-10 month old PDAPP transgenic, a strain that possess a human APP mutation (v717f) under control of the platelet-derived growth factor promoter, were impaired in an object novelty detection task using a spontaneous exploration measure. In contrast, Chen et al. (2000) reported that PDAPP mice showed normal object novelty preference across their lifespan up to 18-21 months of age. The latter authors offer no explanation for the differences between the two studies. However, several procedural differences may account for the

different pattern of results. For example, Chen et al. (2000) used a within-subject longitudinal design and Dodart et al. (1999) used different groups at each age range. The use of a longitudinal design may have reduced the sensitivity of the task to age-dependent changes in amyloid production. However, Dodart et al. (1999) also reported that PDAPP mice showed elevated levels of locomotor activity at 6 and 9 months of age (the ages at which differences in novelty detection were observed). Unfortunately, the contact time with the objects at test was not reported and it is unclear whether the elevated levels of locomotor activity in PDAPP mice interacted with object exploration. Clearly further studies with this strain of mice are required to evaluate the effects of this disruption on object novelty and object-place memory.

Middei et al. (2006) recently published results on Tg2576 object recognition performance at 7 and 14 months of age. Similarly to the findings presented in this chapter they found a deficit in aged Tg2576 mice ability to detect a change in the spatial relocation of familiar objects, and this impairment was also evident at 7 months of age. However, their results also present a discrepancy with our findings, as they demonstrated that the aged wild type mice were not sensitive to the spatial relocation of familiar objects or a novelty manipulation.

The authors acknowledged that these are anomalous results, particularly in terms of the spatial deficit, as the same wild type animals demonstrated place learning in a subsequent plus maze task. Middei et al. (2006) reasoned that this dissociation represented a motivational deficit in the wild type animals. The object recognition task was non-appetitive whilst the animals received a food reward in the plus maze task. Consideration of the task demands of Middei et al.'s (2006) object recognition experiment however, offers an alternative explanation. The test procedure was more than double the length of the experiments reported in this chapter. The spatial test

phase was conducted between the 32nd and 37th minute of the experiment, and the novelty task was conducted between 48th and 53rd minutes into the testing procedure. The observed deficits in the spatial and novelty tasks may not reflect age related impairment in object-place memory or novelty detection per se, but demonstrate age related sensitivity to habituation to the general experimental environment. A similar explanation can also be applied to why the aged Tg2576 mice in Meddei et al's (2006) task demonstrated impairment in novelty detection, whilst the same aged animals ran in experiment 4 (phase 1) did not present impairment, even with the introduction of a 24-hour delay.

Middei et al. (2006) adopted a different scoring procedure to the one used in the experiments detailed in this chapter. Novelty (spatial or object identity) was calculated on the basis of a preference ratio, whereby the amount of contact time made with a particular object in the previous trial was subtracted from the contact made with it during the subsequent test phase. For the object identity phase of the experiment this meant that novelty detection was calculated on the basis of a preference for a different object, that was previously located in the position of the novel item. This left the data open to bias through uncontrolled object preferences. If the mice were particularly interested in the said familiar object prior to its removal before the novelty phase, then this would have deflated the object novelty scores. In contrast the scoring method adopted in Experiment 4 can be considered a pure measure of novelty detection. For this reason it can be argued that the results presented in this chapter offer a more robust analysis of Tg2576 behaviour in an object recognition paradigm.

Middei et al. (2006) also report that the transgenic mice made consistently more contact with the objects than the wild type animals. This can be attributed to yet

another experimental caveat. The majority of objects were placed in the periphery of the maze in the Middei et al. (2006) experiment. The habituation data revealed that the transgenic mice spent persistently more time in the periphery and this was significantly different from wild type behaviour at 14 months of age. The increased contact time scores may therefore be a consequence of the Tg2576 bias to remain in the periphery of the maze where the majority of objects were located.

The contact time differences observed in Middei et al.'s (2006) experiment present an intriguing discrepancy with the contact time data from Experiment 4. A comprehensive analysis of the exploratory behaviour of the mice during the sample phases of Experiment 4 revealed that the transgenic mice made significantly less contact with the objects during the exposure phases of the relative recency compared to the control mice (mean 77.3 and 137.6, respectively) $F(1,18) = 11.18, p < 0.05$ and object in-place (mean 56.1 and 88.9 respectively) $F(1,18)=6.39, p <0.05$ tasks. In light of these results, it could be argued that the object-place deficit observed in Tg2576 animals in Experiment 4 (phase4) reflects an uncontrolled sampling bias. This argument seems unlikely however, as it would not explain the unimpaired sensitivity to relative recency demonstrated by the transgenic mice in Experiment 4 (phase 3). Nevertheless, this difference in contact time represents a considerable caveat in our experimental results. The initial set of experiments in Chapter 4 was conducted to address this issue.

3.2 Experiment 5: Impaired "episodic-like" object memory in adult APP_{swe} transgenic mice

3.2.1 Introduction

Recent evidence suggests that mice and rats form integrated memories of the spatial and temporal context in which objects are presented (Dere et al., 2005a, 2005b; Kart-Teke et al., 2006). Consistent with dual processing theories of recognition memory (e.g., Aggleton & Brown, 1999; Mumby, 2001; Sutherland & Rudy, 1989) Good et al., (2007a) recently reported that normal rats explored an object presented earlier in a sequence and in a different location at test relative to other objects that possessed only one of these properties. These results are consistent with the notion that normal rats form an integrated memory of the spatio-temporal properties of objects (see also Fortin et al., 2004; Kart-Teke et al., 2006). In contrast to control rats, rats with hippocampal lesions failed to form an integrated memory of the spatio-temporal properties of objects (i.e., they showed impaired memory for "what," "where," and "when" objects were presented; cf. Clayton et al., 2001; Morris, 2001). These results provide support for theories that posit a central role for the hippocampus/medial temporal lobe region in a conjunctive process that binds an object to the spatio-temporal context in which it was presented. Neuropsychological and neuropathological evidence indicates that during the early stages of Alzheimer's disease medial temporal lobe function is compromised (e.g., Twamley et al., 2006). Although AD is associated with deficits in several cognitive domains, impairments in episodic or declarative memory processes are an acknowledged feature of the early stages of the disease. Nevertheless, the precise relationship between the

neuropathological processes underlying AD and memory function remains unclear. One leading hypothesis has proposed that the development of senile plaques, which are formed by aberrant processing of amyloid precursor protein (APP), is an important step in synaptic, neuronal, and cognitive deterioration (Hardy, 2006). Consistent with the amyloid hypothesis, mice possessing human APP mutations develop impairments in hippocampus dependent spatial memory tasks with age (e.g., Hsiao et al., 1996; cf. Janus et al., 2001). Several influential neurobiological theories of hippocampal function have highlighted the important contribution that spatial information plays in episodic memory (e.g., Burgess, 2002; Gaffan, 1991; Moscovitch et al., 2006; O'Keefe & Nadel, 1978; Smith & Mizumori, 2006). An immediate implication of this view is that APP transgenic mice should show an impaired integrated memory for the spatio-temporal properties of events or items. The present set of experiments, therefore, had two main aims: (a) to determine whether control mice are able to form an integrated memory of the spatio-temporal properties of objects (see also Dere et al., 2005a, 2005b) and (b) to characterize the effects of the APPswe mutation in adult Tg2576 mice on memory for objects and their spatio-temporal properties. Given the pattern of results presented in the previous study we anticipated that exploratory activity in Tg2576 would be sensitive to manipulation of the temporal order of the objects but not the spatial location of the objects. That is, object exploration would reflect the order of the objects but that this property of the objects would not interact with information about the location of the objects.

3.2.2 Methods

Subjects

10 transgenic male mice were compared to 10 male wild type mice to ensure that age and background strains were comparable. All animals were housed either individually or in same-sex mixed genotype litter groups of 2 to 4 animals. Details of mouse breeding, genotyping and maintenance of the colony were similar to those described previously in Chapter 2. All mice were 10-12 months old at the start of behavioural testing and were naïve to the apparatus and procedures used in the present study. The mice were maintained on a 12-h light/dark cycle and had *ad libitum* access to food and water throughout behavioural testing.

Apparatus

The mice received exposure to the objects in a square arena (82 cm x 82 cm), which was constructed from laminated plywood. The walls measured 30 cm in height and were made of clear Perspex that was covered externally with white card. The arena was placed on a stand that elevated the arena 30 cm above the floor of the room and was situated in the centre of a quiet testing room, with a variety of extramaze cues. The floor and walls of the arena were wiped down with 70% ethanol (with distilled water) between each trial. A camera was suspended from the ceiling, 90 cm above the centre point of the arena, and was attached to a video recorder (Panasonic Model Number NV-MV20), monitor and a RISC-PC computer. The movement of the animals in the maze was tracked and recorded using EthoVision (Noldus, Wageningen, Holland).

Objects

All objects used in this series of experiments were sourced from the same collection as that described previously in this chapter, section 3.1.2, page 94. To briefly reiterate, all objects were made from a nonporous robust material. They were of approximate size, and each object was heavy enough to withstand the investigative behaviour of the mice. The objects were wiped down with 70% ethanol in distilled water before they were placed into the maze to eliminate any possible odour cues.

Behavioural Procedures

Habituation

The mice received two days of acclimatization to the test arena for 10 minutes each day. The mice then received two consecutive days of testing on each of the tasks depicted in Figure 3.2.1. The tasks were presented in the order shown in Figure 3.2.1 (proceeding from top to bottom) and were each separated by a 2-day rest period. Each test used a novel set of objects and the nature of the target items was counterbalanced.

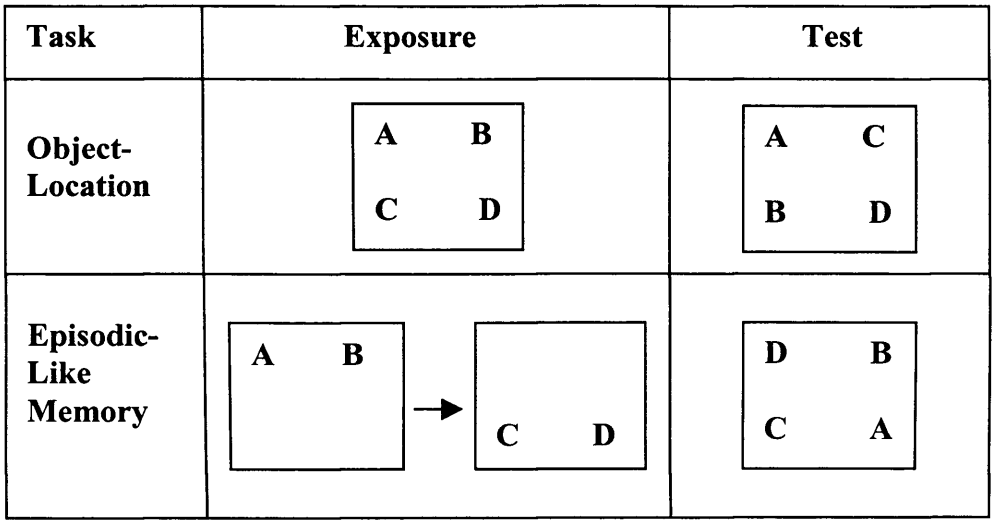


Figure 3.2.1 Summary of the behavioural procedures used for Experiment 5 phase 1 (Object-Location) and Experiment 5 phase 2 (Episodic-like memory). The design first assessed the tendency of mice to explore an object located in a different spatial position with respect to an exposure phase. Phase 2 separately examined the memory for the spatio-temporal properties of objects (i.e. memory for 'what' was presented 'where' and 'when').

Experiment 5 phase 1: Object-location memory

To establish the influence of the APPswe mutation on spatial memory in the 10-12 month old mutant mice, we first examined memory for object locations (cf. Experiment 4). Object exploration was measured as a function of the time the animal spent attending to (actively sniffing or interacting with) the object at a distance no greater than 2 cm (Ennaceur & Delacour, 1988). Object exploration was not scored if the animal was in contact with, but not facing the object or if it sat on the object or used it as a prop to look around or above the object. As well as analysing raw contact

times, the data for Experiment 5 (phase 1 and 2) were converted into a preference ratio measure (of the form: time spent exploring mismatch object(s)/time spent exploring all objects). Preference ratios above .5 indicate that the mouse was exploring the mismatch object(s) more than the other object(s). Analysis of preference is less subject to influence of variability in the individual mice's duration of contacts with the objects.

Four different objects were placed in the middle of the central four squares of the arena, approximately 20 cm apart (centre to centre) and approximately 30 cm from the walls (see Figure 3.2.1; A, B, C, & D). As previously described in Experiment 4, the mouse was placed into the arena at the mid point between the four objects. During the sample phase the animal was allowed to explore the objects for 10 minutes whereupon it was removed and placed in a holding cage. The arena and all the objects were thoroughly cleaned with 70% alcohol. The same four objects were then repositioned in the maze. Two objects were replaced in the same position that they had occupied during the sample phase; the remaining two objects switched positions. The spatial displacement took place on a diagonal plane such that the top left object during exposure would be placed in the bottom right position during the test phase and vice versa. The direction of the diagonal switch top left – bottom right versus top right – bottom left, was counterbalanced across genotype and day. After a two-minute retention interval the mouse was then placed between the four objects and allowed to explore the new arrangement for 10 minutes. The animals received two sessions of testing conducted on separate days. Novel objects were used in each session.

Experiment 5 phase 2: Episodic- like memory

After a two-day rest interval the mice were then tested on the spatio-temporal context test of recognition memory. Each mouse received two (10 minute) sample stages (see Figure 3.2.1) separated by a 2-minute interval during which they were placed in a holding cage. In the first sample stage, half of the mice in each group were presented with two different novel objects (A & B) located in the top two central squares of the arena, approximately 20 cm apart and approximately 30 cm from the side walls (their position left or right was counterbalanced within groups). The remaining mice received presentations of two different novel objects, one in each of the lower two central squares of the arena (again with the positions counterbalanced). During the interval, when the mouse was placed in a holding cage, the first set of objects were removed, the maze cleaned and the objects replaced with two different novel objects (C & D) located in the central squares that were unoccupied in the first sample trial. The mouse was then released from the centre of the arena and allowed to explore these objects for a further 10 minutes. Each mouse was then placed in a holding cage for 2 minutes, during which time copies of each of the 4 objects (A, B, C & D) were placed in the arena. Two of the objects (B & C), one from the first sample array and one from the second, were placed in the same locations used during the sample stage. The remaining two objects (A & D) exchanged positions. The identities of the pairs of objects that switched locations and those that did not was counterbalanced as was the identities served as either B or C and A and D. For example, 5 transgenic and 5 control animals are exposed to objects A and B first, and 5 transgenic and 5 control mice are exposed to objects C and D first. Half of all the animals at the test phase have a top left- versus bottom- right switch, and half a top

right- versus bottom –left switch. This would mean that an object (A, B, C, or D) would have changed its relative location and have been seen least recently on either four (2 tg+ and 2 tg-) or six (3 tg+ and 3 tg-) trials per test session. The mouse was then reintroduced into the arena and allowed to explore the object array for a further 10 minutes. We predicted that an object that had been presented recently and was placed in the same spatial location (i.e., object C; see Figure 3.2.1) would elicit the least exploratory activity and that the object that had been presented earlier in a sequence and was presented in a different spatial location during the test stage (i.e., object A; see Figure 3.2.1) would generate the most exploratory activity. The animals received two sessions of testing conducted on separate days. Novel objects were used in each session.

3.2.3. Results

Experiment 5 phase 1: Object-location memory

The time spent exploring the objects during the exposure stage for transgenic and wild type mice was 37.85 (SE= 8.94) and 41.57 (SE= 4.96), respectively. A t-test confirmed that was no significant difference between these means, $t(18)=0.33$, $p>0.74$. Figure 3.2.2 shows the mean exploration time with test objects that were either located in the same position (B & C) or in different positions (A and D; see Figure 3.2.1). Wild type mice were more likely to explore the objects presented in a different position than those presented in the same position. However, this preference was not apparent in Tg2576 mice. An ANOVA revealed that there was no significant main effect of group, $F(1,18)=4.02$, $p>0.05$, there was a significant main effect of object, F

(1,18)=11.63, $p<0.01$, and a significant interaction between these factors, F (1,18)=7.62, $p<0.05$. Tests of simple main effects revealed a significant preference for objects located in a different position in control mice, F (1,18)=10.22, $p<0.01$, but revealed no such preference in Tg2576 mice, F (1,18)=0.21, $p>0.59$. There was also a significant main effect of group for contact with the objects located in a different position, F (1,31)=10.22, $p < 0.01$, but no significant differences in contact times with the objects located in the same position, F (1,31)=0.22, $p>0.10$. This analysis was confirmed by an analysis of preference ratios. The mean preference ratio for wild type mice and Tg2576 mice was 0.66 (SE= ± 0.02) and 0.47 (SE= ±0.04) respectively, t (18)= 3.37, $p < 0.01$. In addition, the performance of the control mice, but not transgenic mice, differed significantly from 0.5, t's (9)= 6.36, and 0.67, $p<0.05$ and $p>0.10$, respectively.

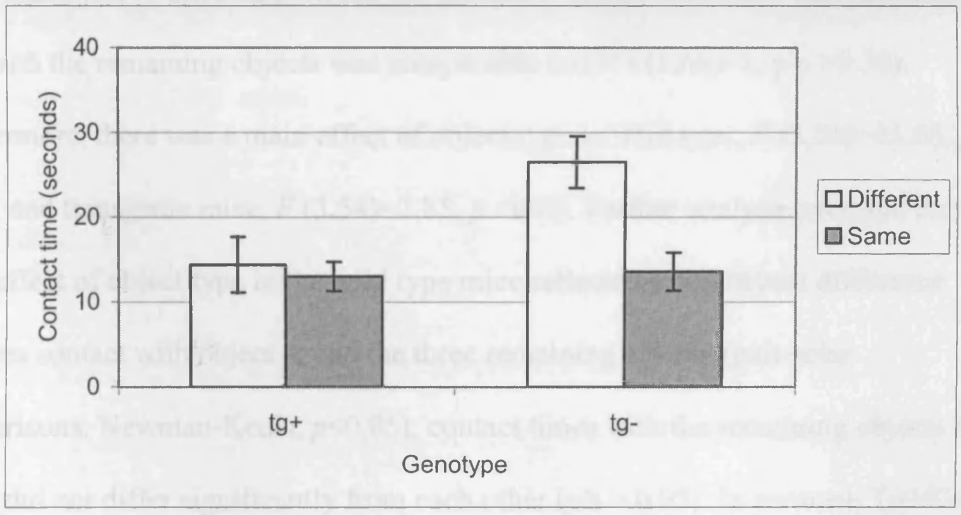


Figure 3.2.2 The mean contact time shown by transgenic (tg+) and wild type (tg-) mice with objects presented in either the same or a different spatial location. Error bars represent the standard error of the mean.

Experiment 5 phase 2: Episodic-like memory

The mean total time spent exploring the objects across the sample phases of the task for wild type and Tg2576 mice was 36.22 (SE= 7.16) and 21.22 (SE=2.47). A t-test confirmed there was no significant difference between means ($t(18)=1.98$, $p>0.05$). During the test stage, wild type mice spent more time exploring object A (see Figure 3.2.3), which was presented both earlier in the sequence and in a different location relative to the remaining objects (B, C & D). In contrast, transgenic mice showed a preference for exploring objects that were presented earlier in the sequence (A and B) irrespective of whether they had changed spatial location. An ANOVA revealed a significant main effect of group, $F(1,18)=6.02$, $p< 0.05$, a main effect of object, $F(3,54)=14.26$, $p < 0.001$ and a significant interaction between these factors, $F(3,54)=3.65$, $p<0.05$. Simple main effects analysis revealed that the mean time exploring object A differed between the groups, $F(1,68)=16.74$, $p< 0.01$, but contact time with the remaining objects was comparable (all F's $(1,68)<1$, p's >0.30). Furthermore, there was a main effect of object type in wild type, $F(3,54)=15.06$, $p< 0.001$, and transgenic mice, $F(3,54)=2.85$, $p <0.05$. Further analysis revealed that the main effect of object type in the wild type mice reflected a significant difference between contact with object A and the three remaining objects (pair-wise comparisons: Newman-Keuls, $p<0.05$); contact times with the remaining objects B, C and D did not differ significantly from each other (p's > 0.05). In contrast, Tg2576 mice showed greater contact times with objects presented earlier in the sequence than objects presented recently, irrespective of changes to their spatial location (Av C & D; B v C & D, Newman-Keuls, $p<0.05$). Importantly, this indicates that Tg2576 mice were able to discriminate between the objects and their exploratory performance was

influenced primarily by the temporal sequence in which the objects were presented and not by memory for their spatial location (cf. Experiment 4).

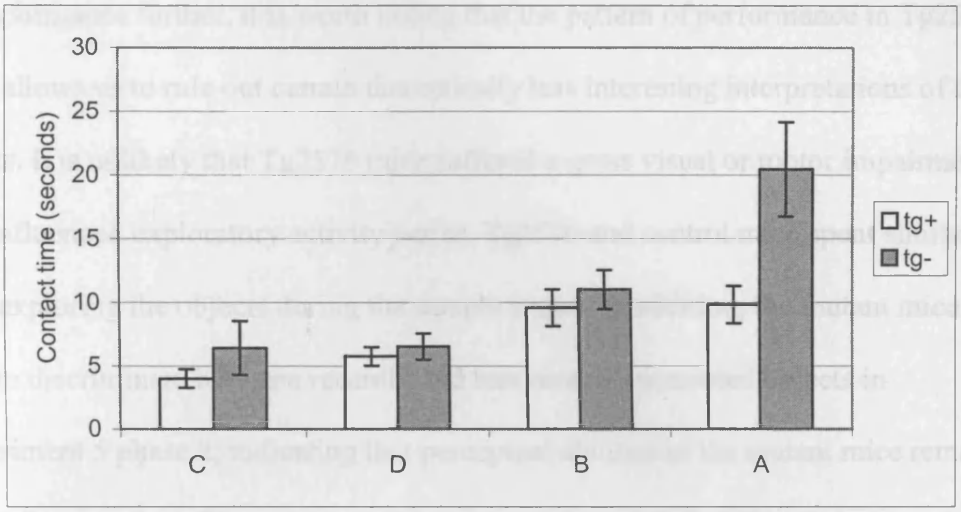


Figure 3.2.3 The mean contact time with individual objects made by transgenic (tg+) and wild type (tg-) mice during the spatio-temporal recognition memory test. Object A was presented earlier in a sequence and in a different location. Error bars represent the standard error of the mean.

3.2.4. Discussion

The present study demonstrated that, similar to rats (Good et al., 2007a), control mice show increased exploration of an old object that was presented in a different spatial location (see also Good et al., 2007b). These results are consistent with the view that the spatial and temporal properties of the objects interacted to influence performance and is consistent with the view that rodents are able to form an integrated memory of the spatio-temporal context in which an object was presented. In contrast, the exploratory activity of the Tg2576 mice was influenced only by

temporal memory as we predicted. The Tg2576 mice failed to react to changes in the spatial location of the objects and thus to integrate this information with the temporal sequence of the objects. Before considering the theoretical implication of the pattern of performance further, it is worth noting that the pattern of performance in Tg2576 mice allows us to rule out certain theoretically less interesting interpretations of the results. It is unlikely that Tg2576 mice suffered a gross visual or motor impairment that influenced exploratory activity *per se*. Tg2576 and control mice spent similar time exploring the objects during the sample stages. In addition, the mutant mice were able to discriminate between recently and less recently presented objects in Experiment 5 phase 2, indicating that perceptual abilities of the mutant mice remained intact. Furthermore, Experiment 4 demonstrated that adult Tg2576 mice showed comparable memory with control mice for object familiarity. In the same study, adult Tg2576 mice showed normal memory for the temporal order of objects but impaired memory for the location of objects when assessed independently. The present study has replicated and extended this analysis by showing that (a) wild type mice, unlike adult Tg2576 mice, are able to form an integrated memory of the spatial and temporal properties of objects (see also Dere et al., 2005a, 2005b) and (b) in a simultaneous test of memory for object location and temporal order, the exploratory activity of Tg2576 mice was influenced primarily by the temporal order of the objects. These findings represent the first demonstration that adult Tg2576 mice are unable to integrate "what" "where" and "when" (episodic-like) information. The spatial nature of this deficit underlines the critical role of spatial information in episodic (and episodic-like) memory processes (cf. Burgess, 2002; Gaffan, 1991; O'Keefe & Nadel, 1978).

Although a direct comparison with memory impairments in patients with AD should be regarded with caution, it is interesting to note that the pattern of impaired

visuospatial memory in Tg2576 mice finds parallels with memory deficits in patients during the early stages of the disease. For example, object-place memory tasks are highly sensitive to AD pathology, and these tasks are able to discriminate patients with AD from other patient groups, for example, depressive patients and patients with fronto-temporal dementia (Lee, et al., 2003; Swainson et al., 2001). Indeed, it has been proposed that such tasks may provide an accurate preclinical marker for AD-related cognitive decline (Blackwell et al., 2004).

Experiment 4 demonstrated that adult Tg2576 mice are able to perform an object novelty/familiarity task as well as control mice. It is also interesting to note that familiarity-based forms of recognition memory, although impaired, are superior to episodic dependent forms of recognition memory in patients during the early stages of the disease (Dalla Barba, 1997; Camus et al., 2003; Fleischman et al., 2005; Gallo et al., 2004; Karlsson, et al., 2003; Knight, 1998; Willems, et al., 2002). However, it should be acknowledged that other APP transgenic mice are impaired in object novelty detection (e.g., Dodart et al., 1999). Although the reasons for this discrepancy are unclear, there are several factors, such as strain differences and differences in experimental methodology, see discussion of Experiment 4.

In conclusion, by using a novel behavioural procedure, we have shown that (in wild type mice) memory for the spatial and temporal features of objects interact to influence exploratory behaviour (cf. Aggleton & Brown, 1999; Dere et al., 2005a, 2005b; Good et al., 2007a,b). Furthermore, the results suggest that the aberrant processing of APP disrupted memory for the spatio-temporal properties of objects. The precise mechanism by which aberrant APP processing influences cognitive function remains unclear. Recent evidence suggests that the accumulation of soluble oligomers of amyloid beta may be sufficient to cause functional (synaptic) deficits

prior to overt cell death (see Catalano et al., 2006, for a recent review). Indeed, Lesné et al. (2006) recently reported that the accumulation of a 56-kDa soluble amyloid assembly was associated with impaired memory, independent of plaques or cell loss, in middle-age (6–14 months old) Tg2576 mice. The presence of memory deficits in 10–12 month old mice in the present study is consistent with this hypothesis and indicates that aberrant processing of APP can disrupt visuo-spatial memory processes that may be integral to the formation of episodic-like memories.

Exploration and Visuospatial memory in Tg2576 mice

A number of anomalies in the experimental designs of the object recognition paradigm detailed in Chapter 3 needed to be addressed before further tests exploring the Tg2576 visuospatial (object-place association) deficit could be implemented. The initial part of Chapter 4 examined genotypic differences in the amount of contact time made with the objects during a 10 minute sample phase observed in the present study. The second part of chapter presents a series of experiments that were designed to characterise the specific nature of the visuospatial deficit observed in the Tg2576 mice reported in Chapter 3.

4.1 Experiment 6: Pre-training habituation to the arena and objects.

4.1.1 Introduction

The first set of experiments detailed in Chapter 4 evaluated exploratory activity in an open field in Tg2576 mice, both with and without objects presented in the arena. This followed the surprising outcome of the first phase of habituation where the Tg2576 mice showed lower levels of contact with the objects during the initial stages of pre-training. Similar to the habituation protocol conducted in Experiment 4, measures of activity were taken and the contact time made with each object was measured. Middei et al. (2006) reported that in a circular open field, Tg2576 mice spent significantly more time in the periphery than in the centre of the maze, at both 7

months and 14 months of age. Wild type mice in comparison only demonstrated this trait of anxiety at 7 months of age. The amount of time spent by the animals in different regions of the maze has important implications for interpretation of the effects of object manipulations on exploration. We therefore assessed the effects of the APPswe mutation on exploratory activity in the arena and towards objects.

4.1.2 Method

Subjects

11 male transgenic mice and 11 male wild type mice were used in this experiment. They were sourced from the breeding colony of Tg2576 mice at the School of Psychology, University of Wales. Breeding and maintenance information have previously be detailed in chapter 2.1. All the mice were 16 months of age at the start of Experiment 6. The same animals were also used in Experiments 7 and 8. At the start of Experiment 6 all mice were naive to the open field arena and to the objects and procedures used in the spontaneous object recognition task.

Apparatus

Open field

The open field arena has previously been described in Chapter 3, section 3.1.2, page 93. The experiment was conducted in the same testing room, and the extramaze cues detailed previously, were provided. The central location of the maze remained constant. The same cleaning protocol, using 70% alcohol (in distilled water) to wipe down the walls and floor of the arena and objects was also adopted.

Objects

All objects used in this series of experiments were sourced from the same collection as that described in Chapter 3, section 2.1.2, page 94.

Tracking equipment

The same tracking equipment and set up was used as in Chapter 3. A camera was suspended from the ceiling 90 cm above the centre point of the arena and was attached to a video recorder (Panasonic Model No. NV-MV20), monitor, and a computer. The movement of the animals in the maze was tracked using EthoVision (Noldus, Wageningen, Holland). The same scoring protocol detailed in chapter 3 was adopted. Object exploration was defined as the time spent attending to (actively sniffing or interacting with) the object at a distance no greater than 2 cm (Ennaceur & Delacour, 1988). Object exploration was not scored if the animal was in contact with but not facing the object or if it sat on the object or used it as a prop to look around or above the item. Each object was assigned a zone and a keyboard button to identify it. Pressing the key signified the beginning or end of investigative behaviour. EthoVision recorded the total exploration time for each target zone.

Behavioural Training

All mice received 10 minutes of free exploration of the maze for 2 consecutive days. During this period no objects were presented in the arena. This was then immediately followed by 2 days of object habituation. Prior to the start of each object habituation trial, two identical objects were placed in the middle of the arena approximately 10 cm apart. The position of the target zones remained the same

throughout object habituation. Each animal was allowed to explore the objects for 10 minutes and was then removed from the arena and placed back into its holding cage. After this initial period of habituation, all mice received a further period of 3 days of habituation to the arena with a pair of novel objects after it emerged that Tg2576 mice showed lower contact times with the objects than the wild type mice. The arena was divided into different areas (see Figure 4.1.3) in order to evaluate how long the mice were spending in the centre of the arena compared to the periphery. A potential explanation as to why the transgenic mice had lower contact times with the objects is that they were displaying neophobia. Heightened anxiety in the transgenic animals may have led them to favour the areas at the periphery of the apparatus. The amount of time the animals spent in the corner squares of the arena was also evaluated.

4.1.3 Results

Habituation

Figure 4.1.1 demonstrates that the control mice systematically made more contact with the objects over two days of habituation. This interpretation was confirmed in an ANOVA that revealed a main effect of genotype $F(1,20)= 6.55, p < .05$, a main effect of day $F(1,20) = 13.72, p < .001$ and a nonsignificant interaction between these factors $F(1,20) = .45, p > .50$. Both transgenic and wild type mice displayed habituation to the objects over the two days; the control animals however, spent significantly longer exploring the objects on both days. Analysis of the animal's locomotor activity (total distance moved in centimetres) indicated that this bias is not due to hyperactivity in the Tg2576 mice (see figure 4.1.2). An ANOVA conducted on

the locomotor activity obtained from the 4 day period of habituation (first two days without objects) revealed no main effect of genotype, $F(1,20) = 1.59$, $p > .10$, no main effect of day $F(3,60) = .86$, $p > .10$ and a nonsignificant interaction between these factors $F(3,60) = .17$, $p > .50$.

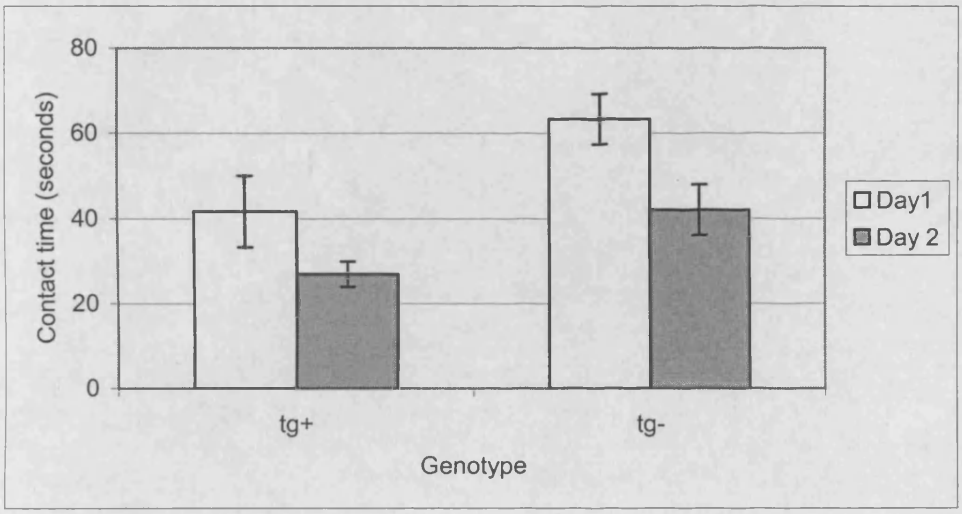


Figure 4.1.1 The mean contact time in seconds for transgenic (tg+) and wild type (tg-) mice with a pair of novel objects over 2 days of habituation. The same set of objects was presented each day. Error bars show the standard error of the mean.

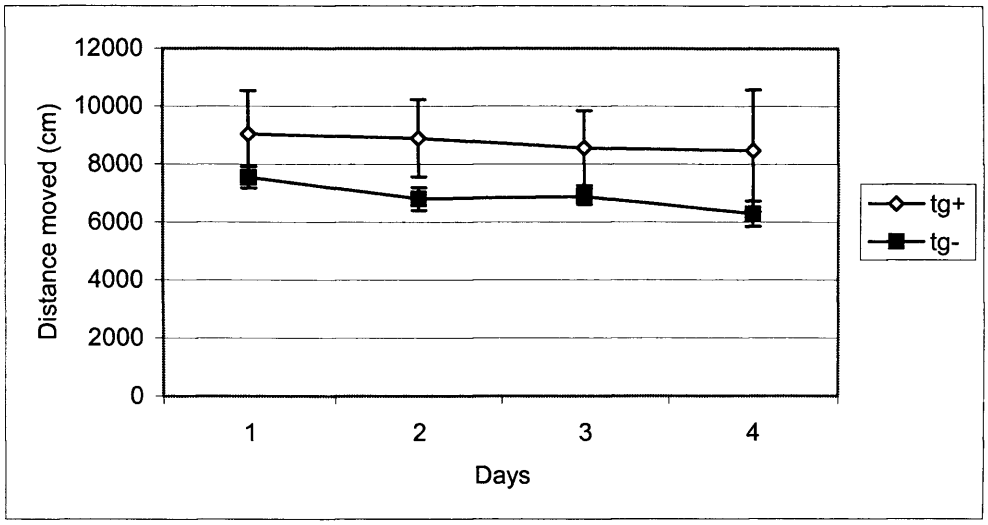


Figure 4.1.2 The mean distance moved in centimetres (cm) for transgenic (tg+) and wild type (tg-) mice over a 4 day period of habituation (last two days with objects present in the arena). Error bars show the standard error of the mean.

Figure 4.1.3 demonstrates the division of the maze into corner, peripheral and central zones during the habituation analysis. Automated scores using the Etho Vision tracking equipment were used to calculate the total amount of time each animal spent in the zones during the 10 minute period of habituation per day. Figure 4.1.4 demonstrates the average amount of time the transgenic and wild type mice spent in each of the 3 zones across the 4 day habituation period. Zone preference was divided into the first two days of habituation to the open field and then the subsequent two days with objects (see figure 4.1.4). An ANOVA conducted on the open field data revealed no main effect of genotype $F(1,20) = .290$, $p>.50$, a main effect of zone $F(2,40)$ 63.07, $p < .001$, a main effect of day $F(1,20)$ 109.79, $p < .001$, a significant zone by genotype interaction $F(2,40) = 4.405$, $p < .05$, a nonsignificant day by genotype interaction $F(1,20) = .270$, $p>.05$, a nonsignificant zone by day interaction $F(2,40) = .406$, $p > .50$, and a nonsignificant genotype by day by zone interaction,

$F(2,40) = 2.07, p > .10$. Subsequent tests of simple main effects showed that there was a significant difference in the amount of time the animals spent in the 4 corners of the arena $F(1,40) = 8.54, p < .01$ but no differences in the groups between time spent in the periphery and the centre $F(1,40) = .98, p > .10$, $F(1,40) = 3.7, p > .05$, respectively. An ANOVA conducted on the last two days of habituation (with objects) revealed no main effect of genotype $F(1,20) = 2.12, p > .10$, a main effect of zone $F(2,40) = 11.02, p < .001$, no main effect of day, a nonsignificant genotype by zone interaction $F(2,40) = 1.69, p > .10$, a nonsignificant day by genotype interaction $F(1,20)$ .526, $p > .10$, a significant day by zone interaction $F(2,40) = 3.83, p < .05$, and a nonsignificant genotype by day by zone interaction $F(2,40) = .70, p > .50$. Subsequent tests of simple main effects showed that on average the amount of time the animals spent in the corners and centre area of the maze differed over the two days but the amount of time they spent in the periphery remained constant $F(1,20) = 8.2, p < .01$, $F(1,20)$ 4.39, $p < .05$, $F(1,20)$ .07, $p > .50$. Visual inspection of figure 4.1.4 shows that both the transgenic and wild type animals spent longer in the centre of the maze on the third day of habituation compared to the fourth, and spent longer in the corners of the maze on the fourth day of habituation compared to the previous day. This complements the contact time scores (see Figure 4.1.1) that demonstrates that both wild type and transgenic mice spent less time in contact with the objects in the centre of the maze on the fourth day of habituation.

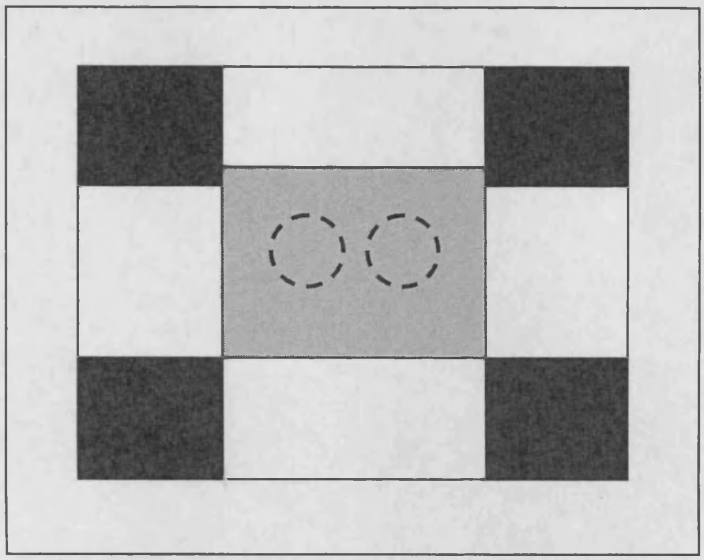


Figure 4.1.3 Zone distribution in the open field during habituation. Black squares represent corner zones, the white oblongs represent the periphery and the grey square represents the centre zone. The dashed black circles indicate the location of the objects on the last two days of the initial habituation, and during all three days of the subsequent rehabituation.

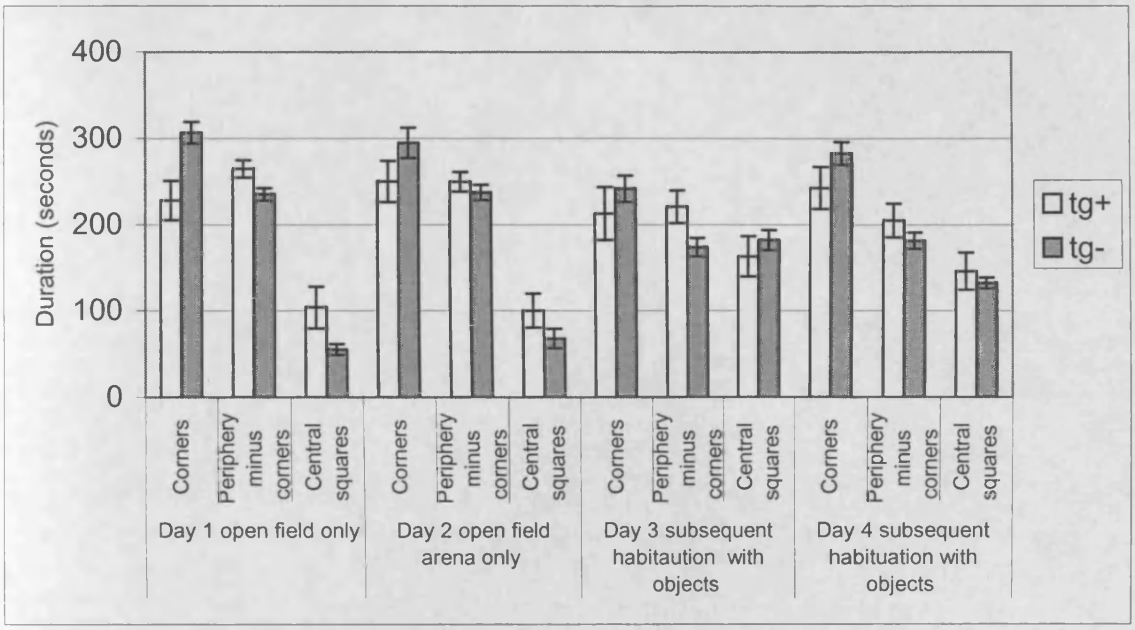


Figure 4.1.4 The mean time transgenic (tg+) and wild type (tg-) mice spent in different areas of the maze (corner squares, periphery, and central zone) during the initial 4 days of habituation with and without the presentation of objects. Error bars show the standard error of the mean.

Rehabituation

All animals subsequently underwent 3 days of rehabituation to the open field and a novel set of objects. Figure 4.1.5 illustrates that the differences in contact time between the two groups persisted across all three days of rehabituation. This interpretation was confirmed in an ANOVA that showed a main effect of genotype $F(1,20) = 23.2, p < .001$, no main effect of day $F(2,40)=1.79, p > 10$, and a nonsignificant interaction between these factors $F(2,40) = 2.35, p > .10$. Analysis of the animals' locomotor activity across the 3 day period of rehabituation also indicated that the Tg2576 mice did not display hyperactivity (see figure 4.1.6). An ANOVA

conducted on the locomotor scores (total distance moved in centimetres) revealed no main effect of genotype $F(1,20) = 2.03, p > .10$, a main effect of day $F(2,40) = 3.79, p < .05$, and a nonsignificant interaction between these factors $F(2,40) = 2.22, p > 10$. The transgenic and wild type animals showed decreasing locomotor activity by day 3 of rehabituation, but the distance covered by the mice was not significantly different between the genotypes.

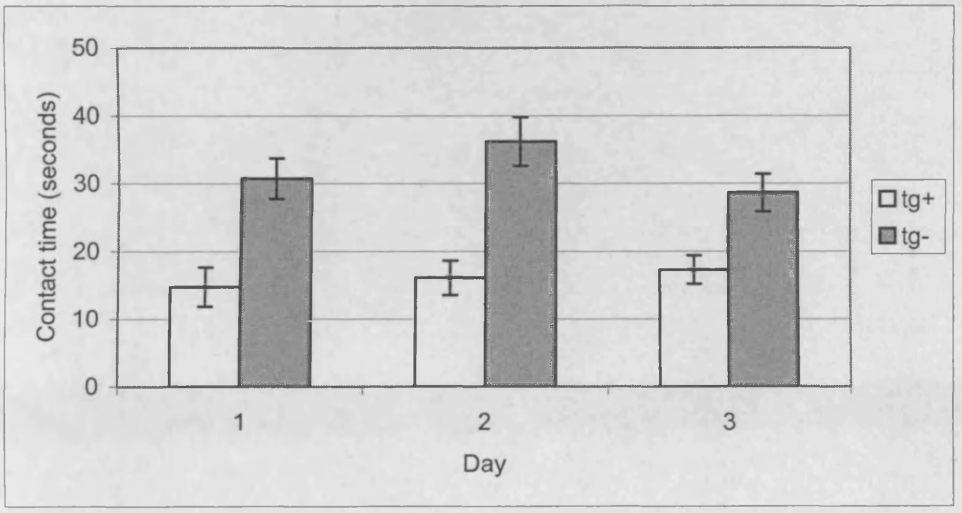


Figure 4.1.5 The mean contact time in seconds for transgenic (tg+) and wild type (tg-) mice with a pair of novel objects over 3 days of re-habituation. The same set of objects was presented each day. Error bars show the standard error of the mean.

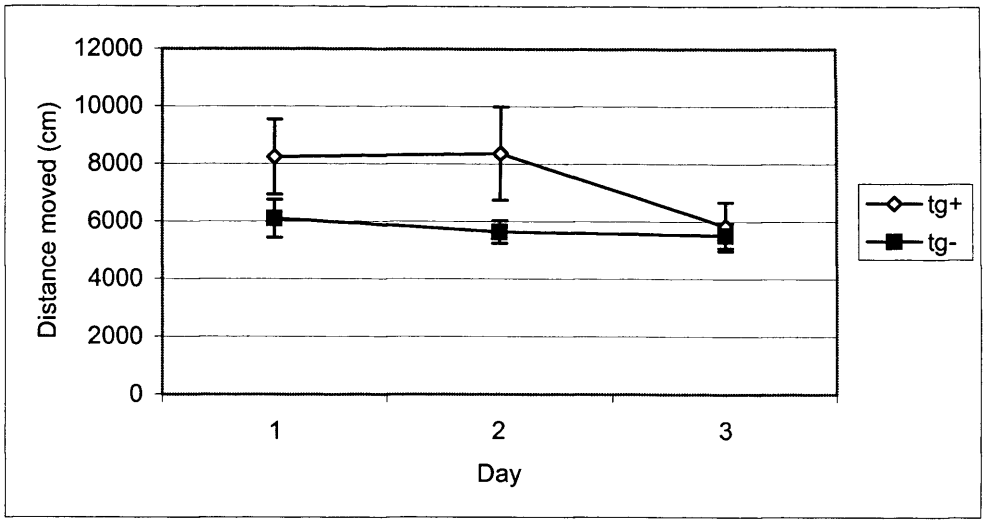


Figure 4.1.6 The mean distance moved in centimetres (cm) for transgenic (tg+) and wild type (tg-) mice over a 3 day period of rehabituation. Error bars show the standard error of the mean.

Figure 4.1.7 shows the average amount of time the transgenic and wild type mice spent in each of the zones in the arena (corner squares, periphery, central area) across the 3 day rehabituation period. An ANOVA conducted on the duration data revealed a main effect of genotype $F(1,20) = 6.6, p < .05$, a main effect of zone $F(2,40)$ 31.01, $p < .001$, no main effect of day, $F(2,40) = 1.76, p > .10$, a significant zone by genotype interaction $F(2,40) = 4.89, p < .05$, a nonsignificant day by genotype interaction $F(2,40) = 1.78, p > .10$, a nonsignificant location by day interaction $F(4,80) = 2.48, p > .05$, and a nonsignificant genotype by zone by day interaction $F(4,80) = 1.34, p > .10$. Subsequent tests of simple main effects showed that the wild type animals spent a significantly greater proportion of time in the corners $F(1,40) = 6.73, p < .05$, whilst the transgenic mice spent more time in the periphery $F(1,40) = 7.92, p < .05$. However all animals spent a comparable amount of time in the centre of the arena $F(1,40) = .02, p > .50$.

The preceding analyses suggest that the differences in object contact times between Tg2576 and wild type mice were not caused by gross changes in locomotor activity *per se* or gross differences in the way that activity was distributed throughout the arena.

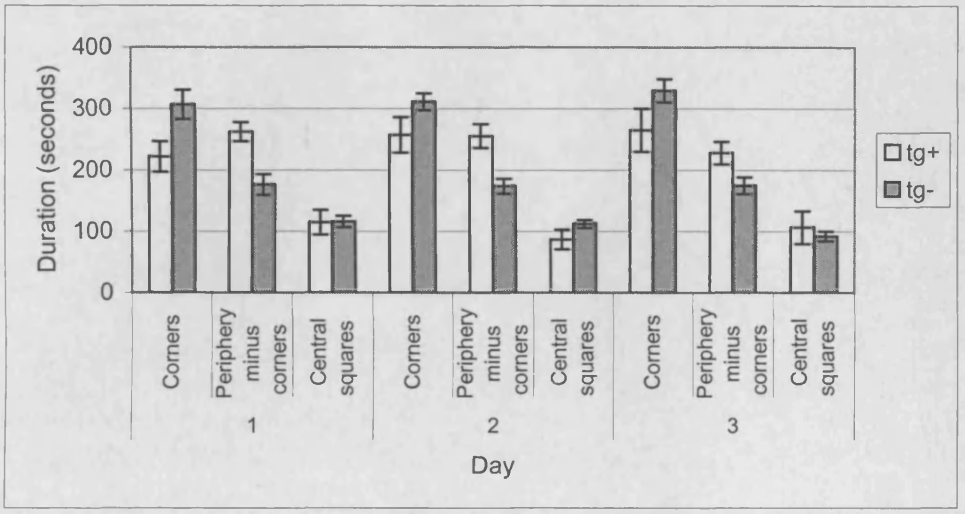


Figure 4.1.7 The mean time transgenic (tg+) and wild type (tg-) mice spent in different areas of the maze (corner squares, periphery, and central zone) during the 3 days of rehabituation with objects. Error bars show the standard error of the mean.

4.1.4 Discussion

The results from Experiment 6 demonstrate that the transgenic mice consistently spent less time investigating a pair of objects than the wild type controls. This cannot be attributed to hyperactivity, as the animals moved around the arena to a similar extent. These results do not correspond with the findings of Middei et al. (2006) or the locomotor data from Experiment 1. In this experiment a measure of total distance moved in centimetres was taken as the locomotor score. Experiment 1 adopted a strategy similar to that used in Middei et al.'s (2006) study, whereby the arena was divided into equal proportions, and the number of lines crossed was taken as a measure of locomotor activity. The difference in levels of activity may therefore reflect a difference in the methods used in measuring locomotor activity. Alternatively the animals in Experiment 1 were 12-14 months of age, whilst the animals in these experiments were over 16 months of age, so the difference in activity levels may reflect an effect of aging in the Tg2576 mice.

Both the wild type mice and transgenic animals spent a comparable amount of time in the centre of the arena where the objects were located. Unlike the bias demonstrated in Middei et al.'s (2006) study (see discussion Experiment 4), a genotypic difference in the amount of time spent in the area where the objects were located, cannot explain the difference in contact time scores observed in Experiment 6.

To verify the accuracy of the experimenters scoring, a comparison between the manually obtained data and the automated scores provided by Etho Vision was conducted for each of the habituation stages. Etho vison automatically recorded when the mouse was in the target zone. This was a gross measure and did not differentiate between when the mouse was facing and engaging with the object or when it was

running past the object or sat with it's back to it. The results revealed that manually obtained scores significantly correlated with the automated scores (wild type initial habituation data r = 0.56 $p<.01$, transgenic initial habituation data r = 0.49, $p<.05$, wild type rehabituation data r = 0.7 , $p < .01$, transgenic rehabituation data r = 0.55, $p < .01$). This revealed that an experimental bias in the manually recorded scores cannot account for the genotypic difference in contact time. It also suggests that when the mice were in the target zone they were investigating the object.

In order to equate the transgenic and control mice exposure to the object we adopted a yoked exposure method, whereby transgenic and wild type animals were paired. The transgenic mice received ten minutes during the sample phase to investigate the objects and their total investigative time was recorded. The wild type partners of these mice would be removed from the sample stage once they had accumulated the same amount of contact time with the objects as their mutant counterparts. The advantage to this would be that the amount of actual contact with the objects per se would be equated across groups. The obvious disadvantage to this would be that the wild type animals would have less overall exposure to the object arrays in the arena.

4.2 Experiment 7: Evaluating Tg2576 object-familiarity, object-in place and object location memory using a yoked sample procedure.

4.2.1 Introduction

Chapter 3 reported that adult Tg2576 mice were impaired in detecting a change in the spatial location of objects in an open field arena. In this procedure two

objects exchanged location between the sample and test trials while two other objects remained in the same location. In contrast, Tg2576 mice were as sensitive as control mice to the presentation of a novel object. These results suggested that Tg2576 are impaired in forming a representation of the spatial organisation of objects in an arena but not impaired in discriminating familiar from novel items. However, two issues remain unresolved.

The first concerns the extent to which the APPswe mutation selectively disrupts processes specific to memory for location information as opposed to object identity. In the study conducted in Chapter 3, object novelty was assessed using a two-item object array, whereas memory for object location was assessed using a four-item object array. Given that the role of the hippocampus in object recognition memory is controversial (Aggleton & Brown, 1999; Clark, Zola & Squire, 2000; Mumby, 2001; Brown & Aggleton, 2001; Broadbent et al., 2004; Ainge et al., 2006), it remains possible that the disruptive effects of the APPswe mutation in Tg2576 mice on recognition memory may have been underestimated by using a small sample size. More specifically, object recognition memory involving more complex (4-item) arrays may be more sensitive to the APPswe mutation than memory for 2-item arrays. In order to determine whether the size of the object array interacted with object memory in Tg2576 mice, Experiment 7 examined object novelty and object-location associations using comparable 4-item object arrays.

The second issue concerns the nature of the object-location memory deficit in aged Tg2576 mice. A study conducted by Dix and Aggleton (1999) highlighted the fact that animals may be able to form memories of different components of the spatial organisation of objects. This distinction was exemplified by two different object-location transformations. In one manipulation, where the absolute locations of objects

were changed, familiar objects were moved to locations that were previously unoccupied by an object. Under these conditions, normal rats showed a preference for exploring the object placed in a novel location. In a second manipulation, the object in-place condition, two objects were moved to new locations by simply switching their locations, while two other objects remained in the same positions occupied during the sample trial. Once again, normal rats showed a preference for exploring the two objects that had exchanged locations. Dix & Aggleton (1999) suggested that the former task may reflect memory for the spatial organization of objects in the arena, but only the latter task required memory for specific object-location associations or configurations.

In order to determine the nature of the object memory impairment in Tg2576 mice, we examined memory for the spatial organisation of object arrays using a variety of manipulations. Phases 2 and 3 of Experiment 7, investigate the absolute location versus object in-place distinction highlighted by Dix and Aggleton (1999). Specifically in phase 3 the metric relationships between the object and the walls were altered by moving two objects to two novel (previously unoccupied) locations while the remaining object remained in the same location relative to the sample trial. In Experiment 9, two objects were moved to new (previously unoccupied) locations while the metric properties of the array (that is the object-object distance and shape of the landmark array) remained consistent between the sample and test trial. In order to ensure that the mice remained sensitive to changes in absolute novelty a control test was carried out in Experiment 7, phase 4 in which two novel items were placed in novel spatial locations, while the familiar objects remained in the same familiar locations occupied during the exposure stage.

4.2.2 Method

Subjects

The same 11 transgenic and wild type male mice used in the Experiment 6 were ran in all phases of experiment 7. To counteract any motivational or aging affects the animals were ran in two groups. Each group comprised an equal number of transgenic and wild type mice. Group A completed the phases in numerical order from Phase 1 to phase 4 (Phase 1: object novelty task; phase 2 object-in place memory task; phase 3 familiar objects in novel location task; phase 4 novel object in novel location task). Group B were ran in the reverse order. The mean age for each of these phases was 17.5 months. Each phase lasted 2 days with a 2 day break between each stage. The order of object set used in each experimental phase was counterbalanced across day and genotype. All mice were 16 months of age at start of Experiment 7.

Apparatus

Details of the open field, objects, testing environment and recording equipment have been briefly outlined in the method section of Experiment 6. For more comprehensive details please see the method section 3.1.2 of Experiment 4, pages 93-94.

Experiment 7 – phase 1: Object Novelty

Four different objects were placed in the middle of the central four squares of the arena (approximately 10 cm apart). Animals were always transferred from a holding cage and placed in the centre of the arena. This start location remained the same throughout behavioural testing. The mouse was placed into the arena at the mid point between the four objects. During the sample phase, the transgenic animal was allowed to explore the objects for 10 minutes whereupon it was removed and placed in a holding cage for a 2 minute delay. During the retention interval two of the objects were replaced by novel objects. The arena, two sample objects and two novel objects were wiped down with 70% alcohol (in distilled water) and then placed in the arena. The two familiar objects were replaced in the same locations used in the sample phase; the two novel objects were positioned in the remaining target zones. The novelty switch was manipulated on a diagonal plane such that if the top-left object during exposure was replaced with a novel item then the bottom-right position would also locate a novel object during the test phase and vice versa. The direction of the diagonal switch top left—bottom right versus top right—bottom left, was counterbalanced across genotype and day. The mouse was then placed back into the arena with the test set of objects for a further 10 minutes. For both the sample and test phases, the animal's exploratory behaviour was scored with the same criterion as previously described in Experiment 4. Each wild type was yoked with a transgenic to ensure that the total contact time accumulated during exposure to the sample object array was equivalent. This meant that the wild type animal was removed from the sample phase once it had accumulated the same total amount of contact with the

objects as its transgenic partner. Both wild type and transgenic animals received a fixed 10 minute test stage.

Experiment 7 phase 2: Object-in place memory

Four different objects were placed in the same arrangement as that of the object novelty test and the mouse was placed into the arena at the mid point between the four objects. During the sample phase, transgenic mice were allowed to explore the objects for 10 minutes while wild types were removed as soon as they had accumulated the equivalent total contact time as their yoked transgenic counterpart. Once the sample phase was complete the animal was removed and placed in a holding cage for a 2 minute retention period. The arena and all 4 objects were then thoroughly cleaned with 70% alcohol and repositioned in the maze. Two objects were replaced in the same position that they had occupied during the sample phase; the remaining two objects switched positions. The relative spatial shift was conducted in the same manner as the novelty switch detailed above and the object-in place experiment described in Experiment 4. The diagonal direction was counterbalanced across genotype and day. After the 2 minute retention interval the mouse was then placed between the four objects and allowed to explore the new arrangement for 10 minutes. The test phase lasted 10 minutes irrespective of genotype. The same procedure was repeated the following day with a new set of objects, the order of which were counterbalanced across day and genotype.

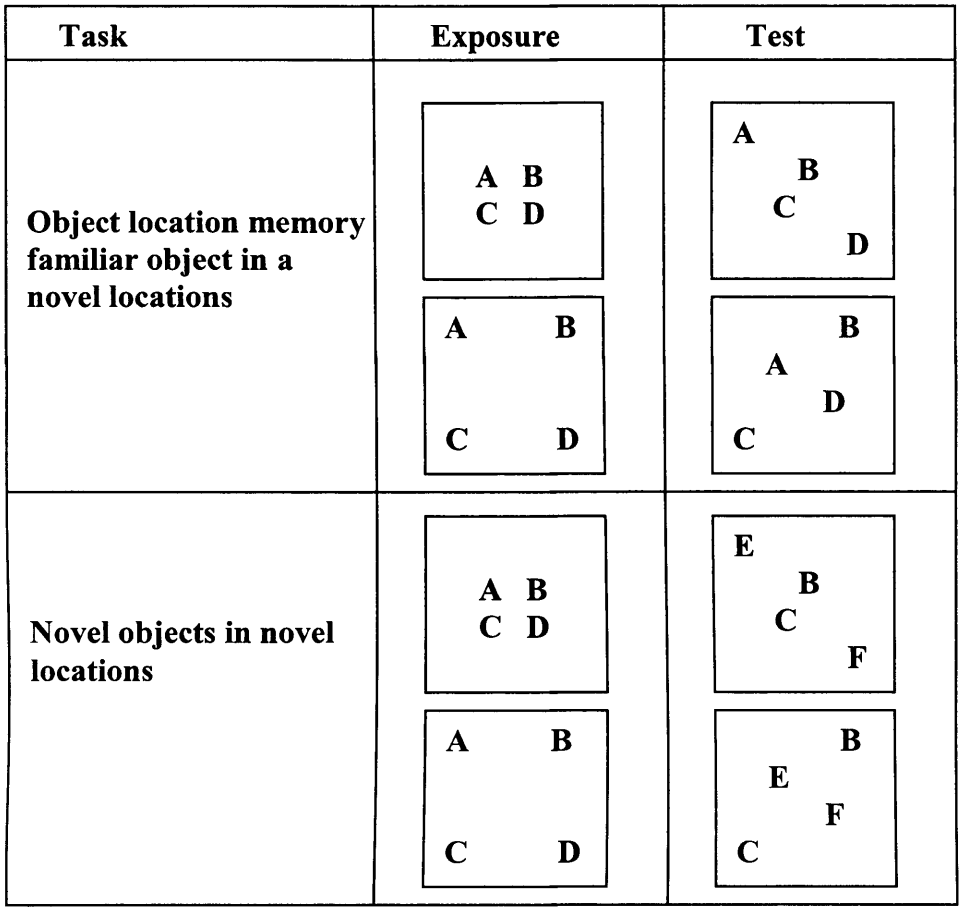


Figure 4.2.1 Summary of the behavioural procedures used for Experiments 7 phase 3 (Object location memory; familiar objects in novel locations) and Experiment 7 phase 4 (novel objects in novel locations). The design in both tasks used different exposure arrays. The objects were either placed in the periphery or the centre of the arena. At test objects were either moved towards the centre or towards the corners depending on the arrangement during the sampling stage. Sample arrangement was kept constant across trial and task for each animal.

Experiment 7 phase 3: Object location memory; familiar object in a novel location.

To determine whether aged Tg2576 mice are able to detect the movement of objects to novel locations half the mice were initially exposed to 4 objects in the corner squares of the arena, the other half sampled objects in the central positions of the arena as described previously. This difference in sample arrangement was counterbalance across genotype but remained constant across day and for conditions in Experiment 7 phase 4. The spatial change took place on a diagonal plane. Depending on the sample array the target objects were either moved out towards the periphery or moved into the centre squares appropriate for a diagonal shift. If animals were allocated to the expanding array condition (i.e., objects initially in the centre and then two objects moved out to the corner squares) the objects were moved so that they were approximately 5 cm away from the walls. If the animals were allocated to the shrinking array condition then they started with all four objects in the corner squares and then two objects moved into a central position approximately 15 cm in from the walls. It is important to note that irrespective of the sample array the configuration of the test objects was the same; two objects were in the centre and two in the periphery (see figure 4.2.1). A yoking procedure was also adopted in this phase of the experiment. Each wild type was paired with a transgenic mouse and was removed from the arena during the sample phase, once it had accumulated the same amount of contact time as its transgenic partner.

Experiment 7 phase 4: Novel objects in novel locations.

The procedure was identical to that described above except that two novel items were placed in novel spatial locations, while the familiar objects remained in the same familiar locations occupied during the exposure stage (see figure 4.2.1). All other aspects of the procedure were identical to those described in the preceding paragraph. This final phase was introduced as a control measure in the event that the transgenic mice were not sensitive to the manipulation in phase 3, familiar objects being moved to unfamiliar locations.

4.2.3 Results

	Object Novelty	Object In Place	Familiar object novel location	Novel object novel location
tg- Total time in arena	249.92 (29)	243.85 (22.51)	274.05 (19.96)	302.98 (38.46)
tg+/tg- Total contact time with Objects	37.98 (4.03)	28 (2.88)	45.31 (4.9)	33.98 (5.05)

Table 2 The average amount of time (in seconds) wild type (tg-) mice spent in the arena during the exposure phases of Experiment 7. Standard error of the mean is shown in brackets. All transgenic (tg+) mice spent 600 seconds in the area. The average amount of contact time (in seconds) accumulated with all four objects during exposure is also presented. As this was a yoked procedure these values are the same for the tg+ and their tg- counterparts. Standard error of the mean is shown in brackets.

As shown in Table 2 the wild type mice spent approximately fifty percent less time in the arena than the transgenic mice during the exposure phases of the experiments. Presumably if the wild type animals were allowed to spend 600 seconds in the arena like their transgenic counterparts then they would have accrued greater contact times. Indeed as will be demonstrated shortly, the wild type animals spent significantly longer with the objects during the full 10 minutes allowed in the test phase of each experiment. However, the important thing to bear in mind is that despite having their exposure time capped during the initial phase of the experiment, a detriment was not seen in their performance during the test phase.

Object Novelty

Figure 4.2.2 shows the mean contact in seconds made by transgenic and wild type mice with the novel and familiar objects. Inspection of this figure indicates that although Tg2576 mice showed lower contact times than wild type mice they nevertheless showed a preference for exploring the novel versus the familiar objects. This was confirmed by an ANOVA which revealed a main effect of group $F(1,20)= 7.72$, $p < 0.05$, a main effect of cue, $F(1,20)= 27.56$, $p < 0.05$ and no interaction between these factors, $F(1,20)=2.80$, $p>0.10$. We also evaluated the preference shown by the wild type and Tg2576 mice using a preference ratio (of the form time spent with novel objects/ time spent with all objects); a preference ratio of 0.5 indicates no preference. The mean preference ratio for wild type and Tg2576 mice is shown in Figure 4.2.6. Inspection of this figure indicates that both groups showed a similar preference for the novel object and this was confirmed by a t-test which revealed no significant difference between the these means $t(20)=0.11$, $p>0.10$. In addition a one-

sample t-test confirmed that the preference score for wild type and Tg2576 mice was significantly above chance, (minimum $t(10)=4.55$, $ps < 0.01$).

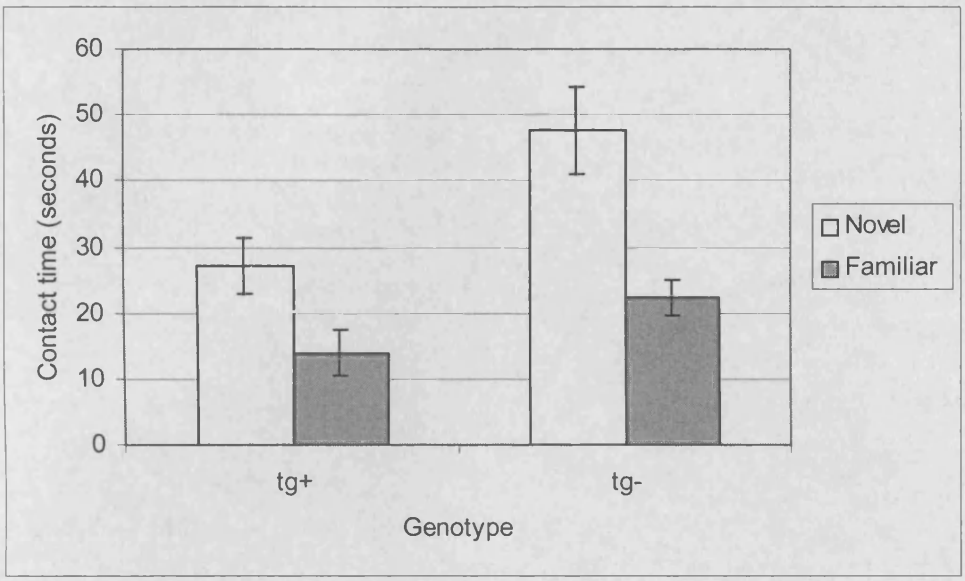


Figure 4.2.2 The mean contact time in seconds for transgenic (tg+) and wild type (tg-) mice with novel versus familiar objects in a yoked procedure. Error bars show the standard error of the mean.

Object-location memory

Figure 4.2.3 shows the mean time in seconds spent by transgenic and wild type mice exploring objects presented in the same or different location. Objects in the different location condition swapped position between exposure and test phases. Inspection of this figure indicates that Tg2576 mice spent less time exploring the objects than wild type mice and did not show a preference for exploring the objects located in a different but familiar location. This was confirmed by an ANOVA which revealed a main effect of group, $F(1,20)=20.65$, $p < 0.01$, a main effect of object

location (Same vs Different) $F(1,20)=8.96$, $p < 0.01$ and a significant interaction between these factors, $F(1,20)=10.70$, $p < 0.01$. Tests of simple main effects revealed a main effect of group in contact time with objects in both the same and different location (minimum, $F(1,33)=4.90$, $p < 0.05$) and a main effect of object location for wild type but not Tg2576 mice $F(1,20) = 19.62$ and 0.03, $ps < 0.01$ and $p>0.10$, respectively). Thus wild type but not Tg2576 mice showed a preference for exploring objects that moved to a different but familiar location. An analysis of the preference ratio (see Figure 4.2.6) confirmed this analysis and revealed a significant difference between the groups $t(20)=2.41$, $p< 0.05$. Furthermore the preference score of the wild type but not the Tg2576 mice was significantly above chance (one sample t-tests: $t(10)= 3.4$ and 0.08, $ps < 0.05$ and $p>0.10$, respectively).

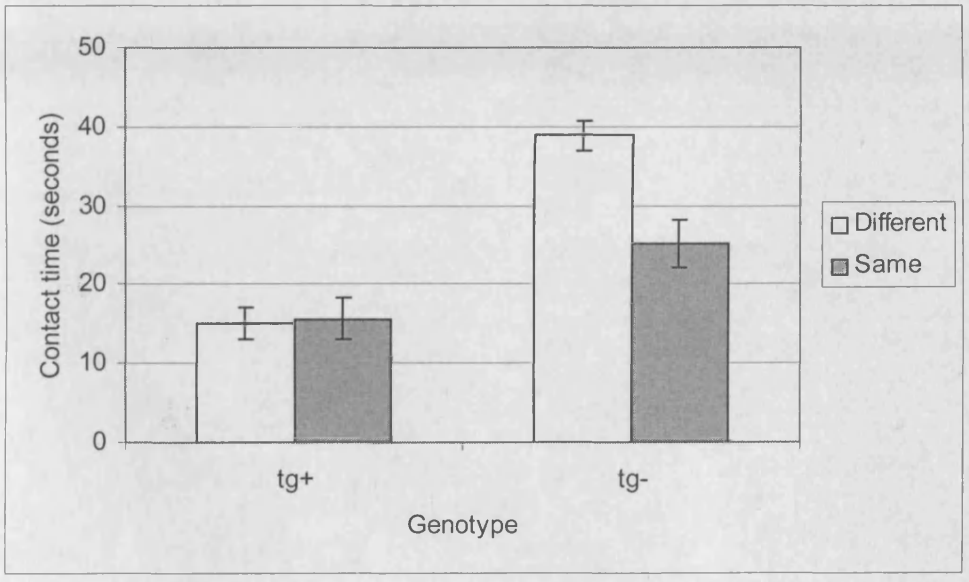


Figure 4.2.3 The mean contact time in seconds for transgenic (tg+) and wild type (tg-) mice with objects located in the same and different positions from the sample trial in a yoked procedure. Objects differed in their relative location. Error bars show standard error of the mean.

Familiar objects in unfamiliar location

Figure 4.2.4 shows the mean contact time in seconds, of the exploration of objects that had moved to a new spatial location or objects that remained in the same location by control and transgenic mice. Inspection of this figure indicates that although control mice spent more time exploring the objects overall, both wild type and transgenic mice showed a preference for exploring objects that had moved to a novel location. An ANOVA confirmed this impression and revealed a main effect of group, $F(1,20)= 28.35$, $p < 0.01$, a main effect of location, $F(1,20)=14.27$, $p < 0.01$ and no significant interaction between these factors, $F(1,20)=1.65$, $p >0.21$. An analysis of the preference ratio (see Figure 4.2.6) revealed no significant difference between the groups $t(20)=0.39$, $p >0.10$. Furthermore, the preferences shown by control and transgenic mice were significantly above chance (one sample t-tests: $t(10)= 3.28$ and 2.33, p's < 0.05. respectively).

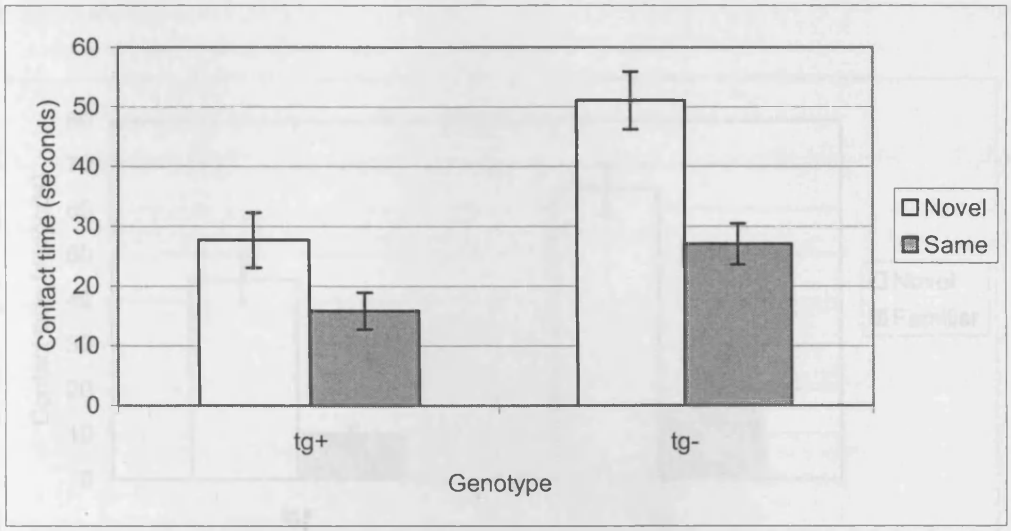


Figure 4.2.4 The mean contact time in seconds for transgenic (tg+) and wild type (tg-) mice with objects located in the same and different positions from the sample trial. Objects were relocated to a novel location. Error bars show standard error of the mean.

Novel objects in unfamiliar locations

Figure 4.2.5 shows the mean time in seconds that control and transgenic mice spent exploring a novel object placed in a novel location. Inspection of this figure indicates that although control mice made more contact with the objects overall, both wild type and transgenic mice showed a preference for exploring the novel object. An ANOVA revealed a main effect of group, $F(1,20)=8.31$, $p < 0.01$, a main effect of object, $F(1,20)=93.83$, $p < 0.001$, and no significant interaction between these factors, $F(1,20)=2.96$, $p>0.10$. An analysis of the preference ratios (see Figure 4.2.6) confirmed that the mean preference ratio did not differ significantly between groups, $t(20)=0.21$, $p>0.10$) and that both groups differed significantly from 0.5, t's$(10)=10.76, 7.8$, p's < 0.05, respectively).

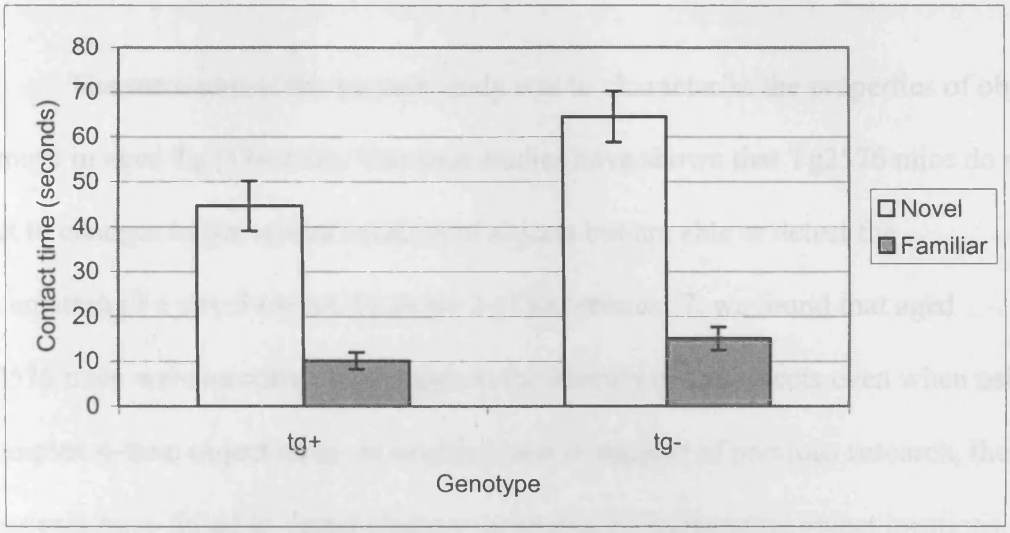


Figure 4.2.5 The mean contact time in seconds for transgenic (tg+) and wild type (tg-) mice with familiar objects located in the same position and novel objects presented in a novel position from the sample trial. Error bars show standard error of the mean.

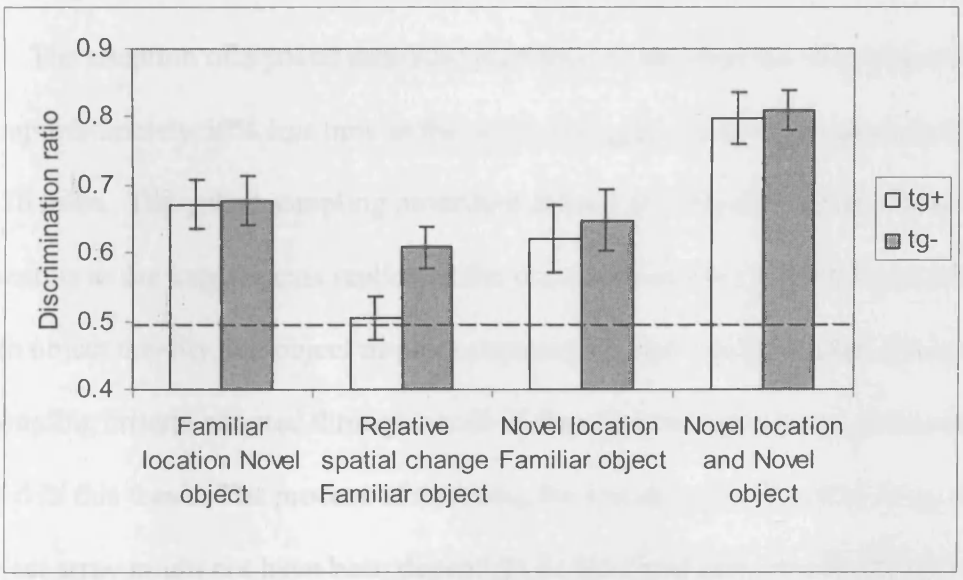


Figure 4.2.6 Discrimination ratios for transgenic (tg+) and wild type (tg-) performance on each of the phases of experiment 7. Error bars show standard error of the mean.

4.2.4 Discussion

The main aim of the present study was to characterise the properties of object memory in aged Tg2576 mice. Previous studies have shown that Tg2576 mice do not react to changes in the spatial location of objects but are able to detect the presentation of a novel object. In phase 2 of Experiment 7, we found that aged Tg2576 mice were sensitive to changes in the identity of the objects even when using a complex 4-item object array. In contrast, and in support of previous research, the transgenic mice failed to detect changes generated by exchanging object locations. We subsequently examined the nature of the spatial representations that were impaired by the APPswe mutation. In contrast to Experiment 7 phase 2, phase 3 revealed that Tg2576 mice were sensitive to changes in the spatial organisation of the objects when the objects were moved to locations previously unoccupied during the sample stage.

The adoption of a yoked sampling procedure meant that the wild type mice spent approximately 50% less time in the arena during the sampling stage than the Tg2576 mice. The yoked sampling procedure did not detrimentally affect the wild type results as the experiments replicated the previous sensitivity of wild type animals to both object novelty and object in-place memory. These results validate the use of the sampling criteria adopted throughout all of the object recognition experiments detailed in this thesis. The process of encoding the spatial and non spatial features of an object array might not have been dependent on proximal contact with the objects. The total amount of time spent in the arena, independent of the animal's location, might have been more important. If this were the case then it would be plausible to suppose that the wild type animals would have demonstrated impaired performance

relative to the Tg2576 mice on the novelty control experiments (Experiment 7 phase 1, experiment 7, phase 4, and showed an impairment in the object location task (Experiment 7 phase 2).

To address why Tg2576 animals have difficulties in detecting certain changes in object location it is necessary to explore the spatial arrangements of objects during the sample and test phases of the re-location experiments in Experiment 7. Switching the relative positions of two objects (Experiment 7, phase 2) did not alter the geometric properties of the overall object array. Inter object distances and angles remained constant, as did the distance between each object and the wall of the arena. To determine that two objects had been relocated a 'sense' of left and right positioning was required, and in a free moving animal, polarising cues would have been needed to determine what represented the top (north) and what constituted the bottom of the arena (south).

The rodent navigation literature suggests that extramaze cues can be used by animals to orient themselves within an environment. The cognitive map theory postulates that the hippocampus stores neural representations of distal landmarks to form an internal map that can guide navigation (O'Keefe & Nadel, 1978). As discussed in the introductory chapter support for this theory is provided by the existence of neurones that fire selectively when an animal occupies certain locations within a familiar open-space (O'Keefe & Dostrovsky, 1971). A sufficient change in environmental features leads to 'remapping' and the creation of a new representation (c.f Kubie & Muller, 1991). Consistent with this idea is the fact that relocation of objects in space changes the firing pattern of place cells (Lenkin-Santini et al., 2005) and these changes are accompanied by re-exploration of the displaced object. The crucial design feature of the relative shift experiment (experiment 7, phase 2) was that

animals needed to establish a specific link between an object and its location in space (see Dix & Aggleton, 1999), and this may be dependent upon the ability of the animal to orient themselves relative to the extramaze environment. Although it is unlikely that the Tg2576 mice harbour a visual impairment, Experiment 8 was conducted as confirmation that genotypic differences in visual acuity cannot account for their impaired performance on the object-in place task. A salient intra-maze cue was provided which could have been used as a point of refernce in the formation of object-place associations.

The question then becomes if Tg2576 failed to detect the relative relocation of an object how did the mutant mice detect an absolute change in object location? The simple answer is that the design of the absolute location shift did not necessitate the ability to learn specific object-location associations. Unlike the square array that never altered in shape or size, the array used in the test phase of the absolute shift (experiment 7 phase 3) differed from the sample configuration in both these features. Configuration cues such as change in inter-object distances, variation in wall to object distances and a change in the overall shape of the array could all be used as strategies to determine a change in object location (cf. Skov-Rackette & Shuttleworth, 2005).

In conditions where normal animals could not orientate themselves with respect to the extramaze cues, rats were found to be unable to identify a relative change in object position, but could detect an absolute change in object location. Skov-Rackette & Shuttleworth (2005) concluded that this was the result of detecting a change in inter-object distances. They do not specify why specific objects obtained higher levels of re-exploration as opposed to greater exploration of the object set as a whole. A similar argument can be applied to any explanation given to re-exploration on the basis of a change in the overall shape of an array. A variation in object to wall

distances would be unique to displaced objects, this strategy could account for the specific re-exploration of target objects. However, this would require the animals to have learnt specific object-location associations.

An alternative account is that the Tg2576 mice were able to encode a coarse overall representation of the object array that allowed them to detect novelty in the group arrangement of landmarks. For example, mice could have become accustomed to experiencing objects in the centre of the array and consequently detected a novel change when they experienced an object in the periphery, independent of the object's identity. In line with this idea, Experiment 9 was conducted to assess the sensitivity of the Tg2576 to detect an absolute spatial change. Experiment 9 was conducted to see whether the Tg2576 ability to detect the relocation of familiar objects to novel locations could be generalised to conditions whereby the relocations were confined to the maze periphery. However, before considering this aspect of object memory further we addressed one final issue. More specifically whether the failure to identify that two objects had been transposed reflected an impairment in using extramaze cues to provide a directional heading for orienting the animals with respect to the object array.

4.3 Experiment 8: Object in place memory with an intra maze cue card.

4.3.1 Introduction

Despite the fact that Experiments 1 and 2 presented in Chapter 2 demonstrate that aged Tg2576 mice can use extramaze cues to guide behaviour, Experiment 8 was conducted to address the possibility that a failure to use a directional heading

provided by extramaze cues may have contributed to the object-place impairment. An intramaze cue was therefore introduced into the arena to polarise the environment.

4.3.2 Method

Subjects

The 11 transgenic and 8 wild type male mice ran in Experiments 6 and 7 were used in Experiment 8.

Apparatus

Details of the open field, objects, testing environment and recording equipment have been outlined in the method section of Experiment 4. For comprehensive details please see the method section of Experiment 4 section 3.1.2 pages 93 – 94.

Behavioural Training

All experimental details are the same as for Experiment 7 phase 2; however one wall of the arena was covered in black card in the cue card condition. The standard version of the object-in place task was repeated as a control for any possible training effects. Mice were run in two cohorts. Half the mice completed the polarized cue version of the task before the standardized version, and the other half were ran in the reverse order. Each phase lasted 2 days with a 2 day break between each stage. The order of object set used in each experimental phase was counterbalanced across day and genotype. All animals were 19 months age at the start of experiment 8. Prior to each test the animals underwent a habituation procedure as previously described in

Experiment 6. Habituation prior to the polarized cue test incorporated a black card on one side of the maze's walls. Following habituation animals received a 2 day break before commencing with the test procedures. Analysis of the habituation procedure revealed that the wild type mice were making significantly more contact with the objects during habituation. To control for this, and to maintain comparable task design with Experiment 7, a yoking procedure was also conducted during the sample phases of Experiment 8. For details please see the method section of Experiment 7.

4.3.3 Results

Genotypic differences in contact time persisted during the habituation to the standard arena. An ANOVA revealed a main effect of genotype, $F(1,17) = 9.1, p < .01$, no main effect of day, $F(1,17) = 1.82, p > .10$, and a nonsignificant interaction between these factors $F(1,17) = .93, p > .10$. In the cue card version of the arena an ANOVA revealed no main effect of genotype, $F(1,17) = 2.15, p > .10$, no main effect of day, $F(1,17) = 3.32, p > .05$, and a non significant interaction between these factors $F(1,17) = 1.79, p > .10$. A yoking procedure was adopted during the cue-card version of the task despite the nonsignificant differences in the contact times, to ensure that the results were comparable to the control condition.

Figure 4.3.1 shows the mean time in seconds spent by transgenic and wild type mice exploring objects presented in the same or different locations. Objects in the different location condition swapped position between exposure and test phases. During both phases of the task a salient intra maze cue was present on one side of the arena's wall. Inspection of this figure indicates that Tg2576 mice spent less time exploring the objects than wild type mice and did not show a preference for exploring the objects located in a different but familiar location. This was confirmed by an

ANOVA which revealed a main effect of group, $F(1,17)=25.19$, $p < 0.01$, a main effect of object location (Same vs Different; $F(1,17)= 16.78$, $p < 0.01$) and a significant interaction between these factors, $F(1,17)=9.8$, $p < 0.01$. Tests of simple main effects revealed a main effect of group in contact time with objects in both the same and different location (minimum, $F(1,30)=6.02$, $p < 0.05$) and a main effect of object location for wild type but not Tg2576 mice $F(1,17) = 22.55$ and 0.55, $ps< 0.01$ and $p>0.10$, respectively). Thus wild type but not Tg2576 mice showed a preference for exploring objects that moved to a different but familiar location. An analysis of the preference ratio confirmed this analysis and revealed a significant difference between the groups $t(17)=2.53$, $p< 0.05$. Furthermore the preference score of the wild type but not the Tg2576 mice was significantly above chance (one sample t-tests: $t(7)= 4.6$ $p < .01$ and $t(10) =1.44$, $p> .50$, respectively).

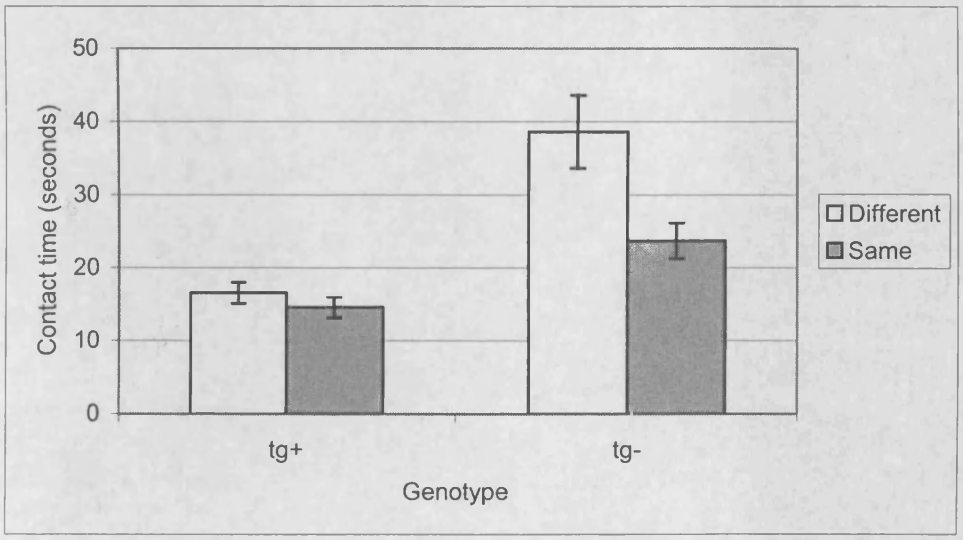


Figure 4.3.1 The mean contact time in seconds for transgenic (tg+) and wild type (tg-) mice with objects located in the same and different positions from the sample trial in the cue-card version of the maze. Objects differed in their relative location. Error bars show standard error of the mean.

Figure 4.3.2 shows the mean time in seconds spent by transgenic and wild type mice exploring objects presented in the same or different location in a replication of the standard version of the task. Objects in the different location condition swapped position between exposure and test phases. Inspection of this figure indicates that Tg2576 mice spent less time exploring the objects than wild type mice and did not show a preference for exploring the objects located in a different but familiar location. This was confirmed by an ANOVA which revealed a main effect of group, $F(1,17)=19.01, p < 0.01$, a main effect of object location (Same vs Different) $F(1,17)= 14.04, p < 0.01$ and a significant interaction between these factors, $F(1,17)=17.83 , p < 0.01$. Tests of simple main effects revealed a main effect of group in contact time with objects in different locations ($F(1,26) =34, p < 0.01$) but not the same location ($F(1,26) = 3.52, p > .05$) and a main effect of object for wild type but not Tg2576 mice $F(1,17) = 27.43$ and 0.13, $ps< 0.01$ and $p>0.50$, respectively. Thus wild type but not Tg2576 mice showed a preference for exploring objects that moved to a different but familiar location. An analysis of the preference ratio confirmed this analysis and revealed a significant difference between the groups $t(17)=4.62, p< 0.01$. Furthermore the preference score of the wild type but not the Tg2576 mice was significantly above chance (one sample t-tests: $t(7)= 4.83$ $p < .01$ and $t(10) = - 0.68$, $p> .50$, respectively).

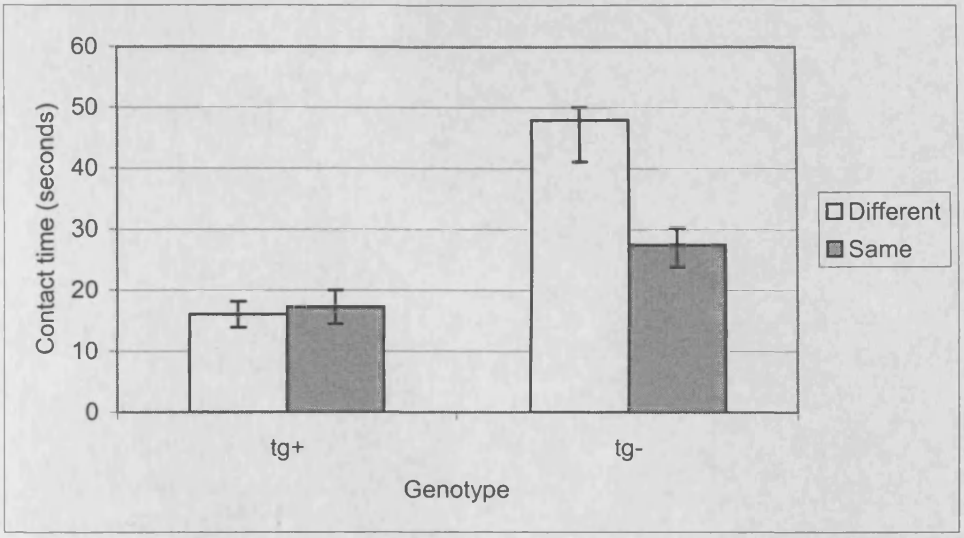


Figure 4.3.2 The mean contact time in seconds for transgenic (tg+) and wild type (tg-) mice with objects located in the same and different positions from the sample trial in the standard version of the task. Objects differed in their relative location. Error bars show standard error of the mean.

4.3.4 Discussion.

Discussion of Experiment 7 phase 2 suggested that animals may fail to detect change as they cannot orient themselves with respect to the extramaze environment. Experiment 8 demonstrates that the provision of an intra maze polarising cue did not improve Tg2576 ability to detect a relative change in the location of a familiar object. The animals did not run into the objects or the side of the walls. Therefore it is unlikely that the animals were not able to see the locale polarising cue. Consistent with the previous argument it would seem that Tg2576 mice are unable to use prominent polarising cues in the extra or intra maze environment to enable them to form stable representations of the location of an object within an array. Alternatively

the deficit in Tg2576 performance on this version of the object-in place task might suggest that the animals did not use the polarizing wall.

4.4 Experiment 9: Object-in place and object-location memory in a parallelogram object array.

4.4.1 Introduction

The series of tests presented in Experiment 7 examined memory for object location when objects were transferred to either a location previously occupied by an object or to a location that had never been occupied by an object. In order to establish the generality of these effects, for example the salience of an object in the centre versus periphery of the maze, the present experiment investigated the same type of spatial changes but using a different-shaped array of objects. Interpretation of the results from phase 3 of Experiment 7 suggests that Tg2576 animals may have access to a coarse representation of the environment to detect the novel location of a familiar object. With reference to the design of Experiment 7 phase 3, the novel relocation of a familiar object could have been detected on the basis of a maze peripheral versus maze central discrimination. The parallelogram shape incorporated in Experiment 9 allowed for the locality of the absolute shifts to be confined to the periphery. Experiment 6 highlights that the wild type mice showed a preference for spending time in the corners of the maze. This would mean that their sensitivity to detecting a change in the relocation of an object might be biased if the transfer took the form of a corner-to-corner change. To ensure that ability to detect the relative relocation of objects assessed within this new array was not preferentially biased towards wild type

performance, a vertical shift was incorporated. Objects in the centre of the peripheral areas of the maze also switched places in the object-in place version of the task. As previously mentioned in the introduction of Experiment 7, the peripheral location of objects might favour spatial processing (cf. Cressant et al., 1997; 1999). Previous experiments that have assessed Tg2576 ability to detect the relative relocation of an object have used an object array presented in the central area of the maze. Experiment 9 permitted an evaluation of this ability when all the objects were in the periphery of the maze.

4.4.2 Method

Subjects

This experiment used the 11 transgenic and 8 wild type mice previously used in Experiments 6 (habituation), 7 (4 object square array) and 8(cue card experiment). An additional 8 more male transgenic and 4 more male wild type mice were also ran in this experiment aged 23 months. These mice had previous experience of object recognition procedures but were naïve with respect to the manipulations carried out in Experiment 9. All of the mice were experimentally naive to the objects used in Experiment 9. The average age of the mice was 21 months old. The order that the experiments were ran was counterbalanced. Half the animals received the absolute location change followed by the diagonal and then vertical relative shift experiments, the other half were ran in the reverse order. Each experiment lasted 2 days with a 2 day break between each stage. The order of object set used in each experimental phase was counterbalanced across day and genotype.

Apparatus

Details of the open field, objects, testing environment and recording equipment have been briefly outlined in the method section of Experiment 4. For more comprehensive details please see the method section of Experiment 4, section 3.1.2, pages 93 –94.

Behavioural Training

Habituation

All animals underwent an initial habituation period as described in Experiment 6. After habituation, all animals received a 2 day break before commencing with the test procedures. Analysis of the habituation results revealed that a genotypic difference in contact time was still prevalent. Therefore a yoked sample procedure was also conducted in this current set of experiments, whereby a wild type animal was paired with a transgenic mouse and it was removed from the sample phase of the experiment when it accumulated the equivalent amount of contact time with the objects as its transgenic counterpart.

Parallelogram object- in place and object location memory

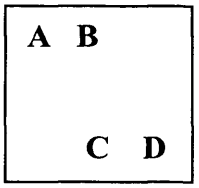
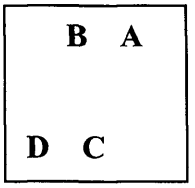
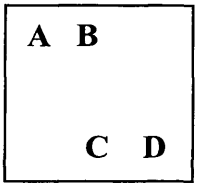
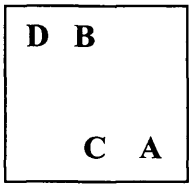
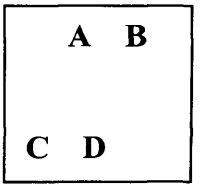
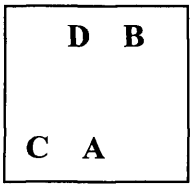
Task	Exposure	Test
Absolute lateral shift task		
Relative diagonal shift task		
Relative vertical shift task		

Figure 4.4.1 Summary of the behavioural procedures used for Experiments 9 phase 1 (absolute lateral task), Experiment 9 phase 2 (relative diagonal shift task) and Experiment 9 phase 3 (relative vertical shift task).

The sample array for each of the phases of Experiment 9 was the same. Two objects were placed in the centre of opposite corner squares - approximately 5cm from the back and side walls. Two objects were also placed in the middle of the outer periphery, in line with each corner object, approximately 5 cm from back/ front wall and approximately 36 cm away from the sidewalls (see figure 4.1.1). For phase 1, the absolute lateral shift task, the two corner objects were relocated to the corners that were empty during the sample phase. Objects were moved to their adjacent corner. They therefore remained 5cm away from the side and back walls but moved approximately 70 cm on a horizontal plane. For phase 2, the relative diagonal shift

task, the two corner objects switched their relative locations. They maintained a position of 5cm from the back and side wall, but had moved approximately 90 cm on a diagonal plane. For phase 3, the vertical relative shift, the central objects switched locations. Objects were still 5 cm from the back/ front wall and 36 cm away from the sidewalls, but had shifted 70 cm on a vertical plane.

4.4.3 Results

Habituation

An ANOVA conducted on the habituation data revealed a main effect of genotype in the amount of contact time made with the objects $F(1,29) = 10.88, p < .05$, a main effect of day, $F(1,29) = 26.04, p < .01$ and a significant interaction between these factors $F(1,29) = 4.36$, $p < 0.05$. A subsequent test of simple main effects revealed that the groups differed significantly in contact on day 1, $F(1,48) = 15.24, p < .01$ but not on day 2 $F(1,48) = 3.03, p > .05$, but both the wild type and tg2576 mice showed a significant decline in object contact over the two days, $F(1,29) = 21.10, p < .01$, $F(1,29) = 5.87, p < .05$, respectively.

Objects in novel locations

The mean time exploring the objects moved to a new unfamiliar location is shown in Figure 4.4.2. An ANOVA revealed a main effect of group, $F(1,29)=14.02, p < 0.01$, a main effect of object $F(1,29)=32.61, p < 0.01$ and a significant interaction between these factors, $F(1,29)= 6.50, p < 0.05$. Tests of simple main effects revealed

a significant difference in contact time with objects in a different location $F(1,47)=20.43, p < 0.01$, a main effect of object type in both Tg2576 and wild type mice Fs$(1,29)=27.84$ and 6.44, ps < 0.05, respectively. An analysis of the preference ratio measure (see Figure 4.4.3) indicated a nonsignificant difference between the means for the wild type and Tg2576 mice, $t(29)=1.12, p >0.01$ and that the performance of wild type ($t(11)=5.29, p < 0.01$) and the Tg2576 mice($t(18)=3.66, p < 0.01$) differed significantly from chance.

Objects in familiar locations.

An ANOVA conducted on the contact time with objects that moved to familiar locations (see Figure 4.4.2) revealed a main effect of group, $F(1,29)= 26.61, p < 0.01$, a main effect of object $F(1,29)=55.47, p < 0.01$ and a significant interaction between these factors, $F(1,29)=48.97, p < 0.001$. Tests of simple main effects revealed a significant difference between the groups in contact time with objects located in the same or different location, $F(1,36)= 51.68$ and 6.46, p's < 0.05, respectively. There was also a significant main effect of object type for wild type $F(1,29)=85.12, p < 0.001$) but not for Tg2576 mice $F(1,29)=0.13, p > 0.10$). An analysis of the preference ratio data (see figure 4.4.3) confirmed this analysis and revealed a significant difference between the means for wild type and Tg2576 mice, $t(29)=5.59, p < 0.01$. Furthermore the performance of the wild type ($t(11)=8.23, p < 0.05$, but not the Tg2687 mice ($t(18)=0.55, p > 0.10$) differed significantly from chance.

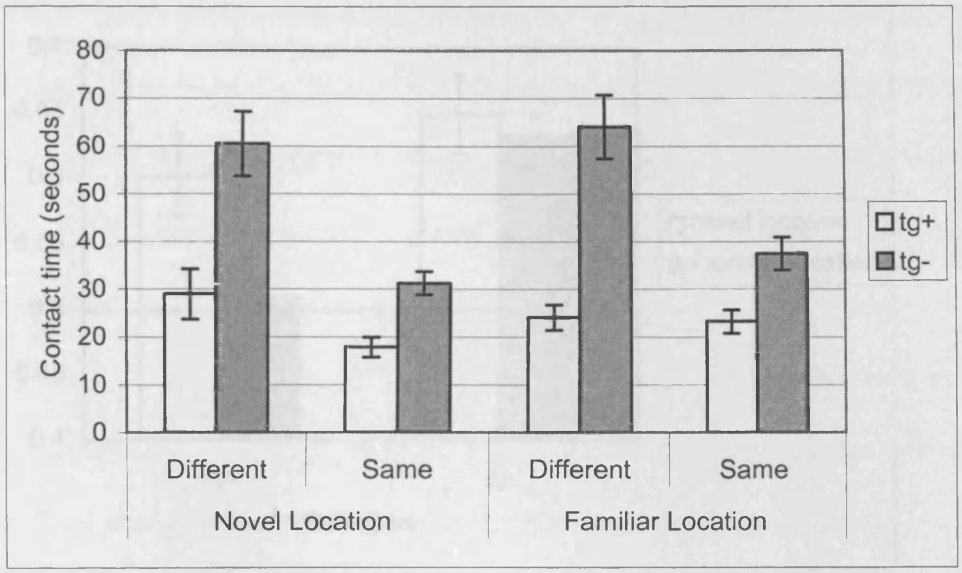


Figure 4.4.2 The mean contact time in seconds for transgenic (tg+) and wild type (tg) mice with objects located in the same and different positions from the sample phase. Objects have either moved to a novel location or changed their relative position and have therefore been repositioned in a familiar location. Error bars show standard error of the mean.

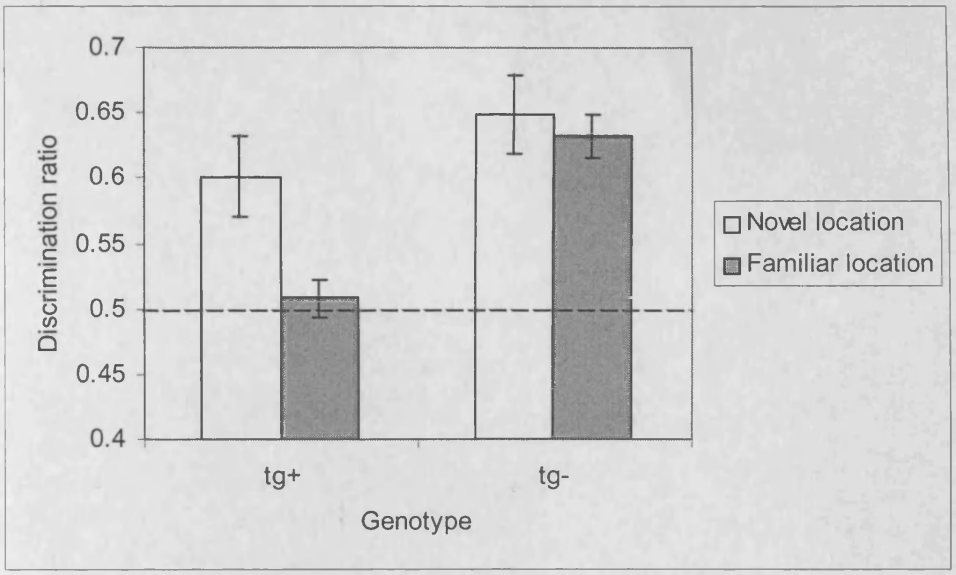


Figure 4.4.3. Discrimination ratios for transgenic (tg+) and wild type (tg-) performance on each of the phases (objects either moved to a novel location or changed their relative position and have therefore been repositioned in a familiar location) of Experiment 9. Error bars show standard error of the mean.

4.4.4 Discussion

In Experiment 9, we replicated in older mice, the findings of Experiments 4 and 7 that showed that Tg2576 mice explored objects placed in previously unoccupied locations but did not explore objects that exchanged spatial locations, even though the spatial transformations maintained the metric relationships between the objects and the walls of the arena.

The mechanism(s) by which animals form a spatial representation of their environments has been the subject of considerable theoretical and empirical research. One view that has gained support is that spatial memory reflects the integration of a hierarchy of distinct types of information (e.g., the integration of crude topological

information with metric information that specifies the distances and angles between landmarks; Gallistel, 1990b; Poucet, 1993). It remained possible therefore that the APPswe mutation in aged Tg2576 mice may spare certain components of spatial memory. Indeed, recent evidence suggests that the detection of these different spatial transformations may be supported by different neural systems. Thus, using similar procedures to those described in Experiments 7 and 9, Goodrich-Hunsaker et al., (2005) reported that lesions of the dorsal hippocampus impaired exploratory activity generated by changing the distance between objects (i.e., moving objects to previously unoccupied locations). In contrast, rats with dorsal hippocampal lesions showed normal exploration of objects generated by exchanging the spatial locations of objects. These authors suggested that memory for metric, but not topological, relations are impaired by hippocampal lesions. Furthermore, Goodrich-Hunsaker et al. (2005) also showed that rats with parietal cortex lesions displayed the reverse pattern of deficits. Thus, rats with parietal lesions showed increased exploration of objects following a change in the metric relationship between objects but not when the topological relationship between the objects was altered.

Parron et al. (2006) recently provided further empirical support for this distinction. These authors investigated the effects of hippocampal-entorhinal disconnection, using a cross-lesion approach, on object exploration. In their study the objects were moved to a novel location (i.e., one previously unoccupied by an object during the sample stage). Consistent with Goodrich-Hunsaker et al. (2005), Parron et al. (2006) reported that animals with contralateral lesions of the hippocampus and the entorhinal cortex failed to explore an object moved to a new location. This suggests that an interaction between the hippocampus and the entorhinal cortex is required for the detection of changes in the metric properties and/or the spatial location of objects.

In phase 3 of Experiment 7 we found that Tg2576 mice were sensitive to changes in object locations when the object array was expanded or contracted along one dimension but not when the topological relationship between the objects was altered. One interpretation of these findings, based on the work described previously, is that the APPswe mutation may disrupt spatial memory processes supported by the parietal cortex (cf. Goodrich-Hunsaker et al., 2005). However, the spatial change in Experiment 7 phase 3 confounded a change in the location of the objects as specified by extramaze cues with a change in the metric relationship between the objects and the perimeter of the environment. Experiment 9, therefore, examined whether a change in the metric relationship between the objects and/or the walls was critical for the Tg2576 mice to detect the movement of an object to a novel location. The distance between the objects and the walls of the arena remained the same during the sample and test trials irrespective of whether the objects exchanged location (a topological change) or moved to novel locations. Similar to the results of Experiment 7 phase 2 and phase 3 we found that Tg2576 mice explored objects that moved to novel (previously unoccupied) locations but did not explore objects that exchanged spatial locations. This would suggest that the detection of changes in the metric relationships between objects and/or the perimeter of the arena is not a necessary condition for Tg2576 mice to react to changes in object location.

One explanation of these results can be developed from the analysis of object – location associations offered by Dix and Aggleton (1999). These authors suggested that the exploratory activity elicited by objects that exchanged spatial locations reflected memory for specific object-location associations. That is, an integrated representation of the specific properties of objects together with information about the location of the item. In contrast, a representation that specifies the position of objects

independent of their identity may be sufficient to generate exploratory activity caused by the movement of objects to a location that was previously unoccupied. This analysis leads us to suggest that aged Tg2576 mice are able to form a crude representation of the spatial layout of their environment. That is, Tg2576 mice may be able to acquire information that specifies the position of items within the environment (i.e., the centre versus the periphery of an arena; see also Bizon et al., 2007). However, this information is not integrated with specific object identity information that permits the formation of specific object-location associations. Furthermore, the observation that Tg2576 mice are able to detect object novelty replicates our previous findings in Experiment 4 in which 14-month old wild type and Tg2576 mice showed a similar capacity for object novelty detection with delays of up to 24 hours. These results suggest that the processes supporting object identity are intact and independent from those supporting spatial memory.

Several studies have suggested that the hippocampus may form part of the neural system that integrates spatial and object information. For example, unit recording studies have shown that the firing properties of hippocampal pyramidal place cells are controlled by landmark arrangements in a circular arena when placed against the sides of the walls but not when the objects are located in the centre of the arena (Cressant et al., 1997, 1999). However, Rivard et al. (2004) showed that when a polarising cue was made available, a number of hippocampal pyramidal cells fired in proximity to an object and continued to fire when the object was moved and ceased firing when the object was removed from the apparatus. In addition, these cells became active when the object appeared in a new environment despite the fact that place cells remapped between environments. These results suggest that object or landmark features are represented in the hippocampal neural code and can be

independent of the code for specific spatial environments. A recent study by Lenck-Santini et al. (2005) examined the effects of object-novelty and object-location transformations on place cell activity. The authors rotated two objects to new locations relative to a cue card that provided directional information. Under these conditions, fields near the objects remapped and underwent unpredictable rotations or ceased firing. Place fields that were distal to the objects changed in relatively minor ways. The replacement of one of the objects with a novel object, however, had no effect on the place fields either near or distal to the objects. These findings suggest that the spatial configuration of the objects relative to the cue card exert control over local place fields. We are not aware of any published studies that have examined the effects of exchanging the location of familiar objects on the firing properties of hippocampal cells. Nevertheless, the study of hippocampal pyramidal cell activity suggests that information about objects and their location are represented in the hippocampal network.

The findings from unit recording studies are consistent with a number of current computational theories that characterize the hippocampus as mediating between pattern separation and completion processes (e.g., Gluck & Myers, 1993; O'Reilly & Rudy, 2001; Rolls & Kesner, 2006). In a recent review, Knierim et al. (2006) highlighted the importance of these processes in the formation of configural representations of objects and places and their potential contribution to episodic memory. More specifically, they postulated that information from the perirhinal cortex conveys object related information and inputs from the postrhinal cortex conveys spatial information into the hippocampus. These data streams are then integrated in the dentate gyrus (DG) and the CA3 region of the hippocampus. Interestingly, recent evidence indicates that spine density in the DG and inputs from

the entorhinal cortex are compromised, structurally and functionally, during development in Tg2576 mice (Jacobsen et al., 2006; Dong et al., 2007; see also Chapman et al., 1999). These anatomical changes may thus provide a substrate for the impairments in specific object-place associations in Tg2576 mice; perhaps by disrupting the integration of object and place information in the DG-CA3 network. Further work is required to evaluate this proposal.

Recent evidence from studies of human medial temporal lobe (MTL) lesions offers further support for the view that memory for location and specific object-location conjunctions can be dissociated. Olson et al. (2006) reported that MTL patients showed accurate recognition memory for recently presented objects and the locations occupied by objects. In contrast, memory for object/location combinations was selectively impaired in MTL patients. Using a similar task, Mitchell et al. (2000) showed that hippocampal activation and recognition performance was poorer during conjunctions of object and places in aged, but not in younger, adults. We are not aware of studies using a similar procedure with AD patients. However, visuospatial paired associate learning is sensitive to AD pathology and indeed this task can discriminate between AD and depression, fronto-temporal dementia and questionable dementia (Swainson et al., 2001; Blackwell et al., 2004).

In conclusion, our results indicate that aged APPswe mutant mice are able to form memories of objects and the spatial layout of objects in an environment but are unable to integrate this information. This pattern of deficits may reflect anatomical and functional changes in the connectivity of the entorhinal-hippocampal networks. Taken together with other recent research, the findings from Experiment 9 suggest that aberrant APP processing in Tg2576 mice contributes to impairments in conjunctive memory processes (Good et al., 2007b). However, further experiments

are required to determine whether this impairment extends beyond integrating spatial and object information (e.g., see Gluck et al., 2006).

In this chapter a précis of the findings reported in the experimental chapters is presented. The cognitive deficits observed in the transgenic mice will be discussed in the context of AD pathogenesis and symptoms, and in broader terms of rodent lesion and navigation data. Finally, directions for future research shall be discussed.

5.1. Place versus Response learning in the Tg2576 mouse.

The experiments presented in Chapter 2 examined spatial and non-spatial maze learning in adult Tg2576 mice. The purpose of these experiments was to establish the influence of the mutation on processing extramaze cues. Transgenic mice were impaired in acquisition of a T-maze FCA task at 16 months of age consistent with other published reports of deficits in spatial navigation in adult Tg2576 mice (Hsaio et al., 1996; King et al., 1999; Kotilinek et al., 2002; Westerman et al., 2002; Chapman et al., 1999; see also Corcoran et al., 2002). A deficit in allocentric processing was also demonstrated by Tg2576 performance in a reference memory task in a plus maze (Experiment 3). In contrast, transgenic mice were able to acquire a simple room discrimination (Experiment 1), suggesting that Tg2576 mice were able to process at least some features of their extramaze environment. Furthermore, adult Tg2576 mice were able to use this contextual information to successfully guide their responses on a T-maze left-right discrimination (Experiment 2). Tg2576 mice learnt the task at an equivalent rate to the wild type mice, suggesting that deficits in spatial navigation cannot be attributed to task difficulty *per se* or a gross perceptual impairment in the mutant mice. These results are consistent with the hypothesis that,

although Tg2576 mice are able to process extramaze cues, they are impaired in forming allocentric representations of the environment.

Aged Tg2576 mice were able to adopt a response strategy in a plus maze task (Experiment 3). The demonstration of impaired place but preserved response learning in Tg2576 mice is consistent with dual process theories of spatial navigation such as the Locale and Taxon systems described in O'keefe and Nadel's (1978) Cognitive Map Theory. The results from Chapter 2 coupled with this dichotomised concept of spatial navigation, complements the Alzheimer literature. Typically patients in the early stages of the disease are unimpaired on simple route learning, where stimulus-response relationships can be utilized, but show an inability to process allocentric spatial information (e.g., Kavic et al., 2006). Ideothetic processes have also been found to be unimpaired in early stage Alzheimer patients (Kalová et al., 2005). The mice in the response task could have utilized an inertial sense of direction, and/or ideothetic processes to guide their behaviour. Ideothetic processes, in the form of path integration, were not evaluated in these mice, although it would be of great interest to do so.

5.2 Tg2576 performance in a spontaneous object recognition paradigm and episodic-like memory task.

The object recognition paradigm allowed us to manipulate object variables such as; object type 'what', temporal presentation of object 'when' and position/location of object 'where' in such way that 'episodic-like' memory could be assessed in this transgenic model of Alzheimer's disease.

The results of the object recognition study show that adult mutant mice were able to discriminate between familiar and novel objects with delays of up-to 24 hours at a level comparable to that of control mice. Adult Tg2576 and control mice were also able to discriminate the relative familiarity of two objects. In contrast to their normal performance on object recognition and relative recency, Tg2576 mice failed to investigate objects that had changed their relative spatial positions. The novelty and recency results indicate that the deficit in object-place memory was unlikely to be the result of some non-specific effect of the mutation on novelty detection *per se* or impaired memory for object information. Although it is unlikely that the Tg2576 mice harbour a visual impairment Experiment 8 incorporated a salient intra-maze cue to confirm that genotypic difference in visual acuity cannot account for their impaired performance on the object-in place task.

Experiment 5 demonstrated that aged Tg2576 mice were unable to form an integrated memory of the spatio-temporal context in which objects are presented, their behavior being predominantly influenced by the temporal order in which the objects were presented. This deficit can be considered to reflect a primary deficit in processing or memory for the spatial location of objects.

Object-place memory tasks are highly sensitive to AD pathology and it has been suggested that such tasks may provide an accurate preclinical marker for AD-related cognitive decline (Blackwell et al., 2004). It is interesting therefore to note that the pattern of impaired memory in Tg2576 mice presented in experiments 4 and 5 are analogous with the memory deficits in patients during the early stages of the disease.

5.3 Generalised effects of Tg2576 transgene on performance in spontaneous object recognition tests.

Chapter 4 details a set of experiments that allowed us to evaluate the object recognition paradigm in a more systematic manner. Aged transgenic animals were spending significantly lower amounts of time in contact with the objects in comparison to their littermate controls. Experiment 6 comprised a detailed analysis of 19 month old Tg2576 behaviour in the open field arena during 10 minute long habituation sessions. The results from Experiment 6 demonstrate that the transgenic mice consistently spent less time investigating a pair of objects than the wild type controls.

One possible explanation for the differences in contact times, although speculative, is that the mechanisms supporting sustained attention generated by novelty may be compromised in aged Tg2576 mice. This issue requires further investigation. In an effort to match the exposure of the mice to the object arrays we yoked the exposure of wild type mice to individual Tg2576 mice and also evaluated performance as a preference ratio. During the test stages we observed comparable changes in exploratory preferences in wild type and Tg2576 mice for certain object manipulations across a range of values. The fact that the deficit in Tg2576 mice was specific to certain objects manipulations and not others argues against a general, non-specific, impairment related to differences in object contact time.

5.4 Evaluating Tg2576 object-familiarity, object-in place and object location memory using a yoked sample procedure.

Using the yoked procedure we were able to replicated previous results, Tg2576 mice were sensitive to object novelty but were unable to detect a change in the relative repositioning of a familiar object. Experiment 7 phase 3 demonstrated that Tg2576 mice could detect the relocation of a familiar objects to a novel location, suggesting that certain components of spatial memory may be spared in the mutant mice. This task could be solved using a coarse representation of the environment to detect the change – i.e. all objects were in centre and two are now in the periphery (or vice versa). Alternatively metric information in terms of changes in inter-object and object-wall distances/angles could have influenced their behaviour.

Experiment 9 used a parallelogram shape to control for the metric changes in the spatial array, and Tg2576 mice were still able to detect that they had not seen a familiar object in a location before but were unable to detect a topological change, when two objects exchanged location. One explanation of these results is that Tg2576 manifest an impairment in integrated representations of specific properties of objects together with information about the location of the item, harbouring an impairment in object-location associations (see Dix & Aggleton, 1999). In contrast, Tg2576 mice can detect spatial change based on a representation that specifies the position of an item independent of object identity.

5.5 Relevance to theories of spatial navigation.

The Tg2576 impairment in the formation of object-location associations is indicative of hippocampal impairment (Gilbert & Kesner, 2002). This view complements the role of the hippocampus described in theories of spatial navigation. O'Keefe and Nadel (1978) suggest that hippocampal place cells form the neural correlate of an allocentric representation of space based on the configural association of landmarks. Similarly, Jacobs and Schenk's (2003) Parallel Map Theory (PMT) suggests that hippocampal integrity is fundamental to the formation of fine grain representation of the environment (sketch map), which utilises specific object location associations in the animals' proximal surroundings. The ability of Tg2576 mice to detect absolute changes to an objects lateral position in the maze periphery (experiment 9), suggests that the mice can use cues, for example distal visual cues or room geometry, to orientate themselves within the arena. These findings complement the results from experiment 1, in demonstrating that extra maze cues can be used to guide their behaviour in an open field.

The parietal cortex has also been demonstrated to play a role in processing proximal environmental cues (e.g. Save et al., 2005) and in path integration in rodents (Save et al., 2001). Interestingly Save et al. (2005) suggest that the parietal cortex contributes to the formation of a fine-grained representation of the environment (c.ref Sketch Map). Although the authors do not provide a detailed account of how the contribution may take place, their previous work has implicated the parietal cortex as having a role in combining visual spatial and ideothetic information (Save & Poucet 2000*b*). Interestingly, the spatial deficits observed in Alzheimer patients characterised by an inability to link landmarks with routes, have also been attributed

to parietal dysfunction (Monacelli et al., 2003, but also see Burgess et al., 2006). Save and Poucet (2000*a*) argue that the association between cue location and ideothetic information is critical in proximal environments, as self-movement dramatically alters the perception of an object. Jacobs and Schenk (2003) argue that such a mechanism is important to the formation of a sketch map, as the dramatic effect movement has on the perception of local cues, permits the calculation of metric relationships between the proximal landmarks.

5.6 Further directions

An aspect of spatial navigation that has not been evaluated in this thesis is an inertial sense of direction. It has been suggested that the hippocampus integrates inertial information to form an on-line representation of the animal's location (McNaughton et al., 1996). The ability to use self-motion cues to guide behaviour is disrupted by hippocampal lesions in mice (Gorny et al., 2002). It would therefore be of interest to assess this function in the Tg2576 model. Indeed, experimental observations have noted that wildt type mice tended to make more entries into the start arm of the T-maze during habituation in Experiment 3 and spend more time running up and down the length of the start arm during acquisition (this was an observation and has not been statistically verified). Such behaviours resemble the formation of a 'home-base' – the point of reference from which subsequent self movement is calibrated (Gorny et al., 2002). The lack of such responding in mutant mice may be indicative of a hippocampal-dependent path integration deficit. Further experiments are required to determine whether the APPswe mutation disrupts specific navigation strategies in these mice.

It is also of interest to note that Tg2576 mice can acquire a response strategy in a conditional plus maze task (Experiment 3) but do not adopt an effective place strategy in the FCA task. A possible explanation may be reflected in the perseverative tendencies of aged Tg2576 mice (personal communications Barnes, 2004). The plus maze task is somewhat simpler than FCA in that the mouse can continually turn in the same direction and gain a reward, whereas the latter task requires it to alternate its response in reaction to the direction it has previously ran. The deficit in FCA performance demonstrated by Tg2576 mice in experiment 2a might represent an inability to modulate behavioural responses. Experiment 3 in contrast exploits a tendency to perseverate. Further experiments are needed to determine whether Tg2576 mice have a deficit in response inhibition.

Experiments 1 and 2 demonstrated that Tg2576 mice can use extramaze cues to guide behaviour under appetitive conditions. Although somewhat tenuous, it could be argued that a genotypic difference in motivation hindered spatial processing in Tg2576 mice during their performance in the non-appetitive relative shift experiment. Indeed Middei et al. (2006) offer a similar explanation for why aged wild type failed to perform in a similar task. Future experiments could evaluate spatial processing of object arrays under appetitive conditions, where one array signals the presence of reward, and the relative displacement of two object signals the absence of a reinforcer.

Another point to consider is that the Tg2576 deficit in object-place learning may not necessarily indicate a deficit in spatial processing per se. It is possible that Tg2576 mice are simply unable to form any kind of association with an object. One way to test this would be to pair objects with odours and then present a mismatch of these pairings at test. Gilbert and Kesner (2002) found that hippocampal lesion rats

were unable to learn object-place and odour-place associations in this way, but were able to form object-odour associations. A similar task could be conducted with the Tg2576 mice.

In conclusion the experiments presented in this thesis demonstrate that Tg2576 mice can use information provided in the extramaze environment to guide their behaviour. Tg2576 mice cannot however use distal visual cues to form an allocentric representation of the environment. This may be defined more specifically as an inability to form object-location associations.

Aggleton, J.P., & Brown, M.W. (1999). Episodic memory, amnesia, and the hippocampla-anterior thalamic axis. *Behavioural Brain Science, 22(3),* 444-489.

Aggleton, J.P., Hunt, P.R., & Rawlins, J.N.P. (1986). The effects of hippocampal lesions upon spatial and non-spatial tests of working memory. *Behavioural Brain Research, 19*, 133-146.

Aguirre, G.K., (2003). Topographical disorientation: a disorder of way-finding disorder. In Neurological Foundations of Cognitive Neuroscience, D'Esposito M (ed). MIT Press: Cambridge; 89-108.

Ainge, J.A., Heron-Maxwell, C., Theofilas, P., Wright, P., de Hoz, L., & Wood, E.R. (2006). The role of the hippocampus in object recognition in rats: examination of the influence of task parameters and lesion size. *Behavioural Brain Research, 167,* 183-195.

Albert, M.S. (1996). Cognitive and neurological markers of early AD. *Proceeding of National Academy of Sciences, USA, 93.* 13547-13551.

Allen, K., Potvin, O., Dore, F.Y., Goulet, S. (2004). Modulation of the impairment of hippocampectomized rats on the radial-arm maze cue task by visual characteristics and subicular damage. *Behavioral Neuroscience, 118(6),* 1214-1224.

Alyan, S., & McNaughton, B.L. (1999). Hippocampectomized rats are capable of homing by path integration. *Behavioural Neuroscience, 113(1),* 19-31.

Alzheimer, A. (1907). Über eine eigenartige Erkrankung der Hirnrinde. [A peculiar disease of the cerebral cortex.] Allg Z Psychiatrie Psychisch-Gerlichtlich Med 64: 146-148.

Andlin-Sobocki, P., Jonsson, B., Wittchen, H,-U., & Olsen, J. (2005). Cost of disorders of the brain in Europe. *European journal of neurology 12 (Suppl 1)* 1-27.

Andra, K., Abramowski, D., Duke, M., Probst, A., Wiederhold, K. H., Burki, K., Goedert, M., Sommer, B., & Staufenbiel, M. (1996). Expression of APP in transgenic mice: a comparison of neuron-specific promoters. *Neurobiology of Aging*, 17, 183-190.

Arendash, G.W., Gordon, M.N., Diamond, D.M., Austin, L.A., Hatcher, J.M., Jantzen, P., DiCarlo, G., Wilcock D., & Morgan, D. (2001). Behavioral assessment of Alzheimer's transgenic mice following long-term Abeta vaccination: task specificity and correlations between Abeta deposition and spatial memory. *DNA and Cell Biology, 20(11),* 737-744.

Arendash, G.W., King, D.L., Gordon, M.N., Morgan, D., Hatcher, J.M., Hope, C.E., & Diamond, D.M. (2001). Progressive, age-related behavioural impairments in transgenic mice carrying both mutant amyloid precursor protein and presenilin-I transgenes. *Brain Research, 891,* 42-53.

Armstrong, D.M., Bruce, G., Hersh., L.B., & terry, R.d. (1986). Choline acetyltransferase immunoreactivity in neuritic plaques in Alzheimer brain. *Neuroscience letters, 71(2),* 229-234.

Arriagada, P.V., & Growdon, J.H., Hedley-Whyte, T., & Hyman, B.T. (1992). Neurofibrillary tangles but not senile plaques parellel duration and severity of Alzheimer's disease, *Neurology, 42,* 631-639.

Baddeley, .D., & Hitch, G.J. (1974). Working Memory. In G.H. Bower (Ed.), *The psychology of learning and motivation* (Vol 8). London. Academic press.

Bannerman, D.M., Good, M.A., Butcher, S.P., Ramsay, M., & Morris, R.G.M. (1995). Distinct components of spatial learning revealed by prior training and NMDA receptor blockade. *Nature, 378,* 182-186.

Bannerman, D.M., Grubb, M., Deacon, R.M., Yee, B.K., Feldon, J., Rawlins, J.N., (2003). Ventral hippocampal lesions affect anxiety but not spatial learning. *Behavioural Brain Research, 139(1-2),* 197-213.

Bannerman, D.M., Lemaire, M., Yee, B.K., Iversen, S.D., Oswald, C.J., Good, M.A., Rawlins, J.N. (2002). Selective cytotoxic lesions of the retrohippocampal region produce a mild deficit in social recognition memory. *Experimental Brain Research, 142(3),* 395-401.

Bannerman, D.M., Yee., B.K., Good, M.A., Heupel, M.l., Iverson, S.D., & Rawlins, J.N.P. (1999). Double dissociation of function within the hippocampus: A comparison of dorsal, vetral, and complete hippocampal cytotoxic lesions. *Behavioural Neuroscience, 113,* 1170-1188.

Bannerman, D.M., Yee, B.K., Lemaire, M., Wilbrecht, L., Jarrad, L., Iversen, S.D., Rawlins, P., & Good, M.A. (2001). The role of the entorhinal cortex in two forms of spatial learning and memory. *Experimental Brain Research, 141,* 281-303.

Banquet, J.P., Gaussier, P., Ouoy, M., Revel, A., & Burnod, Y. (2005). A hierarchy of associations in hippocampo-cortical systems: cognitive maps and navigation strategies. *Neural Computation. 17(6),* 1339-1384.

Barnes, P., Hale, G., & Good, M. (2004). Intramaze and extramaze cue processing in adult APPswe Tg2576 transgenic mice. *Behavioral Neuroscience, 118(6),* 1184-1195.

Becker, J.T., Wess, J., Hunkin, N.M., & Parkin, A.J. (1993). Use of temporal context information in Alzheimer's disease. *Neuropsychologia, 31(2),* 137-143.

Bizon, J., Prescott, S., & Nicolle, M.,M. (2007). Intact spatial learning in adult Tg2576 mice. *Neurobiology of Aging, 28(3),* 440-446.

Blackwell A.D., Sahakian B.J., Vesey R., Semple J.M., Robbins T.W., & Hodges J.R. (2004). Detecting dementia: novel neuropsychological markers of preclinical Alzheimer's disease. *Dementia and geriatric cognitive disorders, 17 (1-2),* 42-48.

Blair, H.T., & Sharp, P.E. (1996). Visual and vestibular influences on head-direction cells in the anterior thalamus of the rat. Behavioural Neuroscience, 110(4), 643-60.

Blennow, K., de Leon, M.J., & Zetterberg, H. (2006). Alzheimer's Disease. *Lancet, 368,* 387-403.

Blodgett, H.C. & McCutchan, K. (1947). Place versus response learning in the simple T-maze. *Journal of Experimental Psychology, 37,* 412-422.

Bobinski, M., de Leon, M.J., Tarnawski, M., Wegiel, J., Bobinski, M., Reisberg, B., Miller. D.C., & Wisniewski, H.M. (1998). Neuronal and volume loss in CA1 of hippocmapal formation uniquely predicts duration and severity of Alzheimer's disease. *Brain Research, 805,* 267-269.

Bouchard, R.W., & Rossor, M. (1996). Chapter 3: Typical clinical features . *in: Clinical Diagnosis and Management of Alzheimer's Disease* . Gauthier, S. (Ed.). Martin Duntiz Ltd.

Braak, H., & Braak E. (1991). Neuropathological staging of Alzheimer-related changes. *Acta Neuropathalogia Scandinavica, 82,* 239-259.

Braak, H., & Braak E. (1995). Staging of Alzheimer's disease-related neurofibrillary changes. *Neurobiology of Aging, 16(3),* 271-284.

Broadbent, N.J., Squire, L.R., & Clark, R.E. (2004). Spatial memory, recognition memory, and the hippocampus. *Proceedings of National Academy of Sciences, USA, 101(40),* 14515-14520.

Brown, M.W., & Aggleton, J.P. (2001). Recognition memory: what are the roles of the perirhinal cortex and hippocampus? *Nature Reviews Neuroscience 2(1),* 51-56.

Burgess, N. (2002). The hippocampus, space, and viewpoints in episodic memory *Quarterly Journal of Experimental Psychology (A),* 55, 1057-1080.

Burgees, N., Trinkler, I., King, J., Kennedy, A., & Cipolotti, L. (2006). Impaired allocentric spatial memory underlying topographical disorientation. *Reviews in Neurosciences, 17(1-2),* 239-251.

Buzsáki, G. (2005). Theta rhythm of navigation: link between path integration and landmark navigation, episodic and semantic memory. *Hippocampus, 15(7),* 827-840.

Cacucci, Chapman & O'Keefe (Society for Neuroscience Abstracts, 2005)

Camus, J. F., Nicolas, S., Wenisch, E., Morrone, I., Blanchard, F., & Bakchine, S. (2003). Implicit memory for words presented in short texts is preserved in Alzheimer's disease. *Psychological Medicine*, 33, 169-174.

Chapman, P.F., White, G.L., Jones, M.W., Cooper-Blacketer, D., Marshall, V.J., Irizarry, M., Younkin, L., Good. M.A., Bliss, T.V.P., Hyman, B.T., Younkin, S.G., and Hsiao, K.K. (1999). Impaired synaptic plasticity and learning in aged amyloid precursor protein transgenic mice. *Nature Neuroscience, 2 (3),* 271-276.

Chen, G., Chen, K.S., Knox, J., Inglis, J., Bernard, A., Martin, S.J., Justice, A., McConlogue, L., Games, D., Freeman, S.B., Morris & R.G. (2000). A learning deficit related to age and beta-amyloid plaques in a mouse model of Alzheimer's disease. *Nature 408(6815),* 975-979.

Cheng, K. (1986). A purely geometric module in the rat's spatial representation, *Cognition, 23(2),* 149-78.

Cheng, K., Newcombe, N.S. (2005). Is there a geometric module for spatial orientation? Squaring theory and evidence. *Psychological Bulletin Review, 12(1),* 1-23.

Cherrie, M.M., Mendez, M., & Perryman, K. (2001). Route learning performance in Alzheimer's disease patients. *Neuropsychiatry, Neuropsychology and Behavioural Neurology, 14,* 159-168.

Citron, M., Oltersdorf, T., Haass, C., McConlogue, L., Hung, A.Y., Seubert, P., Vigo-pelfrey, C., Lieberburg , I., & Selkoe, D.J. (1992). Mutation of the ß-amyloid precursor protein in familial Alzheimer's disease increases ß-protein production. *Nature, 360,* 672-674.

Citron, M., Vigo-pelfrey, C., Teplow, D.B., Miller, C., Scheneck, D., Johnson, J., Winbald, Venizelos, N., Lannfelt, L., & Selkoe, D.J. (1994). Excessive production of amyloid ß-protein by peripheral cells of systematic and presystomatic patients carrying the Swedish familial Alzheimer disease mutation. *Proceedings of the national Academy of Science of the USA,* 91, 11993-11997.

Citron, M., Westaway, D., Xia, W., Carlson, G., Diehl, T., Levesque, G., Johnston-Wood, K., Lee, M., Seubert, P., Davis, A., Kholodenko, D., Motter, R., Sherrington, R., Perry, B., Yao, H., Strome, R., Lieberburg, I., Rommens, J., Kim, S., Schenk, D., Fraser, P., St George-Hyslop, P., & Selkoe, D.J. (1997). Mutant pesenilins of Alzheimer's disease increase production of 42-residue amyloid ß-protein in both transfected cells and transgenic mice. *Nature Medicine, 3,* 67-72.

Clark, R.E., Zola, S.M., & Squire, L.R. (2000). Impaired recognition memory in rats after damage to the hippocampus. *Journal of Neuroscience, 20,* 8853-8860.

Clayton, N. S,. Griffiths, D. P., Emery, N. J., & Dickinson, A. (2001). Elements of episodic-like memory in animals. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences, 356,* 1483-1491.

Cole, G.M, & Frautschy, S.A. (2006). Alzheimer's amyloid story finds its star. *Trends Mol Med. 12(9),* 395-396.

Corcoran, K.A., Lu, Y., Turner, R.S., & Maren, S. (2002). Over expression of hAPPswe Impairs Rewarded Alternation and Contextual Fear Conditioning in a Transgenic Mouse Model of Alzheimer's Disease. *Learning & Memory, 9,* 243-252.

Crawley, J. N., Belknap, J. K., Collin,s A., Crabbe, J. C., Frankel, W., Henderson, N., Hitzemann, R. J., Maxson, S. C., Miner, L. L., Silva, A.J., Wehner, J. M., Wynshaw-Boris, A., Paylor, R. (1997). Behavioral phenotypes of inbred mouse strains: implications and recommendations for molecular studies. *Psychopharmacology (Berlin), 132,*107-124.

Cressant, A., Muller, R.U., & Poucet, B. (1997). Failure of centrally placed objects to control the firing fields of hippocampal place cells. *Journal of Neuroscience, 17,* 2531-2542.

Cressant, A., Muller, R.U., & Poucet, B. (1999). Further study of the control of place cell firing by intra-apparatus objects. *Hippocampus, 9,* 423-431.

Dalla Barba, G. (1997). Recognition memory and recollective experience in Alzheimer's disease. *Memory, 5(6),* 657-672.

De Hoz, L., Knowx, J., & Morris, R.G. (2003). Longitudinal axis of the hippocampus: both septal and temporal poles of the hippocampus support water maze spatial learning depending on the training protocol. *Hippocampus, 13(5),* 587-603.

Delatour, B., & Gisquet-Verrier, P. (1996). Prelimbic cortex specific lesions delayed variable response tasks in the rat. *Behavioral Neuroscience, 110(6),* 1282-1298.

Delatour, B., & Gisquet-Verrier, P. (2000). Functional role of rat prelimbic-infralimbic corticies in spatial memory: evidence for their involvement in attention and behavioural flexibility. *Behavioural Brain Research, 109(1),* 113-128.

Dere, E., Huston, J. P., & De Souza Silva, M. A. (2005*a*). Episodic-like memory in mice: simultaneous assessment of object, place and temporal order memory. *Brain Research and Brain Research Protocols, 16,* 10-9.

Dere, E., Huston, J. P., & De Souza Silva, M. A. (2005*b*). Integrated memory for objects, places, and temporal order: evidence for episodic-like memory in mice. *Neurobiology of Learning & Memory, 84*, 214-21.

Desai, A.K., & Grossberg, G.T. (2005). Diagnosis and treatment of Alzheimer's disease. *Neurology, 28 (3),* 34-39.

Dix, S.L., & Aggleton, J.P. (1999). Extending the spontaneous preference test of recognition: evidence of object-location and object-context recognition. *Behavioural Brain Research*, 99, 191-200.

Dodart, J.C., Meziane, H., Mathis, C., Bale, K.R., Paul, S.M., & Ungerer, A. (1999). Behavioral disturbances in transgenic mice overexpressing the V717F beta-amyloid precursor protein. *Behavioural Neuroscience, 113(5),* 982-990.

Dong, H., Goico, B., Martin, M., Csernansky, C.A, Bertchume, A., & Csernansky, J.G. (2004). Modulation of hippocampal cell proliferation, memory, and amyloid plaque deposition in APPsw (Tg2576) mutant mice by isolation stress. *Neuroscience, 127(3),* 601-609.

Dong, H., Martin, M.V., Chambers, S., & Csernansky, J.G. (2007). Spatial relationship between synapse loss and beta-amyloid deposition in Tg2576 mice. *The Journal of Comparative Neurology, 500,* 311-21.

Douglas, R.J. (1966). Cues for spontaneous alternation. *Journal of Comparative and Physiological Psychology, 62,* 171-183.

Dudchenko, P.A. (2001). How do animals actually solve the T-maze. *Behavioral Neuroscience, 115(4),* 850-860.

Dudchenko, P.A., & Davidson, M. (2002). Rats use a sense of direction to alternate on T-mazes located in adjacent rooms. *Animal Cognition, 5,* 115-118.

Duff, K., Eckman, C., Zehr, C., Yu, X., Prada, C.M., Perez-tur, J., Hutton, M., Buee, L., Harigaya, Y., Yager, D., Morgan, D., Gordon, M.N., Holcomb, L., Refolo, L., Zenk, B., Hardy, J., & Younkin, S. (1996). Increased amyloid – ß42(43) in brains of mice expressing mutant presenilin 1. *Nature, 383, 710-713.*

Eacottt, M.J., & Norman, G. (2004). Integrated memory for object, place, and context in rats: a possible model of episodic-like memory? *Journal of Neuroscience, 24(8),* 1948-53.

Eichenbaum, H., Dudchencko, P., Wood, E., Shapiro, M., & Tanila, H. (1999). The hippocampus, memory and place cells: Is it spatial memory of memory space? *Neuron, 23*, 209-226.

Eichenbaum, H., Stewart, C., & Morris, R.G. (1990). Hippocampal representation in place learning. *Journal of Neuroscience, 10(11),* 3531-42.

Ennaceur, A., & Delacour, J. (1988). A new one-trial test for neurobiological studies of memory in rats. 1: Behavioural data. *Behavioural Brain Research, 31(1),* 47-59.

Ennaceur, A., Neave, N., & Aggleton, J.P. (1997). Spontaneous object recognition and object location memory in rats: the effects of lesions in the cingulated cortices, the medial prefrontal cortex, the cingulum bundle and the fornix. *Experimental Brain Research, 113(3),* 509-519.

Etienne, A.S., & Jeffery, K.J. (2004). Path integration in mammals. *Hippocampus, 14(2),* 180-192.

Eysenck, M.W., & Keane, M.T. (1995). *Cognitive psychology: A student's handbook* (3rd edition). UK: Psychology Press.

Ferbinteanu, J., & McDonald, R.J. (2001). Dorsal/ventral hippocampus, fornix, and conditioned place preference *Hippocampus. 11(2),* 187-200.

Ferbinteanu, J., Ray, C., & McDonald, R.J. (2003). Both dorsal and ventral hippocampus contribute to spatial learning in Long-Evans rats. *Neuroscience Letters 345(2),* 131-135.

Fleischman, D. A., Wilson, R. S., Gabrieli, J. D., Schneider, J. A., Bienias, J. L., & Bennett, D. A. (2005). Implicit memory and Alzheimer's disease neuropathology. *Brain, 128,* 2006-2015.

Forstl, H., & Kurz, A. (1999). Clinical features of Alzheimer's disease. European Archives of Psychiatry and clinical neuroscience, *249,* 288-290.

Fortin, N.J., Agster, K.L., & Eichenbaum, H.B. (2002). Critical role of the hippocampus in memory for sequences of events. *Nature Neuroscience, 5(5),* 458-462.

Fortin, N.J., Wright, S.P., & Eichenbaum, H. (2004). Recollection-like memory retrieval in rats is dependent on the hippocampus. *Nature, 431,* 188-91.

Fox, N.C., Warrington, E.K., Seiffer, A.L., Agnew, S.W. & Rossor, M.N. (1998). Presymptomatic cognitive deficits in individual at risk of familial Alzheimer's disease: a longitudinal prospective study. *Brain, 121,* 1631-1639.

Frautschy, S.A., Yang, F., Irrizarry, M., Hyman, B., Saido, T.C., Hsiao, K., & Cole, G.M. (1999). Microglial response to amyloid plaques in APPsw transgenic mice. Comment in *American Journal of Pathology, 154(6),* 307-317.

Gaffan, D. (1991) Spatial organization of episodic memory. *Hippocampus,*1, 262-264.

Gallistel, C.R. (1990*a*). *The organization of learning.* MIT Press, Cambridge, MA.

Gallistel, C.R. (1990*b*). Representations in animal cognition: an introduction. *Cognition. 1990, 37(1-2),* 1-22.

Gallo, D. A., Sullivan, A. L., Daffner, K. R., Schacter, D. L., & Budson A. E. (2004). Associative recognition in Alzheimer's disease: evidence for impaired recall-to-reject. *Neuropsychology, 18,* 556-563.

Garcia, M.F., Gordon, M.N., Hutton, M., Lewis, J., McGowan, E., Dickey, C.A., Morgan, D., & Arendash, G.W. (2004). The retinal degeneration (rd) gene seriously impairs spatial cognitive performance in normal and Alzheimer's transgenic mice. *Neuroreport, 15,* 73-777.

Gerlai, R. (1996). Gene-targeting studies of mammalian behavior: is it the mutation or the background genotype? *Trends in Neuroscience, 19,* 177-181.

Gilbert, P.E., & Kesner, R.P. (2002). Role of the rodent hippocampus in paired-associate learning involving associations between a stimulus and a spatial location. *Behavioral Neuroscience, 116(1),* 63-71.

Gilbert, P.E., & Kesner, R.P. (2003). Localization of function within the dorsal hippocampus: the role of the CA3 subregion in paired-associate learning. (2003) *Behavioral Neuroscience, 117(6),* 1385 – 1394.

Gilbert, P.E., & Kesner, R.P. (2004). Memory for objects and their locations: the role of the hippocampus in retention of object-place associations. *Neurobiology of Learning and Memory, 81(1),* 39-45.

Gluck, M.A. & Myers, C.E. (1993). Hippocampal mediation of stimulus representation: a computational theory. *Hippocampus, 3,* 491-516.

Gluck, M.A., Myers, C.E., Nicolle, M.M., & Johnson, S. (2006). Computational models of the hippocampal region: implications for prediction of risk for Alzheimer's disease in non-demented elderly. *Current Alzheimer Research, 3,* 247-57.

Goedert, M. (1993). Tau protein and the neurofibrillary pathology of Alzheimer's disease. *Trends in Neuroscience, 16,* 460-465.

Good, M.A., Barnes, P., Staal, V., McGregor, A, & Honey, R.C. (2007*a*). Context-but not familiarity-dependent forms of object recognition are impaired following excitotoxic hippocampal lesions in rats. *Behavioral Neuroscience, 121(1)* 218-223.
Good, M.A., Hale, G., & Staal, V. (2007*b*) Impaired "episodic-like" object memory in adult APPswe transgenic mice. *Behavioral Neuroscience, 121,* 443-448.

Goodrich-Hunsaker, N.J., Hunsaker, M.R., & Kesner, R.P. (2005). Dissociating the role of the parietal cortex and dorsal hippocampus for spatial information processing. *Behavioral Neuroscience, 119,* 1307-1315.

Gorny, J.H., Gorny, B., Wallace, D.G & Whishaw, I.Q. (2002). Fibria-Fornix lesions disrupt dead reckoning (homing) component of exploratory behaviour in mice. *Learning and Memory. 9(6),* 387-394.

Gothard, K.M., Skaggs, W.E., Moore, K.M., & McNaughton, B.L. (1996). Binding of hippocampal CA1 neural activity to multiple reference frames in a landmark-based navigation task. *Journal of Neuroscience, 16(2),* 823-35.

Graf, P., & Schacter, D.L. (1985). Implicit and explicit memory for new association in normal and amnesic subjects. *Journal of experimental psychology: Learning memory and cognition, 11,* 501-518.

Gregory, G.C., Macdonald, V., Schofield, P.R., Kril, J.J., & Halliday, G.M. (2006). Difference in regional brain atrophy in genetic forms of Alzheimer's disease. *Neurobiology of Aging, 27,* 387-393.

Haass, C., Lemere, C.A., Capel, A.M., Seubert, P., Schenk, D., Lannfelt, L., & Selkoe, D.J. (1995). The Swedish mutation causes esrly-onset Alzheimer's disease by ß-secretase cleavage within the secretory pathway. *Nature medicine, 1,* 1291-1296.

Haass, C., & Selkoe, D.J. (1993). Cellular processing of ß-amyloid precursor protein and the genesis of amyloid ß-peptide. *Cell, 75,* 1039-1042.

Hafting, T., Marianne, F., Molden, S., Moser, M-B., & Moser, E.1. (2005). Microstructure of a spatial map in the enthorinal cortex. *Nature, 463, (11)*, 801-806.

Hagan, J.J., Verheijck, E.F., Spigt, M.H., & Ruigt, G.S. (1992). Behavioural and electrophysiological studies of entorhinal cortex lesions in the rat. *Physiol Behaviour, 51(2),* 255-266.

Hale, G., & Good, M. (2005). Impaired Visuospatial Recognition Memory but Normal Object Novelty Detection and Relative Familiarity Judgements in Adult Mice Expressing the APPswe Alzheimer's Disease Mutation. *Behavioural Neuroscience, 19(4),* 884-891.

Hamann, S., Monarch, E.S., & Goldstein, F.C. (2002). Impaired fear conditioning in Alzheimer's disease. *Neuropsychologia, 40,* 1187-1195.

Hardy, J. (1997). Amyloid, the presenilins and Alzheimer's disease. *Trends in Neuroscience, 20,* 154-159.

Hardy J. (2006). Has the amyloid cascade hypothesis for Alzheimer's disease been proved? *Current Alzheimer Research, 3,* 71-73.

Hart, S., & Semple, J.M. (1990). *Neuropsychology and the Dementias.* Lawrence Erlbaum Associates.

Head, E., Callahan, H., Muggen, B.A., Cotman, C.W., & Milgram, N.W. (1988). Visual-discrimination learning ability and β-Amyloid accumulation in the dog. *Neurobiology of Aging, 19(5),* 415-425.

Higgins, G.A, & Jacobsen, H. (2003). Transgenic mouse model of Alzheimer's disease: phenotype and application. *Behavioural Pharmacology, 14,* 419-438.

Hock, J., & Lamb, Jr, B. (2001). Transgenic mouse models of Alzheimer's disease. *Trends in Genetics 17 (10)* 7- 12.

Holcomb, L., Gordon, M.N., McGowan, E., Yu, X., Benkovic, S., Jantzen, P., Wright, K., Saad, I., Mueller, R., Morgan, D., Sanders, S., Zehr, C., O'Campo, K., Hardy, J., Prada, C.M., Eckman , C., Younkin, S., Hsiao, K., & Duff, K. (1998). Accelerated Alzheimer-type phenotype in transgenic mice carrying both mutant amyloid precursor protein and presenilin 1 transgenes. *Nature Medicine, 4,* 97-100.

Hsiao, K., Chapman, P., Nilsen, S., Eckman, C., Harigaya, Y., Younkin, S., Yang, F., & Cole, G. (1996). Correlative Memory Deficits, Aβ Elevation, and Amyloid Plaques in Transgenic Mice. *Science, 274,* 99-102.

Hyman, B.T., Van Hoesen, G.W., Damasio, A.R., & Barnes, C.L. (1984). Alzheimer's disease: Cell specific pathology isolated the hippocampal formation, *Science,* 225, 1168-1170.

Jacobs, L.F., & Schenk, F. (2003). Unpacking the cognitive map: the parallel map theory of hippocampal function. *Psychological Review, 110(2),* 285-315.

Jacobsen, J.S., Wu, C.C., Redwine, J.M., Comery, T.A., Arias, R., Bowlby, M., Martone, R., Morrison, J.H., Pangalos, M.N., Reinhart, P.H., & Bloom, F.E. (2006). Early-onset behavioral and synaptic deficits in a mouse model of Alzheimer's disease. *Proceedings of the National Academy of Sciences, USA, 103,* 5161-5166.

Janus, C., Chishti, M.A., & Westaway, D. (2000). Transgenic mouse models of Alzheimer's disease. *Physiology & Behaviour, 73,* 873-886.

Janus, C., Phinney, A.L., Chishti, M.A., & Westaway, D. (2001). New developments in animal models of Alzheimer's disease. *Current Neurology and Neuroscience Reports,* 1, 451-7.

Jarrard, L.E. (1983). Selective hippocampal lesions and behaviour. Effects of Kainic acid lesions on performance of place and cues tasks. *Behavioural Neuroscience, 97,* 873-889.

Jarrard, L.E. (1989). On the use of ibotenic acid to lesion selectively different components of the hippocampal formation. *Journal Neuroscience Methods, 29(3)* 251-259.

Jönsson, L., & Berr, C. (2005). Cost of dementia in Europe. *European journal of neurology 12 (Suppl 1)* 50 -53.

Jung, M.W., Wiener, S.I. & Mc Naughton, B.L. (1994). Comparison of spatial firing characteristics of units in dorsal and ventral hippocampus of the rat. *Journal of Neuroscience, 12*, 7347-56.

Kalovà, E., Vlcek, K., Jarolomovà, E., & Bures, J. (2005). Allothetic orientation and sequential ordering of places is impaired in early stages of Alzheimer's disease: corresponding results in real space tests and computer tests. *Behavioral Brain Research, 159(2),* 175-186.

Karlson, T., Johanson, I., Adolfsson, R., Nilsson, L.G., & Dubus, S. (2003). Recognition memory in Alzheimer's disease. A demonstration of a remarkable memory capacity in Alzheimer's disease. *Dementia and Geriatric Cognitive Disorders, 15(1),* 6-9.

Karpiak, S.E. (1983). Ganglioside treatment improves recovery of alternation behavior after unilateral entorhinal cortex lesion. *Experimental Neurology. 81(2),* 330-339.

Kart-Teke, E., De Souza Silva, M.A., Huston, J.P., & Dere, E. (2006). Wistar rats show episodic-like memory for unique experiences. *Neurobiology of Learning & Memory, 85*, 173-82.

Kavcic, V., & Duffy C.J. (2003). Attentional dynamics and visual perception: mechanisms of spatial disorientation in Alzheimer's disease. *Brain, 126*, 1173-1181.

Kavcic, V., Fernandez, R., Logan, D., & Duffy, C.J. (2006). Neurophysiological and perceptual Correlates on navigational impairment in Alzheimer's disease. *Brain (129),* 736-746.

Kawarabayashi, T., Younkin, L.H., Saido, T.C., Shoji, M., Hsiao-Ashe, K., & Younkin, S.G. (2001). Age-Dependent Changes in Brain, CSF, and Plasma Amyloid β Protein in the Tg2576 Transgenic Mouse Model of Alzheimer's Disease. *Journal of Neuroscience, 21(2),* 372-381.

Kearns, M.J., Warren, W.H., Duchon, A.P., & Tarr, M.J. (2003). Path integration from optic flow and body senses in a homing task. *Perception, 31(3),* 349-347.

Kesner, R.P., Gilbert, P.E., & Barua, L.A. (2002). The role of the hippocampus in memory for the temporal order of a sequence of odors. *Behavioral Neuroscience, 116(2),* 286-290.

Kesner, R.P., & Ragozzino, M.E. (2003). The role of the prefrontal cortex in object-place learning: a test of the attribute specificity model. *Behavioural Brain Research, 146,* 159-165.

King, D.L., & Arendash, G.W. (2002). Behavioural characterisation of the Tg2576 transgenic model of Alzheimer's disease through 19 months. *Physiology and Behaviour, 75,* 627-642.

King, D.L., Arendash, G.W., Crawford, F., Sterk, T., Menendez, J., & Mullan, M.J. (1999). Progressive and gender-dependent cognitive impairment in the APP(SW) transgenic mouse model for Alzheimer's disease. *Behavioural Brain Research, 103,* 145-162.

Knierim, J.J., Kudrimoti, H.S., & McNaughton, B.L. (1998). Interactions between idiothetic cues and external landmarks in the control of place cells and head direction cells. *Journal of Neurophysiology , 80(1),* 425-46.

Knierim, J.J., Lee, I., & Hargreaves, E.L. (2006). Hippocampal place cells: parallel input streams, subregional processing, and implications for episodic memory. *Hippocampus*, *16,* 755-764.

Knight, R.G. (1998). Controlled and automatic memory process in Alzheimer's disease. *Cortex, 34*, 427-435.

Kobayashi, D.T., & Chen, K.S., (2005). Behavioural phenotypes of amyloid-based genetically modified mouse models of Alzheimer's disease. *Genes, Brain and Behavior, 4*, 173-196.

Kolb, B. (1984). Functions of the frontal cortex of the rat: A Comparative review. *Brain Research Reviews, 8,* 65-98.

Kolb, B. (1990*a*). Posterior parietal and temporal associations cortex. In B. Kolb & R. C. Tees (Eds). *The cerebral cortex of the rat* (pp. 459-471). Cambridge, MA: MIT Press.

Kolb, B. (1990*b*). Prefrontal cortex. In B. Kolb & R. C. Tees (Eds). *The cerebral cortex of the rat* (pp. 437-458). Cambridge, MA: MIT Press.

Kosik, K.S., Orecchio, L.D., Binder, L., Trojanowski, J.Q., Lee, V.M., & Lee, G. (1988). Epitotes that span the tau module are shared with paired filaments. *Neuron, 1,* 817-825.

Kotilinek, L.A., Bacskai, B., Westerman, M., Kawarabayashi, T., Younkin, L., Hyman B.T., Younkin, S., & Ashe, K.H. (2002). Reversible memory loss in a mouse transgenic model of Alzheimer's disease. *Journal of Neuroscience*, *22*, 6331-6335.

Kubie, J.L., & Muller, R.U. (1991). Multiple representations in the hippocampus. *Hippocampus*, Jul;1(3), 240-242.

Kumaran, D., & Maguire, E.A. (2005). The human hippocampus; cognitive maps or relational memory? *The journal of neuroscience, 25 (31),* 7254-7259.

Lee, A.C.H., Rahman, S., Hodges, J.R., Sahakian, B.J., & Graham, K.S. (2003). Associative and recognition memory for novel objects in dementia: implications for diagnosis. *European journal of Neuroscience, 18,* 1660-1670.

Lehman, E.J., Kulnane, L.S., & Lamb, B.T. (2003). Alterations in beta-amyloid Production and deposition in brain regions of two transgenic models. *Neurobiology of Aging, 24(5),* 645-653.

Lehtovirta, M., Soininen, H., Helisalmi, S., Mannermaa, A., Helkala, E.L., Hartikainen, P., Hanninen, T., Rynanen, M., & Riekkinen, P.J. (1996). Clinical and neuropsychological characteristics in familial and sporadic Alzheimer's disease: Relation to apolipoprotein E polymorphism. *Neurology, 46,* 413-419.

Lemere, C.A., Blusztajn, J.K., Yamaguchi, H., Wisniewski, T., Saido, T.C., & Selkoe, D.J. (1996). Sequence of deposition of heterogeneous amyloid β-peptides and APOE in Down Syndrome: Implications for initial events in amyloid plaque formation. *Neurobiology of disease, 3,* 16-32.

Lenck-Santini, P.P., Muller, R.U., Save, E., Poucet, B. (2002). Relationships between place cell firing fields and navigational decisions by rats. *Journal of Neuroscience, 22(20),* 9035-9047.

Lenkin-Santini, P.P., Rivard, B., Muller, R.U., & Poucet, B. (2005). Study of CA1 Place Cell Activity and Exploratory Behaviour Following Spatial and Nonspatial Changes in the Environment. *Hippocampus, 15,* 356-369.

Lenck-Santini, P.P., Save, E., & Poucet, B. (2001). Evidence for a relationship between place-cell spatial firing and spatial memory performance. *Hippocampus, 11(4),* 377-90.

Lesné, S., Koh, M.T., Kotilinek, L., Kayed, R., Glabe, C.G., Yang, A., Gallagher, M., Ashe, K.H. (2006). A specific amyloid-beta protein assembly in the brain impairs memory. *Nature, 440 (7082),* 352-357.

Maaswinkel, H., & Jarrard, L.E., Whishaw, I.Q. (1999). Hippocampectomized rats are impaired in homing by path integration. *Hippocampus, 9(5),* 553-61.

Mapstone, M., Steffenella, T.M., & Duffy, C.J. (2003). A visuospatial variant of mild cognitive impairment: getting lost between aging and AD. *Neurology, 60(5),* 802-808.

Marshall, V.J., McGregor, A., Good, M., & Honey, R.C. (2004). Hippocampal lesions modulate both associative and nonassociative priming. *Behavioral Neuroscience, 118(2),* 377-382.

Masters, C.L., Simms, G., Weinman, N.A, Multhaup G., McDonald B.L., & Beyreuther, K. (1985). Amyloid plaque core protein in Alzheimer disease and Down syndrome *Proceedings of the National Academy of Sciences, USA 82*, 4245-4249.

McNaughton, B.L., Barnes, C. A., Gerrard, J.L., Gothard, K., Jung, M.W., Knierm, J.J., Kudrimoti, H., Quinn. Y., Skagga, W.E., Suster, M., & Weaver, K.L., (1996). Deciphering the hippocampal polyglot: The hippocampus as a path integration system. *The Journal of Experimental Biology, (199),* 173-185.

McNaughton, B.L., Battaglia, F.P., Jensen, O., Moser, E.I., & Moser, M.B. (2006). Path integration and the neural basis of the 'cognitive map'. *Nature Reviews Neuroscience 7(8),* 663-78.

Middei, S., Daniele, S., Caprioli, A., Ghirardi, O., Ammassari-Teule, M. (2006). Progressive cognitive decline in a transgenic mouse model of Alzheimer's disease overexpressing mutant hAPPswe. *Genes Brain Behaviour. 5(3),*249-56.

Middei, S., Geracitano, R., Caprioli, A., Mercuri, N., & Ammassari-Teule, M. (2004*a*). Preserved frontal-striatal plasticity and enhanced procedural learning in a transgenic mouse model of Alzheimer's disease overexpresing mutant $_{h}APP_{swe.}$ *Learning and memory, 11(4),* 447-452.

Middei, S., Restivo, L., Sgobio, C., Passino, E., & Ammassari-Teule, M. (2004*b*). Reversible inactivation of hippocampus and dorsolateral striatum in c57BL/6 and DBA/2 inbred mice failed to show interaction between memory systems in these genotypes. *Behavioural Brain Research. 154(2),* 527-537.

Mitchell, J.B., & Laiacona, J. (1998). The medial frontal cortex and temporal memory: test using spontaneous exploratory behaviour in the rat. *Behavioural Brain Research, 97(1-2),* 107-113.

Mitchell, K.J., Johnson, M.K., Raye, C.L., & D'Esposito, M. (2000). fMRI evidence of age-related hippocampal dysfunction in feature binding in working memory. *Brain Research. Cognitive Brain Research, 10,* 197-206.

Mizumori, S.J., McNaughton, B.L., Barnes, C.A., Fox, K.B. (1989). Preserved spatial coding in hippocampal CA1 pyramidal cells during reversible suppression of CA3c output: evidence for pattern completion in hippocampus. *Journal of Neuroscience, 9(11),* 3915-28.

Monacelli, A.M., Cushman, L.A., Kavcic, V., & Duffy, C.J. (2003). Spatial disorientation in Alzheimer's disease: the remembrance of things passed. *Neurology, 61(11),* 1491-7.

Morris, R.G.M. (1981). Spatial localisation does not require the presence of local cues. *Learning and Motivation, 12,* 238-260.

Morris, R.G. (2001). Episodic-like memory in animals: psychological criteria, neural mechanisms and the value of episodic-like tasks to investigate animal models of neurodegenerative disease. *Philosophical Transactions of the Royal Society, London, B, 356,* 1453-1465.

Morris, R.G., Anderson, E., Lynch, G.S., & Baudry, M. (1986). Selective impairment of learning and blockade of long-term potentiation by an N-methyl-D-aspartate receptor antagonist, AP5. *Nature, 319(6056),* 774-776.

Morris, R.G.M., Garrud, P., Rawlins, J.N.P., & O'Keefe, J. (1982). Place navigation impaired in rats with hippocampal lesions. *Nature, 297,* 681-683.

Moscovitch, M., Nadel, L., Winocur, G., Gilboa, A., & Rosenbaum, R.S. (2006). The cognitive neuroscience of remote episodic, semantic and spatial memory. *Current Opinion in Neurobiology, 6,* 179-190.

Mount, C., & Downton, C. (2006). Alzheimer disease: progress or profit? *Nature Medicine, 7,* 780-784.

Mullan, M., Crawford, F., Axelman, K., Houlden, H., Lilius, L., Winbald, B., & Lannfelt, L. (1992). A pathogenic mutation for probable Alzheimer's disease in the APP gene at the N-terminus of ß-amyloid. *Nature genetics, 1,* 345-347.

Mumby, D.G. (2001). Perspectives on object-recognition memory following hippocampal damage: lessons from studies in rats. *Behavioural Brain Research, 127(1-2),* 159-181.

Mumby, D.G., Gaskin, S., Glenn, M.J., Schramek, T.E., & Lehmann, H. (2002). Hippocampal damage and exploratory preferences in rats: Memory for objects, places, and contexts. *Learning and Memory. 9(2),* 49-57.

Nagahama, Y., Okina, T., Norio, S., Matsuzaki, S., Yamauchi, H., Nabatame, H., & Matsuda, M. (2003). Factor structure of a modified version of the Wisconsin Card Sorting Test: An analysis of executive deficit in Alzheimer's Disease and mild cognitive impairment. *Dementia and Geriatric Cognitive Disorders, 16(2),* 103-112.

Nestor, P.J., Scheltens, P., & Hodges, J.R. (2004). Advances in the early detection of Alzheimer's disease. *Nature Medicine, 10,* 34-41.

Nunan, J., & Small, D.H. (2000). Minireview: Regulation of APP cleavage by α, β and γ- secretases. *FEBS Letters, 483,* 6-10.

O'Brien, H.L., Tetewsky, S.J., Avery, L.M., Cushman, L.A., Makous, W., & Duffy, C.J. (2001). Visual mechanisms of spatial disorientation in Alzheimer's disease. *Cerebral Cortex 11(11),* 1083-92.

Obnibene, E., Middei, S., Daniele, S., Adriani, W., Ghirardi, O., Caprioli, A., & Laviola, G. (2005). Aspects of spatial memory and behaviour disinhibition in Tg2576 transgenic mice as a model of Alzheimer's disease. *Behavioural Brain Research, 156,* 225-232.

O'Keefe, J. & Burgess, N. (2005). Dual phase and rate coding in hippocampal place cells: theoretical significance and relationship to entorhinal grid cells. *Hippocampus, 15(7),* 853-66.

O'Keefe, J., & Conway, D.H. (1978). Hippocampal place units in the freely moving rat: why they fire where they fire. *Experimental Brain Research, 31(4),* 573-590.

O'Keefe, J., & Dostrovsky, J. (1971). The hippocampus as a spatial map: Preliminary evidence from activity in freely moving rat. *Brain research (34)* 171-175.
O'Keefe, J., & Nadel, L. (1978). *The Hippocampus as a Cognitive Map.* Oxford University Press.

O'Keefe, J., & Speakman, A. (1987). Single unit activity in the rat hippocampus during a spatial memory task. *Experimental Brain Research. 68(1)* 1-27.

Olson, I.R., Page, K., Moore, K.S., Chatterjee, A., & Verfaellie, M. (2006). Working memory for conjunctions relies on the medial temporal lobe. *Journal of Neuroscience, 26,* 4596-601.

Olton, D.S., Becker, J.T., & Handelmann, G.E. (1979). Hippocampus, space and memory. *The Behavioural and Brain Sciences, 2,* 313-366.

Olton, D.S., Walker, J.A., & Gage, F.H. (1978). Hippocampal connections and spatial discrimination. *Brain Research, 139(2),* 295-308.

O'Reilly, R.C., & Rudy, J.W. (2001). Conjunctive representations in learning and memory: principles of cortical and hippocampal function. *Psychological Review, 108,* 311-345.

Oswald, C.J., Bannerman, D.M., Yee, B.K., Rawlins, J.N., Honey, R.C., Good, M. (2003). Entorhinal cortex lesions disrupt the transition between the use of intra- and extramaze cues for navigation in the water maze. *Behavioural Neuroscience, 117(3),* 5880595.

Packard, M.G. (1999). Glutamate infused posttraining into the hippocampus or caudate-putamen differentially strengthens place and response learning. Proc. Natl. Acad. Sci, *96*: 12881-12886.

Packard, M.G., & McGaugh, J.L. (1996). Inactivation of the hippocampus or caudate nucleus with lidocaine differentially affects expression of place and response learning. *Neurobiology of learning and memory, 65*, 65-72.

Pai, M-C., & Jacobs, W.J. (2004). Topographical disorientation in community-residing patients with Alzheimer's disease. *International Journal Of Geriatric Psychiatry, 19,* 250-255.

Parkinson, J.K., Murray, E.A., & Mishkin, M. (1998). A selective mnemonic role for the hippocampus in monkeys: memory for the location of objects. *Journal of Neuroscience, 8(11),* 4159-67.

Parron, C., Poucet, B., & Save, E. (2006). Cooperation between the hippocampus and the entorhinal cortex in spatial memory: a disconnection study. *Behavioural Brain Research, 170,* 99-109.

Passini, R., Rainville, C., Marchand, N., & Joanette, Y. (1995). Wayfinding in dementia of the Alzheimer type: planning abilities. *Journal of Clinical Experimental Neuropsychololgy, 17(6),* 820-32.

Passino, E., Middei, S., Restivo, L., Bertaina Anglade, V., & Ammassari-Teule, M. (2002). Genetic approach to variability of memory systems: Analysis of place Vs response learning and Fos-related expression in hippocampal and striatal areas of C57BL/6 and DBA/2 mice. *Hippocampus. 12,* 63-75.

Perry, R.J., & Hodges, J.R. (2002). Differentiating frontal and temporal variant frontotemporal demential and Alzheimer's disease, *Neurology, 54,* 2277-2284.

Peuskens, H., Sunaert, S., Dupont, P., Van Hecke, P., & Orban, G.A. (2001). Human brain regions involved in heading estimation. *Journal of Neuroscience, 21(7),* 2451-2461.

Poldrack, R.A., & Packarf, M.G., (2003). Competition among multiple memory systems: converging evidence from animal and human brain studies. *Neuropsychologia, 41(3),* 245-251.

Pompl, P.N., Mullan, M.J., Bjugstad, B., & Arendash, G.W. (1999). Adaption of the circular platform spatial memory task for mice; use in detecting cognitive impairment in the APP_{sw} transgenic mouse model of Alzheimer's disease. *Journal of Neuroscience Methods, 87,* 87-95.

Potvin, O., Allen, K., Thibaudeau, G., Dore, F.Y., & Goulet, S. (2006). Performance on spatial working memory tasks after dorsal or ventral hippocampla lesions and adjacent damage to the subiculum. Behavioural Neuroscience, *120(2),* 413-422.

Poucet, B. (1989). Object exploration, habituation, and response to a spatial change in rats following septal or medial frontal cortical damage. *Behavioral Neuroscience, 103(5),* 1009-16.

Poucet, B. (1990). A further characterization of the spatial problem-solving deficit induced by lesions of the medial frontal cortex in the rat. *Behavioral Brain Research, 41(3),* 229-237.

Poucet, B. (1993). Spatial cognitive maps in animals: new hypotheses on their structure and neural mechanisms. *Psychological Review, 100,* 163-182.

Poucet, B., & Thinus-Blanc, C., & Muller, R.U. (1994). Place cells in the ventral hippocampus of rats. *Neuroreports, 5(16),* 2045-8.

Pouzet, B., Welzl, H., Gubler, M.K., Broersen, L., Veenman, C.L., Feldon, J., Rawlins, J.N., & Yee, B.K. (1999). The effects of NMDA-induced retrohippocampal lesions on performance of four spatial memory tasks known to be sensitive to hippocampal damage in the rat. *European journal of Neuroscience, 11(1),* 123-140.

Ramirez, J.J., & Stein, D.G. (1984). Sparing and recovery of spatial alternation performance after entorhinal cortex lesions in rats. *Behavioural Brain Research, 13(1),* 53-61.

Redish, A.D., & Touretzky, D.S. (1997). Cognitive maps beyond the hippocampus. *Hippocampus, 7(1),* 15-35.

Reed, J.M., & Squire, L.R. (1997). Impaired recognition memory in patients with lesions limited to the hippocampal formation. *Behavioural Neuroscience, 111(4),* 667-675.

Reeves, T.M., & Smith, D.C. (1987). Reinnervation of the dentate gyrus and recovery of alternation behavior following entorhinal cortex lesions. *Behavioural Neuroscience. 101(2),* 179-186.

Rivard, B., Li, Y., Lenck-Santini, P.P., Poucet, B., & Muller, R.U. (2004). Representation of objects in space by two classes of hippocampal pyramidal cells. *Journal of General Physiology, 124,* 9-25.

Rizzo, M., Anderson, S.W., Dawson, J., Myers, R., & Ball, K. (2000). Visual attention impairments in Alzheimer's Disease, *Neurology, 54,* 1954-1959.

Rogers, J.L., Hunsaker, M.R., & Kesener, R.P. (2006). Effects of ventral and dorsal CA1 subregional lesions on trace fear conditioning. *Neurobiology of Learning and Memory, 86(1),* 72-81.

Rogers, J.L., & Kesner, R.P. (2006). Lesions of the dorsal hippocampus or parietal cortex differentially affect spatial information processing. *Behavioural Neuroscience, 120(4),* 852-860.

Rolls, E.T., & Kesner, R.P. (2006). A computational theory of hippocampal function, and empirical tests of the theory. *Progress in Neurobiology, 79,* 1-48.

Roullet, P., Lassalle, J.M., Jegat, R. (1993). A study of behavioral and sensorial bases of radial maze learning in mice. *Behavioural and Neural Biology, 59(3),*173-179.

Samsonovich, A.V., & Ascoli, G.A. (2005). A simple neural network model of the hippocampus suggesting its pathfinding role in episodic memory retrieval. *Learning and Memory, 12(2),* 193-208.

Samsonovich, A., & McNaughton, B.L. (1997). Path integration and cognitive mapping in a continuous attractor neural network model. *Journal of Neuroscience, 17(15),* 5900-5920.

Save, E., & Moghaddam, M. (1996). Effects of lesions of the associative parietal cortex on the acquisition and use of spatial memory in egocentric and allocentric navigation tasks in the rat. *Behavioural Neuroscience, 110(1),* 74-85.

Save, E., Paz-Villagran, V., Alexinsky, T., & Poucet, B. (2005). Functional interaction between the associative parietal cortex and hippocampal place cell firing in the rat. *European Journal of Neuroscience. 21(2),* 522-30.

Save, E., Poucet., B., Buhot, M-C., & Foreman, N. (1992). Object Exploration and Reactions to Spatial and Nonspatial Changes in Hooded Rats Following Damage to the Parietal Cortex of Hippocampal formation. *Behavioral Neuroscience, 106,* 3 447-456.

Save, E., & Poucet, B. (2000*a*). Involvement of the hippocampus and associative parietal cortex in the use of proximal and distal landmarks for navigation. *Behavioural Brain Research, 109(2),* 195-206.

Save, E., & Poucet, B. (2000*b*). Hippocampal-parietal cortical interactions in spatial cognition. *Hippocampus, 10(4),* 491-499.

Save, E., Poucet, B., & Guazzelli, A. (2001). Dissociation of the Effects of Bilateral Lesions of the Dorsal Hippocampus and Parietal Cortex on Path Integration in the Rat. *Behavioral Neuroscience, 115(6),* 1212-1223.

Schenk, F., & Morris, R.G. (1985). Dissociation between components of spatial memory in rats after recovery from the effects of retrohippocampal lesions. *Experimental Brain Research, 58(1),* 11-28.

Schroeder, J.P, Wingard, J.C., & Packard, M.G. (2002). Post-training Reversible Inactivation of Hippocampus Reveals Interference Between Memory Systems. *Hippocampus 12,* 280 – 284.

Selkoe, D.J. (1994). Normal and Abnormal psychology of the β-amyloid precusor protein. *Annual Review of Neuroscience, 17,* 489-517.

Selkoe, D.J. (2001). Alzheimer's disease: Genes, proteins and therapy. *Physiological Reviews, 81,* 741-766.

Simon, E., Leach, L., Winocur, G., & Moscovitch, M. (1994). Intact primary memory in mild to moderate Alzheimer disease: indices from the California Verbal Learning Test. *Journal of Clinical and Experimental Neuropsychology,16,* 414-22.

Skov-Rackette, S.I., & Shuttleworth, S.J. (2005). What do rats learn about the geometry of Object Arrays? Tests with Exploratory Behaviour. *Journal of Experimental Psychology: Animal Behaviour Processes, 31(2),* 142-154.

Smith, D.M., & Mizumori, S.J. (2006). Hippocampal place cells, context, and episodic memory. *Hippocampus,16*, 716-729.

Smith, P.E.M. (1998). *Key Topics in Neurology.* Oxford: Bios Scientific Publishers Ltd.

Spires, T, L., & Hyman, B.T. (2005). Transgenic Models of Alzheimer's Disease: Learning from Animals. *The journal of the American Society for Experimental NeuroTherapeutics, 2*, 423-437.

Steffenach, H-A., Witter, M., Moser, M-B., & Moser, E.I. (2005). Spatial memory in the rat requires the dorsolateral band of the enthorhinal cortex. *Neuron 45(2),* 301-313.

Stepankoya, K., Fenton, AA., Pastalkova, E., Kalina, M., Bohbot, V.D. (2004). Object-location memory impairment in patients with thermal lesions to the right or left hippocampus. *Neuropsychologia, 42(8),* 1017-1028.

Storandt, M., Kaskie, B., & Von Dras, D.D. (1998). Temporal memory for remote events in healthy aging and dementia. *Psychology of Aging, Mar;13(1),* 4-7.

Sullivan, E.V., & Sagar, H.J. (1989). Nonverbal recognition and recency discrimination deficits in Parkinson's disease and Alzheimer's disease. *Brain, 122(6),* 1503-1517.

Sutherland, R.J & Rudy, J.W. (1989). Configural association theory: The contribution of the hippocampus to learning, memory and amnesia. *Psychobiology, 17*, 129-144.

Sutherland, R.J., & Rudy, J.W. (1991). Exceptions to the rule of space. *Hippocampus, 3*, 250-252.

Swainson R, Hodges J.R., Galton C.J., Semple J., Michael A., Dunn B.D., Iddon J.L., Robbins T.W., & Sahakian B.J. (2001). Early detection and differential diagnosis of Alzheimer's disease and depression with neuropsychological tasks. *Dementia and Geriatric Cognitive Disorders, 12(4),* 265-280.

Swerdlow, R.H. (2007). Is aging part of Alzheimer's disease, or is Alzheimer's disease part of aging? *Neurobiology of Aging, 28(10)* 1465-80.

Tagliavini, F., & Pilleri, G. (1983). Neuronal counts in basal nucleus of meynert in Alzheimer's disease and in simple dementia. *Lancet, I,* 469-470.

Tanzi, R.E. (2005). The synaptic Aβ hypothesis of Alzheimer disease. *Nature Neuroscience,* 8, 977-979.

Telford, L., Howard, I.P., & Ohmi, M. (1995). Heading judgments during active and passive self-motion. *Experimental Brain Research, 104(3),* 502-10.

Tendolkar, I., Schoenfeld, A., Golz, G., Fernández, Kühl, K.P., Ferszt, R., & Heinze, H.J. (1999). Neural correlates of recognition memory with and without recollection in patients with Alzheimer's disease and health controls. *Neuroscience Letters, 263,* 45-48.

Tetewsky, S.J., & Duffy, C.J. (1999). Visual loss and getting lost in Alzheimer's disease. *Neurology, 52(5),* 958-965.

Thinus-Blanc, C., Save, E., Rossi-Arnaud, C., Tozzi, A., & Ammassari-Teule, M. (1996). The differences shown by C57BL/6 and DBA/2 inbred mice in detecting spatial novelty are subserved by a different hippocampal and parietal cortex interplay. *Behavioural Brain Research, 80(1-2),* 33-40.

Tsien, J.Z., Huerta, P.T., & Tonegawa, S. (1996). The essential role of hippocampal CA1 NMDA receptor-dependent synaptic plasticity in spatial memory. *Cell, 87(7),* 1327-38.

Tolman E.C., Ritchie, B.F., & Kalish, D. (1946). Studies in spatial learning.I. Orientation and the short-cut. *Journal of Experimental Psychology*, 36, 13-24.

Tomidokoro, Y., Harigaya, Y., Matsubara, E., Ikeda, M., Kawarabayshi, T., Shirao, S., Ishiguro, K., Okamoto, K., Younkin, S.G., & Shoji, M. (2001). Brain Aß amyloidosis in APPsw mice induces accumulation of presenilin-1 and tau. *Journal of the Neurological sciences 11,* 205-242.

Touretzky, D.S., & Redish, A.D., (1996). Theory of rodent navigation based on interacting representations of space. *Hippocampus, 6(3),* 247-470.

Tsodyks, M.. (1999). Attractor neural network models of spatial maps in hippocampus. *Hippocampus, 9(4),* 481-489.

Twamley, E.W., Ropacki, S.A. & Bondi, M.W. (2006). Neuropsychological and neuroimaging changes in preclinical Alzheimer's disease. *Journal of the International Neuropsychological Society, 12*, 707-735.

Van Hoesen, G.W., & Hyman, B.T. (1990). Hippocampal formation: anatomy and the patterns of pathology in Alzheimer's disease. *Progress in Brain Research*, 83, 445-457.

Vas, C.J. et al. (2001). Alzheimer's Disease: The Brain Killer. World Health Organization.

Wagner, A.R. (1981). SOP: A model of automatic memory processing in animal behaviour. In N.E. Spear & R.R. Miller (Eds.), *Information processing in animals: Memory mechanisms* (pp.5-47). Hillsdale, NJ: Lawrence Erlbaum Associates Inc.

Welsh, K.A., Butters, N., Hughes, J.P., Mohs, R.C., & Heyman, A. (1992). Detection and staging of dementia in Alzheimer's disease. *Archives of neurology, 49,* 448-452.

Wen, G.Y., Yang, S.Y., Kaczmarski, W., He, X.Y., & Pappas, K.S. (2002). Presence of hydroxysteroid dehydrated type 10 in amyloid plaues (APs) OF Hsiao's APP-Sw transgenic mouse brains, but absence in Aps of Alzheimer's brains. Brain Research, *954,* 115-122.

Westerman, M.A., Cooper-Blacketer, D., Mariash, A., Kotilinek, L., Kawarabayashi, T., Younkin, L.H., Carlson, G.A., Younkin, S.G., & Ashe, K.H. (2002). The Relationship between Aβ and Memory in the Tg2576 Mouse Model of Alzheimer's Disease. *Journal of Neuroscience, 22,* 1858-1867.

Whishaw, I.Q. (1998). Place learning in hippocampal rats and the path integration hypothesis. *Neuroscience Biobehaviour Review, 22(2),* 209-220.

Whishaw, I.Q., & Brooks, B.L. (1999). Calibrating space: exploration is important for allothetic and idiothetic navigation. *Hippocampus, 9(6),* 659-657.

Whishaw, I.Q., & Gorny, B. (1999). Path integration absent in scent-tracking fimbria-fornix rats: evidence for hippocampal involvement in "sense of direction" and "sense of distance" using self-movement cues. *Journal of Neuroscience, 19(11),* 4662-4673.

Whishaw, I.Q., & Jarrard, L.E. (1995). Similarities vs. differences in place learning and circadian activity in rats after fimbria-fornix section or ibotenate removal of hippocampal cells. *Hippocampus, 5 (6),* 595-604.

Whishaw, I.Q., & Maaswinkel, H. (1988). Rats with fimbria-fornix lesions are impaired in path integration: a role for the hippocampus in "sense of direction". *Journal of Neuroscienc., 18(8),* 3050-3058.

Wilcock, D.M., DiCarlo, G., Henderson, D., Jackson, J., Clar Ugen, K.E., Gordon M.N., & Morgan, D. (2003). Intracranially administered anti-Abeta antibodies reduce beta-amyloid deposition by mechanisms both independent of and associated with microglial activation. *Journal of Neuroscience, 23(9),* 3745-3751.

Willems, S., Adam, S., & Van der Linden, M. (2002). Normal mere exposure effect with impaired recognition in Alzheimer's disease. *Cortex, 38(1),* 77-86.

Wilson, M.A., & Tonegawa, S. (1997). Synaptic plasticity, place cells and spatial memory: study with second generation knockouts. *Trends in Neuroscience, 20(3),* 102-106.

Winters, B.D., Forwood, S.E., Cowell, R.A., Saksida, L.M., & Bussey, T.J. (2004). Double dissociation between the effects of peri-postrhinal cortex and hippocampal lesions on tests of object recognition and spatial memory: heterogeneity of function within the temporal lobe. *Journal of Neuroscience, 24(26),* 901-908.

Woodruff-Pak, D.S., Papka, M., Romano, S., & Li, Y.T. (1996). Eyeblink classical conditioning in Alzheimer's disease and cerebrovascular dementia. Neurobiology of Aging, *17,* 505-512.

World Health Report (2001). Burden of mental and behavioural disorders. In Prentice T. (Ed.), *The World Health Report 2001. Mental Health: New Understanding, New Hope. (pp. 19-46):* World Health Organisation.

Yen, S.H., Dickson, D.W., Crowe, A., Butler, M., & Shelanski, M.L. (1987). Alzheimer's neurofibrillary tangles contain unique epitotes and epitotes in common with heat-stable microtubule associated proteins, tau and MAP2. *American journal of Pathology, 126,* 81-91.